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# AOMC–JMS 2024

AOMC-JMS 2024

Joint Conference of
The 22nd Annual Meeting of Asian and Oceanian Myology Center and
The 10th Annual Meeting of Japan Muscle Society

Dates
**September 12 (THU)–15 (SUN), 2024**

Venue
**Nara Prefectural Convention Center, Japan**

## Program and Abstract

# We chase the *miracles* of science to improve people's lives

私たちは人々の暮らしをより良くするため、科学のもたらす奇跡を追求します。

**サノフィ株式会社**
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# Welcome Message

Dear Colleagues and Friends,

On behalf of the Asian Oceanian Myology Center (AOMC) and the Japan Muscle Society (JMS), it is my great honor to welcome you to the AOMC-JMS 2024 Joint Conference. This year's meeting, held from September 12th to 15th in the beautiful city of Nara, promises to be an extraordinary gathering of minds dedicated to the field of neuromuscular research and patient care.

The AOMC-JMS 2024 is not just a conference; it is a convergence of shared goals, diverse perspectives, and the relentless pursuit of excellence. Here, we celebrate the progress we have made in understanding neuromuscular disorders, and we embrace the challenges that lie ahead with renewed vigor and collaboration.

Our program this year is rich with innovative research presentations, educational sessions, and ample opportunities for networking. We have brought together a distinguished group of speakers who will share their latest findings and insights, stimulating discussions that will undoubtedly inspire new ideas and collaborations.

We are pleased to announce that there will be three independent collaboration sessions: one with the World Muscle Society (WMS), one with TREAT-NMD, and one with the Japanese Society of Neuropathology. I would like to extend my heartfelt thanks to WMS and the TREAT-NMD Alliance for their support in sending three and one internationally well-known experts from outside the Asian-Oceanian region, respectively. Their participation will greatly enrich our conference and provide valuable global perspectives.

Nara, with its serene temples and historic charm, offers a perfect backdrop for this meeting. Beyond the academic sessions, I encourage you to explore the cultural heritage of this ancient city, which has been the heart of Japan’s spiritual and artistic traditions.

I am particularly excited about the diverse topics we will cover this year, reflecting the multidisciplinary nature of our field. From molecular biology to clinical applications, and from patient management to cutting-edge technologies, AOMC-JMS 2024 is designed to provide a comprehensive overview that will benefit both seasoned experts and young investigators.

As we gather here, let us remember that our ultimate goal is to improve the lives of patients affected by neuromuscular diseases. Through our combined efforts, we can push the boundaries of what is possible in research and clinical practice.

Thank you for your participation and contribution to making AOMC-JMS 2024 a landmark event. I look forward to the fruitful discussions, the exchange of ideas, and the forging of new collaborations that will emerge from this conference.

Warm regards,

Ichizo Nishino, MD, PhD

Congress Chair, AOMC-JMS 2024

President, Asian Oceanian Myology Center (AOMC)

# Schedule

## Day 1/Thu, September 12th

### Room2

Oral Session
2F 201+202

10:25-10:30 **Educational Programme Opening Remarks**

10:30-12:00 **Educational Programme 1**

12:10-13:00 **Luncheon Seminar 1**
Emerging Science in SMA Treatment for Better Outcomes
Sponsored By: Biogen Japan Ltd.

13:10-14:40 **Educational Programme 2**

14:50-16:20 **Educational Programme 3**

16:30-18:00 **Educational Programme 4**
[WMS Collaborate session]

18:00-18:05 **Educational Programme Closing Remarks**

## Day 2/Fri, September 13th

### Room1

Oral Session
1F Convention Hall C

8:30-9:00 **Opening Ceremony**

9:10-9:55 **Keynote Lecture1**
Emerging Therapies for Thymidine Phosphorylase and Thymidine Kinase 2 Deficiencies

9:55-10:40 **Keynote Lecture2**
Precision medicine in Duchenne muscular dystrophy: exon skipping therapies and innovative models for personalised drug development

10:50-12:20 **Symposium 1**
**[AOMC-TREAT-NMD collaboration]**
Clinical trials in NMD
- Challenges we face

12:30-13:20 **Luncheon Seminar 2**
Long-Term Treatment Strategies for Myasthenia Gravis
Sponsored By: Alexion Pharma G.K.

13:30-15:00 **Symposium 2**
Link between Research, Clinical trials, and Patient registries

15:10-16:40 **Symposium 3**
Myositis Update

16:50-18:20 **Symposium 4**
Current Status of the Neuromuscular Field in New Member and Expectedto-be New Member Countries

### Room2

Oral Session
2F 201+202

Chairs: Yoshihide Sunada
Ichizo Nishino
Speaker: Michio Hirano

Chairs: Satish V. Khadilkar
Kazuma Sugie
Speaker: Yoshitsugu Aoki

10:50-12:20 **Oral Session 1**

12:30-13:20 **Luncheon Seminar 3**
Generalized Myasthenia Gravis Seminar -Entering a New Stage -
Sponsored By: UCB Japan Co. Ltd.

13:30-15:00 **Oral Session 2**

15:30-16:40 **Sponsored Symposium**
Multidisciplinary Approach to Muscular Dystrophy Management
Sponsored By: Pfizer Japan Inc.

16:50-18:20 **Symposium 5**
Recent advances in sarcopenia and frailty studies

19:00-21:00 **AOMC Board meeting**
(1F 107+108)

### Poster

Poster Session
2F 203+204

9:00-13:00* **Poster Set-up**
*All JMS Student Award candidates must have their posters displayed by noon.

13:00-18:30 **Poster Round**

18:30-19:45 **JMS Student Award Session/ Poster Session**

## Day 3/Sat, September 14th

### Room1
Oral Session
1F Convention Hall C

8:00-8:50
**Morning Seminar**
Exploring Neu-REFIX Beta Glucans: Mechanisms and Effects in Reducing Muscle Fibrosis and Fatigue
Sponsored By: GN Corporation Co Ltd, Japan

9:00-10:30
**Symposium 6**
Muscle atrophy and hypertrophy

10:40-12:10
**Symposium 7**
Muscle stem cells in Development, Regeneration and Homeostasis

12:35-13:25
**Luncheon Seminar 4**
The diagnosis and treatment of treatable childhood-onset neuromuscular diseases
Sponsored By: CHUGAI PHARMACEUTICAL CO., LTD.

13:30-15:00
**Symposium 8**
Muscle Mechanosensing and Metabolic Dynamics during Physical Activities

15:10-16:40
**Symposium 10**
Perspectives on skeletal muscle and organ interactions in homeostatis and disease

16:50-18:50
**JSNP Joint Symposium**
Pathomechanism of OPDM and related disorders

19:00-20:30
**Networking Dinner**
(2F 205+206)

### Room2
Oral Session
2F 201+202

9:00-10:30
**AOMC Young Investigator Award Session**

10:40-12:30
**JMS Young Investigator Award Session**

13:30-15:00
**Symposium 9**
Treatable Neuro Muscular Disorders

15:10-16:40
**Symposium 11**
Other myopathies

17:00-18:30
**Oral Session 3**

### Poster
Poster Session
2F 203+204

9:00-18:00
**Poster Round**

## Day 4/Sun, September 15th

### Room1
Oral Session
1F Convention Hall C

8:00-8:50
**JMS Student Award Session**

9:00-10:30
**Symposium 12**
Advancing Next-Generation Therapeutic Modalities for Muscular Disorders

10:40-12:10
**Symposium 13**
New technologies and models to facilitate muscle research

12:20-13:10
**Luncheon Seminar 5**
Treatment Strategies for Generalized Myasthenia Gravis
Sponsored By: argenx Japan K.K.

13:20-14:20
**Award and Closing ceremony**

### Room2
Oral Session
2F 201+202

9:30-12:10
**Clinical Pathological Conference**

12:20-13:10
**Luncheon Seminar 6**
Updates on GNE Myopathy Treatment in Japan
Sponsored By: Nobelpharma Co., Ltd

### Poster
Poster Session
2F 203+204

9:00-13:00
**Poster Round**

13:00-15:00
**Poster Removal**

# Access Map

## Nara Prefectural Convention Center

1-691-1 Sanjooji, Nara 630-8013 Nara, Japan（TEL: +81-0742-32-2290）
https://www.nara-cc.jp/english/


Nara Kotsu Bus Nara-shicho-mae — Immediately after getting off

Kintetsu Shin-Omiya Stn. — About 10 min. on foot

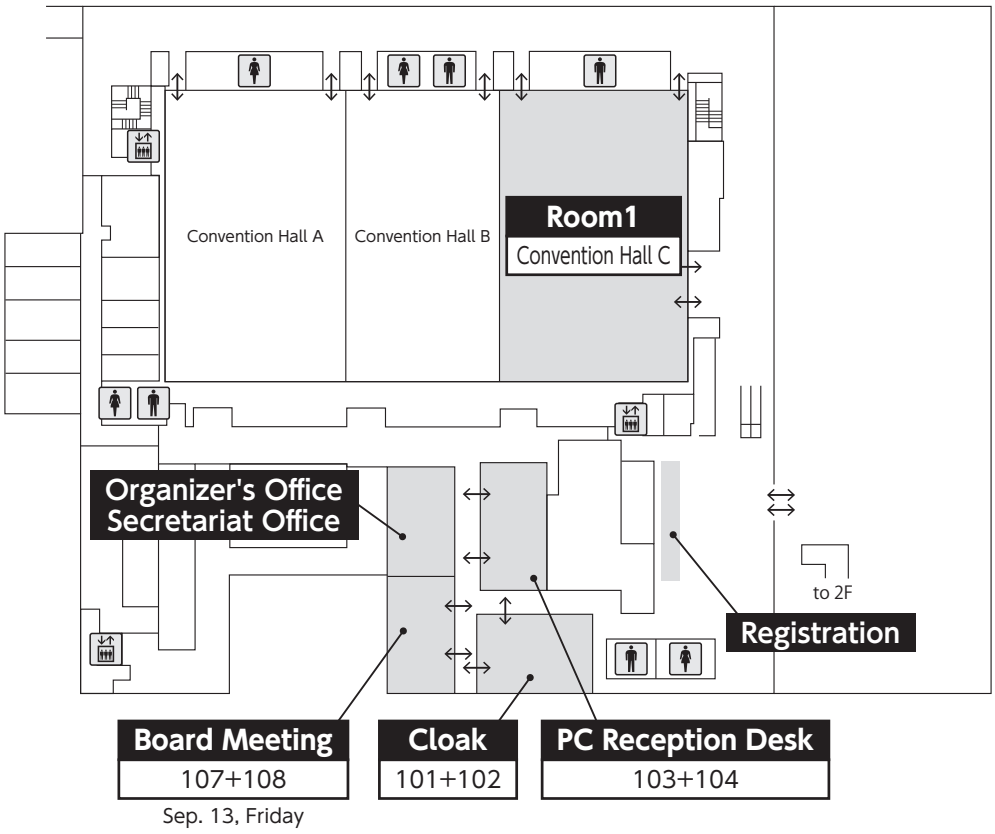
JR Nara Stn. — About 15 min. on foot

| | | | |
|---|---|---|---|
| From Kansai International Airport | by Airport Bus | About 100 min. ※ | |
| From Osaka International Airport (Itami) | by Airport Bus | About 60 min. | |
| From JR Kyoto Stn. | by JR | About 50 min. | JR Nara Stn. |
| From JR Shin-Osaka Stn. Osaka Stn. | by JR | About 60 min. | JR Nara Stn. |
| From Kintetsu Nagoya Stn. | by Kintetsu Limited Express | About 2h 30 min. | Kintetsu-Nara Stn. |
| From Kintetsu Kyoto Stn. | by Kintetsu | About 45 min. | Kintetsu Shin-Omiya Stn. |
| | by Kintetsu Limited Express | About 35 min. | Kintetsu-Nara Stn. |
| From Osaka-Namba Stn. | by Kintetsu | About 35 min. | Kintetsu Shin-Omiya Stn. → Kintetsu-Nara Stn. |

**Nara Prefectural Convention Center**

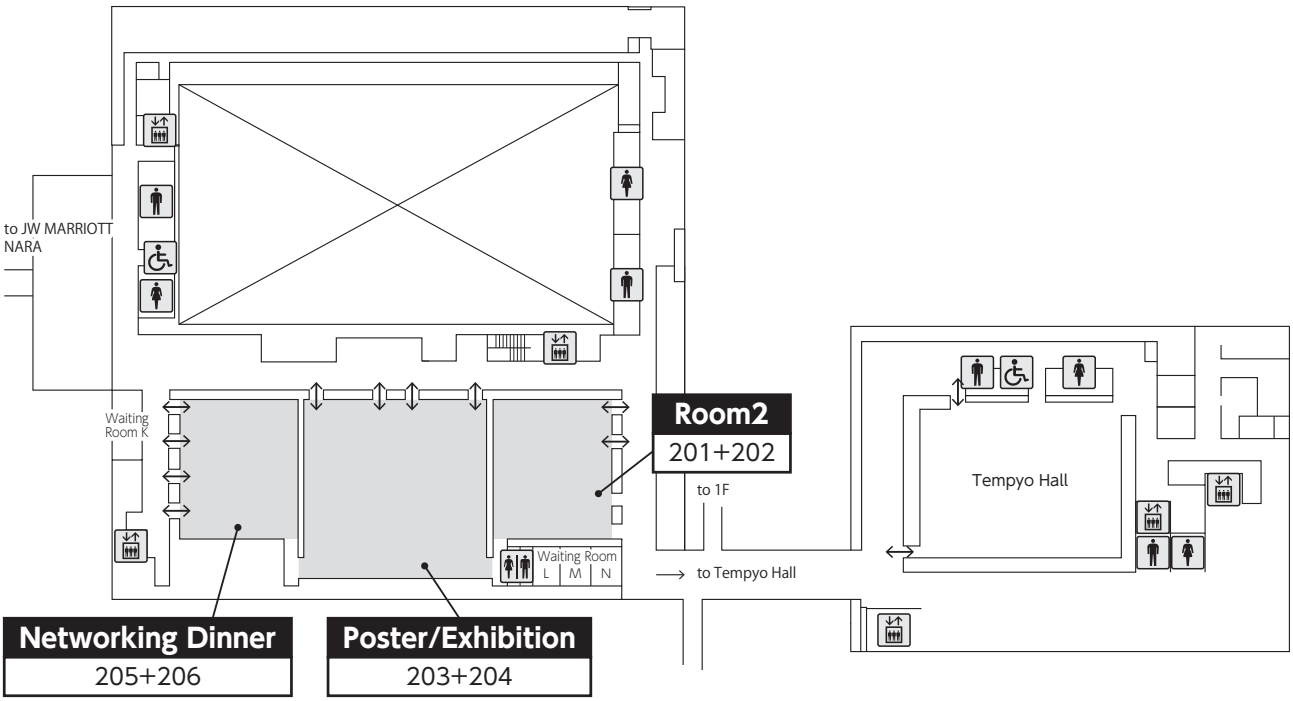
# Floor Map

## 1F




Sep. 13, Friday

## 2F




Sep. 14, Saturday
19:00-20:30

# General Information

**Date:** September 12-15, 2024
**Theme:** For a new chapter of myology in the region
**Venue:** Nara Prefectural Convention Center, Japan
https://www.nara-cc.jp/english/index.html#access
**Congress Chair:** Ichizo Nishino, MD, PhD
President, Asian Oceanian Myology Center (AOMC)
Department of Neuromuscular Research National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

## 1. Important Dates

Opening Ceremony
Date & Time: 08:30-09:00, September 13, 2024
Location: Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Networking Dinner
Date & Time: 19:00-20:30, September 14, 2024
Location: 205+206, 2F, Nara Prefectural Convention Center

JMS Student Award Session (Oral Presentation)
Date & Time: 08:30-09:00, September 15, 2024
Location: Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Closing Ceremony
Date & Time: 13:20-14:20, September 15, 2024
Location: Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

## 2. Registration for Participation

### 2-1. Request for On-line Registration

The AOMC-JMS 2024 employs the same online registration system for “Early Bird Registration” and “Regular and Onsite Registration.” To prevent congestion at the registration desk, please register to participate through the online registration system on the AOMC-JMS2024 website in advance.

**【Registration Period】**
Early Bird Registration:
From April 8 to 23:59 JST on July 10, 2024 (Closed)
Regular and Onsite Registration:
From July 11 to 15:00 JST on September 15, 2024

* Please note that the online registration system will close at 15:00 (JST) on Sunday, Sep 15.
* Credit card payment only.

# General Information

【Registration Fees】

| Category | Early Bird | Regular and Onsite |
|---|---|---|
| Regular Participants | 20,000JPY | 25,000JPY |
| Undergraduate Students,<br>Graduate Students & Residents | 10,000JPY | 15,000JPY |
| Industry | 30,000JPY | 40,000JPY |

*Discount registration is available for Undergraduate Students, Graduate Students & Residents. Those registering in this category must upload a certificate, such as a work certificate, practice license, or student ID.

*Fee changes might occur depending on the situation.

**2-2. How to Participate**

1) Please print your name card from the AOMC-JMS2024 my page, and have it with you. Please come to the registration desk to pick up your neck strap and a commemorative gift. All delegates must wear a name badge for identification. According to the venue's regulation, you are requested to wear your badge during all sessions of the congress.
2) If you forget to bring your name card, please provide your receipt or present the receipt on your mobile screen to the reception staff. After the staff confirms your registration, you will be given a blank name card. Please fill in your name and wear it at all times.

【Registration Desk】

Location: Entrance Lobby, 1F, Nara Prefectural Convention Center

Opening Hours:

| Sep 12 (Thu.) | Sep 13 (Fri.) | Sep 14 (Sat.) | Sep 15 (Sun.) |
|---|---|---|---|
| 9:30~16:30 | 7:30~16:30 | 7:30~16:30 | 7:30~12:00 |

**2-3. Luncheon Seminars**

No tickets are required to participate in the Luncheon Seminars. Admission is on a first-come, first-served basis. Please visit the session room directly if you want to participate.

Boxed lunches will be provided at the seminar by the sponsors.

Please note that the number of vegetarian/halal boxed lunches is limited on a first-come, first -served basis.

## 3. Simultaneous Interpretation

All sessions will be in English.

Simultaneous interpretation is not available.

## 4. Etiquette

1) Please turn your mobile phone to silent/vibration mode in the conference room to minimize disruption to the forum proceedings.
2) You should always carry your valuables with you. Also, please do not leave personal belongings in the conference room overnight, as the room may be used for other functions. Neither the venue, organizer, secretariat, nor staff can be held responsible for any loss.

# General Information

## 5. For Chairs of Oral Sessions

Please be seated in the “Next Chair” seat at the front right corner of your session room at least **15 minutes** before the session starts.
During your session, please strictly control the progress and ensure each presentation adheres to the designated time allocation.

## 6. For Speakers of Oral Sessions

All speakers (including those using their laptops) are requested to stop by the PC Reception Desk to submit their presentation data at least **45 minutes** before their session.
Please pay attention to your session date and time and be on time.
Please be aware of and strictly control your presentation time.

**【PC Reception Desk】**
Location: 103+104, 1F, Nara Prefectural Convention Center
Opening Hours:

| Sep 12 (Thu.) | Sep 13 (Fri.) | Sep 14 (Sat.) | Sep 15 (Sun.) |
|---|---|---|---|
| 9:30~16:00 | 7:30~16:30 | 7:30~16:30 | 7:30~13:00 |

### 6-1. Notes on Oral Presentation

PC projectors will be used for presentations. Please see the details below.

1) Only computers with Windows 10 OS are provided at the venue.
2) Please bring your presentation data on a USB flash drive to the PC Reception Desk. As a precautionary measure, we recommend that you double-check beforehand if the data stored on the USB drive works properly on a different PC.
3) A monitor, a mouse, and a clicker are provided on the podium. Speakers should operate the peripheral devices for PowerPoint by themselves.
4) If you use a Macintosh computer to make your presentation, please bring your own laptop with your presentation data, or check if the data works properly on a Windows 10-based PC before the conference to prevent any technical issues that may arise between the Windows and Macintosh operation systems.
5) It is recommended that speakers who include video or sound files in their presentations bring and use their own laptops.
6) Please stop by the PC Reception Desk at least **45 minutes** before your session to submit your presentation data.
7) Presentations should adhere to the Code of Research Conduct and Research Ethics and rules on protecting personal information.
8) PowerPoint Presenter View cannot be used during the presentation. If you need a script for your presentation, please print it out and bring it to the venue. No printers are available for note printing in the venue.

### 6-2. Instructions for Preparing Presentation Data

1) Monitor screen size: Wide XGA (16:9)
2) Please make your presentation data in English.

# General Information

**[If you bring your data on a USB flash drive]**

1) Please ensure that your presentation will display correctly on the systems with the following pacifications:
   OS: Windows 10
   Software: PowerPoint 2019/2021
   Monitor screen size: Wide XGA (16:9)
2) Fonts: Please use standard fonts of Windows 10 (OS) (e.g., Helvetica, Arial, Times New Roman) on your presentation slides, as unusual fonts may not be correctly displayed on the computers in the session rooms.
3) Movies: If you embed videos in your PowerPoint presentation data, please also bring the video files that can be played using the codecs in Windows Media Player's default settings. MP4 format is recommended. Please save the video data together in the same folder to maintain the links with PowerPoint.
4) Images: Please ensure images in your presentation data are in JPEG/TIFF/BMP format. Do not include images in the standard Macintosh PICT format.
5) Graphs: To create graphs, please use standard PowerPoint functions or Excel graphs. If you use other software, please paste the exported graph data into an image format described in the "Images" section above.
6) Anti-Virus Check: Please check the files using the latest anti-virus software before submitting them to the PC Reception Desk.
7) Data Submission: All speakers are requested to submit their presentation data to the PC Reception Desk. The data will be temporarily stored on the server at the PC Reception Desk and on the computers in the respective session room, and deleted after the conference.

**[If you bring your own laptop]**

1) Speakers using their own laptops MUST HAVE an AC adapter.
2) Speakers using their own laptops MUST HAVE a VGA D-sub 15pin female output or HDMI cable. Some laptops require a particular video output cable for the D-sub 15pin to connect to external monitors and data projectors.
   Please note that we are not equipped with that specific cable; for bring one if needed.
3) Please turn off beforehand the modes that will hinder presentation, such as the screen-saver and energy-saving modes.
4) You should have your data backed up in case of computer trouble.
5) After having your computer/presentation data checked at the PC Reception Desk, please bring your computer to the operation desk near the "Next Chair" seat in your session room no later than **15 minutes** before your presentation. Mirroring will be conducted at the operation desk, so please use the monitor, the mouse, and the clicker on the podium that the Secretariat provides to operate your slides. Please remember to get your laptop back from the operation desk after your presentation.
6) Please check your laptop using the latest anti-virus software before bringing it to the PC Reception Desk.

### 6-3. Conflict of Interest (COI) disclosure

1) At the AOCM-JMS2024, all speakers are requested to disclose all conflicts of interest (COI).
2) Whether or not a speaker has any conflicts of interest (COI), they must include a COI disclosure slide on the first page of their presentation.
3) Please note that the presentations without the COI disclosure will not be allowed. Visit the website for more detailed policies and COI slide templates.

# General Information

## 7. For Chairs of Poster Session

Please stop by the Reception Desk **15 minutes** before the session.
During the poster session, please strictly control the progress and ensure each presentation adheres to the designated time allocation.

## 8. For Speakers of Poster Presentations

### 8-1. Presentation Time:

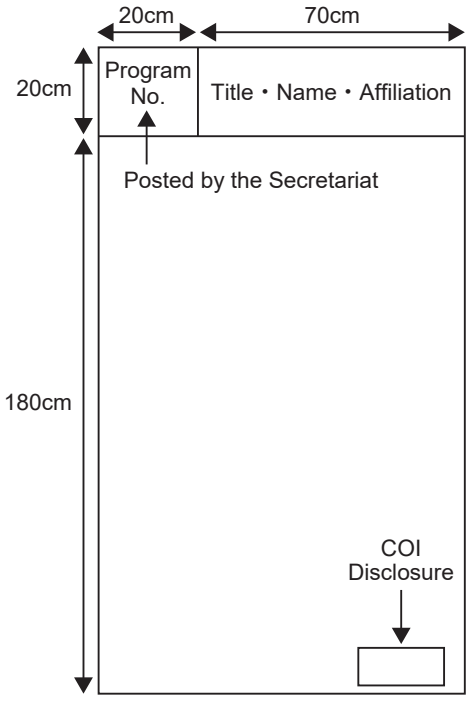
A three (3)-minute flash talk for each presentation.

### 8-2. Schedules for Poster Presentations

| Sep 12 (Thu.) | Sep 13 (Fri.) | Sep 14 (Sat.) | Sep 15 (Sun.) |
|---|---|---|---|
| - | 9:00~13:00*<br>Poster Set-up<br>13:00~18:30<br>Poster Round<br>18:30~19:45<br>Presentation | 9:00~18:00<br>Poster Round | 9:00~13:30<br>Poster Round<br>14:00~15:00<br>Poster Removal |

*All SA candidates must have their posters displayed by **noon**.
*All posters will be displayed until the last day of the congress.

### 8-3. Poster Preparation Instructions




1) The anticipated size of poster panels is 210 cm tall × 90 cm wide.
   Please create a poster so that it will fit inside the panel.
2) The Secretariat will prepare the panel with only the presentation number at the top.
3) Pins to attach posters to the panels will be provided at the conference.
4) Speakers are responsible for writing the presentation title, speaker name(s), and affiliation(s) horizontally within a 20 cm by 70 cm space.
5) In a large, easy-to-understand manner, the posters should present the Main Points, Purpose, Methods, Results, and Discussion, all in that order.
6) The text should be written in a large font that can be read from two to three meters away, while the diagrams should be at least 20 cm on one side and include a title and a brief explanation.
7) Whether or not a speaker has conflicts of interest (COI), they must include their COI disclosure on the poster. For more details, please refer to Section 6-3 above.
   Please note the COI disclosure is a MUST; presentations without a COI disclosure will not be allowed.
8) The panels cannot be written or drawn on directly, nor can items be glued to them.
9) Please make your poster in English.
   <Poster Printing Service>
   For participants who cannot post their posters by themselves, Organizing Committee offers

the option to use a Poster Printing Service. Please refer to the AOMC-JMS 2024 website for more details.

### 9. For YIA Nominees

<First-round Selection: Abstract Evaluation>
Evaluation method: Scoring by selection committee members
Number of abstracts to be selected: five (5) for AOMC-YIA and six (6) for JSM-YIA
Candidates who pass the first round will be notified in advance.
(Abstracts not selected for the second round will be presented in the general oral or poster sessions.)

<Second-round Selection: Oral Presentation on Day 3 (September 14)>
Evaluation method: Voting by members of the selection committee
Presentation time: twelve (12) minutes, Q&A time: six (6) minutes
Result Announcement: The Most Outstanding YIA and the Excellent YIA will be announced at the Award and Closing Ceremony. All second-round candidates, please attend.

### 10. For JMS Student Award Nominees

<First-round Selection: Poster Presentation on Day 2 (September 13)>
Evaluation method: Poster evaluation by selection committee members
(Day 2, September 13, 18:30-20:30)
Posters must be displayed by noon on Day 2 (September 13). (Failure to do so will result in exclusion from the selection process.)
A mark will inform those selected for the next round on their posters between 19:30 and 20:00 on the day of the presentation.

<Second-round Selection: Oral Presentation on Day 4 (September 15)>
Evaluation method: Voting by selection committee members
Presentation time: seven (7) minutes, Q&A time
(to be adjusted according to the number of abstracts selected)
Result Announcement: The Most Outstanding SA and the Excellent SA will be announced at the Award and Closing Ceremony.

All SA candidates must have their presentation slides ready for the second-round selection.
Oral presentations during the second-round selection will be in the ascending order of the abstract ID numbers.
Failure to attend the second-round selection will result in disqualification from the competition.
All second-round candidates, please attend the Award and Closing Ceremony.

# AOMC- JMS 2024 Organizing Committee

**Host**
Japan Muscle Society (JMS)

## Ichizo Nishino

*Congress Chair*

Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

## Kazuma Sugie

*Secretary General*

Department of Neurology, Nara Medical University, Japan

## Nobuyuki Eura

*Secretary Office*

Department of Neurology, Nara Medical University, Japan

## Yoshitsugu Aoki

*Program Committee Chair*

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

# Local Program Committee

| | |
|---|---|
| Masayuki Nakamori | Department of Neurology, Yamaguchi University Graduate School of Medicine, Japan |
| Akiyoshi Uezumi | Division of Cell Heterogeneity, Medical Institute of Bioregulation, Kyushu University, Japan |
| Madoka Mori-Yoshimura | Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Japan |
| Satoru Noguchi | National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan |
| Shinichiro Hayashi | Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan |
| Yuko Shimizu-Motohashi | Department of Child Neurology, National Center of Neurology and Psychiatry, Japan |
| Norio Motohashi | Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan |
| Keisuke Hitachi | Division for Therapies against Intractable Diseases, Center for Medical Science, Fujita Health University, Japan |
| Hiroaki Mitsuhashi | Department of Bioengineering, School of Engineering, Tokai University, Japan |
| Takashi Yamada | Department of Physical Therapy, Sapporo Medical University, Japan |
| Tetsuya Takeda | Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan |
| Masaki Inada | Department of Biotechnology and Life Science, Faculty of Engineering, Tokyo University of Agriculture and Technology, Japan |
| Tomoya Uchimura | Department of Clinical Application, Center for iPS Cell Research and Application, Kyoto University, Japan |
| Ai Shima | Graduate School of Information Science and Technology Department of Mechano-Informatics, the University of Tokyo, Japan |
| Naoki Suzuki | Department of Rehabilitation Medicine, Tohoku University, Japan |
| Keiko Ishigaki | Department of Pediatrics, Tokyo Women's Medical University, School of Medicine, Japan |
| Takashi Kurashige | Department of Neurology, National Hospital Organization Kure Medical Center and Chugoku Cancer Center, Japan |

# International Program Committee

| | |
|---|---|
| Sophelia Hoi-Shan Chan | Department of Paediatrics and Adolescent Medicine, Paediatric Neurology Division, The University of Hong Kong, Hong Kong SAR |
| Umapathi Thirugnanam | Department of Neurology and Neuro ophthalmology National Neuroscience Institute, Singapore |
| Jantima Tanboon | Department of Pathology, Mahidol University,Thailand |
| Merrilee Needham | Fiona Stanley Hospital, Murdoch University and Notre Dame University, Australia |

# Local Advisory Board

| | |
|---|---|
| Shin'ichi Takeda | National Center of Neurology and Psychiatry, Japan |
| Tatsushi Toda | Department of Neurology, Graduate School of Medicine, The University of Tokyo, Japan |
| Katsuhisa Ogata | National Hospital Organization Higashisaitama Hospital, Japan |
| Yukiko K. Hayashi | Department of Pathophysiology, Tokyo Medical University, Japan |
| Motoi Kanagawa | Ehime University Graduate School of Medicine, Japan |
| So-ichiro Fukada | Graduate School of Pharmaceutical Sciences, Osaka University, Japan |
| Hidetoshi Sakurai | Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan |
| Takayuki Akimoto | Waseda University, Japan |
| Tsuyoshi Matsumura | Department of Neurology, National Hospital Organization Osaka Toneyama Medical Center, Japan |
| Hirofumi Komaki | National Center of Neurology and Psychiatry, Japan |
| Yoshihide Sunada | Kawasaki Medical School, Japan |

# AOMC Executive Board

| | | |
|---|---|---|
| **President** | Ichizo Nishino | Japan |
| **Vice President** | Andrew J. Kornberg | Australia |
| **Secretary** | Satish V. Khadilkar | India |
| **Assistant Secretary** | David Hutchinson | New Zealand |
| **Treasurer** | Rajesh Benny | India |
| **Member** | Raymond L. Rosales (Immediate Past President) | Philippines |
| | Atchayaram Nalini | India |
| | Merrilee Needham | Australia |
| | Gina Ravenscroft (Elect) | Australia |
| | Yun Yuan | China |
| | Chuanzhu Yan | China |
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| | Luh Ari Indrawati | Indonesia |
| | Shahriar Nafissi | Iran |
| | Yukiko K. Hayashi | Japan |
| | Katsuhisa Ogata | Japan |
| | Tatsushi Toda | Japan |
| | Yoshitsugu Aoki (Elect) | Japan |
| | Kazuma Sugie (Elect) | Japan |
| | Young-Chul Choi | Korea |
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| | Jong-Hee Chae | Korea |
| | Khean Jin Goh | Malaysia |
| | Kum Thong Wong | Malaysia |
| | Ohnmar | Myanmar |
| | Khine Mi Mi Ko (Elect) | Myanmar |
| | Sara Khan | Pakistan |
| | Ludwig F. Damian | Philippines |
| | Josiah Chai | Singapore |
| | Umapathi Thirugnanam | Singapore |
| | Yuh-Jyh Jong | Taiwan |
| | Yung-Ting Kuo | Taiwan |
| | Charungthai Dejthevaporn | Thailand |
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| | Jantima Tanboon | Thailand |

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| | Kazuma Sugie | Nara Medical University, Japan |
| | Naohiro Hashimoto | National Center for Geriatrics and Gerontology, Japan |
| | Shin'ichi Takeda | National Center of Neurology and Psychiatry, Japan |

# AOMC Peer review committee

| | |
|---|---|
| Ichizo Nishino | Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Japan |
| Kazuma Sugie | Department of Neurology, Nara Medical University, Japan |
| Nobuyuki Eura | Department of Neurology, Nara Medical University, Japan |
| Yoshitsugu Aoki | Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan |
| Masayuki Nakamori | Department of Neurology, Yamaguchi University Graduate School of Medicine, Japan |
| Akiyoshi Uezumi | Division of cell heterogeneity of Medical Institute of Bioregulation, Kyushu University, Japan |
| Yuko Shimizu-Motohashi | Department of Child Neurology, National Center of Neurology and Psychiatry, Japan |
| Norio Motohashi | Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan |
| Atchayaram Nalini | Neurology, National Institute of Mental Health and Neurosciences, India |
| Hui Xiong | Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, China |
| Sophelia Hoi-Shan Chan | Department of Paediatrics and Adolescent Medicine, Paediatric Neurology Division, The University of Hong Kong, China, Hong Kong SAR |
| Anna Cho | Department of Pediatrics, Seoul National University Bundang Hospital Seoul National University College of Medicine, Republic of Korea |
| Luh Ari Indrawati | Department of Neurology, Dr. Cipto Mangunkusumo Hospital, Universitas Indonesia, Indonesia |
| Sara Khan | Department of Neurology, Aga Khan University Hospital, Pakistan |
| Wen-Chen Liang | Department of Pediatrics, Kaohsiung Medical University Hospital, Taiwan |
| Jantima Tanboon | Department of Pathology, Mahidol University, Thailand |
| Wenhua Zhu | Huashan Hospital, Fudan University, China |

# JMS Peer review committee

# AOMC- JMS 2024 Faculty

**Keynote Lecture 1**

## Michio Hirano

*USA*

H. Houston Merritt Neuromuscular Research Center, Department of Neurology, Columbia University Irving Medical Center, New York, USA

**Keynote Lecture 2**

## Yoshitsugu Aoki

*Japan*

Department of Molecular Therapy, National Institute of Neuroscience National Center of Neurology and Psychiatry (NCNP), Japan

**Educational Programme 1**

## Wen-Chen Liang

*Taiwan*

Departments of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

## Hui Xiong

*China*

Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

# AOMC- JMS 2024 Faculty

## Atchayaram Nalini

*India*

National Institute of Mental Health and Neurosciences, Bengaluru, India

**Educational Programme 2**

## Satish V. Khadilkar

*India*

Department of Neurology, Bombay Hospital Institute of Medical Sciences, India

## Kinji Ohno

*Japan*

Nagoya University of Arts and Sciences, Japan

## Teerin Liewluck

*USA*

Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

**Educational Programme 3**

## Kimiko Inoue

*Japan*

NHO Osaka Toneyama Medical Center, Japan

## Yuka Ishikawa

*Japan*

National Hospital Organization Hokkaido Medical Center, Japan

## Atsuhito Takeda

*Japan*

Department of Pediatrics, Hokkaido University Graduate School of Medicine, Japan

**Educational Programme 4**

## Jordi Alberto Diaz-Manera

*UK*

John Walton Muscular Dystrophy Research Center, Newcastle University, UK

# AOMC- JMS 2024 Faculty

## Werner Stenzel

*Germany*

Department of Neuropathology, Charite University Hospital, Germany

## Alessandra Ferlini

*Italy*

Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

**Symposium 1 [AOMC-TREAT-NMD collaboration]**

## Annemieke Aartsma-Rus

*Netherlands*

Leiden University Medical Center, Leiden, the Netherlands

## Alessandra Ferlini

*Italy*

Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

## Masanori P. Takahashi

*Japan*

Osaka University Graduate School of Medicine, Japan

**Symposium 2**

## Jong-Hee Chae

*Republic of Korea*

Department of Genomic Medicine & Pediatrics Seoul National University College of Medicine, Republic of Korea

## Oranee Sanmaneechai

*Thailand*

Faculty of Medicine, Siriraj Hospital Mahidol University, Thailand

## Sophelia HS Chan

*Hong Kong*

The University of Hong Kong, Department of Paediatrics and Adolescent Medicine, Neurology Division, Hong Kong

# AOMC- JMS 2024 Faculty

## Yuko Shimizu-Motohashi

*Japan*

National Center of Neurology and Psychiatry, Department of Child Neurology, Japan

**Symposium 3**

## Werner Stenzel

*Germany*

Department of Neuropathology, Charite University Hospital, Germany

## Jantima Tanboon

*Thailand*

Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

## Tahseen Mozaffar

*USA*

Department of Neurology and Pathology, University of California, Irvine, USA

## Wei Wang

*China*

Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

## Satoshi Yamashita

*Japan*

Department of Neurology, International University of Health and Welfare Narita Hospital, Japan

## Naoko Okiyama

*Japan*

Department of Dermatology, Graduate School of Medicine and Dental Sciences, Tokyo Medical and Dental University, Japan

**Symposium 4**

## Si Tri Le

*Vietnam*

University Medical Center of Ho Chi Minh City, Vietnam
Alhadi Neuromuscular Diseases Center, Imam Hussein Health and Education Administration, Iraq

# AOMC- JMS 2024 Faculty

## Abdulnasir Hussin Ameer

*Iraq*

Alhadi Neuromuscular Centre Karbala, Iraq
Alhadi Neuromuscular Diseases Center, Imam Hussein Health and Education Administration, Iraq

## Altynshash Jaxybayeva

*Kazakhstan*

Astana Medical University, Astana, Kazakhstan

## Sanjaya Shanthiputhra Mandadige Fernando

*Sri Lanka*

Department of Paediatric Neurology, Ladyridgeway Hospital for Children, Ministry of Health Sri Lanka, Sri Lanka

## Esmatullah Hamed

*Afghanistan*

French Medical Institute for Mothers and Children, Afghanistan

## Hidenori Arai

*Japan*

National Center for Geriatrics and Gerontology, Japan

## Hiroshi Asahara

*Japan*

Tokyo Medical and Dental University, Japan
Scripps Research, La Jolla, CA, USA

## Tatsuya Yoshizawa

*Japan*

Cell Biology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan

## Tatsuya Hosoi

*Japan*

Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Japan

# AOMC- JMS 2024 Faculty

**Symposium 6**

## Satoru Takahashi

*Japan*

Department of Anatomy and Embryology, Institute of Medicine, University of Tsukuba, Japan

## Masaki Inada

*Japan*

Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Japan

## Hirohiko Hohjoh

*Japan*

Graduate School of Medicine, Juntendo University, Japan

## Yuki Saito

*Japan*

Sapporo Medical University, Japan

## So-ichiro Fukada

*Japan*

Graduate School of Pharmaceutical Sciences, Osaka University, Japan

## Yuji Hara

*Japan*

School of Pharmaceutical Sciences, University of Shizuoka, Japan

## Yusuke Ono

*Japan*

Department of Muscle Development and Regeneration, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan
Muscle Biology Laboratory, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Japan

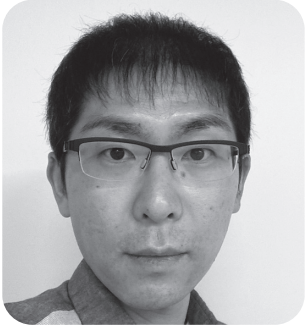
## Hidetoshi Sakurai

*Japan*

Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan

# AOMC- JMS 2024 Faculty

**Symposium 8**



## Naoki Ito

*Japan*

Brain-Skeletal Muscle Connection in Aging Project Team, National Center for Geriatrics and Gerontology, Japan

## Ayaka Tabuchi

*Japan*

The University of Electro-Communications, Japan

## Kohei Kido

*Japan*

National Institute of Advanced Industrial Science and Technology (AIST), Japan

## Yasuhiro Sawada

*Japan*

National Rehabilitation Center for Persons with Disabilities, Japan

## Chuanzhu Yan

*China*

Neuromuscular Center and Department of Neurology, Qilu Hospital, Shandong University, China

## Akinori Uruha

*Japan*

Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan

## Hirofumi Komaki

*Japan*

Department of Child Neurology, Translational Medical Center, National Center of Neurology and Psychiatry, Japan

## Yuh-Jyh Jong

*Taiwan*

Graduate Institute of Clinical Medicine, Kaohsiung Medical University; Departments of Pediatrics and Laboratory Medicine, KMU Hospital, Taiwan

# AOMC- JMS 2024 Faculty

**Symposium 10**

## Naoomi Tominaga

*Japan*

Department of Clinical Laboratory Science, Graduate School of Medicine, Yamaguchi University, Japan

## Naoyuki Kawao

*Japan*

Kindai University Faculty of Medicine, Japan

## Koji Ohashi

*Japan*

Nagoya University Graduate School of Medicine, Japan

## Chihiro Tohda

*Japan*

Section of Neuromedical Science, Institute of natural Medicine, University of Toyama, Japan

## Matthew Watts

*Australia*

Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research - New South Wales Health Pathology, Westmead Hospital and Sydney Institute for Infectious Diseases, University of Sydney, Sydney, New South Wales, Australia

## Teerin Liewluck

*USA*

Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN, USA

## Michio Inoue

*USA*

Department of Neurology, Washington University School of Medicine, USA

## Gina Ravenscroft

*Australia*

Centre for Medical Research, The University of Western Australia, Nedlands, WA, Australia
Harry Perkins Institute of Medical Research, Nedlands, WA, Australia

# AOMC- JMS 2024 Faculty

## Jordi Alberto Diaz-Manera

*UK*

John Walton Muscular Dystrophy Research Center, Newcastle University, UK

**Symposium 12**

## Takashi Okada

*Japan*

The Institute of Medical Science, The University of Tokyo, Japan

## Masayuki Nakamori

*Japan*

Department of Neurology, Yamaguchi University, Japan

## Motoi Kanagawa

*Japan*

Ehime University Graduate School of Medicine, Japan

## Shinichi Nakagawa

*Japan*

Hokkaido University, Japan

**Symposium 13**

## Akitsu Hotta

*Japan*

Center for iPS Cells and Research Application (CiRA), Kyoto University, Japan

## Yukari Endo

*Japan*

Juntendo University, Institute of Health and Sports Science & Medicine, Japan
Juntendo University, Department of Pharmacology, School of Medicine, Japan
Hospital for Sick Children, Program for Genetics and Genome Biology, Japan

## Hironobu Takahashi

*Japan*

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Japan

# AOMC- JMS 2024 Faculty

## Tomoya Uchimura

*Japan*

Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

**JSNP Joint Symposium**

## Hiroyuki Ishiura

*Japan*

Department of Neurology, Okayama University, Japan

## Takashi Kurashige

*Japan*

Department of Neurology, NHO Kure Medical Center and Chugoku Cancer Center, Japan

## Norifumi Shioda

*Japan*

Department of Genomic Neurology, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University, Japan

## Masashi Ogasawara

*Japan*

Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
Department of Pediatrics, Showa General Hospital, Tokyo, Japan

## Jun Sone

*Japan*

Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Japan

## Rie Saito

*Japan*

Department of Pathology, Brain Research Institute, Niigata University, Japan

**Clinical Pathological Conference**

## Wenhua Zhu

*China*

Huashan Hospital, Fudan University, China

# AOMC- JMS 2024 Faculty

## Josiah Chai

*Singapore*

Department of Neurology, National Neuroscience Institute, Singapore

## Akatsuki Kubota

*Japan*

Department of Neurology, The University of Tokyo, Japan

## Zhaoxia Wang

*China*

Department of Neurology, Peking University First Hospital, China

## Jin-Hong Shin

*Republic of Korea*

Department of Neurology, Pusan National University Yangsan Hospital, Republic of Korea

## Chuanzhu Yan

*China*

Neuromuscular Center and Department of Neurology, Qilu Hospital, Shandong University, China

## Yung-Ting Kuo

*Taiwan*

Department of Pediatrics, Taipei Medical University - Shuang Ho Hospital, Taiwan

## Jantima Tanboon

*Thailand*

Department of Pathology, Mahidol University, Thailand

# Meeting Agenda

## Sep. 12, Thursday

### Room 2

10:25-10:30 Educational Programme Opening Remarks — Ichizo Nishino (Department of Neuromuscular Research National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

10:30-12:00

### Educational Programme 1

Chairs: Sara Khan (Department of Neurology, Aga Khan University Hospital, Pakistan)
Kazuma Sugie (Department of Neurology, Nara Medical University, Japan)

**EP1-1 How to examine infants suspected to have neuromuscular disorders**

Wen-Chen Liang

Departments of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

**EP1-2 Characteristics, natural history and management of LAMA2-related dystrophies: A multi-cohort study from the Asian-Oceanian Network**

Hui Xiong, Lin Ge

Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

**EP1-3 Examination of an adult patient with Muscle and Neuromuscular junctions disorders**

Atchayaram Nalini

National Institute of Mental Health and Neurosciences, Bengaluru, India

12:10-13:00

### Luncheon Seminar 1

Chair: Hirofumi Komaki (National Center of Neurology and Psychiatry)

**Emerging Science in SMA Treatment for Better Outcomes**

**LS1** Yin-Hsiu Chien[1], Takashi Nakajima[2]

[1]National Taiwan University Hospital
[2]NHO Niigata National Hospital

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# Meeting Agenda

13:10-14:40

## Educational Programme 2

Chairs: Chongbo Zhao (Department of Neurology Huashan Hospital, Fudan university, China)
Rajesh Benny (Department of Neurology, Fortis Hospital, India)

**EP2-1** **Making Sense of The Clinical spectrum of Limb Girdle Muscular Dystrophies**

Satish V. Khadilkar
Department of Neurology, Bombay Hospital Institute of Medical Sciences, India

**EP2-2** **Congenital myasthenic syndromes: Review of 38 causal genes**

Kinji Ohno
Nagoya University of Arts and Sciences, Japan

**EP2-3** **The expanding genetic landscape of hereditary rhabdomyolysis: what clinicians need to know**

Teerin Liewluck
Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

14:50-16:20

## Educational Programme 3

Chairs: Katsuhisa Ogata (National Hospital Organization Higashisaitama Hospital, Japan)
Anna Cho (Department of Pediatrics Seoul National University Bundang Hospital
Seoul National University College of Medicine, Republic of Korea)

**EP3-1** **Rehabilitation for progressive neuromuscular disorders: focus on Duchenne muscular dystrophy**

Kimiko Inoue
NHO Osaka Toneyama Medical Center, Japan

**EP3-2** **Pulmonary rehabilitation and management of dysphagia in patients with neuromuscular disorders**

Yuka Ishikawa
National Hospital Organization Hokkaido Medical Center, Japan

**EP3-3** **Pathophysiology and management of cardiomyopathy associated with neuromuscular disease**

Atsuhito Takeda
Department of Pediatrics, Hokkaido University Graduate School of Medicine, Japan

# Meeting Agenda

16:30-18:00

## Educational Programme 4

Chairs: Wen-Chen Liang (Department of Pediatrics, Kaohsiung Medical University Hospital, Taiwan)
Gina Ravenscroft (Harry Perkins Institute, University of Western Australia, Australia)

### WMS Collaborate session

**EP4-1 Muscle Imaging**

Jordi Alberto Diaz-Manera
John Walton Muscular Dystrophy Research Center, Newcastle University, UK

**EP4-2 Differential diagnosis of IBM**

Werner Stenzel
Department of Neuropathology, Charite University Hospital, Germany

**EP4-3 Genetic diagnosis of muscle diseases: from gene-by-gene testing through next generation sequencing toward genomic medicine**

Alessandra Ferlini
Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

18:00-18:05 Educational Programme Closing Remarks — Kazuma Sugie (Department of Neurology, Nara Medical University, Japan)

# Meeting Agenda

## Sep. 13, Friday

### Room 1

8:30-9:00 Opening Ceremony Ichizo Nishino (Department of Neuromuscular Research National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

9:10-9:55

### Keynote Lecture1

Chairs: Yoshihide Sunada (Kawasaki Medical School, Japan)
Ichizo Nishino (Department of Neuromuscular Research National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

**K-1 Emerging Therapies for Thymidine Phosphorylase and Thymidine Kinase 2 Deficiencies**

Michio Hirano

H. Houston Merritt Neuromuscular Research Center, Department of Neurology, Columbia University Irving Medical Center, New York, USA

9:55-10:40

### Keynote Lecture2

Chairs: Satish V. Khadilkar (Department of Neurology, Bombay Hospital Institute of Medical Sciences, India)
Kazuma Sugie (Nara Medical University, Japan)

**K-2 Precision medicine in Duchenne muscular dystrophy: exon skipping therapies and innovative models for personalised drug development**

Yoshitsugu Aoki

Department of Molecular Therapy, National Institute of Neuroscience
National Center of Neurology and Psychiatry (NCNP), Japan

10:50-12:20

### Symposium 1 [AOMC-TREAT-NMD collaboration]

Chairs: Dae-Seong Kim (Department of Neurology, Pusan National University Yangsan Hospital, Korea)
Yukiko K. Hayashi (Department of Pathophysiology, Tokyo Medical University, Japan)

#### Clinical trials in NMD- Challenges we face

**S1-1 The importance of involving patients in therapy development Lessons learned from Duchenne exon skipping**

Annemieke Aartsma-Rus

Leiden University Medical Center, Leiden, the Netherlands

# Meeting Agenda

### S1-2 Therapeutic approaches in Duchenne muscular dystrophy: fall, rise, and challenges

Alessandra Ferlini
Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

### S1-3 Clinical Trial Readiness in Myotonic Dystrophy

Masanori P. Takahashi
Osaka University Graduate School of Medicine, Japan

12:30-13:20

## Luncheon Seminar 2

Chair: Ichizo Nishino (Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

### LS2 Long-Term Treatment Strategies for Myasthenia Gravis

Kimitoshi Kimura
Department of Neurology, Graduate school of Medicine and Faculty of Medicine, Kyoto University

Sponsored By: Alexion Pharma G.K. Medical Affairs Division

13:30-15:00

## Symposium 2

Chairs: Sophelia Hoi-Shan Chan (Department of Paediatrics and Adolescent Medicine, Paediatric Neurology Division, The University of Hong Kong, China, Hong Kong SAR)
Yuko Shimizu-Motohashi (Department of Child Neurology, National Center of Neurology and Psychiatry, Japan)

## Link between Research, Clinical trials, and Patient registries

### S2-1 Current status in South Korea: From patients registry to Clinical trials

Jong-Hee Chae
Department of Genomic Medicine & Pediatrics Seoul National University College of Medicine, Republic of Korea

### S2-2 Interconnection between Research, Clinical Trials, and Neuromuscular Patient Registries in Thailand

Oranee Sanmaneechai
Faculty of Medicine, Siriraj Hospital Mahidol University, Thailand

### S2-3 Advancements in FSHD Research: Insights from the Hong Kong FSHD Patient Registry and Innovative AI Applications

Sophelia HS Chan
The University of Hong Kong, Department of Paediatrics and Adolescent Medicine, Neurology Division, Hong Kong

# Meeting Agenda

### S2-4 Congenital muscular dystrophies and myopathies: Current status in Japan

Yuko Shimizu-Motohashi
National Center of Neurology and Psychiatry, Department of Child Neurology, Japan

15:10-16:40

## Symposium 3

Chairs: Akinori Uruha (Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan)
Jantima Tanboon (Department of Pathology, Mahidol University, Thailand)

## Myositis Update

### S3-1 Myositis classification and pathology: an update (Part 1)

Werner Stenzel
Department of Neuropathology, Charite University Hospital, Germany

### S3-2 Myositis classification and pathology: an update (Part 2) Dermatomyositis, Antisynthetase syndrome, and a few others

Jantima Tanboon
Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

### S3-3 Update on Muscle Imaging in Idiopathic Inflammatory Myopathy (IIM)

Tahseen Mozaffar
Department of Neurology and Pathology, University of California, Irvine, USA

### S3-4 Single-cell analysis of refractory anti-SRP necrotizing myopathy treated with anti-BCMA CAR T-cell therapy

Wei Wang
Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

### S3-5 Unlocking the Mystery: The Pathogenic Role of Anti-cN1A Antibodies in Inclusion Body Myositis

Satoshi Yamashita
Department of Neurology, International University of Health and Welfare Narita Hospital, Japan

### S3-6 Autoimmunity against melanoma differentiation-associated gene 5 induces interstitial lung disease mimicking dermatomyositis in mice

Naoko Okiyama[1], Yuki Ichimura[1,2], Risa Konishi[1,3], Manabu Fujimoto[4]
[1]Department of Dermatology, Graduate School of Medicine and Dental Sciences, Tokyo Medical and Dental University, Japan
[2]Division of Rheumatology, Department of Internal Medicine, Tokyo Women's Medical University, Japan
[3]Department of Dermatology, Faculty of Medicine, University of Tsukuba, Japan
[4]Department of Dermatology, Graduate School of Medicine, Osaka University, Japan

# Meeting Agenda

16:50-18:20

## Symposium 4

Chairs: Umapathi Thirugnanam (Department of Neurology, National Neuroscience Institute, Singapore)
Ohnmar (Department of Neurology, University of Medicine 1/Yangon General Hospital, Myanmar)

### Current Status of the Neuromuscular Field in New Member and Expected-to-be New Member Countries

**S4-1 An Overview of Myology in Vietnam: From AOMC 2017 to the Present**

Si Tri Le

University Medical Center of Ho Chi Minh City, Vietnam

**S4-2 Challenges and lessons learnt in setting up clinical, eletrodiagnostic and pathologic services for neuromuscular diseases in Iraq**

Abdulnasir Hussin Ameer[1,2]

[1]Alhadi Neuromuscular Centre Karbala, Iraq
[2]Alhadi Neuromuscular Diseases Center, Imam Hussein Health and Education Administration, Iraq

**S4-3 Management of neuromuscular diseases in Kazakhstan**

Altynshash Jaxybayeva

Astana Medical University, Astana, Kazakhstan

**S4-4 Emerging from the ashes and founding myology services in Sri Lanka**

Sanjaya Shanthiputhra Mandadige Fernando, Saamir Mohideen

Department of Paediatric Neurology, Ladyridgeway Hospital for Children, Ministry of Health Sri Lanka, Sri Lanka

**S4-5 Neurological Care in Afghanistan with 40 million population faces a rising neurological disease burden, including encephalitis, meningitis, malaria, measles and stroke. Neurological care is limited, with only two neurologists trained at The Aga Khan University. Challenges include poor awareness, inadequate training, and financial constraints. FMIC is working to improve services and establish a residency program**

Esmatullah Hamed

French Medical Institute for Mothers and Children, Afghanistan

# Meeting Agenda

# Sep. 13, Friday

## Room 2

10:50-12:20

### Oral Session 1

Chairs: Nobuyuki Eura (Department of Neurology, Nara Medical University, Japan)
Mariko Taniguchi-Ikeda (Department of Clinical Genetics, Fujita Health University Hospital, Aichi, Japan)

**O-1** **Regulation of skeletal muscle stem cell bioenergetics by a $Mg^{2+}$-permeable ion channel TRPM7**

Kotaro Hirano, Yuji Hara
University of Shizuoka, Japan

**O-2** **Prostaglandin J inhibited cellular proliferation and differentiation in myoblast via reprograming into adipocyte**

Masaru Takatoya[1], Tsukasa Tominari[1,2], Daichi Arai[1,2], Kensuke Shimizu[1], Keisuke Ikeda[1], Michiko Hirata[1], Yoshifumi Itoh[3], Masaki Inada[1]
[1]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Japan
[2]Department of Molecular Therapy, National Center of Neurology and Psychiatry, Japan
[3]Kennedy Institute of Rheumatology, University of Oxford, UK

**O-3** **3-(4-Hydroxy-3-methoxyphenyl) propionic acid mitigates dexamethasone-induced muscle atrophy by attenuating Atrogin-1 and MuRF-1 expression**

Anayt Ulla[1], Md Mizanur Rahman[1], Takayuki Uchida[1], Hiroyuki Kayaki[2], Yosuke Nishitani[2], Susumu Yoshino[2], Hiroshige Kuwahara[2], Takeshi Nikawa[1]
[1]Department of Nutritional Physiology, Institute of Biomedical Sciences, Tokushima University Graduate School, Japan
[2]Research Center, Maruzen Pharmaceuticals Co., Ltd., Japan

**O-4** **Single-Cell RNA Sequencing Analysis Unrevealed Enhanced Platelet Activation and Proinflammatory Response in anti-AChR positive Myasthenia Gravis**

Qi Wen, Shu Zhang, Jingsi Wang, Yaye Wang, Yuwei Da
Beijing Xuanwu Hospital, Capital Medical University, China

**O-5** **Factors Affecting Disease Severity in Adults with Myasthenia Gravis**

Nairong Xie, Haoran Liu, Yuwei Da
Department of Neurology, Xuanwu Hospital, Capital Medical University, Beijing, China

**O-6** **Optimize antibody-RNA conjugate for treating myotonic dystrophy type 1**

Hao Wu, Jingxiang Zheng, Yibo Qiu, Fanyu Yuan, Yafei Xing, Fei Sheng, Liangdong Zhang, Guodong Wang, Hao Hu, Yue Wu
ChainGen Biopharmaceuticals

### O-7 Protein methylation is essential to maintain skeletal muscle strength and function by regulating myosin heavy chain activity

Keisuke Hitachi[1], Hisateru Yamaguchi[2], Motoshi Kaya[3], Kasimchetty Arun[3], Tatsuhiko Ozawa[4], Yuri Kiyofuji[1], Masashi Nakatani[5], Masafumi Inui[6], Ichizo Nishino[7], Kunihiro Tsuchida[1]

[1]Division for Therapies against Intractable Diseases, Center for Medical Science, Fujita Health University, Japan
[2]School of Nursing and Medical Care, Yokkaichi Nursing and Medical Care University, Japan
[3]Department of Physics, Graduate School of Science, University of Tokyo, Japan
[4]Laboratory of Molecular and Cellular Biology, Faculty of Engineering, University of Toyama, Japan
[5]Faculty of Rehabilitation and Care, Seijoh University, Japan
[6]Laboratory of Animal Regeneration Systemology, Department of Life Sciences, School of Agriculture, Meiji University, Japan
[7]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

### O-8 Muscle and diaphragm involvement in GNE Myopathy: Insights from a large cohort and comparative study with other distal myopathies

Wakako Yoshioka, Madoka Mori-Yoshimura, Nobuyuki Eura, Yoshihiko Saito, Yasushi Oya, Hiroyuki Yajima, Shinichiro Hayashi, Yukio Kimura, Noriko Sato, Satoru Noguchi, Ichizo Nishino

National Center of Neurology and Psychiatry

### O-9 The androgen receptor in mesenchymal progenitors regulates skeletal muscle mass via *Igf1* expression in male mice

Hiroshi Sakai[1,2], Hideaki Uno[2], Harumi Yamakawa[2], Kaori Tanaka[3], Aoi Ikedo[1], Akiyoshi Uezumi[4], Yasuyuki Ohkawa[3], Yuuki Imai[1,2]

[1]Division of Integrative Pathophysiology, Proteo-Science Center, Ehime University, Toon, Ehime 791-0295, Japan
[2]Department of Pathophysiology, Ehime University Graduate School of Medicine, Toon, Ehime 791-0295, Japan
[3]Division of Transcriptomics, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka 812-0054, Japan
[4]Divison of Cell Heterogeneity, Medical Institute of Bioregulation, Kyushu University, Higashi-ku, Fukuoka 812-0054, Japan

12:30-13:20

## Luncheon Seminar 3

Chair: Kazuma Sugie (Professor & Chairman Department of Neurology Nara Medical University, Japan)

## Generalized Myasthenia Gravis Seminar -Entering a New Stage -

### LS3 The forefront of gMG treatment - Positioning of Subcutaneous Injections -

Hiroyuki Murai

Professor, Department of Neurology, International University of Health and Welfare

Sponsored By: UCB Japan Co. Ltd.

# Meeting Agenda

13:30-15:00

## Oral Session 2

Chairs: Arada Rojana-udomsart (Neurological Institute of Thailand, Thailand)
Daigo Miyazaki (Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine/Intractable Disease Care Center, Shinshu University Hospital, Japan)

### O-10 Lama1 upregulation prolongs the lifespan of a novel mouse model of LAMA2-related congenital muscular dystrophy

Yidan Liu[1,6], Dandan Tan[1,2], Kaiyue Ma[4,5], Huaxia Luo[1], Jingping Mao[6], Jihang Luo[1,6], Qiang Shen[6], Luzheng Xu[7], Shiqi Yang[1,6], Lin Ge[3,5], Yuxuan Guo[6], Hong Zhang[6], Hui Xiong[1,3]

[1]Department of Pediatrics, Peking University First Hospital, Beijing 102600, China
[2]Department of Neurology, The First Affiliated Hospital, Jiangxi Medical College, Nanchang University, Nanchang 330006, China
[3]Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing 100045, China
[4]Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders, Ministry of Education, Shanghai Jiao Tong University, Shanghai 200030, China
[5]Department of Genetics, Yale School of Medicine, New Haven, CT 06510, USA
[6]State Key Laboratory of Vascular Homeostasis and Remodeling, The Institute of Cardiovascular Sciences, School of Basic Medical Sciences, Peking University Health Science Center, Beijing 100191, China
[7]Medical and Health Analysis Center, Peking University, Beijing 100191, China

### O-11 FKTN variant interpretation through high throughout sequencing assay

Lin Ge[1,2], Kaiyue Ma[2], Monkol Lek[2], Hui Xiong[1]

[1]Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, China
[2]Department of Genetics, Yale School of Medicine, USA

### O-12 A Phase 1 Study of Antisense Oligonucleotide NS-035 in Patients with Fukuyama Congenital Muscular Dystrophy

Go Fujino[1], Asuka Kitamura[1], Akiko Takahashi[1], Meiko Maeda[1], Akatsuki Kubota[1], Yukino Tokuyama[2], Ikue Wada[2], Kazuhiro Kobayashi[3], Hirofumi Komaki[4], Mariko Taniguchi-Ikeda[5], Keiko Ishigaki[6], Tatsushi Toda[1]

[1]Department of Neurology, Graduate School of Medicine, The University of Tokyo
[2]Clinical Research Promotion Center, The University of Tokyo Hospital
[3]Division of Molecular Brain Science, Kobe University Graduate School of Medicine
[4]Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry
[5]Department of Clinical Genetics, Fujita Health University Hospital
[6]Department of Pediatrics, Tokyo Women's Medical University School of Medicine

# Meeting Agenda

## O-13 Unraveling the Pathogenic Mechanisms of B3GALNT2-Related α-Dystroglycanopathy: Insights into Enzymatic Activity and Gene Expression Changes

Xiaona Fu[1], Hui Wang[2,3], Wenjia Chai[2,3], Jihang Luo[4], Xiaoyu Chen[4], Danyu Song[4], Wei Wang[2,3], Jingwei Zhong[2,3], Zhimei Liu[1], Xiao Tong[1], Hui Xiong[1], Xiaotun Ren[1], Jingang Gui[1]

[1]Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[2]Laboratory of Tumor Immunology, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[3]Key Laboratory of Major Diseases in Children, Ministry of Education, Beijing Pediatric Research Institute, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China
[4]Department of Pediatrics, Peking University First Hospital, Beijing, China

## O-14 Mn007 facilitates O-mannosyl glycosylation in Fukuyama muscular dystrophy

Mariko Taniguchi-Ikeda[1], Michiyo Koyanagi-Aoi[2], Tatsuo Maruyama[3], Yoichiro Harada[4], Takashi Aoi[2]

[1]Department of Clinical Genetics, Fujita Health University Hospital, Aichi, Japan
[2]Department of iPS cell applications, Kobe University Graduate School of Medicine, Kobe, Japan
[3]Chemical Science and Engineering, Kobe University Graduate School of Enginee ring, Kobe, Japan
[4]Department of Glyco-Oncology, Osaka International Cancer Institute, Osaka, Japan

## O-15 From basics to translational research for 14-years unravelling the Anti-inflammatory potential of a novel b-glucan; moving to clinic in Duchenne muscular dystrophy

Samuel JK Abraham[1,2,3,4,5,6], Nobunao Ikewaki[7,8], Kadalraja Raghavan[9], Kosagi-Sharaf Rao[10], Koji Ichiyama[2], Rajappa Senthilkumar[2], Senthilkumar Preethy[11], Gary A. Levy[12], Masaru Iwasaki[1]

[1]Centre for Advancing Clinical Research (CACR), University of Yamanashi - School of Medicine, Chuo, Japan
[2]Antony- Xavier Interdisciplinary Scholastics (AXIS), GN Corporation Co. Ltd., Kofu, Japan
[3]Mary-Yoshio Translational Hexagon (MYTH), Nichi-In Centre for Regenerative Medicine (NCRM), Chennai, India
[4]R & D, Sophy Inc., Japan
[5]Levy-Jurgen Transdisciplinary Exploratory (LJTE), Global Niche Corp., Wilmington, DE, USA
[6]Haraguchi-Parikumar Advanced Remedies (HARP), SoulSynergy Ltd., Phoenix, Mauritius
[7]Dept. of Medical Life Science, Kyushu University of Health and Welfare, Japan
[8]Institute of Immunology, Junsei Educational Institute, Nobeoka, Miyazaki, Japan
[9]Dept of Paediatric Neurology, Jesuit Antonyraj memorial Inter-disciplinary Centre for Advanced Recovery and Education (JAICARE), Madurai, India
[10]KL Deemed-to-be University, Vaddeswaram, Andhra Pradesh, India
[11]Fujio-Eiji Academic Terrain (FEAT), Nichi-In Centre for Regenerative Medicine (NCRM), Chennai, India
[12]University of Toronto, Ontario, Canada

## O-16 Becker muscular dystrophy mouse models revealed nNOS reduction with capillary change and decreased type IIa fibers in skeletal muscle

Daigo Miyazaki[1,2], Mitsuto Sato[1,3], Naoko Shiba[4], Takahiro Yoshizawa[5], Akinori Nakamura[6,7]

[1]Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine
[2]Intractable Disease Care Center, Shinshu University Hospital
[3]Department of Brain Disease Research, Shinshu University School of Medicine
[4]Department of Regenerative Science and Medicine, Shinshu University
[5]Research Center for Advanced Science and Technology, Shinshu University
[6]Department of Clinical Research, NHO Matsumoto Medical Center
[7]The Third Department of Medicine, Shinshu University School of Medicine

# Meeting Agenda

### O-17 Cardiac Dysregulation in Duchenne Muscular Dystrophy: An ECG analysis

Sathyaprabha Talakad Narasappa[1], Arjun Krishnamurthy[2], Kaviraja Udupa[1], Inbaraj Ganagarajan[1], Boris W. Kramer[3], Harry W M Steinbusch[4], Nalini Atchayaram[5]

[1]Department of Neurophysiology, National Institute of Mental Health and Neurosciences, Bangalore, India
[2]Department of Computer Sciences, Dept. of CSE, School of Engineering, Dayananda Sagar University, Bangalore
[3]Department of Neonatology, Poznan University of Medical Sciences, Poland
[4]Department of Cellular and Translational Neuroscience, Maastricht University, Maastricht, Netherlands
[5]Department of Neurology, National Institute of Mental Health and Neurosciences, Bangalore, India

### O-18 Caveolin 3 inhibits phosphorylation-dependent activation of sarcolemmal nNOS

Yutaka Ohsawa, Asami Munekane, Kohei Ohkubo, Shin-ichiro Nishimatsu, Yoshihide Sunada

Kawasaki Medical School, Japan

15:30-16:40

## Sponsored Symposium

Chair: Hirofumi Komaki (Director, Translational Medical Center, National Center of Neurology and Psychiatry, Japan)

## Multidisciplinary Approach to Muscular Dystrophy Management

### SS1 Motor Function Assessment in the Treatment of Muscular Dystrophy

Keiko Ishigaki

Associate Professor, Department of Pediatrics, Tokyo Women's Medical University School of Medicine, Japan

### SS2 Cardiac Management of the Patient with Muscular Dystrophy

Mi Kyoung Song

Associate Professor, Pediatrics, Seoul National University Hospital, Republic of Korea

### SS3 Usefulness of Japanese National Registry of Muscular Dystrophy (Remudy)

Harumasa Nakamura

Director, Department of Clinical Research Support, National Center of Neurology and Psychiatry, Japan

### SS4 Multidisciplinary Approach to Management of Muscular Dystrophy Treatment in Korea

Jong-Hee Chae

Professor, Pediatrics, Seoul National University Hospital, Republic of Korea

Sponsored By: Pfizer Japan Inc.

# Meeting Agenda

16:50-18:20

## Symposium 5

Chairs: Akiyoshi Uezumi (Division of cell heterogeneity of Medical Institute of Bioregulation, Kyushu University, Japan)
Satoru Noguchi (Department of Neuromuscular Research, NCNP, Japan)

### Recent advances in sarcopenia and frailty studies

**S5-1 Research update of sarcopenia in Asia**

Hidenori Arai
National Center for Geriatrics and Gerontology, Japan

**S5-2 Athlete Giftedness and Genetics**

Hiroshi Asahara[1,2], Ryo Nakamichi[1,2]
[1]Tokyo Medical and Dental University, Japan
[2]Scripps Research, La Jolla, CA, USA

**S5-3 The diverse functions of NAD-dependent lysine deacylase SIRT7 in the musculoskeletal systems**

Tatsuya Yoshizawa
Cell Biology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan

**S5-4 The Androgen-Androgen Receptor Signaling in skeletal muscle: Insights into the Mechanisms of Sarcopenia Development and Potential Aging Regulation**

Tatsuya Hosoi
Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Japan

19:00-21:00 AOMC Board meeting (1F 107+108)

# Meeting Agenda

# Sep. 13, Friday

## Poster

18:30-19:35

### JMS Student Award Session (Poster)

Chair: Masayuki Nakamori (Department of Neurology, Yamaguchi University, Japan)

**JSA-1 LSMEM2, localized at the neuromuscular junction, modulatesMitochondrial integration in skeletal muscles**

Eman Elrefaei[1,2,3], Satorou Yamazaki[3], Issei Yazawa[3], Yusuke Takahashi[3], Naoki Ito[4], Nozomi Hayashiji[5], Yuya Nishida[3], Ichizo Nishino[6], Takashima Seiji[1], Yasunori Shintani[3]

[1]Osaka University, Medical Biochemistry department, faculty of medicine, Japan
[2]Tanta University, Medical Biochemistry department, Faculty of medicine, Japan
[3]National cerebral and cardiovascular center, Molecular pharmacology department
[4]Brain-Skeletal Muscle Connection in Aging Project Team, Geroscience Research Center, National Center for Geriatrics and Gerontology, Japan
[5]Juntendo Advanced Research Institute, Juntendo University Graduate School of Medicine, Japan
[6]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

**JSA-2 Satellite cells are not indispensable for repair following exercise-induced muscle damage**

Nao Tokuda, Azuma Naito, Nao Yamauchi, Ayaka Niibori, Takashi Yamada

Graduate School of Health Sciences, Sapporo Medical University, Japan

**JSA-3 Reactive oxygen species related DNA damage induced cellular senescence in myoblasts**

Daichi ARAI[1,2], Tsukasa TOMINARI[1,2], Masaru TAKATOYA[1], Urara KASUGA[1], Michiko HIRATA[1], Yoshitsugu AOKI[2], Masaki INADA[1]

[1]Cooperative Major of Advanced Health Science,Tokyo University of Agriculture and Technology, Japan
[2]Department of Molecular Therapy,National Center of Neurology and Psychiatry, Japan

Chair: Motoi Kanagawa (Department of Cell Biology and Molecular Medicine, Ehime University, Japan)

**JSA-4 Overexpression of PGC-1α prevents eccentric contraction-induced muscle damage in a utrophin-independent manner**

Azuma Naito[1], Nao Tokuda[1], Nao Yamauchi[1], Koichi Himori[2], Yuki Ashida[3], Takashi Yamada[1]

[1]Graduate School of Health Sciences, Sapporo Medical University, Japan
[2]Institute for Glyco-core Research, Tokai National Higher Education and Research System, Nagoya University, Nagoya, Japan
[3]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

# Meeting Agenda

## JSA-5 Isometric interval training with high- but not low-intensity contractions improves fatigue resistance in dystrophin deficient muscle

Nao Yamauchi[1], Azuma Naito[1], Nao Tokuda[1], Yuki Ashida[2], Norio Motohashi[2], Yoshitsugu Aoki[2], Takashi Yamada[1]

[1]Graduate School of Health Sciences, Sapporo Medical University, Japan
[2]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

## JSA-6 Next-generation cell and gene therapy

Edvinas Cerniauskas, Uikyu Bang, Eman Taha, Akitsu Hotta, Makoto Ikeya, Hidetoshi Sakurai

CiRA, Kyoto University, Japan

## JSA-7 Inactivation of Aconitase2 under simulated microgravity and analysis of skeletal muscle-specific Aco2-deficient mice

Minori Suzuki[1], Erika Yamano[1], Kota Kishida[1], Miho Takata[1], Kosuke Sugiura[1,2], Yuta Yanagihara[3], Yuuki Imai[3], Kaori Tanaka[4], Iori Sakakibara[5], Madoka Uezumi[6], Akiyoshi Uezumi[6], Anayt Ulla[1], Takayuki Uchida[1], Takeshi Nikawa[1]

[1]Department of Nutritional Physiology, The Medical Nutrition, Graduate School of Tokushima, Japan
[2]Orthopedic Surgery, Institute of Biomedical Sciences, Tokushima University, Japan
[3]Division of Integrative Pathophysiology, Proteo-Science Center, Department of Pathophysiology, Graduate School of Ehime, Japan
[4]Division of Transcriptomics, Medical Institute of Bioregulation, Kyusyu University, Japan
[5]Department Physiology, School of Medicine, Aichi Medical University, Japan
[6]Division of Cell Heterogeneity, Medical Institute of Bioregulation, Kyusyu University, Japan

Chair: Akiyoshi Uezumi (Division of cell heterogeneity of Medical Institute of Bioregulation, Kyushu University, Japan)

## JSA-8 Elucidation of the mechanism by which training improves the engraftment efficiency of transplanted cells in Duchenne muscular dystrophy

Mayuho Miki[1,2], Nana Takenaka-Ninagawa[1], Mio Iwasaki[3], Kenji Murata[4], Sora Kawabata[4], Clémence Kiho Yoshioka Bourgeois[1,2], Megumi Goto[1], Tomoki Aoyama[2], Hidetoshi Sakurai[1]

[1]Department of Clinical application, Center for iPS cell Research and Application, Kyoto University, Japan
[2]Department of Physical Therapy, Human Health Sciences, Graduate School of Medicine, Kyoto University, Japan
[3]Department of Life Science Frontiers, Center for iPS cell Research and Application, Kyoto University, Japan
[4]Graduate Course of Health and Social Services, Saitama Prefectual University, Japan

## JSA-9 BMP signaling controls skeletal muscle cell maturation

Takumi Makino[1], Hidetoshi Sakurai[2], Ryuichiro Sato[1], Yoshio Yamauchi[1]

[1]Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Japan
[2]Center for iPS Cell Research and Application, Kyoto University, Japan

# Meeting Agenda

## JSA-10 A mechanistic analysis of epigenetic disruption in Facioscapulohumeral muscular dystrophy

Takumi Kishimoto, Mitsuru Honda, Hidetoshi Sakurai

Department of Clinical application, Center for iPSCs Research and Application (CiRA), Kyoto University, Japan

Chair: So-ichiro Fukada (Laboratory of Stem Cell Regeneration and Adaptation Graduate School of Pharmaceutical Sciences, Osaka University, Japan)

## JSA-11 RNA-based CRISPRoff silencing to target DUX4 in Facioscapulohumeral muscular dystrophy

Junjie He[1,2], Mitsuru Sasaki-Honda[1], Hidetoshi Sakurai[1]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan
[2]Graduate School of Medicine, Kyoto University, Japan

## JSA-12 Reduced expression of Dok-7 and agrin due to mechanical unloading induces acetylcholine receptor degeneration in type 1 myofibers in mice

Tatsuhiro Yamaguchi[1,2], Kazushige Sasaki[1], Koichi Nakazato[3]

[1]The University of Tokyo, Japan
[2]Japan Society for the Promotion of Science, Japan
[3]Nippon Sport Science University, Japan

## JSA-13 Elucidating the role of Oncostatin M signaling in myoblast proliferation and muscle physiology in wild-type and SOD1G93A mice

Chang Chen[1,2], Iah Saiful[1], Norio Motohashi[1], Akiko Uyeda[3], Rieko Muramatsu[3], Yoshitsugu Aoki[1]

[1]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan
[2]Tokyo Medical and Dental University, Japan
[3]Department of Molecular Pharmacology, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

## JSA-14 Development of in vitro neuromuscular junction model through direct reprogramming of human urine-derived cells from Amyotrophic lateral sclerosis patients

Kazuki Okoshi, Katsuhiko Kunitake, Norio Motohashi, Yoshitsugu Aoki

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan

# Meeting Agenda

18:30-19:45

## Poster Session 1

Chairs: Yuko Shimizu-Motohashi (National Center of Neurology and Psychiatry, Japan)
Takahiro Nakayama (Yokohama Rosai Hospital, Japan)

### Clinical assesment of DMD and the other diseases

**P-1 Health care transition from pediatric to adult care for patients with neuromuscular disorders in Japan: A single center study**

Ayaka Ohno[1], Yuko Shimizu-Motohashi[1], Yoko Takahashi-Kobayashi[1], Ryo Sugiyama[1], Madoka Mori-Yoshimura[2], Kaoru Yamamoto[1], Hisako Yamamoto[1], Noriko Sumitomo[1], Eri Takeshita[1], Takashi Saito[1], Eiji Nakagawa[1,3], Hirofumi Komaki[1,4]

[1]Department of Child Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Japan
[2]Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Japan
[3]Department of Epileptology, National Center Hospital, National Center of Neurology and Psychiatry, Japan
[4]Translational Medical Center, National Center of Neurology and Psychiatry, Japan

**P-2 Automatic calcuation of muscle volume of CT images of leg, using artificial intelligence**

Takahiro Nakayama[2], Joji Kimizuka[1], Fumiya Isaki[1]

[1]Rakuten Socio Business
[2]Yokohama Rosai Hospital

**P-3 Safety of dexmedetomidine anesthesia for muscle and nerve biopsy**

Satoko Ota[1], Akinori Uruha[1], Tomoya Kawazoe[1], Keizo Sugaya[1], Nanae Miyake[2], Shiro Fukuda[2], Hiroaki Matayoshi[2], Kazushi Takahashi[1]

[1]Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan
[2]Department of Anesthesiology, Tokyo Metropolitan Neurological Hospital, Tokyo, Japan

**P-4 Usefulness of ultrasound-guided nerve block in muscle biopsy**

Nozomu Tawara[1], Yoshihiro Shibata[2], Yasushi Maeda[1], Kentaro Hara[1], Chikako Nagatoshi[1], Akiko Fujimoto[1], Masatoshi Ishizaki[1], Ryoichi Kurisaki[1], Yasuto Nishida[1], Hidetsugu Ueyama[1]

[1]Department of Neurology, NHO Kumamoto Saishun Medical Center, Japan
[2]Department of Anesthesiology, NHO Kumamoto Saishun Medical Center, Japan

**P-5 Empowering Progress: Patient Registries and Digital Transformation in Neuromuscular Disorders**

Anna Cho[1], Yun Jeong Lee[2], Jung Hwan Lee[3], Jin-Hong Shin[4], Dajung Kim[5], Yonghyun Kim[5], Jong Hee Chae[6,7]

[1]Department of Pediatrics, Seoul National University Bundang Hospital, Seongnam, Republic of Korea
[2]Department of Pediatric, Kyungpook National University Hospital, Daegu, Republic of Korea
[3]Department of Neurology, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Republic of Korea
[4]Department of Neurology, Pusan National University Yangsan Hospital, Yangsan, Republic of Korea
[5]Humanscape, Seoul, Republic of Korea
[6]Department of Genomic Medicine, Seoul National University Hospital, Seoul, Republic of Korea
[7]Department of Pediatrics, Seoul National University College of Medicine, Seoul, Korea

# Meeting Agenda

## P-6 Two cases of female patients with Duchenne muscular dystrophy caused by DMD gene mutation

Shiqi Yang[1], Jihang Luo[1], Yidan Liu[1], Cuijie Wei[1], Hui Xiong[2]

[1]Peking University first Hospital, China
[2]Beijing Children's Hospital, Capital Medical University, China

## P-7 Activities for patient involvement in Becker muscular dystrophy in Japan

Hiroyuki Shibasaki[1,2], Masaru Torigoe[1], Hikaru Endo[1], Yuko Fukue[1], Yoshitsugu Aoki[2], Akinori Nakamura[3], Hirofumi Komaki[4], Hisanobu Kaiya[1], Tamotsu Takeda[1]

[1]Japan Muscular Dystrophy Association, Tokyo, Japan
[2]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[3]Department of Neurology NHO Matsumoto Medical Center, Nagano, Japan
[4]Translational Medical Center, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan

## P-8 Integrated Ayurveda and Yoga Intervention for Enhancing Functionality and Ambulation in Patients with Muscular Dystrophies: A Single-arm Pilot Feasibility Trial

Dr Umesh Chikkanna[2], Dr. Hemanth Bhargav[3], Dr. Nalini Atchayaram[4], Dr. Kishore Kumar Ramakrishna[1], Dr. Bharath Holla[5]

[1]Dr. Kishore Kumar R, Professor, Department of Integrative Medicine, NIMHANS, Bengaluru, India
[2]Dr. Umesh Chikkanna, Assistant Professor, Department of Integrative Medicine, NIMHANS, Bengaluru, India
[3]Dr. Hemanth Bhargav, Associate Professor, Department of Integrative Medicine, NIMHANS, Bengaluru, India
[4]Dr. Nalini Atchayaram, Professor, Department of Neurology, NIMHANS, Bengaluru, India
[5]Dr. Bharath Holla, Associate Professor, Department of Neurology, NIMHANS, Bengaluru, India

## P-9 Natural history of Becker muscular dystrophy in a Japanese national registry of muscular dystrophy

Hotake Takizawa[1], Madoka Mori-Yoshimura[1], Yuji Takahashi[1], Wakako Yoshioka[2], Ichizo Nishino[2], Harumasa Nakamura[3]

[1]Department of Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan
[3]Department of Clinical Research Support, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan

# Meeting Agenda

18:30-19:45

## Poster Session 2

Chairs: Satoru Noguchi (Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

Seung Ah Lee (Department of Neurology, Ewha Womans University Mokdong Hospital, Republic of Korea)

### Genetic analyses and identification of the variants

**P-10 The burden of consanguineous marriages in Swat Valley, Pakistan**

AMJAD IQBAL, IRFAN ULLAH, Rahmat Ullah, Zaheer Udin, Ubaid Ullah, Zeeshan Khan, Umapathi N.Thirugnanam, Wasil Khan, Mian Ayazul Haq, Muhammad Ayub Khan

Saidu Group Of Teaching Hospital, Swat, Pakistan

**P-11 Aberrant mRNA processing caused by splicing mutations in TTN-related neuromuscular disorders**

Pengfei Lin, Guangyu Wang, Wenjing Wu, Xiaoqing Lv, Chuanzhu Yan

Qilu Hospital of Shandong University, China

**P-12 Novel compound heterozygous mutations in the TTN gene: elongation and truncation variants causing limb-girdle muscular dystrophy type 2J in a Han Chinese family**

Pengfei Lin, Guangyu Wang, Xiaoqing Lv, Ling Xu, Rui Zhang, Chuanzhu Yan

Qilu Hospital of Shandong University, China

**P-13 The Clinical Features and TTN Mutation Spectrum in a Chinese Cohort of Patients with hereditary myopathy with early respiratory failure**

Pengfei Lin[1], Xiaoqing Lv[2], Yuebei Luo[4], Zhe Zhao[3], Chuanzhu Yan[1], Wei Lv[5]

[1]Qilu Hospital of Shandong University, China
[2]Qilu Hospital of Shandong University, Cheeloo College of Medicine, Shandong University
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**P-14 Identification of deep intronic pathogenic variants in autosomal recessive muscle disorders by in silico splicing prediction**

Rui Shimazaki[1], Yoshihiko Saito[1,2], Tomonari Awaya[3,4], Ryo Kurosawa[3], Motoyasu Hosokawa[5], Hiroaki Ohara[3], Shinichiro Hayashi[1], Akihide Takeuchi[5], Masatoshi Hagiwara[3], Satoru Noguchi[1], Ichizo Nishino[1,2]

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[3]Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan
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[5]Department of Developmental Biology and Functional Genomics, Ehime University Graduate School of Medicine, Ehime, Japan

## P-15 The Clinical Features and TCAP Mutation Spectrum in a Chinese Cohort of Patients with Limb-girdle Muscular Dystrophy R7

Pengfei Lin[1,5], Xiaoqing Lv[1], Feng Lin[1], Wenjing Wu[1], Hui Wang[3], Yuebei Luo[4], Zhiqiang Wang[2], Chuanzhu Yan[5], He Lv[3], Sushan Luo[6]

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## P-16 Aberrant mRNA processing caused by splicing mutations in TTN-related neuromuscular disorders

Pengfei Lin, Guangyu Wang, Dandan Zhao, Chuanzhu Yan

Qilu Hospital of Shandong University, Jinan 250012 Shandong, China

## P-17 First reported case of distal arthrogryposis type 2A in Korea with genetic confirmation

Soo-Hyun Kim, Yun Jung Choi, Young-Chul Choi

Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

## P-18 Deletion of exons 6-9 of the ISPD gene causes congenital muscular dystrophy in mice

Jihang Luo[1], Yidan Liu[1], Shiqi Yang[1], Danyu Song[1], Yanbin Fan[1], Hui Xiong[2]

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[2]Beijing Children's Hospital, Capital Medical University, China

## P-19 Clinical and molecular genetic analysis further delineates the phenotypic variability of POMT2-related limb girdle muscular dystrophy type R14

Pengfei Lin[1], Guiguan Yang[1], Meirong Liu[2], Xiaoqing Lv[1], Chuanzhu Yan[1]

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## P-20 Aberrant splicing caused by three intronic mutations in autosomal recessive limb-girdle muscular dystrophy-1

Pengfei Lin, Guangyu Wang, Wenjing Wu, Haoyang Liu, Chuanzhu Yan

Qilu Hospital of Shandong University

## P-21 Phenotype-driven variant prioritization and re-analysis enhances genetic diagnosis of Neuromuscular Disorders

Chun Hing She, Yao Lei, Wanling Yang, Sophelia Hoi Shan Chan

Department of Paediatrics and Adolescent Medicine, The University of Hong Kong

### P-22 Novel *TFG* mutation causes autosomal-dominant spastic paraplegia and defects in autophagy

Pengfei Lin, Ling Xu, Yuying Zhao, Chuanzhu Yan

Department of Neurology and Research Institute of Neuromuscular and Neurodegenerative Diseases, Qilu Hospital of Shandong University, Jinan 250012 Shandong, China

18:30-19:45

## Poster Session 3

Chairs: Shinichiro Hayashi (National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

Takahiro Fujimoto (Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan)

## DMD pathogenesis

### P-23 Investigation of dystrophin localization and function in dog and mouse sperm

Katsura Minegishi, Satomi Shirakaki, Yoshitsugu Aoki

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan

### P-24 Characteristics of the skeletal muscle in Duchenne muscular dystrophy model rat

Karina Kouzaki, Yuki Tamura, Koichi Nakazato

Nippon Sport Science University, Japan

### P-25 Generation and characterization of *DMD*-edited microminipigs: an advanced surrogate for Duchenne muscular dystrophy

Michihiro Imamura[1], Masayoshi Otake[2], Satoko Enya[2], Akihisa Kangawa[2], Masatoshi Shibata[2], Kinuyo Ozaki[3], Koichi Kimura[4], Etsuro Ono[3], Yoshitsugu Aoki[1]

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[4]Departments of Laboratory Medicine/Cardiology, The Institute of Medical Science, The University of Tokyo

### P-26 Muscle stem cells remain quiescent in Duchenne muscular dystrophy

Shinichiro Hayashi[1], Francia Victoria A De Los Reyes[1], So-ichiro Fukada[2], Satoru Noguchi[1], Ichizo Nishino[1]

[1]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP)
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### P-27 Dystrophin short product-specific tag-insertion transgenic mouse line

Takahiro Fujimoto, Kyoko Itoh

Department of Pathology and Applied Neurobiology, Kyoto Prefectural University of Medicine, Graduate School of Medical Science, 465 Kajii-cho, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602-8566, Japan

## P-28 Genetic elucidation of the role of dystrophin isoforms in cognitive processes

Hiroya Ono[1,2,3,4], François Ruby[2], Cassandre Corvo[5], Sébastien Goutal[5], Nicolas Tournier[5], Valentina Taglietti[1], Frederic Relaix[1], Olivier Stettler[1], Laurent Tiret[1,2]

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[5]Laboratoire d'Imagerie Biomédicale Multimodale (BIOMAPS), Université Paris-Saclay, CEA, CNRS, INSERM, Service Hospitalier Frédéric Joliot, 91401, Orsay, France

## P-29 Regional differences in telomere length and their association with disease progression in canine models of Duchenne Muscular Dystrophy

Karin Watanabe[1,2], Mutsuki Kuraoka[2,3], Hitoshi Hatakeyama[4], Toshiaki Yamamoto[5], Eri Takeuchi[2], Katsura Minegishi[2], Yoshiyuki Ohta[1], Yoshitsugu Aoki[2]

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## P-30 Patient-derived iPSC brain organoids as a model for cognitive phenotypes of Duchenne Muscular Dystrophy

Chaitra Sathyaprakash[1], China Tatebori[1], Reiko Terada[1], Katsuhiko Kunitake[1], Daisuke Kawauchi[2], Momoko Watanabe[3], Mariko Taniguchi-Ikeda[4], Hideya Sakaguchi[5], Yoshitsugu Aoki[1]

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## P-31 Investigating the role of AQP4 in abnormal social behavior exhibited by mdx52 mice

Yuki Ashida[1], Hironaka Igarashi[2], Eri Takeuchi[1], Norio Motohashi[1], Masayuki Sekiguchi[1], Yoshitsugu Aoki[1]

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[2]Center for Integrated Human Brain Science, Brain Research Institute, University of Niigata, Niigata, Japan

### P-32 Enhanced fear and anxiety-like behavior associated with Brain Dp427 deficiency in Duchenne muscular dystrophy dogs

Eri Takeuchi[1], Shinichiro Taya[2], Tomoki Nishioka[3], Yuki Ashida[1], Michihiro Imamura[1], Takefumi Kikusui[4], Masayuki Sakiguchi[1,5], Yoshitsugu Aoki[1]

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[3]Division of Cell Biology, International Center for Brain Science, Fujita Health University
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[5]Laboratory of Pharmacology, Faculty of Pharmaceutical Science, Tokyo University of Science

### P-33 Characterizing subcellular localization and developmental expression of dystrophin in mammalian brain models

Simon BENETIERE[1], Yoshie KAMATA[1], Satomi SHIRAKAKI[1], Daisuke KAWAUCHI[2], Chaitra SATHYAPRAKASH[1], Yoshitsugu AOKI[1]

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## Poster Session 4

Chairs: Keiko Ishigaki (Department of Pediatrics, Tokyo Women's Medical University, Japan)
Norio Motohashi (Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan)

## Treatment and therapy for DMD and the other diseases

### P-34 Characterization of disease-specific alterations in metabolites and effects of mesenchymal stromal cells on dystrophic mice

YUKO NITAHARA-KASAHARA[1], Guillermo Posadas-Herrera[1], Kunio Hirai[2], Yuki Oda[2], Noriko Snagu-Miyamoto[3], Yuji Yamanashi[4], Takashi Okada[1]

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[3]Division of Oral and Maxillofacial Surgical, Tokyo Women's medical school
[4]Division of Genetics, The Institute of Medical Science, The University of Tokyo

### P-35 Evaluation of the efficacy of Viltolarsen to Duchenne muscular dystrophy using muscular imaging

Satoshi Kuru

Suzuka National Hospital, Japan

## P-36 Exploration of the current challenges and future directions for optimizing DMD management in Asia from a survey and expert panel discussion

Wen-Chin Weng[3,4,5], Furene Wang[6,7], Hsu-Wen Chou[1], Jocelyn Lim[8], Roy Gomez[2], Sarah Tsai[1], Sophelia HC Chan[9], Ting Rong Hsu[10,11], Yuh-Jyh Jong[12,13,14]

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[6]Khoo Teck Puat-National University Children's Medical Institute, National University Hospital, Singapore
[7]Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[8]Neurology Service, Department of Paediatric Medicine, KK Women's and Children's Hospital, Singapore
[9]Paediatric Neurology Division, Department of Paediatrics and Adolescent Medicine, School of Clinical Medicine, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong
[10]Department of Pediatrics, Taipei Veterans General Hospital, Taipei, Taiwan
[11]Institute of Clinical Medicine, National Yang-Ming University, School of Medicine, Taipei, Taiwan
[12]Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[13]Departments of Pediatrics and Laboratory Medicine, and Translational Research Center of Neuromuscular Diseases, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan
[14]Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan

## P-37 A real-world survey on the treatment patterns and neurologist treatment satisfaction for Duchenne muscular dystrophy patients in Japan

Keiko Ishigaki[1], Mitsuhiro Nagano[2], Daisuke Shima[2], Nate Posner[3], Joseph Cappelleri[3], Anna Talaga[3], Ella Morton[4], Halima Iqbel[4], Emma Chatterton[4], Jonathan DeCourcy[4]

[1]Tokyo Women's Medical University
[2]Pfizer Japan
[3]Pfizer
[4]Adelphi Real World

## P-38 Febuxostat improves DMD phenotype in dystrophin mutant model of mice via enhancement of cellular ATP

Satomi Shirakaki[1], Norio Motohashi[1], Naoyuki Kamatani[2], Yoshitsugu Aoki[1]

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## P-39 Development of a treatment for Duchenne muscular dystrophy based on the Fucosyltransferase 8

Nozomi Hayashiji[1], Tomohiko Fukuda[2], Yoshitsugu Aoki[3], Jianguo Gu[2], Eri Arikawa-Hirasawa[4]

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[3]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry
[4]Research Institute for Diseases of Old Age, Juntendo University Graduate School of Medicine
Background

## P-40 Drug screening to induce jagged1 expression using transgenic zebrafish

Genri Kawahara, Mami Nakayashiki, Yukiko Hayashi

Department of pathophysiology, Tokyo Medical University, Japan

### P-41 Enhancing Antisense-Oligonucleotide Delivery through Muscle Metabolism Regulation

Norio Motohashi, Yuki Ashida, Yoshitsugu Aoki
Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan

### P-42 *In vivo* gene therapy for striated muscle laminopathy

Mariko Okubo[1,2], Astrid Brull[1], Maud Beuvin[1], Nathalie Mougenot[1], Gisele Bonne[1], Anne T.Bertrand[1]
[1]Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France
[2]National Center for Global Health and medicine, Research Institute, Tokyo, Japan

18:30-19:45

## Poster Session 5

Chairs: Hidetoshi Sakurai (Department of Clinical Application Center for iPS Research and Application (CiRA), Kyoto University, Japan)
Wen-Chen Liang (Kaohsiung Medical University, Taiwan)

## LGMD and FSHD

### P-43 Development of a technique to map the transcriptome to histological changes in frozen sections of skeletal muscle at the single-cell level

Nanami Yamada[1,2], Hiroki Ikeda[2], Kazuki Kurimoto[2], Kazuma Sugie[1]
[1]Department of Neurology, Nara Medical University
[2]Department of Embryology, Nara Medical University

### P-44 Dissecting the immunometabolism of delta-sarcoglycan deficient animal model with multimodal mass spectrometry imaging

Maiko Okamura[1], Shinichi Yamaguchi[2], Takushi Yamamoto[2], Koji Okuda[2], Shuji Yamashita[1], Satoru Noguchi[3], Kisaki Amemiya[4], Kenji Minatoya[5], Hidetoshi Masumoto[5], Ichizo Nishino[3], Hatsue Ishibashi-Ueda[4,6], Masaya Ikegawa[1]
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[4]Department of Pathology, National Cerebral and Cardiovascular Center
[5]Department of Cardiovascular Surgery, Graduate School of Medicine, Kyoto University
[6]Department of Diagnostic Pathology, Hokusetsu General Hospital

### P-45 Generation of a zebrafish model of limb-girdle muscular dystrophy (LGMDR6) using genome editing technology

Shohei Majima, Hiroaki Mitsuhashi
Graduate School of Engineering, Course of Applied Science, Tokai University

## P-46 Stress-induced Cardiomyopathy with Aspiration Pneumonia Caused by the Ventilator Disconnection Accident in a Patient with Limb Girdle Muscular Dystrophy

Koichi Kimura[1], Hiroyuki Morita[2], Koki Nakanishi[2], Masao Daimon[3], Tomoko Nakao[2], Megumi Hirokawa[2], Yuriko Yoshida[2], Tetsuhiko Ikeda[4], Yoshitsugu Aoki[5], Norihiko Takeda[2]

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[4]Department of Neurology, NHO Niigata National Hospital, Niigata, Japan
[5]Department of Molecular Therapy, National Institute of Neuroscience, National Centre of Neurology and Psychiatry, Tokyo, Japan

## P-47 The introduction of the FSHD patient association in Japan and the future perspective towards international collaboration

FSHD Japan core members[1,2]

[1]FSHD Japan
[2]The Japan Muscular Dystrophy Association

## P-48 The temporal changes of physiologic functions and gene expressions in drug-induced skeletal muscle specific *DUX4* over expression mice

Takahiro Yoshizawa[1], Daigo Miyazaki[2], Tomohide Takaya[3], Tsutomu Nakada[1], Akinori Nakamura[4], Tomoki Kosho[5]

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[3]Department of Agriculture, Graduate School of Science and Technology, Shinshu University
[4]National Hospital Organization Matsumoto Medical Center
[5]Department of Medical Genetics, Shinshu University School of Medicine

## P-49 *JAG2*-related muscular dystrophy as a rare mimicry of facioscapulohumeral muscular dystrophy (FSHD)

Yuan GAO[1], Amanda CHEUNG[2], Jacky LING[3], Felix WONG[3], Ching-wan LAM[3], Shirley PANG[1]

[1]Division of Neurology, Department of Medicine, Queen Mary Hospital, The University of Hong Kong
[2]Division of Anatomical Pathology, Department of Pathology, Queen Mary Hospital, The University of Hong Kong
[3]Division of Chemical Pathology, Department of Pathology, Queen Mary Hospital, The University of Hong Kong

## P-50 Characteristics of Patient with FacioscapulohumeralMuscularDystrophy in Japanese Nationwide Registry of Muscular Dystrophy (Remudy)

Tsuyoshi Matsumura[1], Hotake Takizawa[2], Wakako Yoshioka[3], Madoka Mori-Yoshimura[2], Yoshihiko Saito[3], Ichizo Nishino[3], Harumasa Nakamura[4]

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### P-51 Facioscapulohumeral Muscular Dystrophy in Taiwan

Chen-Hua Wang[1], Wen-Chen Liang[2,4,5], Chien-Hua Wang[2], Shyh-Shin Chiou[2,3,4], Yuh-Jyh Jong[2,3,5]

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## Poster Session 6

Chairs: Takashi Kurashige (Department of Neurology, NHO Kure Medical Center and Chugoku Cancer Center, Japan)
Theerawat Kumutpongpanich (Division of Neurology, Department of Internal Medicine, Siriraj Hospital, Mahidol University, Thailand)

## Channelopathies and Myotonic dystrophies

### P-52 Tubular Aggregate Myopathy: A Case Series Analysis in a Tertiary Hospital in Taiwan

Chen-Hua Wang[1], Wen-Chen Liang[2,4,5], Wan-Ling Hsiao[2], Yuh-Jyh Jong[2,3,5]

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[4]Department of Pediatrics, School of Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan
[5]Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

### P-53 Excess Desmin expression diminishes muscle contractile function concomitant with alteration of SOCE protein expression in mice skeletal muscle

Satoru Ato[1,2]

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### P-54 Subcellular localization of sarcoplasmic reticulum-related factors in gastrocnemius muscle of aged mice

Yuji Kanazawa[1,2], Tatsuo Takahashi[3], Mamoru Nagano[2], Satoshi Koinuma[2], Takao Inoue[4], Yasufumi Shigeyoshi[2]

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[3]Department of Clinical Pharmacology, Hokuriku University
[4]Department of Pathology, Kindai University Faculty of Medicine

## P-55 Pathological features in hypokalemic periodic paralysis due to *ATP1A2*

Yukako Yae[1,2], Masashi Ogasawara[1,3], Ikuya Nonaka[1], Shinichiro Hayashi[1], Aritoshi Iida[4], Yuko Okamura-Oho[5], Satoru Noguchi[1], Ichizo Nishino[1,4]

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[3]Department of Pediatrics, Showa General Hospital
[4]Department of Clinical Genome Analysis, Medical Genome Center, National Center of Neurology and Psychiatry
[5]Faculty of Human Life Science, Jissen Women's University

## P-56 Clinical and genetic features of patients with paramyotonia congenita in Korea

Yun Jung Choi, Soo-Hyun Kim, Young-Chul Choi

Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea

## P-57 Comprehensive analysis of splicing abnormalities in multiple brain regions of myotonic dystrophy type 1: Comparisons between frontal cortex, temporal cortex, and cerebellum

Manami Hama[1], Aono Fukumoto[1], Kazuki Segawa[1], Yoshiaki Yasumizu[2], Kana Shiotsu[1], Kazuki Yoshizumi[3], Takashi Kimura[3], Tsuyoshi Matsumura[4], Tomoya Kubota[1], Kimiko Inoue[4], Harutoshi Fujimura[4], Masaaki Komatsu[5], Ken Asada[5], Syuzo Kaneko[5], Ryuji Hamamoto[5], Masanori P. Takahashi[1]

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[5]Division of Medical AI Research and Development, National Cancer Center Research Institute, Tokyo 104-0045, JAPAN

## P-58 MRI evaluation of sinusitis complications: Comparison of Myotonic dystrophy type 1 and Amyotrophic lateral sclerosis

Michio Kobayashi, Tomoyuki Hatakeyama, Erika Abe, Chizu Wada, Tadayuki Ishihara, Itaru Toyoshima

Department of Neurology, NHO Akita National Hospital, Akita, Japan

### P-59 Assessment of cognitive function in a Japanese DM2 patient

Shigehisa Ura[1], Mai Miyagishi[1], Kaede Ishikawa[1], Masahiro Wakita[1], Hiroaki Yaguchi[2], Ichiro Yabe[2], Mika Otsuki[3], Narihiro Minami[4], Ichizo Nishino[4,5], Satomi Mitsuhashi[6,7], Tohru Matsuura[8]

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[7]Department of Genomic Function and Diversity,Medical Research Institute,Tokyo Medical and Dental University,Japan
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### P-60 Consideration focusing on caregivers in intervention research through the program for patients with myotonic dystrophy type 1 and their caregivers

HIROTO TAKADA[1], MOMOKO GOTO[1], MAKIKO ENDO[2], KAORI ODAIRA[1], GO KURAUCHI[1], HIROMI SATO[1], SEIKO KON[1], ATSUSHI KOSEKI[3], NORIO WATANABE[4], NORIO SUGAWARA[5], MADOKA MORI[2], HARUMASA NAKAMURA[2], E N KIMURA[6]

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[2]National Center of Neurology and Psychiatry
[3]NHO Hokkaido Medical Center
[4]Graduate School of Medicine Kyoto University
[5]Dokkyo Medical University
[6]Graduate School of Medicine Osaka University

18:30-19:45

## Poster Session 7

Chairs: Toshiaki Takahashi (NHO Sendai Nishitaga Hospital, Japan)
Nobuyuki Eura (Department of Neurology, Nara Medical University, Nara, Japan)

## Distal myopathies

### P-61 Genetic features of Japanese dysferlinopathies

Toshiaki Takahashi[1], Rumiko Izumi[2,3], Naoki Suzuki[2,4], Chikako Yaginuma[5], Naoko Shimakura[2], Naoko Nakamura[1,2], Yasuko Shimosegawa[6], Tomoko Totsune[1], Yoko Sugimura[1], Takahiko Sasaki[7], Masaru Yoshioka[1], Toru Baba[1], Hideki Oizumi[1], Hiroyasu Tanaka[1], Hirtoshi Warita[2], Tetsuya Niihori[3], Takafumi Hasegawa[1], Atsushi Takeda[1], Yoko Aoki[3], Masashi Aoki[2]

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[4]Department of Rehabilitation Medicine, Tohoku University School of Medicine
[5]Department of Clinical Laboratory, National Hospital Organization Sendai Nishitaga Hospital
[6]Department of Neurosurgery, National Hospital Organization Sendai Nishitaga Hospital
[7]Department of Internal Medicine, National Hospital Organization Sendai Nishitaga Hospital

## P-62 Analysis of the distribution of affected muscles in anoctaminopathy

Reoto Ueda[1,2], Hiroto Azuma[1,2], Ai Yamanaka[1,2], Wakako Yoshioka[1], Shinichiro Hayashi[1], Satoru Noguchi[1], Kazuma Sugie[2], Ichizo Nishino[1]

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## P-63 The International Clinical Outcome Study for Dysferlinopathy - 10 years of natural history data

Jinhong Shin[1], Meredith James[2], Heather Hilsden[2], Heather Gordish-Dressman[3], John Day[4], Jerry Mendell[5], Roberto Fernandez Torron[6], Matt Harms[7], Alan Pestronk[8], John Vissing[9], Urvi Desai[10], Madoka Yoshimura[11], Tahseen Mozaffar[12], Tanya Stojkovic[13], Elena Pegoraro[14], Jorge Bevilacqua Rivas[16], Montse Olive[15], Carmen Paradas[17], Maggie Walter[18], Volker Straub[2]

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[2]John Walton Muscular Dystrophy Research Centre, Translational and Clinical Research Institute, The Newcastle upon Tyne Hospitals NHS Foundation Trust and Newcastle University, UK
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[5]Nationwide Children's Hospital, Columbus, OH, USA
[6]Neuromuscular Area, Biodonostia Health Research Institute, Neurology Service, Donostia University Hospital, Donostia-San Sebastian, Spain
[7]Neurology, Columbia University Irving Medical Center, New York, NY, USA
[8]Neurology, Washington University School of Medicine, St Louis, MO, USA
[9]Copenhagen Neuromuscular Center, Department of Neurology, Rigshospitalet, University of Copenhagen,Copenhagen, Denmark
[10]Carolinas MDA Care Center, Atrium Health, Charlotte, NC, USA
[11]Neurology, National Center Hospital, National Center of Neurology and Psychiatry, Tokyo, Japan
[12]Neurology, MDA ALS and Neuromuscular Center, University of California-Irvine, Orange, CA, USA
[13]Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
[14]Neuroscience, University of Padova, Padova, Italy
[15]Neuromuscular Disorders Unit, Neurology Department, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain
[16]Neurología y Neurocirugía Clínica, Clínica Dávila, Santiago, Chile
[17]Neuromuscular Unit, Neurology, Hospital U. Virgen del Rocío/Instituto de Biomedicina de Sevilla, Seville, Spain
[18]Friedrich-Baur-Institute, Ludwig-Maximilians-University of Munich, Germany

## P-64 Analysis of a novel mechanism of extracellular vesicles secretion from skeletal muscle

Kana Tominaga[1], Naoomi Tominaga[2]

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[2]Department of Clinical Laboratory Science, Yamaguchi University Graduate School of Medicine

## P-65 Analysis of anoctaminopathy focusing on inflammatory pathology

Hiroto Azuma[1,2], Reoto Ueda[1,2], Ai Yamanaka[1,2], Shinichiro Hayashi[1], Satoru Noguchi[1]

[1]Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Neurology, Nara Medical University, Nara, Japan

## P-66 Multisystem Proteinopathy in Neurological Disorder

XINGYU XIA[1], Xi Chen[1], Yiming Sun[1], Ming Zhang[2], Kai Qiao[1], Yan Chen[1], Chongbo Zhao[1], Yi Dong[1], Wenhua Zhu[1]

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[2]Department of Medical Genetics, School of Medicine, Tongji University,200090, Shanghai, China

## P-67 Clinicopathological characteristics of Japanese patients with multisystem proteinopathy

Satoshi Yamashita[1,2], Yuji Takahashi[3], Jun Hashimoto[4], Ayuka Murakami[5], Ryoichi Nakamura[5,6], Masahisa Katsuno[5,7], Rumiko Izumi[8], Naoki Suzuki[8], Hitoshi Warita[8], Masashi Aoki[8]

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[8]Department of Neurology, Tohoku University Graduate School of Medicine, Sendai, Japan

## P-68 A pilot, placebo-controlled trial of 6'-sialyllactose in GNE myopathy

Jinhong Shin[2,4], Jaeil Choi[2,3], Joon Ki Jung[3], Lila Kim[3], Yeoung-Eun Park[1,4], Dae-Seong Kim[2,4]

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[2]Pusan National University Yangsan Hospital, Korea
[3]New Drug Develpment, Neuragene
[4]Pusan National University School of Medicine, Korea

## P-69 Mysterious Foot drop in two sisters from swat Pakistan

Amjad Iqbal[1], N.Thirugnanam Umapathi[2], Muhammad Ayub Khan[1], IRFAN Ullah[1], Mian Ayaz Ul Haq[4], Ubaid Ullah[4], Zeeshan Khan[1], Rahmat Ullah[3], Wajeeh Ur Rehaman[1]

[1]Saidu Group Of Teaching Hospital Swat Pakistan
[2]Department of Neurology, at the National Neuroscience Institute Singapore
[3]Bannu Medical College KPK Pakistan
[4]LRH Peshawar Pakistan

## P-70 Long-term analysis of oculopharyngodistal myopathy: Clinical course and electrophysiological evidence of purely neurogenic changes

Nobuyuki Eura[1], Ai Yamanaka[1,2], Akito Tanaka[1], Minako Yamaoka[1], Tomo Shiota[1], Naohiko Iguchi[1], Yukako Nishimori[1], Ichizo Nishino[2], Kazuma Sugie[1]

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## Poster Session 8

Chairs: Tsukasa Tominari (National Center of Neurology and Psychiatry, Japan)
Anna Cho (Seoul National University Bundang Hospital / Seoul National University College of Medicine, Republic of Korea)

### UCMD and tendinopathies

#### P-71 Lipid metabolism is impaired in mEDS mouse model, and their muscle pathology may recover with HFD feeding

Haruto Kushige[1,2], Sakura Shimogaito[2], Fumiyo Saito[3], Ryusuke Momota[5], Risuke Mizuno[4], Hiroshi Sakai[6], Yuuki Imai[6], Manuel Koch[7], Yayoi Izu[8]

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[2]Department of Laboratory Animal Science, Faculty of Veterinary Medicine, Okayama University of Science
[3]Department of Veterinary Toxicology, Faculty of Veterinary Medicine, Okayama University of Science
[4]Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, Okayama University of Science
[5]Department of Human Morphology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences
[6]Division of Integrative Pathophysiology, Proteo-Science Center, Department of Pathophysiology, Graduate School of Medicine, Ehime University
[7]University of Cologne
[8]Laboratory of Comparative Cellular Biology, Nippon Veterinary and Life Science University

#### P-72 Splicing switching of alternative last exons due to a deletion including canonicalpolyadenylation site in *COL6A2* gene causes recessive UCMD

Yoshihiko Saito[1], Rasha El Sherif[2,3], Tomonari Awaya[4], Shinichiro Hayashi[1], Satoru Noguchi[1], Ichizo Nishino[1]

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[2]Myo-Care Neuromuscular Center, Myo-Care National Foundation
[3]School of Medicine, New Giza University
[4]Department of Anatomy and Developmental Biology, Graduate School of Medicine and Faculty of Medicine, The University of Kyoto

#### P-73 Ullrich Congenital Muscular Dystrophy in Taiwan- A Medical Center Experience

Chen-Hua Wang[1], Wen-Chen Liang[2,4,5], Yuh-Jyh Jong[2,3,5]

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[2]Departments of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
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[5]Graduate Institute of Clinical Medicine, College of Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan

## P-74 The last nucleotide substitutions in exons of *COL6A1/2/3* induce exon skipping in collagen VI-related muscular dystrophies

Seung-Ah Lee[1,2], Yoshihiko Saito[2], Rui Shimazaki[2], Shinichiro Hayashi[2], Yu-ichi Goto[2], Satoru Noguchi[2], Nishino Ichizo[2]

[1]Department of Neurology, Ewha Womans University Mokdong Hospital, Ewha Womans University College of Medicine, Seoul, Republic of Korea
[2]Department of Neuromucular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

## P-75 Differentially expressed genes detected in the rat crural fascia after lengthening contractions using RNA-sequence analysis

Hiroki Ota[1,2], Kimiaki Katanosaka[3], Toru Taguchi[1,2]

[1]Dept. Phys. Ther., Fac. Rehabil., Niigata Univ. Health Welfare, Niigata, Japan
[2]Inst. Human Mov. Med. Sci., Niigata Univ. Health Welfare, Niigata, Japan
[3]Dept. Biomed. Sci., Col. Life Health Sci., Chubu Univ., Kasugai, Japan

## P-76 Retinoic acid receptor agonists have dual and opposing effects on injury-induced tendon ossification in a mouse Achilles tenotomy model

Masashi Isaji[1], Shugo Yonehara[1], Haruo Sasaki[1], Shinya Kondo[1], Takahiro Nakagawa[1], Masatoshi Amako[2], Keisuke Horiuchi[1]

[1]Department of Orthopedic Surgery, National Defense Medical College
[2]Department of Rehabilitation Medicine, National Defense Medical College Hospital

## P-77 Visualization of dynamic interactions between cartilaginous and tendinous/ligamentous primordia during musculoskeletal development

Xinyi Yu[1], Ryosuke Kawakami[2], Shinsei Yambe[1], Yuki Yoshimoto[1], Takako Sasaki[3], Haruhiko Akiyama[4], Taiji Adachi[5], Takeshi Imamura[2], Chisa Shukunami[1]

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[2]Department of Molecular Medicine for Pathogenesis, Graduate School of Medicine, Ehime University, Ehime, Japan
[3]Department of Biochemistry II, Faculty of Medicine, Oita University, Japan
[4]Department of Orthopaedic Surgery, Gifu University Graduate School of Medicine, Gifu, Japan
[5]Laboratory of Biomechanics, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

## P-78 Generation and characterization of conditional Mkx knockout mouse

Lin Liu, Tomoki Chiba, Hiroshi Asahara

Department of Systems BioMedicine, Graduate school of Medical and Dental Sciences, Tokyo Medical and Dental University

## P-79 Sclerostin is a modulator of mineralization degree and stiffness profile in the fibrocartilaginous enthesis for mechanical tissue integrity

Shinsei Yambe[1], Yuki Yoshimoto[2], Koichiro Maki[3], Xinyi Yu[1], Shigenori Miura[1], Toshihide Mizoguchi[4], Taiji Adachi[3], Chisa Shukunami[1]

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# Meeting Agenda

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## Poster Session 9

Chairs: Ito Mikako (Nagoya University, Graduate School of Medicine, Japan)
Tetsuya Takeda (Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan)

### NMJ, Myasthenia Gravis and Centronuclear diseases

**P-80 Early Effect of Efgartigimod in Generalized Myasthenia Gravis: A Single Center Experience from China**

Jingsi Wang, Qi Wen, Shu Zhang, Yaye Wang, Nairong Xie, Haoran Liu, Yuting Jiang, Qinyao Liu, Yan Lu, Li Di, Min Wang, Min Xu, Hai Chen, Suobin Wang, Wenjia Zhu, Xinmei Wen, Jianying Duo, Yue Huang, Yuwei Da
Department of Neurology, Xuanwu Hospital, Capital Medical University

**P-81 Changes in the Treatment of MG and the Therapeutic Effectiveness of FcRn Antagonists**

Takao Kiriyama, Hironori Shimizu, Akito Tanaka, Hitoki Nanaura, Kana Hamada, Tomohito Ohashi, Naoya Kikutsuji, Naohiko Iguchi, Nobuyuki Eura, Kazuma Sugie
Nara Medical University, Department of Neurology

**P-82 Neuromuscular blockade attenuates cramp-like muscle contraction induced by an infusion of hypertonic saline in rats**

Toru Taguchi[1,2], Hiroki Ota[1,2]
[1]Department of Physical Therapy, Faculty of Rehabilitation, Niigata University of Health and Welfare
[2]Institute of Human Movement and Medical Sciences, Niigata University of Health and Welfare, Japan

**P-83 Identification of a pair of closely related genes encoding muscle cytoplasmic proteins required for NMJ formation**

Akane Inoue-Yamauchi[1], Takahiro Eguchi[1,2], Yuji Yamanashi[1]
[1]The Institute of Medical Science, The University of Tokyo
[2]Present address: Brain-Skeletal Muscle Connection in Aging Project Team, Geroscience Research Center, National Center for Geriatrics and Gerontology

**P-84 Centronuclear Myopathy in *Dnm2* E368K Mice: Behavioral and Pathological Insights**

Genevieve Uy[1], Megumu Ogawa[1], Yukiko Inoue[2], Shinichiro Hayashi[1], Tetsuya Takeda[3], Yoshihiko Saito[1], Shinichiro Hayashi[1], Takayoshi Inoue[2], Ichizo Nishino[1], Satoru Noguchi[1]
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[2]Department of Biochemistry and Cellular Biology, National Center of Neurology and Psychiatry, Kodaira City, Tokyo, Japan
[3]Department of Biochemistry, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan

### P-85 Reconstitution approaches to elucidate pathomechanisms of centronuclear myopathy

Tetsuya Takeda[1], Kenshiro Fujise[1,2], Kohji Takei[1], Satoru Noguchi[3], Ichizo Nishino[3]

[1]Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Okayama, Japan
[2]Departments of Neuroscience and Cell Biology, Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT, USA
[3]National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Kodaira, Tokyo, Japan

### P-86 mTOR Signaling Dysregulation Contributes to Impaired Myogenic Differentiation in Mtm1-knockout C2C12 Cells

Kengo Kora, Atsushi Yokoyama, Naoko Yano, Kinuko Nishikawa, Taisei Kayaki, Satoshi Kajimoto, Junko Takita, Takeshi Yoshida

Department of Pediatrics, Kyoto University Graduate School of Medicine, Kyoto, Japan

18:30-19:45

## Poster Session 10

Chairs: Akitsu Hotta (Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan)
Takashi Yamada (Sapporo Medical University, Japan)

## Metabolic myopathies

### P-87 Telbivudine-induced myopathy and clinicopathological characteristics : Case report

Wannisa Wongpipathpong[1], Nantaporn Srivanitchapoom[1], Jariya Waisayarat[2], Charungthai Dejthevaporn[3]

[1]Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, Thailand
[2]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3]Division of Neurology, Department of Internal Medicine, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

### P-88 Neuromuscular junction dysfunction in a glycogen storage disease

Subramaniam Ganesh, Monica Shukla, Deepti Chugh

Department of Biological Sciences and Bioengineering, Indian Institute of Technology Kanpur, India

### P-89 Glycogen storage myopathies diagnosed by muscle biopsy in Iran, 16 years of experience in a referral center

Yalda Nilipour[1,2]

[1]Neuromuscular research center, Tehran University of Medical Sciences
[2]Pediatric pathology research center, research institute for children's health, Shahid Beheshti university of medical sciences, Tehran, Iran

### P-90 *ETFDH* mutation causes excessive apoptosis and neurite outgrowth defect via Bcl2 pathway

Wen-Chen Liang[1,3], Chuang-Yu Lin[4], Shin-Cheng Chang[1], Ming-Chi Lai[5], Yuh-Jyh Jong[1,2,3]

[1]Departments of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan
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[4]Department of Biomedical Science and Environmental Biology, College of Health Sciences, Kaohsiung Medical University, Kaohsiung, Taiwan
[5]Department of Pediatrics, Chi-Mei Medical Center, Tainan, Taiwan

### P-91 The novel GAA variant R190G: expanding the spectrum of late onset Pompe disease and possible implications for screening in the Chinese population

Kexin Jiao[1], Edwin H. Jacobs[2], Dongyue Yue[3], Jacqueline A.M.C. Boonman[2], Chongbo Zhao[1], Marianne Hoogeveen-Westerveld[2], W. W. M. Pim Pijnappel[2], Wenhua Zhu[1]

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[2]Department of Clinical Genetics, Erasmus University Medical Center, Rotterdam, The Netherlands
[3]Department of Neurology, Jing'an District Center Hospital of Shanghai, Shanghai, China

### P-92 Knockdown of LAMP-2 in HEK293T cells impairs lysosome homeostasis

Tomo Shiota[1], Kiichi Nakahira[2], Minako Yamaoka[1], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Pharmacology, Nara Medical University

18:30-19:45

## Poster Session 11

Chairs: Yukako Nishimori (Department of Neurology, Nara Medical University, Nara, Japan/Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan)
Yen-Lin Chen (Tri-Service General Hospital, National Defense Medical Center, Taiwan)

## Immune-Mediated Necrotizing Myopathy

### P-93 Asymptomatic hyperCKemia for 40 years: Diagnosis of treatable immune-mediated necrotizing myopathy with anti-SRP antibody

Ryota Ishida, Nobuyuki Eura, Mayu Sugata, Yukako Nishimori, Kazuma Sugie
Department of Neurology, Nara Medical University

### P-94 Challenges in the Diagnosis of Immune-Mediated Necrotizing Myopathy: Focus on EULAR/ACR Classification Criteria

SHOGO KOMAKI[1], AKATSUKI KUBOTA[1], MEIKO MAEDA[1], ASUKA KITAMURA[1], JUN SHIMIZU[2], TATSUSHI TODA[1]

[1]Department of Neurology, Graduate School of Medicine, The University of Tokyo
[2]Department of Physical Therapy, School of Health Sciences, Tokyo University of Technology

### P-95 Myositis associated with antimitochondrial autoantibodies showing early respiratory failure

Kentaro Kawama[1], Shinsuke Tobisawa[1], Akinori Uruha[1], Satoko Ota[1], Hirotake Nishimura[2], Hiromi Onizuka[3], Takashi Komori[4], Kazushi Takahashi[1]

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[2]Department of Pathology, Kawasaki Medical School
[3]Departments of Pathology, Kyorin University School of Medicine
[4]Department of Laboratory Medicine and Pathology (Neuropathology), Tokyo Metropolitan Neurological Hospital

### P-96 Analysis of muscle imaging of patient with anti-mitochondrial M2 antibody-positive myositis

Yukako Nishimori[1,2], Kazuma Sugie[1], Ichizo Nishino[2]

[1]Department of Neurology, Nara Medical University, Nara, Japan
[2]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

### P-97 Clinical characteristic of IMNM patients seen at Neurological Institute of Thailand

Sirilux Angsuwattanakul, Thanes Termglinjan, Arada Rojana-udomsart

Neurological Institute of Thailand, Department of medical Services, Ministry of Public Health, Bangkok, Thailand

### P-98 Clinical, Laboratory, and Radiological Features of Korean Patients with Anti-SRP Immune-Mediated Necrotizing Myopathy

Byoung Joo Choi, Hyung Jun Park, Soo-Hyun Kim, Yunjung Choi, Young-Chul Choi

Department of Neurology, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea

### P-99 PD-L1 inhibitor is a risk factor to cause severe respiratory failure in immune checkpoint inhibitor-associated myopathy

Hironori Shimizu, Masaki Kobayashi, Naohiko Iguchi, Yukako Nishimori, Hitoki Nanaura, Nobuyuki Eura, Takao Kiriyama, Hiroshi Kataoka, Kazuma Sugie

Department of Neurology, Nara Medical University

18:30-19:45

## Poster Session 12

Chairs: Jantima Tanboon (Department of Pathology, Mahidol University, Thailand)
Wenhua Zhu (Huashan Hospital, Fudan University, China)

## Myositis

### P-100 Exploring immunohistochemical expression in idiopathic inflammatory myopathies at a single center in Vietnam

SI LE TRI[2], Thu Phan Dang Anh[1]

[1]University of Medicine and Pharmacy
[2]University Medical Center of Ho Chi Minh city

## P-101 Nuclear actin in antisynthetase syndrome

Jantima Tanboon[1,3], Rui Shimazaki[1], Shinichiro Hayashi[1], Satoru Noguchi[1], Ichizo Nishino[1,2]

[1]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, JAPAN
[2]Department of Genome Medicine Development, Medical Genome Center, National Center of Neurology and Psychiatry, Tokyo, JAPAN
[3]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

## P-102 Interstitial lung disease patients associated with antisynthetase syndrome present myopathic change in electromyography without myositis symptoms: a prospective case series clinical study

Maki Ozaki[1], Naohiko Iguchi[1], Naoki Iwasa[1], Tomoo Mano[1,2], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Rehabilitation Medicine, Nara Prefecture General Medical Center

## P-103 Interstitial Lung Disease in Patients with Idiopathic Inflammatory Myopathies: Data from a Cohort at a Tertiary Care Center in Karachi, Pakistan

Shanawer Khan[1], Bisma Aziz[1], Marib Ghulam Rasool Malik[2], Shajeaa Ali[1], Akbar Shoukat Ali[1], Tariq Gazdar[3], Ali bin Sarwar Zubairi[1], Sara Khan[1]

[1]Department of Medicine, Aga Khan University Hospital, Karachi, Pakistan
[2]Department of Paediatrics and Child Health, Aga Khan University Hospital, Karachi, Pakistan
[3]Autoimmune Labs and Clinic, Karachi, Pakistan

## P-104 Compound muscle action potential of whole-forearm flexors: A clinical biomarker for inclusion body myositis

Tomoo Mano[1,2], Naohiko Iguchi[1], Maki Ozaki[1], Naoki Iwasa[1], Nanami Yamada[1], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Rehabilitation Medicine, Nara Prefecture General Medical Center

## P-105 A patient with IBM-pattern of weakness, history of statin usage, anti-HMGCR positivity and HLA-DRB1*1101 allele

Arada Rojana-udomsart[1], Narupat Suanprasert[1], Duangtawan Thammanichanond[2], Jantima Tanboon[3]

[1]Neurological Institute of Thailand, Department of medical Services, Ministry of Public Health, Bangkok, Thailand
[2]Histocompatibility and Immunogenetics Laboratory, Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3]Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

## P-106 Are MAC Deposits in Amyloid Myopathy a New Entity or a Previously Overlooked Feature?

Xinmei Wen, Li Di, Wenjia Zhu, Hai Chen, Yuwei Da

Department of Neurology, Xuanwu Hospital Capital Medical University, Beijing, China

# Meeting Agenda

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## Poster Session 13

Chairs: Naoki Ito (Brain-Skeletal Muscle Connection in Aging Project Team, National Center for Geriatrics and Gerontology, Japan)
Keisuke Hitachi (Center for Medical Science, Fujita Health University, Japan)

### Sarcopenia

**P-107 Muscle-derived IL-1β regulates EcSOD expression via the NBR1-p62-Nrf2 pathway in muscle during cancer cachexia**

Mitsuharu Okutsu[1], Mami Yamada[1], Eiji Warabi[2], Hisashi Oishi[3]

[1]Graduate School of Science, Nagoya City University
[2]Institute of Medicine, University of Tsukuba
[3]Nagoya City University Graduate School of Medical Sciences

**P-108 Elucidation of muscle integrity mechanisms supported by heterogeneity of mesenchymal progenitors**

Madoka Uezumi[1], Shinichiro Hayashi[2], Satoru Noguchi[2], Ichizo Nishino[2], Tamaki Kurosawa[3], Takeshi Nikawa[4], Akiyoshi Uezumi[1]

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[3]Laboratory of Veterinary Pharmacology, Department of Veterinary Medical Sciences, Graduate School of Agriculture and Life Sciences, Tokyo University, Japan
[4]Department of Nutritional Physiology, Institute of Biomedical Sciences, Graduate School of Tokushima University, Japan

**P-109 Characterization of de-nitration activity present in injured muscle extract**

Junri Miyamoto[1], Alaa Elgaabari[1,2], Kahona Zushi[1], Sakiho Tanaka[1], Mako Nakamura[1], Takahiro Suzuki[1], Ryuichi Tatsumi[1]

[1]Department of Animal and Marine Bioresource Sciences, Graduate School of Agriculture, Kyushu University, Fukuoka, Japan
[2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

**P-110 Age-related changes in the capillary network of skeletal muscles**

Sakurako Mihara[1], Yuri Yamashita[1,2], Aurelien Kerever[1,2], Kazuteru Murakoshi[1], Satoshi Nakada[3], Eri Arikawa-Hirasawa[1,2,3]

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[2]Aging Biology in Health and Disease, Graduate School of Medicine, Juntendo University
[3]Japanese Center for Research on Women in Sport, Graduate School of Health and Sports Science, Juntendo University

**P-111 Effectiveness of Curcumin in Sarcopenia: A Systematic Review**

Maria Grace De Guzman[1], Jojo Evangelista[2], Steve Milanese[3], Raymond Rosales[4,5]

[1]Graduate School, University of Santo Tomas, Manila, Philippines
[2]Department of Neuroscience and Behavioral Medicine, University of Santo Tomas, Manila, Philippines
[3]International Centre for Allied Health Evidence, Adelaide SA, Australia
[4]Research Center for Health Sciences, Faculty of Medicine & Surgery, University of Santo Tomas, Manila, Philippines
[5]Department of Neuroscience and Brain Health, Metropolitan Medical Center, Manila, Philippines

### P-112 Effect of Curcumin Supplementation on Rat Skeletal Muscle Morphology and AMPK Levels

Maria Grace De Guzman[1], Veatrix Myrtle Cruz[2], Raymond Rosales[3,4]

[1]Graduate School, University of Santo Tomas, Manila, Philippines
[2]Unilab Medical Affairs, Biological Science Department, Mandaluyong City, Philippines
[3]Research Center for Health Sciences, Faculty of Medicine & Surgery, University of Santo Tomas, Manila, Philippines
[4]Department of Neuroscience and Brain Health, Metropolitan Medical Center, Manila, Philippines

### P-113 Muscle p62 inhibits CXCL13 expression and protects against aging-induced systemic inflammation

Mami Yamada[1], Eiji Warabi[2], Hisashi Oishi[3], Mitsuharu Okutsu[1]

[1]Nagoya City University Graduate School of Science
[2]Tsukuba University Institute of Medicine
[3]Nagoya City University Graduate School of Medical Science

### P-114 Establishing senescent skeletal muscle cell model to explore the sarcopenia mechanism in mitochondrial bioenergetic changes and set-up the platform for drug screening

Yu Xin Goh[1], Chin Ying Shih[2], Zi Wen Liang[2], Yung Ting Kuo[2,3]

[1]Department of Neurology, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, New Taipei City, Taiwan
[2]Department of Pediatrics, Shuang Ho Hospital, Ministry of Health and Welfare, Taipei Medical University, New Taipei City, Taiwan
[3]Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University

18:30-19:45

## Poster Session 14

Chairs: Naoki Suzuki (Department of Neurology, Tohoku University, Japan)
Yutaka Ohsawa (Kawasaki Medical School, Japan)

## SMA and ALS

### P-115 Two-year efficacy of risdiplam administration for spinal muscular atrophy

Toshio Saito[1], Hisahide Nishio[2]

[1]Division of Child Neurology, Department of Neurology, National Hospital Organization Osaka Toneyama Medical Center
[2]Faculty of Rehabilitation, Kobe Gakuin University

## P-116 Long-Term Impact of Nusinersen on Motor and Electrophysiological Outcomes in Adolescent and Adult Spinal Muscular Atrophy: Insights from a Multicenter Retrospective Study

Ningning Wang[1,2,3], Ying Hu[4], Kexin Jiao[1,2,3], Nachuan Cheng[1,2,3], Jian Sun[1,2,3], JinXue Tang[6], Jie Song[1,2,3], Chong Sun[1,2,3], Tao Wang[7], Kai Wang[4], Kai Qiao[1,2,3], Jianying Xi[1,2,3], Chongbo Zhao[1,2,3], Liqiang Yu[5], Wenhua Zhu[1,2,3]

[1]Department of Neurology, Huashan Hospital, Fudan University, Shanghai, China
[2]National Center for Neurological Disorders (NCND), Shanghai, China
[3]Huashan Rare Disease Center, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China
[4]Department of Neurology, The First Affiliated Hospital of Anhui Medical University, Anhui, China
[5]Department of Neurology, The First Affiliated Hospital of Soochow University, Suzhou, China
[6]Qilin District People's Hospital, Qujing City, Yunnan Province
[7]State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University

## P-117 Intrathecal injection of nusinersen in adolescent and adult patients with spinal muscular atrophy: focusing on adverse effect

Wan Ling Hsiao, Wen Chen Liang, Yuh Jyh Jong

Kaohsiung Medical University Hospital

## P-118 WITHDRAWAL

## P-119 Postural changes in respiratory and diaphragm function in amyotrophic lateral sclerosis based on neurophysiological examination

Naohiko Iguchi, Tomoo Mano, Naoki Iwasa, Maki Ozaki, Nanami Yamada, Naoya Kikutsuji, Tomohito Ohashi, Kazuma Sugie

Department of Neurology, Nara Medical University

## P-120 Postural facial deformation and virtual fit of non-invasive ventilation mask in amyotrophic lateral sclerosis

Sooyeon Kim[1], Rayu Yun[1], Jiwon Yun[1], Sungchul Huh[1], Heecheon You[2], Wonsup Lee[2]

[1]Department of Rehabilitation Medicine, Pusan National University Yangsan Hospital
[2]Department of Industrial and Management of Engineering, Pohang University of Science and Technology

## P-121 SESSION CHANGE

## P-122 Analysis of aberrant phase separation of RNA-binding proteins associated with ALS

Hitoki Nanaura[1], Minako Yamaoka[1], Eiichiro Mori[2], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Future Basic Medicine, Nara Medical University

# Meeting Agenda

18:30-19:45

## Poster Session 15

Chairs: Masaki Inada (Department of Biotechnology and Life science, Tokyo University of Agriculture and Technology, Japan)

Motoyasu Hosokawa (Department of Developmental Biology and Funcutional Genomic Ehime University, Graduate School of Medicine, Japan)

### Experimental Atrophy

**P-123 SFPQ maintains skeletal muscle mass through regulating aerobic metabolism**

Motoyasu Hosokawa[1,2], Kei Iida[3], Jun Tanihata[4,5], Shin'ichi Takeda[5], Masatoshi Hagiwara[2], Akihide Takeuchi[1]

[1]Department of Developmental Biology and Functional Genomics, Ehime University Graduate School of Medicine
[2]Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University
[3]Faculty of Science and Engineering, Kindai University
[4]Department of Cell Physiology, The Jikei University School of Medicine
[5]Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry

**P-124 The impact of ketogenic diets blended with medium-chain triacylglycerols on skeletal muscle metabolism during atrophy**

Harune Murakami[1], Yuna Izumi-Mishima[1], Erina Oya[1], Sonoko Yasui-Yamada[1], Rie Tsutsumi[1], Kazuhiro Nomura[1], Hiroshi Sakaue[1,2]

[1]Department of Nutrition and Metabolism, Institute of Biomedical Sciences, Tokushima University Graduate School
[2]Diabetes Therapeutics and Research Center, Tokushima University

**P-125 Microgravity inhibits myoblast proliferation by reduced intracellular $Ca^{2+}$ levels due to suppression of extracellular $Ca^{2+}$ uptake**

AYAKA ICHIHARA[1,2], Yuki Enoki[1], Kazuaki Matsumoto[1], Susumu Minamisawa[2], Jun Tanihata[2]

[1]Division of Pharmacodynamics, Keio University Faculty of Pharmacy
[2]Division of Aerospace Medicine, Department of Cell Physiology, The Jikei University School of Medicine

**P-126 Stroke -prone spontaneously hypertensive rats exhibit delayed skeletal muscle recovery from disuse atrophy by suppression of ribosomal protein S6 phosphorylation**

Takao Inoue[1], Yuji Kanazawa[2], Nobuyuki Mizuguchi[3], Osamu Maenishi[4], Masatomo Kimura[4,5], Man Hagiyama[1], Azusa Yoneshige[1], Akihiro Wada[1], Takaaki Chikugo[4], Tatsuki Itoh[6], Takao Satou[4], Akihiko Ito[1]

[1]Department of Pathology, Kindai University Faculty of Medicine
[2]Department of Physical Therapy, Faculty of Health and Medical Sciences, Hokuriku University
[3]Kindai University Life Science Research Institute
[4]Department of Diagnostic Pathology, Kindai University Hospital
[5]Department of Diagnostic Pathology, Hashimoto Municipal Hospital
[6]Department of Food Science and Nutrition, Faculty of Agriculture, Kindai University

# Meeting Agenda

## P-127 Ratio of skeletal muscle resident cells fluctuates during immobilization-induced muscle atrophy and subsequent recovery

Yuki Miwa[1], Masaki Yoda[4], Yoshiyuki Takahashi[3], Osahiko Tsuji[1], Keisuke Horiuchi[2], Kota Watanabe[1], Masaya Nakamura[1]

[1]Department of Orthopedic Surgery. Keio University School of Medicine
[2]Department of Orthopedic Surgery. National Defense Medical College
[3]Department of Orthopedic Surgery. Saiseikai Yokohama City Eastern Hospital
[4]Department of Pathology. The Jikei University School of Medicine

## P-128 SMAD2 ubiquitination regulates skeletal muscle mass and tissue remodeling

Yuki Yamasaki[1], Keita Sakamoto[1], Asushi Kubo[2,3], Keisuke Hitachi[4], Masafumi Inui[1]

[1]Laboratory of Animal Regeneration Systemology, Department of Life Sciences, School of Agriculture, Meiji University
[2]Laboratory of Stem Cell Regeneration and Adaptation, Graduate School of Pharmaceutical Sciences, Osaka University
[3]Department of Molecular and Cellular Biology, Institute of Development, Aging and Cancer, Tohoku University
[4]Division for Therapies against Intractable Diseases, Center for Medical Science, Fujita Health University

## P-129 Gene-transcriptome analysis of hypertrophic skeletal muscles induced by 2 *g* hypergravity in mice

Tsukasa Tominari[1,2], Masaru Takatoya[1], Daichi Arai[1,2], Michiko Hirata[1], Yoshifumi Itoh[3], Yoshitsugu Aoki[2], Dai Shiba[4], Masaki Inada[1]

[1]Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology
[2]Department of Molecular Therapy, National Center of Neurology and Psychiatry
[3]Kennedy Institute of Rheumatology, University of Oxford
[4]JEM Utilization Center, Japan Aerospace Exploration Agency (JAXA)

## P-130 Mkl1/2 inhibits muscle atrophy by blocking the GR/FoxO axis

Atsushi Kubo[1,2]

[1]Laboratory of Stem Cell Regeneration and Adaptation, Graduate School of Pharmaceutical Sciences, Osaka University
[2]Department of Molecular and Cellular Biology, Institute of Development, Aging and Cancer, Tohoku University

## P-131 Roles of the fibrinolytic system in skeletal muscle atrophy induced by mechanical unloading

Takashi Ohira[1,2], Yoko Ino[2], Naoyuki Kawao[1], Yuya Mizukami[1], Kiyotaka Okada[1], Osamu Matsuo[1], Hisashi Hirano[2], Yayoi Kimura[2], Hiroshi Kaji[1]

[1]Department of Physiology and Regenerative Medicine, Kindai University Faculty of Medicine
[2]Advanced Medical Research Center, Yokohama City University

## P-132 The effects of treadmill exercise mainly performed by forelimb on atrophy and mitochondrial adaptations in immobilized hindlimb muscle in mice

Tatsuya Matsumoto, Wenxin Wang, Tomoyasu Kadoguchi, Takeru Inaba, Yuki Morita, Yumiko Takahashi

The University of Tokyo

### P-133 Mechanical stress may suppress myotube atrophy linked to cancer cachexia via androgen receptors

Masahiro Iwata[1], Mitsuhiro Fujiwara[2], Shingo Matsuo[1], Yuki Kimata[1], Mana Fukase[1], Isshin Matsuura[1], Yuji Asai[1]

[1]Department of Rehabilitation, Faculty of Health Sciences, Nihon Fukushi University
[2]Department of Physical Therapy, School of Health Science, Toyohashi SOZO University

18:30-19:45

## Poster Session 16

Chairs: Takayuki Akimoto (Faculty of Sport Sciences, Waseda University/Center for Disease Biology and Integrative Medicine, The University of Tokyo, Japan)
Takahiko Sato (Fujita Health University, Japan)

## Muscle development, regeneration & homeostasis

### P-134 Analysis of mouse embryo skeletal muscle cell lineage by single nuclei RNAseq

Yutaro Kawa, Masafumi Inui

Laboratory of Animal Regeneration Systemology, Department of Life Sciences, School of Agriculture, Meiji University

### P-135 Novel Functional Analysis of the Cholesterol Regulatory Factor ABCA1 in Skeletal Muscle Homeostasis

Hayataka Takase[1], Makoto Shimizu[2], Sumiko Dohmae[3], Yoshio Yamauchi[1], Ryuichiro Sato[1], Takashi Sasaki[1]

[1]The University of Tokyo
[2]Ochanomizu University
[3]Chubu University

### P-136 Adrenaline Resistance in Obese Skeletal Muscle Impairs Exercise Metabolism

Kazuhiro Nomura[1,2,3], Yu Hirata[2], Yuna Izumi-Mishima[1], Sonoko Yamada[1], Anna Krook[3], Juleen Zierath[3], Hiroshi Sakaue[1,4], Wataru Ogawa[2]

[1]Department of Nutrition and Metabolism, Institute of Biomedical Sciences, Tokushima University Graduate School
[2]Department of Diabetes and Endocrinology, Kobe University Graduate School of Medicine
[3]Department of Physiology and Pharmacology, Karolinska Institutet
[4]Diabetes Therapeutics and Research Center, Tokushima University

### P-137 Analysis of planar cell polarity protein Vangl2 expression in skeletal muscle stem cells

Tadahiro Nagaoka, Keisuke Hitachi, Kunihiro Tsuchida

Division for Therapies against Intractable Diseases, Center for Medical Science, Fujita Health University

### P-138 Expression of microRNA-24 improves regeneration in fast-twitch muscle

Fusako Sakai-Takemura[1,2], Ye Huang[1], Shuaibang Yuan[1], Minjung Lee[1,3], Takayuki Akimoto[1]

[1]Faculty of Sports Science, Waseda University
[2]JSPS Restart Postdoctral Fellowship
[3]Cellular and Molecular Biotechnology Research Institute, National Institute of Advanced Industrial Science and Technology

### P-139 The role of microRNA-140 in skeletal muscle

Takayuki Akimoto[1,2], Jaehoon Shin[1], Masataka Nakamura[2], Shuaibang Yuan[1], Minjung Lee[1], Shigeru Miyaki[3], Hiroshi Asahara[4], Takashi Ushida[2]

[1]Faculty of Sport Sciences, Waseda University
[2]Center for Disease Biology and Integrative Medicine, The University of Tokyo
[3]Medical Center for Translational and Clinical Research, Hiroshima University Hospital
[4]Graduate School of Medical and Dental Sciences, Tokyo Medical and Dental University

### P-140 Doxorubicin irreversibly impairs skeletal muscle regeneration

Shinya Kondo, Shugo Yonehara, Haruo Sasaki, Masashi Isaji, Takahiro Nakagawa, Keisuke Horiuchi

Department of Orthopedic Surgery, National Defense Medical College

### P-141 Myoblast differentiation induced expression of complement regulatory proteins and CD59 expression on the membrane of myotubes was the uniquely clustering pattern

Kazuo Iwasa, Karin Kuo, Rena Takahashi, Yuka Nagaoka, Miwa Imai, Takao Hirai

Department of Health and Medical Sciences, Ishikawa Prefectural Nursing University

### P-142 Development of an early and non-invasive method for predicting skeletal muscle stem cell induction efficiency utilizing culture supernatants

Naoya Inoue[1], Miki Hojo[1], Toru Natsume[2], Shungo Adachi[2], Hidetoshi Sakurai[1]

[1]Sakurai Group. Center of iPS Research and Application(CiRA), Kyoto University
[2]National Institute of Advanced Industrial Science and Technology

### P-143 Acceleration of the development of myotubes from MYOD1-overexpressed human iPS cells using a new simple method

Eiji Wada[1], Nao Susumu[1], Yuya Okuzaki[2,3], Akitsu Hotta[2], Hidetoshi Sakurai[2], Yukiko Hayashi[1]

[1]Department of Pathophysiology, Tokyo Medical University
[2]Center for iPS Cell Research and Application (CiRA), Kyoto University
[3]Graduate School of Bioagricultural Sciences, Nagoya University

### P-144 Detection of microRNA leakage by stretch stimulation model using patient-derived iPS muscle cells

Satoshi Nakada[1], Yuri Yamashita[2], Hidetoshi Sakurai[3], Eri Arikawa-Hirasawa[1,2]

[1]Japanese Center for Research on Women in Sport, Juntendo University Graduate School of Health and Sports Science
[2]Research Institute for Diseases of Old Age, Juntendo University Graduate School of Medicine
[3]Center for iPS Cell Research and Application, Kyoto University

# Meeting Agenda

18:30-19:45

## Poster Session 17

Chairs: Ai Shima (Department of Mechano-Informatics, The University of Tokyo, Graduate School of Information Science and Technology, Japan)

Tomoya Uchimura (Department of Clinical Application Center for iPS Cell Research and Application, Kyoto University, Japan)

### Myokine and New technologies

**P-145 Heat acclimation modifies skeletal muscle functions in mice**

Yuka Kudo, Nozomi Yazawa, Yuho Mizuseki, Keigo Murata, Taku Nedachi

Graduate School of Life Sciences, Toyo University

**P-146 Comprehensive Analysis of Stress-Dependent Subcellular Localization Changes in Proteins**

Takahide Matsushima[1], Yuki Naito[1], Tomoki Chiba[1], Ryota Kurimoto[1], Koji Ochiai[2], Koichi Takahashi[2], Naoki Goshima[3,4], Hiroshi Asahara[1]

[1]Department of Systems BioMedicine, Tokyo Medical and Dental University
[2]Laboratory for Biologically Inspired Computing, RIKEN Center for Biosystems Dynamics Research
[3]Molecular Profiling Research Center for Drug Discovery, National Institute of Advanced Industrial Science and Technology
[4]Department of Human Science, Faculty of Human Science, Musashino University

**P-147 Development of Controllable Stretching and Continuous Force Measurement System for *in vitro* Engineered Skeletal Muscle Tissue**

Shota Noda, Louis Sterker, Jun Sawayama, Shoji Takeuchi

The University of Tokyo

**P-148 Regulation of myokines by nutrition and heat stimulation in C2C12 myotubes**

Nozomi Yazawa, Yuka Kudo, Yuho Mizuseki, Keigo Murata, Taku Nedachi

Graduate School of Life Sciences, Toyo University

**P-149 Regulatory mechanism of skeletal muscle-derived IL-6 expression: Impact on skeletal muscle-brown adipose tissue amino acid metabolism**

Mizuki Sugiuchi[1], Yuna Izumi-Mishima[1], Manaka Tsutsumi[1], Tetsuya Shiuchi[2], Yuko Okamatsu[3], Takeshi Yoneshiro[4], Sonoko Yasui-Yamada[1], Rie Tsutsumi[1], Kazuhiro Nomura[1], Hiroshi Sakaue[1,5]

[1]Department of Nutrition and Metabolism, Institute of Biomedical Sciences, Tokushima University Graduate School
[2]Department of Physiology, Institute of Biomedical Sciences, Tokushima University Graduate School
[3]Laboratory of Biochemistry, Faculty of Veterinary Medicine, Hokkaido University
[4]Division of Molecular Physiology and Metabolism, Tohoku University Graduate School of Medicine, The University of Tokyo
[5]Diabetes Therapeutics and Research Center, Tokushima University

**P-150 Fatty acid-dependent myokine expression in mouse skeletal muscle**

Yuho Mizuseki[1], Yuri Ishiuchi-Sato[1], Ayaka Shinozaki[2], Erika Hiraiwa[2], Taku Nedachi[1,2]

[1]Graduate school of Life Science, Toyo University
[2]Faculty of Life Science, Toyo University

# Meeting Agenda

**P-151** **Consideration of fusion mechanisms based on characterization of myoblast migration, morphology, membrane stiffness, and cytoplasmic fluidity**

Motoshi Kaya
Department of Physics, University of Tokyo

**P-152** **Biofabrication of cultured meat using a hollow fiber bioreactor**

Minghao Nie, Ai Shima, Shoji Takeuchi
The University of Tokyo

**P-153** **An Uncommon Case of Inclusion Body Myositis: Upper Limb Girdle Muscle Atrophy and Weakness as Initial Manifestations**

Zhaoxia Wang, Hongyan Qiu
Peking University First Hospital

# Meeting Agenda

# Sep. 14, Saturday

## Room 1

8:00-8:50

### Morning Seminar

Chair: Masaru Iwasaki (Vice-President, University of Yamanashi, Kofu, Japan)

### Exploring Neu-REFIX Beta Glucans: Mechanisms and Effects in Reducing Muscle Fibrosis and Fatigue

**MS** **Oral administration of Neu-REFIX Beta 1,3-1,6 Glucan reduces skeletal muscle fibrosis and fatigue in dystrophic mice**

Yoshitsugu Aoki

Director, Department of Molecular Therapy, National Institute of Neuroscience
National Center for Neurology and Psychiatry, Kodaira, Tokyo, Japan

Sponsored By: GN Corporation Co Ltd, Japan

9:00-10:30

### Symposium 6

Chairs: Masaki Inada (Tokyo University of Agriculture and Technology, Japan)
Naoki Suzuki (Rehabilitation Medicine, Tohoku University, Japan)

### Muscle atrophy and hypertrophy

**S6-1** **Elucidation of Skeletal Muscle Regulation Mechanisms Using Mouse Space Experiments**

Satoru Takahashi

Department of Anatomy and Embryology, Institute of Medicine, University of Tsukuba, Japan

**S6-2** **Differential gene responses for slow-twitch and fast-twitch muscles in muscular atrophy and hypertrophy**

Masaki Inada

Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Japan

**S6-3** **MicroRNA having an effect on muscle differentiation and muscle hypertrophy**

Hirohiko Hohjoh

Graduate School of Medicine, Juntendo University, Japan

### S6-4 Diverse roles of cellular senescence in skeletal muscle inflammation and regeneration

Yuki Saito[1], Takako S. Chikenji[2]
[1]Sapporo Medical University, Japan
[2]Hokkaido University, Japan

10:40-12:10

## Symposium 7

Chairs: Shinichiro Hayashi (National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)
So-ichiro Fukada (Graduate School of Pharmaceutical Sciences, Osaka University, Japan)

## Muscle stem cells in Development, Regeneration and Homeostasis

### S7-1 Molecular mechanisms regulating behaviors of muscle satellite cells in response to intrinsic and extrinsic factors

So-ichiro Fukada
Graduate School of Pharmaceutical Sciences, Osaka University, Japan

### S7-2 Role of mechanosensitive ion channels in muscle regeneration

Yuji Hara, Kotaro Hirano
School of Pharmaceutical Sciences, University of Shizuoka, Japan

### S7-3 Functional heterogeneity in the activated satellite cell population

Yusuke Ono[1,2]
[1]Department of Muscle Development and Regeneration, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan
[2]Muscle Biology Laboratory, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Japan

### S7-4 Development of Cell Therapy for DMD by iPSC-derived Muscle Stem Cell (iMuSC)

Hidetoshi Sakurai
Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan

12:35-13:25

## Luncheon Seminar 4

Chair: Hiroyuki Awano (Research Initiative Center, Organization for Research Initiative and Promotion, Tottori University)

### LS4 The diagnosis and treatment of treatable childhood-onset neuromuscular diseases

Keiko Ishigaki
Department of Pediatrics, Tokyo Women's Medical University School of Medicine

Sponsored By: CHUGAI PHARMACEUTICAL CO., LTD.

# Meeting Agenda

13:30-15:00

## Symposium 8

Chairs: Takashi Yamada (Department of Physical Therapy, Sapporo Medical University, Japan)
Norio Motohashi (Department of Molecular Therapy, National Institute of Neuroscience National Center of Neurology and Psychiatry (NCNP), Japan)

### Muscle Mechanosensing and Metabolic Dynamics during Physical Activities

**S8-1** **Age-related dysfunction of $NAD^+$ metabolism and $Ca^{2+}$ signaling in skeletal muscle as a cause of anabolic resistance**

Naoki Ito

Brain-Skeletal Muscle Connection in Aging Project Team, National Center for Geriatrics and Gerontology, Japan

**S8-2** ***in vivo* intracellular calcium ion dynamics regulating exercise-induced muscle damage: Spatio-temporal characteristics and its underlying mechanism**

Ayaka Tabuchi

The University of Electro-Communications, Japan

**S8-3** **Intramuscular Regulation of Post-Exercise Glucose Clearance**

Kohei Kido

National Institute of Advanced Industrial Science and Technology (AIST), Japan

**S8-4** **Deciphering the mechanisms underlying the positive effects of exercise on organismal homeostasis from mechanobiological perspectives, thereby developing novel therapeutic/preventative strategies for a variety of diseases and disorders**

Yasuhiro Sawada[1], Naoyoshi Sakitani[2]

[1]National Rehabilitation Center for Persons with Disabilities, Japan
[2]National Institute of Advanced Industrial Science and Technology, Japan

15:10-16:40

## Symposium 10

Chairs: Keisuke Hitachi (Center for Medical Science Fujita Health University, Japan)
Tomoya Uchimura (Dept. of Clinical Application Center for iPS Cell Research and Application, Kyoto University, Japan)

### Perspectives on skeletal muscle and organ interactions in homeostatis and disease

**S10-1** **The role of muscle-derived extracellular vesicles in aging**

Naoomi Tominaga, Yuta Miyagi, Saki Horie

Department of Clinical Laboratory Science, Graduate School of Medicine, Yamaguchi University, Japan

# Meeting Agenda

### S10-2 Crosstalk between skeletal muscle and bone

Naoyuki Kawao
Kindai University Faculty of Medicine, Japan

### S10-3 The role of exercise-induced myokines in cardiovascular homeostasis

Koji Ohashi, Noriyuki Ouchi
Nagoya University Graduate School of Medicine, Japan

### S10-4 Skeletal muscle atrophy-induced cognitive dysfunction: its mechanism and protective strategy

Chihiro Tohda, Tsukasa Iki
Section of Neuromedical Science, Institute of natural Medicine, University of Toyama, Japan

16:50-18:50

## JSNP Joint Symposium

Chairs: Takashi Kurashige (Department of Neurology NHO Kure Medical Center and Chugoku Cancer Center, Japan)
Jun Sone (Institute for Medical Science of aging Aichi Medical University, Japan)
Commentator: Akiyoshi Kakita (Niigata University, Japan)

## Pathomechanism of OPDM and related disorders

### Noncoding repeat expansions in OPDM and related disorders and their mechanisms

Hiroyuki Ishiura
Department of Neurology, Okayama University, Japan

### RAN translation and RNA foci: Causing differentiation of phenotype

Takashi Kurashige
Department of Neurology, NHO Kure Medical Center and Chugoku Cancer Center, Japan

### RNA G-quadruplexes cause neuronal dysfunction in trinucleotide CGG repeat diseases

Norifumi Shioda
Department of Genomic Neurology, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University, Japan

### Muscle pathology of OPDM - how to differentiate from OPMD

Masashi Ogasawara[1,2], Nobuyuki Eura[1,3], Ichizo Nishino[1]
[1]Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Pediatrics, Showa General Hospital, Tokyo, Japan
[3]Department of Neurology, Nara Medical University, Nara, Japan

### Neuronal intranuclear inclusion disease

Jun Sone
Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Japan

# Meeting Agenda

## Neuropathology of OPDM

Rie Saito, Akiyoshi Kakita
Department of Pathology, Brain Research Institute, Niigata University, Japan

# Meeting Agenda

# Sep. 14, Saturday

## Room 2

9:00-10:30

### AOMC Young Investigator Award Session

Chairs: Shahriar Nafissi (Department of Neurology, Tehran University of Medical Sciences, Iran)
Yuh-Jyh Jong (Department of Pediatrics and Laboratory Medicine, Kaohsiung Medical University Hospital, Taiwan)

**AY-1** **Visualization of degenerative processes of the myofibers on muscle pathology in OPDM based on single nucleus RNA-seq data**

Ai Yamanaka[1], Nobuyuki Eura[1], Shinichiro Hayashi[1], Kazuma Sugie[2], Satoru Noguchi[1], Ichizo Nishino[1]

[1]Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Neurology, Nara Medical University, Nara, Japan

**AY-2** **Detection of STR expansions on a neuromuscular gene panel using STRipy improves diagnostic rate of ataxia**

Chiara Lai Folland[1], Carolin Scriba[1], Michael Black[2], Rebecca Gooding[2], Nigel Laing[1], Mark Davis[2], Gianina Ravenscroft[1]

[1]Centre for Medical Research, University of Western Australia, Harry Perkins Institute of Medical Research, Perth, Western Australia, Australia
[2]Department of Diagnostic Genomics, Department of Health, PathWest Laboratory Medicine, QEII Medical Centre, Nedlands, Western Australia, Australia

**AY-3** **Characterizing the Cell-Cell Interaction in Inclusion Body Myositis**

Francia Victoria De Los Reyes, Shinichiro Hayashi, Satoru Noguchi, Ichizo Nishino

Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

**AY-4** **4qA D4Z4 methylation test as a valuable complement for differential diagnosis in patients with FSHD-like phenotype**

Xingyu Xia[1], Nachuan Cheng[1], Yiqi Liu[1], Dongyue Yue[2], Mingshi Gao[3], Kexin Jiao[1], Ningning Wang[1], Bochen Zhu[1], Chong Sun[1], Jie Song[1], Chong Yan[1], Sushan Luo[1], Jie Lin[1], Jiahong Lu[1], Chongbo Zhao[1], Wenhua Zhu[1]

[1]Department of Neurology, Huashan Hospital, Fudan University, Shanghai 200040, China
[2]Department of Neurology, Jing'an District Center Hospital of Shanghai, Shanghai, 200040, China
[3]Department of Pathology, Huashan Hospital, Fudan University, Shanghai 200040, China

**AY-5** **Identification of key gene functions impaired in dystrophinopathy by transcriptomic analysis of patient-derived iPSC-cardiomyocytes**

Jeffrey Lui[1], Stephen Yin Cheng[1], Anna Hing Yee Law[1], Sheng Zhu[1], Hung Fat Tse[1,2], Godfrey Chi Fung Chan[1], Yiu Fai Cheung[1], Sophelia Hoi-Shan Chan[1]

[1]Department of Paediatrics and Adolescent Medicine, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR
[2]Division of Cardiology, Department of Medicine, School of Clinical Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, HKSAR

# Meeting Agenda

10:40-12:30

## JMS Young Investigator Award Session

Chairs: Tohru Hosoyama (National Center for Geriatrics and Gerontology, Japan)
Genri Kawahara (Department of pathophysiology, Tokyo Medical University, Japan)

### JY-1 Serglycin promotes skeletal myogenesis through EZH2 degradation in satellite cells

Katsuhiko Kunitake, Norio Motohashi, Yuki Ashida, Yoshitsugu Aoki

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

### JY-2 Macrophage HGF-denitration activity: a key to youthful muscle regeneration

Alaa Elgaabari[1,2], Junri Miyamoto[1], Takahiro Maeno[1], Kahona Zushi[1], Mako Nakamura[1], Takahiro Suzuki[1], Ryuichi Tatsumi[1]

[1]Department of Animal and Marine Bioresource Sciences, Graduate School of Agriculture, Kyushu University, Fukuoka, Japan
[2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

### JY-3 Generation of an FSHD1 mouse model carrying FSHD1-derived chromosome 4q35 using mouse artificial chromosome

Yosuke Hiramuki[1], Ichizo Nishino[2], Hiroyuki Kugoh[1], Yasuhiro Kazuki[1]

[1]Department of Chromosome Biomedical Engineering, School of Life Science, Faculty of Medicine, Tottori University, Japan
[2]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

### JY-4 The role of muscle glucocorticoid receptor signaling in accelerating obesity, glucose intolerance, and aging-related metabolic changes

Hiroki Yamazaki[1], Masaaki Uehara[2], Akiko Kuribara-Souta[3], Motohisa Yamamoto[2], Yasuaki Kabe[4], Makoto Suematsu[5], Kazuhisa Tsukamoto[1], Hirotoshi Tanaka[6]

[1]Division of Endocrinology and Metabolism, Department of Internal Medicine, Teikyo University School of Medicine, Japan
[2]Department of Rheumatology and Allergy, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, Japan
[3]Department of Cell Processing and Transfusion, IMSUT Hospital, Institute of Medical Science, The University of Tokyo, Japan
[4]Department of Biochemistry, Keio University School of Medicine, Japan
[5]Central Institute for Experimental Medicine and Life Science, Japan
[6]Department of Rheumatology, Kitasato University Kitasato Institute Hospital, Japan

### JY-5 Modeling cell type specific and sporadic DUX4 gene expression in FSHD

Mitsuru Sasaki-Honda[1,2], Hidetoshi Sakurai[1], Alvaro Rada-Iglesias[2]

[1]CiRA, Kyoto University, Japan
[2]IBBTEC, University of Cantabria, Spain

### JY-6 Establishment of Cell Transplantation Therapy Aimed at Ameliorating Ullrich Congenital Muscular Dystrophy~Exploration of Cell Sources for Transplantation~

Megumi Yokomizo (Goto)[1], Nana Takenaka[1], Kiho Clemence Yoshioka[1,2], Mayuho Miki[1,2], Hidetoshi Sakurai[1]

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan
[2]Graduate School of Human Health Sciences, Kyoto University, Japan

13:30-15:00

## Symposium 9

Chairs: Khean-Jin Goh (Department of Neurology University of Malaya, Malaysia)
Sara Khan (Department of Neurology Aga Khan University Hospital, Pakistan)

## Treatable Neuro Muscular Disorders

### S9-1 State of the art in lipid storage myopathy

Chuanzhu Yan
Neuromuscular Center and Department of Neurology, Qilu Hospital, Shandong University, China

### S9-2 Current landscape of sporadic late-onset nemaline myopathy

Akinori Uruha
Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan

### S9-3 Gene-Targeted Therapy for Duchenne Muscular Dystrophy: Clinical Development Update

Hirofumi Komaki
Department of Child Neurology, Translational Medical Center, National Center of Neurology and Psychiatry, Japan

### S9-4 Challenges and Opportunities in the Treatment of Spinal Muscular Atrophy

Yuh-Jyh Jong
Graduate Institute of Clinical Medicine, Kaohsiung Medical University; Departments of Pediatrics and Laboratory Medicine, KMU Hospital, Taiwan

# Meeting Agenda

15:10-16:40

## Symposium 11

Chairs: Raymond L. Rosales (Research Center for Health Sciences-Faculty of Medicine and Surgery University of Santo Tomas, Philippines)

Charungthai Dejthevaporn (Division of Neurology, Department of Medicine Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand)

### Other myopathies

**S11-1** ***Anncaliia algerae*** **infection: potentially fatal, unclear epidemiology**

Matthew Watts

Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research - New South Wales Health Pathology, Westmead Hospital and Sydney Institute for Infectious Diseases, University of Sydney, Sydney, New South Wales, Australia

**S11-2** **Electrodiagnostic and myopathologic correlation in critical illness myopathy**

Teerin Liewluck

Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN, USA

**S11-3** **Muscle Chaperonopathies: From Clinical Presentation to Molecular Mechanisms**

Michio Inoue

Department of Neurology, Washington University School of Medicine, USA

**S11-4** **Ten years of disease gene discovery and diagnostics in neurogenetic diseases**

Gina Ravenscroft[1,2]

[1]Centre for Medical Research, The University of Western Australia, Nedlands, WA, Australia
[2]Harry Perkins Institute of Medical Research, Nedlands, WA, Australia

**S11-5** **VCP Myopathy**

Jordi Alberto Diaz-Manera

John Walton Muscular Dystrophy Research Center, Newcastle University, UK

19:00-20:30 Networking Dinner (2F 205+206)

# Meeting Agenda

17:00-18:30

## Oral Session 3

Chairs: Mariko Okubo (Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France/National Center for Global Health and medicine, Research Institute, Tokyo, Japan)

Satoshi Yamashita (International University of Health and Welfare Narita Hospital, Japan)

### O-19 High-Risk Screening for Late-Onset Pompe Disease in China: An Expanded Multicenter Study

Bochen Zhu[1,2,3], Kexin Jiao[1,2,3], Xueli Chang[4], Wenhua Zhu[1,2,3]

[1]Department of Neurology, Huashan Hospital Fudan University, Shanghai, China
[2]National Center for Neurological Disorders (NCND), Shanghai, China
[3]Huashan Rare Disease Center, Shanghai Medical College, Huashan Hospital, Fudan University, Shanghai, China
[4]Department of Neurology, First Hospital, Shanxi Medical University, Taiyuan, China

### O-20 FORTIS Update: Biomarker Results and Up to 2 Years Safety and Exploratory Efficacy in a Phase 1/2 Open-Label Clinical Study of AT845 Gene Replacement Therapy for Late Onset Pompe-Disease

Chieri Hayashi[3], Tahseen Mozaffar[2], Nicola Longo[1], Mark Walzer[3], Achim Steup[3], Julie Coats[3,4], Jordi Diaz-Manera[5]

[1]University of Utah, Salt Lake City, UT, USA
[2]University of California Irvine, Irvine, CA, USA
[3]Astellas Pharma Global Development, Northbrook, IL
[4]Astellas Gene Therapies, San Francisco, CA, USA
[5]John Walton Muscular Dystrophy Research Centre, Newcastle University and Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK

### O-21 Novel mutations and genotype-phenotype correlation in a multicenter cohort of GNE myopathy in China

Kexin Jiao[1], Jialong Zhang[1], Qiuxang Li[2], Xiaoqing Lv[3], Chongbo Zhao[1], Daojun Hong[6], Zhe Zhao[4], Zhiqiang Wang[5], Wenhua Zhu[1]

[1]Department of Neurology and Rare Disease Center, Huashan Hospital, Fudan University, and National Center for Neurological Disorders (NCND), Shanghai 200040, China
[2]Department of Neurology and National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410000, China
[3]Department of Neurology and Research Institute of Neuromuscular and Neurodegenerative Diseases, Qilu Hospital of Shandong University, Jinan, Shandong 250012, China
[4]Department of Neuromuscular Disease, Third Hospital of Hebei Medical University, Hebei 050000, China
[5]The First Affiliated Hospital of Fujian Medical University, Fujian 350000, China
[6]Department of Neurology and Department of Medical Genetics, The First Affiliated Hospital of Nanchang University, Nanchang 330006, China;

### O-22 Development of sarcomere-observable mice for analyzing skeletal myofibril degeneration

Satoru Noguchi[1], Megumu Ogawa[1], Fuyu Kobirumaki-Shimozawa[2], Yukiko Inoue[3], Takayoshi Inoue[3], Norio Fukuda[2]

[1]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan
[2]Department of Cell Physiology, The Jikei University School of Medicine. Tokyo, Japan
[3]Department of Biochemistry and Cellular Biology, National Institute of Neuroscience, NCNP, Tokyo, Japan

## O-23 Immune Mediated Megaconial Myopathy (IMMM): A Novel Subtype of Autoimmune Myopathy Featuring Giant Mitochondria

Teerin Liewluck[1], Ashley Santilli[1], Oliver Ni[2], Margherita Milone[1], Duygu Selcen[1], Anahit Mehrabyan[3], Arjun Seth[4], Christine Hsieh[5], Wasim Raslan[6], Moayd Alkhalifah[7], Raed Alenezi[8], Stefan Nicolau[9], Pannathat Soontrapa[10]

[1]Muscle Pathology Laboratory and Division of Neuromuscular Medicine, Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA
[2]Department of Laboratory Medicine and Pathology, Hennepin Healthcare, Minneapolis, Minnesota, USA
[3]Department of Neurology, University of North Carolina, Chapel Hill, North Carolina, USA
[4]Department of Neurology, Northwestern University, Chicago, Illionois, USA
[5]Division of Rheumatology, Department of Medicine, Northwestern University, Chicago, Illionois, USA
[6]Department of Pathology and Laboratory Services, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia
[7]Department of Neurology, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia
[8]Department of Medicine, Johns Hopkins Aramco Healthcare, Dhahran, Saudi Arabia
[9]Center for Gene Therapy, Nationwide Children's Hospital, Columbus, OH, USA
[10]Department of Medicine, Siriraj Hospital, Mahidol University, Bangkok, Thailand

## O-24 Alterations in Cerebrospinal Fluid Metabolite Profiles in Patients with Spinal Muscular Atrophy

Wei Zhuang[1], Minying Wang[2], Zhehui Chen[2], Meifen Luo[2], Wanlong Lin[1], Xudong Wang[3], Mei Lu[2]

[1]Department of Pharmacy, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China
[2]Department of Pediatrics, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China
[3]Department of Xiamen Newborn Screening Center, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China

## O-25 The open-label phase 4 RESPOND study evaluating nusinersen in children with spinal muscular atrophy (SMA) previously treated with onasemnogene abeparvovec: Interim clinical, neurofilament, and safety results

Angela Paradis[1], Crystal Proud[2], Richard Finkel[3], Julie Parsons[4], Riccardo Masson[5], Nancy Kuntz[6], Richard Foster[7], Wenjing Li[1], Sowmya Chary[1], Jihee Sohn[1], Bora Youn[1], Stephanie Fradette[1]

[1]Biogen, Cambridge, MA, USA
[2]Children's Hospital of The King's Daughters, Norfolk, VA, USA
[3]St. Jude Children's Research Hospital, Memphis, TN, USA
[4]Children's Hospital Colorado, Aurora, CO, USA
[5]Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Milano, Italy
[6]Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA
[7]Biogen, Maidenhead, Berkshire, UK

## O-26 Prospective study on clinical outcomes and health-related quality of life in spinal muscular atrophy patients receiving nusinersen or risdiplam

Michael Kwan Leung Yu[1], Hayley Hoi Ning Ip[2], Pui Kei Cheng[2], Shirley Ng[3], Wilfred Hing Sang Wong[1], Sophelia Hoi Shan Chan[1,2,3]

[1]Department of Paediatrics and Adolescent Medicine, The University of Hong Kong, Hong Kong Special Administrative Region, China
[2]Department of Paediatrics and Adolescent Medicine, Queen Mary Hospital, Hong Kong Special Administrative Region, China
[3]Department of Paediatrics and Adolescent Medicine, Hong Kong Children's Hospital, Hong Kong

## O-27 Dynamic Changes in Cerebrospinal Fluid Metabolites as Predictors of Nusinersen Efficacy in Spinal Muscular Atrophy Patients: A Prospective Cohort Study

Wei Zhuang[1], Minying Wang[2], Zhehui Chen[2], Meifen Luo[2], Wanlong Lin[1], Xudong Wang[3], Mei Lu[2]

[1]Department of Pharmacy, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China
[2]Department of Pediatrics, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China
[3]Department of Xiamen Newborn Screening Center, Women and Children's Hospital, School of Medicine, Xiamen University, Xiamen, China

# Meeting Agenda

# Sep. 15, Sunday

## Room 1

8:00-8:50

### JMS Student Award Session

Chairs: Hiroshi Sakai (Ehime University)
Mikako Ito (Nagoya University Graduate School of Medicine, Japan)

9:00-10:30

### Symposium 12

Chairs: Yoshitsugu Aoki (Department of Molecular Therapy, National Institute of Neuroscience National Center of Neurology and Psychiatry, Japan)
Masayuki Nakamori (Department of Neurology Yamaguchi University, Japan)

### Advancing Next-Generation Therapeutic Modalities for Muscular Disorders

**S12-1 Development of immune tolerance-inducing gene therapy using mesenchymal cells**

Takashi Okada
The Institute of Medical Science, The University of Tokyo, Japan

**S12-2 Pentatricopeptide repeat protein targeting CUG repeat RNA ameliorates RNA toxicity in myotonic dystrophy type 1**

Masayuki Nakamori
Department of Neurology, Yamaguchi University, Japan

**S12-3 CDP-ribitol prodrug treatment ameliorates *ISPD*-deficient muscular dystrophy**

Motoi Kanagawa
Ehime University Graduate School of Medicine, Japan

**S12-4 Exon skipping therapy using a novel chimeric RNA fused with 4.5SH**

Shinichi Nakagawa
Hokkaido University, Japan

# Meeting Agenda

10:40-12:10

## Symposium 13

Chairs: Tsukasa Tominari (National Center of Neurology and Psychiatry, Japan)
Ai Shima (University of Tokyo, Japan)

### New technologies and models to facilitate muscle research

**S13-1 Skeletal muscle delivery tools of CRISPR-Cas genome editing**

Akitsu Hotta

Center for iPS Cells and Research Application (CiRA), Kyoto University, Japan

**S13-2 Investigating and Developing Treatments for Triadopathies Using Zebrafish Pre-Clinical Models**

Yukari Endo[1,2,3], James Dowling[3,4]

[1]Juntendo University, Institute of Health and Sports Science & Medicine, Japan
[2]Juntendo University, Department of Pharmacology, School of Medicine, Japan
[3]Hospital for Sick Children, Program for Genetics and Genome Biology, Canada
[4]University of Toronto, Department of Molecular Genetics, Canada

**S13-3 Production of human muscle tissues based on cell sheet-based tissue engineering**

Hironobu Takahashi

Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Japan

**S13-4 Disease modeling of Duchenne muscular dystrophy for the functional analyses by muscle training using patient-derived iPSC in vitro**

Tomoya Uchimura, Hidetoshi Sakurai

Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

12:20-13:10

## Luncheon Seminar 5

Chair: Masanori Takahashi (Lab of Clinical Neurophysiology, Dept. Clinical Laboratory and Biomedical Sciences, Osaka University Graduate school of Medicine)

### Treatment Strategies for Generalized Myasthenia Gravis

**LS5-1 Clinical impact of efgartigimod, the first approved anti-neonatal Fc receptor inhibitor for MG in Japan**

Shigeaki Suzuki

Department of Neurology, Keio University School of Medicine, Japan

# Meeting Agenda

**LS5-2** **Efgartigimod therapy in patients with myasthenia gravis of working age: challenges encountered by patients with MG at work and when caring for children**

Akitoshi Takeda
Department of Neurology, Osaka Metropolitan University

Sponsored By: argenx Japan K.K.

13:20-14:20 AOMC-YIA, JMS-YIA & SA Award Ceremony/
JMS Young Researcher Encouragement Award Speech/Closing Ceremony

13:20-14:20

**Award and Closing ceremony**

# Meeting Agenda

## Sep. 15, Sunday

### Room 2

9:30-12:10

### Clinical Pathological Conference

Chairs: Ichizo Nishino (National Center of Neurology and Psychiatry, Japan)
Kum Thong Wong (University of Malaya, Malaysia)

**Discussant**

Wenhua Zhu
Huashan Hospital, Fudan University, China

Josiah Chai
Department of Neurology, National Neuroscience Institute, Singapore

Akatsuki Kubota
Department of Neurology, The University of Tokyo, Japan

Zhaoxia Wang
Department of Neurology, Peking University First Hospital, China

Jin-Hong Shin
Department of Neurology, Pusan National University Yangsan Hospital, Republic of Korea

Chuanzhu Yan
Neuromuscular Center and Department of Neurology, Qilu Hospital, Shandong University, China

Yung-Ting Kuo
Department of Pediatrics, Taipei Medical University - Shuang Ho Hospital, Taiwan

Jantima Tanboon
Department of Pathology, Mahidol University, Thailand

**CPC-1 A 52 year old man with episodic respiratory distress**

Atchayaram Nalini
Department of Neurology, National Institute of Mental Health and neuro Sciences, Bengaluru, India

**CPC-2 A boy with Progressive Lower Limb Weakness for 1 Year**

Bochen Zhu
Huashan Hospital, Fudan University, China

**CPC-3 A case of rhabdomyolysis with severe respiratory failure and unusual muscle biopsy findings**

Tingjun Dai, Chuanzhu Yan
Department of Neurology, Qilu Hospital of Shandong University, Jinan, China

### CPC-4 A 52-year-old man presented with a four-year history of slow-progressing proximal muscle weakness

Wannisa Wongpipathpong[1], Jariya Waisayarat[2], Charungthai Dejthevaporn[3]

[1]Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samutprakan, Thailand
[2]Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[3]Division of Neurology, Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand

### CPC-5 A 18-year old young man with shoulder girdle weakness and an unexpected pathological finding

Qiang Gang[1,2], Jing Chen[1,2], Meng Yu[1,2], Wei Zhang[1,2], Zhaoxia Wang[1,2], Yun Yuan[1,2]

[1]Department of Neurology, Peking University First Hospital, Beijng, China
[2]Beijing Key Laboratory of Neurovascular Disease Discovery, Beijing, China

### CPC-6 A 29-year-old previously healthy woman presenting with asymmetrical muscle weakness and exercise intolerance following her last pregnancy 3 years ago

Yalda Nilipour

Department of Pathology, Shahid Beheshti university of medial sciences, Tehran, Iran

### CPC-7 The “strong’ bones and weak muscles: A diagnostic odyssey

Prasana Nair Gengadharan[1], Cheng Yin Tan[1], Khean Jin Goh[1], Kum Thong Wong[2]

[1]Neurology Unit, Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia
[2]Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

### CPC-8 A 4-Year-Old Boy with Progressive Muscle Weakness

Xiaona Fu

Department of Neurology, Beijing Children’s Hospital, Capital Medical University, National Center for Children’s Health, China

12:20-13:10

## Luncheon Seminar 6

Chair: Ichizo Nishino (National Center of Neurology and Psychiatry)

## Updates on GNE Myopathy Treatment in Japan

### LS6-1 Recent Insights into GNE Myopathy: Genotype-Phenotype Correlations, Disease Progression, and Therapeutic Approaches Beyond Sialic Acid Supplementation

Wakako Yoshioka

National Center of Neurology and Psychiatry

# Meeting Agenda

## LS6-2 A New Era in the Treatment of Distal Myopathy: Expectations and Future Prospects for Aceneuramic acid

Naoki Suzuki
Tohoku University Graduate School of Medicine

Sponsored By: Nobelpharma Co., Ltd

# AOMC-JMS 2024

Keynote Lecture

Educational Programme

Symposium

JSNP Joint Symposium

Sponsored Symposium

Luncheon Seminar

Morning Seminar

AOMC Young Investigator Award Session

JMS Young Investigator Award Session

JMS Student Award Session

# Keynote Lecture1

9:10-9:55, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Yoshihide Sunada (Kawasaki Medical School, Japan)
Ichizo Nishino (Department of Neuromuscular Research National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

## K-1

## Emerging Therapies for Thymidine Phosphorylase and Thymidine Kinase 2 Deficiencies

Michio Hirano
H. Houston Merritt Neuromuscular Research Center, Department of Neurology, Columbia University Irving Medical Center, New York, USA

Among more than 400 genetically distinct mitochondrial diseases, deficiencies of thymidine phosphorylase (TPase) and thymidine kinase 2 (TK2) stand out as two disorders with therapies currently under investigation. Both are disorders of mitochondrial DNA (mtDNA) maintenance. TPase deficiency presents clinically in childhood or adolescence as a multisystemic disease, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), a devastating progressive disease with cachexia, ptosis, chronic progressive external ophthalmoplegia, gastrointestinal dysmotility, peripheral neuropathy, and leukoencephalopathy on brain MRI. Mortality is high with median survival at about age 38 years-old. Drs. Ichizo Nishino, Antonella Spinazzola, and Ramon Marti, as postdoctoral fellows in my laboratory, demonstrated that in MNGIE, TYMP mutations cause TPase deficiency, which, in turn, produces toxic accumulations of the nucleosides thymidine and deoxyuridine in blood and tissues. Excesses of these pyrimidine nucleosides cause unbalances of the deoxynucleoside triphosphate (dNTPs) in mitochondria with excess deoxythymidine triphosphate (dTTP) and lack of deoxycytidine triphosphate (dCTP) that, in turn, lead to pathogenic depletion, deletions, and site-specific point mutations of mtDNA. As a proof-of-principal, we demonstrated that platelets infused into MNGIE pateints, can transiently restore TPase activity and decrease plasma levels of thymidine and deoxyuridine. Allogeneic hematopoietic stem cell (bone marrow) and liver transplantations have been performed to attempt to permanently restore TPase activity. When successful, transplantation therapy has led to biochemical and partial clinical improvements. Nevertheless, due to severity of MNGIE, many patients are not suitable for transplantation. Enzyme replacement therapy is currently under investigation as an alternative approach.

In contrast to MNGIE, TK2 deficiency typically presents as a childhood onset progressive myopathy with early respiratory insufficiency and dysphagia. Early onset before age 2 years is associated with rapid progression and early mortality compared to late onset after age 12 years. Deficiency of the intramitochondrial TK2 enzyme leads to lack of dTTP and dCTP, which cause mtDNA depletion and deletions. In a Tk2 knockin mouse model, we demonstrated that oral administration of deoxythymidine and deoxycytidine transiently improves mitochondrial dNTP pools, delays disease onset, and prolongs survival of Tk2 mutant mice. Together with international colleagues, 2011-12, we started to administer deoxythymidine and deoxycytidine under compassionate use (expanded access) to TK2 deficient patients, who have shown stabilization or clinical improvements with improved survival compared to historical untreated patients. This deoxynucleoside therapy has been licenced to a pharmaceutical company that is conducting a phase 2 clinical trial. Based upon these preliminary studies, we think that deficiencies of TPase and TK2 may be treatable mitochondrial diseases.

# Keynote Lecture2

9:55-10:40, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Satish V. Khadilkar (Department of Neurology, Bombay Hospital Institute of Medical Sciences, India)
Kazuma Sugie (Nara Medical University, Japan)

## K-2

## Precision medicine in Duchenne muscular dystrophy: exon skipping therapies and innovative models for personalised drug development

Yoshitsugu Aoki
Department of Molecular Therapy, National Institute of Neuroscience
National Center of Neurology and Psychiatry (NCNP), Japan

Duchenne muscular dystrophy (DMD) is an incurable X-linked disorder arising from mutations in the DMD gene. Due to its genetic diversity, precision medicine approaches are deemed indispensable. These approaches are tailored to effectively address the mutation-specific requirements within different patient subgroups.

Among the most advanced approaches is splicing modulation therapy, known as exon skipping, using antisense oligonucleotides (ASOs). These synthetic molecules bind to specific pre-mRNA sequences to ameliorate the disrupted reading frame of the DMD gene. We have recently developed Viltolarsen, an exon 53 skipping drug, in collaboration with Nippon Shinyaku Co. Ltd. Our ongoing work involves developing exon-skipping drugs for exon 44 (NS-089/NCNP-02), exon 50 (NS-050/NCNP-03), and exon 51 (NS-051/NCNP-04). Our drugs collectively target up to 31% of DMD cases, demonstrating considerable potential for effective disease management. When coupled with advanced drug delivery systems, exon skipping harbours the potential to transform DMD from a fatal condition into a manageable disorder. However, challenges entail the need for highly specific antisense sequences customised to each mutation and issues related to broad applicability across various mutations and sustainability in terms of cost, sequence specificity, and enormous effort.

To address these challenges, the computational tool eSkip-Finder was formulated to streamline the design of personalised splicing modulation strategies, consequently enabling their implementation in N-of-1 trials - highly individualised clinical trials for rare or unique mutations – and individualised treatments for pre-mRNA splicing-associated rare diseases. Complementary technologies, including patient-derived stem cells and AI-driven tools, have been integrated into therapy development processes to augment these mutation-specific approaches further. Human urine-derived stem cells (UDCs) offer a non-invasive, accessible source of patient-specific mesenchymal cells, crucial for disease modelling, drug screening, and drug development. These cells retain vital epigenetic information and replicate disease-specific phenotypes, thus supporting AI-driven in silico therapeutic discovery and facilitating "clinical trials in a dish" to evaluate therapies in non-invasive, patient-specific settings. Regulatory support, such as the FDA Modernization Act 2.0, allows for alternatives to animal testing. It propels these innovations by encouraging alternatives to traditional testing methods, thereby expediting the development of precision therapies encompassing ASO-mediated exon skipping, UDC-based models, and computational tools like eSkip-Finder.

Our laboratory has pioneered human brain organoids replicating DMD's cognitive symptoms. Combined with DMD animal models, these models provide pivotal insights into disease pathophysiology and invaluable preclinical and clinical research tools. We deliver a comprehensive precision medicine approach by integrating these models with cutting-edge molecular and computational technologies, bridging the gap between laboratory research and patient care. While precision medicine in muscle disorders, particularly DMD, exhibits excellent promise, ongoing advancements are imperative to fully realise the potential presented by personalised and more effective therapies.

# Educational Programme 1

10:30-12:00, Sep 12 (Thu), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Sara Khan (Department of Neurology, Aga Khan University Hospital, Pakistan)
Kazuma Sugie (Department of Neurology, Nara Medical University, Japan)

## EP1-1

## How to examine infants suspected to have neuromuscular disorders

Wen-Chen Liang

Departments of Pediatrics, Kaohsiung Medical University Hospital, Kaohsiung Medical University, Kaohsiung, Taiwan

Neuromuscular diseases (NMDs) are the disorders involved in lower motor neuron, peripheral nerve, neuromuscular junction, and muscle. Vast majority of NMDs are hereditary, degenerative, and rare. However, the clinical manifestations of NMDs are quite heterogeneous, which often leads to the difficulty making accurate diagnosis of MNDs. Especially for infants, as they cannot express by themselves and it is also difficult to perform neurological examinations thoroughly on them, making early diagnosis of NMDs might be more challenging. “Observation” of appearances, movements and behaviors thus also plays a critical role to take part in the diagnostic course. Herein I would like to introduce some points for attention about how to observe and examine the babies suspected to have NMDs.

## EP1-2

# Characteristics, natural history and management of LAMA2-related dystrophies: A multi-cohort study from the Asian-Oceanian Network

Hui Xiong, Lin Ge

Department of Neurology, Beijing Children's Hospital, Capital Medical University, National Center for Children's Health, Beijing, China

**Summary**

**Background** LAMA2-related muscular dystrophies (LAMA2-RDs), resulting from mutations in the LAMA2 gene, exhibit a clinical spectrum ranging from severe, early-onset, and progressive manifestations to milder, late-onset forms. We sought to systematically assess the phenotypic characteristics and natural history of patients with LAMA2 deficiency.

**Methods** We did an international, multicenter, cohort study, analyzing retrospective data from Jan 1, 2003, to April 15, 2024, from patients with LAMA2 deficiency followed up in AOMC membership hospitals in 9 countries globally. The key inclusion criterion were clinical symptoms and genetically confirmed LAMA2 deficiency. There were no exclusion criteria. Our primary objectives were to (1) describe the natural history of patients diagnosed with LAMA2-RDs across Asia and Oceania and (2) identify the genotype-phenotype correlations in a large and well-characterized cohort. Patients diagnosed with LAMA2-CMD and LAMA2-LGMD were both statistically analyzed. Other objectives were to (1) optimize clinical management of LAMA2-RD patients focusing on the prevention and treatment of complications and (2) select relevant clinical and functional outcome measures for reaching clinical trial-readiness.

**Findings** The study encompasses a Chinese multi-center cohort with 149 cases and an international multi-center cohort with 74 cases, covering countries/regions including China, Taiwan, Myanmar, Pakistan, Thailand, Malaysia, Indonesia, Iran, and South Korea. The longest follow-up recorded is 21 years. Standardized case report forms (CRF) were systematically collected, reviewed, and evaluated. After data filtering, a total of 156 LAMA2-CMD and 28 LAMA2-LGMD patients were included (26 patients have passed away). We provide a detailed description and comparison of the cohorts from the following aspects including neurodevelopmental characteristics, genetic analysis, multi-system characteristics, neuroimaging findings and neurocognitive function and outcomes.

**Interpretation** Our description of characteristics of LAMA2 deficiency in a large patient cohort provides knowledge that might inform clinical management and future evaluation of therapies.

## EP1-3

## Examination of an adult patient with Muscle and Neuromuscular junctions disorders

Atchayaram Nalini
National Institute of Mental Health and Neurosciences, Bengaluru, India

Lower motor neurons syndromes result from abnormalities of the lower motor neurons, that includes anterior horn cells of spinal cord, spinal motor roots, motor fibres of the peripheral nerves, neuromuscular junction, motor nuclei of cranial nerves and Voluntary muscle. Many primary muscle disorders but symptoms are few: Pain and weakness is most frequent, Limpness, Fatigue, Wasting, Myokymia / fasciculations, Palpable tenderness, Hypertrophy / atrophy of muscles, cramps, myotonia and contractures. Apart from the core limb muscle manifestations, additional features like cranio-bulbar muscle weakness, cardiac dysfunction, gastro-intestinal symptoms, peripheral neuropathy, visual / hearing impairment can co-exist. Distribution of muscle weakness could be classically in limb-girdle pattern, proximo-distal or only distal. In muscular dystrophy: Preferential muscle weakness / Selectivity is characteristic in the majority. The topography of involvement is evaluated as: Deltoid vs. Pectorals; Biceps vs. Triceps; Iliopsoas vs. Gluteus maximus; Hip abductors vs. adductors; Quadriceps vs. Hamstrings. A pattern emerges in Dystrophinopathies: Pectorals, Triceps, Gluteus maximus, Gluteus Medius and Quadriceps are weaker than their antagonistic muscles. The reverse pattern is true for several LGMDs. Typically, there is muscle hypertrophy in muscular dystrophies but several of them have atrophy rather than hypertrophy. In myopathies, generally the muscle mass is poor, abnormal facies, slender habitus, no preferential muscle weakness and proximo-distal weakness. Muscle diseases with hypertrophy: Duchenne / Becker muscular dystrophy, LGMD's, FSHD. Early and prominent contractures are: EDMD, LGMD IB, Bethlem myopathy, rigid spine syndrome, Calpainopathy, congenital muscular dystrophies. Associated cardiac abnormalities commonly in Dystrophinopathies, Saroglycanopathies, Myofibrillar myopathies, EDMD. Prominent muscle atrophy is noted in: Dsyferlinopathies, Myofibrillar myopathies, GNE Myopathy, Calpainopathy. Based on these basic principles of disease manifestations and examination findings a fairly good bedside phenotyping is possible in most primary muscle diseases. This helps in targeted genetic testing as well.

# Educational Programme 2

13:10-14:40, Sep 12 (Thu), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Chongbo Zhao (Department of Neurology Huashan Hospital, Fudan university, China)
Rajesh Benny (Department of Neurology, Fortis Hospital, India)

## EP2-1

## Making Sense of The Clinical spectrum of Limb Girdle Muscular Dystrophies

Satish V. Khadilkar
Department of Neurology, Bombay Hospital Institute of Medical Sciences, India

The expansion of the spectrum of limb girdle muscular dystrophies (LGMDs) in recent years means that neurologists need to be familiar with the clinical clues that can help with their diagnosis. The LGMDs comprise a group of genetic myopathies that manifest as chronic progressive weakness of hip and shoulder girdles. Their inheritance is either autosomal dominant (LGMD1) or autosomal recessive (LGMD2). Their prevalence varies in different regions of the world; certain ethnic groups have documented founder mutations and this knowledge can facilitate the diagnosis. The clinical approach to LGMDs uses the age at onset, genetic transmission, and clinical patterns of muscular weakness. Helpful clinical features that help to differentiate the various subtypes include: predominant upper girdle weakness, disproportionate respiratory muscle involvement, distal weakness, hip adductor weakness, 'biceps lump' and 'diamond on quadriceps' sign, calf hypertrophy, contractures, and cardiac involvement. Almost half of patients with LGMD have such clinical clues. Investigations such as serum creatine kinase, electrophysiology, muscle biopsy and genetic studies can complement the clinical examination. In this presentation, I shall discuss diagnostic clinical pointers and comment on the differential diagnosis and relevant investigations, using illustrative case studies.

## EP2-2

## Congenital myasthenic syndromes: Review of 38 causal genes

Kinji Ohno
Nagoya University of Arts and Sciences, Japan

Congenital myasthenic syndromes (CMS) are a heterogeneous group of disorders characterized by impaired neuromuscular signal transmission due to pathogenic germline variants in genes expressed at the neuromuscular junction (NMJ). A total of 38 genes have been reported in CMS. The 38 genes can be classified into 14 groups according to the pathomechanical, clinical, and therapeutic features of CMS patients. Recently identified groups of CMS are characterized by identification of unexpected involvement of defective NMJ signal transmission in other diseases especially in developmental disorders and skeletal disorders. Mild to moderate myasthenic features were likely to be underestimated by the presence of severer phenotypes in the other organs including the central nervous system and the skeletal system. Measurement of compound muscle action potentials (CMAPs) elicited by repetitive nerve stimulation (RNS) is required to diagnose CMS. In some groups of CMS, abnormal CMAPs are observed only in high frequency RNS but not in low frequency RNS. One of recently identified forms of CMS is caused by defective release of synaptic vesicles at the NMJ and mimics Lambert-Eaton myasthenic syndrome (LEMS). In the LEMS-like CMS, incremental CMAPs are observed in high frequency RNS, as in LEMS. As clinical and electrophysiological features are usually insufficient to identify a defective gene, and an extensive genetic study is required for accurate diagnosis. From a pharmacological point of view, cholinesterase inhibitors are effective in most groups of CMS, but are contraindicated in some groups of CMS including COLQ-CMS and slow-channel CMS. Similarly, ephedrine, salbutamol (albuterol), amifampridine are effective in most but not all groups of CMS. The speaker will also address the pathomechanisms of variants in AGRN, DOK7, CHRNG, and GFPT1 that we recently analyzed.

## EP2-3

## The expanding genetic landscape of hereditary rhabdomyolysis: what clinicians need to know

Teerin Liewluck

Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, Minnesota, USA

Rhabdomyolysis is a clinical syndrome characterized by the breakdown of skeletal muscle fibers, leading to the release of intracellular contents, including creatine kinase (CK), into the bloodstream. While there is no universally accepted threshold for defining rhabdomyolysis, a CK level approximately 10 times the upper limit of normal or baseline is commonly used as a benchmark. Acquired causes of rhabdomyolysis, such as trauma, medications, and excessive exercise, are more prevalent than genetic etiologies. However, in patients presenting with recurrent rhabdomyolysis or in the absence of clear acquired etiologies, an underlying genetic myopathy should be considered. Genetic contributors to rhabdomyolysis encompass a range of conditions, including metabolic myopathies (e.g., muscle glycogenoses, disorders of fatty acid oxidation and lipid metabolism, and mitochondrial disorders), disorders of excitation-contraction coupling ( *RYR1*, *CACNA1S* and *SCN4A* myopathies), and specific muscular dystrophies (e.g., *FKRP*-opathy, *GMPPB*-opathy, dystrophinopathy, and *ANO5*-opathy). Recently identified genetic factors, such as pathogenic variants in *OBSCN, MLIP* and *MYH1*, further expand the understanding of this condition. Although rhabdomyolysis is a relatively uncommon manifestation of mitochondrial disorders, certain genes (e.g., *MT-CYB, TANGO2, ISCU*, and *FDX2*) are notable exceptions. Evaluating patients between episodes of rhabdomyolysis, utilizing baseline neurologic exam findings and CK levels, can aid in distinguishing the various genetic etiologies. It is important to recognize that normal muscle biopsy or needle EMG findings do not exclude the presence of an underlying myopathy in patients with suspected hereditary rhabdomyolysis. With advancements in molecular genetics, next-generation sequencing has become the first-tier diagnostic approach, while muscle biopsy remains valuable in cases of suspected mitochondrial disorders or when genetic tests yield negative results or variants of uncertain significance (VUS). In the latter scenario, muscle biopsy can be instrumental in validating the pathogenicity of the identified VUS. Identifying the underlying genetic cause is crucial for guiding dietary or activity modifications and phamarcotherapy aimed at reducing the frequency of rhabdomyolysis episodes.

# Educational Programme 3

14:50-16:20, Sep 12 (Thu), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Katsuhisa Ogata (National Hospital Organization Higashisaitama Hospital, Japan)
Anna Cho (Department of Pediatrics Seoul National University Bundang Hospital
Seoul National University College of Medicine, Republic of Korea)

## EP3-1

## Rehabilitation for progressive neuromuscular disorders: focus on Duchenne muscular dystrophy

Kimiko Inoue
NHO Osaka Toneyama Medical Center, Japan

Rehabilitation for the patients with progressive neuromuscular disorders includes
(1) To evaluate the patient's ability correctly,
(2) To ensure ability of daily life (ADL) and quality of life (QOL) for the patients as much as possible, and
(3) To consider how to deal with future functional decline in the patients.
Nowadays gene therapy and the drugs that modify disease progression are available, so that rehabilitation will be increasingly important for maintaining condition and giving good prognosis of the patients.
This lecture will focus on Duchenne muscular dystrophy, including motor functional assessment, rehabilitation (physical therapy) according to the stage of disease progression and management of respiratory failure.

## EP3-2

## Pulmonary rehabilitation and management of dysphagia in patients with neuromuscular disorders

Yuka Ishikawa
National Hospital Organization Hokkaido Medical Center, Japan

We followed 98 patients with Duchenne muscular dystrophies (DMD) in our hospital on Oct. 1st 2023. The number of patients in each age group was 23 aged 9-19 years, 28 aged 20-29years, 31 aged 30-39 years, and 16 aged 40-53 years including 6 patients aged 50 years or older. The number of patients using noninvasive ventilation (NIV) for 20 hours or more was 0 cases, 12 cases (42.9%), 29 cases (93.5%), and 16 cases (100%) in each group. The number of patients who continue oral intake was 23 (100%), 24 (85.7%), 30 (96.8%), and 13 (81.3%) in each group. So, Oral intake using NIV and mechanical insufflation exsufflation (MIE) was possible in more than 80% of patients with DMD in all age groups.

However, patients with neuromuscular disease are at high risk of gastrointestinal problem including severe abdominal distention when applying NIV or MIE. Because many studies have highlighted the strong relationship between gut microbiota and skeletal muscle. For example, genetic disruption of dystrophin expression led to morphological gastrointestinal tract alterations, weakened the gastrointestinal tract digestion, and gastric dysfunction in the DMD pigs (Zou X, et al. Cell Biosci. 2021 Jul 15;11(1):131). And the decrease of free fatty acid receptor 2 in the ileum might reveal a dysregulation of the gut microbiota short-chain fatty acid chain production and bioavailability for skeletal muscles in mdx mice (Jollet M, et al. Am J Pathol. 2024;194:264-79).

To ameliorate abdominal distention when using NIV or MIE, drugs that accelerate gastrointestinal transit, changes in the ventilator settings may helpful (Blokhuis AM, et al. Neuromuscul Disord. 2024;40:31-7). Usually, volume control mode is more likely to cause abdominal distension than pressure control mode. Sometimes changing type of ventilator could reduce aerophagia with maintain proper percutaneous blood gas, because the air supply algorithm differs depending on the machine.

Despite the risk of bloating, airway clearance technique including MIE should be introduced to prevent further exacerbation of lung pathology due to aspiration and to keep thoracic compliance as lung volume recruitment (LVR) Patients with Neuromuscular ventilatory weakness and bulbar dysfunction could continue oral intake and avoid respiratory failure and aspiration pneumonia with NIV and MIE. However, there is clear need for more research on prevention and treatment of GI problem in order to provide the best optimal respiratory and nutritional management.

## EP3-3

## Pathophysiology and management of cardiomyopathy associated with neuromuscular disease

Atsuhito Takeda
Department of Pediatrics, Hokkaido University Graduate School of Medicine, Japan

Secondary cardiomyopathy in children is often caused by inborn errors of metabolism or neuromuscular diseases. The prognosis is extremely poor in cases that develop early in infancy. Those with onset in infancy to school age develop latent disease, and heart failure becomes apparent in adulthood or later. Myocardial involvement varies depending on the underlying disease, and the main pathological conditions are abnormal energy metabolism, accumulation of complex lipids, and cytoskeletal dysfunction. Each of these pathologies has a different mode of development of cardiac damage, age of onset of cardiomyopathy, and prognosis. In this lecture, we will discuss the different types of pediatric secondary cardiomyopathy, and how the changes over time affect the clinical picture of the disease. In addition, the recent development of diagnostic imaging in the field of cardiology has been remarkable, and it is now possible to diagnose myocardial properties. Early diagnosis of latent cardiac lesions in childhood through diagnostic imaging and prophylactic intervention can be expected to reduce the onset and progression of heart failure. Finally, I would like to mention that there is a world of ultrastructure, tissue biochemistry, and molecular genetics that can only be revealed by myocardial tissue, and I would like to discuss the future role of myocardial biopsy.

# Educational Programme 4

## WMS Collaborate session

16:30-18:00, Sep 12 (Thu), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Wen-Chen Liang (Department of Pediatrics, Kaohsiung Medical University Hospital, Taiwan)
Gina Ravenscroft (Harry Perkins Institute, University of Western Australia, Australia)

## EP4-1

## Muscle Imaging

Jordi Alberto Diaz-Manera
John Walton Muscular Dystrophy Research Center, Newcastle University, UK

Muscle imaging has gained popularity as an useful tool for the diagnosis and follow-up of patients with neuromuscular diseases in recent years. In this presentation, I will do an overview of the different imaging modalities that can be applied to patients in clinics and in a research setting. The talk will be mainly focused in muscle MRI. We will discuss the different MRI sequences available including semiquantitative and quantitative sequences. Moreover, we will present the main imaging patterns observed in different diseases. I will show recent results from research projects that have applied different modalities of artificial intelligence to the analysis of muscle MRI. In the second part of the talk, I will review the rationale behind using quantitative muscle MRI for the follow-up of patients in natural history studies and clinical trials.

## EP4-2

## Differential diagnosis of IBM

Werner Stenzel
Department of Neuropathology, Charite University Hospital, Germany

Inclusion body myositis is a clinically and pathologically well described entity among the so called ‘Idiopathic inflammatory myopathies’. The disease has some peculiar and highly characteristic features in many aspects, e.g. it only occurs in adults and has never been seen in children, IBM may be associated with other immune-compromised conditions such as viral diseases and autoimmune diseases. On a morphological level, the gold-standard of diagnostic accuracy has been the ultrastructural evidence of tubulofilaments in myonuclei and in- or juxtaposed to vacuoles in the cytoplasm. Presence of vacuoles has long been the cornerstone of the firm diagnosis of IBM. However, there are numerous diseases in which one can identify vacuoles that look very similar to those in IBM e.g. rimmed vacuoles such as hereditary and /or distal myopathies. Ultrastructural evidence of tubulofilaments have since been reported in certain other non-IBM disease entities as well.
This talk will summarize the current diagnostic standard to IBM and discuss the morphological parameters also in the context of clinical data. Additionally, frequent differential diagnoses of IBM will be highlighted as well.

## EP4-3

## Genetic diagnosis of muscle diseases: from gene-by-gene testing through next generation sequencing toward genomic medicine

Alessandra Ferlini
Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Genetic diagnosis in neuromuscular diseases (NMDs) is now an integral part of the diagnostic flowchart and has played a fundamental role in NMD care and treatment since being compulsory to get a genotype definition and, when suitable, to access personalized therapies.
Next-generation sequencing (NGS) is now routinely applied to NMD patients for genetic definition, leading to a remarkable amelioration of genetic diagnosis, via new variants, genes, or new phenotypes discovery.
NGS allows high parallelism and throughput runs but it cannot however always ensure an exhaustive mutation detection, since some mutation types (as copy number variations and dynamic mutations) often escape its identification. Therefore, under some specific circumstances (example prenatal testing) or for some mutation types (example dynamic mutations) standard, gene-by-gene molecular genetic tests are still needed.
Among NGS strategies, gene panels, whole exome sequencing, and whole genome sequencing are widely used to identify gene pathogenic variants as well as to discover new disease genes. These three approaches have “pros and cons”, and their use might be finely tuned, depending on the diagnostic demand. Beside genotype definition, RNA profile is very important to define functional meaning of variants of uncertain significance and allows to solve many unsolved cases or better define mutation mechanisms.
The new non-invasive prenatal diagnostic testing (NIPT) can identify a variety of recurrent (or de novo) mutations at the prenatal age, simply sequencing the DNA foetal fraction, circulating in the maternal blood.
These outstanding achievements have readdressed the genetic diagnosis concept and its application to the vaster approach consisting on genetic screening. This includes genetic newborn screening (gNBS), meaning analysing all neonates for a potentially infinite variety of gene variations at birth, and pre-conceptional screening (PCS), which can detect carriers of gene mutations before conception. gNBS and PCS applications in human health will be challenging, while expected to positively impact the rare diseases’ (RDs) diagnostic journey. Nevertheless, both gNBS and PCS, which are the core of genomic medicine, bring many complexities to be solved, especially ethical, privacy-related, and, not lastly, costs. We expect that during the next decade(s) genomic medicine will impact on (not only) NMD patients, by providing an early and accurate diagnosis, which can radically change the NMD diagnostic pipeline and make equitable approach to care and treatments, in a sustainable, ethical, and cost-effective framework. The Screen4Care EU-IHI project (GA No 101034427) is acknowledged.

# Symposium 1 [AOMC-TREAT-NMD collaboration]

## Clinical trials in NMD- Challenges we face

10:50-12:20, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Dae-Seong Kim (Department of Neurology, Pusan National University Yangsan Hospital, Korea)
Yukiko K. Hayashi (Department of Pathophysiology, Tokyo Medical University, Japan)

## S1-1

### The importance of involving patients in therapy development Lessons learned from Duchenne exon skipping

Annemieke Aartsma-Rus
Leiden University Medical Center, Leiden, the Netherlands

Duchenne muscular dystrophy is a progressive muscle wasting disease that occurs in ~1 in 5000 newborn boys. The disease is caused by pathogenic variants that disrupt the open reading frame of the dystrophin gene. The dystrophin protein provides muscle fibers with stability during exercise by connecting the actin cytoskeleton to the extracellular matrix. Lacking dystrophin, Duchenne patients suffer from accumulating muscle damage, which eventually results in loss of muscle tissue and function. When pathogenic variants maintain the open reading frame, internally deleted but partially functional dystrophins can be produced. These variants are found in Becker muscular dystrophy, which has a later onset and slower progression compared to Duchenne.
The exon skipping approach aims to allow Duchenne patients to make Becker-like dystrophins. This is achieved with antisense oligonucleotides (ASOs) that target specific exons during pre-mRNA splicing of dystrophin transcripts. Skipping an exon will enlarge the deletion, but restore the reading frame.
Proof-of-concept for ASO-mediated dystrophin restoration was provided in cultured cells, animal models and patients. In this lecture I will highlight which crucial elements were not in place when clinical development started, ultimately leading to an unsuccessful marketing authorization application. I will also outline how the field has implemented changes to improve stakeholder dialogues, develop more predictive model systems and build infrastructure and datasets needed. This example serves as a paradigm for rare disease therapy development, allowing proactive preparation of models, tools and initiating stakeholder identification early.

## S1-2

## Therapeutic approaches in Duchenne muscular dystrophy: fall, rise, and challenges

Alessandra Ferlini
Unit of Medical Genetics, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

Duchenne muscular dystrophy (DMD) is a severe X-linked neuromuscular rare disease due to pathogenic variations in the dystrophin (DMD) gene, which lead to the reduction or absence of dystrophin protein. DMD is characterized by progressive weakness of skeletal, respiratory, and cardiac muscles with different phenotypes running from the severe Duchenne type to the Becker milder type, including other intermediate phenotypes and the isolated dilated cardiomyopathy. Deletions are the most common molecular defects (57%), followed by small mutations (32%), duplications (11%), and small rearrangements. Lack or reduction of the dystrophin protein causes a cascade of pathological events which lead to failure of regeneration and replacement of muscle cells with adipose and fibrous tissue. Several gene corrective strategies are ongoing to restoring dystrophin protein via translation read-through therapy, exon skipping, or vector-mediated gene therapy. Antisense oligoribonucleotides (AON) approach was granted orphan drug designation and is approved in the USA and other countries. AON molecules are safe and very well tolerated and target nuclear DMD pre-mRNA to skip a specific exon flanking the deleted region. This approach restores the reading frame of the transcript and thus allows the production of an internally deleted but partially functional dystrophin protein. Approximately 55% of total DMD mutations causative for DMD would be eligible for some form of exon skipping therapy. Third generation AONs have been synthesized by functionalizing the RNA strand using peptides to increase muscle uptake and are currently in clinical trials. Gene therapy has raised great expectation in the DMD community since offers a “universal, disease specific” treatment, and a few trials were started. Some results have been published or disclosed by pharma-companies, and failures in reaching primary endpoints occurred. Among drugs used to treat DMD boys, those acting on dystrophin epigenetics are intriguing since of their mechanism of action which suggests their possible use in association with other treatments. The results of DMD therapies offers many aspects for reflections and encourages the DMD community to “take to good and address the bad”, thus, to learn through failures. Despite all these therapeutic approaches target DNA or RNA, knowledge about mechanisms underlying DMD transcription and translation remain poor, although being outstanding for designing DNA or RNA targeted therapies. Therefore, basic research is still needed for DMD and should be encouraged as well as the translational research.

## S1-3

## Clinical Trial Readiness in Myotonic Dystrophy

Masanori P. Takahashi
Osaka University Graduate School of Medicine, Japan

Myotonic dystrophy is the most common hereditary muscle disease in adults. Most are type 1 (DM1) in Japan, and the overall prevalence is similar to those in Europe and the US. The genetic cause of DM1 is an abnormal expansion of the CTG repeat in the untranslated region of the *DMPK* gene. Over the past 20 years, research has shown that an accumulation of repeat-expanded RNA in the nucleus causes quantitative abnormalities in RNA-binding proteins, resulting in splice defects of various genes across different organs.

Advances in understanding the disease have driven the move toward therapeutic development. Thus, for the past decade, we have been vigorously working on establishing a patient registry, clinical research network, and outcome measures and conducting natural history studies, all crucial for the therapeutic development of rare diseases.

In 2014, we launched the DM patient registry as part of the national registry of muscle diseases (Remudy). This registry collects comprehensive data, including mandatory and recommended items of the Naarden dataset, and cooperates with TREAT-NMD. The data is available to researchers and industries and is used for clinical trial recruitment.

Since 2014, a network of clinical institutions for DM research has been formulated and maintained under public grant funding. This network has promoted registry utilization, developed a Japanese version of patient-reported outcomes, and conducted cross-sectional and retrospective studies to better understand the disease. In 2021, our network launched a three-year prospective natural history study to establish outcome measures. Of note is that one institution in the network recently joined the END-DM1 natural history study.

Drug development for DM1 is progressing with two approaches: nucleic acid drugs targeting repeat-expanded RNA and small molecule drugs inhibiting the binding of splice regulator proteins to the repeats. Nakamori et al. identified erythromycin, which acts in a latter mechanism, through a drug repositioning strategy. A phase 2 trial, conducted as an investigator-initiated clinical trial, was completed as scheduled despite the pandemic, thanks to the use of the registry. The drug was well tolerated, and some biomarkers showed promising signs. Abroad, several early-phase trials have been conducted by pharmaceutical companies, with some progressing to phase 3. Our efforts on clinical readiness with an international collaboration may have led to a recent decision by a company to include Japan in its global phase 3 clinical trial.

# Symposium 2

## Link between Research, Clinical trials, and Patient registries

13:30-15:00, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Sophelia Hoi-Shan Chan (Department of Paediatrics and Adolescent Medicine, Paediatric Neurology Division, The University of Hong Kong, China, Hong Kong SAR)
Yuko Shimizu-Motohashi (Department of Child Neurology, National Center of Neurology and Psychiatry, Japan)

### S2-1

### Current status in South Korea: From patients registry to Clinical trials

Jong-Hee Chae

Department of Genomic Medicine & Pediatrics Seoul National University College of Medicine, Republic of Korea

## S2-2

## Interconnection between Research, Clinical Trials, and Neuromuscular Patient Registries in Thailand

Oranee Sanmaneechai
Faculty of Medicine, Siriraj Hospital Mahidol University, Thailand

In Thailand, the integration of research, clinical trials, and neuromuscular patient registries forms a cornerstone of advancing healthcare for neuromuscular disorders. This talk delves into the essential connections between these elements, emphasizing their collective impact on improving patient outcomes and fostering medical innovation.

Research on neuromuscular disorders in Thailand provides foundational knowledge, generating data and insights that guide clinical practices. This research is pivotal in understanding disease mechanisms, identifying potential therapeutic targets, and developing new treatments. Clinical trials serve as a bridge between this research and patient care, rigorously evaluating the safety and efficacy of new interventions. By conducting well-designed clinical trials, researchers can translate theoretical findings into practical therapies that benefit patients.

Neuromuscular patient registries play a vital role in this interconnected framework. These registries systematically collect detailed information on patients' conditions, treatments, and outcomes. In Thailand, neuromuscular patient registries enable longitudinal studies that track the progression of diseases, monitor treatment responses, and identify long-term trends. The data from these registries is crucial for informing research, optimizing clinical trials, and refining treatment protocols.

The synergy between research, clinical trials, and patient registries enhances the overall effectiveness of neuromuscular healthcare. Research generates new hypotheses and identifies promising therapies, clinical trials validate these therapies, and patient registries provide real-world data to support and improve them. This cyclical process accelerates the development of effective treatments and ensures they are tailored to the needs of Thai patients.

Moreover, the integration of these components supports the reimbursement from Thai universal health care coverage under rare disease subgroup.

In conclusion, the interconnectedness of research, clinical trials, and neuromuscular patient registries is crucial for the advancement of neuromuscular healthcare in Thailand. This integrated approach fosters innovation, enhances patient care, and ensures that medical practices are continually informed by the latest scientific evidence. Strengthening these connections will enable Thailand to improve its neuromuscular healthcare system, benefiting patients and contributing to global medical knowledge.

## S2-3

## Advancements in FSHD Research: Insights from the Hong Kong FSHD Patient Registry and Innovative AI Applications

Sophelia HS Chan

The University of Hong Kong, Department of Paediatrics and Adolescent Medicine, Neurology Division, Hong Kong

Facioscapulohumeral muscular dystrophy (FSHD) is the third most common form of muscular dystrophy, characterized by progressive muscle weakness that eventually leads to irreversible motor disability. Numerous clinical trials are currently evaluating potential new treatments for FSHD. FSHD type 1 is caused by a contraction of the D4Z4 repeat array on chromosome 4qA variant, while FSHD type 2 is associated with mutations in the SMCHD1, DNMT3B, and LIRF1 genes. From established FSHD patient registries in Europe, the United States, and Asia, we have learned that FSHD type 1 accounts for 95% of cases, and that allele size and disease duration are correlated with more severe progression. The primary symptoms to address for FSHD patients are muscle weakness and mobility issues, which should be the main outcomes in clinical trials investigating treatments.

Launched in March 2021, the Hong Kong FSHD patient registry, as part of the Hong Kong Neuromuscular Disease patient registry, collects data from patients and clinicians in accordance with international agreements, with the goal of facilitating clinical research planning, design, and recruitment. Currently, seventy-three patients with genetically confirmed FSHD type 1 have been enrolled in the Hong Kong FSHD patient registry. This talk will discuss insights from our FSHD cohort and ongoing research, including AI-based facial weakness recognition and muscle MRI studies.

## S2-4

## Congenital muscular dystrophies and myopathies: Current status in Japan

Yuko Shimizu-Motohashi
National Center of Neurology and Psychiatry, Department of Child Neurology, Japan

Congenital muscular dystrophies and myopathies include various diseases with muscle weakness that become apparent at or near birth. Recent advances in molecular genetics have led to a better understanding of the pathogenesis, and clinical trials have been conducted in some of the disease but with approved drugs yet to be developed. Due to the rarity of the diseases, there is a significant distance between the goal that we want to achieve, and the reality of having a significant lack of information on underlying pathology. On such ground, a patient registry for congenital muscular dystrophies and myopathies was launched in 2016 in Japan, aiming to collect epidemiological data as well as natural history data, which may fill these unmet needs.
There are other considerations as well, which include insufficient understanding on long-term clinical courses, and further, technical issues such as difficulty in reaching out to the patients and physicians or sustainability of data collection.
In this symposium, the current status of patient registry will be presented, and will discuss what should be done to further enhance the clinical studies for patients with congenital muscular dystrophies and myopathies in Japan.

# Symposium 3

## Myositis Update

15:10-16:40, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Akinori Uruha (Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan)
Jantima Tanboon (Department of Pathology, Mahidol University, Thailand)

### S3-1

### Myositis classification and pathology: an update (Part 1)

Werner Stenzel
Department of Neuropathology, Charite University Hospital, Germany

Myositis is a group of diseases that is commonly comprising a number of entities also referred to as the ‘Idiopathic Inflammatory Myopathies’. Historically the first descriptions of myositis were referring to a disease with skin symptoms hence called dermatomyositis and one without skin symptoms called Polymyositis. The term poly – derived from ancient Greek polùs meaning ‘many’ already implies that early on people suggested that polymyositis comprises many different origins of myositis. However, in the modern era of research into pathogenetic mechanisms one cannot but try to identify subentities with a uniform pathogenesis and try to decipher common or key molecular disease pathways. Two of those pathways have recently been identified and have entered the scene allowing for subclassification of different forms of myositis. One is the association with autoantibodies which are either nuclear or cytoplasmic and which allow for a good stratification of many patients with myositis. The other one is the type I interferon signature which allows for identification of Dermatomyositis by different means and also for differentiation between DM and anti-Synthetase syndrome on a morphological level.
This presentation gives a wrap-up on the historic developments that led to current classification criteria. It also paves the way for part II of this topic, which is given by Dr. Tanboon who covers the modern aspects of classification in myositis.

## S3-2

## Myositis classification and pathology: an update (Part 2) Dermatomyositis, Antisynthetase syndrome, and a few others

Jantima Tanboon
Department of Pathology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand

Clinical and pathological features of dermatomyositis and antisynthetase syndrome can be overlap. Without credible serological information, dermatomyositis can be mistaken for antisynthetase syndrome or vice versa. In this session, we will focus on the role of muscle pathology in dermatomyositis and antisynthetase syndrome classification. We will review the archetypal pathological features used in the current and/or recently proposed classification and the pathological variations according to their antibody subtypes. Essential histochemical, enzyme histochemical, and immunohistochemical staining panels and their diagnostic value will be discussed. In addition, we will review muscle pathology findings in common myositis-associated antibodies as some of them are recently proposed as a distinct subtype of myositis.

## S3-3

## Update on Muscle Imaging in Idiopathic Inflammatory Myopathy (IIM)

Tahseen Mozaffar
Department of Neurology and Pathology, University of California, Irvine, USA

There have been significant advances in serological and radiological biomarkers for diagnosis of idiopathic inflammatory myopathies. The diagnoses of these inflammatory myopathies can be realiably made using serological markers, especially the autoantibodies. However, muscle imaging has become an important adjunct in aiding diagnosis of inflammatory myopathies. This review will discuss the role of muscle ultrasound, muscle MRI and positron emission tomography (PET) of muscle to aid in the diagnosis and characterize disease progression and extent of muscle involvement in inflammatory myopathies.

## S3-4

## Single-cell analysis of refractory anti-SRP necrotizing myopathy treated with anti-BCMA CAR T-cell therapy

Wei Wang

Department of Neurology, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China

Immune mediated necrotizing myopathy (IMNM) is an autoimmune disorder associated with the presence of autoantibodies, characterized by severe clinical presentation with rapidly progressive muscular weakness and elevated levels of creatine kinase, while traditional pharmacological approaches possess varying and often limited effects. Considering the pathogenic role of autoantibodies, chimeric antigen receptor (CAR)-T cells targeting B cell maturation antigen (BCMA) have emerged as a promising therapeutic strategy. We reported here a patient with anti-signal recognition particle (SRP) IMNM refractory to multiple available therapies, who was treated with BCMA-targeting CAR-T cells, exhibited favorable safety profiles, sustained reduction in pathogenic autoantibodies, and persistent clinical improvements over 18 months. Longitudinal single-cell RNA, BCR, TCR sequencing analysis presented the normalization of immune microenvironment after CAR-T cell infusion, including reconstitution of B-cell lineages, replacement of T-cell subclusters and suppression of overactivated immune cells. Analysis on characteristics of CAR-T cells in IMNM demonstrated a more active expansion of CD8+ CAR-T cells, with a dynamic phenotype shifting pattern similar in CD4+ and CD8+ CAR-T cells. A comparison of CD8+ CAR-T cells in patients with IMNM and those with malignancies collected at different timepoints revealed a more NK-like phenotype with enhanced tendency of cell death and neuroinflammation and inhibited proliferating ability of CD8+ CAR-T cells in IMNM while neuroinflammation might be the distinct characteristics. Further studies are warranted to define the molecular features of CAR-T cells in autoimmunity and to seek higher efficiency and longer persistence of CAR-T cells in treating autoimmune disorders.

## S3-5

## Unlocking the Mystery: The Pathogenic Role of Anti-cN1A Antibodies in Inclusion Body Myositis

Satoshi Yamashita
Department of Neurology, International University of Health and Welfare Narita Hospital, Japan

Inclusion body myositis (IBM) is a refractory muscle disease that predominantly affects the elderly and presents with heterogeneous and diverse clinical features. Autoantibodies against cytosolic 5'-nucleotidase 1A (cN1A) have been discovered in the blood of IBM patients, but their pathogenic role remains unclear.
We actively immunized wild-type C57BL6 mice with three types of mouse cN1A peptides corresponding to previously reported epitope sequences of human cN1A. We examined changes in body weight, motor ability, and histological changes in the peptide-immunized group and the control group. Furthermore, we compared the clinical features of patients who met the IBM diagnostic criteria among 570 suspected cases, based on the presence or absence of anti-cN1A antibodies, gender, age of onset, and disease duration.
Some of the peptide-immunized groups showed significant weight loss and decreased motor ability. In all peptide-immunized groups, we observed an increase in internalized nuclear fibers, infiltration of CD8-positive T cells around or inside muscle fibers, abnormal protein aggregation, and overexpression of p62 and LC3. Of the 353 cases that met the diagnostic criteria, 196 (55.5%) were positive for anti-cN1A antibodies. Logistic regression analysis showed that anti-cN1A antibody-positive patients had a higher frequency of finger flexor weakness. Multiple regression analysis revealed that patients with later onset had shorter disease duration, lower BMI, and lower serum CK levels. Male patients had a higher frequency of onset with finger muscle weakness, while female patients had lower BMI.
Active immunization with cN1A peptides partially reproduced the clinicopathological features of IBM in wild-type mice. This model demonstrates the pathogenicity of anti-cN1A antibodies in causing IBM-like tissue changes. Furthermore, these antibodies, along with gender, age of onset, and disease duration, influence the clinical presentation of IBM, suggesting their potential as a therapeutic target.

## S3-6

# Autoimmunity against melanoma differentiation-associated gene 5 induces interstitial lung disease mimicking dermatomyositis in mice

Naoko Okiyama[1], Yuki Ichimura[1,2], Risa Konishi[1,3], Manabu Fujimoto[4]

[1]Department of Dermatology, Graduate School of Medicine and Dental Sciences, Tokyo Medical and Dental University, Japan
[2]Division of Rheumatology, Department of Internal Medicine, Tokyo Women's Medical University, Japan
[3]Department of Dermatology, Faculty of Medicine, University of Tsukuba, Japan
[4]Department of Dermatology, Graduate School of Medicine, Osaka University, Japan

Anti-melanoma differentiation-associated gene 5 (MDA5) antibody-positive dermatomyositis (DM) is characterized by amyopathic DM with interstitial lung disease (ILD). Patients with anti-MDA5 antibody-associated ILD frequently develop rapidly progression and present high mortality rate in acute phase. The disease mechanism has been unclear due to the lack of suitable animal models. To determine whether autoimmunity to MDA5 involves the establishment of ILD, we immunized C57BL/6 mice with recombinant murine MDA5 whole protein, accompanied with complete Freund's adjuvant (CFA) once a week for 4 times. The immunized mice developed MDA5-reactive T cells and anti-MDA5 antibodies, and slight ILD. After an additional treatment, acute lung injury induced by intranasal administration of polyinosinic-polycytidylic acid [poly (I:C)] mimicking viral infection, the MDA5-immunized mice developed fibrotic ILD representing prolonged respiratory inflammation accompanied by fibrotic changes 2 weeks after poly (I:C)-administration, while the control CFA-treated mice had quickly and completely recovered from the respiratory inflammation induced by the intranasal administration of poly (I:C). Rag-1 knockout mice could not developed the MDA5-induced ILD, while B cell-deficient (μMT) mice did as well as wild-type mice. Adoptive transfer of T cells collected from the MDA5-immunized mice induced ILD change in recipient mice, while that of IgGs from the mice did not. Treatment with anti-CD4 depleting antibody, but not anti-CD8 depleting antibody, suppressed the severity of MDA5-induced fibrotic ILD. Upregulation of type I interferons (IFNs) mRNA was observed temporarily in poly (I:C)-treated MDA5-immunized mice. Moreover, mice with a deficiency of type I IFN receptor could not develop the MDA5-induced ILD. In contrast, upregulation of interleukin (IL)-6 mRNA, which was temporarily observed in control mice treated with poly (I:C) and CFA, was prolonged in poly (I:C)-treated MDA5-immunized mice. Treatment with anti-IL-6 receptor antibody ameliorated the MDA5-induced fibrotic ILD. These results suggested that autoimmunity against MDA5 exacerbates toll-like receptor 3-mediated acute lung injury, and prolongs inflammation resulting in the development of fibrotic ILD. IL-6 may play a key role developing fibrotic ILD in this model. Collectively, autoimmunity against MDA5 induces fibrotic interstitial lung disease in mice. CD4+ T cells, type I IFNs and IL-6 may play essential roles for the pathogenesis of the ILD mimicking anti-MDA5 antibody-positive DM.

# Symposium 4

## Current Status of the Neuromuscular Field in New Member and Expected-to-be New Member Countries

16:50-18:20, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Umapathi Thirugnanam (Department of Neurology, National Neuroscience Institute, Singapore)
Ohnmar (Department of Neurology, University of Medicine 1/Yangon General Hospital, Myanmar)

### S4-1

### An Overview of Myology in Vietnam: From AOMC 2017 to the Present

Si Tri Le
University Medical Center of Ho Chi Minh City, Vietnam

The diagnosis and treatment of muscle diseases in Vietnam have historically faced significant challenges due to the rarity of these conditions, insufficient training for neurologists, and a lack of necessary diagnostic tools.
The AOMC 2017 conference in Singapore marked a pivotal moment, as it was the first time Vietnamese doctors participated in a specialized muscle disease conference. In 2019, Vietnam performed its first muscle biopsy with limited staining techniques and diagnostic capabilities. Thanks to the generous help of Prof. Nishino from NCNP and the Vietnam Neurology Association, over 200 muscle biopsies have been conducted in Vietnam to date, utilizing a full panel of IHC staining for myositis.
Diagnostic support tools for muscle disorders in Vietnam have expanded to include myositis antibody panels, whole-body muscle MRI, muscle biopsy with IHC, and genetic testing. Despite these advancements, challenges persist due to the lack of specialized training and practical experience. Neurologists now face the task of applying paraclinical tests accurately to minimize the financial burden on patients. For instance, well-known diseases such as FSHD or myotonic dystrophy can often be diagnosed through clinical “tips” without resorting to expensive or invasive tests.
In developing countries, opportunities to participate in specialized conferences like AOMC and muscle disease courses are essential for developing this specialty. Local doctors need support to perform diagnoses appropriate to the actual local situation.

## S4-2

## Challenges and lessons learnt in setting up clinical, eletrodiagnostic and pathologic services for neuromuscular diseases in Iraq

Abdulnasir Hussin Ameer[1,2]

[1]Alhadi Neuromuscular Centre Karbala, Iraq
[2]Alhadi Neuromuscular Diseases Center, Imam Hussein Health and Education Administration, Iraq

**Establishing Alhadi Neuromuscular Center in Iraq**

Abdulnasir Hussin Ameer M.B.Ch.B Ph.D Clinical Neurophysiology
Alhadi Neuromuscular Centre, Karbala, Iraq
University of Baghdad College of Medicine

Al-Hadi Center for Neuromuscular Diseases is a project that holds a lot of hope for those suffering from neuromuscular diseases in Iraq and the Middle East. It came as a result of years of efforts by doctors in Iraq, with the help of doctors abroad, and with unlimited support from the Imam Hussein Shrine Health and Education Authority.

Neuromuscular diseases often remain neglected in many countries due to the relatively small number of cases, high costs of diagnosis and treatment, and a lack of specialized professionals. To address this critical healthcare gap, the Imam Hussein Shrine Health and Education Authority has decided to establish the Alhadi Neuromuscular Center in Iraq. This initiative aims to provide comprehensive care and support for patients with neuromuscular diseases. By recruiting highly skilled international professors and specialists, the center will offer state-of-the-art diagnostic and therapeutic services.

Alhadi Neuromuscular Center aspires to be a unique charity institution, serving not only the people of Iraq but also the broader region, ensuring accessible and advanced healthcare for those afflicted by these challenging conditions.

Conclusion:

There will be a greater role for the Muscle Diseases Association in the future to accredit the Al-Hadi Center for training doctors and medical personnel and conducting solid medical research.

## S4-3

## Management of neuromuscular diseases in Kazakhstan

Altynshash Jaxybayeva
Astana Medical University, Astana, Kazakhstan

Neuromuscular disease are group of rare but heterogeneous disjreds and not all doctors are familiar with diseases, naturally leading to wrong diagnoses and inappropriate managements. In Kazakhstan, it is a burning issue now due to an increasing number of patients with muscle diseases although the alertness at doctors is virtually lacking regarding this rare condition. many of these patients have wrong diagnosis like cerebral palsy and have been treated more aggressively then needed. In Kazakhstan, international guidelines for diagnostic and treatment approaches to Duchenne muscle dystrophy and spinal muscular atrophy were just recently accepted, and we started to give patients more or less proper care including hormones, respiratory and nutritional support. nevertheless, other types of muscle diseases are still underdiagnosed or even undiagnosed due to lack of knowledge and absence os system for neuromuscular service in Kazakhstan. That is why attempt to establish a sytem for managment of these conditions is a very crucal issue for the practice in Kazakhstan and will be presented during the session.

## S4-4

## Emerging from the ashes and founding myology services in Sri Lanka

Sanjaya Shanthiputhra Mandadige Fernando, Saamir Mohideen

Department of Paediatric Neurology, Ladyridgeway Hospital for Children, Ministry of Health Sri Lanka, Sri Lanka

Sri Lanka, the pearl of the Indian Ocean, is an island nation with a well-developed healthcare system primarily driven by the ministry of health Sri Lanka. The country's healthcare system focuses on preventive care through its established primary health facilities, while also providing secondary and tertiary care through a hierarchy of government institutions. Healthcare services are provided free of charge at the point of delivery to patients of all ages. Sri Lanka has well trained adult and pediatric neurologists, as well as clinical neurophysiologists. The histopathology services are in the process of development, we have limited facilities for genetic testing. The neurology services in the country are overlooked by the **Association of Sri Lankan Neurologists (ASN).**

We inaugurated our myology services center in 2018. The center operated smoothly until we encountered economic challenges that affected the entire country. Since 2018, we have conducted approximately 30 biopsies annually with a favorable yield. Regrettably, the inconsistent supply of certain stains, including the necessary enzyme, has had a detrimental impact on diagnostic confirmations. As a nation, we are rising from the ashes and are currently in the process of establishing the national myology center in Colombo, the country's capital.

We have initiated a monthly myology clinic at the Lady Ridgeway Hospital for Children, the largest children's hospital in the South East Asian region. Furthermore, we hold monthly multidisciplinary team meetings (MDT meetings) to discuss 2-3 patients with the involvement of all teams in patient management. Patient data is entered into a digital database for future reference and research purposes. Presently, we have approximately 300 children with muscle-related diseases enrolled in the digital database. Our goal is to expand this data entry system to adult services in the near future. For diagnosis, we rely on clinical history and examination, followed by basic blood tests. We are equipped to conduct extended metabolic panels in patients suspected of having an Inborn Error of Metabolism. Clinical neurophysiology assessments are performed as needed. We have the capability to perform open muscle biopsies and utilize the best available stains and histopathology expertise in complemeting and confirming our clinical hypothesis.

A comprehensive management strategy is formulated for each patient during the MDT meetings (neurology team, the rehabilitation team, histopatholoy team, the cadiologist, orthopaedic surgeon, the social worker and many more take part in the discussion and the management), with patients being assessed periodically. Rehabilitation, family assistance, genetic guidance, and palliative care are all crucial components which we have not undermined. The myology services currently in place were developed by the Neuromuscular Subcommittee of the ASN, and we are fully committed to executing our plan with determination and resilience

## S4-5

# Neurological Care in Afghanistan with 40 million population faces a rising neurological disease burden, including encephalitis, meningitis, malaria, measles and stroke. Neurological care is limited, with only two neurologists trained at The Aga Khan University. Challenges include poor awareness, inadequate training, and financial constraints. FMIC is working to improve services and establish a residency program

Esmatullah Hamed
French Medical Institute for Mothers and Children, Afghanistan

Afghanistan is a 40 million populations with highest fertility rate country, struggling to recover from more than 4 decades of conflict and destruction of infrastructure.
Burden of neurological diseases is increasing in Afghanistan due to prevalence of infectious disease and non-communicable diseases and other risk factors.
Viral encephalitis, bacterial and tubercular meningitis, cerebral malaria are the most common neuroinfectious disorders seen, as well as measles, and diphtheria.
Polio and tetanus are still in Afghanistan.
With high prevalence of hypertension, Diabetes Mellitus and pregnancy related complications, Stroke is common and usually not fully investigated in young patients.
Poor maternal and new born care and expanding drug user populations are all indicators of increasing neurological disease burden.
Currently most of neurologic patients are seen by general physicians, and we have limited Neuropsychiatrists who are trained in the country only center, providing training of neurology along with psychiatry in a 3 years training program.
Only two neurologists are practicing in Afghanistan who are trained at The Aga Khan University Hospital, Karachi. And only one neurophysiology diagnostic center is functioning in the country.
Lack of public awareness, poor neurology skills of physicians, limited diagnostic tools and investigations, limited number of neurologists, absence of neurology training center and poor financial situations are biggest challenges facing neurology practice in Afghanistan.
Currently in our center at FMIC there are two neurologists with three medical officers and 5 neurophysiology technologists are operating we had inpatient services stablished in august 2022.
Professor umapathi from Singapore has visited Afghanistan twice and leading efforts locally and internationally to facilitate lunching the residency program and improving neurophysiology lab in our center which we hope to have it established within a year.

# Symposium 5

## Recent advances in sarcopenia and frailty studies

16:50-18:20, Sep 13 (Fri), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Akiyoshi Uezumi (Division of cell heterogeneity of Medical Institute of Bioregulation, Kyushu University, Japan)
Satoru Noguchi (Department of Neuromuscular Research, NCNP, Japan)

## S5-1

## Research update of sarcopenia in Asia

Hidenori Arai
National Center for Geriatrics and Gerontology, Japan

Sarcopenia was first introduced to describe the age-associated decline in skeletal muscle mass and strength. This condition is recognized as a major determinant of frailty, disability, and morbidity, leading to a significant impact on quality of life and healthcare costs. The progressive loss of muscle mass and function not only increases the risk of falls, fractures, and physical disability but also contributes to a higher mortality rate among the aging population. The Asian Working Group for Sarcopenia (AWGS) 2019 consensus updated the clinical guidelines and diagnostic criteria for sarcopenia in Asian populations, recognizing the need for regional adaptations due to differences in body composition and lifestyle. The 2019 AWGS guidelines highlight the importance of combining assessments of muscle mass, muscle strength, and physical performance for diagnosing sarcopenia. Muscle mass measurement is recommended using dual-energy X-ray absorptiometry (DXA) or bioelectrical impedance analysis (BIA), with specific cutoff values provided for the Asian population. Muscle strength is primarily evaluated through grip strength, while physical performance can be assessed using the gait speed test or five-time chair standing test, the short physical performance battery (SPPB).
The pathophysiology of sarcopenia is multifaceted, involving both intrinsic and extrinsic factors. Intrinsic factors include the natural aging process characterized by a reduction in type II muscle fiber size and number, altered satellite cell function, mitochondrial dysfunction, and hormonal changes. Extrinsic factors involve sedentary lifestyles, inadequate protein and caloric intake, and chronic diseases that exacerbate muscle loss through catabolic pathways. The mechanistic pathways underlying sarcopenia involve alterations in protein metabolism leading to an imbalance between muscle protein synthesis and degradation. Increased cytokine levels, such as interleukin-6 and tumor necrosis factor- , contribute to a pro-inflammatory state, further exacerbating muscle catabolism.
The management of sarcopenia according to the AWGS 2019 guidelines focuses on lifestyle interventions, including nutritional supplementation and physical activity. Resistance training is emphasized as the most effective form of exercise to improve muscle strength and increase muscle mass. Nutritional interventions advocate for adequate protein intake, with consideration for vitamin D supplementation in deficient individuals. Early screening and interventions are crucial, especially in populations at higher risk of rapid decline in muscle mass and strength.
Future research directions include the exploration of genetic markers, the development of targeted pharmacological therapies, and the implementation of community-based programs to address the multifactorial nature of sarcopenia and promote healthy aging.

## S5-2

## Athlete Giftedness and Genetics

Hiroshi Asahara[1,2], Ryo Nakamichi[1,2]

[1]Tokyo Medical and Dental University, Japan
[2]Scripps Research, La Jolla, CA, USA

The identification of Mkx as the central transcription factor for tendons marked a significant breakthrough (Yokoyama et al. Dev Cell 2009), shedding light on the elusive mechanisms governing tendon production (Ito et al, PNAS 2010, Suzuki et al, PNAS 2016, Nakamichi et al. Nat Commun 2016). This discovery, coupled with our exploration of Piezo1, a mechano-sensor and Mkx activator, revealed surprising findings. Through mouse models, we found that enhancing Piezo1 activity specifically in tenocytes led to remarkable improvements in jumping ability and maximum speed (Nakamichi et al, Sci Transl Med 2022). Expanding our investigation to human athletes, collaboration with the Athrome Consortium uncovered intriguing insights. By analyzing the frequency of the active PIEZO1 E756del variant in Jamaican sprinters and the general population, we observed a significantly higher occurrence among sprinters (Nakamichi et al, Sci Transl Med 2022). These findings hint at the potential influence of tendon biology on athletic performance. Overall, our research not only deepens our understanding of tendons but also highlights their broader implications for motor function and athleticism. Such insights hold promise for advancing medical interventions and promoting overall societal health and well-being.

## S5-3

## The diverse functions of NAD-dependent lysine deacylase SIRT7 in the musculoskeletal systems

Tatsuya Yoshizawa
Cell Biology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan

Sirtuins (SIRT1-7 in mammals) are nicotinamide adenine dinucleotide (NAD+)-dependent lysine deacetylases/deacylases that regulate a wide variety of biological functions including energy metabolism, stress resistance, tumorigenesis, and aging. Although sirtuins were thought to only act as lysine deacetylases, recent studies have revealed that these enzymes can also remove other acyl-lysine modifications, including propionylation, succinylation, malonylation, myristoylation, and palmitoylation. SIRT1, SIRT6, and SIRT7 are predominantly located in the nucleus, where they regulate the expression of specific genes by deacetylation/deacylation of histones and transcription factors. Most of the previous studies have demonstrated that SIRT1 and SIRT6 are important for maintaining bone and cartilage homeostasis.
Until recently, SIRT7 had been the least studied sirtuin, but it has lately been reported to have many important biological functions, with roles in ribosome biogenesis, the stress response, genome integrity, metabolism, cancer, and aging. We focus on metabolic function of SIRT7 and revealed that SIRT7 serve as an energy-saving factor by acting on liver, white adipose tissue, and brown adipose tissue, in striking contrast to other sirtuins. We also elucidated the molecular function of SIRT7 in bone and cartilage. These our findings and further unpublished data in the musculoskeletal system will be presented.

## S5-4

## The Androgen-Androgen Receptor Signaling in skeletal muscle: Insights into the Mechanisms of Sarcopenia Development and Potential Aging Regulation

Tatsuya Hosoi
Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, Japan

Sarcopenia, a term coined by Rosenberg in 1988, is defined as a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength. With the global aging population, its significance has been increasingly recognized worldwide, leading to substantial advancements in basic and clinical research over the past decade.
Multiple mechanisms have been implicated in the onset of sarcopenia, with particular attention to androgen-androgen receptor (AR) signaling. In our study, we generated a new mouse model termed "fmARKO mice," in which AR is specifically deleted in fast-twitch muscles, and longitudinally analyzed their phenotype from young to old age. The results indicated that AR in skeletal muscle plays a critical role in the maintenance of muscle quality, such as muscle strength and endurance, and influences the slow/fast muscle fiber ratio. While previous reports suggested that AR signaling in skeletal muscle might not be essential for muscle mass maintenance, our study indicated that this role might change with aging. Furthermore, comprehensive gene expression analysis and LC/MS metabolomic analyses suggested a potential contribution of AR-mediated polyamine pathways.
In this presentation, we will also discuss recent insights into the relationship between sex hormones and sarcopenia/frailty.

# Symposium 6

## Muscle atrophy and hypertrophy

9:00-10:30, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Masaki Inada (Tokyo University of Agriculture and Technology, Japan)
Naoki Suzuki (Rehabilitation Medicine, Tohoku University, Japan)

### S6-1

### Elucidation of Skeletal Muscle Regulation Mechanisms Using Mouse Space Experiments

Satoru Takahashi
Department of Anatomy and Embryology, Institute of Medicine, University of Tsukuba, Japan

The effects of microgravity in space on skeletal muscles have been analyzed by various space experiments, but it has been technically difficult to scientifically analyze the threshold of gravity effects. To solve this problem, a small animal breeding apparatus with a centrifuge that can generate artificial gravity in the space environment was installed on the International Space Station. In collaboration with the Japan Aerospace Exploration Agency (JAXA), we used this device to raise mice under several gravity environments and analyzed the effects of gravity on skeletal muscles. As a result, as expected, soleus atrophy and fast muscle fiber change induced by microgravity were completely suppressed in artificial 1g (terrestrial gravity). On the other hand, 1/6g (lunar gravity) suppressed muscle atrophy but not fast muscle fiber change. These results suggest that there is a threshold for the effect of gravity on skeletal muscle, and that muscle atrophy and muscle fiber type change are independently regulated. Therefore, to analyze the molecular mechanisms of skeletal muscle atrophy and fast muscle fiber change, we analyzed the genes that fluctuated in response to the gravitational environment by gene expression analysis. As a result, we identified Large Maf transcription factors that induces Type IIb fast-twitch muscle fiber, which had not been clarified before. In this talk, I would like to discuss the genes that fluctuate in response to the gravitational environment and the regulatory mechanisms of skeletal muscle revealed from the genes.

## S6-2

## Differential gene responses for slow-twitch and fast-twitch muscles in muscular atrophy and hypertrophy

Masaki Inada
Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Japan

Disuse muscular atrophy is known to be induced by the circumstances of physical unloading. The investigation focused on the influence of artificially produced unloading with 1G and loading with 2G hypergravity on mouse muscle mass. Comprehensive RNA-seq analysis of myogenic and/or myo-atrophic genes was conducted in both the slow-twitch fiber-dominant soleus (SOL) and fast-twitch fiber-dominant tibialis anterior (TA) in a mouse model of hindlimb unloading (HLU) and 2G hypergravity using a newly developed centrifuge device.

In atrophied skeletal muscles, the expression of atrogenes was rapidly upregulated, while muscular growth factors such as insulin-like growth factor (IGF) were downregulated after one day duration of HLU in SOL. The myofiber types shifted from slow-twitch fibers to fast-twitch fibers, as observed in the SOL. RNA-seq analysis indicated that ubiquitin-proteasome and autophagy-lysosome system-related genes were positively regulated, especially in slow-twitch fiber-dominant SOL. Pro-apoptotic genes were positively regulated only in the slow-twitch fiber-dominant SOL, while several myogenic, mitochondrial, and angiogenic genes were negatively regulated. The significance of muscle atrophy induced by HLU was attributed to the suppression of transcription factors involved in muscle differentiation, decreased expression of myogenic myokines, reduced mitochondrial function, and defect in muscular angiogenesis, leading to the induction of muscle cell apoptosis in the slow-twitch fiber-dominant SOL.

In hypertrophied skeletal muscles, 2G hypergravity enhanced the mRNA expression of myogenic genes Myod1 and Myf5 in mouse muscles. In contrast, 2G hypergravity tended to downregulate the expression of ubiquitin ligases (Atrogin1 and Murf1) and significantly suppressed the expression of autophagy-related genes (Lc3b, Atg5, Atg7, and Atg16l). RNA-seq analysis revealed that 2G hypergravity increased muscle regulatory factors (Myod1, Myog, Myf5, Myf6, and Pax7), myokines (Igf1, Fstl1, and Sparc), and Yap1/Taz transcription factors. In contrast, the gene expression of protein degradation genes involved in the ubiquitin-proteasome and autophagy-lysosome systems was decreased. Fast glycolytic fiber genes (Myh4 and Myh1) significantly decreased and increased fast oxidative fiber gene (Myh2) under 2G hypergravity that resulted in 2G hypergravity induced a fast-to-slow (glycolytic-to-oxidative) fiber type shift. Hypertrophy in the 2G group was due to increased myogenic gene expression and the suppression of atrophic genes in muscle. These results indicated that muscle mass is regulated by unloading and loading forces under the positive and negative circumstances of disuse and exercise.

## S6-3

## MicroRNA having an effect on muscle differentiation and muscle hypertrophy

Hirohiko Hohjoh
Graduate School of Medicine, Juntendo University, Japan

MicroRNAs (miRNAs) are 21~23-nucleotied-long small non-coding RNAs that are incorporated into the RNA-induced silencing complex (RISC) and function as mediators of gene silencing, in which target messenger RNAs (mRNAs) are suppressed in translation or digested. Thus, miRNAs are involved in the regulation of gene expression by suppressing target genes. Thousands of miRNA genes have been found, and tissue- and developmental stage-specific expression as well as disease-associated expression of miRNAs have been observed. MiRNAs are found not only inside cells but also outside cells. For example, they exist in the blood as cell-free RNAs and circulating in the vascular system. These cell-free miRNAs (cf-miRNAs) are incorporated into small vesicles called extracellular vesicles, which are protected from extracellular nucleases. Cf-miRNAs are thought to play an important role in cell-to-cell communication, and significant associations between cf-miRNAs and diseases have been detected. To investigate the relationship between cf-miRNAs and aging, we examined cf-miRNAs in the blood of young and aged mice and found that muscle-related miRNAs are more abundant in the blood of young mice than aged mice. Further analysis of cf-miRNAs abundant in young mice revealed that miR199, whose association with muscle was unknown, has a strong ability to promote myogenic differentiation and muscle regeneration. We identified the *Lin28b* and *Suz12* genes as targets for miR199 and evaluated the effects of miR199 on muscle in vivo using synthetic miR199 mimics. The results showed that administration of miR199 mimics to aged mice resulted in muscle fiber hypertrophy, and furthermore, administration to mdx mice, a well-known animal model of Duchenne muscular dystrophy (DMD), improved the muscle strength in the mice. The findings suggest that cf-miR-199 in the blood may have anti-aging effects, including hypertrophic effects on aged muscle fibers and suggest its potential as a novel RNA therapeutic agent for DMD and aging-related diseases.

## S6-4

## Diverse roles of cellular senescence in skeletal muscle inflammation and regeneration

Yuki Saito[1], Takako S. Chikenji[2]
[1]Sapporo Medical University, Japan
[2]Hokkaido University, Japan

The phenomenon of cellular senescence, deriving from the Latin "senex" meaning "old," has been pivotal in the field of cell biology since it was observed that cultured cells, once believed to proliferate indefinitely, gradually slow their division rate and ultimately cease to divide. This observation, named "cellular senescence," has catalyzed significant advancements in the discovery of telomeres and the elucidation of cancer suppression mechanisms. Cellular senescence is characterized not merely by a halt in cell division, rendering cells "static," but by a transition to a metabolically active state responsive to stressors. This state is marked by the secretion of a variety of bioactive substances, collectively termed the senescence-associated secretory phenotype (SASP), which endows these cells with dynamic properties. Through SASP, senescent cells play crucial roles in organismal development, tissue repair, and regeneration, while also contributing to chronic inflammation, fibrosis, and aging. Understanding the diverse functions of cellular senescence is thought to contribute to the promotion of healthspan and is gaining significant attention.

In the context of skeletal muscle, several studies have underscored the involvement of cellular senescence in muscle regeneration, chronic inflammation, and aging. Recent research has illuminated how exercise-induced stress on skeletal muscle influences muscle homeostasis, regeneration, and various muscle pathologies, including chronic inflammation and fibrosis, through mechanisms involving cellular senescence. This symposium will explore the tissue repair mechanisms associated with cellular senescence, the role of senescence in age-related diseases, and the regulation of cellular senescence by exercise, particularly focusing on its impact on skeletal muscle repair, incorporating findings from our recent research.

# Symposium 7

## Muscle stem cells in Development, Regeneration and Homeostasis

10:40-12:10, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Shinichiro Hayashi (National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)
So-ichiro Fukada (Graduate School of Pharmaceutical Sciences, Osaka University, Japan)

Outline:
Skeletal muscle possesses remarkable regenerative capabilities, primarily attributed to the presence of resident muscle stem cells (MuSCs). This symposium explores cutting-edge research on MuSCs and their potential applications in regenerative medicine. We will discuss the roles of MuSCs in muscle regeneration and homeostasis, highlighting recent discoveries on their regulation and heterogeneity. Key topics include the artificial expansion of MuSCs for the treatment of muscular disorders, the impact of mechanosensing mechanisms in muscle repair, and the functional diversity within activated satellite cell populations. Additionally, the symposium will explore recent advances of cell therapy applications of MuSC from induced pluripotent stem cells (iPSCs). These advancements in MuSC biology provide significant insights into stem cell dynamics and open new avenues for therapeutic interventions in muscle-related disorders.

## S7-1

## Molecular mechanisms regulating behaviors of muscle satellite cells in response to intrinsic and extrinsic factors

So-ichiro Fukada
Graduate School of Pharmaceutical Sciences, Osaka University, Japan

Muscle satellite cells (MuSCs) play crucial roles in muscle regeneration and hypertrophy. Similar to other types of adult stem cells, MuSCs are retained in a quiescent and undifferentiated state. In this quiescent state, MuSCs require the CalcR, a GPCR whose expression is recognized in osteoclasts. We have demonstrated that the ablation of CalcR in MuSCs resulted in a transient expression of cell-cycle-related genes and a reduction in MuSC number (1). Furthermore, we have elucidated that the Notch target genes, Hey1 and HeyL, exhibit redundant functionalities in maintaining their undifferentiated state (2, 3).

Upon injury, MuSCs escape from the quiescent state, initiate to proliferate, and eventually generate new myofibers. MuSCs also respond to mechanical loading. For instance, mechanical loading above a specific threshold, as induced by exercise or resistance training, instigates MuSC proliferation. Notably, this proliferation does not necessitate myofiber damage. Instead, the activation of mesenchymal progenitors, also termed fibro-adipogenic progenitors (FAPs), via mechanical stimuli is crucial for MuSC proliferation (4, 5). In this process, we discovered that the downregulation of CalcR is also imperative for the proliferation of MuSCs. Additionally, despite the significant reduction of HeyL expression in proliferating MuSCs during regenerative processes, HeyL expression is sustained in proliferating MuSCs subjected to mechanical loading (6). In this symposium, I would like to discuss the mechanisms regulating MuSC behaviors and the potential for the artificial expansion of MuSCs without injury and exercise for treatments of muscular disorders.

1. M. Yamaguchi et al., Calcitonin Receptor Signaling Inhibits Muscle Stem Cells from Escaping the Quiescent State and the Niche. Cell reports 13, 302-314 (2015).
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## S7-2

## Role of mechanosensitive ion channels in muscle regeneration

Yuji Hara, Kotaro Hirano
School of Pharmaceutical Sciences, University of Shizuoka, Japan

Myofibers possess regenerative capacity in response to muscle damage. Muscle satellite cells (MuSCs), which are stem cells that reside on myofibers, play a fundamental role in the regeneration process. MuSCs are presumed to be activated by mechanical stress caused by changes in the microenvironmental niche, upon which they differentiate into myogenic myoblasts that repair myofibers to maintain muscle function. However, the molecular mechanisms underlying muscle regeneration processes dependent on mechanical stimuli remain to be elucidated. In this study, we identified PIEZO1, a mechanosensitive $Ca^{2+}$ channel that is activated by membrane tension, as a key regulator of myofiber regeneration. Using *Piezo1-tdTomato*, a transgenic mouse line that expresses endogenous PIEZO1 fused with tdTomato at the C-terminus, we demonstrated that PIEZO1 accumulates in the midbody during MuSC division. Moreover, MuSC-specific deletion of Piezo1 resulted in impaired muscle regeneration following muscle injury, partly because of the abnormalities in cell division of undifferentiated MuSCs, such as the presence of chromosomal bridges and micronuclei. Taken together, PIEZO1 plays a role in muscle regeneration, such as MuSC division, suggesting that the mechanosensing machinery is central to the maintenance of skeletal muscle homeostasis. In this session, we will also present our preliminary data showing that a series of mechanosensitive ion channels plays individual roles in the MuSCs function.

## S7-3

## Functional heterogeneity in the activated satellite cell population

Yusuke Ono[1,2]

[1]Department of Muscle Development and Regeneration, Institute of Molecular Embryology and Genetics, Kumamoto University, Japan
[2]Muscle Biology Laboratory, Tokyo Metropolitan Institute for Geriatrics and Gerontology, Japan

Muscle satellite cells are mitotically quiescent in healthy adult muscle but are activated in response to stimulation such as muscle injury and proliferate extensively. The majority of activated satellite cells then undergo myogenic differentiation to produce new myonuclei, while others return to a quiescent state to self-renew and replenish the stem cell pool. Accumulating evidence indicate that satellite cells are not a homogeneous population: some exhibit stem cell-like properties, while others do not. Single-cell RNA sequencing analysis revealed that Ankrd1, a MyoD target gene, is heterogeneously expressed in the activated satellite cell population. To visualize the dynamics of Ankrd1 gene expression in satellite cells, we generated an Ankrd1-tdTomato reporter knock-in mouse line. Ankrd1-tdTomato was not detected in quiescent satellite cells but began to be expressed in activated cells when stimulated with mitogen-rich medium, following the expression of MyoD protein. In activated satellite cells, we found that the Ankrd1-tdTomato low-expressing (Ankrd1low) cell population divided slowly after FACS sorting but expanded extensively when passaged. The Ankrd1-tdTomato high (Ankrd1high) cell population exhibited a marked proliferative capacity; however, it was almost depleted following passaging. The Ankrd1low cell population gave rise to both Ankrd1low and Ankrd1high progeny, whereas the Ankrd1high cell population generated only Ankrd1high cells. Importantly, Ankrd1low cells produced a greater number of Dystrophin-positive newly formed myofibers as well as self-renewed cells than Ankrd1high cells when transplanted into mdx mice. Taken together, these results suggest that the Ankrd1low cell population retains the stemness for generating progeny capable of long-term self-renewal, and thus may be a potential therapeutic target for the satellite cell-based regenerative therapy. We will discuss our recent findings on the functional heterogeneity of the activated satellite cell population.

## S7-4

## Development of Cell Therapy for DMD by iPSC-derived Muscle Stem Cell (iMuSC)

Hidetoshi Sakurai
Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan

Induced pluripotent stem cells (iPSCs) have been used in research for the development of treatments for various intractable diseases due to their unlimited proliferative and multipotent differentiation potential. We are aiming to develop novel therapies for intractable muscular diseases using iPS cells by two approaches i.e. cell therapy and drug screening. In this presentation, I focus on the cell therapy research for Duchenne muscular dystrophy (DMD).

We have developed a differentiation method that mimics the developmental stages and have succeeded in inducing fetal skeletal muscle stem cells from iPSC (iMuSCs) that are applicable to cell transplantation. We have found that iMuSC transplantation into DMD model mice restores significant numbers of dystrophin positive myofibers. In addition, some of the cells have been engrafted as satellite cells *in vivo*, and it is expected that the therapeutic effect will continue for a long time.

For aiming clinical application, three issues have remained, i.e. 1) clinical applicable differentiation method by xeno-free system, 2) effective purification method of muscle stem cell and 3) how to assess the efficacy of cell therapy. In this presentation, I focus the recent progress about 1) and 3).

As for the differentiation method by xeno-free system, we have generated a new synthetic matrix named “new generation laminin fragment (NGLF)” as a coating substrate for efficient myogenic differentiation. The NGLF is generated by conjugating domain 1 of perlecan with heparan sulfate chains (HS) to the C-terminus of the laminin E8 fragment. Using NGLFs, hiPSCs are highly promoted to direct differentiation into a paraxial mesoderm state with high-efficiency muscle lineage generation. HS conjugation to the C-terminus of Laminin E8 fragments brings fibroblast growth factors (FGFs) bound to the HS close to the cell surface of hiPSCs, thereby facilitating stronger FGF signaling pathways stimulation and initiating HOX genes expression, which triggers the paraxial mesoderm differentiation of hiPSCs.

As for the efficacy of cell therapy to the motor function, we have recently revealed that more than 10% regeneration of dystrophin positive myofiber was sufficient to prevent severe DMD muscle weakness. Furthermore, the regeneration of dystrophin positive myofibers in DMD model mice mainly ameliorates muscle fatigue tolerance rather than maximal contraction force. Preferential regeneration of oxidative myofibers in DMD muscles might be an explanation of the fatigue tolerance induction.

Finally, I would like to discuss about future direction of cell therapy combined with the genome editing technology for iPSCs.

# Symposium 8

## Muscle Mechanosensing and Metabolic Dynamics during Physical Activities

13:30-15:00, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Takashi Yamada (Department of Physical Therapy, Sapporo Medical University, Japan)
Norio Motohashi (Department of Molecular Therapy, National Institute of Neuroscience National Center of Neurology and Psychiatry (NCNP), Japan)

### S8-1

### Age-related dysfunction of $NAD^+$ metabolism and $Ca^{2+}$ signaling in skeletal muscle as a cause of anabolic resistance

Naoki Ito

Brain-Skeletal Muscle Connection in Aging Project Team, National Center for Geriatrics and Gerontology, Japan

Sarcopenia, the age-related loss of muscle mass and strength, is a serious problem in rapidly aging societies. In addition to the gradual loss of muscle mass and strength, anabolic resistance becomes a socioeconomic problem. Compared to younger individuals, the elderly show impaired nutrition- or exercise-induced activation of protein synthesis and subsequent muscle hypertrophy, which hinders nutrition and exercise therapy. However, the molecular mechanisms underlying anabolic resistance remain unclear. Recently, the importance of nicotinamide adenine dinucleotide ($NAD^+$) metabolism in sarcopenia is being established. In this study, we focused on the relationship between skeletal muscle $NAD^+$ metabolism and $Ca^{2+}$ signaling, and demonstrate the contribution of the age-associated dysfunction of $NAD^+$ metabolism and $Ca^{2+}$ signaling to anabolic resistance.

## S8-2

## *in vivo* intracellular calcium ion dynamics regulating exercise-induced muscle damage: Spatio-temporal characteristics and its underlying mechanism

Ayaka Tabuchi
The University of Electro-Communications, Japan

Skeletal muscle adapts to exercise and pathophysiological events with changing its mass and function. Unaccustomed and intense exercise induces skeletal muscle damage accompanying muscle structural disruption and declines in performance. This exercise-induced skeletal muscle damage is especially evident after exercise with eccentric contraction (ECC) and is severe with populations of muscular diseases. Dysregulation of intracellular calcium ion concentration ($[Ca^{2+}]_i$) is known as one of the primary factors leading to skeletal muscle damage. $[Ca^{2+}]_i$ is tightly regulated by $Ca^{2+}$ handling proteins that sense cellular environmental changes. These cellular mechanisms produce the unique $[Ca^{2+}]$i pattern in spatial and temporal dynamics to initiate the specific cellular process. In skeletal muscle, $[Ca^{2+}]_i$ regulates not only muscle contractions, but is also involved in multiple adaptive responses induced by exercise from fatigue and damage to hypertrophy. Therefore it is important to define the mechanism of controlling $[Ca^{2+}]_i$ dynamics for a better understanding of skeletal muscle adaptations. We have revealed unique $[Ca^{2+}]_i$ dynamics patterns with *in vivo* imaging during the muscle damage process following ECC. During ECC, there was an excessive $[Ca^{2+}]_i$ accumulation induced by two different manners: 1) a mechano-sensing dependent mechanism allowing $Ca^{2+}$ influx from extracellular space, which was significant in the muscle region with greater lengthening than other regions and 2) a stretch independent and longitudinal propagating mechanism possibly supported by the store overload-induced calcium release. Additionally, we focused on the $[Ca^{2+}]_i$ dynamics post-ECC phase and revealed that the ryanodine receptor mediates local and heterogenous $[Ca^{2+}]_i$ patterns and it was highly related to the muscle structural disruption induced by ECC. In my presentation, I would like to discuss how multiple $Ca^{2+}$ regulating mechanisms of skeletal muscle concertedly regulate the [Ca2+]i dynamics depending on or for improving conditions of the skeletal muscle itself.

## S8-3

## Intramuscular Regulation of Post-Exercise Glucose Clearance

Kohei Kido
National Institute of Advanced Industrial Science and Technology (AIST), Japan

AMP-activated protein kinase (AMPK) is a crucial cellular energy sensor found in all human and animal cells, operating as a heterotrimeric complex with catalytic α-subunits and regulatory β- and γ-subunits, each having multiple isoforms. In human skeletal muscle, three heterotrimeric combinations exist (α2β2γ3, α2β2γ1, and α1β2γ1), while mouse skeletal muscle exhibits five (α2β2γ3, α2β2γ1, α1β2γ1, α2β1γ1, and α1β1γ1). Pharmacological activation of AMPK in skeletal muscle enhances glucose uptake, and studies in rodent models and nonhuman primates indicate that selective AMPK activators hold promise as therapeutic agents for hyperglycemia. Previous research shows that ADaM-site-binding small-molecule AMPK activators increase muscle glucose uptake through an AMPKα-dependent but AMPKγ3-independent mechanism, while the nonspecific AMPK activator AICAR, an AMP mimetic, increases muscle glucose uptake via an AMPKγ3-dependent mechanism. AMPK activation in skeletal muscle is triggered by exercise, exhibiting a correlation with both the duration and intensity of the exercise regimen. Despite the AMPKγ3 complex comprising only about 20% of all AMPK complexes, its activation during exercise is notably robust and selective. Both exercise and AICAR enhance AMPKγ3 complex activity by promoting AMPKα-T172 phosphorylation, driven by changes in intracellular AMP and ZMP levels. This led to the hypothesis that the AMPKγ3 complex is critical in regulating glucose uptake during exercise and muscle contractions. However, research involving AMPK-deficient mice has yielded inconsistent results, likely due to methodological variations. Many studies have observed impaired exercise-induced glucose uptake in AMPK knockout mice, often assessing muscle glucose uptake post-contraction rather than during contraction. This observation suggests that AMPK, particularly the AMPKγ3 complex, likely plays a central role in regulating muscle glucose uptake following exercise to aid in replenishing muscle energy stores. Furthermore, it is of great importance to explore the specific pathway by which AMPK activation induces muscle glucose uptake. In this symposium, I will present our latest findings supporting this hypothesis, introduce a potential novel downstream target of AMPK, and discuss the mechanisms of intramuscular glucose uptake regulation following acute exercise.

## S8-4

# Deciphering the mechanisms underlying the positive effects of exercise on organismal homeostasis from mechanobiological perspectives, thereby developing novel therapeutic/preventative strategies for a variety of diseases and disorders

Yasuhiro Sawada[1], Naoyoshi Sakitani[2]

[1]National Rehabilitation Center for Persons with Disabilities, Japan
[2]National Institute of Advanced Industrial Science and Technology, Japan

Moderate exercise is broadly effective in improving symptoms and disorders ranging from musculoskeletal impairments to lifestyle-related diseases such as hypertension, diabetes/metabolic syndrome, and even cognitive impairments. It can truly be said that “Exercise is Medicine.” However, much of the underlying mechanisms behind the effects of exercise remain unclear. Fundamentally, what aspects of exercise contribute to the maintenance and improvement of bodily functions—that is, “what is exercise” in essence—are not well understood.

In exercise therapy for abnormal glucose metabolism, it has been demonstrated that improvements in glucose metabolism occur independently of weight loss. In the training of the quadriceps femoris for knee osteoarthritis, pain relief occurs before muscle strength increases, indicating that bodily movements themselves have beneficial effects on maintaining and improving individual functions. Since exercise actions universally induce local changes and alterations in pressure distribution in the body, they alter the mechanical stress environment in which cells are placed or to which they respond. Thus, the effects of exercise are mediated through mechanical stress applied to cells during exercise—simply put, “Exercise is Mechanical Stress.”

Our previous research efforts aim to demonstrate this, particularly focusing on a paper that shows improvement in hypertension due to mechanical stress on the brain. Furthermore, we will outline future strategies, including the development of novel treatments for muscular dystrophy.

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# Symposium 9

## Treatable Neuro Muscular Disorders

13:30-15:00, Sep 14 (Sat), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Khean-Jin Goh (Department of Neurology University of Malaya, Malaysia)
Sara Khan (Department of Neurology Aga Khan University Hospital, Pakistan)

## S9-1

## State of the art in lipid storage myopathy

Chuanzhu Yan
Neuromuscular Center and Department of Neurology, Qilu Hospital, Shandong University, China

Lipid storage myopathy (LSM), pathologically defined by excessive accumulation of neutral lipid droplets in muscle fibers, represents a heterogeneous group of lipid metabolic disorders. Traditionally, disorders affecting carnitine and the carnitine transport system were identified as the primary causes of LSM. Recently, numerous cases of LSM have been reported worldwide. The most common cause is multiple acyl-CoA dehydrogenation defects (MADD) due to mutations in electron transfer flavoprotein (ETF) or electron transfer flavoprotein dehydrogenase (ETFDH), also known as ETF-ubiquinone oxidoreductase (ETF-QO). The second most common cause is neutral lipid storage disease with myopathy (NLSDM), associated with variants in the patatin-like phospholipase domain-containing 2 (PNPLA2) gene. More recently, pathogenic biallelic variants in the human coenzyme A synthase gene (COASY) have been identified in Chinese LSM patients with previously unknown genetic defects. Although rare, FAD-related LSM caused by defects in riboflavin transport or FAD synthesis has also been reported.

MADD, also known as glutaric aciduria type II, is characterized by dysmetabolism of fatty acids and amino acids. It presents with heterogeneous clinical phenotypes, including neonatal onset forms with (type I) or without (type II) congenital anomalies and a late-onset form (type III) that presents as LSM and occasionally accompanied by encephalopathy. Most patients with the late-onset myopathic form of MADD respond dramatically to riboflavin treatment (RR-MADD), as riboflavin supplementation may stabilize variant ETF-QO protein by restoring FAD homeostasis. To date, more than 800 cases of RR-MADD have been reported worldwide.

Since the PNPLA2 gene mutation was identified as the causative gene of NLSDM in 2007, nearly 90 cases have been reported. Phenotypes of NLSDM include asymptomatic hyperCKemia, pure skeletal myopathy, pure cardiomyopathy, and combined skeletal myopathy and cardiomyopathy. The distinct pathological hallmark for diagnosis is muscle fibers with excessive lipid droplets and rimmed vacuole formation. Currently, NLSDM is untreatable.

The COASY gene encodes a bifunctional enzyme containing 4’-phosphopantetheine adenyltransferase (PPAT) and dephospho-CoA kinase (DPCK) domains, which catalyze the final steps of de novo CoA biosynthesis. Biallelic COASY variants have been associated with severe neurodegenerative diseases. Interestingly, COASY variants were recently found to be a novel genetic cause of LSM in 16 Chinese patients, clinically mimicking RR-MADD.

LSM is the most common pathological phenotype of inherited lipid metabolic disorders. Given the heterogeneity of clinical presentations, LSM and other lipid metabolism defects are likely underdiagnosed. Supplementations of riboflavin, CoQ10, and carnitine, as well as low-fat diets, have demonstrated positive clinical effects for the majority of LSM cases. Early diagnosis through newborn screening combining MS/MS with genetic testing could help keep patients in the preclinical stage, preventing disease onset.

## S9-2

## Current landscape of sporadic late-onset nemaline myopathy

Akinori Uruha
Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan

Sporadic late-onset nemaline myopathy (SLONM) is a rare adult-onset, acquired, muscle disease. SLONM typically manifests with subacute progressive proximal and axial muscle weakness, often with head drop. SLONM is often associated with monoclonal gammopathy or viral infection such as HIV and HTLV-1, although the pathological link remains elusive. This disease can be lethal due to severe respiratory insufficiency. The pathogenesis is poorly understood, with some evidence pointing to plasma cell dyscrasia and autoimmune mechanisms. The pathological hallmark of SLONM is nemaline rods in muscle fibers. There is morphological variation in fibers containing nemaline rods. MHC class I overexpression in muscle fibers is also often observed. As its overexpression tends to be more prominent in SLONM without monoclonal gammopathy, it may be associated with more autoimmune inflammatory features than SLONM with monoclonal gammopathy. Actually, some patients respond to intravenous immunoglobulins, at least partly. In light of monoclonal gammopathy, antiplasma cell dyscrasia therapy, including autologous stem-cell transplantation following high-dose melphalan and chemotherapy, can be dramatically effective for SLONM with monoclonal gammopathy. As SLONM is lethal but is now considered treatable, the significance of early recognition of the disease is increasing, regardless of its rarity. I review here current knowledge about clinical presentation, pathological features, and pathophysiology.

## S9-3

# Gene-Targeted Therapy for Duchenne Muscular Dystrophy: Clinical Development Update

Hirofumi Komaki

Department of Child Neurology, Translational Medical Center, National Center of Neurology and Psychiatry, Japan

Duchenne muscular dystrophy (DMD) is a severe genetic disorder that causes progressive muscle weakness and wasting. Clinical development of novel therapies for DMD is rapidly advancing, offering new hope for improving the lives of patients. Gene-targeted therapies represent a promising approach to treating DMD, and several strategies are currently being investigated.

1) Micro-dystrophin Gene Therapy aims to deliver a shortened, functional version of the dystrophin gene to muscle cells using viral vectors. While micro-dystrophin cannot fully replace the function of full-length dystrophin, it can help protect muscle cells and slow disease progression. This therapy has been approved in some countries, but it carries risks of serious side effects, including kidney injury (TMA), liver dysfunction, cardiomyopathy, and immune-mediated myositis. Additionally, deaths have occurred in clinical trials, highlighting the need for robust monitoring protocols. 2) Exon Skipping Therapy utilizes antisense oligonucleotides to induce the skipping of specific exons from the DMD mRNA, enabling the production of partially functional dystrophin proteins. Clinical development is ongoing for several exon skipping drugs targeting exons 51, 53, 45, and 44, with some already approved for use. While challenges remain in drug delivery, stability, and safety, exon skipping therapy holds promise for personalized medicine due to the flexibility in targeting specific RNA sequences. 3) Genome Editing Therapy, using tools like CRISPR/Cas9, directly corrects the genetic mutations causing DMD, offering a potential cure. This approach is highly anticipated as a definitive treatment for DMD.

Clinical trials typically evaluate new therapies in patient populations carefully selected to optimize the assessment of efficacy and safety. However, once approved, these therapies are used in a broader patient population with varying ages, disease severities, and comorbidities. Gene-targeted therapies, in particular, raise concerns about potential short- and long-term side effects not observed in clinical trials. Therefore, long-term post-market surveillance is crucial to continuously monitor the safety and efficacy of these therapies in real-world settings. This ongoing evaluation will ensure that the true value of these treatments is accurately assessed and that patients receive the best possible care. This presentation will provide a comprehensive overview of the latest clinical developments in gene-targeted therapies for DMD, focusing on the clinical aspects and implications of these promising treatment approaches.

## S9-4

## Challenges and Opportunities in the Treatment of Spinal Muscular Atrophy

Yuh-Jyh Jong

Graduate Institute of Clinical Medicine, Kaohsiung Medical University; Departments of Pediatrics and Laboratory Medicine, KMU Hospital, Taiwan

Spinal muscular atrophy (SMA) is a genetic disease caused by mutations in the SMN1 (survival motor neuron 1) gene, resulting in the loss of motor neurons and leading to progressive muscle wasting and weakness. SMA was once the leading genetic cause of infant death worldwide.

One of the primary challenges in SMA treatment is early diagnosis. Emphasizing the importance of newborn screening programs is crucial, as early detection is vital for the effectiveness of current therapies. On the other hand, the advent of innovative therapies has transformed the SMA treatment landscape. Breakthroughs in gene therapy, particularly the approval of onasemnogene abeparvovec (Zolgensma), and the development of drugs like nusinersen (Spinraza) and risdiplam (Evrysdi), have shown remarkable efficacy in altering the disease course. The speech will highlight the mechanisms of action of these therapies, their clinical outcomes, and ongoing research aimed at improving their effectiveness and delivery.

Additionally, the role of multidisciplinary care in managing SMA will be explored. Integrating physical therapy, nutritional support, respiratory care, and other supportive measures can significantly enhance the quality of life for patients. In conclusion, while SMA presents considerable challenges, particularly in terms of early diagnosis and equitable access to treatment, ongoing advancements in medical research offer promising opportunities. By continuing to innovate and address these challenges, there is hope for improving outcomes and the quality of life for individuals affected by this debilitating condition.

# Symposium 10

## Perspectives on skeletal muscle and organ interactions in homeostatis and disease

15:10-16:40, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Keisuke Hitachi (Center for Medical Science Fujita Health University, Japan)
Tomoya Uchimura (Dept. of Clinical Application Center for iPS Cell Research and Application, Kyoto University, Japan)

Outline:
Organs communicate with each other in a complex way using diverse signaling molecules, including hormones, cytokines, and myokines. Recent research has shed light on the crucial role of myokines and exosomes secreted by skeletal muscle in regulating the function of distant organs such as the brain, heart, and bone via the bloodstream. These discoveries highlight the significant impact of skeletal muscle on overall health and disease progression. This symposium will delve into the fascinating world of crosstalk between skeletal muscle and other organs, featuring presentations by four renowned experts actively engaged in this field. Through their insights, we aim to deepen our understanding of the intricate communication network between skeletal muscle and non-skeletal muscle tissues and develop new collaborations making progress in this field of research.

## S10-1

## The role of muscle-derived extracellular vesicles in aging

Naoomi Tominaga, Yuta Miyagi, Saki Horie
Department of Clinical Laboratory Science, Graduate School of Medicine, Yamaguchi University, Japan

In the year 2020, Japan's elderly population accounted for a staggering 28.9% of its total inhabitants, solidifying its status as a super-aged society. As the world grapples with similar demographic shifts, the implications extend far beyond mere statistics. Aging-related diseases are on the rise, and elucidating their onset mechanisms is one of the important issues. Skeletal muscle accounts for nearly 20% of body weight and is an essential tissue for independent activities of a person. Frailty caused by age-related weakening of skeletal muscles has also been linked to aging-related diseases. The overall frailty prevalence among Japanese elderly individuals is approximately 8.7%. This percentage is lower compared to other countries, however, the pre-frailty is estimated to be around 40.8%. Furthermore, muscle power and force are decreased around 30% during aging. Since muscle makes a big change during aging, the elucidation of muscle aging is an urgent issue such situation. Extracellular vesicles (EVs) are microscale vesicles composed of lipid bilayers released by various cell types, containing proteins, nucleic acids, lipids, and metabolites. Since EVs are involved in intercellular signaling, as they are transported and function between host and recipient cells, EVs are valuable tools for studying cell-cell communication in biological processes, including cancer, autoimmune disease, and neurodegenerative disorder, leading to blood-based biomarkers for early diagnosis disease. We elucidated that cancer-derived EVs have a function to promote brain metastasis by breakdown of blood-brain barrier. Cancer-derived EVs also have a feature of contents such as microRNAs, proteins, and glycoproteins. It is suggested that these features of EVs reflect the properties of donor cells. Therefore, age-related changes in cell properties are predicted to be accompanied by changes in the properties of secreted EVs. We focused on EVs derived from aged and young muscles. In this study, we analyzed age-related changes in the properties of skeletal muscle-derived EVs using primary cells. We found that EVs derived from skeletal muscle change with aging. We would like to discuss EVs related to muscle aging.

## S10-2

## Crosstalk between skeletal muscle and bone

Naoyuki Kawao
Kindai University Faculty of Medicine, Japan

Frailty has been recently recognized as a public health issue. Osteoporosis and sarcopenia are important causes of frailty. Numerous clinical studies reported that sarcopenia is highly comorbid with osteoporosis. Diagnostic criteria for sarcopenia include decreased muscle strength, muscle mass, and physical function, which are related to decreased bone mineral density and increased fracture risk. Factors, such as mechanical stress, endocrine factors, genes, nutritional states, aging, diabetes, and glucocorticoid excess, concurrently affect muscle and bone. Based on the clinical evidence, crosstalk between skeletal muscle and bone has emerged as a novel research field in the past decade. Muscle and bone are interacted as proximate organs that regulate the musculoskeletal function. Increasing evidence suggests that muscle and bone influence each other through humoral factors, such as myokines and osteokines. Myostatin and irisin have been reported as myokines affecting bone. The release of the majority of myokines is regulated by exercise. Moreover, myokine levels were shown to be regulated by various endocrinological abnormalities. Recently, we have been studying effects of renal failure on muscle and bone in mice. Our data indicated that a decrease in irisin levels in the skeletal muscles related to bone loss induced by renal failure in mice. Extracellular vesicles have recently been recognized as a communication tool for cell to cell or organ to organ. Extracellular vesicles contain various proteins and microRNA and contribute to a variety of function by transferring their contents to local and distant cells and organs. Several studies suggest that extracellular vesicles are involved in crosstalk between muscle and bone. We recently reported that muscle cell-derived extracellular vesicles suppressed the osteoclast formation in mouse bone marrow cells and enhanced the osteoblastic differentiation of mouse mesenchymal cells. Moreover, these effects of muscle cell-derived extracellular vesicles were enhanced by shear stress to muscle cells. These results suggest that extracellular vesicles released from muscle cells affect bone metabolism. In this presentation, I would like to introduce our recent findings on the crosstalk between muscle and bone via myokines and extracellular vesicles.

## S10-3

## The role of exercise-induced myokines in cardiovascular homeostasis

Koji Ohashi, Noriyuki Ouchi
Nagoya University Graduate School of Medicine, Japan

Physical exercise benefits a variety of organs, including cardiovascular system and kidney. Recent evidence has shown that skeletal muscle produces a variety of secreted factors, which are called as myokines, and can act on nearby and remote organs. Here, we focus on our recent research about some exercise-induced myokines in cardiovascular homeostasis.

Follisatain like (Fstl) 1 and fibroblast growth factor (FGF) 21, which expression is upregulated by resistance training, protect against ischemic heart disease and chronic kidney disease. Systemic administration of Fstl1 by adenovirus system reduced infarct size area after ischemia reperfusion of left anterior descending coronary artery in wild type (WT) mice by reducing cardiomyocyte apoptosis and inflammation through AMP activated protein kinase (AMPK) dependent mechanism. Fstl1 knockout (KO) mice also showed severer renal dysfunction compared with WT mice in a mouse model of subtotal nephrectomy by reducing inflammatory response via AMPK dependent manner. Intramuscular administration of adenovirus expressing FGF21 increased FGF21 in treated muscle tissue and blood stream, and attenuated cardiac remodeling in a mouse model of myocardial infarction through adiponectin dependent mechanism.

Myonectin is upregulated by endurance training and protects the heart in a mouse model of ischemia reperfusion injury by suppressing cardiomyocyte apoptosis and inflammation. Furthermore, we recently reported that myonectin is downregulated in atrophic skeletal muscle of aged mice at 80 weeks compared with young mice at 20 weeks. Myonectin KO mice showed severer lower limb muscle atrophy induced by sciatic nerve denervation and steroid treatment compared with WT mice. Conversely, supplementation of myonectin protein to WT mice improved muscle atrophy induced by sciatic nerve denervation and steroid treatment compared with vehicle-treated WT mice.

Collectively, we have investigated the role of the exercise-induced myokines, such as Fstl1, FGF21 and myonectin, in cardiovascular system for a long time. These myokines potentially play crucial roles in inter-tissue communication. Thus, the approaches to modulate the production and secretion of these myokines or to enhance their signaling pathways could be a novel therapeutic target of cardiovascular diseases and kidney diseases.

## S10-4

## Skeletal muscle atrophy-induced cognitive dysfunction: its mechanism and protective strategy

Chihiro Tohda, Tsukasa Iki
Section of Neuromedical Science, Institute of natural Medicine, University of Toyama, Japan

Physical inactivity is one of risk factors for Alzheimer's disease (AD). Performing physical exercise is difficult at old age, and thus, decline in physical movement may be a cause of age-associated lowering of the brain function. This study aimed to elucidate the molecular mechanism and onset of the skeletal muscle atrophy-induced acceleration of AD.
Pre-symptomatic young AD model mice (5XFAD) or non-transgenic wildtype mice were used. The bilateral hindlimbs were immobilized by placing them in casts for 14 days. Casting for 2 weeks reduced skeletal muscle weight. At same time, object recognition memory in the cast-attached 5XFAD mice was impaired than that in age-matched wildtype and non-cast 5XFAD mice. The hindlimb muscles were isolated for organ culture. Conditioned media (CM) of each muscle was separated by 2D-PAGE and analyzed by MALDI-TOF MS. Eighty-eight spots were differentially expressed in muscle CM. The most increased spot in the cast-attached 5XFAD muscle CM was hemopexin. Hemopexin levels in the skeletal muscle, plasma, and hippocampus were increased in cast-attached 5XFAD mice. Continuous i.c.v. infusion of hemopexin for 2 weeks induced memory deficits in young 5XFAD mice without casting. Gene microarray analysis of the hippocampus was performed to investigate the molecules involved in the accelerated memory deficit. Lipocalin-2 (Lcn2) mRNA, neuroinflammation-associated factor, was increased in the hippocampus in hemopexin-infused 5XFAD mice than in control mice. LCN2 protein in the hippocampus was localized in the neurons, but not glial cells. Lcn2 mRNA levels in the hippocampus were also increased by cast-immobilization of the hindlimbs. We are now investigating protective strategies for the hemopexin-elicited memory dysfunction.
These findings provide new evidence indicating that skeletal muscle atrophy has an unbeneficial impact on the occurrence of memory impairment in young 5XFAD mice, which is mediated by the muscle secreted hemopexin.

# Symposium 11

## Other myopathies

15:10-16:40, Sep 14 (Sat), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Raymond L. Rosales (Research Center for Health Sciences-Faculty of Medicine and Surgery University of Santo Tomas, Philippines)
Charungthai Dejthevaporn (Division of Neurology, Department of Medicine Faculty of Medicine Ramathibodi Hospital, Mahidol University, Thailand)

## S11-1

### *Anncaliia algerae* infection: potentially fatal, unclear epidemiology

Matthew Watts

Centre for Infectious Diseases and Microbiology, Institute of Clinical Pathology and Medical Research - New South Wales Health Pathology, Westmead Hospital and Sydney Institute for Infectious Diseases, University of Sydney, Sydney, New South Wales, Australia

*Anncaliia algerae* is a pathogenic microsporidian that has emerged as an opportunistic infection in humans. It has caused localized infections, primarily keratitis, and potentially fatal systemic infections. Systemic infections have typically presented as skeletal muscle myositis and, in some cases, there was evidence of cardiac and smooth muscle infection.

In the environment, *A. algerae* has been found to infect insects, including the myocytes of mosquito larval stages. The insect lifecycle is thought to be simple, with no intermediate hosts, where spores or infected or dead hosts are consumed by other insects, and there is amplification in environmental water. The route of transmission to humans is unknown with ingestion or inhalation of spores in untreated water proposed as mechanisms. To date, there is no evidence to indicate transmission though insect bite. Susceptible human hosts include those taking immunosuppressive therapy for rheumatoid arthritis, haematological malignancies, stem cell and organ transplants, and other conditions requiring biological therapies. Cases have been described in North America, Australia, Japan, China and New Zealand, with the majority in the state of New South Wales, Australia.

In humans, systemic infection has usually presented with fever, fatigue and muscle weakness and pain. Creatine kinase will be moderately elevated and patients will have lymphopenia. Diagnosis requires a muscle biopsy with species confirmation by PCR and sequence analysis. Initially, the condition may be responsive to corticosteroids, but then there will be progression without specific treatment. Albendazole and fumagillin have been effective treatments. Other important management includes the reduction of immunosuppression and measures to prevent aspiration pneumonia, due to bulbar muscle weakness. The mortality rate of systemic infection has been approximately 60%. There remain epidemiological questions about this infection. Why has it emerged, what is the means of transmission to humans and what are the most relevant factors for host susceptibility?

## S11-2

## Electrodiagnostic and myopathologic correlation in critical illness myopathy

Teerin Liewluck

Division of Neuromuscular Medicine and Muscle Pathology Laboratory, Department of Neurology, Mayo Clinic, Rochester, MN, USA

Critical illness myopathy (CIM) is an acquired myopathy characterized by weakness and muscle atrophy in patients who are critically ill, typically occurring in the setting of prolonged intensive care unit (ICU) stay, often alongside other complications such as critical illness polyneuropathy. The diagnostic criteria for CIM generally include the presence of muscle weakness, a history of critical illness, and exclusion of other neuromuscular disorders. Electrodiagnostic findings in CIM often reveal prolongation of compound muscle action potential (CMAP) duration and myopathic motor unit potentials. Prolonged CMAP duration is considered pathognomonic for CIM, especially when present in more than 1 motor nerves. Muscle biopsy in CIM typically reveals a preferential loss of myosin, although this finding is present in only 70% of patients. While type II fiber atrophy is more frequently observed, it is not specific to CIM and can occur in various conditions. The correlation between CMAP duration and myosin loss in CIM was not well understood until a recent study demonstrated that longer CMAP durations are predictive of myosin loss. In general, a CMAP duration greater than 8 milliseconds in most motor nerves is considered prolonged. However, the median CMAP durations in CIM patients with preferential myosin loss were found to be significantly longer: 13.4 milliseconds for the fibular nerve, 10 milliseconds for the tibial nerve, and 11.1 milliseconds for the ulnar nerve. In addition to ATPase staining and electron microscopic analysis, myosin loss can also be evaluated by assessing the myosin/actin ratio. A reduced myosin/actin ratio of less than 1.7 is indicative of preferential myosin loss. However, this finding is only present when more than 50% of muscle fibers are affected, reflecting high-grade myosin loss. This threshold emphasizes the need for substantial muscle involvement to establish a definitive diagnosis of CIM based on this biochemical ratio. In CIM, longer CMAP durations and a higher number of muscle fibers with myosin loss may indicate a more severe form of the condition. Notably, CIM patients with high-grade myosin loss have shown a borderline higher mortality rate ($p=0.05$) compared to those with no or low-grade myosin loss. This correlation underscores the potential impact of myosin loss severity on patient outcomes in CIM.

## S11-3

## Muscle Chaperonopathies: From Clinical Presentation to Molecular Mechanisms

Michio Inoue
Department of Neurology, Washington University School of Medicine, USA

Chaperones and cochaperones (cofactors that assist chaperone functions) play a crucial role in the cellular protein homeostasis network. They facilitate the proper folding of proteins, prevent misfolding, and promote degradation processes. Chaperonopathies are diseases caused by mutations in genes encoding chaperones and cochaperones, affecting various tissues, including muscles and nerves.

Skeletal muscle is particularly sensitive to disruptions in protein homeostasis. This sensitivity is likely due to the high demands for protein quality control in post-mitotic, terminally differentiated cells, as well as the mechanical, oxidative, and thermal stress to which muscle cells are exposed. In fact, the 2024 gene table for neuromuscular disorders lists approximately 20 chaperones and cochaperones, highlighting their significance in maintaining muscle integrity.

In this presentation, I will introduce the clinical features and disease mechanisms of muscle chaperonopathies, focusing on well-known conditions such as DNAJB6-related Limb-girdle muscular dystrophy (LGMD) D1 and BAG3-related myofibrillar myopathy. Additionally, I will present our recent discovery of DNAJB4 myopathy, discussing its clinical implications and the insights gained from the patients and laboratory.

## S11-4

## Ten years of disease gene discovery and diagnostics in neurogenetic diseases

Gina Ravenscroft[1,2]

[1]Centre for Medical Research, The University of Western Australia, Nedlands, WA, Australia
[2]Harry Perkins Institute of Medical Research, Nedlands, WA, Australia

More than 500 genes listed in the Neuromuscular Disorders Gene Table have now been associated with neuromuscular diseases. Nevertheless, comprehensive gene panel or clinical exome testing fails to identify a precise molecular diagnosis in >50% of probands. Both the Perth Neurogenetics Research Group and the diagnostic Neurogenetics Unit at PathWest were early adopters of short-read next generation sequencing. This led in the last decade or so, to identification and characterisation of >15 novel human disease genes and extended the phenotype associated with multiple known disease genes. The success of this research has relied heavily on the close collaboration between the Neurogenetic Unit, (a national referral centre for neuromuscular disease molecular diagnosis), the Perth research groups, the Royal Perth Hospital Neurogenetic Clinic, other WA clinicians, and national and international networks. This collaborative has a long history of researching early, including in utero onset, muscle diseases. More recently it has expanded to include long-read, and targeted long-read, sequencing researching late-onset neurodegenerative disorders. Notably, two novel pathogenic repeat alleles in *RFC1* causing CANVAS and prevalent in our Asian and Oceanian geographic region have been identified. This presentation will cover key findings and learnings over the past decade. The critical roles of regular multidisciplinary team meetings bringing together the lexicons of the clinic, genetics and pathology and accurate phenotyping and functional genomics in these discoveries and their accelerating pace. This internationally interconnected research has led to diagnostic improvements and an accurate genetic diagnosis for many patients and families across Australia and around the world.

## S11-5

## VCP Myopathy

Jordi Alberto Diaz-Manera
John Walton Muscular Dystrophy Research Center, Newcastle University, UK

In this talk I will review the main genetic and clinical features of patients with multisystemic proteinopathies (MSPs). The talk will be mainly centered in MSP-1 produced by mutations in the VCP gene. I will describe the mechanism of action of VCP in physiologic conditions and present an overview of the potential pathophysiology of the disease. I will also describe the main clinical presentation described in patients and the recent genotype-phenotype correlations. Common findings on muscle MRI and muscle biopsy will also be described. In the second part of the talk I will do an overview of the other MSP types described, presenting the clinical symptoms and pathologic features of the few families that have been described so far as well as the differential diagnosis of these diseases with other neuromuscular conditions.

# Symposium 12

## Advancing Next-Generation Therapeutic Modalities for Muscular Disorders

9:00-10:30, Sep 15 (Sun), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Yoshitsugu Aoki (Department of Molecular Therapy, National Institute of Neuroscience National Center of Neurology and Psychiatry, Japan)
Masayuki Nakamori (Department of Neurology Yamaguchi University, Japan)

### S12-1

### Development of immune tolerance-inducing gene therapy using mesenchymal cells

Takashi Okada
The Institute of Medical Science, The University of Tokyo, Japan

*In vivo* gene therapy for DMD utilizes adeno-associated virus (AAV) vectors, whereas various issues with activation of the innate immune system have emerged. We have discovered a innate immune response mediated by the TLR9-MyD88 pathway in AAV infection. To control this, we have devised a treatment protocol that utilizes the immunoregulatory function of mesenchymal stromal cells, and developed a new treatment technique that safely maintains high efficacy for a long period of time using a small amount of vector.

## S12-2

## Pentatricopeptide repeat protein targeting CUG repeat RNA ameliorates RNA toxicity in myotonic dystrophy type 1

Masayuki Nakamori
Department of Neurology, Yamaguchi University, Japan

Myotonic dystrophy type 1 (DM1) is an autosomal dominant multi-systemic disorder caused by the expansion of a CTG triplet repeat in the 3′ untranslated region of the *DMPK* gene. It results in the transcription of toxic RNAs that contain an expanded CUG repeat ($CUG^{exp}$). Splicing factors, such as MBNL1, are sequestered by $CUG^{exp}$, thereby disrupting the normal splicing program that is essential for various cellular functions. The pentatricopeptide repeat (PPR) proteins, originally found in plants, regulate RNA in their organelles by binding in a sequence-specific manner. We designed PPR proteins that specifically bind to the hexamer of the CUG repeat RNA (CUG-PPRs) and showed that the CUG-PPR1 could ameliorate RNA toxicity induced by $CUG^{exp}$ in DM1 model cells. A single systemic delivery of the recombinant adeno-associated virus (AAV9) containing CUG-PPR1 demonstrated long-term therapeutic effects on myotonia in DM1 model mice in correlation with the restoration of splicing activity. These results suggest that the systemic delivery of the CUG-specific PPR molecule by AAV9 might be an effective option for the treatment of DM1. Furthermore, this study demonstrated the general potential of PPR molecules that can target pathogenic RNA sequences associated with other RNA-mediated disorders.

## S12-3

## CDP-ribitol prodrug treatment ameliorates *ISPD*-deficient muscular dystrophy

Motoi Kanagawa
Ehime University Graduate School of Medicine, Japan

A group of muscular dystrophy, including Fukuyama congenital muscular dystrophy, is caused by defects in ribitol-phosphate modification, which is crucial for the functional maturation of dystroglycan (DG). Currently, no effective treatments are available for this disease group. *Isoprenoid synthase domain containing* (*ISPD*) encodes an enzyme that synthesizes CDP-ribitol, a donor substrate for ribitol-phosphate modification, and its defects are associated with congenital and limb-girdle muscular dystrophies. To explore therapeutic strategies, we established a mouse model and examined gene therapy and prodrug treatment. Skeletal muscle-selective *Ispd* conditional knockout (cKO) mice showed reduction in CDP-ribitol levels, abnormal glycosylation of DG, and severe muscular dystrophy. AAV gene replacement restored CDP-ribitol levels and rescued the ISPD-deficient pathology. Administration of tetraacetylated CDP-ribitol, which we developed as a prodrug, ameliorated the dystrophic pathology in the cKO mice. These data demonstrate that prodrug treatments can ameliorate muscular dystrophy caused by defects in *ISPD*. Our findings provide proof-of-concept for supplementation therapy with CDP-ribitol and could accelerate the development of therapeutic agents for muscular dystrophy and other neuromuscular diseases caused by glycosylation defects.

## S12-4

## Exon skipping therapy using a novel chimeric RNA fused with 4.5SH

Shinichi Nakagawa
Hokkaido University, Japan

Exon skipping represents a promising therapeutic strategy for treating genetic disorders characterized by pathogenic exons that harbor deleterious mutations. Chemically modified antisense oligonucleotides (ASOs) have been extensively utilized to facilitate the skipping of disease-associated exons, several of which have already been developed into clinical therapeutics. However, the synthesis of these chemically modified nucleotides is economically inefficient, and their frequent administration poses significant challenges for patients.

We have recently identified a rodent-specific noncoding RNA, 4.5SH, that acts as a "natural therapeutic" by antagonizing toxic exons, thus ensuring the survival of mice. Mechanistically, 4.5SH recruit protein complexes to target exons via sequences complementary to the target exons, which induces efficient exon skipping. Notably, the recognition sequences for targeting is programmable, allowing for the artificial design of chimeric RNA molecules capable of inducing exon skipping of specific, disease-associated exons. In this symposium, we will explore the biology of 4.5SH noncoding RNA and discuss ongoing efforts to develop chimeric RNAs that facilitate exon skipping in both in vitro and in vivo models.

# Symposium 13

## New technologies and models to facilitate muscle research

10:40-12:10, Sep 15 (Sun), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Tsukasa Tominari (National Center of Neurology and Psychiatry, Japan)
Ai Shima (University of Tokyo, Japan)

### S13-1

### Skeletal muscle delivery tools of CRISPR-Cas genome editing

Akitsu Hotta
Center for iPS Cells and Research Application (CiRA), Kyoto University, Japan

Duchenne muscular dystrophy (DMD) is an intractable disease that causes atrophy of skeletal muscles throughout the body due to genetic mutations. Although the development of genome editing tools such as CRISPR-Cas9 has made it possible to repair genetic mutations, safe and efficient delivery technology is essential for the application of this technology to *in vivo* gene therapy. Although AAV vectors are commonly used to deliver, they have issues such as the risk of genomic insertion and the inability to administer multiple doses due to immune response. Therefore, we have developed transient protein delivery technology using virus-like particles based on lentivirus and mRNA delivery technology based on lipid nanoparticles to realize genome editing activity in skeletal muscle *in vivo*. Transient delivery of CRISPR-Cas9 RNP reduces off-target mutagenesis risk, and LNP delivery allows multiple dosing. I would like to share our ongoing efforts and challenges of these novel transient delivery systems.

## S13-2

# Investigating and Developing Treatments for Triadopathies Using Zebrafish Pre-Clinical Models

Yukari Endo[1,2,3], James Dowling[3,4]

[1]Juntendo University, Institute of Health and Sports Science & Medicine, Japan
[2]Juntendo University, Department of Pharmacology, School of Medicine, Japan
[3]Hospital for Sick Children, Program for Genetics and Genome Biology, Canada
[4]University of Toronto, Department of Molecular Genetics, Canada

The use of zebrafish (Danio rerio) in human disease research is well-established and no longer novel. Studies employing zebrafish as a model organism have contributed to significant discoveries in muscle disease research. However, zebrafish are less familiar to researchers and clinicians than mice and rats in terms of their use as model animals. This presentation will elucidate the advantages and limitations of using zebrafish in human disease research, illustrated with examples from our experiments.

Firstly, we present the utilization of zebrafish in a large-scale chemical screen. Mutations in RyR1, a component of the skeletal muscle triad, can lead to severe congenital myopathies (*RYR1*-related myopathy; *RYR1*-RM), for which there is currently no effective treatment. A drug discovery pipeline was developed utilizing C. elegans, zebrafish, and mammalian cell models, aiming to identify new therapeutic targets for RYR1-RM. This unique platform, designed to leverage the strengths of each model, shows potential for application to a broad range of neuromuscular diseases.

Secondly, we demonstrate more practical zebrafish models than existing disease mouse models. *CACNA1S*-related myopathy is a congenital myopathy caused by mutated CAV1.1, another essential component of the triad alongside RyR1. In the absence of an appropriate animal model, the only available option was the *Cacna1s*-KO mouse (dysgenic mouse), whose experimental use had been limited by embryonic lethality, making it difficult to assess the morphology of the triad. Our zebrafish model exhibits rapid muscle development, superior observation of triad morphology, and phenotypic changes that resemble characteristic facial alterations of congenital myopathies, represents a promising alternative for further investigation.

In conclusion, the zebrafish model offers significant advantages for early-stage drug screening and genetic analysis thanks to its rapid development, capacity for large-scale breeding, and ease of genetic manipulation. The acceleration of zebrafish application in muscle disease research will facilitate the development of therapeutic strategies.

## S13-3

## Production of human muscle tissues based on cell sheet-based tissue engineering

Hironobu Takahashi
Institute of Advanced Biomedical Engineering and Science, Tokyo Women's Medical University, Japan

In preclinical drug testing, human muscle tissue models that replicates myogenesis including development, growth, and regeneration, will become a powerful tool to better understand the mechanisms of muscle diseases and facilitate the discovery of new drugs for their treatment. However, due to the difficulty in in-vitro maturation of human muscle, a large number of studies have reported on production of rodent muscle tissues. Recently, we have developed a method to produce functional human muscle tissues based on cell sheet-based tissue engineering approach. A thermoresponsive micropatterned substrate regulates the biomimetic alignment of myofiber structures enabling the harvest of the aligned myofibers as a single cell sheet. Moreover, multiple myofiber sheets can be layered on a fibrin-based gel. This gel environment promotes myofiber maturation, provides the tissue an elastic platform for contraction, and allows the attachment of a measurement device. Since this multilayering approach is effective in enhancing the contractile ability of the muscle tissue, our human muscle tissue generates a significantly high contractile force that can be measured quantitatively. The human muscle tissue shows unidirectional contraction from both electrical and chemical stimulation. In addition, their physiological responses to representative drugs can be determined quantitatively in real time by changes in contractile force. For example, our muscle tissue model demonstrated that clenbuterol had a dose-dependent influence on the contractile ability as reported in a previous study. These physiological properties indicate that our engineered muscle tissue will become a promising tissue model for preclinical in-vitro studies in muscle physiology and drug discovery.

## S13-4

## Disease modeling of Duchenne muscular dystrophy for the functional analyses by muscle training using patient-derived iPSC in vitro

Tomoya Uchimura, Hidetoshi Sakurai
Center for iPS Cell Research and Application (CiRA), Kyoto University, Kyoto, Japan

Duchenne muscular dystrophy (DMD) is a progressive muscle degenerating disease caused by a loss of dystrophin protein, and therapeutics are quite limited. DMD is characterized by the decline of muscle performance accompanied with muscle fatigue leading to overused muscles, eventually causing the degeneration of muscle fibers as an onset of the disease. Those muscle-specific primary phenotypes are followed by secondary phenotypes such as inflammatory responses. One of the limitations of disease modeling of DMD is a lack of an appropriate in vitro model that recapitulates a decline in muscle performance, muscle fatigue, and/or degeneration of muscle fibers. The establishment of such a model will accelerate identifying target molecules to prevent the initiation of DMD and developing strategies for drug development.
We have established a combinational myogenic culture system using hiPSCs with electrical-field stimulation (EFS) and collagen gel. Myotubes cultured in this system showed progressed myogenic maturation characterized by sarcomere formation and a response of excitation-contraction coupling. Using the system, we recapitulated a muscle fatigue-like decline in contractile performance without cellular damages by the EFS training program (1Hz, 20V, 2ms, continuous for 2 weeks) using DMD patient-derived iPSCs representing overused muscle. Furthermore, we developed a tetanic training program with EFS (50Hz, 15-20V, 2ms) to mimic actual contraction. When dystrophic myotubes were trained with a tetanic program at 15V, they showed a gradual decline in contraction, representing muscle fatigue, while the contraction of control myotubes was not changed. In addition, when dystrophic myotubes were trained with a tetanic program at 20V, the cells were dramatically degenerated, representing muscle damage, while the damage to control myotubes was minimal. Quantitative analyses of creatine kinases released into the culture media demonstrated a significantly increased amount of CK levels compared to control myotubes, suggesting that dystrophic myotubes are susceptible to contraction-induced damage, which may recapitulate the primary symptom of DMD.
As a conclusion, we have established a new disease model of DMD that fully recapitulates functional phenotypes of the disease, such as muscle fatigue and damage. Utilizing this model would be valuable to investigate the cellular and molecular mechanisms of the pathogenesis of DMD, detect compounds for drug development, and to use it for an in vitro proof-of-concept study.

# JSNP Joint Symposium

## Pathomechanism of OPDM and related disorders

16:50-18:50, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chairs: Takashi Kurashige (Department of Neurology NHO Kure Medical Center and Chugoku Cancer Center, Japan)
Jun Sone (Institute for Medical Science of aging Aichi Medical University, Japan)
Commentator: Akiyoshi Kakita (Niigata University, Japan)

### Noncoding repeat expansions in OPDM and related disorders and their mechanisms

Hiroyuki Ishiura
Department of Neurology, Okayama University, Japan

Oculopharyngodistal myopathy (OPDM) is a muscular disease characterized by ocular, facial, pharyngeal, and distal predominant limb muscle weakness. The cause of OPDM was unknown for a long time after the disease concept was established in 1977 by Satoyoshi and Kinoshita. Recent studies have revealed that OPDM is caused by expansions of CGG or CCG repeats in *LRP12*, *GIPC1*, *NOTCH2NLC*, *RILPL1*, *LOC642361*/*NUTM2B-AS1*, and *ABCD3*. Now, several things have become clear: OPDMs are relatively common in Asia and are less common in Europe and the United States. OPDM occasionally presents with leukoencephalopathy similar to fragile X-associated tremor/ataxia syndrome (FXTAS) and neuronal intranuclear inclusion disease (NIID), diseases also caused by CGG repeat expansions in *FMR1* and *NOTCH2NLC*, respectively. It was proposed that this broad clinical spectrum of diseases caused by CGG/CCG repeat expansions is called FNOP-spectrum disorder after the names FXTAS, NIID, and OPDM. Inclusion bodies are also found in the skin and other organs in the body, as in NIID, although less frequently. Further studies are needed to elucidate the pathogenesis of OPDM and to develop therapeutic strategies.

## RAN translation and RNA foci: Causing differentiation of phenotype

Takashi Kurashige
Department of Neurology, NHO Kure Medical Center and Chugoku Cancer Center, Japan

Non-coding repeat expansions cause several neuromuscular and neurodegenerative diseases, including oculopharyngeal distal myopathy (OPDM) and neuronal intranuclear inclusion disease (NIID). Many genes cause different clinical presentations depending on the repeat length of non-coding repeats as exonic repeat expansions. However, the pathomechanism underlying the determination of clinical phenotypes is still unclear. Interestingly, the clinical presentation of the causative genes of OPDM is less variable than that of other genes causing non-coding repeat diseases. Recently, we reported that a repeat expansion in *LRP12*, a causative gene of OPDM type 1 (OPDM1), is a cause of familial amyotrophic lateral sclerosis (ALS) type 28 (ALS28). ALS is a neurodegenerative disease characterized by the degeneration of motor neurons. A repeat expansion in *C9orf72* is the most common cause. Although the pathomechanism of C9orf72 is thought to be repeat-associated non-AUG (RAN) translation, the pathogenesis of ALS isn't fully understood. Therefore, we evaluated the characteristics of ALS28 patients with repeat expansion in *LRP12* by comparison with OPDM1.
We identified two ALS-affected families, including six individuals with ALS, one individual with OPDM1, and four neurologically healthy participants. We compared them with 1,039 individuals and 40 families in the ALS cohort, 15 individuals with OPDM1 and 853 healthy control participants. Among these patients, we identified a CGG repeat expansion in *LRP12* in five families and two simplex individuals. These ALS28 individuals have 61-100 repeats, in contrast to OPDM1 individuals with 100-200 repeats. The clinical presentation of ALS28 patients differs from that of OPDM1 patients, with the exception of the affected triceps surae muscles.
We investigated muscle tissue and iPS cell-derived motor neurons (iPSMNs) from ALS28 and OPDM1. Muscle tissue from three ALS28 patients showed fiber type grouping and nerve bundles with axonal phosphorylated TDP-43 (pTDP-43) accumulations, but no rimmed vacuoles. Cytoplasmic pTDP-43 is also present in ALS28 iPSMNs. In addition, RNA foci are more prominent in muscle and iPSMNs in ALS28 than in OPDM1, although muscle blind-like 1 aggregates are only observed in OPDM1 muscle.
CGG repeat expansions in *LRP12* cause both OPDM and ALS. Clinical manifestations depend on the repeat length, which may be related to RNA foci formation and RAN translation. RNA foci formation and RAN translation provide insight into the repeat length-dependent switching of clinicopathological presentations.

## RNA G-quadruplexes cause neuronal dysfunction in trinucleotide CGG repeat diseases

Norifumi Shioda

Department of Genomic Neurology, Institute of Molecular Embryology and Genetics (IMEG), Kumamoto University, Japan

Fragile X-related tremor/ataxia syndrome (FXTAS) is a neurodegenerative disease caused by CGG triplet repeat expansions in FMR1, which elicit repeat-associated non-AUG (RAN) translation and produce the toxic protein FMRpolyG. We show that FMRpolyG interacts with pathogenic CGG repeat-derived RNA G-quadruplexes (CGG-G4RNA), propagates cell to cell, and induces neuronal dysfunction. The FMRpolyG polyglycine domain has a prion-like property, preferentially binding to CGG-G4RNA. Treatment with 5-aminolevulinic acid, which is metabolized to protoporphyrin IX, inhibited RAN translation of FMRpolyG and CGG-G4RNA-induced FMRpolyG aggregation, ameliorating aberrant synaptic plasticity and behavior in FXTAS model mice. Thus, we present a novel therapeutic strategy to target G4RNA prionoids.

## Muscle pathology of OPDM - how to differentiate from OPMD

Masashi Ogasawara[1,2], Nobuyuki Eura[1,3], Ichizo Nishino[1]

[1]Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Pediatrics, Showa General Hospital, Tokyo, Japan
[3]Department of Neurology, Nara Medical University, Nara, Japan

Oculopharyngodistal myopathy (OPDM) and oculopharyngeal muscular dystrophy (OPMD) exhibit similar clinicopathological features, such as progressive ptosis, ophthalmoplegia, bulbar muscle weakness, and rimmed vacuoles on muscle pathology. However, OPDM typically presents with more distal muscle weakness, while OPMD shows more proximal muscle weakness. Until recently, there have been no effective tools to differentiate these two disorders aside from genetic analysis.

Recent research has identified several causative genes for OPDM, including *LRP12*, *GIPC1*, *NOTCH2NLC*, *RILPL1*, and *LOC642361/NUTM2B-AS1*. Interestingly, *NOTCH2NLC* has also been associated with neuronal intranuclear inclusion disease (NIID), where skin biopsy is a valuable diagnostic tool due to the presence of p62-positive intranuclear inclusions in fibroblasts, lipid cells, and sweat gland cells.

Given the shared clinicopathological features between OPDM and NIID, we further analyzed skin biopsies from patients with OPDM_LRP12, OPDM_GIPC1, and OPDM_NOTCH2NLC. We found that while not all cases of OPDM_LRP12 showed p62-positive intranuclear inclusions in skin biopsies, all cases of OPDM_GIPC1 and OPDM_NOTCH2NLC did exhibit these inclusions.

Based on these findings, we extended our investigation to muscle pathology, specifically examining p62-positive intranuclear inclusions in the interstitial field and muscle nuclei. Anti-p62 antibody-positive intramyonuclear inclusions were significantly more frequent in OPMD (involving an average of 11.9% ± 1.1% of myonuclei, range 5.9–18.6%) than in OPDM_LRP12, OPDM_GIPC1, and OPDM_NOTCH2NLC (mean 0.9–1.5%, range 0–2.8%, $p < 0.0001$).

Conversely, non-muscle intranuclear inclusions in blood vessels, Schwann cells/pericytes, perineurium, and muscle spindles were frequently seen in OPDM_NOTCH2NLC, followed by OPDM_GIPC1 and OPDM_LRP12, but were absent in OPMD. This differential staining pattern of p62 in non-muscle cells and myonuclei is useful to distinguish OPMD from OPDM_LRP12, OPDM_GIPC1, and OPDM_NOTCH2NLC.

These findings highlight the importance of detailed pathological evaluation in the differential diagnosis of OPDM and OPMD, providing a clearer understanding of their distinct features and aiding in accurate diagnosis.

## Neuronal intranuclear inclusion disease

Jun Sone
Department of Neuropathology, Institute for Medical Science of Aging, Aichi Medical University, Japan

NIID is a neurodegenerative disease in which intranuclear inclusions are found in the nuclei of a wide range of cells, and has long been diagnosed by postmortem autopsy. The pathological characteristic is that intranuclear inclusions stained positively for ubiquitin or p62 are widely found in the nuclei of neurons, glial cells, Schwann cells, and cells in general organs in the central and peripheral nervous systems. Until antemortem diagnosis became possible, the number of reported cases was small and it was considered to be a very rare disease. In addition, it has been reported that the clinical symptoms are diverse, and diagnosis had to be made by autopsy.

However, since we reported in 2011 that NIID can be diagnosed from skin biopsy tissue, the number of cases diagnosed antemortem has been increasing. In particular, many cases of elderly onset of the disease accompanied by dementia have been reported. Linkage analysis and genome analysis using long-read next-generation sequencers were performed on these NIID cases, and the causative gene for NIID was identified as an expansion of the GGC repeat sequence of the *NOTCH2NLC* gene, which was reported in 2019. As a result of this result, it is now possible to diagnose NIID by both skin biopsy and genetic analysis. Segmental duplication has been confirmed in the region where the *NOTCH2NLC* gene exists, making it difficult to analyze using short-read next-generation sequencers or Sanger sequencing methods, and special methods such as PCR must be used.

We examined the clinical findings of 150 adult cases diagnosed with NIID based on both pathological diagnostic findings and NOTCH2NLC gene analysis results. The majority of cases visited the hospital with forgetfulness as the initial symptom, but there was a group in which muscle weakness and peripheral neuropathy were prominent. Furthermore, because there was a group of NIID cases in which forgetfulness or muscle weakness was not prominent, these were classified as the "other" group and examined in three groups: a group showing forgetfulness, a group showing muscle weakness, and a "other" group.

Currently, we are working with a research team from the Ministry of Health, Labor and Welfare of Japan to promote a prospective epidemiological study of NIID, and are collecting data that will contribute to the analysis of the natural history of NIID and the formulation of guidelines. For cases suspected of NIID, we would like to ask for your cooperation in prospective studies.

## Neuropathology of OPDM

Rie Saito, Akiyoshi Kakita
Department of Pathology, Brain Research Institute, Niigata University, Japan

In recent years, a series of discoveries of the genes responsible for oculopharyngodistal myopathy (OPDM) have led to the identification of six gene mutations: CGG repeats in the 5'-untranslated region of *LRP12*, *GIPC1*, *NOTCH2NLC*, *RILPL1* and *LOC642361*/*NUTM2B-AS1*, and a sixth gene (*ABCD3*) will soon be added to this list, namely OPDM1-5 and OPDM6. Although the presence of *NOTCH2NLC* and *LOC642361*/*NUTM2B-AS1* has been reported in OPDM with leukoencephalopathy, the neuropathologic picture of OPDM remains elusive.

In this session, we present an OPDM1 pedigree associated with *LRP12*, showing common OPDM1-related clinical and muscular features. Moreover, autopsy showed coexisting NIID histopathology in almost all organs, except for the skeletal muscles, with abundant round, eosinophilic, ubiquitin- and p62-positive intranuclear inclusions. These inclusions were indistinguishable from those in NIID1. Although clinical information was unavailable, in addition to agonal edematous changes, we observed white matter lesions with perivascular preservation, consistent with previous NIID reports. These findings suggest that the disease process of OPDM1 is not limited to the skeletal muscles and can affect various other organs, possibly the brain as well. In this symposium, we review the neuropathologic features of OPDM, including the present case, to gain a better understanding of the pathomechanisms underlying the similarities and differences among OPDM subtypes.

# Sponsored Symposium

## Multidisciplinary Approach to Muscular Dystrophy Management

15:30-16:40, Sep 13 (Fri), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chair: Hirofumi Komaki (Director, Translational Medical Center, National Center of Neurology and Psychiatry, Japan)

Sponsored By: Pfizer Japan Inc.

## SS1

## Motor Function Assessment in the Treatment of Muscular Dystrophy

Keiko Ishigaki
Associate Professor, Department of Pediatrics, Tokyo Women's Medical University School of Medicine, Japan

Assessment of motor function is important in terms of understanding disease progression and evaluating the effectiveness of treatments developed for muscular dystrophies. However, unlike adults, it is often difficult to apply an appropriate scale for assessing motor function in children because of fluctuations in motivation and mood. Furthermore, children with intellectual disabilities and autistic spectrum disorders such as Duchenne muscular dystrophy (DMD) sometimes have difficulty following the examiner's instructions. The 6-minute walking test and the North Star Ambulatory Assessment (NSAA) were formerly the gold standards, serving as motor function measuring systems for children with DMD, but have various shortcomings: the 6-minute walking test carries a risk of falling, is overly burdensome for patients, and is easily influenced by the child's levels of fatigue and mood. The NSAA is a 17-item rating scale used to assess functional motor abilities in ambulant children with DMD and is probably suitable as a long-term longitudinal motor function assessment scale. The NSAA is still often used as the primary endpoint in clinical trials, but as a short-term measure, such as at one year, it may be inadequate for detecting small changes, since it is only a three-point scale (0-2). In other words, because of the wide range encompassed by one point, the score does not reflect a change from a highly unstable to a more stable movement. For this reason, alternative motor ability evaluation scales have recently been investigated. Timed tests such as the 4-step climbing test and standing time are regarded as the primary endpoints. In Japan, multicenter studies on outcome measures or the natural history of patients with DMD were also conducted to determine appropriate motor function assessment scales. Other measures such as 10-m run/walk time and time to stand are being considered as potentially useful candidates. Natural history studies will become increasingly important in treatment development, but this will require long-term data collection. For this purpose, a simple and time-saving test method is required in clinical practice. Tests such as the time to stand up from the floor and the 10-meter walk test may thus be useful.

## SS2

## Cardiac Management of the Patient with Muscular Dystrophy

Mi Kyoung Song
Associate Professor, Pediatrics, Seoul National University Hospital, Republic of Korea

Many patients with Duchenne muscular dystrophy (DMD) develop ventricular dysfunction during their teenage years. Heart failure and arrhythmias have become leading causes of death in these patients. Consequently, early diagnosis and management of cardiovascular disease are critical for their survival and quality of life. Pharmacological treatments have been developed to reduce or prevent cardiac dysfunction in DMD. Retrospective studies have shown that corticosteroids can improve cardiac function in dystrophinopathies; however, prospective randomized controlled trials are necessary to confirm these findings. Early Angiotensin-converting enzyme inhibitors (ACEIs) effectively reduce mortality and prevent progression to left ventricular (LV) dysfunction in DMD patients. The efficacy of beta-blockers in DMD cardiomyopathy has been less clear, though carvedilol has shown beneficial effects in increasing LV ejection fraction when used with ACEIs. Additionally, aldosterone inhibitors, such as eplerenone, have demonstrated attenuation of progressive LV dysfunction in DMD. For DMD patients with end-stage heart failure, LV assist devices have been demonstrated to be effective and can be used as destination therapy. There have been newer therapeutic strategies including genetic manipulation, and mitochondrial regulation. However, further research is necessary to establish specific therapeutic approaches in DMD cardiomyopathy.

## SS3

## Usefulness of Japanese National Registry of Muscular Dystrophy (Remudy)

Harumasa Nakamura
Director, Department of Clinical Research Support, National Center of Neurology and Psychiatry, Japan

Patient registries play an important role in medical and clinical research by systematically collecting and managing health-related information from defined populations. These databases are invaluable for clinical research, public health monitoring and enhanced patient care.
In clinical practice, patient registries support evidence-based medicine. Physicians and healthcare providers can use registry data to compare patient outcomes across different treatments and interventions, thereby identifying best practice and improving care protocols. It also plays an important role in healthcare policy and planning. Policymakers can use registry data to allocate resources effectively, identify public health priorities and develop targeted interventions. This is particularly important in the management of chronic and rare diseases, where comprehensive data is necessary to develop and implement effective health policies. For example, rare disease registries provide important data to facilitate understanding of diseases, support drug development and inform clinical guidelines.
In recent years, there has been a growing international movement to utilize real data from patient registries in the clinical development of medicines and medical devices. In Japan, activities such as the Clinical Innovation Network are underway, highlighting the role of patient registries as a new development methodology.
Patient registries are an essential tool in healthcare and medical research. Patient registries can improve understanding of disease, improve clinical practice, advance drug development, inform policy decisions and ultimately improve patient outcomes. As healthcare continues to evolve, the importance and utility of patient registries will only increase.
We would like to present the usefulness of patient registries and Remudy in Japan.

## SS4

## Multidisciplinary Approach to Management of Muscular Dystrophy Treatment in Korea

Jong-Hee Chae
Professor, Pediatrics, Seoul National University Hospital, Republic of Korea

Muscular dystrophy (MD) encompasses a group of heterogeneous genetic disorders characterized by progressive muscle weakness and degeneration. This presentation explores the efficacy and necessity of a multidisciplinary approach in the management and treatment of muscular dystrophy, highlighting the integration of various specialties to optimize patient outcomes. Given the complexity and heterogeneity of MD, a single-discipline approach is insufficient to address the multifaceted needs of patients. Thus, a comprehensive treatment plan involving neurologists, rehabilitation doctors, cardiologists, pulmonologists, orthopedic doctors, and genetic counselors, among others, is necessary

The presentation delves into the critical roles played by each discipline. Neurologists are pivotal in the diagnosis and ongoing assessment of disease progression, while rehab doctors focus on maintaining mobility, enhancing daily function, and preventing contractures. Cardiologists and pulmonologists monitor and manage cardiac and respiratory complications, which are common in advanced stages of MD. Genetic counselors provide essential support for patients and families, guiding them through the complexities of genetic testing and implications for future generations.

Moreover, the role of emerging therapies and clinical trials in the multidisciplinary framework is examined. Gene therapy, exon skipping, and other molecular techniques represent promising avenues for altering the disease course. Integrating these novel therapies requires coordination among various specialists to ensure comprehensive care and monitoring of potential side effects.

This presentation also addresses the psychosocial aspects of MD management. The involvement of psychologists and social workers is crucial in supporting patients and their families, addressing the emotional and mental health challenges associated with chronic illness. The presentation emphasizes the importance of patient-centered care, where treatment plans are tailored to individual needs, preferences, and goals, ensuring that patients and their families are active participants in the decision-making process.

I will also present a couple of case studies in our clinic, focusing on Duchenne muscular dystrophy. In conclusion, a multidisciplinary approach is indispensable for the effective management of muscular dystrophy. By fostering collaboration among various healthcare professionals, this approach not only addresses the complex clinical needs of MD patients but also enhances their quality of life.

# Luncheon Seminar 1

## Emerging Science in SMA Treatment for Better Outcomes

12:10-13:00, Sep 12 (Thu), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chair: Hirofumi Komaki (National Center of Neurology and Psychiatry)

Sponsored By: Biogen Japan Ltd.

## LS1-1

## Clinical Experience of Spinal Muscular Atrophy in the Era of Newborn Screening and Treatment

Yin-Hsiu Chien, MD, PhD
National Taiwan University Hospital

## LS1-2

## Neurofeedback Rehabilitation Using Robot-suits Hybrid Assistive Limb (HAL)

Takashi Nakajima, MD, PhD
National Hospital Organization Niigata National Hospital

Spinal muscular atrophy (SMA) is a heterogenous disease affecting people across all age groups with a broad spectrum of phenotypes/severities. Accumulated evidence, nonetheless, has supported that earlier treatment results in better outcomes throughout different SMA types or stages. One such (and as far the most successful) approach is newborn screening – to facilitate diagnosis and enable presymptomatic treatment or treatment immediately after onset. The disease trajectory has significantly changed with the expansion of newborn screening as well as the evolution of therapeutic options now available for SMA. Yet, challenges still remain, including differences in outcome between patients, exploration of effective and consistent measurement of treatment response, or long-term management of physical and motor development. During this seminar, Prof. Yin-Hsiu Chien, National Taiwan University Hospital, and Dr. Takashi Nakajima, National Hospital Organization Niigata National Hospital, will introduce the latest advancements and outlook of SMA treatment and its transforming treatment strategy. Highlights include the experience of newborn and infantile patients with SMA identified through newborn screening in Taiwan and how they have been followed up (Prof. Chien), and the examples of innovative concept of neurorehabilitation for grown-up and adult patients using a robot suit Hybrid Assistive Limb (Dr. Nakajima). Chaired by Dr. Hirofumi Komaki, National Center of Neurology and Psychiatry, emerging evidence and learning for pursuing lifelong better outcomes in patients with SMA will be discussed.

# Luncheon Seminar 2

12:30-13:20, Sep 13 (Fri), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chair: Ichizo Nishino (Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)

Sponsored By: Alexion Pharma G.K. Medical Affairs Division

## LS2

## Long-Term Treatment Strategies for Myasthenia Gravis

Kimitoshi Kimura
Department of Neurology, Graduate school of Medicine and Faculty of Medicine, Kyoto University

Myasthenia gravis (MG) is an autoimmune disease that affects approximately 30,000 people in Japan and more than 700,000 people worldwide. In most cases, the neuromuscular junction is targeted by autoantibodies, specifically anti-acetylcholine receptor (AChR) or anti-muscle-specific tyrosine kinase (MuSK) antibodies. It should be noted that pseudo-negative results for anti-AChR antibodies can occur with the current commonly-used detection method. Patients experience fluctuating muscle weakness, which can interfere with their ability to work and perform daily activities. While immunosuppressive drugs can alleviate the symptoms, traditional long-term treatment with a high dose of steroid carries the risk of systemic adverse events. In Japan, only half of the patients achieve “minimal manifestations” (MM) status with 5mg or less of prednisolone. Recently, several biologics have been approved for the treatment of MG, primarily targeting complement C5 or the neonatal Fc receptor (FcRn). These new drugs are expected to improve persistent symptoms in the patients who have not responded well to low-dose steroid and immunosuppressants, requiring occasional fast-acting treatments such as intravenous immunoglobulin (IVIg), plasmapheresis, or intravenous methylprednisolone (IVMP).

Three pathological mechanisms are recognized in MG with anti-AChR antibody: blockade of the binding of acetylcholine to AChR, internalization of AChR, and activation of the complement cascade leading to the formation of membrane attack complex (MAC). All of these mechanisms are triggered by the binding of anti-AChR antibodies. The pathological mechanisms are different between the clones of anti-AChR antibodies even in the same patients. Previous studies have clearly shown that the neuromuscular junction is severely damaged by the formation of MAC, which cannot be easily recovered. C5-targeting therapy prevents such structural damage by nearly completely inhibiting the process of the final complement cascade. On the other hand, FcRn-targeting therapy reduces the amount of circulating pathogenic autoantibodies transiently.

In this seminar, the unmet medical needs and the long-term treatment strategies in MG will be discussed, based on the current clinical guideline and the knowledge of emerging therapeutic options.

# Luncheon Seminar 3

## Generalized Myasthenia Gravis Seminar -Entering a New Stage -

12:30-13:20, Sep 13 (Fri), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chair: Kazuma Sugie (Professor & Chairman Department of Neurology Nara Medical University, Japan)

Sponsored By: UCB Japan Co. Ltd.

## LS3

## The forefront of gMG treatment
## - Positioning of Subcutaneous Injections -

Hiroyuki Murai
Professor, Department of Neurology, International University of Health and Welfare

Recent advances in the treatment of myasthenia gravis (MG) are remarkable. The latest Japanese guidelines for MG/LEMS were published in 2022. In this guidelines, the primary treatment target of MG is set as “minimal manifestation status with prednisolone dose of 5mg or lower (MM-5mg)”. Furthermore, it is advised to avoid high dose oral steroid administration and to perform early fast-acting treatments (EFT). It is known that high dose oral steroids hamper quality of life in MG patients in many ways. EFT enables us to sidestep high dose steroids, and to accomplish MM-5mg easily. Recently many biologics have become available worldwide and several new components are still under development. Several complement (C5) inhibitors and FcRn inhibitors are approved in Japan. Components available in Japan includes eculizumab, ravulizumab and zilucoplan as C5 inhibitors, efgartigimod (IV, SC) and rozanolixizumab as FcRn inhibitors. Among these, zilucoplan, efgartigimod and rozanolixizumab can be delivered via subcutaneous injection. In this seminar, pathogenetic mechanisms of MG, the downside of oral steroids, the novel treatment strategies using steroids, immunosuppressants, EFT and new biologics are discussed.

# Luncheon Seminar 4

12:35-13:25, Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chair: Hiroyuki Awano (Research Initiative Center, Organization for Research Initiative and Promotion, Tottori University)

Sponsored By: CHUGAI PHARMACEUTICAL CO., LTD.

## LS4

## The diagnosis and treatment of treatable childhood-onset neuromuscular diseases

Keiko Ishigaki
Department of Pediatrics, Tokyo Women's Medical University School of Medicine

In recent years, there have been dramatic advances in the development of treatments for neuromuscular diseases, which were once considered intractable. In Japan, enzyme replacement therapy for Pompe disease was approved in 2007, and antisense nucleic acid drugs for Duchenne muscular dystrophy (DMD) in 2020. Notably, the development of treatments for spinal muscular atrophy (SMA) has been particularly remarkable, beginning with the approval of antisense drugs for intrathecal injection in 2017, followed by gene therapies in 2020 and oral small-molecule compounds in 2021. SMA is a degenerative disease of the lower motor neurons that causes muscle atrophy and weakness due to degeneration of the anterior horn cells of the spinal cord. Type I is a severe form that begins in infancy, i.e. before age 6 months. Affected infants are unable to sit up, show rapid disease progression and generally die before 2 years of age without treatment. Type III, in contrast, has a slow onset, manifesting after age 18 months, and affected children do become ambulatory. It is difficult to make an early diagnosis, which is often delayed, of Type III SMA. The clinical symptoms at the time of initial onset are nonspecific, such as an agitated gait and tendency to fall, tendon reflexes are sometimes preserved unlike in Type I, and serum creatine kinase (CK) levels rise to several thousand U/l. Since DMD and childhood-onset Pompe disease, as well as SMA type III, develop in early childhood with similar symptoms such as muscle weakness and hyperCKemia, it is necessary to diagnose these treatable diseases at the appropriate time. The difference in serum CK levels and the characteristic findings of each disease can, to some extent, advance the diagnosis clinically. Electromyography is useful for differentiating among neurogenic diseases, but skeletal muscle imaging can also differentiate between neurogenic and myogenic diseases. Now that therapeutic agents have been developed, prompt aggressive diagnosis is essential, with both pediatricians and pediatric neurologists increasingly seeing patients with neuromuscular diseases in their daily practice. Thus, these diseases are no longer limited to specialized institutions. Herein, we describe the key diagnostic features of treatable neuromuscular diseases, with a focus on SMA.

# Luncheon Seminar 5

## Treatment Strategies for Generalized Myasthenia Gravis

12:20-13:10, Sep 15 (Sun), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chair: Masanori Takahashi (Lab of Clinical Neurophysiology, Dept. Clinical Laboratory and Biomedical Sciences, Osaka University Graduate school of Medicine)

Sponsored By: argenx Japan K.K.

## LS5-1

## Clinical impact of efgartigimod, the first approved anti-neonatal Fc receptor inhibitor for MG in Japan

Shigeaki Suzuki
Department of Neurology, Keio University School of Medicine, Japan

The management of myasthenia gravis (MG) has been improved due to immunotherapy advances, but 20% of individuals with MG are refractory to the conventional therapy, and the need for novel biological drugs remains. The Japanese clinical guidelines for MG published in May 2022 include the concept that treatment is often lifelong and should aim to maintain a sufficient quality of life and mental health. I provide an overview of the therapeutic strategy for generalized MG in Japan, in comparison with the international consensus. The clinical efficacy, safety, and tolerability of efgartigimod, the first approved anti-neonatal Fc receptor inhibitor for MG is now established.

In addition, I recently published the paper to determine the current aspects of efgartigimod treatment for generalized MG in clinical practices in Japan. It included patients with generalized MG in the 2021 survey of Japan Myasthenia Gravis Registry study group (JAMG-R) who received an initial cycle of efgartigimod between May and September 2022. Of 1,343 JAMG-R patients, 36 (2.7%) started efgartigimod (females 68%, mean age 53 years). Their serological profiles were: AChR+, n=19 (53%); MuSK+, n=6 (17%); and seronegative, n=11 (31%). Twenty-six patients (72%) had refractory MG. There were 81 cycles of efgartigimod during the 26-week observation in 34 patients (average, 2.4 cycles). The mean interval between cycles was 5.9 weeks. A continuous 4-weekly infusion of efgartigimod was performed in 65 (80%) of 81 cycles. In the first cycle, the MG-ADL score of the 34 patients decreased significantly from 10.5±4.3 to 6.9±5.1 (p=0.003). Similarly, the mean MG composite and MG-QOL15-r decreased from 18.4±13.6 to 11.8±9.6 (p=0.004) and from 19.2±6.3 to 14.2±8.3 (p=0.007), respectively. Twenty-one (62%) patients were responders. Therapeutic responses were observed in the subsequent cycles. The duration of efgartigimod's effectiveness were varied among the responders; four responders had only a single effective cycle. Significant improvement was observed in the MuSK+ patients. Seven patient's prednisolone dose was reduced. The patients' postintervention status revealed that six patients achieved minimal manifestations. COVID-19 occurred in five patients. I failed to detect clinical or laboratory findings associated with responders.

Efgartigimod can be considered for the treatment of patients with generalized MG who do not achieve minimal manifestations, with a broad flexibility of patient selection and treatment schedules. Efgartigimod is a promising biological drug for patients with moderate to severe generalized MG with or without anti-AChR antibodies in Japan.

## LS5-2

# Efgartigimod therapy in patients with myasthenia gravis of working age: challenges encountered by patients with MG at work and when caring for children

Akitoshi Takeda
Department of Neurology, Osaka Metropolitan University

Myasthenia gravis (MG) is a rare autoimmune disease that causes debilitating muscle weakness owing to impaired neuromuscular transmission. In addition to standard therapies such as corticosteroids and immunosuppressive drugs such as azathioprine*, cyclosporine A, and cyclophosphamide*(*The treatment of myasthenia gravis with these drugs is not authorized in Japan), new strategies such as complement blockade and neonatal Fc receptor antagonism have been proposed. Efgartigimod, a neonatal Fc receptor antagonist, facilitates antibody degradation by targeting the acetylcholine receptor (AChR-Ab) and muscle-specific tyrosine kinase (MuSK) antibodies (MuSK-Ab). In 2024, a subcutaneous injection formulation VYVDURA ® (efgartigimod alfa and vorhyaluronidase alfa) for subcutaneous use was approved for self-injection in Japan. Several patients with MG have difficulty maintaining their daily activity levels owing to insufficient improvement in their condition, especially younger patients, which interferes with their work and child-rearing capacities. Patients with MG of prime working age (20s–50s) are hesitant to undergo hospitalization for treatment. Self-injection therapy with a neonatal Fc-receptor antagonist may be a treatment option for younger patients whose busy schedules make frequent visits difficult. Early detection of signs of exacerbation in working-age patients with MG and the introduction of anti-FcRn antibodies or fragment preparations in an outpatient setting may prevent hospitalization. This presentation will review issues related to the social life of young patients with MG and present the experience of introducing efgartigimod to young patients.

# Luncheon Seminar 6

## Updates on GNE Myopathy Treatment in Japan

12:20-13:10, Sep 15 (Sun), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chair: Ichizo Nishino (National Center of Neurology and Psychiatry)

Sponsored By: Nobelpharma Co., Ltd

### LS6-1

## Recent Insights into GNE Myopathy: Genotype-Phenotype Correlations, Disease Progression, and Therapeutic Approaches Beyond Sialic Acid Supplementation

Wakako Yoshioka
National Center of Neurology and Psychiatry

GNE myopathy (GNEM) is an ultra-rare autosomal recessive distal myopathy caused by pathogenic variants in the GNE gene, leading to progressive muscle weakness. Accurate diagnosis and understanding of the disease course are critical, especially with the recent approval of the Sialic Acid Extended-Release Tablet (SA-ER) as the first drug approved for GNEM in Japan.

A 10-year observational study involving 220 participants from Japan's national registry highlighted significant differences in disease progression among genotypes. The study found that 90% of participants carried either p.D207V or p.V603L variants. p.V603L homozygotes typically lost ambulation after a median of 10 years, while over 90% of p.D207V/p.V603L compound heterozygotes remained ambulatory 20 years post-diagnosis. Early onset correlated with quicker loss of ambulation, regardless of genotype. Respiratory function declined notably in non-ambulant p.V603L homozygotes. These findings have important implications for prognosis and personalized patient care.

Our comprehensive muscle imaging study statistically defined stage-specific muscle involvement patterns and identified specific muscles that progress at each stage. This information may provide more sensitive clinical outcome measures for trials and guide personalizing patient care strategies. Notably, we identified diaphragm thinning as a contributing factor to reduced respiratory function, emphasizing the importance of diaphragm assessment in monitoring disease progression.

Hyposialylation is a primary cause of muscle weakness in GNEM. While SA-ER has shown efficacy in maintaining upper extremity muscle strength in Japanese trials, international clinical trials did not reach statistical significance. The approval of SA-ER marks a significant step for GNEM treatment, but its effectiveness is not yet sufficient, possibly due to difficulty maintaining the SA level at considerable concentration for sufficient period of time. Ongoing research aims to develop additional or alternative therapies. Current clinical trials in other countries are exploring other SA metabolites such as ManNAc and sialyllactose. Ongoing approaches beyond SA supplementation include antioxidant therapies, gene therapies targeting GNE mutations, small molecules to enhance enzyme activity, and cell transplantation. As we advance our understanding of GNEM pathophysiology, these diverse therapeutic approaches offer hope for more effective treatments in the future. Continued research will be crucial in improving the lives of patients with this challenging disorder.

## LS6-2

# A New Era in the Treatment of Distal Myopathy: Expectations and Future Prospects for Aceneuramic acid

Naoki Suzuki
Tohoku University Graduate School of Medicine

GNE myopathy is an extremely rare genetic disorder caused by mutations in the GNE gene, resulting in reduced sialic acid synthesis and progressive muscle weakness. So far, no established treatment has been identified. For the replacement therapy of aceneuramic acid, extended-release formulation of aceneuramic acid (SA-ER) tablets have been developed.

To evaluate the efficacy and safety of SA-ER for GNE myopathy, phase II/III study and the efficacy confirmation study were performed in Japan. These studies involved patients genetically diagnosed with GNE myopathy, allocated in SA-ER and placebo group (n=16:4 in Ph II/III, and n=10:4 in confirmation study, respectively). The evaluation of efficacy includes the primary endpoint of changes in upper extremity composite (UEC) score, with the secondary endpoint including GNE myopathy functional activity scale (GNEM-FAS).

In the phase II/III study, the mean change in UEC at Week 48 was as small as -0.1 ± 3.7 kg in the SA-ER group versus -5.1 ± 3.4 kg in the placebo group with significant difference in two groups (P=0.0013) in the generalized estimating equation test repeated measurement analysis. The least squares mean (LSM) difference (95% CI) between the groups was 4.8 kg (-0.3 to 9.9; P=0.0635). Also, in the efficacy confirmation study, decrease in LSM change in UEC score at Week 48 with SA-ER (-0.115 kg) was numerically smaller as compared with placebo (-2.625 kg), with LSM difference (95% confidence interval) of 2.510 (-1.720 to 6.740) kg. Additionally, plots of GNEM-FAS upper extremity, mobility and total scores separated from each other between the SA-ER group and the placebo group. No clinically significant adverse events were observed in either study.

Two sets of trial reproducibly showed a trend towards slowing of loss of muscle strength and function with orally administered SA-ER, indicating supplementation with sialic acid might be a promising replacement therapy for GNE myopathy.

# Morning Seminar

## Exploring Neu-REFIX Beta Glucans: Mechanisms and Effects in Reducing Muscle Fibrosis and Fatigue

8:00-8:50 , Sep 14 (Sat), 2024
Room 1 (Convention Hall C, 1F, Nara Prefectural Convention Center)

Chair: Masaru Iwasaki (Vice-President, University of Yamanashi, Kofu, Japan)

Sponsored By: GN Corporation Co Ltd, Japan

## MS

## Oral administration of Neu-REFIX Beta 1,3-1,6 Glucan reduces skeletal muscle fibrosis and fatigue in dystrophic mice

Yoshitsugu Aoki

Director, Department of Molecular Therapy, National Institute of Neuroscience
National Center for Neurology and Psychiatry, Kodaira, Tokyo, Japan

**Background:** Recent advances in treatment for Duchenne muscular dystrophy (DMD), a severe, progressive muscle-wasting disease, include exon skipping and gene therapy, but the outcome remains suboptimal. Disease-modifying treatments are routinely employed, which aim to slow the disease's progress but have significant side effects. We have investigated the effects of an immune-modulating biological response modifier, 1-3-1,6 beta glucan, produced by the N-163 strain of *Aureobasidium pullulans*, Neu-REFIX on muscle fibrosis and function in the mdx mouse model of DMD, following its safety and efficacy in immune modulation proven in pre-clinical studies in SD Rats [1], KKAy mice [2], NASH animal model [3] and clinically in healthy male volunteers [4].
**Materials and methods:** Two studies were conducted. In the first study [5], 45 mdx mice were divided into three groups of 15: Group 1 (normal mice), Group 2 (mdx mice - vehicle), and Group 3 (mdx mice administered Neu-REFIX) for 45 days. Biochemical and histological parameters relevant to inflammation and fibrosis of the skeletal muscle were evaluated. In the second study [6], 30 mice were randomized into three groups of 10: Group 1 (normal mice, control), Group 2 (mdx mice -vehicle), and Group 3 (mdx mice administered Neu-REFIX) for 45 days. Fatigue score was assessed using the fore-limb grip test.
**Results:** Both studies confirmed the safety and feasibility of oral administration of Neu REFIX beta-glucan in mdx mice. In study.1, Neu-REFIX group exhibited a significant decrease in plasma ALT, AST, and LDH levels compared to the vehicle group. Plasma levels of TGF-β increased, while that of IL-13 decreased in the Neu-REFIX group. Neu-REFIX β-glucan significantly decreased the skeletal muscle fibrosis and the inflammation score in HE-stained muscle sections. In study. 2, the fatigue score was significantly lower in the mdx-Neu-REFIX group compared to the mdx-vehicle group ($p = 0.04$), indicating better resistance to fatigue in the Neu-REFIX group and there was a slight improvement in forelimb grip strength.
**Conclusion:** Neu-REFIX beta-glucan as a standalone agent significantly reduced skeletal muscle fibrosis and inflammation while enhancing muscle strength evident by a decline of fatigue after a short 45-day period of oral administration in mdx mice. Larger, multicentric clinical studies with Neu-REFIX beta-glucan, either as a standalone agent or as an adjuvant to other therapies, are recommended to include in the standard disease-modifying treatment guidelines for slowing the progression of DMD, following such validations.

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# AOMC Young Investigator Award Session

9:00-10:30, Sep 14 (Sat), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Shahriar Nafissi (Department of Neurology, Tehran University of Medical Sciences, Iran)
Yuh-Jyh Jong (Department of Pediatrics and Laboratory Medicine, Kaohsiung Medical University Hospital, Taiwan)

## AY-1

## Visualization of degenerative processes of the myofibers on muscle pathology in OPDM based on single nucleus RNA-seq data

Ai Yamanaka[1], Nobuyuki Eura[1], Shinichiro Hayashi[1], Kazuma Sugie[2], Satoru Noguchi[1], Ichizo Nishino[1]
[1]Department of Neuromuscular Research, National Center of Neurology and Psychiatry, Tokyo, Japan
[2]Department of Neurology, Nara Medical University, Nara, Japan

Single nucleus RNA-seq (snRNA-seq) enables independent and simultaneous transcriptomic analyses of all cell types in one muscle tissue. The purpose of this study is to establish a method to identify the pathogenic gene signature on patients' muscles, which was indicated by snRNA-seq.

We performed snRNA-seq on frozen muscles from patients with oculopharyngodistal myopathy (OPDM) that pathologically shows a myofiber atrophy and rimmed vacuoles. On pseudo-time trajectory analysis of the snRNA-seq data, two unique pathogenic pathways in OPDM were identified in first-type myonuclei. Of terminal clusters in two pathways, one expressed *NCAM1* and the other expressed *COL19A1*. In the middle clusters on the trajectories, *ANKRD1* was commonly expressed. Then, we performed immunohistochemical staining of these three proteins on frozen muscle sections to evaluate the fiber-type, morphology and distribution of myofibers, which express each gene signature. Both NCAM1 and COL19A1 were observed in atrophic fibers. However, the morphologies of both fibers were different; COL19A1-positive myofibers were extremely smaller (8.0±3.6 μm in diameter) and were clustered similarly to neurogenic changes, while NCAM1-positive myofibers were small angular fibers (11.3±9.4 μm in diameter) and some revealed rimmed vacuoles. In contrast, ANKRD1 was more frequently observed in mild atrophic fast fibers, indicating the pathological process started with the expression of this protein.

By combination analysis of snRNA-seq and immunohistochemical staining, we could visualize the two distinct pathogenic myofibers on muscle pathology, which are related to two distinct pathways on transcriptomic data in OPDM. We propose that this method can help to identify the pathogenic characteristics as muscle biomarkers during muscle degenerative process in chronic myopathies.

## AY-2

# Detection of STR expansions on a neuromuscular gene panel using STRipy improves diagnostic rate of ataxia

Chiara Lai Folland[1], Carolin Scriba[1], Michael Black[2], Rebecca Gooding[2], Nigel Laing[1], Mark Davis[2], Gianina Ravenscroft[1]

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Short tandem repeats (STRs) are repetitive DNA sequences with motifs between 2-6 bp that comprise approximately 7% of the human genome. Expansions of STR regions give rise to over 50 diseases with the majority demonstrating primary neurological or neuromuscular presentations. Despite the prevalence of STR expansion disorders, genetically diagnosing these conditions is complicated by a lack of efficient and comprehensive diagnostic screening approaches. Therefore, efforts have been made to develop tools able to genotype STR expansions in short-read sequencing data. Recently, a tool called STRipy was released which can genotype loci from a catalogue of known pathogenic STR expansions.

We used STRipy to analyse short-read sequencing data from comprehensive targeted gene panels for neuromuscular disorders within the Department of Diagnostic Genomics, PathWest (Perth, Australia). There are two primary panels, neurological and muscle, based on whether the gene is linked to primary neurological or muscular disease.

We tested STRipy on versions 6 and 7 of the ataxia neurological subpanel. Version 6 already included probes covering four ataxia STR expansion loci: *CACNA1A*, *PPP2R2B*, *TBP*, and *NOP56*. Additional probes targeting all relevant STR expansion loci were designed and included in version 7. All STR expansions detected were validated and sized using NATA-accredited PCR-based diagnostic techniques.

We tested a total of 418 and 67 patients with ataxia on version 6 and 7 of the ataxia subpanels, respectively. For version 6, 59 patients (14.1%) had reportable pathogenic variants, including nine patients with pathogenic repeat expansions detected by STRipy. For version 7, 15 patients (22.4%) had reportable pathogenic variants, including three repeat expansions detected using STRipy. Therefore, STRipy contributed 15.3% and 20% of the solved cases from version 6 and 7 of the ataxia subpanels, respectively. STRipy performed best for short pathogenic repeat loci but could not provide accurate genotyping results for large complex repeat expansion loci. However, we demonstrate that STRipy can be used to screen for some large pathogenic expansions, such as *FGF14*.

Here, we demonstrate that screening pathogenic STR expansions using STRipy on a neurological targeted gene panel improves the genetic diagnosis of ataxias. STRipy offers a simple computational method of screening multiple loci in a single test. This may assist with diagnosing atypical phenotypes or cases with onset earlier than expected, which can be troublesome to diagnose using single loci tests. Work is ongoing to explore the diagnostic utility of STRipy in the context of STR expansions associated with muscular disorders.

## AY-3

## Characterizing the Cell-Cell Interaction in Inclusion Body Myositis

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Introduction: Autoimmune myositis (AIM) consists of several subtypes, including inclusion body myositis (IBM), immune-mediated necrotizing myopathy (IMNM), anti-synthetase syndrome (ASS), and dermatomyositis (DM). The key serologic and histopathologic characteristics of these cases have been studied more extensively in recent years. However, the pathomechanism of each subtype, especially the interaction among specific cell types, remains relatively unknown. This study aims to utilize single nucleus transcriptome analysis, a recently developed technique to achieve precise cell-specific gene expression, to elucidate the pathomechanism of IBM by predicting the potential interactions among different cell types.

Methods: We extracted nuclear RNA from frozen muscle biopsy samples from three patients with IBM, along with the samples from 19 patients with various forms of AIM (DM, IMNM, ASS, immune checkpoint inhibitor myositis, and sarcoid myopathy), 16 with different hereditary muscle diseases, and three non-disease controls. We performed single nucleus RNA sequencing, which was followed by comparative bioinformatics analyses.

Results: The predictive analysis that used the transcript expression suggested that the predominant chemokine produced by the macrophages that recruit T cells is *CCL18*, responsible for attracting both naïve and activated T cells in the tissue, followed by *CXCL10* and *CXCL9*. *CCL13* from macrophages likewise attract T cells and macrophages. The macrophages themselves recruit more macrophages in this manner by interacting with *ACKR4*, an atypical chemokine receptor. Interestingly, *CXCR3* from the macrophages may, in turn, be stimulated by *CXCL13* from T cells, potentially promoting macrophage migration. As expected, T cells appear responsible for interferon-γ production which correlates with receptor interaction with *IFNGR2* of the fast- and slow-type myofibers. The downstream signaling from interferon-γ is further supported by the expression of MHC class II genes in the fast-type myofibers. *HLA-DRB1* from these fibers appears to interact with *CD37* on macrophages, which may hypothetically influence macrophage migration since it has been reported to contribute to dendritic cell migration.

Conclusion: The results show that single nucleus transcriptome analysis provides a more multidimensional understanding of the mechanism of myositis by identifying the priority interaction that may be unique to specific cells of interest in certain diseases.

## AY-4

# 4qA D4Z4 methylation test as a valuable complement for differential diagnosis in patients with FSHD-like phenotype

Xingyu Xia[1], Nachuan Cheng[1], Yiqi Liu[1], Dongyue Yue[2], Mingshi Gao[3], Kexin Jiao[1], Ningning Wang[1], Bochen Zhu[1], Chong Sun[1], Jie Song[1], Chong Yan[1], Sushan Luo[1], Jie Lin[1], Jiahong Lu[1], Chongbo Zhao[1], Wenhua Zhu[1]

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Background and objectives:
Facioscapulohumeral muscular dystrophy (FSHD) is a common autosomal inherited myopathy caused by contraction of the D4Z4 repeat unit (RU) size on chromosome 4q35 (1–10 units) or by mutations of D4Z4-binding chromatin modifier. 4q D4Z4 DNA hypomethylation has been proposed as a molecular signature for diagnosis and explanation of disease severity for FSHD type 1 (FSHD1), but further validation in extended population is needed.
Methods:
All clinically suspected FSHD probands who underwent optical genome mapping (OGM) or Molecular combing (MC) testing to determine the D4Z4 repeat size from September 2017 to December 2023 at Huashan Hospital and 17 healthy controls were enrolled. The distal and global methylation levels of the 4qA D4Z4 array were tested in 75 patients and 17 healthy controls using PCR-based bisulfite sequencing (BSS).
Results:
Among 247 patients, 219 were identified as FSHD1, including two cases of somatic mosaicism. Two cases were further identified as FSHD type 2 (FSHD2) with known pathogenic SMCHD1 variants and global hypomethylation. A repeat length-dependent increase was observed in both distal and global D4Z4 methylation levels. Secondary polynomial fitting model showed the better fit degree than linear fitting with lower root mean squared error (RMSE) (6.8097 vs 7.5915) between D4Z4 repeat size and distal methylation level. Distal methylation levels distinguished FSHD1 patients with a sensitivity of 100% and specificity of 95.45% at a cutoff value of 39.66% from controls. Distal hypomethylation level showed a strong correlation with age-corrected clinical severity score (ACSS ) and higher risk for earlier onset age compared to D4Z4 repeat size. Mediation analysis revealed that the influence of distal methylation on ACSS was partially mediated by onset age. Notably, the distal instead of global hypomethylation similar to FSHD1 were observed in three patients presenting a classic FSHD phenotype without contracted 4qA alleles.
Conclusion:
This study further confirms the 4qA D4Z4 methylation level test as a valuable complement for differential diagnosis within FSHD suspected patients as well as those who with complex structural variants. Distal methylation level show strong correlation with clinical severity and partially mediated by onset age.

## AY-5

# Identification of key gene functions impaired in dystrophinopathy by transcriptomic analysis of patient-derived iPSC-cardiomyocytes

Jeffrey Lui[1], Stephen Yin Cheng[1], Anna Hing Yee Law[1], Sheng Zhu[1], Hung Fat Tse[1,2], Godfrey Chi Fung Chan[1], Yiu Fai Cheung[1], Sophelia Hoi-Shan Chan[1]

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Background: Duchenne Muscular Dystrophy (DMD), Becker Muscular Dystrophy (BMD) and X-Linked Dilated Cardiomyopathy (XLDCM) are dystrophinopathy caused by mutations in the dystrophin gene, resulting in dystrophin protein deficiency with different clinical presentations. DMD, the most common and severe form, is characterized by early onset progressive skeletal muscle weakness and later cardiac involvement. BMD, less common than DMD, presents with milder skeletal muscle weakness but can have severe cardiomyopathy. XLDCM, the rarest form, is marked by early onset severe cardiomyopathy but no skeletal muscle weakness during symptom onset. In this study, we used patient-derived induced pluripotent stem cells (iPSCs) of all three subtypes to differentiate into cardiomyocytes (CMs) in vitro to examine the impaired disease pathways and assess transcriptomic differences among DMD, BMD and XLDCM patient-derived iPSC-cardiomyocytes.

Method: Our research team established iPSC lines from four patients with dystrophinopathies (1 DMD, 2 BMD, 1 XLDCM). We differentiated these patient-derived iPSC lines into monolayer cardiomyocytes and conducted RNA-sequencing to analyze their gene expression profiles.

Results: Through gene ontology (GO) analysis of differentially expressed genes (DEGs), we discovered that KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways of cardiomyopathies were enriched in patient-derived IPSC-CM when compared to that from the healthy control. We observed enrichment of KEGG pathways and biological processes including muscle contraction, regulation of actin cytoskeleton, cholesterol and fatty acid metabolisms, cell adhesion and cell-matrix interaction in the patient-derived iPSC-CMs. Notably, DMD patient-derived iPSC-CMs exhibited significantly more dysregulated gene expressions and enriched terms than other dystrophinopathy subtypes. XLDCM and BMD patient-derived iPSC-CMs are relatively similar when comparing their GO enrichment profile. We further identified a few genes (KLF5, ACKR3, CALD1, LOX) that have an emerging role in cardiovascular diseases dysregulated in all patient-derived iPSC-CMs.

Conclusion: Transcriptomic analysis of dystrophinopathy patient-derived iPSC-CMs identified multiple key impaired biological functions in dystrophinopathy. Furthermore, DMD patient-derived iPSC-CMs demonstrated the most severe dysregulated transcriptomic profile among all dystrophinopathy subtypes. Overall, our dystrophinopathy iPSC-CM platform and the generated transcriptome data are valuable resources for disease modelling and the discovery of disease-related genes in dystrophinopathy.

# JMS Young Investigator Award Session

10:40-12:30, Sep 14 (Sat), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Tohru Hosoyama (National Center for Geriatrics and Gerontology, Japan)
Genri Kawahara (Department of pathophysiology, Tokyo Medical University, Japan)

## JY-1

## Serglycin promotes skeletal myogenesis through EZH2 degradation in satellite cells

Katsuhiko Kunitake, Norio Motohashi, Yuki Ashida, Yoshitsugu Aoki
Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan

Elucidating the mechanisms of skeletal myogenesis is crucial for clarifying the pathophysiology of muscular disorders and facilitating muscle regeneration after injury. Enhancer of Zeste Homologue 2 (EZH2) is a catalytic subunit of Polycomb Repressive Complex 2 that regulates stem cell differentiation via transcriptional repression. In muscle satellite cells, EZH2 contributes to maintaining the chromatin of muscle genes in a repressed state, whereas its downregulation leads to the transcriptional activation of the myogenic program. However, the precise mechanism of triggering this EZH2 downregulation after muscle injury remains unclear. Here, we show that serglycin around satellite cells promotes their myogenesis through EZH2 degradation. We found CD90-positive human urine-derived cells (UDCs), which we previously reported as a cell population with a high potential for myogenic differentiation, excreted more serglycin than CD90-negative UDCs. Adding recombinant serglycin to the culture medium significantly enhanced the fusion index of *MYOD1*-converted UDCs compared to untreated ones. Moreover, our immunoblotting data showed that serglycin activated the CD44-p38-EZH2 pathway, resulting in EZH2 degradation by phosphorylation of threonine 372. As a next step, to confirm whether serglycin could activate the same pathway in mouse satellite cells and promote muscle differentiation, we intramuscularly injected serglycin into the injured muscles of wild-type mice. As a result, in serglycin-injected mice, the percentage of embryonic myosin heavy chain positive fibers evaluated by immunohistochemistry showed its peak on day 5 which was earlier than day 8 in the control group. Simultaneously, serglycin-injected mice indicated significantly high muscle torque both on day 5 and 8 compared to the control group. Taken together, serglycin can potentially enhance regeneration in injured muscles by degrading EZH2 in satellite cells. Our findings should pave the way for developing regenerative medicine approaches using serglycin to mitigate skeletal muscle damage.

## JY-2

## Macrophage HGF-denitration activity: a key to youthful muscle regeneration

Alaa Elgaabari[1,2], Junri Miyamoto[1], Takahiro Maeno[1], Kahona Zushi[1], Mako Nakamura[1], Takahiro Suzuki[1], Ryuichi Tatsumi[1]

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[2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

Our recent study showed that myogenic stem satellite cell activator HGF (hepatocyte growth factor) undergoes tyrosine nitration/dysfunction during aging (Elgaabari *et al.*, *Biochem. Biophys*. *Rep.* 2022, https://doi.org/10.1016/j.bbrep.2022.101295, Elgaabari *et al.*, *Aging Cell* 2024, https://onlinelibrary.wiley.com/doi/10.1111/acel.14117), leading to an emerging *in vitro* finding that nitrated HGF can restore its function through de-nitration activity up-regulated in muscle tissues at 1-day post light and heavy cardiotoxin (CTX)-muscle injuries (1-dpi) in young (3-month-old), but not old mice (24-month-old). Here we show that infiltrated pro-inflammatory macrophage (M1) may mediate denitrase up-regulation to manipulate injury-induced oxidative stress. Briefly, cell lysates and conditioned media from M1 cultures isolated from young mice, but not old mice, exhibit robust de-nitration activity. Notably, depletion of macrophage population by intravenous injection of clodronate liposome before injury resulted in a significant reduction in the de-nitration activity (assayed at 1-dpi). The subsequent experiments showed the bidirectional feedback between HGF nitration/denitration dynamics and macrophage multi-functions. Transwell migration assay demonstrated that injured-muscle extract of young mice stimulates monocyte infiltration while old injured-muscle extract does not possibly due to inefficient chemo-attractivity of nitrated HGF. Additionally, HGF, not nitrated HGF, activates young macrophages to promote their proliferation and migration for successful wound closure as revealed by BrdU-incorporation and wound healing assays, while old macrophages didn't show responsive sensitivity to HGF/c-met signaling and exhibited rapid cell-death. Overall, a possible mechanism centered on M1 as a major source of denitrase production/secretion may maintain HGF activity, thereby preserving the functions of satellite cells and macrophages for successful muscle regeneration, which are significantly disrupted in aging. The present work highlights the first comprehensive molecular implication of the extracellular HGF nitration/de-nitration dynamics in an age-associated muscular disorder, although a denitrase gene(s) is still unknown. This mechanism could be applied to develop pharmaceutical strategies aimed at restoring HGF function in human, pet, and animal health sciences to: combat age-related muscle atrophy with impaired regeneration (including sarcopenia and frailty), guide the extension of healthy life expectancy, and importantly in improving animal welfare and sustaining food security through meat-animal production. Finally, HGF displays pleiotropic functions for regeneration/ repair of a variety of tissues/organs. Therefore, therapeutic applications of HGF have been tested in various diseases including liver cirrhosis, chronic renal failure, lung fibrosis, myocardial infarction, arteriosclerosis obliterans, ALS (Lou Gehrig's disease), and acute spinal cord injury. Prevention and recovery of HGF nitration/dysfunction may be important perspective to improve the therapeutic effects of investigational HGF-drugs as well as their tissue-specific delivery.

## JY-3

# Generation of an FSHD1 mouse model carrying FSHD1-derived chromosome 4q35 using mouse artificial chromosome

Yosuke Hiramuki[1], Ichizo Nishino[2], Hiroyuki Kugoh[1], Yasuhiro Kazuki[1]

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[2]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan

Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disorder characterized by asymmetric muscle weakness of face, shoulder, and upper arm. DUX4 gene is conserved in primates and is located in D4Z4 macrosatellite repeats in the subtelomeric regions of chr4q35. DUX4 is expressed in testis and early embryo, while it is normally repressed in somatic tissues including skeletal muscle. The D4Z4 repeat contraction in FSHD1 causes hypomethylation of D4Z4 resulting in abnormal DUX4 and DUX4 target genes expression in skeletal muscle. However, it remains unclear how the tissue-specific DUX4 expression is genetically and epigenetically regulated, and there is no FSHD animal model carrying megabase-size DNA including telomeric regions. Here, we generated two mouse ES cells carrying a total of 5 Mb of the FSHD1-derived chr4q35 containing normal-sized D4Z4 (control allele) or FSHD1-sized D4Z4 (disease allele) on mouse artificial chromosome (chr4q35-MAC) at multiple steps including chromosome transfer, CRISPR/Cas9 genome editing, and Cre/loxp translocation. The ES cells carrying chr4q35-MAC control allele showed D4Z4 hypermethylation, while those carrying disease allele showed hypomethylation resulting in DUX4 expression. RNA-seq analysis identified differentially expressed genes including DUX4 target genes in ES cells carrying disease allele compared to ES cells without chr4q35-MAC. Using these ES cells, FSHD1 model mice carrying the chr4q35-MAC disease allele were generated. This FSHD1 mouse model could be useful not only to understand molecular mechanisms for the tissue-specific and repeat size-dependent DUX4 expression but also to develop treatments for inhibiting DUX4 and its target genes expression.

## JY-4

# The role of muscle glucocorticoid receptor signaling in accelerating obesity, glucose intolerance, and aging-related metabolic changes

Hiroki Yamazaki[1], Masaaki Uehara[2], Akiko Kuribara-Souta[3], Motohisa Yamamoto[2], Yasuaki Kabe[4], Makoto Suematsu[5], Kazuhisa Tsukamoto[1], Hirotoshi Tanaka[6]

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Metabolic crosstalk from skeletal muscle to multiple organs is important for maintaining homeostasis, and its dysregulation is thought to cause various diseases; however, it is unclear what factors influence muscle metabolism in pathological contexts and what effects they have throughout the body.

Glucocorticoids exert their effects through glucocorticoid receptors (GRs) present in various tissues. Muscle GR signaling promotes muscle proteolysis, which is physiologically important for supplying energy substrates throughout the body in response to starvation. On the other hand, chronic glucocorticoid administration often induces muscle atrophy and metabolic disorders such as diabetes and central obesity. We hypothesized that such metabolically unfavorable phenotypes originate from muscle GR signaling, interacting with GR signaling in other organs than skeletal muscle. To examine the role of muscle glucocorticoid signaling in systemic changes, we generated muscle fiber-specific GR-knockout (GRmKO) mice.

We first used GRmKO in a model wherein chronically administered corticosterone (CORT) in drinking water leads to obesity. Fat accumulation in liver and adipose tissue, muscle atrophy, hyperglycemia, and hyperinsulinemia induced by CORT treatment improved in GRmKO mice. Such CORT-induced fat accumulation was alleviated by suppressing insulin production (streptozotocin injection), indicating that hyperinsulinemia enhanced by muscle GR signaling promotes obesity. In addition, hyperinsulinemia counteracted muscle atrophy by intervening in muscle GR signaling.

We next used GRmKO in the ob/ob obesity model wherein plasma CORT levels were comparable to control mice. Glucose intolerance and obesity were improved in GRmKO mice, indicating that muscle GR signaling contributes to obesity-related metabolic changes, regardless of systemic glucocorticoid levels.

Finally, we investigated the contribution of muscle GR signaling to aging-related metabolic changes. Aged mice (104-week-old) showed higher plasma CORT levels than 26-week-old mice. The aging-related reduction of muscle mass was observed in GRmKO and control mice, but it was slightly alleviated in GRmKO. In addition, the aging-related systemic fat accumulation, hyperinsulinemia, and the elevation of inflammatory markers were significantly suppressed in GRmKO, suggesting that some aging-related metabolic changes originate from accelerated muscle GR signaling. Surprisingly, the lifetime in GRmKO was longer than control mice.

Collectively, the metabolism regulated by muscle GR signaling is closely related to the changes throughout the body, in the glucocorticoid excess model, in the ob/ob obesity model, and in aged mice. Our findings provide new insight for the treatment of obesity and diabetes, and aging-related unfavorable metabolic abnormalities, by targeting muscle GR signaling.

## JY-5

## Modeling cell type specific and sporadic DUX4 gene expression in FSHD

Mitsuru Sasaki-Honda[1,2], Hidetoshi Sakurai[1], Alvaro Rada-Iglesias[2]

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[2]IBBTEC, University of Cantabria, Spain

Facioscapulohumeral muscular dystriphy(FSHD) is a group of polygenic diseases, epigenetically characterized by abnormally hypomethylated large tandem D4Z4 repeats, allowing ectopic and toxic transcriptional leakage of early embryonic transcription factor DUX4 from the last repeat unit, in patients' muscle cells. It is already known that DUX4 gene is activated in a cell type and differentiation specific manner and sporadically with low frequency even in FSHD muscle cells. However, the upstream regulatory mechanism behind this unique expression pattern has not been well explained. First, we modeled the DUX4 expression pattern by using patient-derived iPSCs, genetic editing and a DUX4 reporter system, showing myogenic differentiation-driven sporadic DUX4 activation, which frequency was suppressed by the SMCHD1 mutation repair and increased by induction of homozygous identical mutation in FSHD2 clones. Interestingly, genetic deletion of the TAD boundary around FRG1 gene also increased DUX4 expression level and activation frequency, indicating potential regulatory element(s) beyond the boundary. We also identified some FSHD-specific ATAC-seq peaks and differentiation-specific H3K27ac peaks beyond the boundary that seem independent of DUX4 binding. These observations support the hypothesis that long-range 3D contact across the TAD boundary can contribute to DUX4 activation, which may integrate the previous separated knowledges on DUX4-centered FSHD pathology and abnormal 3D chromatin contacts to D4Z4 locus in the FSHD chromosomes and provide a new clue to develop another strategy to silence DUX4 expression in the pathological context.

## JY-6

# Establishment of Cell Transplantation Therapy Aimed at Ameliorating Ullrich Congenital Muscular Dystrophy~Exploration of Cell Sources for Transplantation~

Megumi Yokomizo (Goto)[1], Nana Takenaka[1], Kiho Clemence Yoshioka[1,2], Mayuho Miki[1,2], Hidetoshi Sakurai[1]

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Ullrich congenital muscular dystrophy (UCMD) is a progressive muscle disorder characterized by early-onset muscle weakness, muscle atrophy, and eventual respiratory failure, caused by mutations in the *COL6A1, A2*, and *A3* genes resulting in deficiency of type VI collagen (COL6). Currently, symptomatic treatments are common for UCMD, and a curative treatment has not been established. Therefore, this study focused on mesenchymal stem cells (MSCs), known to secrete various factors including COL6, with the aim of establishing a more effective treatment for UCMD.

MSCs originate from various tissues and are known to possess different properties. In this study, bone marrow-derived MSCs (BM-MSCs) and adipose tissue-derived MSCs (Ad-MSCs), both clinically applied in various diseases, and induced pluripotent stem cell-derived MSCs (iMSCs), which have shown improvement in skeletal muscle regeneration in experiments with UCMD model mice despite not being clinically applied, were used and their therapeutic effects were compared and evaluated.

BM-MSCs, Ad-MSCs, and iMSCs were transplanted into the tibialis anterior (TA) muscle of UCMD model mice, and histological analysis revealed that the BM-MSC transplantation group showed superior replenishment of COL6, while the iMSC transplantation group excelled in skeletal muscle regeneration. Additionally, significant fibrotic areas were observed in the BM-MSC transplantation group at 12 weeks after transplantation, raising safety concerns. Therefore, iMSCs were suggested to be the most suitable for UCMD treatment.

Furthermore, exploration of the differences in properties of each MSC suggested that IGF2 secreted from iMSCs plays a significant role as one of the factors in muscle differentiation as indicated by co-culture experiments with UCMD model mouse-derived muscle stem cells and each MSCs, knockdown IGF2 in iMSC experiments, and IGF2 supplementation experiments. Elucidating the role of IGF2 could provide valuable insights into the molecular mechanisms of muscle regeneration and serve as a criterion for the selection of the best clone, particularly iMSC, for the treatment of UCMD patients, and the results of this experiment are therefore a valuable contribution to the future development of UCMD treatment.

# JMS Student Award Session (Poster)

18:30-19:35, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chair: Masayuki Nakamori (Department of Neurology, Yamaguchi University, Japan)

## JSA-1

## LSMEM2, localized at the neuromuscular junction, modulatesMitochondrial integration in skeletal muscles

Eman Elrefaei[1,2,3], Satorou Yamazaki[3], Issei Yazawa[3], Yusuke Takahashi[3], Naoki Ito[4], Nozomi Hayashiji[5], Yuya Nishida[3], Ichizo Nishino[6], Takashima Seiji[1], Yasunori Shintani[3]

[1]Osaka University, Medical Biochemistry department, faculty of medicine, Japan
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In addition to the canonical metabolism-regulating function, AMP-activated protein kinase (AMPK) has non-canonical functions, in which AMPK spatiotemporally phosphorylates specific sets of substrates. Recently, we identified LSMEM2, a novel substrate of AMPK in the heart. LSMEM2 is a membrane protein localized at the intercalated disc (ICD), whose function is currently under investigation. Interestingly, LSMEM2 is also expressed in the skeletal muscles. As skeletal muscles lack a homophilic intercellular junction corresponding to the ICD in the heart, predicting the role of LSMEM2 in skeletal muscles is difficult. In this study, we identified that LSMEM2 is expressed in skeletal muscles, specifically at the neuromuscular junction (NMJ). LSMEM2-knockout mice showed no histological abnormalities, suggesting that LSMEM2 is not essential for skeletal muscle development. The overexpression of full-length wild-type or C-del mutant of LSMEM2 led to the tubular aggregate formation with functional abnormality in male mice. RNA-sequencing revealed that the genesets of mitochondrial oxidative phosphorylation and vesicle-mediated transport are enriched in LSMEM2 overexpression. Furthermore, histological analysis demonstrated the accumulation of subsarcolemmal mitochondria with abnormal features in LSMEM2-overexpressing skeletal muscles. The study findings suggest that LSMEM2 may play a role in the pathogenesis of skeletal muscle diseases. Further studies are needed to elucidate the molecular mechanisms of LSMEM2.

## JSA-2

## Satellite cells are not indispensable for repair following exercise-induced muscle damage

Nao Tokuda, Azuma Naito, Nao Yamauchi, Ayaka Niibori, Takashi Yamada
Graduate School of Health Sciences, Sapporo Medical University, Japan

Purpose: Unaccustomed exercise involving eccentric contractions leads to muscle fiber damage and prolonged force depression. Muscle satellite cells (MuSC) have been considered essential for tissue repair and functional recovery following exercise-induced muscle damage (EIMD); however, the evidence supporting this assertion is not sufficiently demonstrated. Here, we investigated the role of MuSC during recovery from EIMD using MuSC-deficient mice.
Methods: Tamoxifen was administered to 16-week-old Pax7CreER+/-/RosaDTA/DTA and Pax7+/+/RosaDTA/DTA mice to generate MuSC-deficient (MuSC-) and MuSC-non-deficient (MuSC+) mice, respectively. The left plantar flexor muscles were subjected to either 100 eccentric contractions (MuSC-/EIMD, MuSC+/EIMD groups) or 1.2% barium chloride (BC) injection (MuSC-/BC, MuSC+/BC groups). Maximum isometric torque of plantar flexors was measured using supramaximal electrical stimulation from pre-treatment to day 35 post-treatment. Muscle samples were collected at various time points post-treatment.
Results: In MuSC- mice, the number of Pax7-positive cells in the plantaris muscle decreased by approximately 90% compared to MuSC+ mice. Evans blue dye-positive fibers were markedly increased in the gastrocnemius muscle of both MuSC-/EIMD and MuSC+/EIMD groups at 3 days post-treatment. Remarkably, regardless of MuSC presence, muscle contractile function followed a similar recovery process and was almost fully restored by day 35 post-EIMD. At that moment, there were no significant differences in total muscle fiber number or mean fiber Feret's diameter between MuSC-/EIMD and MuSC+/EIMD groups. Additionally, central nucleated fibers were observed in approximately 20% of total fibers in the MuSC+/EIMD group but were rare in the MuSC-/EIMD group. Conversely, at day 35 post-BC treatment, muscle weight and torque were almost fully recovered in MuSC+/BC mice, while remaining at a very low level in MuSC-/BC mice.
Discussion: Our findings show that, unlike a conventional pharmacological injury model, EIMD can be repaired through MuSC-independent mechanisms. Recent findings by Roman et al. (Science, 2021) suggest that muscle repair after 'physiological' damage relies on myonuclear migration for cellular reconstruction. Additionally, although BC treatment results in the loss of almost all myonuclei, it has been shown that muscle fibers and myonuclei partially survive following EIMD. Therefore, it appears that surviving myonuclei exhibit repair capabilities beyond their nuclear domain following EIMD, enabling effective tissue repair even in the absence of MuSC.
Conclusion: MuSC are not essential for tissue repair processes following EIMD.

## JSA-3

# Reactive oxygen species related DNA damage induced cellular senescence in myoblasts

Daichi ARAI[1,2], Tsukasa TOMINARI[1,2], Masaru TAKATOYA[1], Urara KASUGA[1], Michiko HIRATA[1], Yoshitsugu AOKI[2], Masaki INADA[1]

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Sarcopenia is defined as an age-related loss of skeletal muscle mass and function. Sarcopenia is caused by several factors such as aging, DNA damage, γH2AX-induced ROS(reactive oxygen species) and senescence-associated secretory phenotype (SASP) production. Previous studies indicated that cellular senescence in satellite cells is implicated as a mediator of sarcopenia. In this study, we examined whether cellular senescence induced by DNA damage-produced ROS interact myogenic differentiation in C2C12. Cellular senescence was induced by treating busulfan, one of the alkylating agents that induced DNA damage in various cancer therapies. As a result, busulfan suppressed C2C12 cell growth associated with upregulating the mRNA expression of cell cycle arrest senescent genes such as Cdkn1a (p21) and Tp53 (p53) and increasing the phosphorylation of p53 and histone H2AX (γH2AX). Senescence-associated β-galactosidase (SA-β-gal)-positive cells were also increased by treating busulfan, suggesting that busulfan induces cellular senescence in C2C12. We cloned the busulfan-treated ROS-induced model of senescent C2C12 and analyzed several markers of cellular senescence. Senescent C2C12 showed a decrease in cell proliferation with increasing mRNA expression of senescent genes such as p21 and p53. Busulfan upregulated the mRNA expression of SASP, including inflammatory cytokines IL-1a and IL-6. Busulfan also increased ROS production associated with upregulating the mRNA expression of ROS-generating enzymes, Nox1 and Nox4, and downregulating that of anti-oxidative factors, Nrf2 and Catalase. Busulfan also inhibited myotube formation of C2C12 and downregulated the mRNA expression of myogenic markers genes including Myogenin, Myomaker, Myomerger and Myosin heavy chain. In conclusion, we suggested that busulfan-induced cellular senescence of C2C12 resulted in the suppression of cell proliferation and myogenic differentiation. These results indicated that the establishment of a cellular senescent model of myoblast that is presenting DNA damage-mediated ROS production induced senescence (p21, p53) and SASP (IL-1a, IL-6) genes expression and senescent phenotype.

Chair: Motoi Kanagawa (Department of Cell Biology and Molecular Medicine, Ehime University, Japan)

## JSA-4

## Overexpression of PGC-1α prevents eccentric contraction-induced muscle damage in a utrophin-independent manner

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[OBJECTIVE] It is generally accepted that slow-twitch oxidative (type I) and fast-twitch oxidative (type IIa) muscle fibers are more resistant to eccentric contraction (ECC)-induced injury than fast-twitch glycolytic (type IIb/IIx) muscle fibers. Here, we investigated the mechanism underlying fiber-type specific differences in susceptibility to ECC with focusing on the role of peroxisome proliferators-activated receptor-γ co-activator (PGC) -1α, a master regulator of oxidative phenotype. Furthermore, we tested whether utrophin (UTRN) is involved in enhancing the damage resistance of fast-twitch glycolytic muscle fibers through PGC-1α overexpression.

[METHODS] C57BL/6 wild-type (WT), skeletal muscle-specific PGC-1α overexpression (PGC-1α Tg), UTRN Knockout (UTRN KO), and PGC-1α Tg/UTRN KO mice were exposed to 100 repeated damaging ECCs in vivo. The plantar flexor muscles were stimulated supramaximally via a pair of surface electrodes every 4 s under isoflurane anesthesia. ECCs comprised forced dorsiflexion from 0° to 40° at 150°/s combined with electrical stimulation (45 V, 0.5 ms monophasic rectangular pulse, 50-Hz stimulation frequency). Maximum isometric plantar flexion torque (MIT) was measured before, immediately (REC0), 1 day (REC1), and 3 days (REC3) after damaging ECCs. The gastrocnemius muscles were excised for histological and biochemical analyses.

[RESULTS] The proportion of myosin heavy chain IIb in the gastrocnemius muscle was ~90% in WT and UTRN KO mice and ~70% in PGC-1α Tg and PGC-1α Tg/UTRN KO mice. In contrast, myosin heavy chain I were almost absent (< 3%) in all groups. In both the WT+ECC and UTRN KO+ECC groups, MIT was decreased at REC0 and remained depressed at REC3 compared with the preinjury values. Evans blue dye (EBD) signal in fast-twitch glycolytic fiber was observed at REC3 accompanied by the increased autolysis of calpain 1. Conversely, in both the PGC-1α Tg+ECC and PGC-1α Tg/UTRN KO+ECC groups, the decrease in MIT was partially prevented at REC0 and was almost completely recovered at REC1. At REC3, there was little EBD signal in fast-twitch glycolytic fibers with no changes in autolysis of calpain 1.

[DISCUSSION] We demonstrated that overexpression of PGC-1α prevents membrane damage in fast-twitch glycolytic fibers and improves force recovery following ECCs. Previous studies have shown that PGC-1α drives the expression of UTRN and efficiently rescues the dystrophin-deficient muscle and has thus generally been assumed to explain the beneficial effects of PGC-1α. However, in the present study, PGC-1α overexpression prevented ECC-induced membrane damage and prolonged force depression even in UTRN KO mice. This suggests that a pathway independent of UTRN expression plays an important role in PGC-1α-mediated enhancement of damage resistance in fast-twitch glycolytic fibers.

[CONCLUSION] The PGC-1α pathway is crucial for conferring resistance to damage resulting from ECCs in fast-twitch glycolytic muscle fibers, irrespective of UTRN involvement.

## JSA-5

# Isometric interval training with high- but not low-intensity contractions improves fatigue resistance in dystrophin deficient muscle

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[OBJECTIVE] In normal mouse skeletal muscles, interval training-mimicking neuromuscular electrical stimulation in the form of isometric contractions (ISO) induces improvements in aerobic capacity and muscle endurance, with greater gains with high-intensity (Hi-ISO) than low-intensity contractions (Lo-ISO). In this study, we investigated the role of contraction intensity during ISO interval training in the endurance adaptation of mdx52 mice, an animal model for Duchenne muscular dystrophy (DMD).

[METHODS] Male mdx52 mice (4- to 7-week-old) were divided into high-intensity ISO (100 Hz stimulation, Hi-ISO) and low-intensity ISO (20 Hz stimulation, Lo-ISO) groups. Plantar flexor muscles were stimulated with in vivo supramaximal electrical stimulation every other day for 4 weeks (a total of 15 sessions).

[RESULTS] In non-trained muscles of mdx52 mice, decreased muscle endurance was accompanied by reduced citrate synthase activity, expression of PGC-1α, and mitochondrial respiratory chain complex II, and an increase in the percentage of Evans Blue dye-positive area. Hi-ISO, but not Lo-ISO, markedly improved fatigue resistance and restored all these alterations in mdx52 mice. Furthermore, an acute session of Hi-ISO, but not Lo-ISO, led to increased phosphorylation of p38 MAPK and mRNA levels of PGC-1α, which were prevented by the p38 MAPK inhibitor SB203580. In contrast, phosphorylation of AMPK was increased in both the Hi-ISO and Lo-ISO groups.

[DISCUSSION] We previously demonstrated that the same Lo-ISO protocol conducted with normal mice results in increased mitochondrial content and fatigue resistance accompanied by upregulation of PGC-1α. However, in this study, neither of these improvements was observed following Lo-ISO in mdx52 mice. The reason for this discrepancy is unclear, but it may be attributed to differences in molecular responses to exercise between normal and dystrophic muscle. In this regard, mechanistic experiments with an acute session of ISO revealed that p38 MAPK, a regulatory factor of PGC-1α responsive to mechanical stress, was activated by Hi-ISO but not by Lo-ISO in mdx mice. Moreover, inhibition of p38 MAPK prevented the increase in PGC-1α mRNA expression induced by Hi-ISO in mdx52 muscles. Thus, these findings suggest the p38 MAPK serves as an important upstream factor for the ISO-induced increase in PGC-1α and, hence, mitochondrial content in mdx52 mice.

[CONCLUTION] Contraction intensity plays a crucial role in ISO interval training-induced improvement of endurance in dystrophin-deficient muscles, which is lined to mitochondrial biogenesis mediated by contraction intensity-dependent activation of the p38 MAPK/PGC-1α pathway.

## JSA-6

## Next-generation cell and gene therapy

Edvinas Cerniauskas, Uikyu Bang, Eman Taha, Akitsu Hotta, Makoto Ikeya, Hidetoshi Sakurai
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Cell supplementation and gene editing are two very potent therapeutic strategies for monogenic diseases. However, neither is without their intrinsic flaws. Cell transplantation is complex and impermanent, while gene editing is often difficult to administer. A new hybrid strategy overcomes these limitations by manufacturing and delivering concentrated gene editing machinery in situ together with all the transient benefits of cell transplantation.

NanoMEDIC extracellular nanovesicle platform is a novel and very promising macromolecule delivery system which can selectively package the desired cargo for transfer to other cells in an exosome-like viral entry protein assisted strategy. Previously, large amounts of purified vesicles were needed when administering them alone. This treatment risks provoking the immune response as well as the loss of vesicles in the periphery. Vesicle production and secretion in a local microenvironment solves these issues.

Human Leukocyte Antigen knockout iPSC-derived mesenchymal stem cells will be used as NanoMEDIC producer cells. A Duchenne muscular dystrophy mouse model will be the first model to be tested to validate the approach. Upon commencement of this hybrid therapy, exon skipping is induced to restore dystrophin levels. A rapid tissue healing facilitated by mesenchymal stem cell paracrine effects is expected to follow.

## JSA-7

# Inactivation of Aconitase2 under simulated microgravity and analysis of skeletal muscle-specific Aco2-deficient mice

Minori Suzuki[1], Erika Yamano[1], Kota Kishida[1], Miho Takata[1], Kosuke Sugiura[1,2], Yuta Yanagihara[3], Yuuki Imai[3], Kaori Tanaka[4], Iori Sakakibara[5], Madoka Uezumi[6], Akiyoshi Uezumi[6], Anayt Ulla[1], Takayuki Uchida[1], Takeshi Nikawa[1]

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Disuse muscle atrophy and osteoporosis are induced in unloaded environments such as bed-ridden and spaceflight. Our laboratory has conducted space experiments to elucidate the mechanisms of disuse muscle atrophy. As a result, accumulation of cis-aconitic acid, an intermediate product of the TCA cycle, was confirmed, suggesting inactivation of its catalytic enzyme, Aconitase 2 (ACO2). A reduction in ACO2 activity in skeletal muscle was also confirmed in an experimental mouse model of disuse muscle atrophy. However, the relationship between ACO2 inactivation and disuse muscle atrophy is still unclear. To investigate whether ACO2 dysfunction could be the cause of disuse muscle atrophy, mice lacking ACO2 in a skeletal muscle-specific and time-specific manner ( ACO2 cKO mice) were generated and their phenotype was examined. The results showed no significant differences in body weight or muscle strength, but a significant reduction in muscle weight, reduced muscle cross-sectional area and signs of damage and regeneration processes in fast-twitch muscle fibres were observed. However, the RT-PCR results showed a decrease in myodegradation markers and an increase in muscle synthesis markers, and the RNA-seq results showed increased expression of inflammatory factors in particular, suggesting that the activation of inflammatory pathways may have caused muscle atrophy. Furthermore, despite the skeletal muscle-specific defects, the bones were found to be brittle. Bone densitometry showed that Aco2 cKO mice had significantly reduced bone density in the proximal tibial region, and micro-CT analysis further confirmed a significant reduction in bone density in both trabecular and cortical bone. Bone morphometry showed no significant differences, but a trend towards a reduced rate of calcification and bone formation on the bone surface was observed. These results suggest that there is some organ-linkage between skeletal muscle and bone. Therefore, we focused on one endocrine factor that was markedly up-regulated in RNA-seq of skeletal muscle from Aco2 cKO mice. Interestingly, this endocrine factor showed a trend towards increased expression in skeletal muscle of wild-type mice subjected to tail suspension, but decreased expression in cultured myotube Sol8 cells due to simulated microgravity. This endocrine factor was suggested to be secreted from inflammatory or stromal cells infiltrating the skeletal muscle, rather than from the myocytes themselves. As described above, it was suggested that this endocrine factor may be an important factor in microgravity-induced muscle atrophy and osteoporosis, and the mice are currently being analysed.

Chair: Akiyoshi Uezumi (Division of cell heterogeneity of Medical Institute of Bioregulation, Kyushu University, Japan)

## JSA-8

# Elucidation of the mechanism by which training improves the engraftment efficiency of transplanted cells in Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a disease that causes muscle wasting and weakness due to a deficiency of the dystrophin protein (Dys). In our laboratory, we endeavored to develop a cell transplantation therapy in which skeletal muscle progenitor cells with normal Dys expression are transplanted into DMD muscle tissue with the objective of restoring Dys expression. Previous studies indicate that the pathological condition is ameliorated with an increase in Dys-positive fibers. We have previously shown that isometric muscle contraction training (Tr) performed in DMD mice prior to cell transplantation significantly improved the engraftment efficiency of transplanted cells and significantly increased the number of Dys-positive fibers. Therefore, the objective of this study is to elucidate the mechanisms that enhance the engraftment efficiency of transplanted cells when Tr is loaded in DMD mice. Specifically, we performed two analyses to narrow down candidate molecules involved in the mechanisms: 1) single omics analysis using phosphoproteomics analysis, and 2) double omics analysis integrating proteomics and phosphoproteomics analysis.

Proteins were extracted from gastrocnemius muscles of three groups of 5-weeks-old sedentary DMD mice (DMD+Intact), WT mice (WT+Intact), and DMD mice and WT mice 24 hours after Tr loading (DMD+Tr) (WT+Tr) (n=5 per group).

1) Phosphoproteomic analysis was performed on the above samples and a simple comparison was made between DMD+Intact/WT+Intact and DMD+Tr/DMD+Intact. We identified Factor-X as the only transcription factor among the molecules that showed significant changes in DMD under Tr loading. We also found phosphorylation of Factor-X was suppressed in DMD+Tr, as it has reported that phosphorylation of Factor-X suppresses skeletal muscle fusion, we considered that the suppression of Factor-X phosphorylation by Tr loading on DMD is likely to be involved in promoting cell engraftment efficiency.

2) Enrichment analysis was performed on the proteomic data for four groups. The results showed a population of proteins involved in cell adhesion was significantly more abundant in DMD than in WT, except for the Intact group, only when Tr loading was added. Network analysis of this population identified Factor-Y and Factor-Z as having high betweenness centrality. Finally, integration of these results with phosphoproteomic data narrowed down Cascade-X, suggesting that "Tr loading of DMD muscle may contribute to improved cell engraftment of transplanted cells by enhancing cell adhesion in the host muscle.

We are currently validating the above narrowed down molecules in vitro and hope to elucidate the responsible molecules and their upstream and downstream signaling cascades in the future.

## JSA-9

## BMP signaling controls skeletal muscle cell maturation

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[Background]

Skeletal muscle is the largest organ in our body, accounting for 40% of body mass in healthy-weight individuals and is responsible for locomotion activity, whole-body metabolism, and energy homeostasis. Therefore, its loss affects to mortality in various pathogenic conditions. Although much knowledge about skeletal muscle has been made by cultured skeletal muscle cells such as C2C12 and L6 cells, these cultured cells are relatively immature compared to skeletal muscle cells in vivo because they do not acquire a sarcomere, the smallest unit for muscle contraction.

The mechanisms of skeletal muscle cell differentiation have been well characterized, yet the skeletal muscle cell maturation process, including the formation of sarcomere, remains largely unknown. Although it is known that signaling through TGF-β superfamily proteins such as TGF-β, Myostatin (GDF-8) and BMP, regulate myocyte differentiation, their involvement in the maturation of muscle cells is not fully understood. In this work, we investigated changes in TGF-β and BMP signals during the myogenic differentiation in three different models.

[Method]

We utilized C2C12 cells, primary muscle satellite cells isolated from mouse gastrocnemius muscle (MuSCs), and human iPS cell-derived myocytes (hiPSC-myocytes) to study the involvement of TGF-β superfamily signaling in skeletal muscle cell maturation. TGF-β superfamily signaling was detected by western blotting and RT-qPCR. The sarcomere formation was visualized by immunofluorescence staining and transmission electron microscopy analysis. The contractile ability of myocytes was evaluated by electrical pulse stimulation.

[Results]

In MuSCs derived myotubes, the most mature skeletal muscle cell, BMP signaling was markedly reduced after differentiation, however, in immature skeletal muscle cells such as C2C12 myotubes and hiPSC-myocytes, BMP signaling remained active. In addition, gene expressions of BMP receptors were higher in immature myocytes. Furthermore, the blockade of BMP signaling by an inhibitor of BMP receptors in post-differentiated myocytes induced sarcomere formation and improved contractile activity.

[Conclusion]

This study suggests that BMP signaling regulates skeletal muscle cell maturation, particularly sarcomere formation. We also found that inhibition of BMP signaling by a small molecule can promote the maturation of skeletal muscle cells.

## JSA-10

## A mechanistic analysis of epigenetic disruption in Facioscapulohumeral muscular dystrophy

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Department of Clinical application, Center for iPSCs Research and Application (CiRA), Kyoto University, Japan

Facioscapulohumeral muscular dystrophy (FSHD) is a genetic muscle disease that primarily affects the muscles of the upper body. It is inherited in an autosomal dominant manner, and the cause is disruption of epigenetic regulation in the D4Z4 repeat region of chromosome 4. There are mainly two types of FSHD: FSHD1 shows shortened repeat regions (<10), and FSHD2 does not show shortened repeat regions but is known to have mutations in SMCHD1 or DNMT3B. Both types show DNA hypomethylation in the D4Z4 region and the relaxing chromatin in this region ectopically induce DUX4 expression only in muscle cells. The molecular mechanism of disruption of epigenetic regulation in FSHD is still not understood.

Therefore, we focused on pre-implantation embryos, which are generally in a state of global DNA hypomethylation. Analysis of published data of human early embryogenesis confirmed that DNA methylation is introduced from pre- to post-implantation in the D4Z4 region.

The aim of this study is establishing early embryogenesis model by human iPSC for elucidating DNA methylation mechanism in D4Z4 region. Conventional human iPS cells are classified as primed pluripotency, with properties comparable to post-implantation embryos. On the other hand, since naïve iPS cells correspond to pre-implantation embryos, we considered them to be suitable as a model for DNA hypomethylation.

We generated naïve iPS cells from three types of prime iPS cells: healthy cells, FSHD2-derived (SMCHD1 mutant) cells, and gene repair cells, and confirmed DNA hypomethylation in the D4Z4 region. Furthermore, we also confirmed the expression of DUX4 due to DNA hypomethylation in the D4Z4 region. Next, we performed repriming to convert naive iPS cells to primed iPS cells. As a result, DNA methylation in the D4Z4 region was confirmed in healthy and gene-repaired cells, but not in FSHD 2-derived cells. Interestingly, repriming suppressed DUX4 expression in all cells, including FSHD2-derived cells.

To elucidate the molecular mechanism of DNA methylation in the D4Z4 region that occurs during the repriming process, we will conduct a D4Z4 region-specific proteomics analysis. We will analyze proteins that specifically accumulate in the D4Z4 region using a method called engineered DNA-binding molecule-mediated chromatin immunoprecipitation (enChIP). This analysis reveals why DNA methylation is not introduced during early development in FSHD-derived iPS cells.

Chair: So-ichiro Fukada (Laboratory of Stem Cell Regeneration and Adaptation Graduate School of Pharmaceutical Sciences, Osaka University, Japan)

## JSA-11

## RNA-based CRISPRoff silencing to target DUX4 in Facioscapulohumeral muscular dystrophy

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Facioscapulohumeral muscular dystrophy (FSHD) is a progressive skeletal muscle disorder caused by abnormal DUX4 expression, due to DNA hypomethylation of the D4Z4 repeats. Sustainable DUX4 suppression is supposed to be a promising therapeutic strategy to prevent disease progression. Previously we proved that CRISPR-based epigenetic editing strategy achieved sustainable DUX4 silencing in FSHD1 and FSHD2 patient-derived iPSCs models by co-expression of dCas9-effectors (KRAB, DNA methyltransferase enzymatic domains) with transient sgRNA delivery. When considering clinical application, it is a hurdle to package multiple dCas9-effectors and transiently co-deliver them into cell. Here, we utilized the published fusion protein CRISPRoff, which enables inheritable epigenetic silencing by transient transfection. CRISPRoff and sgRNA plasmids were transiently transfected into iPSCs followed by cell proliferation and myogenic differentiation. As expected, DUX4 were repressed in an administration frequency-dependent manner, tolerant to cell division and differentiation. DNA methylation was upregulated after administration. These data demonstrate that transiently repeated administrations of CRISPRoff/sgRNA ameliorate D4Z4 epigenetic dysregulation and robustly suppress DUX4. To further investigate the possibility of clinical application, we applied RNA-based CRISPRoff system in in vitro differentiated myocytes. Significant DUX4 suppression was achieved by RNA-based treatment, indicating successful induction of epigenetic editing in myocytes. To conclude, this study demonstrated the utility of RNA-based epigenetic editing for DUX4 suppression in FSHD myocyte.

## JSA-12

# Reduced expression of Dok-7 and agrin due to mechanical unloading induces acetylcholine receptor degeneration in type 1 myofibers in mice

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INTRODUCTION: Aging and amyotrophic lateral sclerosis lead to the neuromuscular junction (NMJ) degeneration and myofiber atrophy preferentially in fast-type motor units. On the other hand, mechanical unloading such as tail suspension causes preferential atrophy of slow-twitch myofibers, but it is not clear whether it involves a similar type-specific NMJ degeneration. In this study, we aimed to elucidate the effects of tail suspension on NMJ for each myofiber type.
METHODS: Male C57BL/6J mice (12-13 weeks old) were divided into control (CON), tail suspension (TS), and cast immobilization with suspension (IMS) groups. TS mice were suspended by their tail to reduce mechanical load on hindlimb muscles. IMS mice were suspended with their hindlimbs immobilized in casts, with the ankle joint angle secured at 90˚ to moderately stretch the lower hindlimb muscles. After 20 days of intervention, muscle wet weight, myofiber size, NMJ morphology, and the expression of proteins and mRNAs related to NMJ formation were analyzed.
RESULTS: The wet weight of the gastrocnemius muscle was significantly lower in IMS than in TS, whereas that of the soleus was significantly lower in TS than in IMS. In the plantaris muscle, type 2a fibers were significantly smaller in TS than in IMS, but type 2b fibers were significantly smaller in IMS than in TS. In the soleus muscle, type 1 and 2a fibers were significantly smaller in TS than in IMS, but type 2x and 2b fibers were not significantly different between the groups. The fluorescent intensity of acetylcholine receptors (AChRs) in the soleus muscle was significantly lower in TS than in CON and IMS, and within TS, it was significantly lower in type 1 than in type 2 fibers. The protein expression of Dok-7, which promotes MuSK (muscle-specific kinase) phosphorylation and induces AChR clustering, was significantly higher in IMS than in CON and TS in the plantaris, and was significantly lower in the TS than in CON and IMS in the soleus. The mRNA expression of agrin, which also leads to MuSK phosphorylation, was not significantly different between the groups in the plantaris but was significantly lower in TS than in IMS in the soleus.
CONCLUSION: Our results suggest that tail suspension reduces Dok-7 and agrin expression and causes postsynaptic AChR degeneration especially in type 1 myofibers. We also found that these changes can be mitigated by passively stretching the muscles, suggesting that AChR degeneration is induced by mechanical unloading rather than a decrease in neuromuscular activity.

## JSA-13

# Elucidating the role of Oncostatin M signaling in myoblast proliferation and muscle physiology in wild-type and SOD1G93A mice

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease characterized by progressive degeneration of motor neurons, leading to severe muscle atrophy. Mutations in the superoxide dismutase 1 gene (SOD1) are the second most frequent cause of familial ALS. Currently, no cure exists for the disease, and ongoing research is focused on identifying disease mechanisms and finding drugs. Recently, we have found that the serum derived from ALS model SOD1G93A-mice significantly inhibited cell proliferation and myotube growth compared to wild-type serum. Additionally, we performed RNA-Seq analysis using wild-type primary myoblasts treated with the serum from SOD1G93A mice and identified several up-regulated receptors in SOD1G93A serum-treated myoblasts. After conducting multiple experiments, we could identify a specific ligand, Oncostatin M (OSM), which regulates myoblast proliferation and myotube growth. Serum OSM levels were elevated in SOD1G93A mice compared to wild-type mice after 100 days of age, and OSM treatment could suppress myoblast proliferation and myotube growth. To further analyze the effects of OSM in vivo, we introduced OSM to wild-type mice and examined its effects on muscle and overall physiology.

In our study, we administered OSM intraperitoneally to four groups of 5-6-week-old C57BL/6J mice for 4 weeks with different concentrations. After OSM administration, we measured the muscle mass, myofiber diameter, and muscle performance, including absolute muscle torque and grip strength. The absolute torque test showed increased muscle force production in the OSM-treated mice, which was supported by increased muscle weights of the quadriceps and gastrocnemius muscles. Muscle diameter also displayed an increase. Although OSM induced muscle hypertrophy, it up-regulated the genes associated with muscle atrophy, including Atrogin-1. This contradiction prompts further investigation into the OSM-induced signaling pathways and their influence on muscle hypertrophy and atrophy. We will also investigate the impact of blocking OSM signaling on muscle atrophy and disease progression in SOD1G93A-mice.

## JSA-14

# Development of in vitro neuromuscular junction model through direct reprogramming of human urine-derived cells from Amyotrophic lateral sclerosis patients

Kazuki Okoshi, Katsuhiko Kunitake, Norio Motohashi, Yoshitsugu Aoki
Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan

[Introduction]
Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons, leading to muscle atrophy, paralysis and eventually death, typically from respiratory failure, within 2–5 years of diagnosis. The loss of neuromuscular junctions (NMJ) is observed as one of the earliest pathological events in both familial and sporadic forms of ALS, a phenomenon known as the dying-back theory. It is essential to develop animal or cultured cell models, including iPS cells, for studying the unclear mechanisms of pathological NMJ in ALS. Compared to NMJ from ALS patient-derived iPS cells, NMJ reprogrammed directly from patient somatic cells are expected to better recapitulate the pathophysiology of late-onset ALS due to the retention of epigenetic ageing hallmarks. However, no NMJ model has yet been reported in which both motor neurons and myotubes are directly reprogrammed by somatic cells collected from the same donors. To date, we have reported the efficient protocol for directly reprogramming human urine-derived cells (UDCs), acquired noninvasively, to myotubes. Here, we aimed to establish human NMJ models by co-culturing myotubes and motor neurons derived from UDCs.
[Methods&Results]
We introduced the MYOD1 gene to UDCs using a retroviral vector. These cells were then differentiated into myotubes via the activation of the Wnt signaling pathway by GSK-inhibitor. At day 14 after differentiation, we observed an elevation in the mRNA expression level of the late muscle regulatory factor MYHC and the skeletal muscle-specific sodium ion channel Nav1.4. Simultaneously, we produced motor neuron spheroids by introducing multiple neuron-specific transcription factors to UDCs cultured in non-adherent V-bottom plates. Neurite outgrowth was observed from day 5 after gene introduction, and a neural network was formed by day 7. At day 14, the mRNA and protein expression of motor neuron markers including HB9 and ChAT were positive in the cells. We then attempted to generate UDC-derived NMJ models by co-culturing both types of cells in a single plate. At day 7 after initiation of the co-culture, we confirmed acetylcholine receptor clusters by immunocytochemistry and spontaneous muscle contractions by video motion analysis. In NMJ generated from ALS patient-derived UDCs, cytoplasmic TDP43 aggregates in motor neurons known as a disease-specific phenotype were detected at day 14.
[Conclusion]
We successfully established a human ALS-NMJ model from the same donor-derived myotubes and motor neurons. The new NMJ modelholds great potential for elucidating the key mechanism of the dying-back theory in ALS, a significant step forward in our understanding of this devastating disease.

# Oral Session 1

10:50-12:20 , Sep 13 (Fri), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Nobuyuki Eura (Department of Neurology, Nara Medical University, Japan)
Mariko Taniguchi-Ikeda (Department of Clinical Genetics, Fujita Health University Hospital, Aichi, Japan)

## O-1

## Regulation of skeletal muscle stem cell bioenergetics by a $Mg^{2+}$-permeable ion channel TRPM7

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Skeletal muscles maintain homeostasis through their inherent high regenerative capacity of myofibers. Muscle resident stem cells, also known as muscle satellite cells (MuSCs), are important for skeletal muscle regeneration. Following injury, MuSCs are activated, exiting quiescence and entering the cell cycle to proliferate and differentiate into myoblasts, which then fuse to form regenerating myofibers. As MuSCs respond rapidly to muscle injury for muscle regeneration, the primary trigger for these events is thought to be the influx of divalent cations, such as $Ca^{2+}$ and $Mg^{2+}$; however, the molecular entity that transduces these ions for activating MuSCs remains unclear. In this study, we identified transient receptor potential melastatin 7 (TRPM7), a $Mg^{2+}$-permeable mechanosensitive ion channel, to be a key regulator of MuSC function. Using fluorescent $Mg^{2+}$-specific indicators, we observed an increase in $Mg^{2+}$ concentration in MuSCs from regenerating muscles in a TRPM7-dependent manner. MuSC-specific deletion of *Trpm7* resulted in a loss of the early responses of MuSCs, such as retraction of quiescent projections and AP-1 gene induction, and abolished the regeneration capacity after muscle injury. In addition, *Trpm7*-deficient MuSCs exhibited a decreased number of MuSCs, an attenuated ability to enter the cell cycle, and defects in mitochondria during muscle regeneration. Mechanistically, *Trpm7* deficiency attenuates the protein kinase B/mammalian target of rapamycin (AKT-mTOR) signaling pathway during the regeneration process; supplementation of excessive $MgCl_2$ in the culture medium inhibited this attenuation. Further, our results indicated that TRPM7 alerts MuSCs following distal injury via promoting MuSC transition from the $G_0$ to $G_{Alert}$ phase. Together, the results indicate that $Mg^{2+}$ influx via TRPM7 acts as a molecular determinant for MuSC activation, which governs skeletal muscle regeneration.

## O-2

# Prostaglandin J inhibited cellular proliferation and differentiation in myoblast via reprograming into adipocyte

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Prostaglandin D2 (PGD2) contributes to muscular inflammation and atrophy in Duchenne muscular dystrophy. Previous studies indicated that the PGD2 metabolite such as PGJ2 is involved in adipogenic activation in mesenchymal cell lineages; however, the role of PGD2 metabolites PGJs (PGJ2, Δ12-PGJ2 and 15d-PGJ2) in myogenic differentiation is still unknown. In this research, we investigated the effects of PGJs on cellular proliferation and myogenesis using C2C12 myoblast. C2C12 cultured in growth and/or differentiation media containing PGJs to examine cell proliferation, cell cycle and myotube formation. We analyzed the mRNA and protein expression by qPCR and western blotting analysis. In cell proliferation analysis, PGJ2, Δ12-PGJ2 and 15d-PGJ2 suppressed C2C12 proliferation. The mRNA expression of myogenic genes such as MyoD, Myogenin, and Myh1 was decreased, whereas that of adipogenic genes such as Pparg, Cebpa and Adiponectin, was increased. 15d-PGJ2 exhibited the most myogenic inhibitory effect compared with PGD2, PGJ2 and Δ12-PGJ2. In apoptosis assay, 15d-PGJ2 induced cleavage form of caspase-3 in myotube of C2C12. These data indicated that PGD2 negatively regulates the myogenesis via increasing the concentration of 15d-PGJ2, PGJ2 and Δ12-PGJ2. C2C12 showed changes in the differentiation pathway of cells by PGJs, including suppressed expression of myogenic transcription factors such as MyoD and Myogenin as well as Myosin heavy chain. Furthermore, treatment of PGJs promoted the production of cleaved caspase-3, an activated form involved in apoptosis, thereby inhibiting myogenic differentiation. Since an increase in marker expression for adipocyte differentiation was observed with PGJs treatment, it is suggested that PGJs may inhibit muscular proliferation and differentiation through regression and reprograming changes to adipocyte differentiation in myoblast.

## O-3

## 3-(4-Hydroxy-3-methoxyphenyl) propionic acid mitigates dexamethasone-induced muscle atrophy by attenuating Atrogin-1 and MuRF-1 expression

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**Objective:** Muscle atrophy is a progressive deterioration of skeletal muscles' mass and function. Denervation, disuse, aging, microgravity, glucocorticoid drugs etc. are the prominent causes of this disease. Currently, it's a growing concern for aged population and severely affect the quality of life. Polyphenols and their metabolites have been reported to exert positive effects on muscle health. Therefore, this study investigated the effect of a potent antioxidant polyphenol, ferulic acid's metabolite 3- (4-hydroxy-3-methoxyphenyl) propionic acid (HMPA), on attenuating muscle atrophy induced by dexamethasone (Dex). Dex is a glucocorticoid drug used in inflammatory diseases, however its higher or prolong use induces muscle atrophy by retarding muscle protein synthesis and encouraging muscle protein breakdown.

**Material and Method:** The effects of HMPA on Dex-induced muscle atrophy was assessed in mouse C2C12 skeletal myotubes and C56BL/6J female mice. Myotube diameter, muscle weight and myosin heavy chain (MyHC) protein content (fast and slow types of MyHC) were measured. The mRNA expression of ubiquitin ligases Atrogin-1, MuRF-1, and their upstream transcription factor, KLF15 (involved in muscle protein breakdown) were quantified by real-time PCR. Total Foxo3a (T-FoxO3a) and phosphorylated FoxO3a (P-FoxO3a), the upstream molecule of ubiquitin ligases, were quantified by western blotting. Myotubes were further treated with Dex and or HMPA in FoxO3a and KLF15 knockdown myotubes to reveal the possible mechanism of action of HMPA. Data were analyzed by one-way analysis of variance (ANOVA) method.

**Results:** HMPA significantly prevented Dex-induced reduction of myotube diameter, gastrocnemius (GA) and tibialis anterior (TA) muscle weight, and fast- type MyHC contents in cells and animals. Moreover, increased mRNA expressions of ubiquitin ligases Atrogin-1 and MuRF-1 by Dex were significantly reversed by HMPA treatment along with their upstream molecule KLF15. Furthermore, Dex-mediated T-FoxO3a and P-FoxO3a protein expression were attenuated by HMPA. Interestingly, the beneficial effects of HMPA were abolished in KLF15 and FoxO3a knockdown cells, which indicates that HMPA regulates its beneficial effects via KLF15-FoxO3a axis.

**Conclusion:** The obtained results from this study suggest that the metabolite of ferulic acid, HMPA, has favorable ameliorating effects on Dex-induced muscle atrophy and can contribute into the development of functional foods for muscle health.

## O-4

## Single-Cell RNA Sequencing Analysis Unrevealed Enhanced Platelet Activation and Proinflammatory Response in anti-AChR positive Myasthenia Gravis

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Background: Myasthenia gravis (MG), an autoimmune disorder, is characterized by autoantibodies targeting the neuromuscular junction, indicative of dysregulated immune responses potentially arising from innate immunity activation.
Methods: We performed single-cell RNA sequencing (scRNA-seq) on peripheral blood mononuclear cells (PBMCs) samples from healthy controls (HC) and anti-AChR positive MG patients before and after immunotherapy, to investigate the transcriptome signature of platelets and their interaction with the immune system. Platelet activation was assessed by surface expression of P-selectin (CD62P) and soluble CD40L (sCD40L) release, alongside morphology examination via transmission electron microscopy. Flow cytometry was utilized to analyze platelet aggregation with leukocytes. The impact of platelets on naïve CD4+ T cell responses was investigated through in vitro co-cultures and immunofluorescence techniques.
Results: Anti-AChR positive MG patients exhibited an increased count and activation of platelets. This was corroborated by the transcriptional profile, morphological alterations, augmented CD62P surface expression, and elevated plasma levels of sCD40L, which were notably diminished upon reaching minimal clinical status (MMS) following immunomodulatory therapy. In addition, these activated platelets demonstrated increased interactions with leukocytes, primarily forming aggregates with neutrophils, positively associated with MG severity and subsided following treatment. In vitro-cultures revealed that MG platelets favored CD4+ T cell proliferation, Th1 cell responses and suppressed regulatory T (Treg) cell activity. Further intervention with RANTES (CCL5) neutralizing antibodies offers a counterbalance to these proinflammatory alterations.
Conclusions: Our study uncovered increased platelet activation in anti-AChR positive MG patients, manifested with a propensity for enhanced adhesion to leukocytes and promotion of a proinflammatory CD4+ T cell phenotype.
Keywords: Myasthenia gravis, platelets activation, platelet-leukocyte aggregation

## O-5

## Factors Affecting Disease Severity in Adults with Myasthenia Gravis

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**Background** Myasthenia gravis (MG) is a rare neuromuscular antibody-mediated autoimmune disease with complex etiology and affected by multiple factors. However, previous studies primarily focused on the influence of demographic and clinical characteristics rather than environmental factors. The correlation between these factors and MG severity has not been systematically investigated.
**Objective** To explore the association of demographic, clinical and environmental factors with disease severity in adult patients with MG.
**Methods** In the cross-sectional multicenter study, adult patients registered in the national clinical MG registry from February 2017 to December 2021 were reviewed. We collected data on demographic, clinical and environmental factors, including latitude, socioeconomic status and lifestyle. MG activities of daily living (MG-ADL) scores and quantitative MG (QMG) scores at baseline were gathered to evaluate the symptom severity. The score of MG-ADL 6 and QMG 11 were cut-off points used and patients were classified into mild and moderate to severe disease categories. Logistic models were used to examine the relationship between variables and disease activity.
**Results** A total of 2469 patients were included. The median MG-ADL score was 3.0 (interquartile range [IQR] 1.0-6.0) and QMG score 6.0 (IQR 3.0-9.0). Multivariate analysis revealed that higher MG-ADL scores were associated with underweight, generalized symptoms at onset, thymoma and high latitude (odds ratio [OR] 1.77, 1.78, 1.60 and 1.82; P =0.004, <0.001, =0.001 and 0.001), and inversely correlated with overweight or obesity, thymectomy and high monthly household income (OR 0.73, 0.62 and 0.76; P =0.004, 0.002 and 0.045). Higher QMG scores were associated with female sex, generalized symptoms at onset, thymoma and high latitude (OR 1.74, 2.47, 1.92 and 1.52; P <0.001, <0.001, <0.001, =0.039), and inversely correlated with overweight or obesity, thymectomy and high monthly household income (OR 0.75, 0.56 and 0.59; P =0.016, =0.001, <0.001). No statistical significance was observed for onset age, disease duration, treatment, comorbidities, autoimmune comorbidities, employment, education, smoking, alcohol consumption and physical activity.
**Conclusion** Increased disease activity was correlated with generalized symptoms at onset, thymoma and high latitude, and inversely associated with overweight or obesity, thymectomy and high monthly household income. Further studies are warranted to explore factors affected MG and their underlying mechanisms.
**Keywords** myasthenia gravis, disease severity, environment

## O-6

## Optimize antibody-RNA conjugate for treating myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a progressive disease that affects skeletal, smooth and cardiac muscles and is characterized as muscle weakness, myotonia and shorter life span. With no approved disease-modifying therapy, DM1 is affecting ~1:20,000 people worldwide and causes substantial disease burden.

DM1 is a monogenic disease, inherited in an autosomal dominant manner, and caused by CTG expansion in the noncoding region of DMPK gene. Muscle targeted silencing of aberrantly transcribed CUG bearing DMPK mRNA by antibody-RNA conjugate (ARC) is one of the therapeutic approaches under development and shows promise of alleviating myotonic symptoms in early stage clinical trials for DM1.

We carefully review the target product profile of existing ARC therapeutics and optimize the drug design. In brief, our proprietary ARC, CGB1001 demonstrates 1) up to 5x improvement in DMPK silencing efficiency in human muscular cells, 2) 7-10x more of siRNA delivery into skeletal muscles, 3) 10x lowering in transferrin competition by a distal antibody binding epitope, and 4) higher yield and purity of ARC through a proprietary manufacturing process vs. existing ARC therapeutics.

The features enable us to develop CGB1001 with better efficacy, less anemia risk, better purity and improved affordability. CGB1001 has recently completed a 12 weeks toxicology studies in non-human primates and shows good tolerability and safety profile. CGB1001 is on schedule to a first-in-human trial by the end of 2024.

## O-7

# Protein methylation is essential to maintain skeletal muscle strength and function by regulating myosin heavy chain activity

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Protein methylation has been studied mainly in histones and other nuclear proteins. Therefore, the impact of lysine methylation on cytoplasmic proteins remains largely unknown. In the context of skeletal muscle contraction, which relies on the interaction between myosin and actin proteins, the methylation of these proteins has been an intriguing area of research for over 50 years, yet its biological significance has remained elusive. Here, our investigation into the protein lysine methyltransferase, referred to as Mettl21e, has shed new light on this topic. Through a series of proteomic analyses, immunostaining, and Western blot analyses, we have uncovered that Mettl21e functions as a skeletal muscle myosin methyltransferase. Specifically, our findings indicate that the methylation of skeletal muscle myosin heavy chain is entirely dependent on Mettl21e activity. *Mettl21e* knockout mice exhibited reduced steady-state muscle strength and muscle mass compared to wild-type littermates. Additionally, they displayed a diminished capacity for overload-induced muscle hypertrophy. Although human *METTL21E* is annotated as a pseudogene in the database, we successfully isolated the functional human *METTL21E* and confirmed its molecular function in human primary myoblast and muscle tissue. Furthermore, we revealed a significant reduction in *METTL21E* expression levels in muscle samples from individuals with muscle disorder compared to healthy controls. Our results strongly indicate that the methylation of myosin heavy chain, modified by Mettl21e, serves as a novel molecular mechanism intricately regulating skeletal muscle function and also imply a potential link between protein methylation and the pathogenesis of human muscle disease.

## O-8

## Muscle and diaphragm involvement in GNE Myopathy: Insights from a large cohort and comparative study with other distal myopathies

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GNE myopathy (GNEM) is an autosomal recessive distal myopathy caused by pathogenic variants in the GNE gene. Accurate diagnosis and understanding of the disease course are critical requirements as Sialic Acid Extended-Release Tablet (SA-ER) has just become the first drug approved for GNEM in Japan, although it is difficult to accumulate data in this ultra-rare disease.
The objectives of this study are: 1) to identify the muscles that are useful in differentiating GNEM from other distal myopathies, which would be helpful in selecting appropriate genetic testing, 2) to establish a whole-body disease progression profile that would identify muscles useful for disease monitoring at each stage of the disease, and 3) to evaluate the diaphragm to determine the relationship with respiratory dysfunction.
We assessed the degree of fat infiltration in each muscle using the modified Merculi scores on CT and MRI images of patients with GNEM (n=96), oculopharyngodistal myopathy (OPDM, n=54), VCP myopathy (n=11), hereditary myopathy with early respiratory function (HMERF, n=10) and FLNC myopathy (n=6) and compared by dimensionality reduction analyses using UMAP and PCA. In the lower limbs, the vastus lateralis/medialis/intermedius (VL/VM/VIM), gluteus maximus/minimus (GMa/GMi) and short head of the biceps femoris muscles (SBF) muscles help to differentiate GNEM from OPDM, VCP myopathy and FLNC myopathy. Meanwhile, in the trunk muscles, the subscapularis (SubS) muscles are particularly effective in distinguishing GNEM from OPDM. The progression profile showed that around 5 years after onset, the posterior thigh and anterior lower leg muscles were mainly replaced by fat, and after a further 10 years, the trunk and quadriceps were also replaced by fat. We also found a correlation between thinness of diaphragms and reduced respiratory function in GNEM patients.
In conclusion, we present muscles that serve as valuable discriminators between GNEM and other distal myopathies (VL/VM/VIM, GMa/GMi, SBF, SubS) to help clinicians make a prompt and accurate diagnosis. In addition, our identification of diaphragm thinning as a contributing factor to reduced respiratory function, which is vital, highlights the emerging importance of diaphragm assessment in monitoring disease progression.

## O-9

## The androgen receptor in mesenchymal progenitors regulates skeletal muscle mass via *Igf1* expression in male mice

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Androgens exert their effects primarily by binding to the androgen receptor (AR), a ligand-dependent nuclear receptor. While androgens have anabolic effects on skeletal muscle, previous studies reported that AR functions in myofibers to regulate skeletal muscle quality, rather than skeletal muscle mass. Therefore, the anabolic effects of androgens are exerted via extra-myofiber cells or tissues. In this context, the cellular and molecular mechanisms of AR in mesenchymal progenitors, which play a crucial role in maintaining skeletal muscle homeostasis, remain largely unknown. In this study, we demonstrated expression of AR in mesenchymal progenitors and found that targeted AR ablation in mesenchymal progenitors reduced limb muscle mass in mature adult, but not young or aged, male mice, although fatty infiltration of muscle was not affected. The absence of AR in mesenchymal progenitors led to remarkable perineal muscle hypotrophy, regardless of age, due to abnormal regulation of transcripts associated with apoptosis and extracellular matrix organization. Additionally, we revealed that AR in mesenchymal progenitors regulates the expression of insulin-like growth factor 1, which can increase skeletal muscle mass in a paracrine manner. These findings indicate that the anabolic effects of androgens regulate skeletal muscle mass via, at least in part, AR signaling in mesenchymal progenitors.

# Oral Session 2

13:30-15:00, Sep 13 (Fri), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Arada Rojana-udomsart (Neurological Institute of Thailand, Thailand)
Daigo Miyazaki (Department of Medicine (Neurology and Rheumatology), Shinshu University School of Medicine/ Intractable Disease Care Center, Shinshu University Hospital, Japan)

## O-10

## Lama1 upregulation prolongs the lifespan of a novel mouse model of LAMA2-related congenital muscular dystrophy

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LAMA2-related congenital muscular dystrophy (LAMA2-CMD), characterized by laminin-α2 deficiency, is debilitating and ultimately fatal. To date, no effective therapy has been clinically available. Laminin-α1, which shares significant similarities with laminin-α2, has been proven as a viable compensatory modifier. To evaluate its clinical applicability, we established a Lama2 exon-3 deletion mouse model (dyH/dyH). dyH/dyH exhibited early lethality and typical LAMA2-CMD phenotypes, allowing evaluation of various endpoints. In dyH/dyH mice treated with synergistic activation mediator (SAM)-based CRISPRa-mediated Lama1 upregulation (total dose: 1.0 × 10^11 vector genomes/mouse), a nearly doubled median survival was observed, as well as improvements in weight and grip. Significant therapeutical effects were also demonstrated in MRI, serum biochemical indices, and muscle pathology studies. We confirmed that treating LAMA2-CMD with LAMA1 upregulation is feasible and early intervention can alleviate symptoms and extend lifespan. Additionally, we revealed limitations of LAMA1 upregulation, including high-dose mortality and non-sustained expression, which require further optimization in future studies.

## O-11

## FKTN variant interpretation through high throughout sequencing assay

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Interpretation of disease-causing genetic variants remains a challenge in human genetics. Current costs and complexity of deep mutational scanning methods hamper crowd-sourcing approaches toward genome-wide resolution of variants in disease-related genes. Our framework, utilize the CRISPR Prime Editing and develop a high throughout approach to screen the functional output of variants within FKTN, addresses these issues by offering simple and cost-effective saturation mutagenesis, as well as streamlining functional assays to enhance the interpretation of unresolved variants.
We used CRISPR Prime Editing technology for the first time to efficiently edit mutations in the FKTN gene in myoblasts, and developed a new high-throughput functional assay for detecting glycosylation levels, building a framework which could be further expanded to other α-DGpathies. We enhanced clinical variant interpretation in dystroglycanopathies. Our approach opens new directions for enabling variant-to-function insights for disease genes in a manner that is broadly useful for crowd-sourcing implementation across standard research laboratories.

## O-12

# A Phase 1 Study of Antisense Oligonucleotide NS-035 in Patients with Fukuyama Congenital Muscular Dystrophy

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Fukuyama congenital muscular dystrophy (FCMD) is an autosomal recessive disease that primarily affects the skeletal muscles, brain, and eyes. FCMD is most commonly caused by a 3-kb retrotransposal insertion into the 3' untranslated region of the *fukutin* gene, resulting in aberrant mRNA splicing (exon-trapping). In collaboration with Nippon Shinyaku, we discovered an antisense oligonucleotide, NS-035, that can prevent this exon-trapping, recovering normal *fukutin* mRNA expression and protein function in both mouse models and cells of FCMD patients. To obtain regulatory approval for NS-035, we initiated the first-in-human phase 1 study of NS-035 in patients with FCMD. This was a two-center, open-label, uncontrolled, dose-escalation clinical trial comprising four cohorts. Twelve patients with FCMD (aged 5–10 years) carrying homozygous or compound heterozygous variants in the *fukutin* gene were included, with three patients in each cohort. The study was initiated in cohort 1. D-mannitol alone was administered intravenously once during the premedication phase, followed by simultaneous doses of NS-035 and D-mannitol administered intravenously once weekly for 12 weeks during the treatment phase. The dose of D-mannitol was fixed at 500 mg/kg in all cohorts, while NS-035 was increased stepwise from cohorts 1 to 4 (1.6, 6.0, 20, and 40 mg/kg). The primary endpoint was safety, and the secondary endpoints were pharmacokinetics and efficacy (glycosylation rate of alpha-dystroglycan [DG], expression of glycosylated alpha-DG, exon-trapping inhibition efficiency, evaluation of gross motor function, and changes in blood CK value). Following institutional review board approval in June 2021, the trial was initiated in August 2021. This study is currently in progress with the final patient (patient 12). This study is progressing smoothly and is scheduled for completion in 2024.

## O-13

# Unraveling the Pathogenic Mechanisms of B3GALNT2-Related α-Dystroglycanopathy: Insights into Enzymatic Activity and Gene Expression Changes

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Background: B3GALNT2 mutations cause α-Dystroglycanopathy (α-DGP), characterized by muscular dystrophy, brain malformations, and developmental delay. Despite the scarcity of reported cases, the underlying pathogenic mechanisms remain underexplored.

Methods: In this study, we analyzed clinical phenotypes and genotypic characteristics in 3 newly reported Chinese patients alongside 28 previously documented patients with B3GALNT2-related α-DGP. Using patient-derived skin fibroblasts, α-dystroglycan (α-DG) glycosylation levels were investigated by wheat germ agglutinin (WGA) pull-down and immunoblot analysis. The binding capacity of α-DG to laminin protein was assessed through laminin overlay and immunofluorescence staining. B3GALNT2 gene expression at both the mRNA and protein levels was evaluated by real-time PCR and immunoblot. Differentially expressed genes at the mRNA level between normal controls and patients were identified by mRNA microarray analysis. Additionally, B3GALNT2 proteins were expressed and purified in vitro, and a novel method to assess B3GLANT2 enzyme activity was introduced.

Results: B3GALNT2-related α-DGP patients commonly presented with psychomotor development delay and abnormal white matter signals on brain MRI. Phenotype-genotype correlations were not evident in this patient cohort. Reduced α-DG glycosylation levels were observed, leading to impaired α-DG binding with laminin. Notably, mutant B3GALNT2 did not exhibit significant reductions in mRNA and protein expression levels but showed distinct structural alterations and a marked decrease in enzyme activity compared to the wild-type protein. Furthermore, both mRNA and protein levels of the CHST10 gene were upregulated in patients relative to the normal control group.

Conclusions: This study expands our knowledge of the genetic and clinical spectrum of B3GALNT2-related α-DGP. It also reveals that reduced enzymatic activity of B3GALNT2 and upregulation of CHST10 may be contributory factors to B3GALNT2-related α-DGP. These findings advance our understanding of the pathogenic mechanisms underlying this rare disorder and provide insights into potential therapeutic strategies for B3GALNT2-related α-DGP in the future.

## O-14

## Mn007 facilitates O-mannosyl glycosylation in Fukuyama muscular dystrophy

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Fukuyama congenital muscular dystrophy (FCMD) is a severe, intractable genetic disease that affects the skeletal muscle, eyes, and brain. It is attributed to a defect in alpha dystroglycan O-mannosyl glycosylation. We have previously reported that the basic polycyclic compound Mannan-007 (Mn007) restored alpha DG glycosylation in both brain and muscle models tested, and partially rescued the abnormal RG migration observed in FCMD cortical organoids. To investigate the mechanism of action of Mn007, expression analysis and proteomics were performed. As a result, several candidate genes were identified. Furthermore, the development of a matriglycan elongation assay system demonstrated that Mn007 enhanced the enzymatic activity of a glycosyltransferase. By elucidating the mechanisms of action of Mn007 in FCMD, we aim to facilitate the practical application of low molecular weight chemical treatments for FCMD.

## O-15

# From basics to translational research for 14-years unravelling the Anti-inflammatory potential of a novel b-glucan; moving to clinic in Duchenne muscular dystrophy

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Background:
Duchenne Muscular Dystrophy (DMD), for which no definitive cure currently exists, primarily relies on disease-modifying agents, significantly targeting inflammation to slow down the disease progress. In our research evaluating the comparative effects of novel 1-3,-1,6-beta-glucans produced by two distinct strains of Aureobasidium pullulans, namely AFO-202 and N-163 (Neu REFIX) on the immune systems, we unraveled the anti-inflammatory, anti-fibrotic, and immune-modulatory potentials of N-163 b-Glucan found worthy evaluating in DMD.
Materials and Methods:
Six studies were conducted, three pre-clinical and three clinical: Preclinical studies: Study 1 in KK-Ay mice, Study 2 in SD rats, and Study 3 in STAM-NASH mice, comparing the effects of AFO-202 beta-glucan and Neu REFIX. Clinical studies: Study 1 involved healthy Japanese male volunteers, while Study 2 focused on Covid-19 patients for 15 days and Study 3, was also on Covid-19 patients but for 30 days, which investigated the efficacy of combination of AFO-202 b-glucan and Neu REFIX.
Results:
Preclinical study 1: Neu REFIX group yielded the highest reduction of non-esterified fatty acids (NEFA). Preclinical study 2 demonstrated an increase in the lymphocyte-to-C-reactive protein ratio (LCR), indicating beneficial immune modulation in Neu-REFIX arm. Preclinical study 3: Neu-REFIX decreased hepatic fibrosis and inflammation significantly. Clinical study 1: Profound decrease in total and LDL cholesterol, a decrease of CD11b, serum ferritin, galectin-3 and fibrinogen, was observed in Group II in which Neu-REFIX was added to AFO-202 produce. Clinical study 2 showed sustained decrease in C-reactive protein (CRP) and ferritin levels only in the treatment arm (Neu REFIX added to AFO-202). IL-6 exhibited a higher decrease in the treatment group compared to the control. Clinical study 3: lymphocyte-to-C-reactive protein ratio (LCR) and leukocyte-to-C-reactive protein ratio (LeCR) increased significantly, and neutrophil-to-lymphocyte ratio (NLR) decreased significantly in Group 3 (Neu REFIX added to AFO-202).
Conclusion:
The findings from these six studies indicate that N-163 beta glucan (Neu REFIX) exhibits safety and superior anti-inflammatory, anti-fibrotic, and immune-modulatory properties. In the NASH animal model, Neu REFIX demonstrated significant anti-inflammatory effects at the tissue level, corroborated by biochemical parameter analyses in other studies. These collective evidence provide a basis for considering Neu REFIX as a potential disease modifying drug adjuvant in our subsequent preclinical and clinical investigations of DMD. Long-term, multi-centric validation studies will be instrumental in evaluating the candidacy of Neu REFIX as a drug or drug adjuvant for DMD.

## O-16

# Becker muscular dystrophy mouse models revealed nNOS reduction with capillary change and decreased type IIa fibers in skeletal muscle

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Becker muscular dystrophy (BMD) severity varies associated with the genotype of *DMD*; however, the underlying mechanisms of the severity of each BMD exon deletion remain unclear. Based on the natural history of BMD by Nakamura et al. (Ann Clin Transl Neurol, 2023), we produced BMD mice having ex45-47 del. (Δ45-47): the most frequent deletion, ex45-48 del. (Δ45-48): the second most frequent and mild phenotype, and ex45-49 del. (Δ45-49): the severer phenotype than others, in *DMD* gene, and we examined the phenotypes of these BMD mice.

All three BMD mice showed muscle weakness, muscle degeneration, and fibrosis, but these changes appeared at different times for each exon deletion, consistent with the severities obtained by the natural history study of human BMD. Unlike *mdx* mice which showed diffuse muscle changes, BMD mice showed site-specific muscle degeneration, especially in the muscle portion containing high amounts of type IIa fibers, and demonstrated a selective reduction of type IIa fibers. Furthermore, BMD mice showed morphological capillary changes around type IIa fibers and reduced sarcolemmal neuronal nitric oxide synthetase (nNOS) expression. These results suggest that changes in capillary formation caused by reduced sarcolemmal nNOS expression may be related to the mechanism of skeletal muscle degeneration and type IIa fiber reduction in BMD mice.

It is hypothesized that vascular dysfunction accompanied by sarcolemmal nNOS reduction is involved in the pathomechanisms of muscle impairment in human DMD. Our findings suggest that vascular hypothesis may be valid also in muscle impairment in human BMD.

## O-17

## Cardiac Dysregulation in Duchenne Muscular Dystrophy: An ECG analysis

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Background: Duchenne Muscular Dystrophy (DMD) is a progressive X-linked recessive disorder characterized by severe muscle degeneration and premature death, often due to cardiac complications. Despite the prevalence of arrhythmogenic cardiomyopathy in DMD, the utility of Electrocardiogram (ECG) analysis in detecting subclinical cardiac dysregulation remains underexplored. This study aims to explore the alterations in Lead II ECG parameters in children with DMD, potentially indicating an elevated risk of Sudden Cardiac Death (SCD).

Methods: In this cross-sectional study, Lead II ECG recordings from 54 genetically confirmed DMD patients were compared against 31 age-matched healthy controls. Parameters analyzed included PR interval, QRS duration, QT and QTc intervals, Tp-Te interval, and P, Q, R, S, and T wave amplitudes, utilizing Labchart pro 8 software and the Hamilton-Tompkins QRS detection algorithm. Statistical analysis was conducted using independent samples t-test to compare differences between groups.

Results: The study revealed significant ECG alterations in the DMD group compared to controls, including reduced PR interval, prolonged QRS and QT intervals, decreased QTc, and increased Tp-Te interval. Additionally, significant increases in P, Q, R wave amplitudes, and ST height were observed, indicative of atrial hypertrophy and potential ventricular arrhythmias.

Conclusions: Lead II ECG analysis in children with DMD demonstrates critical alterations suggestive of subclinical cardiac dysregulation, highlighting a potential non-invasive marker for early detection of cardiac involvement. These findings emphasizes the importance of regular cardiac monitoring in DMD patients to mitigate the risk of SCD through timely intervention and highlight the need for further research into the pathophysiological mechanisms underlying these ECG changes.

## O-18

## Caveolin 3 inhibits phosphorylation-dependent activation of sarcolemmal nNOS

Yutaka Ohsawa, Asami Munekane, Kohei Ohkubo, Shin-ichiro Nishimatsu, Yoshihide Sunada
Kawasaki Medical School, Japan

Mutations of the caveolin 3 gene cause autosomal dominant limb-girdle muscular dystrophy (LGMD)1C. In mice, overexpression of mutant caveolin 3 leads to loss of caveolin 3 and results in myofiber hypotrophy in association with activation of neuronal nitric oxide synthase (nNOS) at the sarcolemma. Here, we show that caveolin 3 directly bound to nNOS and suppressed its phosphorylation-dependent activation at a specific residue, Ser1412 in the nicotinamide adenine dinucleotide phosphate (NADPH)-flavin adenine dinucleotide (FAD) module near the C-terminus of the reduction domain, in vitro. Constitutively active nNOS enhanced myoblast fusion, but not myogenesis, in vitro. Phosphorylation-dependent activation of nNOS eventually occurred in muscles from caveolin 3-mutant mice and LGMD1C patients. Consitently, mating with nNOS-mutant mice exacerbated myofiber hypotrophy in the caveolin 3-mutant mice. Notably, regenerating myofibers after cardiotoxin injury became hypotrophic with reduced myoblast fusion in nNOS-mutant mice. Administration of NO donor increased myofiber size and the number of myonuclei in the caveolin 3-mutant mice. Exercise also increased myofiber size accompanied by phosphorylation-dependent activation of nNOS in both wild-type and caveolin 3-mutant mice. These data indicate that caveolin 3 inhibits phosphorylation-dependent activation of nNOS, which leads to myofiber hypertrophy via enhancing myoblast fusion. Hypertrophic signaling by nNOS phosphorylation could act in a compensatory manner in caveolin 3-deficient atrophic muscles.

# Oral Session 3

17:00-18:30 , Sep 14 (Sat), 2024
Room 2 (Room 201+202, 2F, Nara Prefectural Convention Center)

Chairs: Mariko Okubo (Sorbonne Université, Inserm, Institut de Myologie, Centre de Recherche en Myologie, Paris, France/ National Center for Global Health and medicine, Research Institute, Tokyo, Japan)
Satoshi Yamashita (International University of Health and Welfare Narita Hospital, Japan)

## O-19

## High-Risk Screening for Late-Onset Pompe Disease in China: An Expanded Multicenter Study

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Late-onset Pompe disease (LOPD) typically presents with insidious onset and non-specific early symptoms, leading to frequent diagnostic delays. The spectrum of genetic mutations in Chinese LOPD patients differs from that seen in the rest of the world, and previous research suggests difference in high-risk screening profiles. However, the clinical picture of adolescent LOPD is underrepresented. Thus, this study aimed to expand the cohort for high-risk screening and investigate the correlation between acid alpha-glucosidase (GAA) gene mutations and clinical phenotypes.

Inclusion criteria were amended according to previous studies: (1) patients aged ≥1 year; (2) undiagnosed myopathy patients with at least one of the following: (a) serum creatine kinase (CK) levels exceeding 1.5 times the upper limit of normal (ULN) at least twice within a month, (b) weakness in axial and/or proximal limb muscles, or (c) unexplained respiratory distress. From April 2021 to April 2022, 31 medical centers across China assessed GAA activity using dried blood spots (DBS) and tandem mass spectrometry (MS/MS). Patients with GAA activity below lower normal limit underwent next-generation sequencing (NGS) of the GAA gene, and the clinical-genetic correlations of diagnosed patients were analyzed.

A total of 726 individuals were screened, with 44 (6.1%) exhibiting reduced GAA activity among whom 16 (2.2%) were diagnosed with LOPD. At the time of diagnosis, most patients (13/16) exhibited varying degrees of respiratory system involvement, and all confirmed cases showed significant weakness in axial and proximal limb muscles. Among patients with onset ≤16 years (4/16), CK levels averaged 5.9±2.2 times ULN, with a c.2238G>C mutation frequency of 12.5% (1/8). For those with onset >16 years (12/16), CK levels were at 1.9±1.0 times ULN, with a c.2238G>C mutation frequency of 45.8% (11/24). Additionally, three previously unreported GAA mutations were identified: c.521A>G (p.E174G), c.839_840insCC (p.R281fs), and c.1444 C>T (p.P482S).

Overall, Chinese patients with LOPD exhibit more pronounced respiratory involvement and less frequent hyperCKemia. Patients with onset before age 16 show higher CK levels but a lower frequency of the c.2238G>C mutation. Different early high-risk screening standards should be established for children/ adolescents and adults with LOPD.

## O-20

# FORTIS Update: Biomarker Results and Up to 2 Years Safety and Exploratory Efficacy in a Phase 1/2 Open-Label Clinical Study of AT845 Gene Replacement Therapy for Late Onset Pompe-Disease

Chieri Hayashi[3], Tahseen Mozaffar[2], Nicola Longo[1], Mark Walzer[3], Achim Steup[3], Julie Coats[3,4], Jordi Diaz-Manera[5]

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Pompe disease is a rare, autosomal recessive disease in which a deficiency of lysosomal acid alpha-glucosidase (GAA) causes accumulation of glycogen, resulting in damage to skeletal and cardiac muscles. AT845 (AAV8-eMCK-hGAA) is a gene therapy in clinical development for late onset Pompe disease (LOPD) that expresses the human GAA (hGAA) gene specifically in muscle tissues. FORTIS (NCT04174105) is an ongoing phase 1/2, multicenter, open-label, ascending dose, first-in-human clinical trial to determine the safety and tolerability and exploratory efficacy of AT845 in adults with LOPD. So far 6 participants have received a one-time intravenous infusion of AT845 at either the 3x10^13 vg/kg (n=2) or 6x10^13 vg/kg (n=4) dose level. We present data up to 2 years of follow-up for the first 4 participants dosed. All four participants showed evidence of AT845 vector transduction and GAA activity in the muscle. Three of the 4 participants who have completed at least 2 years of follow-up have stopped enzyme replacement therapy (ERT) and have remained off for at least 89 weeks to 121 weeks. Forced vital capacity and the 6-minute walk test were stable up to 2-years post-dosing. The patient-reported outcomes PROMIS-Fatigue and Rasch-built Pompe-specific Activity (R-PAct) were also stable up to 2 years post-dosing, including following ERT withdrawal. Urine Hex4 (Glc4) and serum CK levels were variable but relatively stable post-dosing, except for one participant whose serum CK level increased after 6 months. Infusions were generally well-tolerated. Three of four participants developed transient, steroid responsive transaminitis that was mild to moderate and deemed possibly related to AT845. A grade 2 peripheral sensory neuropathy event was reported in one of four participants who received the 6x10^13 vg/kg dose and was designated as a serious adverse event (SAE) due to medical significance. In summary, participants in the ongoing FORTIS study have remained clinically stable based on assessment of Pompe disease key functional endpoints and safety endpoints, while off ERT for at least 89 weeks to 121 weeks.

## O-21

# Novel mutations and genotype-phenotype correlation in a multicenter cohort of GNE myopathy in China

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Abstract:

Background: GNE myopathy is a rare autosomal recessive disorder caused by pathogenic variants in the GNE gene, which is essential for the sialic acid biosynthesis pathway.

Objective: This multi-center study aimed to delineate the clinical phenotype and GNE mutation spectrum in Chinese patients, enhancing our understanding of the genetic diversity and clinical manifestation across different populations.

Methods: We retrospectively analyzed GNE variants from 113 patients, integrating this data with external GNE variants from online databases for a global perspective on GNE mutations, examining their consequences, distribution, ethnicity, and severity.

Results: Our mutation analysis revealed 101 distinct GNE mutations, with 38.6% being previously unreported. Sanger sequencing revealed two patients (P48, P58) with deep intronic variant(DIV) c.862+870C>T , while whole genome sequencing (WGS) uncovered three more mutations: c.52-8924G>T, c.1505-12G>A, and c.1445_1447dup, respectively. Predominantly, missense mutations, particularly the c.620A>T variant, were observed, highlighting it as a key pathogenic variant in the Chinese population. Comparative studies with Japanese, Korean, and Jewish cohorts showed later onset ages in Chinese patients, suggesting a milder phenotype influenced by ethnic and geographical factors.

Conclusions: The study emphasizes the importance of comprehensive genetic screening, including advanced techniques like WGS and Nanopore long reads sequencing to capture the full spectrum of GNE mutations. Remarkably, patients with the p.D207V mutation had a significantly delayed progression to wheelchair dependence and ambulation loss. Given the common mutation p.D207V, GNE myopathy's milder phenotype might lead to underdiagnosis in China.

Key words: GNE myopathy, genotype-phenotype correlation, novel mutations

## O-22

# Development of sarcomere-observable mice for analyzing skeletal myofibril degeneration

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Sarcomere is a repeating unit between two Z-lines in contractile apparatus (myofibrils) in striated muscles. It is functionally shortened and extended during contractile cycles in myofibers. There are some skeletal myopathies, such as myofibrillar myopathy, nemaline myopathy, and proximal and distal arthrogryposis, in which sarcomeric structures are primarily affected, leading to myofibril disruption and myofiber degeneration. Overload contraction also reported to cause partial disruption of sarcomeric structures. Thus, by monitoring the sarcomeres in vivo, it will enable to know an initial event on these diseases and better characterize the pathological process of the diseases. In current study, we generated the transgenic mouse harboring the Cre-inducible expression cassette of Actn2-AcGFP, which is localized at Z-line in skeletal muscles. By long-read sequencing, we determined the structure, copy number and the integrated locus of the transgene in genome of the transgenic mouse. Two copies of the expression cassettes of Actn2-AcGFP were integrated at chr13: 103,703,108 with 38-kb fragment of E. coli genome. After cross mating with Acta1 promoter-Cre mouse, the fluorescence signal was strongly detected in skeletal muscles in transgenic mouse. On single fibers isolated from the gastrocnemius, Z-lines as well as costameres were highlighted with fluorescence. The inserted fragment from E. coli genome was unlikely to affect the mouse phenotypes, and the localization of Actn2-AcGFP. These results indicated that this mouse will be a good tool to monitor sarcomere status in vivo as well as to trace myofibril degeneration process related to the diseases chronologically. We will generate various myopathic models with this transgene and observe the myofibril structures during disease progression.

## O-23

# Immune Mediated Megaconial Myopathy (IMMM): A Novel Subtype of Autoimmune Myopathy Featuring Giant Mitochondria

Teerin Liewluck[1], Ashley Santilli[1], Oliver Ni[2], Margherita Milone[1], Duygu Selcen[1], Anahit Mehrabyan[3], Arjun Seth[4], Christine Hsieh[5], Wasim Raslan[6], Moayd Alkhalifah[7], Raed Alenezi[8], Stefan Nicolau[9], Pannathat Soontrapa[10]

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Objectives: To describe a new subtype of autoimmune myopathy, immune mediated megaconial myopathy (IMMM), pathologically characterized by giant mitochondria (megaconia).

Methods: We reviewed the Mayo Clinic Muscle Pathology database to identify patients with megaconial muscle pathology, subacute progressive weakness and hyperCKemia, clinically resembling myositis. We recruited one patient from other institute, who had similar clinicopathological features.

Results: We reported five patients with onset of weakness ranging from 19 to 44.5 (median 37.75) years old. All patients had proximal weakness, elevated creatine kinase levels (1214 to 5920 U/L), necrotizing myopathy pathology, and non-necrotic myofibers harboring multiple large fuchsinophilic granules overreactive for oxidative enzymes, consistent with giant mitochondria. Electron microscopic study performed in one patient confirmed the presence of markedly enlarged mitochondria. Immunohistochemical studies conducted in 4/5 patients showed sarcolemmal MHC-1 and C5b9 immunoreactivities. All patients had negative myositis specific/associated antibodies, including SRP and HMGCR antibodies. Giant mitochondrial pathology resembles that of megaconial congenital muscular dystrophy, an infantile-to-childhood onset muscular dystrophy due to biallelic CHKB mutations, and selenium deficient myopathy. Sequencing of CHKB in 4 patients was unrevealing. Selenium level was mildly low in 1/ 3 tested patients. Immunomodulatory therapy improved weakness and hyperCKemia in 4 treated patients, including a patient with mild selenium deficiency. Interestingly, all patients had coexisting pancreatic diseases (3 cystic fibrosis related exocrine pancreatic insufficiency, 1 pancreatic cancer and 1 pancreatitis).

Conclusion: IMMM is a subset of seronegative immune mediated necrotizing myopathy with uniqute myopathological features and common concomittant pancreatic disorders. The presence of giant mitochondria on muscle biopsy of adult patients with subacute progressive weakness and hyperCKemia should prompt clinicians to consider IMMM. Immunomodulatory therapy should be contemplated in the absence of CHKB mutations. Given its potential paraneoplastic nature, a search for an underlying malignancy is crucial.

## O-24

# Alterations in Cerebrospinal Fluid Metabolite Profiles in Patients with Spinal Muscular Atrophy

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**Introduction:** Spinal muscular atrophy (SMA) is a neurodegenerative disorder characterized by metabolic dysregulation. While previous studies have explored the metabolomic profiles of SMA in various contexts, research on cerebrospinal fluid (CSF) metabolomics in comparison to healthy controls remains limited. CSF metabolomics offers insights into central nervous system function and patient outcomes. This study aims to examine CSF metabolite profiles in untreated SMA patients to enhance our understanding of metabolic dysregulation in SMA.

**Methods:** This case-control study involved 15 SMA patients and 14 control subjects. CSF samples were collected, and untargeted metabolomics analysis was performed to identify metabolites in both groups.

**Results:** Analysis revealed 118 significantly differentially abundant metabolites between SMA patients and controls, with 27 metabolites having a variable importance for the projection (VIP) ≥1.5. The top 5 differential metabolites included N-acetylneuraminic acid (VIP=2.38, Fold change=0.43, $P$=5.49×10-5), 2,3-dihydroxyindole (VIP=2.33, Fold change=0.39, $P$=1.81×10-4), lumichrome (VIP=2.30, Fold change=0.48, $P$=7.90×10-5), arachidic acid (VIP=2.23, Fold change=10.79, $P$=6.50×10-6), and 10-hydroxydecanoic acid (VIP=2.23, Fold change=0.60, $P$=1.44×10-4). Cluster analysis indicated that these metabolites mainly clustered in protein and amino acid metabolism, as well as lipid metabolism categories.

**Conclusion:** The study highlights the intricate metabolic disruptions in SMA, affecting various metabolic pathways, especially in amino acid and lipid metabolism. N-acetylneuraminic acid emerges as a potential treatment target for functional improvement in SMA. Further research is needed to elucidate the underlying mechanisms and identify therapeutic targets associated with metabolic dysregulation in SMA.

## O-25

# The open-label phase 4 RESPOND study evaluating nusinersen in children with spinal muscular atrophy (SMA) previously treated with onasemnogene abeparvovec: Interim clinical, neurofilament, and safety results

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Background/Methods:
RESPOND (NCT04488133) is a single-arm study evaluating 12-mg nusinersen in children with SMA aged ≤36 mo who previously received onasemnogene abeparvovec (OA) and had suboptimal clinical status in ≥1 of 4 domains (motor function, swallowing/feeding ability, respiratory function, other) at baseline per the investigator. Changes from baseline in total HINE-2 score and plasma neurofilament light chain (NF-L) concentrations were examined by age at first nusinersen (≤9 vs >9 mo) dose and *SMN2* copy number in participants reaching Day 183 (D183) by the data cut (15Nov2022). Neurofilaments were included as a biomarker of disease activity and treatment response. Safety data were evaluated as aggregate in all participants receiving ≥1 nusinersen dose.
Results:
At the data cut, 38 participants were on study for a median ~230 days. Of the 29 reaching D183, 14 (48%) were ≤9 mo at first nusinersen dose with 2 *SMN2* copies (group [G] 1; median [range] age: 7.7 [3.4–9.8] mo), 12 (41%) were >9 mo with 2 *SMN2* copies (G2; 16.3 [11.0-33.3] mo), and 3 (10%) were >9 mo with 3 *SMN2* copies (G3; 30.8 [29.2–35.7] mo). All were symptomatic at OA dosing (median [range] age at OA: 1.7 [0.7–5.1], 2.7 [0.8–6.9], 17.5 [13.6–24.3] mo in G1, G2, G3 respectively). At baseline, 76% had ulnar CMAP amplitude ≤1 mV. Mean (SD) change from baseline to D183 in HINE-2 scores was 5.4 (2.6) in G1 and 5.2 (2.7) in G2 (not calculated in G3 due to small sample size). Baseline NF-L was elevated compared to similarly aged neurologically healthy children, with greater elevation among those with 2 *SMN2* copies (medians 112.9, 122.9, 47.5 pg/mL in G1, G2, G3 respectively). Mean NF-L decreased from baseline to D183 by 70.0%, 77.8%, and 42.0% in G1, G2, and G3, respectively, which was consistent when examined by age and time from OA dosing. Adverse events (AEs) occurred in 31/38 (82%) participants, mostly infections/infestations (63%) and respiratory, thoracic, and mediastinal disorders (26%). Two (5%) participants had investigator-determined nusinersen-related AEs (mild proteinuria), which resolved. Thirteen (34%) had serious AEs; none nusinersen-related. Longer-term data from more participants will be presented.
Conclusions:
Most participants had low ulnar CMAP amplitude and elevated NF-L at baseline. HINE-2 scores increased through D183. There was a consistent reduction in NF-L after nusinersen initiation, indicating slowing of axonal injury and neurodegeneration. Reported interim safety outcomes are consistent with the established safety profile of nusinersen.

## O-26

# Prospective study on clinical outcomes and health-related quality of life in spinal muscular atrophy patients receiving nusinersen or risdiplam

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Introduction
Spinal muscular atrophy (SMA) is a hereditary condition with progressive motor neuron degeneration causing muscle weakness, respiratory insufficiency, swallowing difficulty, musculoskeletal deformities and premature death. The introduction of nusinersen in 2018 and risdiplam in 2022 has improved the survival for paediatric SMA patients in Hong Kong.

Methods
This prospective study recruited patients who received at least 0.5 years of nusinersen or risdiplam between May 2018 and May 2024. All patients followed the recommended standard of care, and underwent comprehensive evaluations, including clinical outcomes, motor function, and health-related quality of life (HRQOL) study. Patient-reported clinical changes were also collected.

Results
Thirty-one patients initiated nusinersen treatment (SMA1:10; SMA2:13; SMA3:7; Pre-symptomatic:1), with eight switching to risdiplam due to progressive scoliosis, intrathecal access issues, or nusinersen side effects (SMA1:4; SMA2:3; SMA3:1). Four patients began risdiplam treatment (SMA1:1; SMA2:3). Median age at treatment initiation were 6.2 years (range:0.09-24.4 years) for nusinersen, and 12.2 years (range:6.85-27.7 years) for patient switching from nusinesren to risdiplam. Treatment-naïve patients started risdiplam at a median age of 10.2 years (range:0.68-13.4 years).

Common adverse reactions associated with nusinersen included headache (8.1%). In the risdiplam group, one patient reported a transient rash.

After a median of 3 years of nusinersen treatment (range:0.50-7.00 years), motor function improved in SMA1 and SMA3patients (CHOP-INTEND:27.0 vs 29.0,$p$=0.014; 6MWT:105 vs 125m,$p$=0.063), while SMA2patients showed a slight decrease (RULM:20.0 vs 17.0,$p$=0.001; HFMSE:17.0 vs 14.0,$p$=0.541). Parents of nusinersen patients reported significant HRQOL improvement in the PedsQLTM-Family Impact Module Questionnaires (50.7 vs 59.7,$p$=0.002). After a median of 2.5 years of risdiplam treatment (range:0.64-2.61 years) following nusinersen treatment, patients maintained motor function (ATEND:21.0 vs 23.0,$p$=0.156; MFM32:3.5 vs 3.0, $p$=0.656), with no significant HRQOL changes (Family Impact:49.7 vs 59.0,$p$=0.313). Patients treated solely with risdiplam had similar motor functions (ATNED:24.0 vs 24.0,$p$=0.750; MFM32:14.0 vs 16.0,$p$=1.000), with a median treatment duration of 1.63 years (range:0.91-2.71 years). Parents and patients across all treatment groups perceived improvements in motor, respiratory, feeding, and speaking functions. Among symptomatic SMA patients started on the disease-modifying treatment, a quarter required ventilator support ,experienced progression of scoliosis or hip dislocation/subluxation, and two required full gastrostomy feeding. All patients had low bone mineral density.

Conclusion
Our findings support the safety and efficacy of nusinersen and risdiplam for paediatric SMA1, SMA2, and SMA3 patients, and an updated standard of care guideline is urgently needed. A more sensitive questionnaire to collect parents/patient-reported clinical changes are also essential.

## O-27

# Dynamic Changes in Cerebrospinal Fluid Metabolites as Predictors of Nusinersen Efficacy in Spinal Muscular Atrophy Patients: A Prospective Cohort Study

Wei Zhuang[1], Minying Wang[2], Zhehui Chen[2], Meifen Luo[2], Wanlong Lin[1], Xudong Wang[3], Mei Lu[2]

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**Background:** Nusinersen is the preferred therapeutic intervention for spinal muscular atrophy (SMA). However, substantial interindividual variability in treatment response remains a challenge. Therefore, this study aims to investigate the dynamic changes in cerebrospinal fluid (CSF) following nusinersen administration, with the goal of identifying potential biomarkers or alternative indicators predictive of treatment efficacy.

**Methods:** This prospective nested cohort study included SMA patients who started nusinersen therapy between 2021 and 2022. Patients were categorized into two cohorts based on motor score improvement after ten months of treatment: a robust response group and a suboptimal response group. CSF samples collected before the first injection, before the second, third and sixth injections were subjected to non-targeted metabolomics analysis. Comparative analysis of dynamic trend variances between the two cohorts was performed.

**Results:** Thirteen SMA patients were enrolled, with seven showing substantial functional improvement and six showing limited response. A total of 466 CSF metabolites were identified and included in the analytical framework. Examination of temporal concentration dynamics revealed an upregulation of sphingosine in the poor response cohort, while a decreasing trend was observed in the favorable response cohort. Notably, sphingosine represents a key metabolite within the sphingolipid pathway, suggesting potential involvement in neuronal reparative mechanisms.

**Conclusions:** Sphingosine, located within the sphingolipid metabolic cascade, emerges as a prospective biomarker for prognostication of nusinersen efficacy. However, validation of these findings requires future clinical trials with larger sample sizes, complemented by basic studies using in vivo and in vitro modalities.

# Poster Session 1

## Clinical assesment of DMD and the other diseases

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Yuko Shimizu-Motohashi (National Center of Neurology and Psychiatry, Japan)
Takahiro Nakayama (Yokohama Rosai Hospital, Japan)

### P-1

### Health care transition from pediatric to adult care for patients with neuromuscular disorders in Japan: A single center study

Ayaka Ohno[1], Yuko Shimizu-Motohashi[1], Yoko Takahashi-Kobayashi[1], Ryo Sugiyama[1], Madoka Mori-Yoshimura[2], Kaoru Yamamoto[1], Hisako Yamamoto[1], Noriko Sumitomo[1], Eri Takeshita[1], Takashi Saito[1], Eiji Nakagawa[1,3], Hirofumi Komaki[1,4]

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**Background** Recently, advancements in medical management have improved the prognosis of patients with neuromuscular disorders, making health care transition from pediatric to adult care increasingly important. In Japan, the Japanese Society of Pediatrics issued recommendations for health care transition in 2014. However, there is limited data on the current status of health care transition for patients with neuromuscular disorders.

Aim To characterize the current status of health care transition for patients with neuromuscular disorders at our institution.

**Subjects** Patients who visited the pediatric neurology department of our hospital and reached the age of 18 on or after April 1, 2014. Cases were included if their last visit was at age 18 or older and their medical records were accessible via electronic medical records.

**Methods** A retrospective study. The following aspects were investigated: presence of epilepsy, neurodevelopmental disorders, motor function and medical devices (tracheotomy, ventilator, tube feeding). Patients currently attending only adult departments were classified as the transition-completed group, while those attending only pediatric departments or both pediatric and adult departments were classified as the transition-incomplete group.

**Results** The study included 254 cases, with a male-to-female ratio of 193:61 and an age range of 18-27 years (median age 21 years). The primary diagnoses were as follows: 217 cases of muscular diseases, 9 cases of motor neuron diseases, 5 cases of neuromuscular junction disorders, 17 cases of peripheral nerve disorders, and 7 cases of other conditions. There were 43 cases in the transition-completed group (group C), 202 cases in the transition-incomplete group (group I), 3 cases of patients who had terminated their visits, and 6 cases with unknown status. The percentages of patients with epilepsy were 0% and 4.5% in groups C and I respectively; patients with neurodevelopmental disorder complication were 23.3% and 19.3%, patients with tracheotomy were 4.7% and 4.5%, patients using a ventilator were 18.6% and 37.1%, patients on tube feeding were 4.7% and 9.4%, walkers were 72.1% and 36.1%, sitters were 27.9% and 58.9%, non-sitters were 0% and 4.5%.

**Conclusion** We obtained information on the current status of health care transition from pediatric to adult care for patients with neuromuscular disorders in Japan.

## P-2

# Automatic calcuation of muscle volume of CT images of leg, using artificial intelligence

Takahiro Nakayama[2], Joji Kimizuka[1], Fumiya Isaki[1]
[1]Rakuten Socio Business
[2]Yokohama Rosai Hospital

Objective: To automate the measurement of muscle volume using muscle CT images, there is a limitation to the accuracy of muscle tissue discrimination by the estimation functions. We aim to build an artificial intelligence (AI) deep machine learning framework that combines the training data obtained by processing the target image with the related data of the patients and their arithmetic processing image data. We refined it by convolutional neural network(CNN) model into the AI.

Methods: Tissue-specific images were calculated from muscle CT images of the thighs and lower legs of 25 patients with muscle disease, 6 patients with neurogenic diseases, and 14 patients with non-neurological diseases (average age 54+/-14.7 years). The image processing was optimized using an AI CNN model. The positional data, the disease data, their ages and their sex were also incorporated into a CNN model. Finally, the muscle volume of thigh and lower legs was calculated, and the disease group was diagnosed by the AI, for the 7 patients without training data. The results were compared with the previously calculated using MATLAB and manual elimination process.

Result: The results of skeletal muscle volume of the 7 patients measured by the AI was almost the same as that by the manual process. The standard errors of both measurements were under 5 %.

Discussion: We succeeded in optimizing the image processing for each image from the subjects, and it was considered that muscle mass measurement using CT can be automated by optimizing deep learning. The small amount of the errors supported the reliability of the quality of the measurement.

We have no conflict of interest to disclose in relation to the presentation.

## P-3

## Safety of dexmedetomidine anesthesia for muscle and nerve biopsy

Satoko Ota[1], Akinori Uruha[1], Tomoya Kawazoe[1], Keizo Sugaya[1], Nanae Miyake[2], Shiro Fukuda[2], Hiroaki Matayoshi[2], Kazushi Takahashi[1]

[1]Department of Neurology, Tokyo Metropolitan Neurological Hospital, Japan
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Pain during muscle and nerve biopsy is often an annoying complication for patients. Dexmedetomidine, an α2-adrenorecptor agonist, has analgesic as well as sedative effects with less respiratory depression. We retrospectively evaluated the safety of dexmedetomidine in 100 patients who underwent anesthesia with the agent during muscle or nerve biopsy at our hospital from 2019 to 2023 (63 muscle and 37 nerve biopsies; an average age of 61.8 [17 - 84] years old). The major adverse events were bradycardia (heart rate < 50 bpm, 20/98 [20%]) and hypotension (systolic blood pressure < 70 mmHg, 6/100 [6%]). No cases had to be interrupted due to these adverse events, though six patients were treated for the events with supplemental fluid administration, legs elevation, and dose reduction. Any significant desaturation was not recorded. Overall, dexmedetomidine can be safely applied for muscle and nerve biopsy.

## P-4

## Usefulness of ultrasound-guided nerve block in muscle biopsy

Nozomu Tawara[1], Yoshihiro Shibata[2], Yasushi Maeda[1], Kentaro Hara[1], Chikako Nagatoshi[1], Akiko Fujimoto[1], Masatoshi Ishizaki[1], Ryoichi Kurisaki[1], Yasuto Nishida[1], Hidetsugu Ueyama[1]

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<Background> Local anesthesia is the anesthetic of choice for muscle biopsies, but it does not control the severe pain associated with cutting muscle tissue. General and spinal anesthesia can significantly reduce pain, but there is a risk of anesthesia-related complications in neuromuscular diseases. Ultrasound-guided nerve block may reduce the risk of anesthetic complications and pain during muscle biopsy. <Objective> We aimed to investigate the effect of ultrasound-guided nerve block on pain reduction and safety during muscle biopsy.

<Methods>We evaluated the biopsy site, nerve block site, type of anesthetic, anesthetic dose, time from start of nerve block to start of biopsy, biopsy time, pain during muscle biopsy, and anesthetic complications in nine cases of muscle biopsy using ultrasound-guided nerve block from April 2022 to April 2024. <Results> Brachial plexus block was chosen for 5 patients with biceps brachii biopsy and femoral nerve block for 4 patients with quadriceps biopsy. The anesthetic used was 1% xylocaine in 8/9 patients. The mean time from start of nerve block to start of biopsy was 14.7 minutes. There were no complications such as nerve or vascular injury or infection associated with the nerve block, and only 1/9 of the patients complained of pain during the biopsy (visual analogue scale was 3/10).

<Conclusion>Ultrasound-guided nerve block is safe and significantly reduces pain during muscle biopsy.

## P-5

# Empowering Progress: Patient Registries and Digital Transformation in Neuromuscular Disorders

Anna Cho[1], Yun Jeong Lee[2], Jung Hwan Lee[3], Jin-Hong Shin[4], Dajung Kim[5], Yonghyun Kim[5], Jong Hee Chae[6,7]

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Research on neuromuscular disorders has advanced through gene discoveries, with some gene therapies already in clinical use and trials ongoing. Establishing patient registries is crucial now for quicker diagnosis, improved care, enhanced collaboration, and accelerated research and treatment development. The electronic patient-reported outcome (ePRO) and electronic case report form (eCRF) systems are widely applied in many clinical researches with their ability to provide cost savings, longitudinal data tracking, and flexibility. They enhance clinical and research operations through efficient data collection, accuracy, real-time data accessibility, increased patient engagement, and data integration.

We have established a registry for Korean patients with neuromuscular disorders by integrating ePRO and eCRF through a mobile platform. The registry encompasses Duchenne muscular dystrophy (DMD), spinal muscular dystrophy (SMA), congenital muscular dystrophy, congenital myopathy, and congenital myasthenic syndrome. The database including both clinical and molecular genetic data has been constructed in the form of an eCRF. For the global data integration, the information for DMD and SMA has been merged into the TREAT-NMD Core Datasets. At the time of initial registration, patients install a mobile ePRO application, simultaneously completing survey data collection. The survey information includes demographic details, current health status, and willingness to participate in clinical trials, and consent for enrollment in the TREAT-NMD Global Registry Network. These data are synchronized with the eCRF system through the patient-specific registry numbers. Combining ePRO with eCRF enhances the depth and quality of information by directly involving patients in reporting their data. This comprehensive approach not only enhances data management efficiency but also maximizes the benefits of the registry through the establishment of a two-way communication system.

## P-6

# Two cases of female patients with Duchenne muscular dystrophy caused by DMD gene mutation

Shiqi Yang[1], Jihang Luo[1], Yidan Liu[1], Cuijie Wei[1], Hui Xiong[2]
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[2]Beijing Children's Hospital, Capital Medical University, China

Objective: Dystrophinosis (dystrophinopathy) is a group of myopathies caused by DMD gene mutations, including Duchenne/Becker muscular dystrophy (Duchenne muscular dystrophy,DMD/Becker muscular dystrophy,BMD), etc. DMD gene encodes dystrophin (dystrophin). Among them, the most severe disease is the Duchenne type (DMD), and the lighter is the Becker type (BMD). Two female patients with Duchenne muscular dystrophy caused by DMD gene variant are reported.
Methods: The clinical characteristics of two female patients with DMD caused by DMD gene mutation were analyzed and summarized.
Results: Patient 1,6 years and 6 months old,was admitted to our hospital at 3 years and 10 months because of "3 months of creatine kinase elevation"; Patient 2,5 years and 3 months old,was admitted to our hospital at 2 years and 3 months because of "2 years of creatine kinase elevation". The patient 1 has good motor development and weakness of both lower limbs after 7 months. The patient 2 has delayed motor development, walking alone at the age of 1 year and 4 months, running at the age of 1 year and 8 months, not jumping, and holding up and down stairs at the age of 1 year and 6 months. The language, intelligence, vision and hearing of the two patients were normal, and no seizures occurred.Their parents were not married to close relatives, and they had no family history of neurogenetic diseases. Physical examination: two cases had gastrocnemius pseudohypertrophy, Gowers sign positive, the rest of the nervous system examination were negative. Auxiliary examination: patient 1 creatine kinase (CK)10986IU/L, patient 2 creatine kinase (CK)20439IU/L. Muscle MRI of patient 1 showed patchy slightly hyperintensity in lipid compression sequence and diffusion sequence of muscle groups in anterior, external and posterior groups of left thigh. And patient 2 showed mild fat infiltration and obvious muscle edema. Muscle biopsy in patient 2 revealed muscular dystrophy-like pathological changes, with neutrophil infiltration, negative expression of most muscle fibers at the Dystrophin-N and C ends. Genetic testing revealed duplication of patient 1 DMD gene exon18-20 of unknown origin; patient 2 DMD gene heterozygous variation c.575_576ins TATT maternal origin. The X chromosome inactivation test found that 85% of the X chromosome was non-random inactivation, which was a moderate inactivation deviation.
Conclusion: This paper summarizes the clinical characteristics of two female patients with Duchenne muscular dystrophy caused by DMD gene mutation, and further deepens the understanding of the clinical phenotype of the disease caused by DMD gene mutation.

## P-7

# Activities for patient involvement in Becker muscular dystrophy in Japan

Hiroyuki Shibasaki[1,2], Masaru Torigoe[1], Hikaru Endo[1], Yuko Fukue[1], Yoshitsugu Aoki[2], Akinori Nakamura[3], Hirofumi Komaki[4], Hisanobu Kaiya[1], Tamotsu Takeda[1]

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Japan Muscular Dystrophy Association (JMDA) is the organization that has been active nationwide since 1965, to support patients with neuromuscular diseases and their families and promote the welfare of them, as well as contribute to the establishment of treatment and their research and development. Recently, Patient and Public Involvement (PPI) in research has been internationally important, and it is necessary to promote PPI for each type of muscular dystrophy, which is intractable and rare diseases. On the other hand, there was no organization for Becker Muscular Dystrophy (BMD), which is a dystrophinopathy caused by sarcolemmal dystrophin deficiency due to DMD gene pathogenic variants and has poor prognosis and high variability in symptoms, signs, and rate of disease progression.

Therefore, in order to promote PPI for BMD, we established the group for BMD patients in JMDA two years ago, with the BMD specialist and researcher as advisor, and focused on initiatives that contributed to PPI. For BMD patients to understand their disease medically and socially, we provided opportunities for interaction between patients, disseminated information on BMD and their research and development by our website and seminars.

As a result, it was found that interactions between patients functioned as peer support, and that there were many problems and questions regarding social life, systems, genetics, and cardiac function tests. The website and seminars focused especially on helping patients accurately and objectively understand the results of the BMD natural history research, which led to improved QOL of medical and lifestyle, and motivation for PPI.

Through the activities of the patient group, the patient literacy is considered to be an important foundation for promoting PPI, as there are few places to provide accurate information and few clinical trials for BMD. In the future, we would like to promote research and development of treatments by improving the patient literacy through continued patient group activities, and by collaborating with clinicians and researchers to disseminate the accurate and objective information on BMD needs.

## P-8

# Integrated Ayurveda and Yoga Intervention for Enhancing Functionality and Ambulation in Patients with Muscular Dystrophies: A Single-arm Pilot Feasibility Trial

Dr Umesh Chikkanna[2], Dr. Hemanth Bhargav[3], Dr. Nalini Atchayaram[4],
Dr. Kishore Kumar Ramakrishna[1], Dr. Bharath Holla[5]

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[5]Dr. Bharath Holla, Associate Professor, Department of Neurology, NIMHANS, Bengaluru, India

Muscular dystrophies (MD) are a genetically and clinically heterogeneous group of neuromuscular diseases that cause progressive weakness and skeletal muscle breakdown. Although the current standard of care includes corticosteroids and physical therapy, add-on integrative and traditional medicine has been increasingly sought. This single-arm pre-post study assessed the feasibility and potential benefits of add-on integrated ayurveda and yoga treatment (IAYT) protocol in patients with muscular dystrophy.

Twenty-two patients diagnosed with MD (two patients with Becker's muscular dystrophy, four patients with limb-girdle muscular dystrophy, and sixteen patients with Duchenne muscular dystrophy) on conventional treatments were recruited. The IAYT, comprising of Ayurveda (Panchakarma procedures) and Yoga (Set of asana and pranayama), was administered to the patients in an in-patient setting for 21 days. Functional motor outcome measure and functional ambulation profile (FAP) were assessed using North Star Ambulatory Assessment (NSAA) scale and GAITRite device (For spatiotemporal gait parameters' measurement), respectively, at the baseline, on the 21st day and after six months.

The mean age of the patients was 13.40 + 9.34 years. NSAA scores showed significant improvement in functionality ($p < 0.05$) at 21 days, which was sustained till follow-up. FAP scores showed a slight improvement in stride length and walking velocity but were not statistically significant. The protocol was observed to be safe and feasible, and no deterioration was reported. The study suggests that this protocol can aid in improving MD patients' gait disturbances and activities of daily living. However, a randomized controlled design with larger sample sizes and longer follow-ups is warranted to comment on the sustained effects of IAYT in MDs.

## P-9

# Natural history of Becker muscular dystrophy in a Japanese national registry of muscular dystrophy

Hotake Takizawa[1], Madoka Mori-Yoshimura[1], Yuji Takahashi[1], Wakako Yoshioka[2], Ichizo Nishino[2], Harumasa Nakamura[3]

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Objective: The objective of this study was to analyze the genotype and phenotype of Becker muscular dystrophy (BMD) using data from Remudy, a Japanese neuromuscular disease patient registry system. Methods: A total of 270 male patients with dystrophin abnormalities and confirmed mutations in the DMD gene who were ambulatory at 17 year of age and enrolled in Remudy from July 2009 to January 16, 2023, were analyzed with respect to genotype, history of steroid use, gait function, cardiac function, and respiratory function. Results: The mean age at enrollment in Remudy was 29.4 ± 15.9 years (range 5-76, median 27). The mean duration of enrollment was 6.0 ± 4.2 years (range 0-13.1, median 6), and the mean number of updates was 4.0 ± 3.0 (range 0-12, median 3). The most prevalent genetic mutation was exon deletion accounting for 72.2% of cases, followed by point and other minor mutations constituted 20.7% of cases, duplications accounted for 6.7%, and combined deletions and duplications accounted for 0.4%. Among the patients, 10.4% were using steroids at the time of enrollment, 2.6% had previously used steroids but were not currently using them, and 87.0% had never used steroids. At enrollment, 80.0% of participants were ambulatory. The median age at loss of ambulatory function was 54 years. Of the 146 patients, excluding the 124 with unmeasured respiratory function, 46 (31.5%) had a %FVC < 80 or were on a ventilator at enrollment. The median age of decline in respiratory function was 56 years. At enrollment, 106 of 268 patients (39.6%) had a left ventricular ejection fraction of less than 55% or were diagnosed with of heart failure by their physician. The median age at which cardiac function was impaired was 41 years. Patients with exon deletion mutations had a more favorable prognosis for ambulatory, respiratory, and cardiac function than those with exon duplications or small mutations. Conclusion: The results of our study indicate that patients with BMD exhibit phenotypic diversity related to genetic variation.

# Poster Session 2

## Genetic analyses and identification of the variants

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Satoru Noguchi (Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)
Seung Ah Lee (Department of Neurology, Ewha Womans University Mokdong Hospital, Republic of Korea)

### P-10

### The burden of consanguineous marriages in Swat Valley, Pakistan

AMJAD IQBAL, IRFAN ULLAH, Rahmat Ullah, Zaheer Udin, Ubaid Ullah, Zeeshan Khan, Umapathi N.Thirugnanam, Wasil Khan, Mian Ayazul Haq, Muhammad Ayub Khan
Saidu Group Of Teaching Hospital, Swat, Pakistan

INTRODUCTION
Consanguineous marriages between closely related family member are common in many underserved regions of the global south, including Pakistan. Attempts to reduce this and alter long-established cultural norms are hampered by the lack of pre-marital genetic counselling and testing in these countries
Method:
This is a cross-sectional study from Sept 2023 to May 2024 . All patients presenting to the out-patient department of Swat General Hospital with suspected genetic neurological disorders have been included in this study. Patients underwent a thorough clinical and electrodiagnostic evaluation. Acquired, especially treatable, diseases are ruled out by simple investigations that are available in this hospital. Every patient answers a formal questionnaire with detailed clinical, demographic, and family information.
Results and discussion:
A total of 145 patients have been included in this study. 92 patients have consanguineous parents ‘
Of these 28 patients have peripheral neuropathy, 23 myopathy, 5 channelopathy, and 14 hereditary spastic paraparesis. Central nervous system disorders include leukodystrophies (n=12), ataxias (21), movement disorders (31) and epilepsy (11).
Conclusion; -63% of genetic diseases are related to consanguinity. Tackling intra-family marriages is therefore a “low -lying fruit”- an inexpensive health-care intervention to reduce the burden of neurological diseases in under-resourced regions like Swat Valley, Pakistan.

## P-11

## Aberrant mRNA processing caused by splicing mutations in TTN-related neuromuscular disorders

Pengfei Lin, Guangyu Wang, Wenjing Wu, Xiaoqing Lv, Chuanzhu Yan
Qilu Hospital of Shandong University, China

Mutations in the TTN gene have been reported to be responsible for a range of neuromuscular disorders, including recessive distal myopathy and congenital myopathy (CM). Only five splicing mutations have been identified to induce aberrant mRNA splicing in TTN-related neuromuscular disorders. In our study, we described detailed clinical characteristics, muscle pathology and genetic analysis of two probands with TTN-related autosomal recessive neuromuscular disorders. Besides, we identified two novel intronic mutations, c.107377+1 G > C in intron 362 and c.19994-2 A > G in intron 68, in the two probands. Through cDNA analysis, we revealed the c.107377+1 G > C mutation induced retention of the entire intron 362, and the c.19994-2 A > G mutation triggered skipping of the first 11 bp of exon 69. Our study broadens the aberrant splicing spectrum of neuromuscular disorders caused by splicing mutations in the TTN gene.

## P-12

## Novel compound heterozygous mutations in the TTN gene: elongation and truncation variants causing limb-girdle muscular dystrophy type 2J in a Han Chinese family

Pengfei Lin, Guangyu Wang, Xiaoqing Lv, Ling Xu, Rui Zhang, Chuanzhu Yan
Qilu Hospital of Shandong University, China

Limb-girdle muscular dystrophy (LGMD) is a group of clinically heterogeneous muscle disorders commonly manifesting proximal limb girdle muscle weakness. There have been more than 30 subtypes of LGMD associated with causative genes and limb-girdle muscular dystrophy type 2J (LGMD2J) is caused by mutations in the TTN gene. We report a Han Chinese family with LGMD2J. The proband and his sister both presented with weakness in the
proximal lower limbs bilaterally. Muscle biopsy and genetic analysis were performed. Muscle biopsy of the proband showed dystrophic changes accompanied by rimmed vacuoles. Whole-exome sequencing identifed novel compound heterozygous mutations in the TTN gene, including elongation (c.107962_107963delAT, p.I35988Sfs*26) and truncation (c.99125_99128dupACAG, p.S33043Rfs*9) variants in the proband and his sister. Both two variants have never been reported. Notably, we are the frst to identify an elongation mutation in the TTN gene, broadening the genetic mutation spectrum of LGMD2J. Several variants in the last exon of the TTN gene have been reported, one of which was associated with LGMD2J. Besides, LGMD2J should be distinguished from other myopathies caused by mutations in the TTN gene. The pathogenesis of and specifc curative methods for LGMD2J remain to be further elucidated.

## P-13

# The Clinical Features and TTN Mutation Spectrum in a Chinese Cohort of Patients with hereditary myopathy with early respiratory failure

Pengfei Lin[1], Xiaoqing Lv[2], Yuebei Luo[4], Zhe Zhao[3], Chuanzhu Yan[1], Wei Lv[5]

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Hereditary myopathy with early respiratory failure (HMERF) typically presents in adulthood. Fifteen disease causing variants are recorded in the Human Gene Mutation Database ; these variants have been reported from various countries, demonstrating a wide geographical distribution. Here, we report 13 Chinese patients diagnosed with HMERF and summarize their clinical and pathological features. Our findings will help expand the clinical and genetic spectrum of HMERF in China.

We retrospectively analyzed the clinical findings, findings of muscle imaging, targeted next-generation sequencing, muscle histology, immunohistochemistry, and electron microscopy of 13 patients diagnosed with HMERF at three center in China. In addition, we searched the PubMed and China National Knowledge Infrastructure (CNKI) databases to search and collect data from previous reports pertaining to Chinese patients with HMERF.

The findings were compared with those of previously reported patients. In Chinese patients with HMERF, the presenting symptom was either muscle weakness (8/13) or respiratory failure (2/13); the mean age at onset was 33.33± 15.68 years (mean± SD, range 14–54). Genotype c.95195C>T is very common and it is a hot spot mutation in China. The clinical manifestation of c.95195C>T was milder than c.95358C>G or c.95358C>A (p.N31786K); the phenotype of c.95135G>A showed relatively mild manifestations in China.

Muscle MRI showed involvement of erector spinae, semitendinosus and anterior muscles of calf in four patients (Figure 1).

The common pathological features were fiber variation, increase in internal nuclei and endomysial fibrosis, rimmed vacuoles and cytoplasmic bodies. On immunohistochemical examination, the cytoplasmic bodies stained positive for calpain-3, p53, and programmed death ligand 1 (Figure 2). Electron microscopy showed layering as well as edema of the capillary basement membrane, swelling of capillary endotheliocytes, cytoplasmic bodies, distorted sarcomere architecture, glycogen pool, and subsarcolemmal accumulation of mitochondria and lysosomes (Figure 2).

Detection of necklace-like cytoplasmic bodies on muscle biopsy in combination with respiratory failure is highly suggestive of HMERF; such patients should be subjected to genetic testing to confirm the diagnosis. In this study, we characterized the clinicopathological features of 13 Chinese patients with HMERF, which helps expand the phenotype and genotype spectrum of HMERF in China. Genotype c.95195 C>T is very common in China. Some of the patients developed respiratory failure as the initial symptom and consulted physicians in the respiratory department. This indicates that physicians in the respiratory department should have a high suspicion index for HMERF in patients who present with type II respiratory failure. Further studies are required to elucidate the precise cause of HMERF.

## P-14

# Identification of deep intronic pathogenic variants in autosomal recessive muscle disorders by in silico splicing prediction

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Introduction: After the development of next-generation sequencers, the genetic search for hereditary diseases mainly focused on exonic regions of the genes. However, we were able to diagnose only 50% of the patients through exonic analysis, suggesting the presence of pathogenic variants within deep intron. However, the identification of pathogenic variants in deep intronic regions is still challenging. (In particular, it is very difficult to identify those in the autosomal recessive cases. For example, in compound heterozygotes with one exonic missense variant and another deep intronic variant, the transcript derived from the allele with intronic variant has aberrant splicing and often undergoes nonsense mediated RNA decay. The SpliceAI, a new in silico tool, is widely used to predict splicing consequence from genetic variants. This study aims to identify the deep intronic pathogenic variants leading to splicing abnormalities by using SpliceAI. We focused on the cases in which biallelic disorders were suspected and one missense variant were identified.

Method: We selected patients who were suspected to suffer from an autosomal recessive disorder based on clinical information and identified one exonic variant in the candidate genes. The cohort included 23 patients diagnosed with Limb-Girdle Muscular Dystrophy (7), rhabdomyolysis (7), nemaline myopathy (3), centronuclear myopathy (2), merosin-deficient congenital muscular dystrophy (1), lipid storage disease (1) and sarcoglycanopathy (1). SpliceAI analysis was applied to genome data of the corresponding genes, setting a delta-score threshold of >0.2 as indicative of splicing alterations. RNA-seq was also performed on frozen muscle samples.

Result: Among the 23 cases, a total of 83 intronic variants were identified in the corresponding genes (DYSF, CAPN3, ENO3, LAMA2 and RYR1) in 11 patients. Of these, 3 variants in DYSF, LAMA2, or RYR1 gave high delta scores for splicing alterations in SpliceAI prediction, all of which induced pseudo-exon activation or alternative splicing-acceptor site generation on RNA-seq. The remaining 80 variants showed no significance in SpliceAI and revealed no mRNA structural changes on RNA-seq.

Conclusion: SpliceAI effectively identified the variants causing splicing changes in candidate genes without any false-positive and false-negative findings. This highlights its utility in pre-evaluation of the identified variants using only whole genome sequencing data, before applying RNA analyses. Moreover, this tool c ould be instrumental in discovering novel causative genes.

## P-15

## The Clinical Features and TCAP Mutation Spectrum in a Chinese Cohort of Patients with Limb-girdle Muscular Dystrophy R7

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Limb-girdle muscular dystrophy R7 (LGMDR7, OMIM 601954), also known as limb-girdle muscular dystrophy 2G, is a subtype of progressive muscular dystrophy. In this study, we screened 30 Chinese LGMDR7 patients to determine the clinical, pathological, mutational, and radiological spectrum of LGMDR7 patients in China.

We screened 30 LGMDR7 patients from Peking University First Hospital, Huashan Hospital, Qilu Hospital, China-Japan Friendship Hospital, Fujian Medical University, and Kaohsiung Medical University Hospital. This cohort included two previously reported patients from Xiangya Hospital, six patients from Qilu Hospital of Shandong University, four patients from Kaohsiung Medical University Hospital, one patient from China-Japan Friendship Hospital and four patients from Fujian Medical University. The patient inclusion criteria included the following: (1) progressive muscle weakness; (2) DNA sequencing revealed recessive mutations in TCAP; and (3) myopathic changes in muscle biopsy. All recruited patients met criteria 1 and 2, with or without 3. Patient performed CK value detection in the hospital. We collected the detailed clinical findings, muscle strength results, CK values, magnetic resonance imaging (MRI) examination results, pathological characteristics, and genetic analysis results for the 30 patients. Muscle strength was assessed by the Medical Research Council (MRC) grading scale.

We searched the PubMed database for previous reports about LGMDR7 patients in foreign countries. The keywords used for the database search were as follows: [LGMDR7/limb-girdle muscular dystrophy R7/ limb-girdle muscular dystrophy]. We reviewed detailed information about onset age, CK level, ethnicity, and mutations.

This is the largest scale report on LGMDR7 in the Chinese population and in the world. We analysed the clinical and genetic findings of 30 Chinese LGMDR7 patients, the radiological findings in six patients and the morphopathological findings in 16 patients. The c.26_33dupAGGTGTCG, c.165dupG, and c.110+5G>A mutations are common mutations in the Chinese population. Compared to the other populations, the age of onset occurred later in the Chinese population. Furthermore, we summarized the typical morphological changes in Chinese LGMDR7 patients, such as internal nuclei, lobulated fibres, and scattered rimmed vacuoles. These findings further expand the clinical, pathological, and genetic characteristics of Chinese LGMDR7 patients.

## P-16

## Aberrant mRNA processing caused by splicing mutations in TTN-related neuromuscular disorders

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The TNNT1 gene encoding the slow skeletal muscle TnT has been identified as a causative gene for nemaline myopathy. TNNT1 nemaline myopathy is mainly characterized by neonatal-onset muscle weakness, pectus carinatum and respiratory insufficiency. Herein, we report on a Chinese girl with TNNT1 nemaline myopathy with mild clinical phenotypes without thoracic deformities or decreased respiratory function. Muscle biopsy showed moderate to marked type 1 fiber atrophy and nemaline rods. Next-generation sequencing identified the compound heterozygous c. 587dupA (p. D196Efs*41) and c. 387+5G>A mutations in the TNNT1 gene according to the transcript NM_003283.4. RNA sequencing revealed complete exon 9 skipping caused by the c.387+5G>A mutation. Through quantitative PCR, we found that both the truncation c. 587dupA (p. D196Efs*41) and the splicing c.387+5G>A mutations triggered nonsense-mediated mRNA decay (NMD). Western blotting showed the residual amount of the truncated TNNT1 protein by deletion of exon 9, which may ameliorate the disease to some extent.

## P-17

## First reported case of distal arthrogryposis type 2A in Korea with genetic confirmation

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Introduction: Distal arthrogryposis type 2A, known as Freeman-Sheldon syndrome (FSS), is caused by pathogenic variants in MYH3. It is marked by multiple congenital contractures in the upper and lower limbs, as well as the facial muscles. And it is the most severe form of distal arthrogryposis. Patients with distal arthrogryposis type 2A display unique facial features such as small oral openings, prominent nasolabial folds, sunken eyes, a high narrow palate, and an H-shaped chin dimple. Here, we present a Korean patient with distal arthrogryposis type 2A.

Case Report: A 36-year-old male was referred to the neurology department due to progressive limb motor weakness and respiratory difficulties. His mother's sister had died at the age of 10 from cardiomyopathy but was not diagnosed with distal arthrogryposis. After birth, he exhibited facial anomalies and clubfoot. He was evaluated at another hospital for progressive weakness and congenital anomalies, and subsequently underwent surgery to correct limb deformities. Despite the surgery, his symptoms gradually worsened, and he developed dilated cardiomyopathy at age 15. At that age, he also presented with respiratory difficulty and underwent a tracheostomy. By the age of 20, he required a ventilator. Physical examination revealed contractures in both hands and feet, a small mouth (microstomia) with pursed lips, ocular hypertelorism, micrognathia, clubfoot, generalized muscle atrophy, and proximal dominant weakness with low forced vital capacity. His serum creatine kinase (CK) level was normal. Electrodiagnostic studies showed axonal neuropathy. We conducted targeted sequencing for multiple neuromuscular genes and identified a pathogenic variant (c.2015G>A, pArg672His) in MYH3.

Conclusion: This patient exhibited the clinical and genetic characteristics of distal arthrogryposis type 2A. Our case represents the first reported instance of distal arthrogryposis type 2A in Korea with genetic confirmation.

## P-18

## Deletion of exons 6-9 of the ISPD gene causes congenital muscular dystrophy in mice

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OBJECTIVE: The ISPD gene (OMIM: 614631) encodes cytidine diphosphate ribitol synthase (CDP-L-ribitol pyrophosphorylase A), which is one of the causative genes of dystroglycanopathy (DGP), a disease associated with anti-myasthenia gravis. In the preliminary study of our group, the deletion of exon 6-9 of ISPD gene was found to be a founder mutation. In order to investigate the effect of ISPD exon 6-9 deletion on the enzymatic activity of ISPD and the mechanism leading to the occurrence of DGP, we constructed a mouse model of ISPD exon 6-9 deletion and carried out a preliminary study on the mouse model.
METHODS: The ISPD exon 6-9 deletion mouse model was constructed using CRISPR/Cas9 technology, and the preliminary study of the mouse model was conducted.
RESULTS: (1) ISPD exon 6-9 deletion mouse model was successfully constructed using CRISPR/Cas9 technology. The ISPD exon 6-9 deletion mouse model was successfully constructed using CRISPR/Cas9 technology. The sgRNAs were designed in intron5 and intron9, respectively, and large fragments knocked out exons 6-9 in mice, and the deletion resulted in a code-shift mutation with a knockout size of about 47.5 kb. (2) The WB and qPCR results of the C-terminal end of ISPD confirmed the absence of the C-terminal structural domain in KO mice, demonstrating that the model is a C-terminal deletion mouse model, and the WB and qPCR results of the N-terminal of ISPD confirmed that the N-terminal structural domain was unaffected by the C-terminal knockout. (3) WB and immunofluorescence staining of α-DG confirmed that KO mice were congenital muscular dystrophy mice caused by α-DG deficiency.
CONCLUSION: In this paper, the ISPD exon 6-9 deletion mouse model was constructed through the basis of previous research and preliminary studies were conducted on this model.

## P-19

## Clinical and molecular genetic analysis further delineates the phenotypic variability of POMT2-related limb girdle muscular dystrophy type R14

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Background The POMT2 gene, which encodes protein O-mannosyltransferase 2, is crucial for the initial stage of α-dystroglycan glycosylation. Mutations in POMT2 are responsible for severe congenital muscular dystrophies, such as Walker-Warburg syndrome, muscle-eye-brain disease, and limb-girdle muscular dystrophy R14 (LGMDR14). This article retrospectively analyzed the clinical, pathological and genetic data of three patients diagnosed with LGMDR14. Aberrant mRNA processing analysis was subsequently conducted to investigate the pathogenic mechanism of the mutation. We employed innovative bioinformatic techniques of molecular dynamics to assess the impact of identified POMT2 mutations on protein structure and function and sought to establish a connection between these variants and their phenotypic manifestations.

Results Three LGMDR14 patients from unrelated Chinese families were recruited. Adult onset age and proximal muscle weakness were common in our patients. They all showed myopathic lesions on electromyography and decreased α-dystroglycan expression on muscle biopsy. One patient was found to have severe cardiomyopathy and mild cognitive impairment, highlighting the importance of monitoring both the heart and brain activity. Genetic sequencing revealed that patient 1 harbored compound heterozygous c.1006+1G>A and c.295C>T variants of the POMT2 gene, patient 2 harbored c.1261C>T and c.700_701insCT variants, and patient 3 harbored c.812C>T and c.170G>A variants. Among these mutations, the variants c.700_701insCT, c.812C>T and c.170G>A have never been reported before. RNA sequencing revealed that the c.1006+1G>A mutation could cause retention of the first 26 bp of intron 8 by inducing recognition of new donor splice sites. Pyrosequencing revealed that both the frameshift mutation c.700_701insCT and the splicing mutation c.1006+1G>A triggered nonsense-mediated mRNA decay. Molecular dynamics indicated that the c.1006+1G>A, c.700_701insCT and c.170G>A variants could generate truncated protein structures and change the stability and function of the POMT2 protein.

Conclusions Our study summarizes the clinical and genetic characteristics of three newly diagnosed adult-onset LGMDR14 patients, expanding the genetic spectrum of POMT2 mutations. Moreover, this study also broadens the understanding of the anomalous mRNA regulation of POMT2 caused by splicing mutations and introduces a new bionformatic method for predicting the effect of POMT2 variants on protein structure using molecular dynamics.

## P-20

## Aberrant splicing caused by three intronic mutations in autosomal recessive limb-girdle muscular dystrophy-1

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Autosomal recessive limb-girdle muscular dystrophy-1 (LGMDR1) is the most common subtype of autosomal recessive limb-girdle muscular dystrophy caused by biallelic mutations in the CAPN3 gene. Hundreds of mutations have been identified in LGMDR1 patients, and some studies demonstrated aberrant splicing as pathogenic mechanism in LGMDR1 patients carrying intronic mutations. Here, we describe three LGMDR1 probands carrying three distinct splicing mutations: c. 2185-14T>G in intron 20, c. 1193+30G>A in intron 9 and c. 1194-9 A>G in intron 9 in the CAPN3 gene. The c. 2185-14T>G and c. 1193+30G>A mutations were first reported. Through RNA analysis, we found the three splicing mutations induced aberrant splicing of the CAPN3 mRNA. Notably, the c. 2185-14T>G mutation was located in the polypyrimidine tract of intron 20 and induced pseudoexonization of the entire intron 20. Quantative PCR assay showed reduced CAPN3 mRNA level in two probands, suggesting the aberrant splicing triggered the nonsense mediated mRNA decay. Western blotting showed reduced expression of both 94-kDa and 60-kDa calpain 3 bands in the two probands. Our study broadens aberrant splicing spectrum of CAPN3 mRNA caused by intronic mutations.

## P-21

## Phenotype-driven variant prioritization and re-analysis enhances genetic diagnosis of Neuromuscular Disorders

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Background and aims:
Neuromuscular disorders (NMDs) represent a group of diseases that are both clinically and genetically diverse. With the continued decrease in the cost of genome sequencing, over 680 genes have been identified for this group of diseases. This enabled comprehensive genomic analysis but also complicated variant pathogenicity interpretation, making variant prioritization a critical step in genomic analysis. Conventional variant prioritization relied only on genotype calls and may not be effective for large scale analysis. In this study, we implemented an approach of phenotype-driven variant prioritization for the identification of disease-causing variants in a NMD cohort collected in Hong Kong.

Methods:
Whole genome sequencing was performed on a cohort of 32 patients, with parental sequencing performed for consented families. These patients had negative findings in previous NMD gene panels or whole exome sequencing. Leveraging deep phenotype profiling from patient clinical records, we initially used Exomiser to prioritize candidate variants based on conventional filters and genotype-phenotype correlations under different inheritance models. With the hope of maximizing diagnostic yield, the sequencing data was subsequently re-analysed using more updated versions of ClinVar and variant interpretation databases.

Results:
Our phenotype-driven variant prioritization approach effectively ranked the disease-causing genes among the top three in 13 cases (40.6%) by phenotypic consistency, including 6 cases of TTN and one case of CACNA1S, DMD, MFN2, POMT1, TBCK1, COL6A2 and LAMA2 each. Subsequent re-analysis of the sequencing data revealed an additional 4 pathogenic variants in TTN in 2 patients. For instance, previous Varsome pathogenicity predictions incorrectly regarded an A to C transversion in TTN as a variant of unknown significance (VUS) because of an incorrect assumption of the possible mode of inheritance of TTN. This was rectified in the more updated version of Varsome where both dominant and recessive inheritance patterns were considered, together with a more updated ClinVar database.

Conclusions:
Our study underscores the significance of phenotype-driven variant prioritization in routine genome sequencing data analysis, particularly in the context of rapidly expanding NMD gene list. This approach effectively prioritized candidate variants with high phenotypic consistency, offering a valuable strategy to overcome the complexity of large variant pools in NMD genes. Furthermore, we demonstrated the importance of routine re-analysis of genome sequencing data using more up-to-date resources, providing opportunities to maximize the diagnostic yield. By incorporating both phenotype-driven prioritization and re-analysis of genomic data against updated resources, genome sequencing holds promise to alleviate the diagnostic odyssey and significantly improve patient care.

## P-22

## Novel *TFG* mutation causes autosomal-dominant spastic paraplegia and defects in autophagy

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Background Mutations in the tropomyosin receptor kinase fused (*TFG*) gene are associated with various neurological disorders, including autosomal recessive hereditary spastic paraplegia (HSP), autosomal dominant hereditary motor and sensory neuropathy with proximal dominant involvement (HMSN-P), and autosomal dominant type of Charcot-Marie-Tooth disease type 2 (CMT2).

Methods Whole genome sequencing (WGS) and whole-exome sequencing (WES) was utilized, followed by Sanger sequencing for validation. Haplotype analysis was performed to confirm the inheritance mode of the novel *TFG* mutation in a large Chinese family with HSP. Additionally, another family diagnosed with HMSN-P and carrying the reported *TFG* mutation was studied. Clinical data and muscle pathology comparisons were drawn between HSP and HMSN-P patients. Furthermore, functional studies using skin fibroblasts derived from HSP and HMSN-P patients were conducted to investigate the pathomechanisms of *TFG* mutations.

Results A novel heterozygous *TFG* variant (NM_006070.6: c.125G>A (p.R42Q)) was identified and caused pure HSP. We further confirmed that the well-documented recessively inherited spastic paraplegia, caused by homozygous *TFG* mutations, exists in a dominantly inherited form. Although the clinical features and muscle pathology between HSP and HMSN-P patients were distinct, skin fibroblasts derived from both patient groups exhibited reduced levels of autophagy-related proteins and the presence of TFG-positive puncta.

Conclusions Our findings suggest that autophagy impairment may serve as a common pathomechanism among different clinical phenotypes caused by *TFG* mutations. Consequently, targeting autophagy may facilitate the development of a uniform treatment for TFG-related neurological disorders.

# Poster Session 3

## DMD pathogenesis

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Shinichiro Hayashi (National Institute of Neuroscience, National Center of Neurology and Psychiatry, Japan)
Takahiro Fujimoto (Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Japan)

### P-23

### Investigation of dystrophin localization and function in dog and mouse sperm

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Duchenne muscular dystrophy (DMD) is an intractable X-linked muscular dystrophy caused by mutations in the *DMD* gene, which encodes Dystrophin. On the other hand, Dystrophin is expressed in several tissues other than muscles, such as the central and peripheral nervous system, kidney, sperm and testis. However, the localization and function of Dystrophin in sperm and testis, where Dystrophin and its homologue Utrophin are reported to be expressed, have been less elucidated. Interestingly, DMD model mouse mdx3CV, which lacks functional Dystrophin, is reported to exhibit infertility. Dystrophin has several isoforms (Dp), and Dp427 and Dp71 isoforms are expressed in sperm and testis. Recently, Dp427 has been reported to function in the Sertoli cells, suggesting Dystrophin has been involved in the spermatogenesis. However, the role of Dystrophin in sperm locomotion and fertilization is not known.
In order to analyze the function of Dystrophin in sperm, we observed the localization of the Dystrophin and Utrophin in the sperm in detail. We found that Dystrophin localized in the tail (flagellum) and the head (acrosome). Using several DMD mice with different Dp expression patterns, we compared the localization of these isoforms and examined the effect on the locomotive ability of sperm. We further analyzed the dog sperm, which has more similar shape to human sperm. We found that Dystrophin also localized in the acrosome in dog sperm, suggesting the conserved function of Dystrophin in sperm among species. As the next step, we plan to elucidate the molecular mechanism by which Dystrophin regulate sperm locomotion and fertilization.

## P-24

## Characteristics of the skeletal muscle in Duchenne muscular dystrophy model rat

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Introduction & Purpose: Duchenne muscular dystrophy (DMD) is known as inherited skeletal muscle disease which is the deficit of dystrophin. Progressive muscle disruption and muscle strength decrement are major symptoms. Mitochondria plays an important role in skeletal muscle homeostasis. However, it has been unclear how mitochondria contribute to pathological features in the DMD model rat. The purpose of this study is to assess mitochondrial respiratory function, biogenesis, and structure using DMD model rats.
Methods: Male 15-week Wistar-Imamichi rats were used in this study. DMD rats were dystrophin-deficient rats generated by the CRISPR/Cas9 technique. For muscle functional assessments, the right triceps muscles were subjected to measure muscle endurance. Torque measurement was used torque dynamometer originated from our laboratory. After torque measurement, the left gastrocnemius muscle was harvested and used for biochemical analysis and/or mitochondrial respiratory function assessment. For observation of mitochondria morphology, rats were perfused through the left ventricle with 2% paraformaldehyde, 2.5% glutaraldehyde, and 0.1 M phosphate buffer [pH 7.4]. Results: There are no differences in body weight and muscle wet weights between both WT and DMD groups. In successive 10-times isometric contractions, the torque deficit of the DMD was larger than that of the WT ($p<0.05$). Compositions of myosin heavy chain (MHC) isoforms showed that fast-to-slow composition change occurred in DMD group ($p<0.05$). Mitochondrial oxygen consumption rate and reactive oxygen species (ROS)emission were impaired in DMD rats ($*p < 0.05$). There were significant differences in mitochondria dynamics protein. Especially, OPA1 (fusion) and DRP1 (fission) proteins in the DMD group were higher ($p<0.05$) than those of the WT group. In addition, morphological change in mitochondria was observed by using the electron microscope, in the DMD group. Conclusion: We found that fast-to-slow MHC change occurred in DMD rats. Changes in mitochondrial biogenesis and contents related protein expressions were not changed between WT and DMD rats. On the other hand, we found that high expressions of mitochondrial dynamics protein, concomitant with higher expressions of glycolytic enzymes. Also, ununiformed mitochondria, impairment of mitochondrial oxygen consumption, and increase of ROS emission were observed only in DMD rats. Thus, we conclude that impairment of skeletal muscle mitochondria function might contribute to pathological characteristics of DMD.

## P-25

## Generation and characterization of *DMD*-edited microminipigs: an advanced surrogate for Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is an intractable X-linked progressive muscle disease caused by mutations in the *DMD* gene, which leads to premature death due to respiratory and cardiac failure. Animal models such as mice, rats, and dogs have been commonly used in preclinical research on DMD pathogenesis and drug development. While these animal models are practical, it is crucial to consider species-specific differences between humans and animals. Therefore, a suitable surrogate that more accurately reflects human DMD is needed. Microminipigs, with their pronounced physiological similarity to humans and notably compact size amongst pig models, could offer a more representative model for human diseases. In this study, we accomplished precise *DMD* modification in microminipigs by co-injecting embryos with Cas9 protein and a single-guide RNA targeting exon 23 of *DMD*. The *DMD*-edited microminipigs exhibited pronounced clinical phenotypes, including perturbed locomotion and body-wide skeletal muscle weakness and atrophy, alongside augmented serum creatine kinase levels. Muscle weakness was observed at one month of age, respiratory and cardiac dysfunctions emerged by the sixth month, and the maximum lifespan was 30 months. Histopathological evaluations confirmed dystrophin deficiency and pronounced dystrophic pathology in the skeletal and myocardial tissues. Whereas, echocardiography in six- and 12-month-old *DMD*-edited microminipigs showed a progressive decrease in left ventricular ejection fraction compared to wild-type pigs, consistent with the clinical manifestations observed in human patients with DMD. These results indicated that the *DMD*-edited microminipig is an unprecedentedly suitable model for studying human DMD. The model stands as a distinct and crucial tool in biomedical research, offering a deep understanding of disease progression and enhancing therapeutic assessments, with the potential to influence forthcoming treatment approaches.

## P-26

## Muscle stem cells remain quiescent in Duchenne muscular dystrophy

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In Duchenne muscular dystrophy (DMD), the absence of dystrophin has been known to cause chronic muscle damage, impaired myofiber repair, fibrosis, and muscle weakness. However, the precise molecular mechanisms behind the pathogenesis of DMD and its effects on each cell type is still unclear. To shed light on this, we conducted a study using single nuclei RNA sequencing (snRNA-seq) on frozen muscle biopsy samples from both DMD patients (aged 0, 1, 5, 8, and 11 years) and controls (aged 7 and 10 years). Through unsupervised clustering analysis, we identified 10 distinct cell clusters which were present in both the DMD and control muscles with a concomitant decrease in the proportion of myofiber nuclei and an increase in most the other cell types in DMD. We analyzed the involvement of DMD-specific signaling pathways in each cell type and discovered that the DMD myonuclei displayed an increase in the Foxo signaling and atrophy-related gene expression, which is consistent with the histopathological analysis. Interestingly, we found that muscle stem cells were in a more quiescent state, as evidenced by the upregulation of quiescent marker genes in DMD muscle compared to control muscles. Furthermore, we peformed snRNA-seq on muscle tissues from D2-mdx mice, which exhibited a similar pathology to DMD. Consistent with the results of the DMD muscle, elevated Foxo signaling was observed in the D2-mdx myonuclei and muscle stem cells. Finaly, we found that treatment of D2-mdx mice with the Foxo inhibitor enhanced muscle stem cell activation and muscle regeneration. These findings offer new valuable insights that can lead to a better understanding of muscular dystrophy and guide future treatment strategies of satellite cell re-activation.

## P-27

## Dystrophin short product-specific tag-insertion transgenic mouse line

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Duchenne/Becker muscular dystrophy (DMD/BMD) manifest progressive muscular dystrophy and associate to non-progressive central nervous disorder. Physiological and pathological roles of the responsible gene, dystrophin, in the brain remain elusive due to the presence of multiple dystrophin products, mainly full-length dystrophin, Dp427, and the short product, Dp71. A major difficulty in dystrophin studies is that it is almost impossible to detect exclusively Dp71 by immunohistological techniques because the primary amino acid sequence of Dp71 except its hexapeptide at the NH2-terminus is identical with them of longer dystrophin products. Recently, we have generated a Dp71-specific hemagglutinin (HA) peptide tag-insertion mice to enable specific detection of intrinsic Dp71 expression by anti-HA tag antibodies. This transgenic mouse line is useful for histological and cytological analyses as well as biochemical interactome applications. Here, we introduce Dp71 expression profile and its interacting protein profile in the adult and embryonic mouse brains. We believe the novel Dp71 transgenic mouse provides valuable basic information to understand the role of Dp71 possibly relating to the central nervous disorder observed in DMD/BMD.

## P-28

## Genetic elucidation of the role of dystrophin isoforms in cognitive processes

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Focusing on Duchenne muscular dystrophy (DMD) and Becker muscular dystrophy (BMD), our study examines the neurological abnormalities and cognitive deficits often observed in affected patients. Despite the presence of several treatments for muscle symptoms, current strategies do not adequately address these challenges. The severity of neuropsychological impairment varies depending on the genomic region of the mutation and its effect on dystrophin isoform expression in the nervous system.

Our research takes a multifaceted approach. First, we use automated tracking system to perform behavioral phenotyping and characterize behavioral and cognitive abnormalities in three rat strains that serve as DMD/BMD models and are similar to human mutations targeted for gene therapy. Second, we identify non-invasive biomarkers, including brain imaging, tailored to specific mutation types. In addition, we identify cell types that express the dystrophin isoforms Dp427, Dp140 and Dp71/40, providing critical insights into the molecular landscape of neuronal deficits. In addition, we investigate mutation-specific molecular mechanisms at the cellular level to understand the complex pathways underlying cognitive impairment in DMD/BMD.

Our findings demonstrate anxious behavior in two DMD rat strains, correlating with reduced cerebellar metabolism and loss of Dp427 expression in Purkinje cells. This highlights the importance of further investigating the role of Dp427 in the molecular organization of the vermis, particularly in relation to anxiety disorders. In addition, Dp140 deficiency significantly affects socialization, with Dp140-dependent synaptic organization likely to play a key role.

Once therapeutic targets have been identified through molecular analysis, drug candidates will be tested in our various rat strains to assess their efficacy against each specific dysfunction.

## P-29

# Regional differences in telomere length and their association with disease progression in canine models of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an X-linked muscle disorder characterized by myofiber degeneration and regeneration. During the dystrophic course, muscle atrophy is progressively exacerbated owing to myofiber regeneration reduction, suggesting the involvement of cellular senescence associated with telomere genes . Telomere genes are specific repetitive sequences located at the ends of chromosomes that shorten with each cell division, and are responsible for cytostasis when shortened to limitation length. It has been postulated that muscle satellite cells as myogenic cells lapse telomere regulation by excessive turnover of myofibers in the dystrophic pathology, leading to deterioration of myogenesis. Shortening of telomere gene length in skeletal and cardiac muscles of DMD patients has been previously reported, but these relationships to pathogenesis remain unclear. In the present study, we examined telomere gene length of muscle samples in CXMDJ, a canine dystrophic model that has shown a severe phenotype similar to DMD, to reveal the relation with the disease course. Relative telomere gene length (RTL) was measured with real-time quantitative PCR in the diaphragm and tibialis cranialis muscles of dystrophic and healthy dogs (1-10 years of age, dystrophic; n=9 and 7, healthy; n=10 and 11 in the diaphragm and tibialis cranialis, respectively), and was analyzed correlation with age and histological fibrosis. There was no significant difference between RTL of dystrophic and healthy dogs, but a trend toward age-related shortening of RTL in the diaphragm of dystrophic dogs. We then compared RTL of skeletal muscles (diaphragm, temporalis, biceps femoris) and cardiac muscles (left and right ventricular and septum walls) derived from the identical dystrophic dog (10 years of age). We found that RTL was the greatest shortening in the diaphragm, showing similar level to those in both left and right ventricular walls. From these results, telomere gene length might shorten differently in muscle regions according to the severality and progressive phases. Real-time quantitative PCR in the present study was performed using DNA samples extracted from whole muscle tissue, and estimated total amount of telomere gene in entire tissue cells including a large number of fibroblasts in fibrosis, thus resulting in no significant difference. In order to target muscle satellite cells and myofibers, we have analyzed telomere gene length of specific cells by capturing signal intensity on tissue sections with fluorescence *in situ* hybridization.

## P-30

# Patient-derived iPSC brain organoids as a model for cognitive phenotypes of Duchenne Muscular Dystrophy

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Duchenne muscular dystrophy (DMD) is an early onset, X-linked neuromuscular disease chiefly characterised by skeletal muscle degeneration due to the loss of a vital structural protein, dystrophin. DMD gene mutations leading to early, out-of-frame stop codons or non-sense mutations, cause loss-of-function of dystrophin protein. In addition to loss of ambulation and cardiac deficits, ~30% of DMD patients exhibit cognitive deficits such as autism spectrum disorder (ASD) and memory impairment. Patients with mutations downstream of intron 44 exhibit loss of Dp140, a shorter dystrophin variant highly expressed during embryonic development of the cerebral cortex. Our group recently demonstrated Dp140 as a crucial modulator of glutamatergic synaptic transmission in the basolateral amygdala of adult mdx52 mice, which lack both Dp140 and full-length (Dp427) dystrophin isoforms. The latter regulates GABAergic subunit clustering at the post-synaptic membrane, while the role of Dp140 in the human brain remains unclear. Cerebral brain organoids differentiated from human pluripotent stem cells (ESCs or iPSCs) are self-organising, 3D models that recapitulate localised neuronal architecture of developing human cortical regions. We found that DP140 mRNA expression increases throughout WT human embryonic stem cell-derived cerebral organoid differentiation, mirroring human data. Thus, we hypothesise that these organoids are a powerful model for investigating early brain-related pathophysiological changes in DMD. Using human urine-derived cells, a non-invasive method of obtaining mesenchymal stem cells, we have generated iPSCs from DMD patients diagnosed with ASD and mutations both upstream and downstream of intron 44. These DMD iPSC-cerebral organoids exhibit decreased GABAα2 subunit immunostaining, previously observed mdx mouse lacking Dp427. We have generated dissociated monolayer cultures, exhibiting neuronal network reformation and synaptic marker expression. We aim to perform single cell RNA sequencing to assess DMD isoform expression across cell types in cerebral organoids, as well as pathway analysis in DMD organoids compared to WT. This will allow us to identify the yet unknown cellular expression of DP140, and gain a deeper understanding of human-specific neuronal defects associated with Dp140 deficiency in DMD, but also ASD. Finally, our organoids show potential as a platform for screening novel therapies which may treat both muscle and cognitive phenotypes in DMD.

## P-31

## Investigating the role of AQP4 in abnormal social behavior exhibited by mdx52 mice

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Duchenne muscular dystrophy (DMD) is an X-linked disorder caused by mutations in the *DMD* gene, characterized by progressive muscle wasting and weakness. While abnormalities in skeletal and cardiac muscle function are primarily observed, cognitive impairments, including autism spectrum disorder (ASD), are found in approximately 30% of DMD patients. In particular, the absence of Dp140, a short dystrophin isoform expressed in the brain, has been reported to be clinically associated with cognitive impairments in DMD patients. We previously reported that mdx52 mice, lacking Dp140 and Dp427, a full-length dystrophin isoform, exhibited ASD-like social behavioral abnormalities. In contrast, such abnormalities were not observed in mdx23 mice, which lacked Dp427 but retained Dp140. Moreover, the absence of Dp140 led to a decrease in glutamate release from pyramidal neurons in the basolateral amygdala, suggesting a potential link between Dp140 expression and ASD-like behavior. However, since the localization of Dp140 expression in the brain remains unclear, the link between Dp140 and social behavioral abnormalities is not fully understood.
Recently, aquaporin (AQP) 4, a water transport channel, has been reported to play a crucial role in waste clearance and the maintenance of neuronal excitability in the central nervous system. AQP4 may have functional and structural relevance with Dp140, as it binds to the C-terminus of dystrophin via α1-syntrophin. Therefore, we hypothesized that a deficiency of Dp140 could affect the function of AQP4 and result in defects in the nervous system. To test this hypothesis, we administered TGN-073, an AQP4 facilitator, to mdx52 mice to confirm whether AQP4 activation affects social behavior. TGN-073 was administered intraperitoneally at a dose of 200 mg/kg body weight into wild-type and mdx52 mice at 8 weeks of age. No significant effects of TGN-073 administration were observed in either type of mouse 30 minutes after administration. However, at 2 hours post-administration, a significant improvement in social behavioral abnormalities was observed in mdx52 mice ($P = 0.0108$). Thus, the activation of AQP4 through TGN-073 administration indicates a possible amelioration in ASD-like social behavioral abnormalities associated with Dp140 deficiency. Moving forward, we plan to explore the mechanism through which social behavioral abnormalities in mdx52 mice are ameliorated by AQP4 activators and to elucidate the specific functions of Dp140 in the central nervous system.

## P-32

# Enhanced fear and anxiety-like behavior associated with Brain Dp427 deficiency in Duchenne muscular dystrophy dogs

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Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease that presents progressive muscle wasting caused by a mutation in the DMD gene, which encodes dystrophin protein. DMD is often comorbid with cognitive deficits and psychiatric symptoms such as learning disabilities, high anxiety levels, and autism spectrum disorder. In the brain, Dp427 dystrophin isoform is localized to a subset of GABAergic postsynapses. We previously reported that the number of $GABA_A$ receptor clusters was reduced in the basolateral amygdala (BLA) of dystrophic *mdx* mice. This resulted in impaired GABAergic transmission in the BLA and a freezing response to threat. However, the characterization of brain Dp427 deficiency and its impact on brain phenotypes still need to be better understood. We aim to clarify the molecular mechanism of brain Dp427 and the effects of its lack on brain phenotypes in the DMD canine model. Canine X-linked muscular dystrophy in Japan ($CXMD_J$), a middle-sized and emotionally expressive model of DMD, is suitable for examining the neurobehavior associated with brain Dp427 deficiency.

To understand the effect of brain Dp427 deficiency, we first performed behavioral tests in $CXMD_J$. In the open field test, $CXMD_J$ showed anxiety-like behavior compared to their wild-type littermates. $CXMD_J$ exhibited a higher startle response than their wild-type littermates. It is postulated that the absence of Dp427 in the amygdala may contribute to the increased levels of anxiety and fear levels in $CXMD_J$. In addition, the heart rate variability measurements revealed decreased parasympathetic nerve activity in $CXMD_J$. For further study, we perform a synaptosome proteomic analysis of the amygdala to analyze the biochemical alteration resulting from Dp427 deficiency. We anticipate elucidating the molecular mechanisms underlying the psychiatric symptoms caused by Dp427 deficiency.

## P-33

# Characterizing subcellular localization and developmental expression of dystrophin in mammalian brain models

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The DMD gene, localized in the X chromosome, when mutated, leads to truncation and subsequent loss-of-function of dystrophin protein, manifesting in Duchenne Muscular Dystrophy (DMD). Loss of full-length muscle dystrophin (Dp427m) leads to muscular dystrophy and cardiac impairment. Additionally, around 30% of DMD patients have neurological comorbidities such as ADHD, autism spectrum disorder (ASD) or intellectual disabilities. Recently, HASHIMOTO Y. et al. (2022) demonstrated that mice lacking the expression of Dp140 (a shorter dystrophin isoform), alongside ASD-like behavior, presented a decrease of glutamatergic transmission in the medial prefrontal cortex-basolateral amygdala and a decrease in VGLUT1 in the pre-synaptic region. Using brain organoids differentiated from human embryonic stem cells, it was observed that Dp140 expression gradually increased over time, suggesting a role during brain development (SATHYAPRAKASH et al., unpublished). However, the functions of brain dystrophins during brain development are not currently known. Learning more about their timing of expression alongside their subcellular localization should give precious insights into their potential roles during this period, leading to a better understanding of the mechanisms that cause neurological comorbidities in DMD. Using brains organoids generated from wild type and DMD patient induced pluripotent stem cells alongside embryonic mouse forebrain at different time points (E11.5, E14.5, E18.5), this study aims to further determine brain dystrophin subcellular localization of Dp427c, and Dp140, which may provide clues to its function during mammalian brain development.

# Poster Session 4

## Treatment and therapy for DMD and the other diseases

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Keiko Ishigaki (Department of Pediatrics, Tokyo Women's Medical University, Japan)
Norio Motohashi (Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan)

### P-34

### Characterization of disease-specific alterations in metabolites and effects of mesenchymal stromal cells on dystrophic mice

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**Introduction:** Duchenne muscular dystrophy (DMD) is a genetic disorder caused by mutations in the dystrophin-encoding gene that leads to muscle necrosis and degeneration with chronic inflammation during growth, resulting in progressive generalized weakness of the skeletal and cardiac muscles. We previously demonstrated the therapeutic effects of systemic administration of mesenchymal stromal cells (MSCs) in DMD animal models. We showed preservation of long-term muscle function and slowing of disease progression. However, little is known regarding the effects of cell therapy on the metabolic abnormalities in DMD. Therefore, we aimed to investigate the mechanisms underlying the immunosuppressive effects of MSCs and their influence on DMD metabolism.
**Methods:** A comprehensive metabolomics-based approach was employed, and an ingenuity pathway analysis was performed to identify dystrophy-specific metabolomic impairments in the *mdx* mice to assess the therapeutic response to our established systemic MSCs-mediated cell therapy approach.
**Results and Discussion:** We identified DMD-specific impairments in metabolites and their responses to systemic MSCs treatment. Our results demonstrate the feasibility of the metabolomics-based approach and provide insights into the therapeutic effects of MSCs in DMD. Our findings could help to identify molecular marker targets for therapeutic intervention and predict long-term therapeutic efficacy.

## P-35

# Evaluation of the efficacy of Viltolarsen to Duchenne muscular dystrophy using muscular imaging

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Background
We previously proposed muscle volume index (MVI) and %MVI as indicators for quantitatively evaluating residual muscle volume patients with muscular dystrophy using CT.
Purpose
We tried to evaluate the efficacy of Viltolarsen in Duchenne muscular dystrophy (DMD) using %MVI.
Method
We retrospectively compared the muscle CTs in two Viltolarsen-treated DMD patients (case 1, 2) to those in untreated DMD patients. Case 1 had been on Viltolarsen therapy since the age of 9 years and underwent muscle CT at the ages of 10,12, and 13 years. Case 2 had been on Viltolarsen therapy since the age of 15 years old and underwent muscle CT at the ages of 16 and 17 years. %MVI was calculated at thigh and calf levels according to the reported manner.
Results
%MVI in case1 was better than mean %MVI of age-matched non-treated DMD patients at thigh and calf levels. %MVI in case2 was better than mean %MVI of controls at thigh level, and almost equal to mean %MVI of controls at calf level.
Conclusions
The present study suggested that Viltolarsen might be effective to DMD in terms of muscle volume measured by CT.

## P-36

# Exploration of the current challenges and future directions for optimizing DMD management in Asia from a survey and expert panel discussion

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Background: Advances in Duchenne muscular dystrophy (DMD) multidisciplinary care have significantly prolonged life expectancy. The advent of emerging treatments such as gene therapy will further transform disease trajectory. However, while these innovative treatments hold promise, they also present complex challenges of implementation, cost justification and healthcare financing across the heterogeneous healthcare systems of Asia.
Objective: To understand current DMD management in Asia and explore varying practices, unmet needs and future directions for more standardized care in the region.
Methods: Pediatric neuromuscular experts from Hong Kong, Singapore, and Taiwan were identified, and a survey was administered to understand current approaches in clinical practice in regard to DMD. A follow-up meeting was held in September 2023 to discuss the survey findings and further elucidate opinions ranging from DMD diagnosis and assessment to treatment and management best practices. Perspectives and attitudes towards emerging treatments, including gene therapy, were elicited and consolidated by the experts.
Results: Genetic profiling has been universally adopted by experts as the diagnostic tool of choice. Steroid therapy is recognized as the cornerstone of DMD care, but there are wide differences in its initiation and cessation, dosages and management of side effects. Gene therapy has the potential to significantly improve outcomes in patients with DMD. However, its adoption will likely be hampered by affordability, uncertainties on durability and safety, and inadequate infrastructure for gene therapy infusion centers. Governments and manufacturers must work together to improve these aspects. At the same time, longer-term data will help allay anxieties about efficacy and safety. Establishing a centralized DMD registry garnered strong support from the experts as it will shed light on unique DMD subtypes in the Asian population, promote standardization of treatment protocols, and facilitate the conduct of clinical trials and collection of real-world data. These invaluable insights will in turn help develop more sustainable funding frameworks and inform policy decisions within our regional healthcare systems.
Conclusions: DMD care in Asia is characterized by substantial heterogeneity, with financial and reimbursement issues playing a major role in management decisions. A centralized, regional patient registry has the potential to address these concerns by providing a valuable source of information.

## P-37

# A real-world survey on the treatment patterns and neurologist treatment satisfaction for Duchenne muscular dystrophy patients in Japan

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Introduction: Duchenne muscular dystrophy (DMD) is a genetic disorder associated with progressive muscle degeneration due to a lack of dystrophin. Current treatment for people with DMD facilitates in managing symptoms; however, there are no approved curative treatments. Here, we aimed to understand the current DMD treatment landscape in Japan.

Methods: Data were drawn from the Adelphi DMD Disease Specific Programme™, a cross-sectional survey with retrospective data collection of physicians and their DMD patients conducted in Japan from November 2022 – April 2023. Physicians completed online questionnaires for consecutive consulting DMD patients, reporting demographics, treatment history and satisfaction, and reasons for treatment choice. Analyses were descriptive.

Results: Overall, 28 neurologists provided data on 90 male DMD patients. Mean [standard deviation; SD] patient age was 17.8 [7.5] years, 94% were not in employment, and 76% were non-ambulatory. Overall, 70% of patients were prescribed treatment for their DMD at the time of survey completion. Prednisone/Prednisolone (P/P; 51%) and viltolarsen (16%) were the most widely used treatments. The most common physician-reported reason for treatment choice was to slow disease progression for both P/P (59%) and viltolarsen (79%) patients. Treatment satisfaction was reported for 46% of P/P and 79% of viltolarsen patients. Reasons for dissatisfaction with P/P were a lack of efficacy (67%) and the number of side effects experienced (38%). Reasons for dissatisfaction with viltolarsen were due to the number of side effects experienced (71%) and the frequency of administration (29%). P/P was reported that it could be improved by long-term proven efficacy (41%). Physicians reported that viltolarsen could be improved through its suitability for paediatric patients (57%).

Conclusion: P/P was the most common treatment in Japan followed by viltolarsen. Over half (54%) of the physicians expressed a lack of satisfaction prescribing patients with P/P, with around one-fifth lacking satisfaction with viltolarsen. The number of side effects was a leading reason for lack of satisfaction for both treatments. Neurologist dissatisfaction with current treatment in Japan, combined with the need to slow down disease progression, highlights an unmet need for treatment that limits side effects and maintains long-term efficacy.

## P-38

# Febuxostat improves DMD phenotype in dystrophin mutant model of mice via enhancement of cellular ATP

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Duchenne muscular dystrophy (DMD) is an X-linked neuromuscular disease, characterized by progressive degeneration of both skeletal and cardiac muscle, leading to premature death from cardiomyopathy and respiratory failure. It is caused by a mutation in the DMD gene that codes dystrophin protein crucial for structural support for muscle fibers and cell signaling capabilities . Lack of functional dystrophin compromises cellular myogenic function, eventually causing muscle atrophy. Currently, there are no curative therapies, and the standard of care, including steroids, only slows disease progression. Alternatives including gene therapies that target the genetic cause of the disease have limitations in their efficiency, necessitating combined therapies to maximize efficacy. A strong body of studies evidences multi-level mitochondria dysfunction, associated with dystrophin deficiency, as an important etiology of DMD in both patients and animal models. Myofibers without functional dystrophin experience recurrent muscle damage enabling calcium overload and negatively affecting the glycolytic pathway, ADP/ATP cycling, and citric acid cycle, resulting in mitochondrial swelling, uncontrolled mitochondrial reactive oxygen species (ROS) production, and reduced ATP synthesis. These environmental stresses depleted mitochondria mass and disrupted mitochondria accumulate, further inhibiting the biogenesis, leading to chronic inflammation and progressive fibrosis. Accordingly, ATP-enhancing and mitochondria/ROS-targeting molecules emerge as potential therapeutic approaches for DMD.
Febuxostat (FBX), an inhibitor of xanthine oxidase (XO), can divert hypoxanthine into the salvage pathway, thereby preventing ATP depletion. The increase in available ATP reduces stress on mitochondria and decreases ROS production. Administration of FBX in the dystrophin mutant model of C. elegans has been shown to improve lifespan and disease phenotypes. However, no research examined the effect of FBX in mammalian animal models to bridge such discoveries to clinical application. To fill this gap, we aim to investigate ATP concentration enhancement with FBX and subsequent impact on mitochondria function and disease phenotype using mdx mouse model of DMD. Preliminary findings from mice administered 5mg/kg/day of FBX orally for four weeks showed promising outcomes, including increased succinic dehydrogenase activities in fast-twitch muscles, increased skeletal muscle mass, and improved isometric torque measurements. Further study will confirm the changes in ATP and mitochondrial function and determine whether FBX-induced improvements are sufficient to ameliorate DMD phenotype.

## P-39

# Development of a treatment for Duchenne muscular dystrophy based on the Fucosyltransferase 8

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[4]Research Institute for Diseases of Old Age, Juntendo University Graduate School of Medicine Background

Background

Many proteins and lipids expressed on the surface of cell membranes are glycosylated and acquire their original functions through glycosylation, playing a major role in diverse biological phenomena such as cell differentiation and signal transduction. The glycosyltransferase Fucosyltransferase 8 (Fut8) is the only enzyme that can add a single fucose to the innermost N-acetylglucosamine of an N-linked sugar chain through α1,6 linkage, creating a structure called core fucose. Previously, we have reported that in zebrafish, reduced fut8 leads to abnormal formation of structures called myosepta, which correspond to tendons of muscle fibers and abnormalities in muscle fibers(Cells. 2022). Furthermore, it has been published that reduced Fut8 not only results in smaller regenerating muscle but also in significant regeneration defects, such as ectopic fattening and increased fibrosis. Exogenous L-fucose could increase GDP-fucose, a donor substrate for Fut8, and specifically enhance core fucosylation.

Experimental Methods and Results

In the present study, the efficacy of L-fucose was investigated in a mouse model of cardiotoxin-induced muscle injury and in mdx, a mouse model of Duchenne muscular dystrophy.The muscle injury model was created by injecting 100 µl of 10 uM cardiotoxin into the lower limb muscles of 8-week-old C57BL/6J male mice, and L-fucose was administered at 1 mg/BW(g)/day intraperitoneally and orally. Controls mice received the same amount of L-fucose and saline orally. The percentage of myofibers positive for immunoreactivity to F1.652, a monoclonal antibody to fetal myosin, was examined The percentage of F1.652-positive myofibers was 31.76±5.79% in the non-treated group, whereas 67.90±10.49% in the intraperitoneally administered group and 79.13±4.71% in the orally administered group, significantly increasing the percentage of regenerated muscle (One-way ANOVA. $P<0.01$, $n=5$, each group). Next, 3-week-old male mdx were intraperitoneally administered 1 mg/BW(g) of L-fucose and an equal volume of saline until 8 weeks of age, with the L-fucose group gaining significantly more weight than the control group at 5 weeks of age (9.59±0.78 g saline, 10.84±0.63 g L-fucose. t-test, $p<0.05$, $n=5$, each group). Finally, analysis of the diaphragm at 8 weeks of age showed a significant increase in diaphragm thickness in the group treated with L-fucose (saline 448.11±74.58 um, L-fucose 689.18±105.77 um. t-test, $P<0.05$, $n=3$, each group).

Conclusion

We found that L-fucose administration via increasing core fucosylation significantly promoted muscle regeneration in murine models, possibly leading to a novel treatment for human DMD.

## P-40

## Drug screening to induce jagged1 expression using transgenic zebrafish

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Recent reports have shown that Jagged1 expression ameliorates skeletal muscle phenotype in a dog model of Duchenne muscular dystrophy (DMD) with complete dystrophin deficiency and in the DMD zebrafish model, sapje. We generated jagged1 transgenic (Tg) zebrafish and screened a chemical library to identify drugs that upregulate the expression of zebrafish jagged1.
The EGFP-jagged1-Tg zebrafish generated using the zebrafish jagged1 promoter region showed strong EGFP signals in the brain and spinal cord, and its expression was also observed in skeletal muscle. We screened for drugs that upregulate the expression of jagged1 using this jagged1-Tg zebrafish. We found several drugs that induce zebrafish jagged1 expression from 1,280 drugs in the chemical library. We assayed these candidate drugs to sapje and identified one candidate drug that can improve the abnormal structure of skeletal muscle observed in sapje.
These findings suggested that EGFP-jagged1-transgenic fish is a good tool for screening drugs to improve the muscle phenotype in sapje. To elucidate the molecular pathways and identify candidate drugs to upregulate jagged1 gene expression may be important to contribute for treatments of muscular dystrophies.

## P-41

## Enhancing Antisense-Oligonucleotide Delivery through Muscle Metabolism Regulation

Norio Motohashi, Yuki Ashida, Yoshitsugu Aoki

Department of Molecular Therapy, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Japan

Duchenne muscular dystrophy (DMD) is an inherited muscular disease characterized by degeneration and regeneration of muscle fibers due to dystrophin protein deficiency, leading to progressive muscle atrophy. While therapeutic approaches for DMD have been limited, a treatment to restore dystrophin protein using the exon-skipping strategy with antisense oligonucleotides (ASOs) has been developed. Antisense oligonucleotides using phosphorodiamidate morpholino oligomer (PMO) exhibit high specificity in binding to target RNA and is known for its high safety. Viltolarsen, conditionally approved for medical use in 2020, is a PMO-based drug designed to skip exon 53 of the DMD gene and produce truncated but partially functional dystrophin in DMD patients. However, the major weaknesses of PMO are rapid blood clearance and limited tissue distribution. In particular, there is still a need for further optimization to improve PMO delivery to all skeletal muscles and the heart, as respiratory complications and cardiac dysfunction are the major causes of premature death in DMD patients. To tackle the challenges associated with DMD, we suggest methods to improve exon skipping efficiency in skeletal muscles by using PMO.

Our approach is based on our prior research, which showed that the Mvp-miR-423 pathway is activated and lipid metabolism is enhanced in progressive DMD. This pathway is involved in DMD-related muscle atrophy and can be utilized to regulate the effectiveness of exon skipping. Interestingly, enhancing muscle lipid metabolism may hinder PMO distribution, and we hypothesized that temporarily inhibiting lipid metabolism may enhance PMO delivery in DMD. We have screened potential drugs or natural compounds that inhibit lipid metabolism in C2C12 myoblasts and identified several candidate compounds. We found that some compounds could increase the efficacy of PMO delivery into myoblasts when added simultaneously with PMOs. Furthermore, simultaneous intravenous administration of these compounds with PMOs into DMD model mice resulted in a significant increase in the efficacy of PMO delivery in skeletal muscle. These results suggest that lipid metabolism regulates the efficiency of PMO delivery into skeletal muscle.

## P-42

## *In vivo* gene therapy for striated muscle laminopathy

Mariko Okubo[1,2], Astrid Brull[1], Maud Beuvin[1], Nathalie Mougenot[1], Gisele Bonne[1], Anne T.Bertrand[1]
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*LMNA* mutations induce a group of disorders called laminopathies, most of them affecting striated muscles (SML). All SML present with life-threatening dilated cardiomyopathy, ranging from neonatal period for LMNA-related Congenital Muscular Dystrophy (L-CMD) to an absence of muscle symptoms in isolated dilated cardiomyopathy. L-CMD is the most severe form of striated muscle laminopathy with cardiomyopathy and there is no treatment for L-CMD. We previously reported the phenotype of KI-LmnaK32del mouse model mimicking patient mutation. Mice harbouring this mutation develop an L-CMD phenotype at the homozygous state and isolated cardiomyopathy at the heterozygous state. Taking advantage of our mouse modes, we have previously shown that the disease pathomechanism involves both lamin haploinsufficiency (decrease lamin A/C expression) and dominant negative effect (expression of toxic mutant lamin A/C). Based on these pathophysiological observations, the present study is focused on the evaluation of a therapeutic approach that aim both at restoring the normal lamin A/C expression level and reducing the expression of the mutant lamin A/C. We produced AAV2/9 vectors containing human mature lamin A under control of a CMV promoter either alone, or in combination with shRNA under a H1 promoter that either specifically targets the p.K32del *Lmna* mRNA, or targets both the WT and mutated mRNA. Systemic administration in new-born mice resulted in a significant increase in maximal survival of homozygous mice. In the heart, lamin A protein level was increased, reaching or overpassing that of wild type. Furthermore, liver level of SREBP1 precursor was normalized. By contrast, in heterozygous mice, despite increased lamin A expression in the heart, none of the treatments led to improvement in terms of survival or cardiac function. The absence of therapeutic benefit at long term is neither due to loss of AAV genome particle nor to a loss of its expression with time. Rather, it is due to side effect in the liver, already reported by others, and to inefficient mouse *Lmna* mRNA knock-down. Based on experiments performed on WT and homozygous mouse myotubes in culture, we hypothesise that the lack of knock-down efficacy is due to inefficient shRNA maturation in mutated cells. Future development will need to consider alternative methods to avoid liver targeting and improve mRNA knock-down efficacy.

# Poster Session 5

## LGMD and FSHD

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Hidetoshi Sakurai (Department of Clinical Application Center for iPS Research and Application (CiRA), Kyoto University, Japan)
Wen-Chen Liang (Kaohsiung Medical University, Taiwan)

### P-43

### Development of a technique to map the transcriptome to histological changes in frozen sections of skeletal muscle at the single-cell level

Nanami Yamada[1,2], Hiroki Ikeda[2], Kazuki Kurimoto[2], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Embryology, Nara Medical University

Objective: Muscle fibers are multinucleated syncytia and show heterogeneous gene expression in relation to other cell types and at damaged sites. Therefore, to clarify the pathogenesis of muscle diseases, it is necessary to know the changes in gene expression at the site of pathological change. However, there are few methods to directly map gene expression to pathological images. Direct RNA recovery and quenching for laser capture microdissection (DRaqL) is a method for analyzing gene expression of target cells one by one after microscopic examination of tissue sections. In this study, we analyzed mouse skeletal muscle as a preliminary step in applying DRaqL to human biopsy muscle specimens.
Methods: Fresh frozen sections of mouse tibialis anterior muscle were stained with cresyl violet. In previous observations, we have observed that myofibers can be classified into two types according to the way they are stained by cresyl violet: fibers with darkly stained cytoplasm (darkly stained fibers) and fibers with poorly stained cytoplasm (non-darkly stained fibers). For each staining type, muscle fibers were isolated by laser capture microdissection (24 fibers per staining type), and cDNA was synthesized for each fiber using DraqL-Smart-seq2. Sequencing was performed with Nextseq2000.
Results: We detected a median of 517,000 unique molecular identifiers and a median of 2,740 protein-coding genes per fiber. Thus, we found that DraqL has higher sensitivity than common single-cell transcriptomics methods. Principal component analysis showed marked differences between darkly-stained and non-darkly stained fibers, identifying differences in the transcriptome associated with differences in the staining properties of Cresyl violet. Genes involved in energy metabolism and aerobic respiration contributed to the first and second principal components. Thus, the staining properties of Cresyl violet may be related to differences in the metabolism of each fiber.
Conclusion: DRaqL can link histological differences in muscle fibers and gene expression at the single cell level.

## P-44

# Dissecting the immunometabolism of delta-sarcoglycan deficient animal model with multimodal mass spectrometry imaging

Maiko Okamura[1], Shinichi Yamaguchi[2], Takushi Yamamoto[2], Koji Okuda[2], Shuji Yamashita[1], Satoru Noguchi[3], Kisaki Amemiya[4], Kenji Minatoya[5], Hidetoshi Masumoto[5], Ichizo Nishino[3], Hatsue Ishibashi-Ueda[4,6], Masaya Ikegawa[1]

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Sarcolemmal glycoproteins, alpha-, beta-, gamma- and delta-sarcoglycans (SGCA, SGCB, SGCG, and SGCD) have been shown to form a distinct subcomplex in the dystrophin-glycoprotein complex (DGC). Genetic defects of the sarcoglycans have been identified as the causes of four distinct forms of muscular dystrophies, which are called sarcoglycanopathy. As delta-sarcoglycanopathy (LGMDR6) is very rare, it must be extremely difficult to predict clinical outcome of the SGCD mutation in man. Of note, J2N-k hamsters (J2N-k), which have a defective δ-sarcoglycan (δ-SG) encoding gene, have been utilized as a suitable animal model of dilated cardiomyopathy (DCM). By our histopathological analysis, however, J2N-k's skeletal muscle was also compromised even at 4 weeks of age. In our effort to elucidate molecular basis of pathological entity of J2N-k, spatial multi omics strategy with mass spectrometry imaging (MSI) has been applied. J2N-k and its healthy control J2N-n hamsters (J2N-n), each from 4 to 8 weeks of age, were fed for the experiments. Frozen sectioning of whole thorax, heart, and skeletal muscles of J2N-k and J2N-n were prepared. Matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) was performed to visualize metabolites and low molecular weight compounds as well as peptides and proteins. On the other hand, laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) measurements was performed for elemental imaging. Histopathological features of cardiac muscle of J2N-k were clarified as inflammation, fibrosis, calcification and appearance of multi-nucleated giant cells in the early to mid-stage of their lives. In J2N-k, utilizing MALDI-MSI for proteome analysis, we have detected several proteins specifically distributed among multiple cardiac and skeletal muscle foci such as proteins of thymosin beta family. Furthermore, metabolites such as UDP-GlcNac and cholesterol sulfate were detected on both myocardium and skeletal muscle foci. To further validate these observations at single cell level, here we integrate LA-ICP-MS into proteomic and metabolomic MSI. The determination of the localization and distribution of elements (e.g., C, P, Cu, Fe, Zn, Ca, Mg, Na, and others) in biological tissues was conducted. To better understand robust single-cell information on cardiac and skeletal muscles from J2N-k hamsters in terms of immunometabolism, the current workflows and protocols for multimodal imaging analysis for MALDI-MSI and LA-ICP-MS is a promising strategy. In conclusion, we have developed workflows for multimodal MSI analysis to elucidate immunometabolomic basis of delta-sarcoglycanopathy on J2N-k hamster model.

## P-45

# Generation of a zebrafish model of limb-girdle muscular dystrophy (LGMDR6) using genome editing technology

Shohei Majima, Hiroaki Mitsuhashi
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Background
Zebrafish are excellent vertebrate models to study muscle diseases due to their high numbers of offspring, low maintenance costs, evolutionarily conserved muscle function, the ability to manipulate their gene expression, and ease of observation. We have generated a zebrafish model of autosomal recessive limb-girdle muscular dystrophy-6 (LGMDR6) by knocking out zebrafish δ-sarcoglycan gene (sgcd) using CRISPR/Cas9 technology, and evaluated it as a model of the disease.

Methods
A gRNA against exon 2 of sgcd was designed and injected into fertilized zebrafish eggs with the Cas9 protein. Genome-edited embryos were identified by heteroduplex mobility assay (HMA) and Sanger sequencing. The mRNA sequence transcribed from the genome-edited sgcd gene was also analyzed by Sanger sequencing of the reverse-transcription PCR product. To obtain sgcd -/- embryos, heterozygous sgcd +/- mutants were in-crossed. To assess motor function, spontaneous coiling movements of the embryos at 24 hours post-fertilization (hpf), and spontaneous swimming distances at 5 days post-fertilization (dpf) were measured by Noldus DanioScope and DanioVision, respectively. To confirm the knockout of δ-sarcoglycan protein, Western blotting with anti-δ-sarcoglycan antibody was performed. To examine muscle pathology, skeletal muscle actin fibers and γ-sarcoglycan, dystrophin, β-dystroglycan, β-sarcoglycan, and δ-sarcoglycan were immunostained and observed using confocal laser microscopy.

Results
Sanger sequencing revealed a deletion of 14 bps within exon 2 of the zebrafish sgcd gene in the genome-edited embryos. The same deletion was also identified in the mRNA sequence. This deletion was presumed to cause a frameshift, resulting in the loss of normal δ-sarcoglycan protein. In-crossing of heterozygous sgcd +/- mutants produced abnormal embryos with cardiac enlargement, body curvature, and reduced muscle birefringence at a rate of approximately one-quarter. At 24hpf, there was no significant difference in coiling movements between embryos with normal and abnormal phenotypes, but at 5 dpf, total swimming distance was reduced in embryos with the abnormal phenotype. Phalloidin staining and immunostaining staining revealed muscle degeneration and decreased localization of β-sarcoglycan and γ-sarcoglycan in the myoseptum in the abnormal embryos, while dystrophin and β-dystroglycan were normal. Western blot analysis showed that δ-sarcoglycan was not expressed in the embryos with the abnormal phenotype.

Conclusion
We have successfully generated sgcd knockout zebrafish by genome editing. Our model showed progressive muscle weakness, muscle degeneration, and impaired localization of sarcoglycan complexes similar to the LGMDR6 patients.

## P-46

# Stress-induced Cardiomyopathy with Aspiration Pneumonia Caused by the Ventilator Disconnection Accident in a Patient with Limb Girdle Muscular Dystrophy

Koichi Kimura[1], Hiroyuki Morita[2], Koki Nakanishi[2], Masao Daimon[3], Tomoko Nakao[2], Megumi Hirokawa[2], Yuriko Yoshida[2], Tetsuhiko Ikeda[4], Yoshitsugu Aoki[5], Norihiko Takeda[2]

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CASE REPORT:
A 67-year-old female patient with limb girdle muscular dystrophy was hospitalized due to acute heart failure with aspiration pneumonia. She was using wheelchair to move, receiving tube feeding through the gastrostomy, and underwent artificial ventilation. In her home, she accidentally collapsed from her bed at night and her ventilator tube was disconnected. She got panicked and kept calling out “help me!” for her family as loud as possible, but her family did not notice her call. Six hours later, her family noticed her symptom of chest discomfort with decreased SpO2 of 84% and increased heart rate of 110 bpm. She transferred and admitted to the hospital for further investigation. On admission tests, her chest CT scan showed left pneumonia with bilateral atelectasis. Her laboratory test of BNP was elevated to 984 pg/ml from her baseline 9 pg/ml at the previous month. Her electrocardiogram showed new-onset negative T waves in broad precordal leads of V2 to V6. Her echocardiography showed segmental hypokinesis in the apical lesion with the left ventricular ejection fraction (LVEF) by Teichholz method of 62% (measured only in basal segments) and by Simpson method of 47% (measured including apical lesion). Several weeks treatments using antibiotics and oxygen ventilation had improved her condition. In the next month, BNP level decreased to 18 pg/ml, electrocardiogram became normal, and her echocardiographic finding recovered to normal range with LVEF of 64% by Simpson method. This case indicated that a ventilator disconnection accident can cause strong emotional stress as well as hypoxic incident, leading to stress-induced cardiomyopathy (Takotsubo cardiomyopathy) with aspiration pneumonia.

## P-47

## The introduction of the FSHD patient association in Japan and the future perspective towards international collaboration

FSHD Japan core members[1,2]
[1]FSHD Japan
[2]The Japan Muscular Dystrophy Association

FSHD is one of major types of muscular dystrophies which potentially affects 1 in 8000. Recent numerous efforts made by academic and industry researches have brought advance in development of drug candidates to treat FSHD, some of which are already undergoing clinical trials in some countries. Local and global patient advocacy activities are getting prevalent and highly required to achieve the clinical goals to benefit the patients. However, compared to European and US regions, there are still limited knowleadge available on clinical situations and patient advocacy activities in Asian regions, which can slow down drug accessibility and reduce the chances for patients to obtain and share useful information otherwise to have better quality of life by less efforts. To improve those situations, inspired by other leading groups in the world, we are making diverse efforts for better advocacy including collaboration with clinicians. Here we will introduce our activities and intend to collaborate with other countries' groups especially in Asian regions to make our circumstances more visible.

## P-48

# The temporal changes of physiologic functions and gene expressions in drug-induced skeletal muscle specific *DUX4* over expression mice

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Facioscapulohumeral muscular dystrophy (FSHD) is a genetic muscle disorder caused by abnormal expression of *DUX4* gene. In skeletal muscle, DUX4 was reported as a transcription factor which related with oxidative stress, cell death, inflammation, and suppression of myogenesis. Currently, many studies which analyzed downstream targets of DUX4 in vitro were reported. However, temporal changes of the factors in DUX4-induced muscular weakness in vivo are unclear.

In this study, drug-induced skeletal muscle specific *DUX4* over expression mice (FLEx*DUX4*; ACTA1-MCreM) were used as a model animal of FSHD. Gene expression of *DUX4* was induced by one time of intraperitoneal injection of 10mg/kg tamoxifen. Mice were analyzed before the tamoxifen administration and at 1, 3, 5, and 7 days after the injection. Gastrocnemius muscles were used for gene expression analysis.

Muscular endurance and grip strength were significantly decreased at 5 and 7 days after the injection, respectively. In qRT-PCR, transient and significant increase of *DUX4* was observed at day 3. Gene expressions of myogenesis-related factors were temporary decreased at day 3 and were increased at day 7. Significant changes of gene expressions related with oxidative stress, cell death, and inflammation were observed at day 5.

These findings suggest that suppression of myogenesis precedes other reactions in the process that reaches muscle weakness form *DUX4* expression in vivo.

## P-49

## *JAG2*-related muscular dystrophy as a rare mimicry of facioscapulohumeral muscular dystrophy (FSHD)

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*JAG2*-related muscular dystrophy is a newly described limb-girdle muscular dystrophy. Its clinical presentation, MRI muscle pattern and histopathological findings highly mimic that of facioscapulohumeral muscular dystrophy (FSHD). In this study, we will report a consanguineous family with genetically substantiated *JAG2*-related muscular dystrophy. The clinical presentations, MRI muscle pattern, histopathological features, and molecular characteristics will be presented. The key clinical differences will be compared with that of FSHD. The take-home message from this study is to broaden our differential diagnosis of FSHD. This is also the first Chinese report of *JAG2*-related muscular dystrophy.

## P-50

# Characteristics of Patient with FacioscapulohumeralMuscularDystrophy in Japanese Nationwide Registry of Muscular Dystrophy (Remudy)

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Background: Facioscapulohumeral muscular dystrophy (FSHD) is one of the most prevalent forms of muscular dystrophy. We launched a national patient registry for FSHD as part of Remudy to facilitate future clinical trials in September 2020. This study aims to characterize Japanese FSHD patients by analyzing registry data. Methods: Various clinical data and genetic analysis results, including haplotype information, were collected. Results: 145 patients (66 males and 79 females) enrolled in the registry until October 2023. Of these, 140 had FSHD1, and 5 had FSHD2. 70 patients had another FSHD patient in their family. The mean age at registration and onset were 42.0 and 15.5 years, respectively. Most FSHD patients (n=136) had D4Z4 repeat units shortened to 1-6 repeats and 4qA haplotypes. Specifically, three patients had one repeat unit, 20 patients had two, 41 patients had three, 32 patients had four, 24 patients had five, and 16 patients had six. The median age of disease onset was 10, 8, 10, 12, 19, and 19.5 years, respectively. The proportion of non-ambulatory patients was 26.9%, with a mean age of 37.4 years at which they became non-ambulatory. The percentage of patients who lost ambulation was 60.9% of those with one to two repeats, 27.4% with three to four repeats, and 10.0% with five to six repeats. Respiratory dysfunction was observed in 47%, with 29% requiring mechanical ventilation. Forced vital capacity as percent of predicted was correlated with the number of repeats. Cardiac dysfunction was reported in 3.7%, all of whom had four or fewer repeats. However, no differences in left ventricular ejection fraction were compared by number of repeats. Retinopathy was observed in 5 (3%) and hearing loss in 22 (15%) FSHD1 patients, both with three or fewer repeats. Among all adult female patients with FSHD, a history of pregnancy was reported in 30.7% of those with one to three repeats, 51.4% of those with four to six repeats, and 80% of those with seven or more repeats. Conclusion: In FSHD1, motor function, retinopathy, and hearing loss were influenced by the number of D4Z4 repeats. A notable proportion of patients had previously experienced pregnancy, underscoring the necessity of genetic counseling.

## P-51

## Facioscapulohumeral Muscular Dystrophy in Taiwan

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Background:
Facioscapulohumeral muscular dystrophy (FSHD) is an autosomal dominant disease, causing progressive asymmetric weakness mainly in facial, scapulohumeral muscles and dorsiflexors of the ankle. Current understanding of pathomechanism is associated with the overexpression of Double Homeobox 4 (DUX4) gene, located within each 3.3 kilobase tandemly repeated sequence (D4Z4) repeat array. FSHD type 1 patients carried 1-10 D4Z4 repeats and FSHD type 2 patients carried 11-20 D4Z4 repeats with mutations in the genes related to methylation leading to hypomethylated D4Z4 array. This study aims to investigate the clinical, radiographic and genetic features of Taiwanese FSHD patients.
Methods:
We reviewed the medical records of FSHD patients whose diagnosis were confirmed by non-isotope southern blot analysis with/without whole exome sequencing from 2008 to 2024 in Kaohsiung Medical University Hospital. Eighty-three patients were enrolled.
Results:
Eighty-two patients were diagnosed FSHD1 and one patient FSHD2. In FSHD1 patients, eight patients (9.8%) are early-onset FSHD. Among early onset FSHD, five patients had hearing impairment and five had retinal arterial tortuosity. The number of patients with 1, 2, 3, 4, 5, and 6 D4Z4 repeats was 5, 10, 19, 14, 16, and 12, respectively; one patient had borderline fragment size (8-10 D4Z4 repeat) and 5 patients had compound heterozygous truncated D4Z4 repeats. Positive correlation was noted between D4Z4 unit number and onset age. Restrictive lung disease developed since childhood in some patients with 1-3 D4Z4 unit number whereas patients with 4-6 D4Z4 unit number have relative normal lung function during 3rd-4th decades . In muscle computed tomography imaging, asymmetric pattern and trapezius involvement with bilateral subscapularis sparing were common, and the frequency was 77.6% and 83.7% respectively. Preferential involvement in rectus femoris at thigh level was noted in 28.6% of our patients. The FSHD2 patient was confirmed to have SMCHD1 mutation. The clinical and radiographic feature was similar to FSHD1 patients.
Conclusions:
We observed the inverse correlation between D4Z4 unit number and severity and patients with lower D4Z4 repeat number tend to have earlier onset of age, earlier development of restrictive lung disease and earlier loss of ambulation. High incidence of hearing impairment/ ophthalmologic abnormality was noted in early-onset FSHD. Muscle imaging is an important tool for assisting diagnosis. Monitoring lung function and regular ophthalmology/hearing examination may be important in FSHD patients since childhood, especially in patients with lower D4Z4 repeat numbers.

# Poster Session 6

## Channelopathies and Myotonic dystrophies

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Takashi Kurashige (Department of Neurology, NHO Kure Medical Center and Chugoku Cancer Center, Japan)
Theerawat Kumutpongpanich (Division of Neurology, Department of Internal Medicine, Siriraj Hospital, Mahidol University, Thailand)

### P-52

## Tubular Aggregate Myopathy: A Case Series Analysis in a Tertiary Hospital in Taiwan

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Background:
Tubular aggregate myopathy (TAM) is a rare muscle disease. It is diagnosed by characteristic pathologic feature, subsarcolemmal accumulation of tubular aggregates. TAM could be a hereditary disorder or the consequence of ethyltoxic, anoxia or endocrine disorder. Up to date, many causative genes such as STIM1, ORAI1, GFPT1, DPAGT1, SCN4A, KCJN2 have been identified. The purpose of this research is to investigate the clinical and genetic features of TAM patients in a referral center for neuromuscular diseases.
Methods:
From July 2013 to July 2024, four patients were confirmed the diagnosis of TAM by muscle pathology and whole exome sequencing in Kaohsiung Medical University Hospital. We further analyzed the clinical, pathological and imaging features.
Results:
The onset age ranged from 1.5 years to 4 years. The highest creatine kinase varied from 904 to 3,971 IU/L. Three patients had STIM1 mutations and one patient had KCNJ2 mutation. STIM1 mutation: One patient carried c.910C ＞ T in STIM1, a common mutation of Stormorken syndrome. The other two patients had c. 326A ＞ G mutation. None of them had restrictive lung disease or cardiac involvement. Hypocalcemia was noted in all patients and two patients had osteoporosis since adolescent. The patient with c.910C ＞ T mutation demonstrated all features of Stormorken syndrome, such as miosis, hyposplenia and thrombocytopenia. The other two patients exhibited some of these features but not all. The muscle computed tomography (CT) of two patients showed relatively severe fatty infiltration in satorius.
KCNJ2 mutation: One Andersen-Tawil syndrome patient with c.652C ＞ T in KCNJ2 was confirmed. He had periodic paralysis, ventricular ectopics with prominent U wave and facial dysmorphism. The weakness of this patient is severe (Medical Research Council (MRC) Scale for Muscle Strength of hip: 2) , but muscle CT presented very mild fatty infiltration.
Conclusions:
Early sartorius involvement was noted in the muscle imaging of our patients carrying STIM1 mutations. There is no selective pattern in our KCNJ2 mutated patient and the clinical weakness is not proportional to muscle fatty infiltration. Our case series also highlighted that TAM patients frequently had extra-muscular involvement. Some of the comorbidity required early diagnosis and intervention to improve prognosis. Therefore, complete systemic survey is essential in TAM patients.

## P-53

# Excess Desmin expression diminishes muscle contractile function concomitant with alteration of SOCE protein expression in mice skeletal muscle

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Background: Desmin is known as muscle specific intermediate filament protein that essential for muscle integrity. Des null mutation is also known as the causation of Desminopathy. Meanwhile, Desmin expression is known to upregulate some physiological stimulation (e.g., dietary intake, muscle overload) and aging. However, little is known about how increased Desmin impacted on skeletal muscle function and/or size. Therefore, purpose of this study was to investigate the influence of excess Desmin expression via adeno-associated virus (AAV)-mediated overexpression on skeletal muscle mass and function in mice.
Methods: male 10 wk. old C57BL/6J mice was subjected to AAV injection on bilateral hindlimb. The control vector (AAV6-CMV-EGFP) was injected into the left leg and the Des expression vector (AAV6-CMV-Des) into the right leg. After the AAV injection, muscle contractile capacity mesurement and sampling was performed at 2 wk. and 8 wk. post injection respectively. The obtained muscle samples were used for analysis of muscle contractile protein and SOCE-associated protein expressions.
Results & Discussion: Desmin overexpression reduced the muscle contractility by time dependent manner while it does not influence muscle mass. Muscle contractile protein expression was unaffected by Desmin overexpression during experimental period. On the other hand, STIM1 and Orai1, SOCE-related proteins, expression significantly altered. Stim1 expression was reduced and cleaved Orai1 expression was increased at 8wk. after AAV injection. Our results implicated that Desmin accumulation potentially impairs muscle contractility via the aberrant SOCE proteins expression.

## P-54

# Subcellular localization of sarcoplasmic reticulum-related factors in gastrocnemius muscle of aged mice

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**Objective:** In the skeletal muscles, calcium ions play a role in regulating muscle contraction and mitochondrial metabolism, while the sarcoplasmic reticulum (SR) contributes to calcium ion homeostasis. Although calcium ion homeostasis is impaired in aged muscles, morphological changes and detailed localization of SR-related factors remain uncharacterized. The aim of this study was to identify SR structural changes and determine the localization of SR-related factors in aged muscles.

**Materials and methods:** Three- and twenty-four-month-old C57BL/6J mice were used. Hematoxylin–eosin (HE), Gomori's trichrome, and succinate dehydrogenase (SDH) staining were performed on transverse sections of gastrocnemius muscle to confirm our pathological findings. Transmission electron microscopy (TEM) was performed to observe muscle fiber microstructures. Fluorescence immunostaining (IF) or immunohistochemical staining (IHC) was performed to elucidate the localization of SR-related factors and matrix metalloproteinases (MMPs).

**Results:** In transverse sections of HE-stained gastrocnemius muscle, numerous tears and lakes formed in the cytoplasm of the muscle fibers, with clear or pink material observed inside them in aged mice. Serial sections stained with Gomori's trichrome revealed red staining in regions of tears and lakes observed with HE staining, suggesting the localization of specific structures. SDH staining indicated a slight decrease in areas with abnormal findings, as described above; however, no novel abnormal findings were observed. TEM analysis of these findings revealed SR aggregates (tubular aggregates; TAs) of various shapes, including circular and tubular. We next confirmed by using IF that dihydropyridine receptor α1 (DHPRα1) and SR calcium ion ATPase 1 (SERCA1) were co-localized in TAs. In addition, in TA surroundings, IHC revealed that Junctophilin (JPH)1 and JPH2 co-localizes with MMP2 with a high concordance rate. In this study, these abnormal findings were not observed in young muscles.

**Conclusions:** TAs were observed in the gastrocnemius muscle of aged mice. SR-related factors such as SERCA1 and DHPRα1 were also found to localize to TAs. JPH1 and JPH2 co-localized with MMP2 in the vicinity of TAs. These findings suggest that SR-related factors are localized in and around TAs, some of which may be damaged by MMP2. Further analyses of effects of TAs on muscle function and mechanisms of their appearance are needed.

## P-55

## Pathological features in hypokalemic periodic paralysis due to *ATP1A2*

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The ATP1A2 gene, which encodes the α2 subunit of the Na+/K+ ATPase abundantly expressed in skeletal muscle and brain astrocytes, has been previously shown to cause familial and sporadic hemiplegic migraine and alternating hemiplegia of childhood. To date, one variant of *ATP1A2*, c.2336G>A (p.S779N), has been reported to cause hypokalemic periodic paralysis (HPP) and CNS involvement. HPP is a rare neuromuscular disease characterized by periodic attacks of acute muscle weakness concomitant with low serum potassium. Several variants have been identified in the channel-encoding genes *CACNA1S*, *SCN4A*, and *KCNJ2*, which underlie nearly 70% to 80% of HPP cases. Muscle fiber inexcitability during attacks of paralysis is due to an abnormal depolarizing leak current through mutant voltage sensing domains of either the sarcolemmal voltage-gated calcium, sodium or potassium channels. Interestingly, although *ATP1A2* is a non-channel gene, a similar leak current was observed in p.779N as a common pathomechanism of HPP. We also identified a patient with c.2336G>A in the *ATP1A2*, a 3-year-old girl with HPP, who exhibited typical periodic attacks, and central nervous system abnormalities same as in previous reports. To elucidate the consequences of HPP, especially the muscle pathological features, we compared her condition with those caused by other reported genes. Muscle biopsy of the patient showed marked fiber size variation, scattered necrotic and regenerating fibers, increased internal nuclei, and large vacuolations. Interestingly, tubular aggregate-like deposits were observed at the periphery of the myofibers. In addition, electron microscopy revealed markedly fragmented nuclei with condensed chromatin, collapsed nuclear membrane, and nucleophagy. These characteristic findings, particularly the abnormal nuclear features, were not observed in cases with mutations in other causative genes, suggesting that these changes may be specific to *ATP1A2*-related HPP and the main cause of muscle weakness in our patient.

## P-56

## Clinical and genetic features of patients with paramyotonia congenita in Korea

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Introduction: Paramyotonia congenita is a genetic muscle disorders characterized by episodic muscle cramps and paralysis, significantly exacerbated by cold exposure and exercise, with non-dystrophic changes on muscle biopsy. It results from pathogenic variants in SCN4A. The condition usually manifests in the first decade of life, though symptoms can be variable. We investigated the clinical and genetic features of Korean patients with paramyotonia congenita.

Method: We conducted a retrospective review of medical records within the myopathy database from August 2002 to March 2024. Among the records examined, we identified six patients with pathogenic variants in SCN4A. Then, we analyzed the patient's clinical, laboratory, electrodiagnostic, and genetic findings.

Result: This study analyzed six unrelated patients. A positive family history was noted in two (33%) patients. Among the six patients, five (83%) were male and one (17%) was female. The median age of myotonia onset was 6 years [interquartile range: 4 – 12 years]. Myotonia was presented in all six patients. Muscle stiffness at the onset of exercise was reported in five (83%) patients. Facial muscle involvement was observed in three (50%) patients. Grip myotonia was present in two (33%) patients. Muscle hypertrophy in the lower leg was evident in three (50%) patients. Paradoxical myotonia, and cold sensitivity were observed in three (50%) patients. Creatine kinase elevation was seen in five (83%) patients, with a median value of 539 U/L [interquartile range: 410-706 U/l]. Needle electromyography was performed in five patients, and myotonic discharges were observed in all cases. The following pathogenic variants of SCN4A were identified in each patients: c.1333G>A(p.Val445Met), c.2078T>C(p.Ile693Thr), c.2111C>T(p.Thr704Met), c.3917G>A(p.Gly1306Glu), c.3938C>T(p.Thr1313Met), and c.4109T>A(p.Met1370Lys). Among them, c.4109T>A was novel but classified as a likely pathogenic variant based on the following evidence: 1) located in a mutational hot spot, 2) absent from controls in gnomAD exomes and genomes, 3) novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before, 4) multiple lines of computational evidence support a deleterious effect on the gene or gene product.

Conclusion: Our study elucidates the clinical and genetic features of Korean patients diagnosed with paramyotonia congenita, contributing to the understanding of the manifestation and genetic basis of this disease.

## P-57

# Comprehensive analysis of splicing abnormalities in multiple brain regions of myotonic dystrophy type 1: Comparisons between frontal cortex, temporal cortex, and cerebellum

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Introduction: Myotonic dystrophy type 1 (DM1) is the most common form of muscular dystrophy in adults, impacting various organs, including the eyes, heart, endocrine system, and central nervous system (CNS). CTG-repeat expansion in the 3' untranslated region of the *DMPK* gene leads to the sequestration of RNA binding proteins such as MBNL and resultantly aberrant mRNA splicing.

Among the various symptoms of DM1, CNS symptoms are vital since they significantly affect the quality of life of patients. Despite reports of missplicing involving tau and other molecules in the DM1 brain, the underlying molecular mechanisms remain largely unexplored. While brain imaging and pathology suggest prominent involvement of the temporal lobe, transcriptomic disparities among brain regions remain inadequately characterized.

To address this gap, we undertook a comprehensive transcriptomic analysis encompassing the frontal cortex, temporal cortex, and cerebellum, utilizing postmortem samples obtained from individuals with DM1.

Methods: RNA was extracted from postmortem brain tissue of six DM1 patients and three disease controls (amyotrophic lateral sclerosis). RNA-Seq analysis was conducted utilizing DNBSEQ, followed by quality control, read mapping, and confirmation of read depth. We utilized LeafCutter to compute isoform percent spliced in (PSI) and identify aberrantly spliced clusters by contrasting DM1 with disease controls. Selected findings were validated using RT-PCR with additional samples.

Results & Conclusions: Our analysis revealed 27, 101, and 24 high-confidence aberrantly spliced clusters in the frontal cortex, temporal cortex, and cerebellum, respectively, with a False Discovery Rate (FDR) $<0.01$ and $|\Delta PSI| > 0.1$. Comparative analysis across brain regions within the same individuals unveiled genes exhibiting shared and region-specific splicing alterations. Validation through RT-PCR, including additional samples, corroborated several splicing abnormalities identified via RNA-seq analysis. Notably, our findings underscored the temporal lobe's pronounced involvement, consistent with substantial pathological changes documented in DM1.

## P-58

## MRI evaluation of sinusitis complications: Comparison of Myotonic dystrophy type 1 and Amyotrophic lateral sclerosis

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**Background:** Although sinusitis is sometimes encountered in myotonic dystrophy type 1 (DM1) patients, there have been only a few case reports. We had performed a retrospective magnetic resonance imaging (MRI) study and reported at the 13th International Myotonic Dystrophy Consortium Meeting that 26% of patients had abnormalities suggestive of sinusitis. In this study, we assessed MRI of patients with amyotrophic lateral sclerosis (ALS) and compared the results with those of DM1, because it was unclear whether or not sinusitis is characteristic complication of DM1. **Methods:** DM1 and ALS patients who had undergone brain MRI at NHO Akita National Hospital between January 2014 and December 2021 were enrolled in this study Sinusitis was evaluated using the Lund-Mackay (LM) score, and an LM score of 4 or higher was defined as having abnormal sinuses, in accordance with the previous literature. **Results:** Participants were 53 patients with DM1 and 56 patients with ALS. The mean age was 52 years for DM1 and 73 years for ALS, with ALS being significantly older. 66% (35/53) of DM1 patients and 41% (21/56) of ALS patients were on a respirator, which was significantly higher in DM1 patients. There were no significant differences in the proportion of male/female patients or tracheostomies. The LM score of 4 or higher was 26% (14/53) in DM1 and 21% (12/56) in ALS, showing no significant difference. The mean LM score was 2.4 for both DM1 and ALS. **Discussion:** The frequency of sinus abnormalities on MRI was similar between DM1 and ALS, and disease specificity was not evident. The frequency was about 3 to 4 times higher than that in the general population (7%) in a previous paper, and sinus should be noted as a focus of infection in patients with both DM1 and ALS.

## P-59

## Assessment of cognitive function in a Japanese DM2 patient

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(Objective)
Myotonic dystrophy type1 and type 2 (DM1 and DM2) are two multisystemic disorders with clinical similarities. However, clinical manifestations in DM2 appear to be more variable and are generally milder than those in adult-onset DM1. DM2 is also rare in Japan. A Japanese DM2 patient whose cognitive functioning was evaluated over time is, accordingly, presented.

(Results)
A 34-year-old Japanese woman began to experience difficulty opening her hands at age 17; and at age 18, her symptoms extended to her knees, which she struggled to flex and extend. She has no family history of neuromuscular diseases. Neurologically, she has exhibited only myotonia, which has gradually progressed. However, complications associated with a CK elevation (708 IU/L) and cataracts have been noted, and her musculoskeletal pathology comprises fiber size variation, fibers with internal nuclei, and type 2 fiber atrophy. Repeat-primed polymerase chain reactions demonstrate abnormal CCTG repeats in the CNBP gene, and the number of repeats has been estimated to be approximately 3,700 times on Southern blotting. She was therefore diagnosed with DM2. Her constructional abilities, general intellectual functioning, frontal lobe functioning, language and memory functions were tested at the ages of 23 and 34. Each test revealed no abnormalities.
A Linkage Disequilibrium (LD) block, ~13 kb surrounding the DM2 repeat was identified, including seven SNPs. All available Japanese and Caucasian DM2 individuals were investigated for these informative SNPs in order to assess the founder haplotype. A unique haplotype common to Japanese DM2 pedigrees, distinct from that shared among Caucasians, was found.
(Conclusions) Japanese DM2 patients do not exhibit cognitive dysfunctions. Despite a consistently found repeat expansion of the CNBP gene, additional mechanisms are responsible for variations in the phenotype.

## P-60

# Consideration focusing on caregivers in intervention research through the program for patients with myotonic dystrophy type 1 and their caregivers

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Introduction: We have developed a psychosocial support program for patients with myotonic dystrophy type 1 (DM1) and their caregivers to improve their quality of life (QOL), increase patients' activity level, and decrease caregiving burden. We analyze and discuss the results of the program intervention with focus on the caregivers.

Methods: Psychosocial support program, which included contents for promotion of disease understandings and psychosocial selfcare such as learning ways to cope with symptoms and to increase activity, was conducted on the caregivers by trained medical staffs. Each caregiver created an eco-map (Hartman A, 1978) to reflect on and discuss their caregiving situations and availability of social resources. Caregivers rated their QOL (SF-12v2) and caregiver burden (J-ZBI_8) pre-and post-intervention.

Results: Five caregivers of six patients participated in the study. Their relationships to the patients were two spouses and three parents. One parent participated in the study as a caregiver of two patients. The parents showed improvement in SF-12v2 scores related to emotional and social aspects, and their eco-maps expressed more connections with family, friends and hobby activity relationships. On contrary, the spouses showed no change in QOL scores, although social resources were present on their eco-maps. Median scores of caregiver burden showed a slight improvement from 3.5 at pre-intervention to 3.0 at post-intervention.

Conclusions: Longer caregiving history of the parent caregivers may be a factor in the enrichment of their social resources. Their rich caregiving experiences may be allowing them to take more personal time. Reflective discussions through creation of eco-maps may be related to the improvement of the caregivers' mental status.

# Poster Session 7

## Distal myopathies

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Toshiaki Takahashi (NHO Sendai Nishitaga Hospital, Japan)
Nobuyuki Eura (Department of Neurology, Nara Medical University, Nara, Japan)

### P-61

### Genetic features of Japanese dysferlinopathies

Toshiaki Takahashi[1], Rumiko Izumi[2,3], Naoki Suzuki[2,4], Chikako Yaginuma[5], Naoko Shimakura[2], Naoko Nakamura[1,2], Yasuko Shimosegawa[6], Tomoko Totsune[1], Yoko Sugimura[1], Takahiko Sasaki[7], Masaru Yoshioka[1], Toru Baba[1], Hideki Oizumi[1], Hiroyasu Tanaka[1], Hirtoshi Warita[2], Tetsuya Niihori[3], Takafumi Hasegawa[1], Atsushi Takeda[1], Yoko Aoki[3], Masashi Aoki[2]

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[Objective] Dysferlinopathies are autosomal recessive muscular dystrophies caused by the dysferlin gene (DYSF) deficiency that leads to two main phenotypes, Miyoshi muscular dystrophy (MMD)1 and limb-girdle muscular dystrophy (LGMD)R2. [Methods] We have screened patients for pathogenic variants in DYSF throughout Japan. We analyzed DYSF variant data identified 89 different pathogenic variants in 265 patients and kindred in 225 families. [Results] One hundred and eight families carried homozygous variants and 103 families carried compound heterozygous variants. Five families carried triple variants, and one family carried quadruple variants. In 11 families we could identify variant in only one allele. There were 41 different truncating variants, 31 missense variants, two in frame small indels, 14 splice site variants, and one large deletion including five exons. One hundred and twenty two patients had MMD, 105 for LGMD, one for distal myopathy with anterior tibial onset that was a rare phenotype of dysferlinopathy, and 16 for hyper-CKemia. Although the most common c.2997G>T variant existed in the IdysF domain, the other missense variants were also found most frequently in the IdysF domain. The c.2997G>T variant and other missense variants in the IdysF domain had higher frequency in patients with LGMD than in patients with MMD. On the other hand, the c.3373del variant had higher frequency in patients with MMD. Furthermore, the c.2997G>T variant was related to late onset. [Conclusions] Although there are some relatively prevalent pathogenic variants, DYSF pathogenic variants in Japan are tailor made mutations and distributed along the entire length of the gene. However, the missense variants locate in the IDysF domain as a hotspot. The pathogenic variants associated with phenotype exist.

## P-62

## Analysis of the distribution of affected muscles in anoctaminopathy

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BACKGROUND: Anoctaminopathy and dysferlinopathy are both subtypes of limb-girdle muscular dystrophy caused by impaired membrane repair. Despite similar clinical manifestations, including the pattern of muscle involvement, detailed comparative studies are limited. This study aims to elucidate the distribution of affected muscles in anoctaminopathy compared to dysferlinopathy.

METHODS: We enrolled 15 patients from the NCNP's muscle registry who met all of the following conditions: 1) genetically diagnosed with anoctaminopathy; 2) availability of all clinical, pathological, and imaging data. The comparison group comprised patients with genetically and/or pathologically confirmed dysferlinopathy. Axial muscle CT and/or T1-weighted MRI data were utilized to assess fat infiltration in each muscle, which was scored by modified Mercuri scale. Hierarchical clustering analysis was performed to evaluate obtained data.

RESULTS: The male to female ratio was 12:3 in anoctaminopathy group and 8:7 in dysferlinopathy group. Average of diagnosis age was 45±22 years old in anoctaminopathy. Fifty-three percent (8/15) of anoctaminopathy patients presented with limb muscle weakness. Four of them showed predominantly proximal muscle weakness and two showed predominantly distal muscle weakness. Average serum CK levels were 2,304 ±1,786 IU/L and 6,591 ± 4,181 IU/L, respectively. In the legs, gastrocnemius and soleus were more predominantly affected in anoctaminopathy, and tibialis anterior, extensor digitorum longus and tibialis posterior muscle were spared. In the trunk, the paraspinal and rectus abdominis muscles were involved. Asymmetric fat infiltration of the muscle was observed in 40% of anoctaminopathy and 27% of dysferlinopathy cases, with no significant differences in the distribution of affected muscles between the two conditions.

CONCLUSION: Although imaging findings often show similar patterns of muscle damage in both conditions, specific differences, particularly in lower limb muscle involvement, exist. These results underscore the need for genetic analysis to confirm diagnoses when such muscle damage patterns are observed, indicating the potential presence of either anoctaminopathy or dysferlinopathy.

## P-63

# The International Clinical Outcome Study for Dysferlinopathy - 10 years of natural history data

Jinhong Shin[1], Meredith James[2], Heather Hilsden[2], Heather Gordish-Dressman[3], John Day[4], Jerry Mendell[5], Roberto Fernandez Torron[6], Matt Harms[7], Alan Pestronk[8], John Vissing[9], Urvi Desai[10], Madoka Yoshimura[11], Tahseen Mozaffar[12], Tanya Stojkovic[13], Elena Pegoraro[14], Jorge Bevilacqua Rivas[16], Montse Olive[15], Carmen Paradas[17], Maggie Walter[18], Volker Straub[2]

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Individuals with dysferlinopathies have highly variable clinical presentation presenting significant challenges for trial readiness. The International Clinical Outcome Study for Dysferlinopathy (COS) was the first multi-country natural history study in dysferlinopathy. The study involved 193 participants from 15 sites across eight countries over three years, with some sites collecting data for up to six years (NCT01676077). In the absence of any disease-specific outcome measures, participants completed a variety of standardised assessments of strength and function, PROM, qualitative and quantitative MRI and biomarker studies. The North Star Assessment for limb girdle type muscular dystrophies (NSAD), was developed, validated and quantified dysferlinopathy presentation and progression. The extension study, COSII, recruited 203 participants (119 new) from 16 sites in nine countries. COS2 participants were aged 14-78, (mean 40), 70% ambulant and 56% were female.

Across COS1 and COS2 cohorts, mean follow up time was 43 months (0-117 months). 20 participants have now been followed for 10 years. We analysed annual progression for all participants over a 10 year period using the NSAD, by gender and ambulation status. Mean NSAD total score at first visit was 26.4 (±15.8), at last visit 18.5 (±15.6). There was statistically significant decrease in mean NSAD total score per year of age (95% CI -1.489 to -1.296, p <.001).

Qualitative MRI defined a characteristic pattern of muscle involvement. Quantitative MRI methods including MRS and fat fraction captured change over three years. Utilising NSAD and MRI, COS confirmed Miyoshi and LGMD2B/R2 are not two distinct phenotypes, a critical finding for clinical management, clinical trial population definition and access to disease modifying treatments.

COS1/COS2, conducted with patient advocacy partners, the Jain Foundation, has developed and determined robust outcome measures, defined phenotype and progression, all critical components for clinical trial readiness. This study is supported by the Jain Foundation and is submitted on behalf of the Jain COS Consortium.

## P-64

# Analysis of a novel mechanism of extracellular vesicles secretion from skeletal muscle

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Dysferlin (DYSF) regulates the cell membrane repair of myotubes, and *DYSF* gene mutations cause dysferlinopathy. Using a Human Embryonic Kidney (HEK) cell-based DYSF expression/reporter system, we previously identified 41 missense DYSF mutations that result in DYSF protein instability, degradation, or loss of plasma membrane (PM) localization. However, it was still unknown how each DYSF pathogenic mutation has characteristics. Here, we found that HEK cells and skeletal muscle cells transfected with DYSF pathogenic mutants of the C-terminal region of DYSF accumulate DYSF protein in vesicles and its fusion with the cell membrane is inhibited. Extracellular vesicles (EVs) are reported to be synthesized from the late endosomes, in which intraluminal vesicles progressively accumulate during their maturation into intracellular multivesicular bodies (MVBs). By analyzing CD63 and Rab27 isoforms, which are EVs-related proteins, in these cells using flow cytometry and immunofluorescence, we found the intracellular accumulation of EV-relateproteins in cells transfected with DYSF pathogenic mutations compared to wild type. Therefore, DYSF appears to influence EVs secretion, at least partly, through interaction with EVs-related proteins. Our results could be applied to biomarkers that predict the progression of genetic and age-related muscle diseases.

## P-65

## Analysis of anoctaminopathy focusing on inflammatory pathology

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BACKGROUND: Both anoctaminopathy and dysferlinopathy are limb girdle muscular dystrophies caused by membrane repair impairment , exhibiting overlapping clinical features. Pathologically, inflammatory features such as HLA-ABC expression and sarcolemmal membrane attack complex (MAC) deposition, typically observed in immune-mediated necrotizing myopathy (IMNM), have been reported in dysferlinopathy but not in anoctaminopathy.

METHODS: To clarify the clinicopathological features of anoctaminopathy compared to dysferlinopathy, we reviewed the clinical and pathological findings of 18 cases of anoctaminopathy, 84 of dysferlinopathy, and 36 of IMNM (18 with anti-HMGCR and 18 with anti-SRP antibodies) randomly selected from the muscle registry in NCNP.

RESULTS: Anoctaminopathy predominantly affected males and demonstrated significantly lower serum CK levels (2,365±1,682 IU/L) compared to other groups. Limb muscle weakness was observed in 50% of anoctaminopathy cases, 74% in dysferlinopathy cases and 94% in IMNM cases. Although 1.3% of dysferlinopathy cases and 39% of IMNM cases had dysphagia or dysarthria, no anoctaminopathy patients showed them. Pathologically, more than 70% of anoctaminopathy cases exhibited necrotic fibers and HLA-ABC expression. Notably, sarcolemmal MAC deposition was observed in 22% of anoctaminopathy cases, 45% of dysferlinopathy cases and in 67% of IMNM. Conversely, sarcoplasmic coarse p62 staining was absent in anoctaminopathy and present only in 2.4% of dysferlinopathy cases, though it was present in 94% of IMNM cases.

Conclusion: Anoctaminopathy exhibits some inflammatory features similar to those in dysferlinopathy and IMNM, including sarcolemmal MAC deposition, which suggests activation of the complement pathway. This pathway could be a therapeutic target, as its inhibition has shown to reduce myofiber damage in dysferlinopathy. The rarity of sarcoplasmic coarse p62 staining indicates that different pathological mechanisms may be at play compared to dysferlinopathy and IMNM.

## P-66

## Multisystem Proteinopathy in Neurological Disorder

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Background: Multisystem proteinopathy (MSP) is a pleiotropic group of disorders which might initially presented inclusion body myopathy (IBM), amyotrophic lateral sclerosis (ALS), frontotemporal dementia (FTD), and Paget disease of bone (PDB). The relationship between genotype and phenotype as well as varied clinical characteristic have not been well described systematically. This study aims to retrospectively analyze the genetic spectrum and described the profile of MSP-related patients with neurological disorder in China.
Methods: A total of 949 patients admitted to the Department of Neurology in Huashan Hospital from 2000 to 2023 who were diagnosed with ALS, IBM or dementia with genetic test were enrolled. The characteristic of clinical examination, medical imaging, lab tests, and genetic test were collected.
Results: Altogether twenty-four patients with MSP were found in our cohort. Most of MSP patients were onset at their third to fifth decade, with 28.6% female. Among them, five of them were with a familiar history. The most common MSP-associated mutations were VCP and ANXA11, accounting for 6 and 10 cases, respectively. Others included one case of HNRNP A1, one case of SQSTM1, three case of MATR3, and one case of OPTN. Patients with ALS were more likely presented with initial upper limbs weakness while IBM were initial with lower limbs weakness regardless of the genotype. Cognitive impairment was found in 5/24, within ANXA11, VCP, and OPTN gene mutations. VCP, MATR3 and ANXA11 gene mutation presented the phenotype of both inclusion body myopathy and amyotrophic lateral sclerosis during the follow-up. Additionally, patients with ANXA11 not VCP, were more reported in China than the United States.
Conclusion: These results enrich the understanding of genotype-phenotype relationship in patients who initially present neurological disorders with MSP-associated mutations and highlighted the discriminations among different MSP-related mutations.

## P-67

# Clinicopathological characteristics of Japanese patients with multisystem proteinopathy

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Objective: Multisystem proteinopathy (MSP) is an inherited disorder in which protein aggregates with TAR DNA-binding protein of 43 kDa form in multiple organs. Mutations in *VCP, HNRNPA2B1, HNRNPA1, SQSTM1, MATR3*, and *ANXA11* are causative for MSP. This study aimed to conduct a nationwide epidemiological survey based on the diagnostic criteria established by the Japan MSP study group.

Methods: We conducted a nationwide epidemiological survey by administering primary and secondary questionnaires among 6,235 specialists of the Japanese Society of Neurology.

Results: In the primary survey, 47 patients with MSP were identified. In the secondary survey of 27 patients, inclusion body myopathy was the most common initial symptom (74.1%), followed by motor neuron disease (11.1%), frontotemporal dementia (FTD, 7.4%), and Paget's disease of bone (PDB, 7.4%), with no cases of parkinsonism. Inclusion body myopathy occurred most frequently during the entire course of the disease (81.5%), followed by motor neuron disease (25.9%), PDB (18.5%), FTD (14.8%), and parkinsonism (3.7%). Laboratory findings showed a high frequency of elevated serum creatine kinase levels and abnormalities on needle electromyography, muscle histology, brain magnetic resonance imaging, and perfusion single photon emission computed tomography.

Conclusion: The low frequency of FTD and PDB may suggest that FTD and PDB may be widely underdiagnosed and undertreated in clinical practice.

## P-68

## A pilot, placebo-controlled trial of 6’-sialyllactose in GNE myopathy

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GNE myopathy is a rare inherited muscle disease characterized by initial ankle dorsiflexor weakness and the presence of rimmed vacuoles in muscle pathology. Recessive mutations in the GNE gene cause the disease, and the gene encodes bifunctional enzyme, UDP N-acetyglucosamine epimerase/N-acetylmannosamine kinase. Since the enzyme is responsible for sialic acid production, patients with GNE myopathy are known to have low levels of sialic acid. Efforts to supplement sialic acid with sialic acid itself, or its metabolites have been made. Our previous trial also used 6’-sialyllactose (6SL), a natural source of sialic acid. Based on the promising results obtained from the previous trial, we conducted a subsequent trial with a placebo arm to address the limitations from the prior trial.

Eleven patients with biallelic GNE mutations were enrolled. After a12-week pre-study observation and stratified randomization, five patients were assigned to the 6SL group and six to the placebo group. All participants visited every 12 weeks for 48 weeks for assessments of muscle performance, muscle MRI, a questionnaire, and a 6-minute walk test at each visit. Additionally, biochemical properties were evaluated.

During the trial period, muscle power did not show superiority in the 6SL group compared to the placebo group. Ankle dorsiflexion steadily decreased in both groups throughout the trial period, while other areas neither decreased nor increased in either group. Muscle MRI revealed a significant increase in fat content in the placebo group, whereas in the 6SL group, there were instances of fat content decrease. In the calf region, the 6SL group exhibited a tendency for the increase trend to slow down after 36 weeks. FACS analysis showed a marked decrease in PNA in the 6SL group, and the reduction in the sialic acid-specific lectin SNA was less pronounced compared to the placebo group.

The results of this clinical trial indicate the superiority of the 6SL group compared to the placebo group, despite the lack of statistical significance in muscle strength changes between the two groups. The absence of observed superiority in muscle strength may be attributed to the short duration of the trial, as muscle strength did not decrease even in the placebo group. However, the slowed increase in fat content observed in muscle MRI and the results from flow cytometry suggest that 6SL could be an effective treatment for GNE myopathy.

## P-69

## Mysterious Foot drop in two sisters from swat Pakistan

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Case report

GNE myopathy is inherited myopathy caused by a GNE gene located on chromosome 9p13. We present two Pakistani sisters with a history of distal upper and lower limbs progressive weakness leading to foot drop and finger drop.26-year-old right-handed Reema Bibi is a mother of two kinds her parents are 1st-degree cousins, presented to a primary care physician with painless foot drop. She also developed bilateral upper limbs distal painless mild weakness. She has three sisters and three brothers. Her younger sister Tahira Bibi age 24 also has similar but less severe defects. EMG showed myopathic units in the tibialis anterior and distal upper limb muscles. There were no spontaneous discharges. CPK of Reema was 200 mcg/l and of Tahira was 187 mcg/l. Because of limited resources, muscle MRI has not been performed. Genetic tests are being arranged through the academic network of AOMC. In the meantime, they have been prescribed physical therapy and ankle-foot orthosis.

At follow-up after one month the weakness had not progressed, she has AFO and regular follow-ups with physiotherapy and rehabilitation therapy adherence.

Acknowledgment; - -Pattern recognition of the disease in resource-poor counties can direct a neurologist to disease-specific tests. The need for genetics cannot be overlooked as is the need for neurogenetic disorders.

## P-70

# Long-term analysis of oculopharyngodistal myopathy: Clinical course and electrophysiological evidence of purely neurogenic changes

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Background: Oculopharyngodistal myopathy (OPDM) is an autosomal dominant myopathy with ocular, bulbar, and distal muscle weakness. In Japan, CGG repeat expansion in 5'UTR of *LRP12* is the most frequent causative mutation. Although clinical features of OPDM as myopathy are being established, there are few reports of cases with only electrophysiologically neurogenic changes or long-term follow-up.
Objective: To clarify the natural history of OPDM cases caused by *LRP12* mutations.
Methods: Among patients admitted to our hospital between 2010 and 2023, we extracted the cases with CGG repeat expansion in *LRP12* and reviewed the clinicopathological and electrophysiological findings. The degree of fat replacement on skeletal muscle MRI was evaluated by the modified Mercuri score.
Results: A total of five patients (male 3, female 2) were identified. Three patients had a familial history. The mean age at onset was 46.2 (30-55) years, and the mean follow-up was 8.6 (6-12) years. The onset symptoms were asymmetrical weakness of the distal lower extremities (n=2), dysphagia, ptosis, and facial muscle weakness (n=1). The mean serum CK was 537 (140-828) IU/L. One patient had respiratory failure, and no patients had sensory, cognitive, or cardiac involvement. All patients showed asymmetric fat replacement on skeletal muscle MRI. On electromyography (EMG), three patients showed myogenic changes with low-amplitude and short-duration motor unit action potentials (MUAPs). Muscle pathology from these three patients showed scattered small angular fibers, rimmed vacuoles, and intranuclear inclusions in interstitial cells. On the other hand, two patients revealed purely neurogenic changes on repeated EMG with decreased motor unit recruitment, large-amplitude and long-duration MUAPs in combination with abnormal spontaneous activity, including positive sharp waves, fibrillations, and fasciculation potentials. They also revealed marked asymmetric fat infiltration in limbs or trunk and needed significantly longer time from initial presentation to diagnosis than those with myogenic changes on EMG (6.0 vs 0.67 years, p= 0.04). During the follow-up period, four patients started to use walking aids, and none developed respiratory failure or cardiac complications. No significant correlations were found between repeat size and clinical findings such as the age of onset, time to using a walking aid from onset, sum or annual rate of change of the modified Mercuri Score.
Conclusion: We illustrated the clinical course of five patients with OPDM harboring *LRP12* mutation. Genetic analysis of *LRP12* should be considered in patients with asymmetric distal muscle weakness, even if EMG does not show myogenic changes.

# Poster Session 8

## UCMD and tendinopathies

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Tsukasa Tominari (National Center of Neurology and Psychiatry, Japan)
Anna Cho (Seoul National University Bundang Hospital / Seoul National University College of Medicine, Republic of Korea)

## P-71

## Lipid metabolism is impaired in mEDS mouse model, and their muscle pathology may recover with HFD feeding

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[Background]
Myopathic Ehlers-Danlos syndrome (mEDS) is caused by mutations in the *COL12A1* gene. mEDS patients exhibit muscle weakness and joint hypermobility soon after birth. However, the mechanisms underlying development of the muscle pathology have not been elucidated yet. Our previous report demonstrated that *Col12a1* deficiency increases fast fiber type and decreases slow fiber type in both the soleus and tibialis anterior muscles, suggesting that glycolysis is dominant over beta-oxidation pathway in muscle. Dietary therapy has been reported to alter the energy balance in muscle pathology and potentially improve muscle function. Our study demonstrates the regulatory role of collagen XII in muscle energy metabolism and the potential for therapeutic intervention through lipid intake.
[Results]
15-week-old male wild-type (WT) and *Col12a1* knockout (12KO) mice were fed a normal diet (ND) or high-fat diet (HFD) for 4 weeks. HFD increased body weight in WT but not in 12KO, while food consumption remained unchanged. 12KO on a ND demonstrated elevated creatinine kinase levels in the blood, muscle pathological changes, and decreased grip strength. Interestingly, HFD restored all the muscle pathologies caused by *Col12a1* deficiency. Next, to elucidate the genes influenced by *Col12a1* deficiency and recovered by HFD, muscle microarray analysis was conducted. In 12KO on a ND, the glycolytic pathway was upregulated, and beta-oxidation was downregulated, as expected. HFD restored the gene expression levels related to these metabolic pathways to the levels found in WT on a ND, suggesting that lipid energy is depleted or unusable in *Col12a1* KO muscle. To elucidate fat metabolism, the volume of visceral and subcutaneous fat was measured, and found to be significantly decreased in 12KO, compared to WT on a ND. Additionally, adipocyte diameter was smaller in 12KO compared to WT on a ND. HFD increased adipocyte diameter in WT but not in 12KO. Immunofluorescence analysis demonstrated pericellular localization of collagen XII in adipocytes, and its expression level and area were increased by HFD. Fat microarray analysis demonstrated that genes related to glycolytic pathway were downregulated and those related to lipid metabolism were upregulated in 12KO on a ND, and which were restored by HFD.
[Conclusions and Discussion]
Our data demonstrated that lipid metabolism was impaired in mEDS mice, and that supplying lipids through HFD could recover the muscle pathology. Because collagen XII is localized around the cells, it may play a role in lipid accumulation and/or secretion by regulating pericellular environment, thereby being involved in muscle metabolism.

## P-72

## Splicing switching of alternative last exons due to a deletion including canonicalpolyadenylation site in *COL6A2* gene causes recessive UCMD

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Collagen VI-related myopathy spans a clinical continuum from severe Ullrich congenital muscular dystrophy to milder Bethlem myopathy, caused by genetic variants in *COL6A1*, *COL6A2*, *COL6A3* genes. Our objective is to report newly identified patient with the pathogenic variants restricted to a polyadenylation signal in the 3'-untranslated region which have not been reported in hereditary muscle disease. We performed clinicopathological diagnosis and analysis using whole genome and RNA sequencing. We report Ullrich congenital muscular dystrophy caused by a homozygous deletion, c.*198_*466del, which includes a polyadenylation signal in canonical last exon of the *COL6A2* gene. The parents were consanguineously married and had the heterozygous variant, but they were completely asymptomatic. In patient's muscles, collagen VI was deficient in the sarcolemma, but present in the interstitium, showing the pattern of sarcolemma-specific collagen VI deficiency rather than a pattern of complete deficiency despite the lack of a polyadenylation signal. The RNA sequencing of patient muscle showed that alternative last exons were raised in COL6A2 transcript. Our case provides a valuable example of the mechanism of alternative splicing switches for polyadenylation selection.

## P-73

## Ullrich Congenital Muscular Dystrophy in Taiwan- A Medical Center Experience

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Background:
Collagen VI is a crucial component of the extracellular matrix. Mutations in COL6A1, COL6A2, and COL6A3 result in Ullrich congenital muscular dystrophy (UCMD), which is the most common congenital muscular dystrophy (CMD) in Taiwan. According to clinical manifestations, patients could be stratified into three phenotypes: mild, moderate progressive and early severe. Our study aims to examine two significant complications, scoliosis and restrictive lung disease, in individual sub-groups of UCMD patients in a referral center for neuromuscular diseases.
Methods:
Seventeen patients were enrolled. All of them were confirmed diagnosis by muscle biopsy and/or mutation in COL6A1,2,3 genes from 2005 to 2024 in Kaohsiung Medical University Hospital. We further analyzed their clinical, imaging and molecular features.
Results:
There were 5 females and 12 males in our cohort. Mean follow up duration was 6.3±3.4 years. Four patients (24%), twelve (71%) and one (5%) were categorized as mild, moderate progressive and early severe type, separately. Early severe type was not analyzed due to small patient number and short follow-up time.
Mild type group:
Forced vital capacity (FVC) was constant with no discernible progression. There is only one case had marked scoliosis since adolescence. The remaining three patients had fewer than 20 degrees in Cobb angle. Cobb angle increased at a rate of 1.28 degree per year. One patient lost ambulation during early adulthood and other three patients walked independently.
Moderate progressive type group:
FVC decreased at a rate of 4.05% per year and mean scoliosis progression rate was 4.47 degree per year. Most of them developed rapid scoliosis before 15 years old. Restrictive lung disease seems to develop before scoliosis in our patients. Most patients lost ambulation during 5-13years old.
Conclusions:
Our study characterized Taiwanese UCMD patients, and revealed that intensive surveillance for lung function and spine deformity are essential for patients with UCMD due to relatively rapid progression of these two comorbidities . Different follow-up strategies may be employed for each UCMD subgroup because to the variations in clinical deterioration rates.

## P-74

# The last nucleotide substitutions in exons of *COL6A1/2/3* induce exon skipping in collagen VI-related muscular dystrophies

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*COL6A1, COL6A2*, and *COL6A3* are causative genes for collagen VI-related muscular dystrophies (COL6RD). In collagen VI-related dystrophies in Japan, mono-allelic variants are major causes of the disease, and the variants lead to single amino acid substitution (missense) and those leads to exon skipping account for 57% and 34% of patients, respectively. Interestingly, we had an experience that a single base synonymous substitution at the last nucleotide in exon caused the skipping of mutated exon in COL6RD patient. To consider the current therapeutic strategies, the precise interpretation of the pathogenicity of the identified variants is very important. Therefore, we aimed to investigate the impact of last or second last nucleotide substitutions in exon on aberrant splicing in *COL6A1/2/3.*

Six suspicious pathogenic variants were found in 12 COL6RD patients and three suspicious non-pathogenic variants were found in 7 individuals with other diseases in our cohort, and a neutral variant was also found in gnomAD. We performed both transcript analyses in patients' muscles and in vitro minigene assay of the variants in HeLa or NIH3T3 cells to monitor the precise events and calculate precise skipping rates, which are directly caused by the variants. We also analyzed the abilities of authentic and de novo splicing sites by in silico prediction using SpliceAI.

All of the muscles from the COL6RD patients harboring the variants showed the skipping of mutated exons, and those from the non-COL6RD individuals did not show the exon skipping. Our results also suggested that apparently 10% of exon skipping is related for exhibiting phenotypes. The findings in the minigene assay were essentially identical to those observed in muscles from the patients and non-affected individuals to support that the identified variants are the determinants for exon skipping or not. Furthermore, in minigene assay, one variant induced exon skipping as well as intron retention, which was not observed in patient's muscle, most probably due to post transcriptional regulation.

Our study revealed that single base substitutions at the last nucleotide position of *COL6A1/2/3* could cause exon skipping, rather than amino acid substitution. Moreover, measuring the skipping rate in in vitro assay and in silico prediction allow us to consider the pathogenicity of the variants, which contribute to splicing or amino-acid substitution.

## P-75

# Differentially expressed genes detected in the rat crural fascia after lengthening contractions using RNA-sequence analysis

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Recent studies have shown that fascia covering skeletal muscle plays some roles not only for a supportive tissue but also for a nociceptive sensory organ under physiological conditions. In pathological conditions with mechanical hyperalgesia after exercise (also referred to as “delayed onset muscle soreness”, DOMS), muscle fascia has long been discussed as the source of hyperalgesia. However, genetic alterations in the fascia under DOMS are largely unknown. Using RNA-sequence analysis in the present study, we investigated the gene expression of the muscle fascia in a rat DOMS model. Rats were loaded repetitive lengthening contractions (LC) to the lower leg extensor muscles under isoflurane anesthesia to induce the DOMS model. Twenty-four hours after LC, the crural fascia covering the exercised muscles was collected and snap frozen. Total RNA was extracted from the fascia and checked their quality. A genetic analysis was performed using an mRatBN7.2 database. Gene expression patterns in the fascia were illustrated using a volcano plot to identify differentially expressed genes (DEGs) between the LC and the contralateral side (control). The RNA-sequence analysis revealed that 12 genes were upregulated and 208 genes were downregulated in expression on the LC side compared to the control (FDR < 0.05). Among the upregulated genes, we confirmed significant increase in the mRNA expression of ankyrin repeated domain 1 (Ankrd1), which is already shown to increase after exercise and to be involved in the anti-inflammatory pathway, using real-time reverse transcription PCR. In contrast, the mRNA expression of nerve growth factor and cyclooxygenase-2, which are known to be involved in the development of DOMS, did not change in the fascia after LC. Enrichment analysis in DEGs using Metascape revealed some pathways termed “sarcomere organization” and “muscle tissue development” in upregulated genes. Several pathways termed “epidermis development”, “intermediate filament organization”, “skin epidermis development”, and others, were detected in downregulated genes. In conclusion, several genes and pathways which may be associated with DOMS in the fascia were found in the present study. Further experiments are needed to clarify how these genes and pathways could contribute to mechanical hyperalgesia in DOMS.

## P-76

# Retinoic acid receptor agonists have dual and opposing effects on injury-induced tendon ossification in a mouse Achilles tenotomy model

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Background: Heterotopic ossification (HO) is abnormal bone formation in soft tissues that occurs primarily after injury and major surgery. This condition often causes local pain and restricts joint motion in the affected limb. Currently, there is no effective prophylaxis for HO. Recent studies suggest the use of retinoic acid receptor (RAR) agonists to suppress HO in patients with Fibrodysplasia Ossificans Progressiva, a congenital disorder characterized by progressive ossification of soft tissue, by modulating the aberrant differentiation of mesenchymal stem cells (MSCs). In this study, we aimed to investigate the potential use of RAR agonists in attenuating injury-induced ectopic tendon ossification using a mouse HO model.
Methods: We used an Achilles tenotomy model and adapalene, an RAR agonist, as a mouse model to elucidate the potential use of RAR agonists for prophylaxis against injury-induced HO. Adapalene or vehicle (DMSO) was locally injected at the Achilles tenotomy site immediately after tenotomy. Hindlimbs were harvested 5 weeks after tenotomy for radiographic and histological analysis. Additionally, we conducted in vitro differentiation experiments with MSCs isolated from mouse tendon tissue.
Results: Contrary to our initial hypothesis, administration of RAR agonists throughout the experimental period (5 weeks) accelerated ectopic tendon ossification in our model. Interestingly, in vitro differentiation experiments using tendon-derived MSCs revealed that RAR agonists play opposing roles in osteogenic and chondrogenic differentiation, promoting the former and suppressing the latter. Indeed, we found that RAR agonists suppressed tendon ossification when administered before cartilage nodule formation, but promoted it when administered afterward.
Discussion: Our findings suggest that RAR agonists have a dual and opposing effect on tendon ectopic ossification, depending on the duration and timing of their administration. Further research is warranted to fully understand these complex effects on MSC differentiation and to explore the potential use of RAR agonists for managing injury-induced HO.

## P-77

## Visualization of dynamic interactions between cartilaginous and tendinous/ligamentous primordia during musculoskeletal development

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During musculoskeletal development, each musculoskeletal component initially develops as an individual unit, but then tendons and ligaments integrate each musculoskeletal component into a single functional unit. Scleraxis (Scx) is a member of the Twist subfamily of the basic helix-loop-helix family of transcription factors and is predominantly expressed in tendons and ligaments during development and postnatal growth. Sox9, a high-mobility-group domain transcription factor, is essential for various stages of chondrogenic differentiation. We previously reported that the Scx+/Sox9+ cell population contributes to the establishment of the junction between cartilage and tendons/ligaments during development. In this study, we newly generated ScxTomato transgenic (Tg) mice. By mating ScxTomato Tg mice with Sox9EGFP knock-in mice, we obtained ScxTomato;Sox9EGFP reporter mice that express green and red fluorescence in Scx+ tendons/ligaments and Sox9+ cartilage, respectively. We analyzed the forelimbs of E13.5 or E16.5 embryos optically cleared by the CUBIC method under two-photon or confocal microscopy and found Scx+/Sox9+ cells localized at finger joint primordia and the interface between tendinous/ligamentous and cartilaginous primordia. Further analysis in cryosections revealed that these cells appeared transiently and rapidly differentiated into Scx+ tendon/ligament cells or Sox9+ cartilage cells after birth. Loss of Scx dysregulated the Scx+/Sox9+ progenitor cell fate determination in the chondrotendinous junction, resulting in the formation of a rounded enthesis rather than the control protruding enthesis. By simultaneous detection of red fluorescence in ScxTomato mice and visualization of collagen fiber development by second harmonic generation (SHG) imaging, we could distinguish between mature and immature tendons. In the absence of Scx, tendon primordia failed to become mature and degenerate with severe defects in collagen fiber formation. By utilizing skeletal muscle autofluorescence and fluorescent reporter-expressing tendons/ligaments and cartilage, we found the misguided muscle with morphological changes resulting from loss of deltoid tuberosity in Scx deficient mice. Therefore, our double reporter mouse system would be a unique and powerful tool for a comprehensive understanding of musculoskeletal development.

## P-78

## Generation and characterization of conditional Mkx knockout mouse

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Tendons, which transmit force from muscles to bones, are crucial structures facilitating various body movements including walking, running and jumping. Our previous study demonstrated that transcription factor Mohawk (Mkx) contributes to tendon development and differentiation. However, the role of Mkx in mature tendon and in adult mice remains unclear. Hence, we generated a novel mouse with loxP-flanked allele and Venus-CreER knock-in allele within *Mkx* locus to elucidate the postnatal role of Mkx in tendons. Firstly, Venus-CreER knock-in mice were generated by microinjection to fertilized eggs with plasmid encoding Venus-CreER and Cas9/gRNA complex against *Mkx* start codon. Mice carrying loxP flanked *Mkx* allele were generated by sequential introduction of Cas9/gRNA complex and ssDNA with loxP sequence. Then $Mkx^{Venus\text{-}CreER}$ mice were bred with $Mkx^{flox/+}$ mice to obtain $Mkx^{Venus\text{-}CreER/flox}$ mice. $Mkx^{Venus\text{-}CreER/flox}$ mice, aged 3 days and 6 weeks, were administrated with tamoxifen every other day for four doses. At 10 weeks of age, tendons, ligaments, and muscles were harvested from those mice and genomic DNA analysis confirmed deletion of the flox allele in $Mkx^{Venus\text{-}CreER/flox}$ mice. Transmission electron microscopy analysis revealed that the diameter of Achilles tendon collagen fibrils is significantly reduced in $Mkx^{Venus\text{-}CreER/flox}$ mice. Additionally, RamDA-seq data clarified the postnatal role of Mkx in tendons, ligaments, and muscles. In our study, we successfully generated $Mkx^{Venus\text{-}CreER}$ and $Mkx^{flox/+}$ mice, and $Mkx^{Venus\text{-}CreER/flox}$ mice which received tamoxifen injections showed hypoplasia of Achilles tendon. These mice represent a novel resource for elucidating the postnatal function of Mkx.

## P-79

# Sclerostin is a modulator of mineralization degree and stiffness profile in the fibrocartilaginous enthesis for mechanical tissue integrity

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Tendons and ligaments are dense fibrous connective tissue that integrates musculoskeletal components in vertebrates. The attachment site between tendons/ligaments and bones is called the enthesis where collagen fibers are mineralized and integrated into bone. There are two different types of entheses: fibrous and fibrocartilaginous entheses. Fibrocartilaginous entheses are located at the epiphysis or the apophysis of the bone, consisting of four graded tissue layers including tendon, unmineralized and mineralized fibrocartilage, and subchondral bone with varying degrees of stiffness. The mineral gradient of the enthesis is thought to be particularly important for alleviating stress concentrations at the bone-tendon interface. However, how the formation of such stiffness gradient is controlled remains unclear. In the present study, taking advantage of Kawamoto's method using adhesive cryofilms, we performed histological and atomic force microscopic analyzes on cryosections of the undecalcified hard tissues to examine the formation of the fibrocartilaginous enthesis of the Achilles tendon. Development of mineralized fibrocartilage was followed by the expansion of unmineralized fibrocartilage after the decreased alkaline phosphate activity in the mineralization front. Calcein labelling revealed that the mineralization front in the enthesis extends unidirectionally towards the midsubstance of the Achilles tendon. *Axin2*-lineage analysis of fibrocartilaginous enthesis revealed activation of canonical Wnt/beta-catenin signalling in fibrochondrocytes. We also found that sclerostin, which antagonizes canonical Wnt/beta-catenin and BMP signaling, is expressed in mature mineralized fibrocartilage adjacent to subchondral bone. Sclerostin, the *Sost* gene product, is known to act as a negative regulator of bone formation and favors bone resorption. Patients who are *SOST* deficient suffer from sclerosteosis or Van Buchem disease. We examined the functional role of sclerostin in fibrocartilaginous enthesis formation in *Sost*-deficient mice generated with Platinum TALENs. In the absence of *Sost*, although the morphology of the mineralized fibrocartilaginous enthesis was not significantly affected, mineralization at subchondral bone was increased as compared to that of wild-type mice. We then conducted tissue indentation experiment using AFM in the non-fixed undecalicified enthesis of the Achilles tendon and found that both unmineralized and mineralized fibrocartilage exhibited higher stiffness in *Sost*-deficient mice. Thus, we conclude that sclerostin in mature mineralized fibrochondrocyte acts as a modulator for mechanical tissue integrity of the fibrocartilaginous enthesis.

# Poster Session 9

## NMJ, Myasthenia Gravis and Centronuclear diseases

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Ito Mikako (Nagoya University, Graduate School of Medicine, Japan)
Tetsuya Takeda (Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University, Japan)

### P-80

### Early Effect of Efgartigimod in Generalized Myasthenia Gravis: A Single Center Experience from China

Jingsi Wang, Qi Wen, Shu Zhang, Yaye Wang, Nairong Xie, Haoran Liu, Yuting Jiang, Qinyao Liu, Yan Lu, Li Di, Min Wang, Min Xu, Hai Chen, Suobin Wang, Wenjia Zhu, Xinmei Wen, Jianying Duo, Yue Huang, Yuwei Da

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Background: In a Phase 3 clinical trial, efgartigimod demonstrated an early onset of effect in 57% of patients with acetylcholine receptor (AChR) antibody-positive generalized myasthenia gravis (GMG). Herein, we present our real-world experience with efgartigimod in GMG patients, aiming to describe the efficacy of the initial infusion and identify factors associated with early response to the treatment.
Methods: We recruited 15 patients with AChR antibody-positive GMG who were treated with efgartigimod at Xuanwu Hospital, Capital Medical University between November 2023 and March 2024. Efficacy was assessed with the Myasthenia Gravis Activities of Daily Living (MG-ADL) scale and Quantitative Myasthenia Gravis (QMG) score. Pharmacodynamic effects were analyzed through validated assays of total IgG and AChR antibodies. Evaluation was done at baseline and one week following the initial infusion. The primary endpoint was the proportion of early responders, defined as patients who achieved at least a 2-point improvement in the MG-ADL score within one week of the first infusion.
Results: Of 15 patients treated with efgartigimod, 6 (40.0%) were women, and the mean age was 61.0 (SD 12.5) years. After the first infusion of efgartigimod, participants had a mean score improvement of 3.13 (SD 2.36) on the MG-ADL scale and 4.27 (SD 3.17) on the QMG score ($P < 0.001$); the ocular, bulbar and limb/gross motor subdomain scores were all reduced significantly ($P < 0.05$). Significant reductions in IgG and acetylcholine receptor antibodies were also observed ($P < 0.001$, $P = 0.002$, respectively). Eleven (73.3%) patients were identified as early responders. These patients had a later age of onset ($P = 0.035$), a lower incidence of thymoma ($P = 0.011$) and higher baseline titers of AChR antibodies ($P = 0.019$) compared to non-early responders.
Conclusions: Efgartigimod demonstrated a rapid onset of action in a proportion of patients with AChR-positive GMG. This early response may be associated with the age of disease onset, the presence of thymoma, and baseline titers of AChR antibodies.

## P-81

## Changes in the Treatment of MG and the Therapeutic Effectiveness of FcRn Antagonists

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BACKGROUND: The advent of anti-FcRn antagonists is changing the treatment of MG. However, there is no consensus on which patients are most appropriately treated with FcRn antagonists. We performed a clinical study of MG patients treated with Efgartigimod (EFG), an anti-neonatal Fc receptor immunoglobulin G1 Fc fragment.
METHODS: Among 180 MG patients (69 men and 111 women, 157 anti-AchR antibody, 8 anti-MuSK antibody-positive patients, and 16 antibody-negative patients) who visited our hospital during 7.5 years from November 2017 to April 2024, patients who received non-oral medications (IVIg, therapeutic apheresis, FcRn antagonists and anti-complement therapy) were extracted. Among those patients who used EFG, we examined the duration of disease, reason for use, steroid dosage at the start of the treatment, degree of symptom improvement before and after treatment (QMGs and MG-ADL), and effect of steroid reduction.
RESULTS: Thirty-nine of the MG patients had been treated with the non-oral medications mentioned above during the above period. Sixteen patients (14 anti-AchR ab, 1 anti-MuSK ab, and 1 antibody negative) used EFG after May 2022, when EFG received medical insurance approval in Japan. The mean disease duration of patients treated with EFG was 11 years and 2 months, and the number of cycles of administration ranged from 1 to 15 (mean 4.2 cycles; median 3). The mean dose of prednisolone taken at the start of EFG administration was 11.6 mg/day (5~30 mg/day; median 10), and the mean dose after the last EFG dose was 8.6 mg/day (5~25 mg/day; median 7.25). At 3~6 weeks after the start of treatment, 11/16 patients (68.8%) had improved their QMGs or MG-ADL by 4 points or more (effective cases). Comparing effective and ineffective cases, 6 of 7 patients treated within 4 years of onset were effective cases, and 5 of them achieved MSE (minimal symptom expression: MG-ADL 0 or 1 point). The most common reasons for treatment with EFG were treatment of acute exacerbations and steroid reduction. In addition, EFG was used in 3 patients with a history of anti-complement therapy, 2 patients for additional treatment after therapeutic apheresis for the crisis, 2 patients for initial exacerbations, and 2 patients for acute exacerbations after COVID-19 infection.
DISCUSSION: In recent years, FcRn antagonist is easier and shorter to administer than IVIg, and is increasingly being used as an alternative treatment for acute exacerbations in patients who cannot be hospitalized or who are unable to tolerate plasma exchange or IVIg. It is expected to be more effective when used early in the course of disease and is also useful for steroid reduction.

## P-82

## Neuromuscular blockade attenuates cramp-like muscle contraction induced by an infusion of hypertonic saline in rats

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Muscle cramp is sudden uncontrollable tightening of the muscle with severe pain or discomfort. It is quite common for many people, especially in athletes during sports games and elderlies during sleep. To date, there is no adequate treatment or prevention methods, and the pathological mechanism has not been well understood. First in the current study, we tried to establish a novel experimental model of muscle cramp using electromyographic (EMG) activity induced by a bolus infusion of hypertonic saline into the muscle. Under inhalation anesthesia with 1.5% isoflurane, a needle-type recording electrode that was insulated only at the tip was inserted into the rat tibialis anterior muscle belly at a depth of 3 mm. Using a 30-gauge injection needle attached to an infusion pump, sodium chloride solution (NaCl, either 5% or 0.9%) was continuously administered to the muscle about 1.5 mm apart from the recording electrode at a volume of 100 μl for 1 min. The control period prior to the injection was set for 2 min, and the recording continued up to 10 min after the cessation of the injection. We found that the net number of EMG events was significantly higher in the 5% NaCl group than in the 0.9% NaCl group. Likewise, the EMG amplitude was significantly higher in the group treated with 5% NaCl. Second, we examined the volume effect of 5% NaCl on the cramp-like EMG activity. Different amounts of 5% NaCl (10~100 μl/min) increased the net number and amplitude of EMG events roughly in a volume-dependent manner. Third, we investigated the effect of neuromuscular blockade on the hypertonic saline-induced EMG activity. Pancuronium bromide, which was administered via jugular vein prior to the recording, almost completely suppressed EMG activity induced by 5% NaCl. Considering previous studies that pain induced by intramuscular injection of hypertonic saline in human muscle is typically perceived as “cramping”, and that all muscular C-fiber nociceptors exhibited prominent excitation in response to hypertonic saline injected into rat muscles. In conclusion, hypertonic saline-induced EMG activity observed in the present study could reproduce muscle cramp-like phenomenon, and the model is useful as a basic research of muscle cramp. The hypertonic saline-induced muscle cramp-like phenomenon is probably mediated via nicotinic acetylcholine receptors at the neuromuscular junction.

## P-83

# Identification of a pair of closely related genes encoding muscle cytoplasmic proteins required for NMJ formation

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Motoneuronal control of skeletal muscle contraction requires the neuromuscular junction (NMJ) that forms in the central region of each myotube (including its mature form, a myofiber) as the only synapse between motor neuron and myotube. The formation and maintenance of NMJs is orchestrated by the muscle-specific receptor tyrosine kinase MuSK, which is cooperatively activated by the muscle cytoplasmic protein Dok-7 and the motor neuron-derived secreted protein Agrin. However, mechanisms underlying MuSK-mediated signaling remain largely unknown. Interestingly, many NMJ-related genes, including those required for the formation and maintenance of NMJs such as the genes encoding MuSK and the nicotinic receptor for neuromuscular transmitter acetylcholine (ACh) are transcribed specifically in only a small number of muscle nuclei (“postsynaptic nuclei”) adjacent to the midmuscle, postsynaptic membrane of NMJ. To identify candidate genes required for the formation and maintenance of NMJs, we first identified several genes that were specifically expressed in the central region of myotube. Then, we performed knockdown and depletion analyses of these genes together with their family members, and found that mice lacking both the Cprt-Xa (tentative) gene, which encodes the cytoplasmic protein Xa (tentative), and its closely related gene CprtXb (tentative) in muscles showed severe defects in NMJ formation. In this poster presentation, we will discuss the essential roles of CprtXa and CprtXb in NMJ formation.

## P-84

# Centronuclear Myopathy in *Dnm2* E368K Mice: Behavioral and Pathological Insights

Genevieve Uy[1], Megumu Ogawa[1], Yukiko Inoue[2], Shinichiro Hayashi[1], Tetsuya Takeda[3], Yoshihiko Saito[1], Shinichiro Hayashi[1], Takayoshi Inoue[2], Ichizo Nishino[1], Satoru Noguchi[1]

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Background: Centronuclear myopathy (CNM) is a genetic disorder characterized by a wide spectrum of myopathy and histologically by a centrally placed nucleus. The *Dnm2* variant has been associated with autosomal dominant CNM and many pathological features in CNM have been reported, but key events critical for muscle weakness remains to be determined. This study investigates the neuromuscular phenotypes of *Dnm2* E368K KI mice.

Methods: A genetically modified mouse model was developed, introducing the E368K variant into *Dnm2* locus using CRISPR-Cas9. Comparative phenotypic analysis between wild type and mutant mice included weight, physiologic assessment of motor function, muscle contraction, histopathology and immunohistochemistry. Additionally, the neuromuscular junction was analyzed.

Results: Mutant *Dnm2* E368K mice exhibited notable reduction in body mass, motor performance, and muscle contraction compared to wild type controls. In histopathology in muscle, type 1 fibers in gastrocnemius are extremely atrophied with centrally located nuclei. Immunostaining with dynamin showed distinct localization at the sarcolemma and in between the z-lines, however in atrophic fibers, it was localized towards the center of the muscle fiber. Notably, the neuromuscular junction exhibited less complex architecture.

Conclusions: The *Dnm2* E368K mouse model effectively recapitulates several aspects of CNM pathology, including muscle weakness and atrophy. This study broadens our understanding of the underlying pathomechanism driving skeletal muscle atrophy associated with CNM. These insights may contribute to ongoing therapies that could ameliorate muscle atrophy and enhance the quality of life for individuals affected by CNM.

## P-85

## Reconstitution approaches to elucidate pathomechanisms of centronuclear myopathy

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Centronuclear myopathy (CNM) is a congenital myopathy characterized by centralized nuclei in skeletal myofibers. T-tubules, sarcolemmal invaginations required for excitation-contraction coupling, are disorganized in the skeletal muscles in CNM patients. Previous studies showed that various endocytic proteins are involved in T-tubule biogenesis and their dysfunction is tightly associated with CNM pathogenesis. DNM2 and BIN1 are two causative genes for CNM that encode essential membrane remodelling proteins in endocytosis, dynamin 2 and BIN1, respectively. In this talk, I will overview how the ordered assembly of these membrane remodelling proteins exert their functions in T-tubule biogenesis and discuss how their defective assembly leads to CNM pathogenesis based on our recent studies using reconstitution approaches both in cellulo and in vitro.

## P-86

## mTOR Signaling Dysregulation Contributes to Impaired Myogenic Differentiation in Mtm1-knockout C2C12 Cells

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INTRODUCTION: X-linked myotubular myopathy (XLMTM) is a devastating rare congenital myopathy caused by pathogenic variants in the *MTM1* gene. Due to the involvement of MTM1 in various cellular processes, the skeletal muscle pathomechanism in XLMTM remains unclear. We have previously found the dysregulated lysosomal dynamics and aberrantly activated mTOR signaling were associated with the pathomechanism using a skeletal muscle model derived from XLMTM patient iPS cells. In this study, we used C2C12 cells, a mouse myoblast cell line, to further investigate the role of mTOR signaling in the pathomechanism of XLMTM.

METHODS AND MATERIALS: To examine the impact of mTOR signaling activation on myogenic differentiation, C2C12 cells were transfected with an expression vector of EGFP alone or a combination of EGFP and wild type or mutant RHEB, a key regulator of mTOR complex 1. Mtm1-knockout (KO) C2C12 cell lines were generated using a CRISPR/Cas9 genome editing technique. These cell lines were incubated with differentiation medium, and the myogenic differentiation was evaluated.

RESULTS: Based on the results of flowcytometric evaluation of EGFP, transfection efficiencies were approximately 35%. As previously reported, mutant-RHEB overexpression increased mTOR signaling activation compared to wild type-RHEB overexpression. After incubation with differentiation medium, mutant-RHEB expressing C2C12 cells showed reduced myotube lengths and decreased expression of myosin heavy chain, a myogenic marker, compared to those in wild type cells. Treatment with rapamycin, an mTOR inhibitor, ameliorated these differentiation defects in mutant-RHEB-expressing C2C12 cells, suggesting that mTOR signaling activation was deleterious to normal myogenic differentiation. Furthermore, Mtm1-KO C2C12 cells exhibited aberrantly regulated mTORC1 signaling and impaired differentiation. Notably, treatment with rapamycin significantly improved the differentiation of Mtm1-KO C2C12 cells, confirmed by evaluating the myotube length, myogenic marker expression, and fusion index. In contrast, rapamycin treatment prevented myogenic differentiation in a dose-dependent manner in wild type C2C12 cells. Together, appropriate regulation of mTOR signaling might be critical in normal myogenesis.

CONCLUSION: Our findings suggest that dysregulated mTOR signaling may contribute to the pathomechanism of XLMTM. The ability of rapamycin to rescue the differentiation defects in both mutant-RHEB-expressing and Mtm1-KO C2C12 cells highlighted the potential of targeting mTOR signaling as a therapeutic strategy for XLMTM. These results underscore the importance of further investigating the relationship between MTM1 and mTOR signaling, as it may uncover novel insights into the pathomechanism of XLMTM.

# Poster Session 10

## Metabolic myopathies

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Akitsu Hotta (Center for iPS Cell Research and Application (CiRA), Kyoto University, Japan)
Takashi Yamada (Sapporo Medical University, Japan)

## P-87

## Telbivudine-induced myopathy and clinicopathological characteristics : Case report

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Background
Telbivudine, an L-nucleoside analogue, is one of the mainstay oral antiviral agents used in the treatment of chronic hepatitis B patients. The mechanism of nucleoside analogs (NAs) is to effectively suppress HBV replication and inhibit reverse transcriptase or DNA polymerase. The use of NAs has an excellent safety profile with rare neurological adverse effects, ranging from asymptomatic hyper CK-anemia, fatigue, malaise, and neuropathy to a severe form of myopathy. We herein report a patient with chronic hepatitis B who developed Telbivudine-induced myopathy.

Case Presentation
A 59-year-old man with a three-year history of chronic hepatitis B presented with progressive weakness. The patient had been experiencing myalgia for a year, which was initially misdiagnosed as spinal stenosis. Two months prior presentation, he started having difficulty climbing ladder steps and bilateral leg weakness. The patient had not taken any other medications that could cause his symptoms while taking Telbivudine.

The neurological examination showed proximal muscle weakness, while sensory loss and deep tendon reflex were unremarkable. The investigation revealed a raised serum creatine kinase (CK) of 1109 IU/L (normal range <190 IU/L). Other laboratory investigations were within normal range, including thyroid function test, ANA, myositis-associated antibodies (MAA), and myositis-specific antibodies (MSA). Electromyography (EMG) was performed but did not show any specified myopathic or neuropathic pattern.

Muscle biopsies showed a variation of fiber size (10-102 microns). Atrophic fibers, degenerative/necrotic fibers, regenerating fibers, fibers with vacuoles, and COX-deficient/negative fibers were present. No abnormal fibers, ring, whorl, or split fibers, were identified. Mild to moderately increased lipid accumulation on ORO was noted. No abnormal glycogen accumulation was detected. Scattered small collections of inflammatory cells in perimysium and endomysium were noted.

Immunohistochemistry revealed mildly sarcolemmal staining on major histocompatibility complex HLA ABC and DR (MHC class I and MHC class II). Sarcolemmal labeling of C5b9-MAC in some fibers was noted. MxA did not express on any fiber. P62 showed diffuse fine granular sarcoplasmic staining on some fibers.
Immunohistochemistry revealed mildly CD3+, CD4+, CD8+, CD20+, CD138 and CD68+ lymphocytes infiltrations.

Ultrastructural study on electron microscope showed the interesting findings that suggest mitochondrial damage. The findings do not go against the diagnosis of toxic myopathy.

Following the diagnosis of myopathy, telbivudine was discontinued and replaced with tenofovir. The patient visits the outpatient clinic with improved clinical signs and symptoms.

Conclusion
This case report highlights the clinicopathological changes of Telbivudine-induced myopathy and emphasizes the importance of monitoring serum creatine kinase levels and recognizing myopathic signs and symptoms from oral nucleoside analogs (NAs).

Keywords: Drug-induced myopathy, Telbivudine, Myopathy, Chronic hepatitis B infection

## P-88

## Neuromuscular junction dysfunction in a glycogen storage disease

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Glycogen storage diseases (GSDs) are a growing group of metabolic anomalies that show diverse enzymatic deficiencies in a single metabolic pathway. Lafora disease (LD), a rare adult-onset neurodegenerative disorder classified as a GSD, is caused by autosomal recessive mutations in the EPM2A or EPM2B genes, which encode laforin and malin proteins that regulate glycogen metabolism. Loss of either gene causes Lafora body (LB) glycogen aggregates in the CNS and PNS of LD patients and mouse models. LD patients develop progressive seizures, dementia, dysarthria, ataxia, and respiratory failure due to abnormal glycogen aggregates in tissues. Functional studies of laforin and malin have focused on LB accumulation in the CNS. In LD, abnormal glycogen inclusions in the peripheral nervous system (PNS) and their effects on skeletal muscle impairment have not been studied. Due to CNS and muscle dysfunction, the synaptic interface at the neuromuscular junction (NMJ) may also be involved in LD. In a laforin-deficient mouse model of LD, we investigate the role of neuromuscular junction (NMJ) defects in LD pathology progression. We found impaired NMJ transmission in LD mice's gastrocnemius muscle using repetitive nerve stimulation and compound muscle action potential measurement. Histopathological analysis using immunofluorescence staining shows pre- and post-synaptic irregularities at the NMJ, which change gene expression of NMJ-associated transcripts at the postsynaptic site, essential for endplate acetylcholine clustering. LD animals have reduced motor end-plate area and more fragmented junctions. In LD animals, collateral sprouting compensated for nerve innervation loss. In LD mice, alpha motor neurons in the ventral horn of the spinal cord were significantly reduced, possibly due to increased gliosis and LB accumulation near motor neurons. In addition to pre- and post-synaptic changes, electron microscopy revealed myopathic changes in the gastrocnemius muscle of LD mice, including muscle ultrastructure disorganisation and atrophy. Our study shows neuropathic and myopathic changes impair LD animals' motor behaviour. This suggests that pre- and post-synaptic modifications cause NMJ dysfunction in LD mice. By looking beyond the CNS, we can better understand LD pathophysiology. This allows new muscle dysfunction and CNS-focused therapies to improve LD disease management.

## P-89

# Glycogen storage myopathies diagnosed by muscle biopsy in Iran, 16 years of experience in a referral center

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Introduction

The glycogen storage myopathies (GSD) are caused by enzyme deficiency in the glycogenolytic or in the glycolytic pathway in striated muscle fibers. Metabolic myopathies are a class of rare diseases, many of which are ultra-rare. Most of the GSDs are autosomal recessive and are more prevalent in Iran due to consanguineous marriages.

Method

Muscle biopsy is still an important tool to diagnose patients with metabolic myopathy presentations. Classic Hematoxylin and eosin stain reveal vacuolar myopathy in many GSD cases and PAS staining is particularly useful in GSD. Interpretation of PAS stain is very important as there are multiple artefacts mimicking accumulations of PAS positive materials. Immunohistochemical study of sarcolemmal proteins could be helpful in diagnosis of lysosomal vacuoles in Pompe disease.

Results

We diagnosed multiple cases of GSD revealing vacuolar myopathy by muscle biopsy, however we were able to diagnose other ultra-rare cases of GSD as well by muscle biopsy. In this study we present numbers of common and rare cases of GSD diagnosed by muscle biopsy and we present very interesting images of muscle biopsy in these patients.

Conclusion

Muscle biopsy is still an important tool to diagnose patients with metabolic myopathy presentations.

## P-90

## *ETFDH* mutation causes excessive apoptosis and neurite outgrowth defect via Bcl2 pathway

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The most common mutation in southern Chinese individuals with late-onset multiple acyl-coenzyme A dehydrogenase deficiency (MADD; a fatty acid metabolism disorder) is c.250G>A (p.Ala84Thr) in the electron transfer flavoprotein dehydrogenase gene (*ETFDH*). Various phenotypes, including episodic weakness or rhabdomyolysis, exercise intolerance, and peripheral neuropathy, have been reported in both muscular and neuronal contexts. Our cellular models of MADD exhibit neurite growth defects and excessive apoptosis. Given that axonal degeneration and neuronal apoptosis may be regulated by B-cell lymphoma (BCL)-2 family proteins and mitochondrial outer membrane permeabilization through the activation of proapoptotic molecules, we measured the expression levels of proapoptotic BCL-2 family proteins (e.g., BCL-2-associated X protein and p53-upregulated modulator of apoptosis), cytochrome c, caspase-3, and caspase-9 in NSC-34 cells carrying the most common ETFDH mutation. The levels of these proteins were higher in the mutant cells than in the wide-type cells. Subsequent treatment of the mutant cells with coenzyme Q10 downregulated activated protein expression and mitigated neurite growth defects. These results suggest that the activation of the BCL-2/mitochondrial outer membrane permeabilization/apoptosis pathway promotes apoptosis in cellular models of MADD and that coenzyme Q10 can reverse this effect. Our findings aid the development of novel therapeutic strategies for reducing axonal degeneration and neuronal apoptosis in MADD.

## P-91

# The novel GAA variant R190G: expanding the spectrum of late onset Pompe disease and possible implications for screening in the Chinese population

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Abstract

Background: Pompe disease (OMIM 232300) is a rare autosomal recessive metabolic disorder characteristic by a deficiency of the lysosomal enzyme acid α-glucosidase (GAA) due to pathogenic variations in the GAA gene. Over 560 variants have been identified, leading to a wide phenotypic spectrum ranging from classic infantile to late-onset Pompe disease (LOPD).

Methods: We performed a comprehensive analysis of a Chinese family with LOPD). Family members were assessed through clinical history, laboratory data, muscle pathology, and whole-exome sequencing. In vitro functional studies were performed to characterize the novel variant.

Results: We identified a novel GAA variant c.568C>G, p.(R190G) in trans with the known pathogenic variant c.1082C>T, p.(P361L). The proband exhibited a mild limb-girdle phenotype, with notable proximal weakness and a 20-year history of abnormal gait, without early respiratory involvement. Biochemical analysis showed a profound reduction in GAA enzymatic activity. In vitro studies confirmed a significant decrease in the stability and function of the GAA-R190G variant. Over expression of this variant in HEK293 cells yielded a 5% residual activity of GAA as compared to wild type (WT) GAA. In addition, by Western blotting the 110 kD band of the precursor of GAA was not detectable in medium and the 76 kD band of functional GAA was drastically reduced in cell lysates.

Conclusion: The identification of the novel pathogenic GAA variant c.568C>G, p.(R190G) expands the mutational spectrum of the GAA gene. Symptom variability in LOPD underscores the need for high-risk screening for specific populations.

Keywords: LOPD, novel pathogenic GAA variant, high-risk screening, in vitro functional studies

## P-92

## Knockdown of LAMP-2 in HEK293T cells impairs lysosome homeostasis

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Introduction: Loss-of-function mutations in the lysosome-associated membrane protein 2 (LAMP-2) gene cause Danon disease. Muscles of patients with Danon disease have specific autophagic vacuoles with sarcolemma features. However, it remains unknown how a LAMP-2 mutation affects the autophagy mechanism. Here, we show that LAMP-2 knockdown results in the alteration of mTOR mediated autophagy and lysosomal degradation.

Methods: LAMP-2 knockdown in HEK293T cells was performed by shRNA transfection. The expression of LAMP-2 was detected by western blotting. To evaluate the effects of LAMP-2 knockdown on autophagy, we performed fluorescence imaging and western blotting. Further, LAMP-2 knockdown cells were stained with the lysosome-targeting pH indicator to measure lysosomal acidification.

Results: LAMP-2 knockdown increased the percentage of LC3-positive compartments per cell. The results of western blotting showed that Transcription factor EB (TFEB) and mammalian target of rapamycin (mTOR) significantly upregulated in LAMP-2 knockdown cells. Statistical analyses confirmed the signal intensity of lysosome-targeting pH indicator reduced in LAMP-2 knockdown cells compared to control cells, suggesting that pH was increased by LAMP-2 knockdown. Furthermore, LAMP-2 knockdown reduced the colocalization of LC3 puncta and acid organelles.

Discussion: LAMP-2 knockdown induces the activation of mTOR and TFEB and impairs lysosomal acidification. As TFEB activation occurs on the surface of lysosomes dependent on mTOR, these results suggest that LAMP-2 knockdown dysregulates autophagy via impaired lysosome.

# Poster Session 11

## Immune-Mediated Necrotizing Myopathy

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Yukako Nishimori (Department of Neurology, Nara Medical University, Nara, Japan/Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan)
Yen-Lin Chen (Tri-Service General Hospital, National Defense Medical Center, Taiwan)

### P-93

### Asymptomatic hyperCKemia for 40 years: Diagnosis of treatable immune-mediated necrotizing myopathy with anti-SRP antibody

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Department of Neurology, Nara Medical University

Background: Immune-mediated necrotizing myopathy (IMNM) is a group of idiopathic inflammatory myopathies characterized clinically by progressive proximal muscle weakness, excessively high creatine kinase (CK) levels, and pathologically by active myofiber necrosis with minimal lymphocyte infiltration. IMNM is often associated with anti-signal recognition particle (SRP) or 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) antibodies. IMNM can occasionally follow a chronic course, which may mimic hereditary muscle diseases.
Case: A 59-year-old man was admitted to our hospital with a chief complaint of longstanding high CK and recently noticed difficulty in climbing stairs. Forty years ago, he was first noted to have hyperCKemia on a medical check-up. Since then, he has consistently had elevated CK levels ranging from 5,000 to 10,000, but he did not seek medical attention due to the absence of any symptoms. He had no family history of neuromuscular disorders or consanguineous marriage. He has been undergoing treatment for ulcerative colitis for the past year but has no history of statin use. On admission, he showed mild muscle weakness in the proximal limbs and neck, graded as 4 according to the Medical Research Council scale, and exhibited a lumbar lordotic posture when standing. He did not have dysarthria and dysphagia. On laboratory tests, he was found to have elevated serum CK (6,624 IU/L), and anti-SRP antibody. Spirogram and electrocardiogram did not show any abnormalities. Skeletal muscle MRI revealed edematous changes with high signal intensity on T2-weighted images in the subcutaneous tissue and bilateral adductor longus muscles, instead of selective fat replacement. On muscle pathology, scattered necrotic and regenerating fibers are seen without mononuclear cell infiltration. Immunohistochemistry showed mild expression of major histocompatibility complex (MHC)-I and sarcoplasmic granular expression of p62.
Conclusion: The patient we presented had a long course of 40 years, initially leading to a suspicion of a hereditary muscle disease. In such cases, it is crucial not to overlook treatable inflammatory myopathies. Skeletal muscle MRI findings, including edematous changes without fat replacement, can be a helpful tool for the diagnosis of chronic IMNM. Additionally, recognizing the potential for such long-term, symptom-free progression is important for accurate diagnosis and timely intervention.

## P-94

# Challenges in the Diagnosis of Immune-Mediated Necrotizing Myopathy: Focus on EULAR/ACR Classification Criteria

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Purpose: Immune-mediated necrotizing myopathy (IMNM) is an inflammatory myopathy characterized by subacute progressive muscle weakness and highly elevated serum creatine kinase (CK) levels. However, some cases show chronic courses and scarce inflammatory findings in muscle pathology, making it difficult to distinguish from muscular dystrophy. Considering that IMNM is potentially treated, there is a strong need for an accurate diagnosis. The EULAR/ACR classification criteria of inflammatory myopathies (IIM), widely used in recent years, are superior in terms of sensitivity and specificity, however, some reports suggested that the sensitivity is lower in IMNM than in other IIM. We applied EULAR/ACR classification criteria to IMNM cases to know the ratio of potentially underdiagnosed cases and investigated the clinical and pathological characteristics.

Methods: In total, 71 HMGCR-IMNM cases and 94 SRP-IMNM cases were included. We compared the clinical and pathological characteristics of the groups that were not classified as IIM (“<possible” group) with those classified as IIM (“definite-possible” group) by the EULAR/ACR classification criteria. We measured antibody titers using ELISA, and index values were calculated as the optical density ratio to the negative controls. A follow-up survey of up to 24 months post-muscle biopsy was conducted for the “<possible” group.

Results: According to the EULAR/ACR classification criteria, 26% of HMGCR-IMNM and 20% of SRP-IMNM cases were classified as the “<possible” group. There were no differences in the age of onset or the duration of illness at biopsy between the groups. The “<possible” group had significantly less neck flexor and proximal limb muscle weakness than the “definite-possible” group. Laboratory findings showed that the “<possible” group had lower serum CK and aldolase levels than the “definite-possible” group. There was no difference in the abnormal signals in muscle MRI or spontaneous activities in needle electromyography. On pathological examination, the “<possible” group had fewer necrotic fibers and p62 granular positive fibers than the “definite-possible” group. Antibody titers were not different between the groups in either HMGCR-IMNM or SRP-IMNM. Of the 22 cases in the “<possible” group that could be followed up, all but two SRP-IMNM cases were not classified as IIM by the EULAR/ACR classification criteria during the follow-up period.

Conclusion: Some IMNM cases are clinically and pathologically mild, show no worsening of symptoms over two years, and cannot be classified as IIM by the EULAR/ACR classification criteria. Antibody testing is recommended even in cases with hyperCKemia of unknown etiology, associated with mild clinical and pathological features.

## P-95

## Myositis associated with antimitochondrial autoantibodies showing early respiratory failure

Kentaro Kawama[1], Shinsuke Tobisawa[1], Akinori Uruha[1], Satoko Ota[1], Hirotake Nishimura[2], Hiromi Onizuka[3], Takashi Komori[4], Kazushi Takahashi[1]

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The antimitochondrial M2 antibody (AMA) is widely recognized as a biomarker for primary biliary cholangitis, but it is also detected in some myositis patients. Myositis associated with AMA (AMA+myositis) represent a clinical subtype of inflammatory myopathy, characterized by axial muscle weakness and rarely respiratory failure. Herein, we report two cases with AMA+myositis, presenting with severe respiratory failure while ambulant. One patient was hospitalized due to severe respiratory failure with unknown cause, but soon after he passed away. The autopsy revealed inflammation in the respiratory muscles, and later AMA was found in the serum. Another patient with a nine-year history of muscle weakness of the four limbs and trunk consulted us for respiratory failure requiring mechanical ventilation. The latter responded to corticosteroid therapy and was successfully weaned off the ventilator support. AMA+myositis could be lethal due to severe respiratory failure, but it is potentially treatable. Careful assessment of axial muscles, in addition to AMA measurement, will be a clue to notice the possibility of AMA+myositis.

## P-96

# Analysis of muscle imaging of patient with anti-mitochondrial M2 antibody-positive myositis

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[2]Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan

To characterize the features in a large cohort of patient with anti-mitochondrial M2 antibody (AM2A)-positive myositis, we reviewed muscle imaging and other clinical information of patients whose pathological diagnosis was made at National Center of Neurology and Psychiatry from January 2015 to December 2020. Fat infiltration was examined in 89 patients with T1 weighted image of magnetic resonance imaging (MRI) or computed tomography, and muscle edema was examined in 68 patients with fat-suppression image of MRI. We used modified Mercuri score or myoedema score previously reported and scored each lower limb muscle regarding fat infiltration and edema, respectively. In addition, the patients were categorized into three groups based on disease duration【 less than 1 year (Group1), 1-to-5-year (Group2), and more than 5 years (Group3)】 and muscle imaging were compared to investigate any potential differences. As a result, muscle of gluteus maximus (score: 1.80), semimembranosus (1.75) and long head of biceps femoris (1.56) showed high degree in fat infiltration scoring, whereas gastrocnemius medialis (1.90), soleus (1.80) and vastus lateralis (1.86) showed high degree in edema scoring. Eight patients were accompanied by edema accentuated in periphery of muscles, and five patients showed subcutaneous edema. In Group 3, although no significant difference was obtained, adductor magnus and semimembranosus had relatively high fat infiltration score among lower limb muscles (adductor magnus, semimembranosus: 2.35). We performed muscle imaging analysis using a large cohort of patients with AM2A-postive myositis and clarified the tendency of muscle damaging pattern, suggesting a possibility that the features of muscle image may be useful for diagnosis.

## P-97

## Clinical characteristic of IMNM patients seen at Neurological Institute of Thailand

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Immune mediated necrotizing myopathy (IMNM) affected wide age range. About two third of patients presented with acute or subacute severe symmetrical weakness of proximal arms and legs, however a slowly progressive disease, distal weakness, and muscle atrophy had been reported. We reviewed the clinical course and the laboratory findings including the muscle biopsy of the adult IMNM patients visited Neurological Institute of Thailand during 1 January 2019 to 1 January 2024. Patients were categorized into 3 groups according to their antibody status ; anti-signal recognition particle (SRP) associated IMNM, anti- 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) associated IMNM, and seronegative IMNM. Severity of disease was based on the Medical Research Council (MRC) grade of weakest muscle group.

A total of 30 patients were included, 68% were female. Nineteen patients (63.34%) were anti-SRP positive, 9 patients (33.34%) were found to have anti-HMGCR antibody, while only 1 patient (0.03%) was seronegative. Mean age was 44.5 years, while the median symptom duration was 3 months. Three fourths of patients presented with proximal muscle weakness, whereas one fourths of patients experienced both proximal and distal weakness. Compared to those with anti-HMGCR associated IMNM, patients with anti-SRP associated IMNM had more severe muscle weakness (MRC grade 3-4/5 versus <3/5 in HMGCR and SRP group, respectively). Dysphagia was found in 2 out of 19 anti -SRP associated INMN patients, and in the only one seronegative IMNM patient. Cardiac involvement (as defined by elevated troponin-I) and respiratory insufficiency requiring assisted ventilation was evident in only one patient who has anti-SRP associated IMNM. Serum creatinine kinase level was markedly elevated in both anti-SRP and anti-HMGCR groups with a median level of 40-fold of the upper normal limit. Corticosteroid was initiated within 3 months in all cases. Majority of them (72%) received intravenous corticosteroid, mostly methylprednisolone, follow by oral corticosteroid, the rest (28%) started with only oral prednisolone. All patients received combination therapy. The most common steroid-sparing agents were azathioprine (66.67%) and mycophenolate mofetil (22.22%). Interestingly, rituximab was prescribed in 40% of patients, mostly due to unfavorable outcomes and/or adverse reaction to steroid-sparing agents. At 6 months follow-up, 83% of patients had mild weakness with no functional limitation, 12% required minimal assistance for ambulation, the rest (5%) required assistance for ambulation.

Our small cohort demonstrated that 1) seronegative IMNM was relatively rare 2) anti-SRP associated patients were more severe than those with anti-HMGCR 3) combined treatment, corticosteroid with steroid-sparing agent or rituximab were preferred in our practice.

## P-98

## Clinical, Laboratory, and Radiological Features of Korean Patients with Anti-SRP Immune-Mediated Necrotizing Myopathy

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Objective: Immune-mediated necrotizing myopathy (IMNM) is a subgroup of inflammatory myopathy characterized by subacute proximal weakness and prominent myofiber necrosis with minimal inflammatory cell infiltrate in muscle biopsies. The two principle categories of IMNM, anti-signal recognition particle (SRP) autoantibodies-IMNM and anti-3-hydroxy-3-methylglutaryl-coa reductase autoantibodies-IMNM, currently account for the largest proportion of IMNM and have been extensively described. This study aimed to elucidate the clinical profile and serological markers of Korean patients with anti-SRP autoantibodies-associated IMNM. Methods: We evaluated the presence of anti-SRP antibodies in 114 patients diagnosed with inflammatory myopathy and 107 control subjects, employing an enzyme-linked immunosorbent assay (ELISA). To determine the most effective diagnostic threshold, we conducted receiver operating characteristic (ROC) curve analysis and computed Youden's index. Retrospective analysis of medical records provided comprehensive clinical data, including age at onset, duration of disease, muscle function, and presence of dysphagia, dysarthria, dyspnea, skin manifestations, cardiomyopathy, interstitial lung disease, malignancies, and therapeutic interventions. Serum creatine kinase (CK) levels were also measured. Results: Optimal cut-off values for anti-SRP antibody titers displayed significant variability, with Youden's index peaking at 0.891, indicating that a threshold of 0.49 U/ml yields the highest diagnostic accuracy. Anti-SRP antibodies were not detected in patients with inclusion body myositis, polymyositis, dermatomyositis, or in the healthy cohort. However, 28 of 54 patients with IMNM (12 males, 16 females) tested positive for anti-SRP antibodies. The median anti-SRP antibody titer was 1.9 U/l [interquartile range: 1.0–5.3] in these patients. In the Korean cohort with anti-SRP antibodies, the median age at symptom onset was 43 years [interquartile range: 36 – 52 years], with a median disease duration of 3 months [interquartile range: 2 – 12 months]. The prevalent clinical manifestations included symmetrical proximal muscle weakness in all (100%), neck weakness in 8 (29%), dysphagia in 5 (18%), dyspnea in 4 (14%), dysarthria in 2 (7%), cardiomyopathy in 2 (7%), and interstitial lung disease in 2 (7%). The median CK level stood at 7,262 IU/l [interquartile range: 5,112 – 9,986 IU/l]. There was coexistence of anti-SRP and anti-Ro antibodies in 13 (46%) patients.
Anti-SRP and anti-Ro antibodies coexisted in 13 patients (46%). MRI scans of limb muscles were performed in seven patients (25%). T1-weighted images showed minimal fatty replacement relative to clinical severity, while T2-weighted images revealed muscle atrophy and edema in all muscles in four patients, and predominant edema of the obturator, gluteus, and adductor muscles in three patients.
Conclusions: This study is the first to investigate anti-SRP antibodies in Korean patients with inflammatory myopathy, offering valuable insights into the clinical characteristics of this autoimmune response in IMNM.

## P-99

## PD-L1 inhibitor is a risk factor to cause severe respiratory failure in immune checkpoint inhibitor-associated myopathy

Hironori Shimizu, Masaki Kobayashi, Naohiko Iguchi, Yukako Nishimori, Hitoki Nanaura, Nobuyuki Eura, Takao Kiriyama, Hiroshi Kataoka, Kazuma Sugie
Department of Neurology, Nara Medical University

【Objective】The recent expansion of treatment indications for immune checkpoint inhibitors (ICIs) in cancer therapy has brought various adverse events including neuromuscular diseases. ICI-associated myopathy is often reported to present myocardial and respiratory muscle involvement that leads to serious or even fatal outcomes. The underlying mechanism of ICI-associated myopathy remains unknown, but it is thought to be distinct from other forms of inflammatory myopathies.【Methods】Clinical characteristics of severe cases who required mechanical ventilation due to respiratory muscle paralysis were retrospectively analyzed, compared with those of mild cases without ventilation, in patients with ICI-associated myopathy who are admitted in our hospital as of March 2019.【Results】The subjects were 10 patients (age 70 ± 9.1 years) with ICI-associated myopathy, 4 with lung cancer, 3 with renal cell carcinoma, 1 each with esophageal cancer, gastric cancer, and hepatocellular carcinoma. Seven of the 10 patients received PD-1 inhibitors (5 cases with nivolumab, 2 with pembrolizumab), 3 received PD-L1 inhibitors (2 with durvalumab, 1 with atesolizumab) and 1 received CTLA-4 inhibitors (1 with pilimumab). Proximal muscle weakness and dysphagia were seen in all the patients. Respiratory muscle involvement was present in 6 patients, 4 of whom required mechanical ventilation (defined as severe cases). All of 3 patients with PD-L1 inhibitors developed orthopnea despite the absence of myocardial dysfunction, and had a paradoxical breathing pattern in supine position which was caused by severe paralysis of bilateral diaphragm. Electromyography was performed in 7 cases, 5 of whom showed myogenic changes with active denervation. Muscle biopsy was performed in 2 cases who both showed findings of myositis in which necrotic fibers were distributed in multifocal clusters. Two cases were complicated by myasthenia gravis (2 cases positive for anti-AChR antibody, 2 positive for anti-Titin antibody, and 1 positive for anti-Kv1.4 antibody). The peak CK level was significantly higher in the 4 severe cases (10,604 ± 2,901 U/L) than in the 6 mild cases (3,489 ± 1,845 U/L). Respiratory muscle paralysis in the severe cases progressed even after initiation of steroids or plasmapheresis and resolved over several months.【Conclusion】All patients who received PD-L1 inhibitors exhibited acute supine respiratory failure due to bilateral diaphragmatic paralysis, extremely elevated CK level and poor response against steroid/plasmapheresis even after early intervention, suggesting PD-L1 inhibitor is a risk factor for severe ICI-associated myopathy.

# Poster Session 12

## Myositis

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Jantima Tanboon (Department of Pathology, Mahidol University, Thailand)
Wenhua Zhu (Huashan Hospital, Fudan University, China)

### P-100

### Exploring immunohistochemical expression in idiopathic inflammatory myopathies at a single center in Vietnam

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Introduction:
Our study describes the pathological characteristics of IIM subgroups in a Vietnamese population and highlights the utility of immunohistochemical staining in their diagnosis. Furthermore, this study is the first in Vietnam to comprehensively classify IIM subgroups by incorporating serological testing and muscle biopsy analysis.
Material and Methods:
This retrospective case series study was conducted at the Department of Pathology, University of Medicine and Pharmacy in Ho Chi Minh City, and included 56 patients diagnosed with IIMs between 2019 and 2023. Diagnosis and subgroup classification relied on clinical examination, serological testing (immunoblot assay with 18 antibodies), and muscle biopsy. Histological analysis and immunohistochemistry (assessing HLA-ABC, HLA-DR, C5b-9, Mx1/2/3, and p62 expression) were performed on tissue samples.
Results:
Six categories of inflammatory myopathy were identified: IMNM (58.9%), DM (23.2%), OM (8.9%), ASS (5.4%), IBM (1.8%), and PM (1.8%). The mean age was 49.7 ± 16.1 years, with a female-to-male ratio of 3:1. Antibody testing was positive in 74.4% of cases (32/43). In 53.5% of cases, a single positive antibody aided in subgroup classification, while the remaining cases required correlation with pathology findings due to multiple antibody positives.
Endomysial inflammatory cell infiltrates were present in 62.5% of cases, showing variations across subgroups (ASS 76%, DM 61%, IMNM 57%, OM 100%). Perifascicular atrophy (PFA) was found in 17.8% of cases, predominantly in ASS (100%) and DM (46%). Muscle fiber necrosis was observed in 75% of cases, with a high prevalence in IMNM (94%), OM (80%), DM (61%), and ASS (67%). Rimmed vacuoles were characteristically present in the single IBM case.
Immunohistochemistry revealed positivity rates of 89% for HLA-ABC, 19.6% for HLA-DR, 57% for C5b-9, and 10.7% for Mx1/2/3. Notably, Mx1/2/3 expression was exclusive to DM cases (5/11), and p62 deposits were identified in the single IBM case. Importantly, the combined use of MAC and MHC staining facilitated the detection of IIMs in 96% of patients. Furthermore, HLA-DR positivity was specific to the ASS subgroup in cases exhibiting both skin lesions and PFA on muscle biopsy. This finding highlights the utility of HLA-DR as a marker for differential diagnosis.
Conclusion:
The diagnosis and classification of IIMs should rely on a comprehensive assessment of clinical features, serological testing, and histopathological characteristics. Immunohistochemistry remains a crucial tool in the diagnosis and differentiation of these subgroups, particularly when findings are correlated with serology and clinical presentation.

## P-101

## Nuclear actin in antisynthetase syndrome

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Presence of myonuclear actin inclusion has been proposed as an ultrastructural hallmark of antisynthetase syndrome associated myopathy. However, since the landmark paper, this interesting finding is rarely addressed. This is likely due to obsolete use of electron microscopy in routine myopathological evaluation. A surrogate immunohistochemical marker could be more practical. The propose of this study is to identify incidence, histologic characteristic, sensitivity, and specificity of nuclear actin as a diagnostic marker for antisynthetase syndrome using anti-sarcomeric actin expression. Immunohistochemical positive controls were selected from antisynthetase syndrome ultrastructurally positive for nuclear actin inclusion. To identify positive controls, at least 100 nuclei of 37 antisynthetase syndrome available for electronmicroscopy were evaluated. Immunohistochemical study was performed in the major subtypes of inflammatory myopathies including: antisynthetase syndrome = 208, dermatomyositis = 299, immune-mediated necrotizing myopathy = 199, and inclusion body myositis =100.

## P-102

# Interstitial lung disease patients associated with antisynthetase syndrome present myopathic change in electromyography without myositis symptoms: a prospective case series clinical study

Maki Ozaki[1], Naohiko Iguchi[1], Naoki Iwasa[1], Tomoo Mano[1,2], Kazuma Sugie[1]

[1]Department of Neurology, Nara Medical University
[2]Department of Rehabilitation Medicine, Nara Prefecture General Medical Center

Objective: Antisynthetase syndrome (ASS) can present predominant interstitial lung disease (ILD), myositis and arthritis. Patients with ASS present ILD prior to myositis are often started immunotherapy if they do not have muscle weakness or high serum CK levels. In these cases, myositis may not have been properly evaluated. We investigated whether patients with ASS-associated ILD are affected by subclinical myositis using normalized electromyography (nEMG) and skeletal muscle magnetic resonance imaging (MRI).

Methods: Three patients who had ASS-related ILD but no subjective symptoms of myositis such as muscle weakness are enrolled. Subjective symptoms were assessed using the Inclusion Body Myositis-Functional Rating Scale (IBM-FRS) and Japanese-Health Assessment Questionnaire (J-HAQ) , and muscle strength was graded using Medical Research Council Scores (MRC scores) and grip strength. As a serological evaluation, CK value was evaluated. The objective features of myositis were detected using nEMG and skeletal muscle MRI. This study was approved by the ethics committee of the Nara Medical University School of Medicine.

Results: All three patients had no decline in IBM-FRS nor high serum CK level. They presented with a myopathic motor unit potential pattern on nEMG. Some muscles showed myopathic abnormalities on nEMG but not on MRI. Patients with ASS-associated ILD may already have underlying myositis before subjective symptoms emerge.

Conculsion: Patients with ILD associated with ASS may potentially have comorbid myositis even in the absence of clinical myositis symptoms.

## P-103

# Interstitial Lung Disease in Patients with Idiopathic Inflammatory Myopathies: Data from a Cohort at a Tertiary Care Center in Karachi, Pakistan

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Objectives: Patients with idiopathic inflammatory myopathy (IIM) are at increased risk of interstitial lung diseases (ILD). IIM complicated by ILD is associated with poor outcomes, necessitating early detection and immediate treatment initiation. We aimed to evaluate the occurrence of ILD in IIM in our South Asian population, and to describe the clinical phenotype and associated serological and radiological parameters in our sample population. Secondary aims were to assess treatment response to rituximab.

Methods: Medical charts of patients previously diagnosed with IIM and receiving treatment at our tertiary care centre were retrospectively evaluated. Demographic, clinical, laboratory, and radiographic data was extracted at point of initial IIM diagnosis and during follow-up. The occurrence of IIM relapses (worsening muscle weakness with elevation of creatine kinase greater than 1000) and ILD relapses (worsening dyspnea with forced vital capacity decrease by greater than 10%) in patients taking rituximab were also evaluated. Univariate and multivariate analyses were performed to identify predictive clinical and serological factors associated with IIM-ILD.

Results: Data from 26 IIM patients was retrospectively analyzed as part of our preliminary analysis (13 females, 50.0%). Of these, 19 patients (73.1%) had ILD. 14 patients (53.8%) with IIM were diagnosed with ILD at initial visit, while the remaining 5 (19.2%) developed ILD during follow-up. The mean age at IIM diagnosis was 46.9 ± 13.3 years. Shortness of breath on exertion and cough were the most common initial presenting symptoms (n=14, 53.8%), followed by muscle weakness (n=10, 38.5%). The most common high-resolution computed tomography pattern was non-specific interstitial pneumonia (n=7, 36.8%), followed by usual interstitial pneumonia (n=4, 21.1%). 17 patients (65.4%) demonstrated clinical improvement in their IIM / IIM-ILD symptoms after maintenance therapy with corticosteroids and immunosuppressants. 6 patients (23.1%) were ultimately treated with rituximab after failure of maintenance therapy. Of these, 5 patients demonstrated clinical improvement and no IIM relapse, while 1 patient experienced worsening ILD symptoms. Univariate analysis identified the initial presenting symptoms of arthralgia ($p=0.01$), cough ($p<0.001$), and shortness of breath on exertion ($p<0.01$) to be independently associated with ILD.

Conclusions: IIM patients should be regularly screened for ILD at initial diagnosis and during follow-up. Patients should receive rituximab therapy if maintenance therapy with corticosteroids and immunosuppressants is insufficient. The role of specific autoantibodies as predictors of IIM-ILD in our local population must be further explored, as we plan to do so upon analysis of our complete patient cohort.

## P-104

# Compound muscle action potential of whole-forearm flexors: A clinical biomarker for inclusion body myositis

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Objective: We reported a new electrophysiology techniques of compound muscle action potential (CMAP), to measure flexor muscle strength across the forearm (Mano. 2023). We aimed to investigate the whole forearm flexor muscle (WFFM) CMAP as a potentially highly reproducible quantitative biomarker of inclusion body myositis (IBM) pathology.

Methods: We prospectively enrolled 14 consecutive patients (10 men and 4 women) diagnosed with IBM based on muscle biopsies. We evaluated the baseline-to-peak amplitude of the WFFM CMAP and other quantitative parameters, including grip and pinch strength, Inclusion Body Myositis Functional Rating Scale (IBMFRS) score, and other routine muscle CMAP amplitudes.

Results: The WFFM CMAP was strongly correlated with disease duration and the IBMFRS score. The WFFM CMAP on the more affected side was lower than that on the less affected side. Furthermore, grip power was strongly correlated with the WFFM CMAP, whereas lateral pinch strength was strongly correlated with the WFFM and first dorsal interosseous CMAPs. The 3-point pinch strength was also correlated with the WFFM CMAP.

Conclusions: This study demonstrates that the WFFM CMAP may serve as a biomarker of severity in IBM.

## P-105

## A patient with IBM-pattern of weakness, history of statin usage, anti-HMGCR positivity and HLA-DRB1*1101 allele

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Inclusion body myositis (IBM) affect people who were more mostly older than 40 years old. They had unique clinical presentations such as long finger flexors and quadriceps weakness, and they progressed slowly. Treatment was not effective in IBM. Statin induced immune-mediated necrotizing myopathy (IMNM) also affected seniors. Patients presented with proximal muscle weakness, especially thigh muscle, which could be severe. However, statin induced IMNM patietnts improved significantly with appropriate treatment. Antibody to 3-hydroxy-3-methylglutaryl-coenzyme A reductase (anti-HMGCR) positivity helped the diagnosis. HLA-DRB1*11:01 was the risk allele. We reported here a patient who was referred to Neurological Institute of Thailand for treatment of statin associated INMN but was found to have the IBM-pattern of weakness.

Sixty-years-old male experienced progressive difficulty getting up for 3 years. He had few falls. Pitavastatin for dyslipidemia was stopped a year ago because of weakness. He had been taking anti-retroviral therapy (TLD regimen) for HIV infection. Test for anti-HMGCR by line blot revealed positive. He was then referred to us. Careful physical examination revealed atrophy of left more than right forearm and atrophy of quadriceps muscles. FDPs were mildly weak on the left. Hip flexors, knee extensors were grade 4 while knee flexors were grade 4+. CK was 869 U/L. EMG (right deltoid, triceps, FDI and iliopsoas) showed reduced recruitment and polyphasia. Anti-CN1A was negative. HLA study revealed HLA-DRB1*1101 and HLA-DRB1*1210. Forearm atrophy together with quadriceps weakness/atrophy in this case raised the possibility of IBM.

Muscle biopsy demonstrated mild variation in fiber size. Fibers contained structures suggestive of rimmed vacuoles were common and scattered. Lymphocytic infiltration in endomysium surrounding and invading into non-necrotic fiber was noted in one area. On modified Gomori's Trichrome, ragged red fibers and rimmed vacuoles were highlighted. Several non-ragged red COX-negative fibers were observed. HLA-ABC (HLA-I) was positive in faint diffuse pattern while HLA-DR (HLA-II) was positive in several fibers. MAC staining was positive in secorating sarcolemma, in a few fibers. MxA was negative and, p62 was positive in dot-like/patchy pattern in many fibers.

This patient might be a case of anti-HMGCR IMNM with IBM pathology or a case of IBM with anti-HMGCR positivity. With history of statin usage, anti-HMGCR positivity was the clue that statin triggered his immune response. However, his pattern of weakness was suggestive of IBM and the muscle biopsy findings did not against the diagnosis. HLA-DRB1*1101 allele itself was also associated with other immune mediated conditions such as sarcoidosis. To conclude our findings, careful clinical evaluation was still crucial. Investigations such as antibody testing helped in diagnosis, but muscle biopsy remained required.

## P-106

## Are MAC Deposits in Amyloid Myopathy a New Entity or a Previously Overlooked Feature?

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Objective: Light chain (AL) amyloid myopathy is a rare entity and nonspecific in terms of symptoms and pathological features, except when examined by Congo red staining. There is a great necessity to improve the recognition and diagnostic accuracy in the clinicopathology of light chain (AL) amyloid myopathy.
Methods: We retrospectively reviewed all the AL amyloid myopathy cases diagnosed in our center between 2021 and 2023, including clinical features, laboratory and electrophysiological findings, muscle imaging, and muscle histology, immunohistochemistry (IHC), immunofluorescence (IF), and electron microscopy (EM).
Results: We identified a cohort of five patients with AL amyloid myopathy with myopathic symptoms as initial clinical features (2 female and 3 male). Four patients were found to have light chain restricted amyloidosis, while one patient was identified as heavy and light chain amyloidosis. Muscle pseudohypertrophy and/or macroglossia serve as distinctive signs that set apart this condition from other myopathies. The pathological changes were chronic myopathic process with markedly increased Congo red staining and MAC (C5b-9) staining in perimysial and endomysial regions, as well as blood vessel walls. Non-branching fibrils, confirmed by EM, compressing and disrupting myofiber membranes, stained positive for MAC and light chain.
Conclusions: The presence of elevated MAC staining levels, in the absence of marked inflammatory characteristics, may indicate the potential existence of amyloidosis. Muscle damage might be due to amyloid fibrils destruction or secondary to complement attack.

# Poster Session 13

## Sarcopenia

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Naoki Ito (Brain-Skeletal Muscle Connection in Aging Project Team, National Center for Geriatrics and Gerontology, Japan)
Keisuke Hitachi (Center for Medical Science, Fujita Health University, Japan)

### P-107

### Muscle-derived IL-1β regulates EcSOD expression via the NBR1-p62-Nrf2 pathway in muscle during cancer cachexia

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Oxidative stress contributes to the loss of skeletal muscle mass and function in cancer cachexia. However, this outcome can be at least partially mitigated by an improved endogenous antioxidant defense system. Here, using the well-established oxidative stress-inducing muscle atrophy model of Lewis Lung Carcinoma (LLC) in mice, we demonstrate that extracellular superoxide dismutase (EcSOD) levels increase in the cachexia-prone extensor digitorum longus muscle. LLC transplantation significantly increased IL-1β expression and release from extensor digitorum longus muscle fibres. Moreover, IL-1β treatment of C2C12 myotubes increased NBR1, p62 phosphorylation at Ser351, Nrf2 nuclear translocation, and EcSOD protein expression. Additional studies in vivo indicated that intra-muscular IL-1β injection is sufficient to stimulate EcSOD expression, which is prevented by muscle-specific knockout of p62 and Nrf2 (i.e., in p62 skmKO and Nrf2 skmKO mice, respectively). Finally, because the increase in circulating IL-1β may lead to other potential unwanted outcomes, we demonstrate that targeting this pathway at p62 is sufficient to drive muscle EcSOD expression in an Nrf2-dependent manner. In summary, cancer cachexia increases EcSOD expression in extensor digitorum longus muscle via muscle-derived IL-1β-induced upregulation of p62 phosphorylation and Nrf2 activation. These findings provide further mechanistic evidence for the therapeutic potential of both p62 and Nrf2 to mitigate cancer cachexia-induced muscle atrophy.

## P-108

# Elucidation of muscle integrity mechanisms supported by heterogeneity of mesenchymal progenitors

Madoka Uezumi[1], Shinichiro Hayashi[2], Satoru Noguchi[2], Ichizo Nishino[2], Tamaki Kurosawa[3], Takeshi Nikawa[4], Akiyoshi Uezumi[1]

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Sarcopenia, characterized by age-related loss of muscle mass and strength, is a major social problem, but its etiology remains unknown. Fatty degeneration and fibrosis are hallmarks of aged muscle and are caused by mesenchymal progenitors residing in the interstitium. Furthermore, it has been shown that mice lacking mesenchymal progenitors exhibit phenotypes closely resembling sarcopenia. Therefore, we investigated the age-related changes of mesenchymal progenitors by bulk RNA-seq. Here, we focus on *Nerve growth factor receptor (Ngfr)*, whose expression significantly decreases with aging in mesenchymal progenitors.

Immunofluorescence staining revealed that Ngfr is not expressed in all mesenchymal progenitors, but only in those localized near the neuromuscular junction (NMJ). To clarify the function of Ngfr, we generated mesenchymal progenitor-specific Ngfr knock-out (Ngfr cKO) mice. Intriguingly, Ngfr cKO mice showed reduced muscle weight and altered motor endplate morphology. To further investigate the molecular mechanisms underlying the phenotype of Ngfr cKO mice, we performed single nucleus RNA-seq. This analysis revealed that loss of Ngfr in NMJ-neighboring mesenchymal progenitors, which localizes to the center of the muscle, has a significant impact on the entire myofiber. We will present the progress of these analyses and discuss the importance of heterogeneity in mesenchymal progenitors.

## P-109

## Characterization of de-nitration activity present in injured muscle extract

Junri Miyamoto[1], Alaa Elgaabari[1,2], Kahona Zushi[1], Sakiho Tanaka[1], Mako Nakamura[1], Takahiro Suzuki[1], Ryuichi Tatsumi[1]

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[2]Department of Physiology, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh, Egypt

Our recent study found age-related tyrosine residue nitration (post-translational chemical modification)/ dysfunction of a myogenic stem satellite cell activator HGF (hepatocyte growth factor) that is essential for postnatal muscle growth and regeneration by promoting the cell activation and proliferation (Elgaabari et al., *Biochem. Biophys. Rep.* 31, e101295, 2022, https://doi.org/10.1016/j.bbrep.2022.101295; Elgaabari *et al., Aging Cell* 23, e14041, 2024, https://onlinelibrary.wiley.com/doi/10.1111/acel.14117). The emerging finding led to a possible insight that de-nitration activity may be present in muscle tissue and restore protein structures and functions by removing nitro groups from nitrotyrosines. Limited studies reported that de-nitration has enzymatic traits, although a denitrase gene(s) has not been yet identified.

Here we show physiological characteristics of de-nitration activity present in lower hind-limb muscles of adult mice (3-month-old) with the aim of identifying a denitrase gene(s). When nitrated BSA and carrier-free HGF (prepared by their exposure to peroxynitrite) were incubated with normal un-injured muscle extract under physiological conditions (pH 7.2, 37°C), the nitration level was decreased as visualized by ECL-Western blotting using anti-nitrotyrosine and anti-BSA or anti-HGF α-chain monoclonal antibodies (mAbs), indicating that de-nitration activity may exist in muscle tissue. Importantly, the de-nitration activity was significantly up-regulated early after muscle injury as revealed by comparative assay at 1-day and 7-day post-injury (dpi) after intramuscular injection of 1 μM cardiotoxin (CTX) (1-dpi > 7-dpi).

The subsequent biochemical experiments demonstrated that de-nitration activity is heat-labile (diminished by 2-min boiling), and is attributed to a molecule(s) retained in the fraction that does not pass through a 10-kDa MWCO ultrafiltration membrane since continuous dilution and reconcentration of this fraction enrich the de-nitration activity. To further characterize the denitrase concerned, we employed a 50-kDa MWCO ultrafiltration membrane to suggest that the molecular size of the putative denitrase is approximately over 50 kDa.

Identification of an enzyme (denitrase) and a reaction pathway(s) responsible for the tyrosine de-nitration awaits further study; the mechanism could be applied to develop pharmaceutical strategies for restoring the HGF function in human/pet/animal health sciences to combat age-related muscle atrophy with impaired regeneration (including sarcopenia and frailty). Finally, considering that HGF displays pleiotropic functions for regeneration/ repair of a variety of tissues/organs and hence therapeutic applications of HGF have been tested in various diseases including liver cirrhosis, chronic renal failure, lung fibrosis, myocardial infarction, arteriosclerosis obliterans, amyotrophic lateral sclerosis, and acute spinal cord injury, prevention and recovery of HGF nitration/dysfunction may be important perspectives to improve the therapeutic effects of investigational HGF-drugs as well as their tissue-specific delivery.

## P-110

## Age-related changes in the capillary network of skeletal muscles

Sakurako Mihara[1], Yuri Yamashita[1,2], Aurelien Kerever[1,2], Kazuteru Murakoshi[1], Satoshi Nakada[3], Eri Arikawa-Hirasawa[1,2,3]

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[Objective] The capillary network in skeletal muscle is not only essential for maintaining the morphology and metabolism of skeletal muscle itself, but also play an important role as a secretory pathway for myokine. Previous studies have reported that the number of capillaries in skeletal muscle changes with age or activity level, but the details of the relationship between age-related changes in muscle fiber type and size and capillary structure have not been fully elucidated. Therefore, we investigated the capillary network in different types of skeletal muscle during aging using a three-dimensional evaluation program that we have developed.
[Methods] Capillaries of skeletal muscles collected from 8-9- and 80-81-week-old female mice (C57BL/6, n=3-4) were visualized by perfusion with a fluorescent agent. The skeletal muscles evaluated were quadriceps femoris, tibialis anterior, soleus, and plantaris. After fixation with 4% PFA, longitudinal sections were imaged with a confocal laser microscope, and vascular structures in the 250 x 250 x 50um area were analyzed for volume, diameter, branching point, and curvature using an Imaris filament tracer. In addition, the expressions of *Vegfa*, *Tek*, *Angpt1*, and *Angpt2* in the quadriceps femoris were analyzed by qPCR.
[Results] Body weight, muscle weight, and tibia length increased with age. In mice aged 80-81 weeks, muscle weight per body weight (mg/g) decreased in the quadriceps femoris, tibialis anterior, soleus, and plantaris muscles, while muscle weight per tibial length (mg/cm$^2$) increased in the soleus muscle. In muscle capillaries, the number of branching points and vascular length density were greater in regions located distally and dominated by slow-twitch fibers (the soleus and deep area of the tibialis anterior). There were no significant differences in vascular topology in the 80-81-week-old mice compared to the 8-9-week-old mice. On the other hand, the expression levels of *Angpt1*, and *Angpt2* were increased in 80-81-week-old mice.
[Discussion] When comparing of 8-9-week-old and 80-81-week-old mice, the muscle capillary network showed no obvious morphological changes, but some molecular changes were shown. The age and sex of the mice used in this study were limited. In addition, it should be noted that the morphological data were not analyzed by individual muscle fiber type, but were averaged within muscle tissue. In addition to previous findings, we will discuss adaptations of skeletal muscle capillaries according to myofiber type and fiber size with aging.

## P-111

## Effectiveness of Curcumin in Sarcopenia: A Systematic Review

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Background: Sarcopenia is a generalized disorder of the skeletal muscle associated with an increased likelihood of adverse outcomes and mortality. Sarcopenia is multifactorial, closely associated with aging, and with a progressive decline in skeletal muscle mass, muscle strength, and physical performance. Curcumin is a nutraceutical investigated for its anti-inflammatory and antioxidant properties. It is inexpensive, accessible, and considered a safe and practical approach to help alleviate the symptoms of sarcopenia and improve muscle mass and function. Objective: The purpose of this systematic review was to gather more conclusive evidence on the effectiveness of curcumin for improving muscle strength, performance, and muscle morphology among adults aged 40 and above with sarcopenia. Methods: The review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement. It focused on any interventional studies on curcumin for adults diagnosed with sarcopenia, with the following outcomes: significant improvement in muscle strength and performance, and improvement in muscle morphology. Studies completed until 2024 were included. The databases searched include PubMed, Cochrane Central Register of Controlled Trials (CENTRAL), CINAHL Plus (EBSCOhost), Embase, and Web of Science. Two independent reviewers screened the literature based on the predefined inclusion and exclusion criteria following the PICO process. The quality of evidence and the risk of bias were evaluated utilizing the CASP Randomised Controlled Trial Standard Checklist and the Cochrane Risk of Bias (ROB) tool, respectively. Results: Three RCTs involving 143 participants were included in the review. After 12 weeks of treatment, there was a significant increase in muscle strength, particularly in hand grip, as well as an improvement in performance measures like gait speed and the distance covered before feeling fatigued. The results support that curcumin has the potential to be an effective nutraceutical for improving physical performance and enhancing muscle strength in sarcopenia. Keywords: sarcopenia, curcumin, systematic review

## P-112

# Effect of Curcumin Supplementation on Rat Skeletal Muscle Morphology and AMPK Levels

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Background: Curcumin has been investigated as a potential natural solution to prevent skeletal muscle decline. The emerging research and development of locally sourced curcumin is an opportunity to produce high-quality oral supplements comparable with the existing imported products. Objective: The primary purpose of this study was to determine the effects of oral curcumin administration on skeletal muscle using an animal model that similarly demonstrated the course of human sarcopenia. Methods: Purpose-bred 11- to 12-week-old female Sprague Dawley (SD) rats were used in this study. These animals were chosen because they were born and raised in well-controlled environments for specific uses in biomedical research. Female rats have been selected because they possess less temperature or activity variance, and have more stable behavior compared to the males. To simulate sarcopenia in this animal model, the tail suspension method introduced by Nemoto and Goyagi (2021) was utilized. The tail suspension method involves reduced hind limb function by suspending the animal's tail for the duration of treatment. The SD rats (N=32) were randomized to receive any of the four treatments: (1) low dose curcumin + vehicle; (2) high dose curcumin + vehicle; (3) vehicle only; and (4) control (distilled water). The interventions were further subdivided into 2-week treatment and 4-week treatment. The muscle architecture of the gastrocnemius muscles on both sides were examined for degeneration, necrosis, nuclear orientation, central nucleation, fibrosis, and singular/group atrophy. The presence of connective tissue, fat tissue, and the number of atrophic muscle cells were also determined. Accurate quantitative detection of the rat total AMP-Activated Protein Kinase (AMPK) was performed in the gastrocnemius muscle tissue utilizing the Enzyme-linked immunosorbent assay (ELISA) kit.

## P-113

# Muscle p62 inhibits CXCL13 expression and protects against aging-induced systemic inflammation

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Aging is characterized by chronic and low-grade inflammation, which plays a central role in aging-related diseases, such as obesity, type 2 diabetes, heart failure, atherosclerosis, and muscle atrophy. Among various organs involved in aging-induced inflammation by increased inflammatory cytokines production, skeletal muscle is a major organ in the regulation of inflammatory cytokines levels during aging. However, studies are yet to elucidate to investigate the molecular mechanisms in the regulation of aging-induced inflammatory cytokines production in skeletal muscle. In this study, using the cultured C2C12 myotubes and muscle-specific p62 overexpression (p62mTg) mice, we investigated that transforming growth factor beta 1 (TGF-β1) regulates SRY-box transcription factor 4 (Sox4) that contributes to increased BCA-1/BCL/CXCL13 (CXCL13) expression in C2C12 myotubes. p62mTg mice ameliorated aging-induced inflammation (i.e., spleen enlargement) in comparison to the wild-type littermates mice, which were associated with inhibited CXCL13 mRNA and protein expression in skeletal muscle. The inhibition of CXCL13 expression in aged p62mTg mice is accompanied by suppressing activated TGF-β1 and Sox4 protein expression in the skeletal muscle. These results suggested that p62 overexpression in muscle ameliorated CXCL13 expression is associated with TGF-β1 activation-induced increased Sox4 expression. Our findings provide further evidence for the potential therapeutic targeting of the TGF-β1-Sox4-CXCL13 signaling pathway to mitigate aging-induced inflammation.

## P-114

# Establishing senescent skeletal muscle cell model to explore the sarcopenia mechanism in mitochondrial bioenergetic changes and set-up the platform for drug screening

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**BACKGROUND**

Sarcopenia, also known as muscle wasting, is a clinical syndrome characterized by a decrease in muscle mass and muscle strength, which are common features of the aging process. Generally, after the age of 50 years, people lose an average of 1-2% of their muscle mass and 1.5-5% of their muscle strength each year.

**MATERIAls & METHODs**

I: C2C12 cells are treated with 100uM $H_2O_2$.
II: JC-1 monomer detects changes in cellular membrane potential in the senescent muscle cell model.
III: Using the Seahorse Bioscience system in real-time live samples in the senescent cell model and to screen drugs or compounds (Pyrroloquinoline quinone) for the treatment of sarcopenia.

**RESULTS**

When C2C12 cells are treated with $H_2O_2$ to induce cellular senescent, the results from JC-1 monomer dye and SA-G-Gal staining indicate that the addition of $H_2O_2$ leads to a higher degree of cellular apoptosis compared to the control group. Aging C2C12 cells exhibit decreased ATP production, maximal respiration, proton leak, and spare respiratory capacity compared to the control group. However, the addition of PQQ (Pyrroloquinoline quinone) to aging C2C12 cells improves these parameters.

**CONCLUSIONS**

Using aging cell models, it is possible to investigate molecular and signaling changes within cellular molecular changes, facilitating an understanding of alterations in mitochondrial biofunction during the aging process. Furthermore, it can offer an extension of future animal models.

# Poster Session 14

## SMA and ALS

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Naoki Suzuki (Department of Neurology, Tohoku University, Japan)
Yutaka Ohsawa (Kawasaki Medical School, Japan)

## P-115

## Two-year efficacy of risdiplam administration for spinal muscular atrophy

Toshio Saito[1], Hisahide Nishio[2]

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[Purpose] To evaluate the efficacy of risdiplam administration for spinal muscular atrophy (SMA) over a 2-year period.
[Subjects] Eleven patients with SMA who received risdiplam for approximately 2 years. The disease type was type 2 in 6 cases and type 3 in 5 cases. There were 6 cases of men and 5 cases of women. The average age was 38.0 years. Nine patients used wheelchairs, and two patients were ambulant. Oral intake was possible in all cases. Breathing was maintained in all cases, and 6 cases used NPPV during sleep. The SMN2 copy number in all cases was 3 copies. One patient had previously received nusinersen.
[Method] We analyzed the Hammersmith Functional Motor Scale-Expanded (HFMSE) and revised upper limb module (RULM) at the start of risdiplam administration, 1 year later, and 2 years later. We also collected subjective symptom changes.
[Results] At the start of treatment, HFMSE was 0 to 63 (mean 12.1, SD 23.8), and RULM was 1 to 37 (13.2, 13.0). HFMSE after 1 and 2 years for 9 wheelchair users was 0-4 (1.4, 1.7) at the start, 0-7 (2.3, 2.3) after 1 year, and 0-5 (1.7, 1.8) after 2 years. RULM was 1-18 (7.9, 6.2) at the start, 1-18 (9.6, 5.8) after 1 year, and 1-19 (8.6, 6.1) after 2 years. The HFMSE of the 2 ambulatory patients was 57,63 at the start, 61,64 after 1 year, and 60,63 after 2 years, and the RULM for both was 37 at the start, 1 year, and 2 years later. Seven patients reported a perceived improvement, such as feeling less tired and being able to use their hands more easily, while four patients said there was no change. In one patient, the dose of risdiplam was halved due to stomatitis.
[Conclusion] Motor function showed a tendency to improve after 1 year with risdiplam administration, but it decreased slightly after 2 years. Long-term data collection is necessary. It is also necessary to examine whether improvements in motor function are reflected in daily life.

## P-116

# Long-Term Impact of Nusinersen on Motor and Electrophysiological Outcomes in Adolescent and Adult Spinal Muscular Atrophy: Insights from a Multicenter Retrospective Study

Ningning Wang[1,2,3], Ying Hu[4], Kexin Jiao[1,2,3], Nachuan Cheng[1,2,3], Jian Sun[1,2,3], JinXue Tang[6], Jie Song[1,2,3], Chong Sun[1,2,3], Tao Wang[7], Kai Wang[4], Kai Qiao[1,2,3], Jianying Xi[1,2,3], Chongbo Zhao[1,2,3], Liqiang Yu[5], Wenhua Zhu[1,2,3]

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[6]Qilin District People's Hospital, Qujing City, Yunnan Province
[7]State Key Laboratory of Medical Neurobiology and MOE Frontiers Center for Brain Science, Fudan University

Background: 5q spinal muscular atrophy (SMA) is a progressive autosomal recessive motor neuron disease.
Objective: We aim to assess the effects of nusinersen on motor function and electrophysiological parameters in adolescent and adult patients with 5q SMA.
Methods: Patients with genetically confirmed 5q SMA were eligible for inclusion, and clinical data were collected at baseline (V1), 63 days (V4), 180 days (V5), and 300 days (V6). The patient's treatment efficacy was monitored by encompassing clinical examination, including the Revised Upper Limb Module (RULM), Hammersmith Functional Motor Scale Expanded (HFMSE), 6-Minute Walk Test (6MWT), and percent-predicted Forced Vital Capacity in sitting position (FVC%) and Compound Muscle Action Potential (CMAP).
Results: 54 patients were screened with the mean age at first administration of 27.03 years (range 13–53 years). The HFMSE in the walker subgroup increased significantly from baseline to V4 (mean change 2.3-point, P=0.015), V5 (+3.0, P=0.004) and V6 (+4.2, P=0.005). The patients in both the sitter and walker subgroup had no significant changes in mean RULM between V1 and the following time points. During nusinersen treatment, significant increases in CMAP amplitudes were observed in the trapezius, abductor digit minimi, abductor pollicis brevis, and tibialis anterior post-treatment (P<0.05). Also, patients with RULM≥36 points showed significant CMAP improvements (P<0.05). Our analysis predicted that patients with CMAP of trapezius ≥ 1.76 mV were more likely to achieve significant motor function improvements. The most common adverse reaction was headache, no serious adverse events occurred.
Conclusions: Nusinersen demonstrated a favorable long-term benefit in SMA patients. This is the first report on the CMAP changes in trapezius after treatment in patients with SMA. The CMAP values effectively compensate for the ceiling effect observed in the RULM, suggesting that CMAP could serve as an additional biomarker for evaluating treatment efficacy. However, further research is needed to explore the more long-term changes in CMAP values in treated SMA patients.

## P-117

## Intrathecal injection of nusinersen in adolescent and adult patients with spinal muscular atrophy: focusing on adverse effect

Wan Ling Hsiao, Wen Chen Liang, Yuh Jyh Jong
Kaohsiung Medical University Hospital

Spinal muscular atrophy (SMA) is a rare autosomal recessive neurodegenerative disease resulting from mutations in the SMN1 gene, leading to spinal motor neuron degeneration. This degeneration causes progressive muscle atrophy and weakness. Nusinersen, the first approved disease-modifying therapy for SMA, has shown significant efficacy in improving motor functions. Taiwan government first approved Nusinersen for SMA patients diagnosed within 1 year of age, with treatment starting before age 7 in 2020. This approval was expanded to include more SMA adolescent and adult patients to receive treatment since April 2023.

In this study, we present the experience of intrathecal nusinersen treatment on thirty patients (13 female and 17 male) ranging from 10 to 57 years old (mean age 29.5 years old) who had onset age < 3 years and RULM score ≧ 15. Among these patients, one had SMA type 2, while the others had SMA type 3a. As of May 2024, a total of 162 intrathecal injections were successfully administered. The challenges encountered with intrathecal therapy included obesity and scoliosis, with the highest BMI in our patients being 38 and the largest Cobb's angle measuring 45 degrees. To address these challenges, lumbar X-rays were performed initially, and in cases where scoliosis was observed, preprocedural 3D reconstruction of spinal computed tomography (CT) and spinal ultrasound were utilized for localization. No CT-guided real-time puncture was required. Despite the overall smooth and successful procedure, some patients experienced common side effects associated with lumbar puncture, such as back or injection site pain (57%), post-puncture headache (47%), and dizziness (43%). Only one patient required an epidural blood patch to alleviate post-puncture headache. Considering that this treatment requires lifelong administration, some strategies were implemented to prevent these side effects, including the application of local anesthetics, intravenous hydration, and temporary maintenance of a supine posture immediately after procedure. Encouragingly, all patients reported improvements in their symptoms, with no further complaints of headache were noted after the implementation of these preventive measures.

This treatment method gains acceptance due to successful application with mild adverse effects. Preventive strategies minimize post-procedural discomfort. However, intrathecal injections remain challenging for severely obese or scoliotic SMA patients. Standardized procedures would improve safety and reduce distress.

**P-118**
**WITHDRAWAL**

## P-119

## Postural changes in respiratory and diaphragm function in amyotrophic lateral sclerosis based on neurophysiological examination

Naohiko Iguchi, Tomoo Mano, Naoki Iwasa, Maki Ozaki, Nanami Yamada, Naoya Kikutsuji, Tomohito Ohashi, Kazuma Sugie

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We examined the correlation between Forced vital capacity (FVC) in patients with amyotrophic lateral sclerosis (ALS) measured when the patient was in the sitting and supine positions. Twenty-five patients with sporadic ALS were enrolled. Pulmonary function tests were conducted with the patient in the supine and upright positions. Supine and upright FVC values were compared with other respiratory function parameters. Supine FVC had a strong correlation with the difference in diaphragmatic thicknesses between full expiration and inspiration (ΔDT). Upright FVC had a moderate correlation and correlated with ADL and motor functional score. Supine FVC correlated more strongly with respiratory function than upright FVC. FVC variability, reflecting the difference between supine and upright FVC, correlated with ΔDT, and Medical Research Council (MRC) sum score. The ΔDT/thoracic excursion ratio predicted FVC decline due to postural change more effectively than ΔDT alone. Compared to upright FVC measurements, supine FVC is a more sensitive indicator of respiratory function; therefore, it is important to detect cases in which supine FVC is decreased. Thoracic excursion measurement and diaphragmatic ultrasound effectively detected decreases in supine FVC. Combining these methods, the ΔDT/thoracic excursion ratio identifies FVC decline due to postural shifts to the supine position in patients with ALS. Changes in diaphragm function using the supine FVC and ΔDT should be monitored.

## P-120

# Postural facial deformation and virtual fit of non-invasive ventilation mask in amyotrophic lateral sclerosis

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Background
Use of a non-invasive ventilation (NIV) mask in bulbar amyotrophic lateral sclerosis (bALS) is difficult for several reasons. We used three-dimensional scanning to analyze the facial deformations in different postures in bALS compared to those of healthy participants, then adjusted the masks virtually to examine the possible effects on user compliance.
Materials and Methods
We conducted a study with two groups, i.e., 14 bALS patients and 14 healthy participants aged from 18 to 60 years. Three-dimensional scanning system (3dMD, Atlanta, USA) of facial deformation when changing posture (sitting to supine and sitting to lateral decubitus) were performed. The extent of postural deformations was analyzed using the ALS Functional Rating Scale-Revised subscore and Urimal Test of Articulation and Phonation. In addition, the participants were virtually fitted with an NIV mask to identify possible complications.
Results
The anteroposterior directional difference at the oral area was significantly greater in the bALS group when changing from sitting to supine, and lateral deviation of the oral area was significantly greater compared in the bALS group when changing from sitting to lateral decubitus (Figure 1). The extent of facial deformations, particularly of the oral region, was significantly related to the clinical scores (Table 1). The virtual application of the NIV mask to the participants showed significant changes in the chin, cheek, and nasolabial fold according to the postural change (Figure 2).
Conclusion
We showed that in bALS, use of NIV masks can encounter several difficulties in different postures, and thereby reduce mask compliance. Different regions of the face were prone to complications in different postures in virtual fitting and this effect should be carefully considered.

Keywords: Amyotrophic lateral sclerosis; Non-invasive ventilation; Virtual fit analysis; Facial deformation; 3-dimentional scan

**P-121**
**SESSION CHANGE**

## P-122

# Analysis of aberrant phase separation of RNA-binding proteins associated with ALS

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[Objective] Nuclear import receptors (NIRs) not only transport RNA-binding proteins (RBPs) but also modify phase transitions of RBPs. Arginine-rich poly-dipeptides derived from C9orf72-related ALS/FTD (C9-ALS/FTD) have been found to interact with NIRs and cause nucleocytoplasmic transport deficit, though the molecular basis for the way in which arginine-rich poly-dipeptides affect NIRs function as phase modifiers remains elusive. [Methods] To evaluate the effect of arginine-rich poly-dipeptides on phase modifiers, we performed multiple biochemical and biophysical examinations, including hydrogel binding assay, immunoprecipitation (IP), size-exclusion chromatography (SEC), nuclear magnetic resonance (NMR) and molecular dynamics (MD). [Results] NIRs blocked polymerization of low-complexity domain of FUS/TDP-43 and significantly lost the ability in the presence of arginine-rich poly-dipeptides (**P<0.01). IP confirmed the interaction between NIRs and arginine-rich poly-dipeptides in a cellular environment. SEC revealed the stoichiometry between NIRs and arginine-rich poly-dipeptides. NMR analysis verified by the MD calculation showed that arginine-rich poly-dipeptides target the NLS-binding site (L539, I540, I642 and V643) located at the negatively charged cavity of NIRs. [Conclusions] Arginine-rich poly-dipeptides impede the NIRs function as phase modifiers, highlighting the molecular mechanism for potential C9-ALS/FTD therapeutic targets.

# Poster Session 15

## Experimental Atrophy

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Masaki Inada (Department of Biotechnology and Life science, Tokyo University of Agriculture and Technology, Japan)
Motoyasu Hosokawa (Department of Developmental Biology and Funcutional Genomic Ehime University, Graduate School of Medicine, Japan)

## P-123

## SFPQ maintains skeletal muscle mass through regulating aerobic metabolism

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RNA binding protein SFPQ regulates transcriptional elongation of extra-long genes (Takeuchi et al., *Cell reports*, 2018). Skeletal muscle-specific *Sfpq* knockout mice (KO mice) displayed marked reduction in muscle mass and metabolic myopathy, and investigation of regulatory target genes utilizing primary myotubes revealed that SFPQ governs the expression of long genes related to energy metabolism (Hosokawa et al., *iScience*, 2019).

In further elucidating the link between the reduction in muscle mass and compromised muscle metabolism, transcriptome analysis of skeletal muscle revealed significant enrichment of down-regulated genes in pathways associated with mitochondria in muscle of KO mice. Concurrently, mitochondrial function was reduced upon SFPQ disruption, indicating that SFPQ predominantly regulates mitochondria pathways among the metabolic pathways. Considering that the decrease in muscle mass was caused by a metabolic imbalance due to compromised aerobic metabolism such as lipid metabolism, which is energy efficient, KO mice were fed a high-fat diet (HFD) in order to rectify the imbalance. HFD improved muscle mass reduction and induced the activation of aerobic metabolism-related gene expression in muscle of KO mice, which correlated with changes in gene expression after exercise, including increased expression of PGC1α. HFD partially reversed metabolic imbalance and had a positive effect on skeletal muscle mass in KO mice, supporting that the imbalanced metabolism resulting from aerobic metabolic disruption is responsible for muscle mass reduction in KO mice. Furthermore, the transcriptome changes in KO mice fed on HFD were compared to those in aging human skeletal muscle, showing several similarities. These results will lead to identifying therapeutic target pathways for muscle wasting diseases such as sarcopenia, myopathies, and cachexia.

## P-124

# The impact of ketogenic diets blended with medium-chain triacylglycerols on skeletal muscle metabolism during atrophy

Harune Murakami[1], Yuna Izumi-Mishima[1], Erina Oya[1], Sonoko Yasui-Yamada[1], Rie Tsutsumi[1], Kazuhiro Nomura[1], Hiroshi Sakaue[1,2]

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Background and Aim.

Skeletal muscle atrophy detrimentally affects both quality of life and activities of daily living and bears substantial implications for prognosis. However, the establishment of effective nutritional interventions for muscle atrophy remains elusive. This study aimed to elucidate the effect of medium-chain triacylglycerols (MCTs)-blended ketogenic diets (MCTKD) on skeletal muscle metabolism during atrophy.

Methods and Results.

The study utilized MCTs-blended ketogenic diets containing 42.3% nutritional MCT oils. C57BL/6J mice were fed the experimental diets for 2 days, then subjected to bilateral cast immobilization of hind limbs, while unfixed mice served as controls. Metabolomics analysis revealed higher amounts of glycolytic metabolites and free amino acids in skeletal muscle of normal diet (ND)-fed unfixed mice compared to ND-fed cast-immobilized mice. Conversely, TCA cycle metabolites were decreased in immobilized skeletal muscle. Although 3-hydroxybutyric acid content in skeletal muscle remained unchanged with MCTKD feeding alone, it increased 10 hours after cast immobilization in MCTKD-fed mice. Moreover, the expression of Oxct1, a key enzyme in ketone body metabolism, was upregulated in skeletal muscle of MCTKD-fed mice after 24 hours of cast immobilization, suggesting enhanced ketone body utilization in immobilized skeletal muscle. Additionally, MCTKD feeding suppressed the increase in glycolytic metabolites and free amino acids in immobilized skeletal muscle. Furthermore, MCTKD feeding partially mitigated muscle weight loss in the early phase of cast immobilization. The study also investigated the effects of MCTKD feeding on skeletal muscle metabolism during atrophy in a mouse model of cancer cachexia. BALB/c mice implanted with colon26 cells, a mouse colorectal cancer cell line, were fed MCTKD for 22 days, resulting in alleviation of skeletal muscle atrophy caused by cancer cachexia without concurrent tumor suppression.

Conclusions.

Our results suggest that MCTKD feeding partially prevents skeletal muscle weight loss through alteration of the skeletal muscle metabolism during atrophy.

## P-125

# Microgravity inhibits myoblast proliferation by reduced intracellular $Ca^{2+}$ levels due to suppression of extracellular $Ca^{2+}$ uptake

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【Background】
Muscle atrophy is caused by inactivity and bed rest on Earth and microgravity in space. However, the sensing mechanism of inactivity and how gravity is involved in muscle homeostasis remain unclear. In space experiments, there have been several reports that microgravity suppresses skeletal muscle cell differentiation, but skeletal muscle cell proliferation has not been well studied. Although there have been reports that myoblast proliferation was suppressed under simulated microgravity and a decrease in intracellular $Ca^{2+}$ levels ($[Ca^{2+}]_i$) via decreased TRPC1 expression has been suggested, the actual $[Ca^{2+}]_i$ and other details have not been examined. Therefore, in this study, we hypothesized that microgravity decreases $[Ca^{2+}]_i$ and suppresses cell proliferation, and investigated the effects of changes in $[Ca^{2+}]_i$ on skeletal muscle cell proliferation under microgravity, and also pursued the cause of changes in $[Ca^{2+}]_i$.
【Methods】
C2C12 cells were divided into two groups: one cultured at 1G (CON group) and the other in simulated microgravity (SMG group) using the Gravite®. Cell number (24, 48 hours), percentage of BrdU positive cells (5, 16, 24, 48 hours), and $[Ca^{2+}]_i$ using Fluo4-AM (5, 16, 24, 48 hours) were measured. mRNA expressions of cell cycle markers (Ki67), mechanosensitive $Ca^{2+}$-related ion channels (TRPC1, TRPM7, Piezo1, Piezo2), and other intracellular $Ca^{2+}$ dynamics-related molecules on the plasma membrane (DHPR, Orai1) and sarcoplasmic reticulum membrane (STIM1, IP3R, SERCA1) were analyzed by qPCR at 24 and 48 hours. Rescue experiments were conducted by adding Yoda1 (Piezo1 activator) to the SMG group at 24 hours.
【Results】
In the SMG group, cell number, the percentage of BrdU-positive cells, and Ki67 mRNA expression significantly decreased after 24 hours compared to the CON group. $[Ca^{2+}]_i$ in the SMG group was significantly lower than in the CON group from 16 hours onward. The mRNA expression levels of TRPC1, TRPM7, Piezo1, and Piezo2 were significantly decreased in the SMG group at 48 hours, while mRNA expression levels of other intracellular $Ca^{2+}$ dynamics-related molecules were not changed. In the rescue experiment, adding Yoda1 in the SMG group restored cell number and $[Ca^{2+}]_i$.
【Conclusion】
These results indicate that myoblast proliferation is suppressed due to a reduction in $[Ca^{2+}]_i$ induced by microgravity. Furthermore, it is suggested that this decrease in $[Ca^{2+}]_i$ is primarily due to diminished uptake of extracellular $Ca^{2+}$, with Piezo1 implicated as one potential contributing molecule.

## P-126

# Stroke -prone spontaneously hypertensive rats exhibit delayed skeletal muscle recovery from disuse atrophy by suppression of ribosomal protein S6 phosphorylation

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**Objective:** Skeletal muscle atrophy is a serious problem in older individuals, most of whom have hypertension. We previously reported an inverse correlation between blood pressure and body weight in research using normotensive Wistar-Kyoto rats (WKY), spontaneously hypertensive rats (SHR), and stroke-prone SHR (SHRSP)[1]. We also found that SHRSP presents as a slow-twitch specific hypotrophy[2]. We hypothesized that hypertensive SHRSP may be affected by disuse atrophy and/or recovery from disuse atrophy because slow-twitch skeletal muscles are more susceptible to disuse atrophy than fast-twitch skeletal muscles. We investigated hypertensive rats' responses to disuse atrophy and recovery from disuse atrophy for the possible relationship between hypertension and skeletal muscle disuse atrophy and/or recovery from disuse atrophy.

**Materials and methods:** Twelve-week-old male WKY and SHRSP were subjected to tail suspension for 7 days, followed by reloading for 0, 1, 3, or 7 days. To evaluate disuse atrophy and recovery, changes in weight and cross-sectional area of the soleus were measured, and histological (hematoxylin-eosin and electron microscopy) and western blot analyses were performed.

**Results:** The solei of WKY and SHRSP showed the same rate of atrophy after tail suspension, but recovery after reloading was delayed in SHRSP. WKY, but not SHRSP, exhibited sarcomere structure disruption due to tail suspension followed by necrosis, inflammatory cell infiltration, and edema due to reloading. No significant difference in ubiquitination due to tail suspension was observed in either rat type compared to the non-tail-suspended groups. However, ubiquitination after reloading was delayed in SHRSP. Phosphorylation of ribosomal protein S6 (RPS6), an indicator of protein translation, was elevated (~2-fold) upon reloading in WKY relative to RPS6 phosphorylation in SHRSP. P70-S6 protein kinase 1 (S6K1), which acts upstream of RPS6, was phosphorylated at Thr389 in an mTORC1-dependent manner to the same level in WKY and SHRSP. However, the expression of P60-S6K1, a shorter isoform of P70-S6K1, increased only in WKY. In addition, expression of slow myosin heavy chain due to reloading significantly increased only in WKY.

**Conclusions:** SHRSP exhibited considerably different phenotypes after tail suspension and reloading. In addition to P70-S6K1 phosphorylation, increased P60-S6K1 expression in response to reloading is important to achieve cooperative activation of RPS6 to accelerate protein translation and accelerating recovery.

(1) Inoue et al, (2017) *Exp. Physiol.* 102: 1435-1447.
(2) Inoue et al, (2019) *Life Sci.* 237: 116919.

## P-127

# Ratio of skeletal muscle resident cells fluctuates during immobilization-induced muscle atrophy and subsequent recovery

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【Introduction】Recent advances have elucidated the molecular mechanisms of muscle regeneration. However, understanding of muscle atrophy and the recovery process remains limited. Currently, there are no clinically approved drugs that can accelerate the recovery of atrophic muscles. Therefore, there is an urgent need to develop such treatments. In this study, we aimed to understand the molecular mechanism underlying recovery from muscle atrophy, focusing on mesenchymal progenitor cells (MPCs) and satellite cells (SCs).
【Methods】Hind limbs of 10-week-old mice were immobilized using rubber-coated wire for 2 weeks, after which the immobilization was removed. Muscle weight-to-body weight ratio and muscle fiber cross-sectional area were evaluated for up to 2 weeks after immobilization was removed (total follow-up of 4 weeks). The ratio of MPCs and SCs was evaluated by flow cytometry. Additionally, gene expression and histological analyses were performed using the tibialis anterior muscle.
【Results】The average muscle weight-to-body weight ratio and the cross-sectional area before immobilization were 1.839 mg/g and 1421 μm², respectively. They significantly decreased after 2 weeks of immobilization (1.441 mg/g and 867.4 μm²) and gradually recovered after removal of immobilization (1.596 mg/g and 1127 μm² at the end of follow-up). During immobilization, the ratio of MPCs increased 3-fold compared to basal levels and decreased after immobilization was released. Conversely, the ratio of SCs decreased during immobilization but increased 2-fold after immobilization was released. The expression level of Pdgfra correlated closely with the changes in the MPC ratio.
【Discussion】The inverse correlation between the ratio of MPCs and SCs may imply that they have reciprocal roles in regulating muscle mass during immobilization and recovery. Future research will focus on their functions in muscle atrophy and recovery, utilizing mutant mice that specifically lack MPCs or SCs.
【Conclusion】MPCs and SCs exhibit dynamic fluctuations during the processes of muscle atrophy and subsequent recovery, indicating their potential critical roles in these processes.

## P-128

## SMAD2 ubiquitination regulates skeletal muscle mass and tissue remodeling

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Transforming growth factorβ (TGFβ) signaling pathway negatively regulates skeletal muscle mass during homeostasis, while promotes muscle tissue fibrosis upon injury or denervation. Ubiquitination of SMAD2, an intracellular transducer of TGFβ signaling, is a well-known negative regulation mechanism of TGFβ signaling activity in cell biology field, however, its role in mammalian tissues, including skeletal muscle, has been elusive. Here, to elucidate the role of SMAD2 ubiquitination in vivo, we generated Smad2dPY mice. In Smad2dPY mice, 15 base pairs encoding PY motif, a domain that interacts with the WW domain of the NEDD4 family E3 ligase, are deleted and thus TGFβ signaling is expected to be promoted. No obvious abnormalities in growth or fertility were observed in Smad2dPY mice, indicating that ubiquitination of SMAD2 is not essential for these processes in mice. Skeletal muscle of Smad2dPY mice showed decreased muscle weight and CSA at 12 months of age. In addition, Fibrotic tissue remodeling, induced by cardiotoxin injection, was exacerbated in Smad2dPY mice at 21 days post-injury compared with wild type mice. Primary myoblasts and fibroblasts from Smad2dPY mice displayed increased responsiveness to TGFβ, which may explain the muscle atrophy and promoted traumatic fibrosis observed in Smad2dPY mice. These results indicate that the ubiquitination of SMAD2 is an important regulatory mechanism to maintain TGFβ signaling activity at desired levels in skeletal muscle homeostasis and tissue remodeling.
Next, to analyze the role of Smad2 ubiquitination in muscle satellite cells (MuSCs) activation, we performed single myofiber isolation assay. As a result, the fractions of MYOD-positive and Ki67-positive activated MuSCs were higher in Smad2dPY mice within the first 24hrs of culture. Moreover, the number of cells per single MuSC-derived cluster was significantly higher in Smad2dPY mice after 48hrs of culture, but the difference was not observed at 72hrs of culture. These results imply that the activation process of MuSCs is temporarily promoted in Smad2dPY mice. In this presentation, we would like to discuss the role of SMAD2 ubiquitination in skeletal muscle homeostasis and regeneration.

## P-129

# Gene-transcriptome analysis of hypertrophic skeletal muscles induced by 2 *g* hypergravity in mice

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Mechanical stress is essential for maintaining the musculoskeletal system. Mechanical unloading, such as long-term bed rest and microgravity by space flight, negatively regulates muscle mass and function, whereas mechanical loading, such as exercise, positively affects muscle mass and function. There are numerous reports regarding the analysis of unloading-induced muscle atrophy using rodent disuse models, such as hindlimb suspension, cast immobilization and space flight. Although exercise is widely used as a muscle-loading model, the study of constant loading of hypergravity is limited due to the need for unique equipment for mice. In this study, we performed a comprehensive analysis of gene expression by RNA-seq analysis in mouse skeletal muscles exposed to 2 g hypergravity. Mice were bred under 2 *g* hypergravity for 2 weeks using a gondola-type centrifugal device with a 1.0 m arm attached to mouse cages. As a result, 2 *g* hypergravity significantly increased skeletal muscle weight of the gastrocnemius, tibialis anterior, soleus (SOL), and quadriceps femulis (QF) and volume of calf muscles. We isolated the mRNA from slow-twitch SOL and fast-twitch QF, and analyzed mRNA expression profiles by RNA-seq. RNA-seq revealed that 2 *g* hypergravity alters the expression of 1016 genes in SOL and 271 genes in QF, indicating SOL is more affected by 2 *g* hypergravity than QF. Additionally, atrogenes were downregulated at similar levels in both SOL and QF; however, several myogenic pathways, such as PI3K-AKT and angiogenesis, and mechano-transduction pathways, such as extracellular matrix (ECM) organization and focal adhesion, upregulated in 2 *g*-loaded SOL. In conclusion, 2 *g* hypergravity for 2 weeks induced muscle hypertrophy through elevated several factors for myogenic and mechano-transduction pathways, particularly in slow-twitch SOL as the character of antigravity muscle.

## P-130

## Mkl1/2 inhibits muscle atrophy by blocking the GR/FoxO axis

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*Mkl1* and *Mkl2* are transcriptional coactivators of *Srf*. Analysis of *Mkl2*-KO mice and other studies have shown that Mkl1/2 represses the transcriptional activity of FoxO, which is responsible for muscle atrophy. In fact, overexpression of Foxo1 or Foxo3 in myotubes in vitro induces the expression of a group of genes (Atrogenes) responsible for muscle atrophy, resulting in myotube atrophy. However, when Mkl1/2 was simultaneously expressed, myotube atrophy was not observed and the induction of Atrogenes expression was suppressed. Mkl1/2 also showed an inhibitory effect on muscle atrophy caused by dexamethasone exposure to myotubes. Co-IP and luciferase reporter assays revealed that Mkl1/2 also binds directly to the glucocorticoid receptor (GR) and represses its transcriptional activity. These results suggest that Mkl1/2 acts as a brake of the GR/FoxO cascade in muscle atrophy.

Next, we examined the function of Mkl1/2 in muscle in vivo. Surprisingly, in all mouse models of muscle atrophy examined (aging, sciatic denervation, fasting, dexamethasone administration, and cancer cachexia), *Mkl1* expression was decreased in response to the induction of muscle atrophy. In other words, there is a mechanism by which Mkl1, a brake on muscle atrophy, is eliminated at the onset of muscle atrophy. On the other hand, *Mkl2* gene expression did not change during the induction of muscle atrophy. However, when mice were exercised on a treadmill, the translocation of Mkl2 protein to the nucleus was enhanced. Similarly, electrical stimulation of in vitro myotubes also caused Mkl2 to translocate to the nucleus. Because the repression of FoxO transcriptional activity by Mkl1/2 occurs in the nucleus, exercise may repress the transcriptional activity of FoxO through nuclear translocation of Mkl2.

Finally, Mkl1 was exogenously expressed in skeletal muscle either systemically or locally using adeno-associated virus (MyoAAV.2A). The transduced Mkl1 induced hypertrophy of skeletal muscle and conferred resistance to fasting- or dexamethasone-induced Atrogenes expression. The decrease in muscle mass induced by administration of MyoAAV.2A encoding Foxo1 was also suppressed by the expression of Mkl1. These results revealed a novel mechanism of Mkl1/2-mediated suppression of muscle atrophy.

## P-131

# Roles of the fibrinolytic system in skeletal muscle atrophy induced by mechanical unloading

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Chronic mechanical unloading induces marked skeletal muscle atrophy especially in anti-gravity muscles, such as the soleus and adductor longus muscles. To elucidate the molecular mechanism of this muscle adaptation, we analyzed proteome data on the gastrocnemius (Gast) and soleus muscles of space-flown mice raised under microgravity or artificial 1-*g* for 30 days [1-3]. Consequently, we found that the expression levels of fibrinolysis-related proteins, including plasminogen (Plg), were significantly elevated in the mechanical-unloaded muscles [3]. Next, we investigated the roles of the fibrinolysis system in skeletal muscle atrophy induced by mechanical unloading using mice with Plg gene deficiency ($Plg^{-/-}$) and their wild-type littermates ($Plg^{+/+}$) [3]. Following 21 days of hindlimb suspension, the decrease in the wet weight of the Gast, but not soleus, muscles were accelerated in $Plg^{-/-}$ mice. Additionally, in response to hindlimb unloading, the fiber size of type IIb and IIx fibers in the Gast muscles of $Plg^{-/-}$ mice decreased more prominently than those of $Plg^{+/+}$ mice. Moreover, Plg deficiency significantly increased the expression of autophagy-related markers, beclin1 mRNA and LC3B protein, in the mechanical-unloaded Gast muscles, but did not affect the increase in the gene expression of ubiquitin ligases, atrogin-1 and MuRF1. Neither Plg deficiency nor hindlimb unloading affected the Akt/mTOR pathway in the Gast muscles. These results suggested that Plg deficiency might accelerate protein breakdown via the autophagy-lysosome, but not the ubiquitin-proteasome, system in the mechanical-unloaded Gast muscles. From the above, Plg and the fibrinolysis system might play some protective roles against muscle atrophy, especially of the fast-twitch muscles, induced by mechanical unloading in mice.

References

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[2] Ohira et al., Effects of microgravity exposure and fructo-oligosaccharide ingestion on the proteome of soleus and extensor digitorum longus muscles in developing mice. NPJ Microgravity. 2021, 7(1):34.
[3] Ohira et al., Plasminogen deficiency exacerbates skeletal muscle loss during mechanical unloading in developing mice. J Appl Physiol (1985). 2024, 136(3):643-658.

## P-132

## The effects of treadmill exercise mainly performed by forelimb on atrophy and mitochondrial adaptations in immobilized hindlimb muscle in mice

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**Introduction:** Immobilization-induced muscle atrophy is generally associated with mitochondrial dysfunction including reduction in mitochondrial biogenesis. It has been suggested that exercise induces adaptations not only in exercising muscles but also in other organs through exercise-induced humoral factors. Therefore, we investigated effects of exercise mainly performed by forelimb during hindlimb immobilization period on atrophy and mitochondrial adaptations in hindlimb muscle in mice.
**Methods:** Twelve-week-old male ICR mice were divided into following 3 groups: hindlimb immobilization (IM), hindlimb immobilization and exercise (IM+EX), and control (CON; sedentary without hindlimb immobilization). IM and IM+EX groups underwent bilateral hindlimb immobilization by a cast for 14 days. The IM-EX group performed treadmill exercise (at 10 m/min for 60 min) during immobilization period. Soleus muscle was collected at 24 hours after the last exercise session. Citrate synthase (CS) activity and the protein level of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) in skeletal muscle were measured.
**Results:** At 14 days after hindlimb immobilization induction, body weight and soleus muscle weight were significantly lower in the IM and the IM+EX groups compared to those in the CON group, while there were no significant differences in these between the IM and the IM+EX groups. CS activity (generally used as an index of mitochondrial content) of the soleus muscle was significantly lower in the IM group compared to that in the CON group and the IM+EX groups. Additionally, PGC-1α protein level in the soleus muscle was significantly lower in the IM group compared to the CON group while no significant difference was observed between the IM+EX and the CON groups. PGC-1α protein level tended to be higher in the IM+EX group than that in the IM group ($p=0.09$).
**Conclusion:** Treadmill exercise mainly performed by forelimb during hindlimb immobilization prevented the reduction in CS activity in the immobilized hindlimb muscle.

## P-133

# Mechanical stress may suppress myotube atrophy linked to cancer cachexia via androgen receptors

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Cancer cachexia-associated muscle wasting, as a multifactorial wasting syndrome, is an important factor affecting the long-term survival rate of tumor patients. Skeletal muscle cells/fibers are inherently dependent on mechanical stress in the form of fluid shear stress and contractions (physical exercise). For example, an exposure of skeletal muscles to chronic mechanical loading leads to increased anabolism and fiber hypertrophy, whereas prolonged mechanical unloading results in muscle atrophy. Recent studies have shown that mechanical stress can counteract some deleterious effects of tumor-derived factors on myotubes, however, the underlying anti-cachectic mechanisms remain poorly understood. The present study aims to investigate the anti-cachectic efficacy of mechanical stress and elucidate the underlying mechanism in vitro. A cellular model for cancer cachectic muscle atrophy using C2C12 myotubes was prepared by treating myotubes with a conditioned medium from a culture of Colon-26 cancer cells. During the 48 h of Colon-26 conditioned medium exposure, uniaxial cyclic stretch of 15% at 1/6 Hz was administered, so as to mimic the mechanical stress of exercise. Quantitative real-time polymerase chain reaction and western blotting analysis were used to determine the expression levels of proteolytic genes and proteins, including CCAAT/enhancer-binding protein delta (C/EBPδ), myostatin, and muscle E3 ubiquitin ligase. Androgen production by C2C12 myotubes was measured by enzyme-linked immunosorbent assay. To block the action of androgen receptor, we pretreated C2C12 myotubes with androgen receptor antagonist, flutamide, and androgen receptor siRNA. Our experiments reveal that mechanical stress prevented the thinning of C2C12 myotubes by increasing protein synthesis and suppressing the ubiquitin–proteasome pathway. Both mRNA and protein levels of androgen receptor significantly increased in C2C12 myotubes exposed to mechanical stress. Production of testosterone and dihydrotestosterone from mechanical stress-treated C2C12 myotubes was markedly increased. Of interest, genetic and pharmacological arguments supported a partial implication of the androgen receptor. Signaling analyses suggested a crosstalk between the androgen receptor and the myostatin pathway, possibly via C/EBPδ. Overall, these findings suggest that the activation of androgen receptor signaling could be part of the molecular mechanisms by which mechanical stress ameliorates cancer cachexia-induced muscle wasting.

# Poster Session 16

## Muscle development, regeneration & homeostasis

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Takayuki Akimoto (Faculty of Sport Sciences, Waseda University/Center for Disease Biology and Integrative Medicine, The University of Tokyo, Japan)
Takahiko Sato (Fujita Health University, Japan)

### P-134

### Analysis of mouse embryo skeletal muscle cell lineage by single nuclei RNAseq

Yutaro Kawa, Masafumi Inui

Laboratory of Animal Regeneration Systemology, Department of Life Sciences, School of Agriculture, Meiji University

Movement of the vertebrate body is supported by the connection of muscle, tendon and bone. During the embryonic development, they must connect in the correct configuration to form a functional unit. Skeletal muscles in the mammalian limbs are derived from somite whereas limb tendons, bones and connective tissues are from the lateral plate mesoderm. Consequently, progenitor cells of limb skeletal muscles migrate long distances from their origins to destinations and attach with the appropriate partner during embryonic development. Considering the number and accuracy of muscle-tendon connections in the developing limb, it is reasonable to assume that the local tissue–tissue interactions between myogenic progenitor cells and tenogenic cells take place during developmental processes, but its molecular mechanisms remain unclear. In particular, there have been few reports on signals from tendon to skeletal muscle. We conducted tendon progenitor cell ablation using a mouse model *ScxCreL Tg; Rosa26-DTA* and found that skeletal muscle patterning is significantly altered in this mouse (Ono et al. Development 2023). Our study indicated that instructive signal from tendon that controls skeletal muscle patterning. To elucidate the genes/molecules involved in this process, we performed single-nucleus RNAseq analyses using embryonic limbs (embryonic days 12 and 13). Thirty-one clusters formed including mesenchymal cells and skeletal muscle cell lineages. In the clusters of skeletal muscle cell lineage, myonuclei in the process of differentiation and maturation as well as specialized myonuclei that have been reported recently have been identified. Differentially expressed genes among skeletal muscle clusters were extracted and functional assays using cultured myoblast cells are currently on going. Since clusters of tendon cells were not formed in this analysis, we are continuing our investigation with modified sample preparation method. In this presentation, I would like to introduce and discuss the sample preparation for single nucleus analysis and the data analysis on skeletal muscle cell lineage.

## P-135

# Novel Functional Analysis of the Cholesterol Regulatory Factor ABCA1 in Skeletal Muscle Homeostasis

Hayataka Takase[1], Makoto Shimizu[2], Sumiko Dohmae[3], Yoshio Yamauchi[1], Ryuichiro Sato[1], Takashi Sasaki[1]

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**Background and objectives:** Skeletal muscle regeneration is crucial for addressing various forms of muscle damage triggered by factors like overload, medication, and inflammation. With aging, the regenerative capacity of skeletal muscle declines, impacting quality of life due to reduced muscle mass and strength. Our study aimed to identify a novel regulator activated during muscle damage and regeneration. We observed increased mRNA and protein expression of specific target genes of Liver X Receptor, a nuclear oxysterol receptor, during muscle regeneration. So far, the functions and regulatory mechanisms of proteins involved in cholesterol metabolism in skeletal muscle remain unclear. Furthermore, our study delved into elucidating the functional mechanisms of ATP-binding cassette transporter A1 (ABCA1), a prototypical target gene of LXR, in muscle regeneration and differentiation.

**Methods:** Male mice of the C57BL/6J strain or ABCA1-knockout (ABCA1-KO) mice were intramuscularly injected with cardiotoxin (CTX) to induce muscle damage. Skeletal muscle samples were collected at 7 days post-injury (dpi). Tissue samples were collected from mice aged 2 to 7 months for subsequent analysis. Satellite cells and a mouse muscle cell line (C2C12 cells) were induced to differentiate using medium supplemented with 5% and 2% horse serum, respectively. Satellite cells were isolated from the tibialis anterior muscle (TA) of mice. Gene expression analysis was conducted using real-time quantitative PCR. The diameters of differentiated myotubes were assessed following immunostaining for myosin heavy chain. Skeletal muscle strength was evaluated using Grip-strength and Wire-hanging tests.

**Results:** At 7 dpi, ABCA1-KO mice exhibited a noticeable decrease in skeletal muscle mass compared to their wild-type counterparts. Specifically, examination of hematoxylin and eosin stained sections from ABCA1-KO mice revealed the presence of residual inflammatory sites. Moreover, ABCA1-KO mice demonstrated reduced skeletal muscle mass and strength during aging under physiological conditions. Additionally, a notable increase in plasma creatine kinase levels was observed in ABCA1-KO mice. Particularly, in C2C12 cells, siRNA-mediated knockdown of ABCA1 markedly diminished both muscle differentiation and fusion. These findings underscore the critical role of ABCA1 in preserving skeletal muscle function.

**Conclusions:** This study elucidated that ABCA1, significantly upregulated during muscle regeneration, plays a crucial role in tissue repair and muscle differentiation. ABCA1 primarily functions by facilitating the efflux of cholesterol from intracellular to extracellular compartments. Therefore, these data suggest that regulating cholesterol in skeletal muscle may contribute to preserving muscle function.

## P-136

## Adrenaline Resistance in Obese Skeletal Muscle Impairs Exercise Metabolism

Kazuhiro Nomura[1,2,3], Yu Hirata[2], Yuna Izumi-Mishima[1], Sonoko Yamada[1], Anna Krook[3], Juleen Zierath[3], Hiroshi Sakaue[1,4], Wataru Ogawa[2]

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It is known that there are exercise low-responders among obese individuals who have difficulty losing weight even with exercise. Although a lack of physical activity and an inability to control food intake contribute to obesity, exercise low-responders resistant to exercise still exist even in studies where physical activity and food intake are controlled, suggesting that obese individuals experience a reduction in energy expenditure, making it more difficult for them to lose weight. In fact, obese individuals exhibited a reduced maximal oxygen uptake per skeletal muscle mass during exercise. We found that β2-adrenergic signaling is reduced in skeletal muscle of obese individuals and obese mouse models, leading to a pathological state that can be described as "adrenaline resistance". In this state, the β2-adrenergic signaling activated in skeletal muscle during exercise is impaired, resulting in reduced energy expenditure. In human skeletal muscle, the expression level of the β2-adrenergic receptor gene showed an inverse correlation with BMI and a positive correlation with maximal oxygen uptake. In skeletal muscle of obese mouse models, the expression level of the β2-adrenergic receptor gene was also reduced, and the induction of metabolic gene expression and the increase in energy expenditure during exercise or β2-agonist administration were suppressed compared to control mice. In skeletal muscle of obese individuals or obese mice, the DNA in the promoter region of the β2-adrenergic receptor gene was highly methylated, which may contribute to the mechanism of reduced gene expression. In addition, we generated skeletal muscle-specific β2-adrenergic receptor knockout mice, which exhibited reduced energy expenditure during exercise, characterized as exercise low-responders, and exhibited obesity. These results suggest that the adrenergic signaling in skeletal muscle plays an essential role in improving energy metabolism during exercise. In obese skeletal muscle, epigenetic regulation leads to a state of "adrenaline resistance", which interferes with the improvement in energy metabolism during exercise.

## P-137

## Analysis of planar cell polarity protein Vangl2 expression in skeletal muscle stem cells

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Division for Therapies against Intractable Diseases, Center for Medical Science, Fujita Health University

Skeletal muscle stem cells (MuSCs) play a critical role in muscle regeneration. Understanding the physiology of MuSCs and the mechanisms governing their self-renewal is essential both for comprehending the pathophysiology of age-related sarcopenia and neuromuscular diseases and for developing potential treatments. However, the molecular mechanisms underlying MuSC regulation remain elusive.

In this study, we focus on the planar cell polarity protein Vangl2, which is expressed in MuSCs, and investigate its potential role in maintaining stemness and regulating symmetrical cell division.

To shed light on this mechanism, we employed the mouse myoblast line C2C12 as a model system. Our findings reveal a decrease in Vangl2 protein expression during muscle differentiation induction. Vangl2 protein was observed at cell-cell junctions, where it co-localized with critical junctional proteins such as β-catenin, N-cadherin, and M-cadherin. Furthermore, through co-immunoprecipitation assays using an anti-M-cadherin antibody, we detected an interaction between Vangl2 and M-cadherin, providing evidence for potential molecular crosstalk.

Furthermore, we performed whole mount immunofluorescence of mouse plantaris muscle. Then we confirmed Vangl2 was dominantly expressed in MuSCs and localized along with MuSC / muscle fiber junctions.

Understanding the role of Vangl2 in MuSCs may offer insights into novel therapeutic approaches for muscle-related disorders.

## P-138

## Expression of microRNA-24 improves regeneration in fast-twitch muscle

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MicroRNAs (miRNA) have been shown to play key roles in regulation of skeletal muscle including muscle differentiation, atrophy, and hypertrophy. Our group has found that miRNAs in the miR~23~27~24 cluster are highly expressed in skeletal muscle (JBC, 2011). It has been reported that miR-24 is upregulated during myoblast differentiation and promotes myogenesis in vitro. However, the role of miR-24 in skeletal muscle in vivo is unclear.

In this study, we generated transgenic (Tg) mice over-expressing miR-24 under CAG promotor (chicken β-actin promotor and CMV enhancer) and evaluated their skeletal muscle phenotypes. Compared to wild-type (WT) mice, miR-24 was increased approximately 3-fold in slow-twitch soleus (SOL) and fast-twitch tibialis anterior (TA) muscles. Next, we assessed skeletal muscle mass and fiber type composition at 8 weeks of age. Muscle weight and muscle fiber type composition did not change in all muscles examined compared to those in WT littermates. We then investigated muscle regeneration capacity to inject cardiotoxin (CTX) into SOL and TA muscles. We found that the cross-sectional area of the regenerated fibers was significantly larger only in TA of Tg mice than in WT mice. Furthermore, myoblasts from TA of Tg mice increased their differentiation capacity compared to myoblasts isolated from WT mice. Our results suggest that miR-24 plays an important role in muscle regeneration in fast-twitch muscles.

## P-139

## The role of microRNA-140 in skeletal muscle

Takayuki Akimoto[1,2], Jaehoon Shin[1], Masataka Nakamura[2], Shuaibang Yuan[1], Minjung Lee[1], Shigeru Miyaki[3], Hiroshi Asahara[4], Takashi Ushida[2]

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[Background] We have previously carried out a comprehensive microarray analysis with slow- and fast-twitch muscles to find miRNAs involved in regulation of skeletal muscle plasticity (Wada et al., 2011). We found that miR-140 was expressed in skeletal muscle, dominant in slow-twitch muscle. Interestingly, miR-140 expression was increased in smooth muscle cells by cyclic mechanical loading which mimics muscle activity (Mohamed et al., 2010). These findings may imply a possibility that miR-140 may play crucial roles in skeletal muscle.
[Aim] We aimed to determine the function of miR-140 in skeletal muscle and in exercise-induced muscle adaptation using miR-140−/− mice.
[Results] The body weight as well as muscle weight in miR-140−/− mice were lower than these in wild type mice as reported previously (Mikaki et at., 2010). We found that myosin heavy chain (MyHC) type I expressions were incrased both in fast-twitch and slow-twitch muscles of miR-140−/− mice compared with these of wild type mice. The exercise-induced muscle adaptation in miR-140−/− mice was comparable to that in wild type mice.
[conclusion] Our data suggests that miR-140 plays an important role in slow muscle fiber formation, but not in fast-to-slow fiber type switching induced by endurance exercise. These findings may provide new insight into the field of muscle biology regarding slow fiber formation.

## P-140

## Doxorubicin irreversibly impairs skeletal muscle regeneration

Shinya Kondo, Shugo Yonehara, Haruo Sasaki, Masashi Isaji, Takahiro Nakagawa, Keisuke Horiuchi
Department of Orthopedic Surgery, National Defense Medical College

Background: To date, perioperative chemotherapy has been implemented for various malignant tumors. However, it is unclear whether these chemotherapeutic agents affect soft tissue regeneration at the surgical site. In this study, we aimed to address this issue using one of the standard antitumor drugs, doxorubicin (DOX), in a mouse muscle injury model.
Materials and Methods: 10-week-old male C57BL/6 mice were injected with cardiotoxin into the tibialis anterior muscle to induce muscle injury. Mice were subsequently treated with either an intravenous administration of DOX (DOX group) or PBS (control group). Injured muscles from each group were collected for histologic examination and gene expression analysis to evaluate muscle regeneration. In some experiments, DOX was administered before and after 3 and 7 days of muscle injury.
Results: At the initial stage of muscle regeneration (Day 3), there was no apparent difference in the proliferation of satellite cells, infiltration of inflammatory cells around necrotic tissue, or expression patterns of various genes between the DOX group and the control group. However, the absorption of necrotic tissue and the infiltration of inflammatory cells were significantly prolonged in the DOX group (Day 7 - 14). Furthermore, the injured muscle did not fully recover in the DOX group, with atrophic muscle fibers, interstitial fibrosis, and calcified deposition (Day 28 - 84). When DOX was administered on Day 3 of muscle injury, the duration of inflammation was significantly reduced. Additionally, when DOX was administered after Day 7 or before Day 3 of muscle injury, the effect of DOX was almost negligible.
Discussion: Our results showed that DOX administration not only delays muscle regeneration but also leads to irreversible regeneration impairment. However, the effect of DOX on muscle regeneration can be avoided by administering DOX outside the inflammatory period after muscle injury, indicating that muscle regeneration is susceptible to DOX treatment only for a limited period (Day 0 – 3 of muscle injury in mice). Our study may also suggest that postoperative chemotherapy can be initiated earlier than previously thought in humans.

## P-141

# Myoblast differentiation induced expression of complement regulatory proteins and CD59 expression on the membrane of myotubes was the uniquely clustering pattern

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**Objective**
Myoblasts express various proteins depending on their differentiation status. Membrane complement regulatory proteins are expressed in somatic cells and regulate cytolytic attacks from unwanted complement activation. Myoblasts may express complement regulatory proteins that may be upregulated during myoblast differentiation and muscle regeneration. We investigated the expression levels and patterns of complement regulatory proteins during myoblast differentiation into myotubes.
**Methods**
Human myoblasts were allowed to differentiate into myotubes in differentiation-induction medium. We identified the expression of the membrane complement regulatory proteins CD46, CD55, and CD59. After the induction of differentiation, mRNA and proteins were extracted from the cells on days 0, 2, 4, 6, and 8. mRNA and protein expression were confirmed by RT-PCR using a TaqMan probe and western blotting, respectively. Myotubes were stained with actin to confirm the differentiation status on day 8. Additionally, the expression pattern of each complement regulatory protein in myotubes was detected by immunohistochemistry using monoclonal antibodies against CD46, CD55, and CD59.
**Results**
The mRNA and protein levels of CD46, CD55, and CD59 progressively increased on days 2, 4, and 6 post-differentiation compared to pre-differentiation. However, the increases in the mRNA and protein levels of CD46, CD55, and CD59 stopped until day 6, and the extent of these increases decreased by day 8. Immunohistochemistry showed that CD46 and CD55 were uniformly distributed across the myotube membranes. In contrast, CD59 expression was observed as a clustering pattern in the myotube membranes, unlike CD46 and CD55.
**Discussion**
The expression of complement regulatory proteins is stimulated by initiation of myoblast differentiation. The increased expression of complement regulatory proteins during differentiation may be maintained after maturation in myotubes. Among the complement regulatory proteins, CD46 and CD55 are expressed on the membranes of myotubes and are thought to suppress the complement cascade around myotubes. CD59, which inhibits complement cytolytic activity by binding to C8 and C9 and blocks the assembly of the membrane attack complex (MAC), was clustered at the myotube membrane. It has been suggested that CD59 protects critical areas of the myotube membrane from MAC formation.
Conclusion
Expression of complement regulatory proteins was induced during myoblast differentiation into myotubes. CD46 and CD55 expression was uniformly distributed across the myotube membrane, whereas CD59 expression was localized to the membranes.

## P-142

# Development of an early and non-invasive method for predicting skeletal muscle stem cell induction efficiency utilizing culture supernatants

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Our laboratory has developed a method for inducing human iPSC-derived skeletal muscle stem cells (iMuSCs). iMuSCs have been shown to repair damaged muscle in Muscular dystrophy model mice and are expected to be applied to regenerative medicine. However, iMuSCs induction efficiency (iMuSC %) is unstable. Moreover, it takes more than 80 days to induce iMuSCs, and there is no method to predict the final induction efficiency at an early stage, making it difficult to optimize the protocol. Therefore, the purpose of this study is to develop a newly method for early prediction of iMuSCs % at an early stage. Since iMuSC % sometimes varies even under the same experimental conditions, we adopted a non-invasive method, culture supernatant analysis. Our previous studies are shown that the expression level of muscle-related genes during the myogenic differentiation period (Day17-38) highly correlates with the iMuSCs % at Day82. Therefore, we performed proteome analysis of culture supernatant during this period and successfully identified many proteins whose expression correlated with iMuSC %. Among them, we focused on 3 proteins which have particularly good correlation coefficients. To verify which protein is the better predictive marker, we then quantified its expression in culture supernatants by ELISA and analyzed the correlation with iMuSC %. The results showed that the expression levels of Protein A and B during Day 27-38 showed significantly high correlation with iMuSCs %. Thus, Protein A and B may be promising marker proteins for early and non-invasive prediction of iMuSCs %. However, additional subsequent validation using muscular disease-specific iPS cells showed no correlation between protein expression and iMuSC %. We are currently reexamining the method for measuring the induction efficiency.

## P-143

# Acceleration of the development of myotubes from MYOD1-overexpressed human iPS cells using a new simple method

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Human induced pluripotent stem cells (hiPSCs) are able to differentiate into tissue-specific cell types, and have great potentials as reliable resources for clinical and research applications. Several protocols have been developed to generate differentiated skeletal muscle cells from hiPSCs. Despite the advantages of efficient and rapid induction into muscle cells using transgenic procedures, developed myotubes are relatively immature and not recapitulate several aspects of striated muscle fibers. In previous studies, culture conditions have been modified and adapted using growth factors and several types of media. Additionally, bioengineering culture techniques are applied to improve the degree of myotube maturation, however, adaptation of these skeletal muscle tissue engineering requires an electrical device or fabrication method, and is still challenging to apply for high-throughput drug screening. Here, we established a new monolayer myogenic differentiation culture method to maximize the advantages of a previously established MYOD1-overexpression strategy for hiPSCs. Briefly, we developed a combination protocol using classical 2% horse serum/DMEM and the myotube fusion medium (SKM-03+, Myocea) which improved myotube formation with multi-nuclei and robust sarcomere structures using hiPSCs derived from three healthy donors, a patient with congenial muscular dystrophy caused by LMNA-mutation (G33del), and its isogenic control generated by the CRISPR/Cas9 system. One of the benefits using LMNA patient-derived iPSCs to study the genotype-phenotype correlations is to analyze nuclear shape abnormalities in differentiated muscle cells. We demonstrated that nuclear shape abnormalities including nuclear elongation in myotubes were specific phenotypes in the LMNA hiPSCs, which were recovered in the isogenic control cells. Promoting myotube maturation using this new protocol increased the percentage of nuclear abnormalities up to 18%, and the abnormally elongated myonuclei were pronounced in the LMNA hiPSCs. In short, the new culture method would be widely applicable for MYOD1-overexpression model of hiPSCs derived skeletal muscle cells for studying of a variety of muscle diseases, and also for high-throughput screening for investigating potential drugs for treatment.

## P-144

# Detection of microRNA leakage by stretch stimulation model using patient-derived iPS muscle cells

Satoshi Nakada[1], Yuri Yamashita[2], Hidetoshi Sakurai[3], Eri Arikawa-Hirasawa[1,2]

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[Purpose] Micro RNA (miR), which leaks from cells into the extracellular fluid, changes depending on the state of the cells and has attracted attention as a biomarker that reflects disease. In skeletal muscle, miR is anticipated to be utilized for screening patients with muscle diseases and determining the effects of treatment. However, to date, studies have mainly focused on miR concentrations in patient serum, with very few studies conducted in vitro. In this study, we constructed a mechanical stress model using myotubes derived from induced pluripotent stem cells (iPSCs) of patients with muscle diseases and evaluated miR leakage.

[Methods] The study employed iPS cells derived from a patient with Duchenne muscular dystrophy (DMD strain) and repaired DMD iPS cells with repaired genetic mutation (repaired strain). These cells were differentiated into myocyte cells by forced MyoD expression using tet-ON system and seeded onto a culture substrate, decellularized skeletal muscle sheet (DSMS). Subsequently, myocyte cells were induced to differentiate into myotube cells using low-growth factor medium. Myotube cells formed on DSMS were subjected to repeated uniaxial stretch stimulation at a stretch rate of 20% for 90 minutes at 1 Hz. Culture supernatants were sampled before and 24 hours after stretch stimulation, and miR was quantified.

[Results] miR-22, miR-95a, and miR-206 in the culture supernatant were found to be elevated in the DMD strain following an stretch stimulation. In addition, miR-1, miR-30a, miR-133a, miR-133b, miR-193b, miR-208b, and miR-378a in the culture supernatant were up-regulated by stretch stimulation in both strains, but the DMD strain exhibited a more pronounced up-regulation. Both strains exhibited upregulation of miR-30c and miR-483 in the culture supernatant upon stretch stimulation and no difference between strains. This suggests that the miR leakage is more strongly induced by stretch stimulation in the DMD strains.

[Summary] Ten miRs were upregulated in response to mechanical stress-induced cell damage in disease lines, suggesting that these miRs may serve as biomarkers of muscle disease in vitro. These miRs have the potential to be used to determine the efficacy of drugs in vitro.

# Poster Session 17

## Myokine and New technologies

18:30-19:45, Sep 13 (Fri), 2024
Poster (Room 203+204, 2F, Nara Prefectural Convention Center)

Chairs: Ai Shima (Department of Mechano-Informatics, The University of Tokyo, Graduate School of Information Science and Technology, Japan)
Tomoya Uchimura (Department of Clinical Application Center for iPS Cell Research and Application, Kyoto University, Japan)

### P-145

### Heat acclimation modifies skeletal muscle functions in mice

Yuka Kudo, Nozomi Yazawa, Yuho Mizuseki, Keigo Murata, Taku Nedachi
Graduate School of Life Sciences, Toyo University

Repeated heat exposure induces heat acclimation (HA), which promote the improvement in heat tolerance. It has been shown that HA induces structural and functional changes in thermo-effector organs such as skin. Although skeletal muscle is known to be a vulnerable organ to heat, limited studies have examined whether skeletal muscle function is affected by HA. In this study, we investigate whether HA influences changes in skeletal muscle function and myokine regulation.
To construct a mouse HA model, C57BL6 mice were repeatedly exposed to short-term heat stimulation for 14 days (HA mice). Compared to control mice, HA mice showed significant decreases in body weight, and a significant induction of *Cox2* gene expression in skeletal muscle was observed as previously reported. Gene expressional analysis showed that the expression of selective water channels, aquaporin 1 (*Aqp1*) and 4 (*Aqp4*), was induced in skeletal muscle of HA mice, which suggested water transport abilities via these channels may increase. We also found the acute heat-dependent *Hspa1a* gene expression was suppressed by prior HA. This result suggests that HA may also control the heat shock responses in skeletal muscle.
In addition, we performed extensive myokine expression analysis in animal and cellular models to assess whether HA affects the expression profile of myokines. By utilizing mouse HA model, we found serum CCL5 concentration was significantly decreased in HA mice. Moreover, we found that CCL5 expression and secretion from C2C12 cells was gradually decreased with each heat stimulation. Together, these results suggested that myokine CCL5 was downregulated by HA both in vivo and in vitro.
In conclusion, our present findings suggest that HA in mice potentially modulates water transports and heat shock responses. These may be important for the adaptation process of skeletal muscles against acute heat stress. Furthermore, we suggested that the HA-dependent myokines may transduce thermal signals to the other tissues/organs, which suggested that the HA-dependent myokines may become an useful biomarker to assess HA levels in animals.

## P-146

# Comprehensive Analysis of Stress-Dependent Subcellular Localization Changes in Proteins

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Understanding the localization of these proteins is crucial for elucidating their functions. Existing databases typically provide information on the subcellular localization of proteins in mammalian cells under steady-state conditions. However, the localization of many proteins dynamically changes in response to various stresses. Using our advanced microscopy system and machine learning programs, we aim to systematically and objectively compile databases on the intracellular dynamics of nearly all proteins during various critical biological events, such as stress and inflammation, as well as cell differentiation. Initially, we focused on oxidative stress, analyzing the localization changes of 8,055 proteins under such conditions. As a result, it was suggested that approximately 1,000 proteins form stimulus-dependent foci, implicating the involvement of specific motifs. Additionally, some proteins were indicated to be involved in the regulation of primary cilium retraction during muscle differentiation.

## P-147

# Development of Controllable Stretching and Continuous Force Measurement System for *in vitro* Engineered Skeletal Muscle Tissue

Shota Noda, Louis Sterker, Jun Sawayama, Shoji Takeuchi
The University of Tokyo

In this study, we propose a novel culture system that combines automatic stage control and micro force sensors for continuous measurement of contractile function in engineered skeletal muscle constructs.

**Introduction**

Three-dimensional engineered skeletal muscle tissue is a well-established method for studying the contractile function of skeletal muscle in vitro, with promising applications in regenerative medicine and drug discovery. Previous studies have formed mature muscle tissue through long-term culture and assessed contractile functions such as force-frequency and force-length relationships. However, because both ends of the tissue are fixed, micro-posts or cantilevers could not adjust their length, and the invasive measurement with force sensors disrupted continuous assessment. Here, we propose an engineered skeletal muscle construct culture system that combines automatic stage control and micro force sensors, enabling continuous measurement of contractile function while applying stimuli to promote muscle construct maturation.

**Materials and methods**

The construction process of the skeletal muscle tissue involves three 3D-printed devices: a mold, an anchor, and a baseplate. After assembling these three devices, C2C12 cells suspended in ECM gel were seeded into the mold. After gelation in the incubator, the mold was removed. The tissue, attached to a pair of anchor devices, was then mounted onto the culture system. The tissue was positioned between a fixed end and a force sensor attachment, allowing the sensor to measure the forces at both ends. One end of the tissue was controlled by an automatic X-stage to provide stretching stimulation. The tissue was cultured for 7 days, during which passive tension, contractile forces, and mechanical load during stretch were evaluated.

**Results**

To investigate the development of the skeletal muscle tissue, passive tensions and contractile forces were measured. We observed that passive tensions increased as the construct width decreased. Additionally, we applied 100 Hz electrical stimulation and measured peak-to-peak tetanic contractility. The tissue showed no signal until day 4 but began to contract from day 7, with contractions becoming stronger on day 9. These results indicate that this system allows for continuous measurements of passive and contractile forces throughout the culture period. The results of stretch stimulation by the automatic X-stage indicated that the same stretch strain tends to increase its mechanical load during the culture period.

**Conclusion**

These findings demonstrate that our culture system holds potential for analyzing the development of skeletal muscle tissue under electrical and load-adjustable stretch stimulation, with potential applications as a novel tool in regenerative medicine and drug discovery.

## P-148

## Regulation of myokines by nutrition and heat stimulation in C2C12 myotubes

Nozomi Yazawa, Yuka Kudo, Yuho Mizuseki, Keigo Murata, Taku Nedachi
Graduate School of Life Sciences, Toyo University

Skeletal muscle secretes proteins and peptides called myokines, which play important roles in communication between skeletal muscle and other organs. We previously identified C-C motif chemokine ligand 5 (CCL5) and C-X-C chemokine ligand 10 (CXCL10) as novel myokines that expression is significantly reduced by exercise. More recently, we reported that the expression of CXCL10 and CCL5 was induced in response to increases in ambient glucose availability and was decreased by heat stimulation. Thus, skeletal muscles respond to these multiple stimuli, thereby control the secretion levels of CCL5 and CXCL10. However, the impacts of interaction of these different stimuli on the myokine expression are not fully understood. In this study, we focused on myokines, CCL5 and CXCL10, and attempted to elucidate their expressional regulation in response to combined stimuli.

Fully differentiated C2C12 myotubes were cultured either under DMEM (low glucose (LG: 1.0 g/L glucose) or DMEM high glucose (HG: 4.5 g/L glucose) conditions. Concurrently, the cells were exposed to short-term heat stimulation (42℃ for 3 h) and continuously incubated at 37℃ for 21 h. AMP kinase activation and the induction of Heat shock proteins were clearly identified by each stimulus. As expected, both CCL5 and CXCL10 expression was significantly induced by HG. Heat stimulation reduced the expression of both myokines to the same extent under either HG or LG conditions. These suggested the impact of heat stimulation on the myokines was more potent and counteracted the influence of ambient glucose levels on the expression of CCL5 and CXCL10.

Many reports suggested the association of serum levels of CCL5 and CXCL10 and numerous physiological and pathophysiological states. For example, high CCL5 levels promote immune cell migration and is suggested to be involved in the chronic inflammation. High CXCL10 levels are implicated in inhibiting angiogenesis. Although further studies are required, our results may suggest that thermotherapy, targeting skeletal muscles, may control serum CCL5 and CXCL10 levels in both healthy and obese individuals, thereby controlling the inflammatory states and/or angiogenesis.

## P-149

# Regulatory mechanism of skeletal muscle-derived IL-6 expression: Impact on skeletal muscle-brown adipose tissue amino acid metabolism

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Background and Aim.

We have previously shown that cast immobilization induced thermogenesis in brown adipose tissue (BAT) by utilizing free amino acids present in skeletal muscle. Muscle-derived Interleukin-6 (IL-6) has been shown to stimulate branched chain amino acid (BCAA) metabolites in skeletal muscle. These data suggest that the amino acid metabolism, facilitated by muscle-derived IL-6, in the interaction between skeletal muscle and brown adipose tissue plays a crucial role in maintaining body temperature in endotherms, but the mechanism of muscle-derived IL-6 remains unclear. This study attempted to elucidate the regulatory mechanisms of skeletal muscle-derived IL-6 as a regulator of the skeletal muscle-BAT amino acid metabolism.

Methods and Results.

We first investigated the b-adrenergic receptor stimulation on IL-6 gene expression and amino acid metabolism in skeletal muscle by performing an acute cold tolerance test at 4°C for 4 h in IL-6 KO mice. Following acute cold exposure, there was a significant increase in

IL6 expression in skeletal muscle. In wild-type (WT) mice, the amounts of (BCAAs in skeletal muscle were notably elevated after cold exposure, whereas no such increase was observed in IL-6 KO mice. Additionally, administration of the β2-adrenergic receptor agonist clenbuterol in mice also led to an increase in IL6 expression in skeletal muscle. Furthermore, the treatment with noradrenaline or clenbuterol to C2C12 myotubes resulted in enhanced expression of IL-6 genes. Moreover, the amounts of BCAAs in C2C12 myotubes were increased following treatment with noradrenaline, clenbuterol or recombinant IL-6. We then investigated the mechanisms underlying how IL-6 increases BCAA concentrations in myocytes, focusing on polyamine metabolism, which is involved in the autophagy pathway. The amounts of spermidine, a type of biogenic polyamine, in C2C12 myotubes was increased by b-adrenergic receptor stimulation or by IL-6 stimulation.

Conclusions.

Muscle-derived IL-6, triggered by b-adrenergic receptor stimulation, regulates free BCAA concentrations in skeletal muscle. These acute metabolic responses in myocyte might play a crucial role in BAT thermogenesis and maintaining body temperature in endotherms.

## P-150

## Fatty acid-dependent myokine expression in mouse skeletal muscle

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Skeletal muscle plays a crucial role in physical activity and has the highest basal metabolic rate in the body. Additionally, recent studies have demonstrated that skeletal muscle functions as a secretory organ. Proteins and peptides secreted by skeletal muscle, collectively termed myokines, have been shown to be widely involved in the metabolism of various targets in an endocrine, paracrine, or autocrine manner. Regulation of myokine expression and secretion has been extensively studied in relation to exercise, but studies exploring the relationship between myokines and nutrition and metabolism are limited. Numerous studies have demonstrated that fatty acids, a key nutritional component, exhibit distinct physiological effects based on their carbon chain length and degree of saturation. Notably, medium-chain fatty acids, which are rich in coconut oil and MCT Oil, have been implicated in the inhibition of obesity and the prevention of type II diabetes. This study aims to analyze the regulation of myokine expression and secretion in mouse skeletal muscle in response to various fatty acids.

To evaluate the effects of increased fatty acid intake, we analyzed changes in myokine gene expression in various skeletal muscles of C57BL/6J mice fed a normal or a high-fat diet for 10 weeks. We found that the expression of several myokines, including Chemokine (C-C motif) ligand 5 (CCL5) and chemokine (C-X-C motif) ligand 10 (CXCL10) was significantly induced in skeletal muscles of mice fed a high-fat. We then examined the expression levels of CCL5 and CXCL10 in differentiated C2C12 myotubes, treated with the long-chain saturated fatty acid palmitate, the long-chain unsaturated fatty acid palmitoleic acid or the medium-chain saturated fatty acid capric acid. Results showed that treatment with 1 mM palmitate significantly increased the gene expression of both myokines, but this effect was attenuated by the addition of palmitoleic acid. Conversely, treatment with 1 mM capric acid slightly increased the gene expression of CXCL10, but to a lesser extent than 1 mM palmitate. Similarly, capric acid had no apparent effects on the gene expression of CCL5.

These findings suggest that the expression of myokines CCL5 and CXCL10 in skeletal muscle is differentially regulated by the fatty acid composition of the surrounding environment, specifically the saturation status and carbon chain length of the fatty acids.

## P-151

## Consideration of fusion mechanisms based on characterization of myoblast migration, morphology, membrane stiffness, and cytoplasmic fluidity

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The process of myoblast differentiation and fusion is an essential step in myofiber formation. Understanding this fusion mechanism provides important information for understanding muscle regeneration and hypertrophy. Numerous studies have investigated the mechanisms of muscle regeneration and hypertrophy using genetic engineering and molecular biology approaches. In this study, we will investigate the mechanism of muscle cell fusion from a biophysical perspective. Characteristics of fusion muscle cells are extracted by evaluating cell kinematics and morphology using machine learning models, and by evaluating membrane stiffness using fluorescent probes. Based on these parameters, we will consider the stability of the cell morphology from a mechanical point of view and elucidate why the myotube can elongate in one direction. We will also focus on cytoplasmic fluidity at the interface between two cells during cell fusion. Recently, we have visualized the dynamics of the cytoplasm at the fusion interface using a fluorescent probe and obtained the time constant of the displacement, which showed an extremely slow value on the order of minutes. We will continue to investigate this mechanism.

## P-152

## Biofabrication of cultured meat using a hollow fiber bioreactor

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The University of Tokyo

Advances in in-vitro biofabrication techniques for creating cultured meat from animal cells have been progressing, though mimicking the texture of traditional meat at centimeter scales remains difficult. A new method employing a hollow fiber bioreactor (HFB) has been introduced to tackle this issue. The HFB uses semipermeable hollow fibers that serve as artificial blood vessels, distributing nutrients and oxygen evenly throughout the tissue, and incorporates microfabricated anchors that help align cells. This system's active perfusion enables centimeter-scale chicken muscle tissue to exhibit higher levels of protein markers and sarcomere development throughout the tissue, enhancing both texture and flavor. Looking ahead, scaling this method with industrial robots could significantly impact not just the cultured meat industry but also tissue engineering for creating large artificial organs.

## P-153

## An Uncommon Case of Inclusion Body Myositis: Upper Limb Girdle Muscle Atrophy and Weakness as Initial Manifestations

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Peking University First Hospital

Inclusion body myositis (IBM) typically affects the deep finger flexors and knee extensors. This case report highlights an uncommon presentation related to IBM, beginning with bilateral upper limb girdle muscle atrophy and weakness while sparing the deep finger flexors and knee extensors. It provides an opportunity to reconsider the differential diagnosis in patients with underlying IBM. In cases of uncommon presentations, early comprehensive investigations, including muscle biopsy, specific antibody testing, muscle MRI, and genetic testing, are essential.

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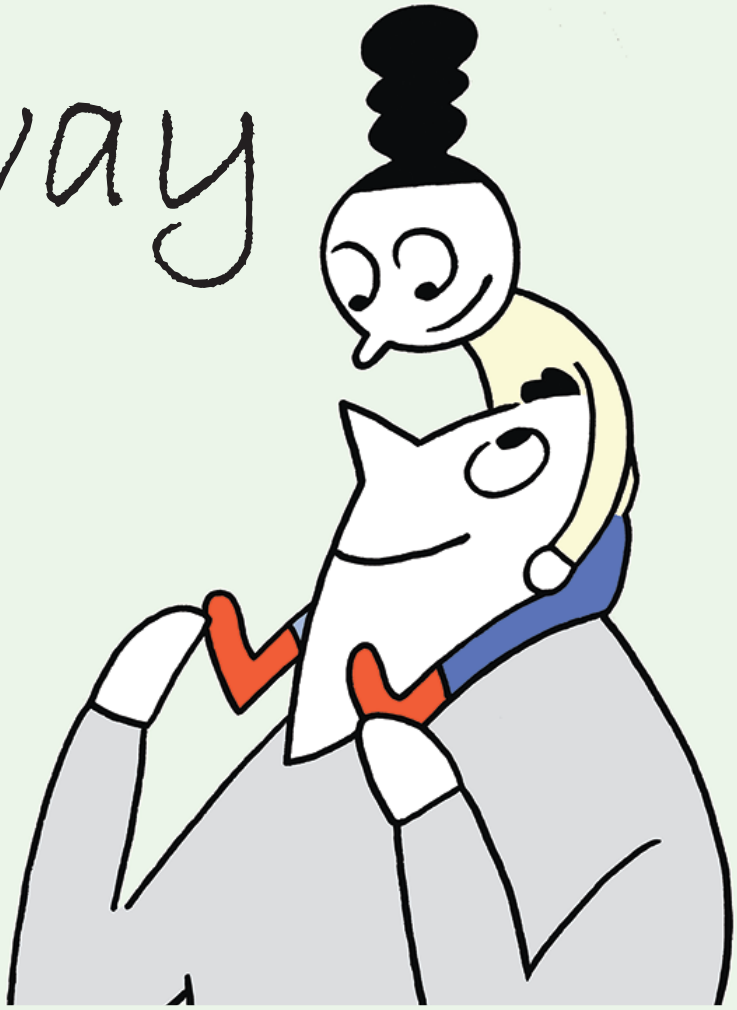

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