IDMC-11

INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING

September 5th - 9th, 2017
San Francisco, California USA

Table of Contents

Welcome	2
Venue Information	3
Conference Committees	9
Sponsors	10
Chairs and Invited Speakers Index	12
Invited Speakers	13
Program at a Glance	26
Detailed Program	30
Platform Presentation Abstracts	36
Poster Abstracts	82
Author Index	246
Attendees Contact Information	276
Notes	293

Welcome to

IDMC-11

September 5-9, 2017
San Francisco, California

John W. Day, M.D., Ph. D.

Katharine Hagerman, Ph.D.

The International Myotonic Dystrophy Consortium remains the premier forum fostering interaction and collaboration of clinicians, pharmaceutical companies and investigators of all types working to understand, treat, and ultimately control this most common form of muscular dystrophy.

The IDMC first met in 1997, five years after identifying the pathogenic DM1 CTG expansion, and four years before finding the DM2 CCTG expansion. Given the confusion regarding the pathogenic mechanisms of these non-coding repeats, the IDMC began as a way to help clarify the novel underlying genetic mechanisms through improved communication and collaboration among international investigators. In the subsequent gatherings in Europe, Asia and North America, the IDMC has maintained the same mission and goals, and at each meeting welcomes many new investigators and clinicians to help clarify myotonic dystrophy clinical care and research.

IDMC-11 is focusing on one of the primary pathogenic mechanisms discovered to play a role in DM1 and DM2 – effects of the repeat expansions on RNA processing. Investigators of RNA mechanisms in other diseases will help inform the consortium, and initiate discussions on best paths forward. Also, IDMC-11 has been jointly organized with the Myotonic Dystrophy Foundation, and on Saturday, September 9, a joint conference of patients, investigators, clinicians and pharmaceutical industry representatives will focus on common goals and priorities regarding the CNS aspects of DM.

We welcome you to San Francisco for IDMC-11. We hope you thoroughly enjoy the meeting and that it fulfills its goals of expanding collaborations and facilitating research that will help us conquer the effects of myotonic dystrophy.

John W. Day, M.D., Ph. D.
Katharine Hagerman, Ph.D.

Venue Information

San Francisco War Memorial

401 Van Ness Avenue
San Francisco

Virtually all IDMC-11 sessions will take place in the newly-remodeled Beaux-Arts Herbst Theatre at the San Francisco War Memorial, and all coffee breaks and lunches during the week will be served in the Green Room. Posters will be presented in the 4th floor Bryan Education Studio. The San Francisco War Memorial is located across from San Francisco's iconic City Hall, the world's 4th-largest free-standing dome.

Herbst Theatre

Hyatt Regency San Francisco

The following IDMC-11 events will be held at the Hyatt Regency San Francisco, the official IDMC-11 conference hotel:

- IDMC-11 attendee breakfasts offered Tuesday through Saturday, which are included in attendee registration fees
- Saturday IDMC awards program
- Joint IDMC-Myotonic Dystrophy Foundation panel session, entitled Bringing the Patient Voice to CNS-Targeting Drug Development in Myotonic Dystrophy, and related lunch discussion
- Drug Development Roundtable hosted by MDF at 7 a.m. on Saturday. Breakfast provided.

The Hyatt Regency San Francisco is also the location of the 2017 MDF Annual Conference, which will be held Friday and Saturday. IDMC-11 registrants are invited to attend MDF Annual Conference sessions Saturday afternoon, including the Industry Updates general session and breakout sessions focused on DM and Sleep; DM, Cancer and Aging; Psychology and DM; Opiates and Anesthesia; and the MDF Annual Conference Closing Dinner. An additional ticket is necessary for the dinner, and can be reserved on the IDMC-11 registration website.

IDMC-11
INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING
September 5th - 9th, 2017 • San Francisco, California. USA

Transportation

Transportation from Local Airports to San Francisco

IDMC-11 registrants will arrive in San Francisco at either the San Francisco International Airport (SFO) or the Oakland International Airport (OAK). Visitors seeking shuttle service and transportation to the Hyatt Regency San Francisco hotel and around the city have a variety of options, including taxi service, airport shuttles and public transportation.

SuperShuttle

SuperShuttle van service operates 24 hours a day, seven days a week, and provides door-to-door service between San Francisco, OAK and SFO. At SFO, go to the upper level (median strip or courtesy island) and look for the blue and yellow van. The cost of the service is $17 one-way, per person. For information, call 1-800-258-3826. Reservations can be booked with the Hyatt Regency San Francisco hotel.

GO Lorrie's Shuttle

Operates 4:00-23:30, seven days a week. Door-to-door service is available between San Francisco International Airport, the Oakland International Airport and the Hyatt Regency San Francisco hotel. The cost of the service is $17 one-way, per person. At the airport, look for the white and green vans entitled "GO Lorrie's" at the shuttle pickup area. For information, call 415-334-9000. Reservations are required for transportation from the hotel to the airport. Reservations can be booked with the Hyatt Regency San Francisco hotel.

Bay Area Rapid Transit (BART)

BART service is available from stations located at both SFO and OAK, to just outside the Hyatt Regency San Francisco. Embarcadero Station Hours: Monday to Friday: 4:23-0:26 Saturday: 6:00-0:26 Sunday: 8:00-0:26.

From San Francisco International Airport (SFO), the total cost is $8.65 each way. Once at the SFO BART station, take any San Francisco-bound (northbound) train to Embarcadero Station. Take the Drumm Street exit; the Hyatt Regency San Francisco hotel is located directly outside the station. BART trains are available every 15-20 minutes.

From Oakland International Airport (OAK), the total cost is $10.05 one-way. The Oakland Airport BART station is located across from the Terminal 1 baggage claim area and a short walk from Terminal 2. Board a San Francisco/ Daly City-bound train to "Embarcadero Station." Take the Drumm Street exit and the Hyatt Regency San Francisco hotel is located directly outside the station. BART trains run every 15-20 minutes.

Taxi

The cost of the service ranges from $40.00-$45.00 one way from SFO.

Ride-sharing Apps

The cost for a private ride from San Francisco airports to San Francisco using ride-sharing apps, such as Uber or Lyft, will range from $30.00-$40.00.

Transportation between the Hyatt Regency San Francisco Hotel and the Conference Venue

There are several ways to travel from the Hyatt Regency San Francisco hotel to the Herbst Theatre located in the San Francisco War Memorial, including the following options:

- MUNI Local Transit System Bus/Train rates: $2.25
- Ride the F Line historic streetcars from the Hyatt Regency San Francisco hotel to the "Van Ness" station stop. Walk north to the Herbst Theatre located in the San Francisco War Memorial. Cost is approximately $2.50 per adult rider.

Taxi

Take a taxi from the Hyatt Regency San Francisco hotel to the Herbst Theatre located in the San Francisco War Memorial. The cost of a taxi will be between $8.00-$14.00.

Ride-sharing Apps

The cost for a private ride between the conference hotel and the San Francisco War Memorial using ride-sharing apps such as Uber or Lyft will range from $8.00-$10.00.

Exploring San Francisco

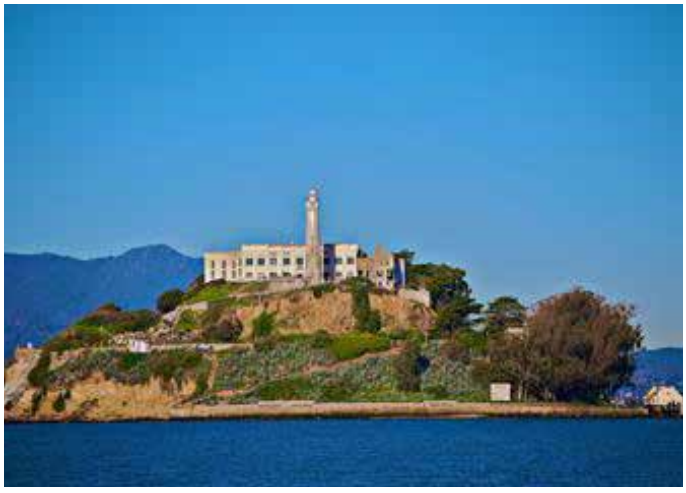
What does San Francisco have to offer? The sights and scenery. The one-of-a-kind events and world-class food. The welcoming people, the diversity and rich history.

The Golden Gate Bridge

The Golden Gate Bridge, the most famous bridge in the world, manages to impress even the most experienced travelers with its stunning 1.7-mile span. Approximately 120,000 automobiles drive across it every day. A pedestrian walkway also allows the crossing on foot, and bikes are allowed on the western side. The Golden Gate Bridge is said to be one of the most photographed things on Earth.

The Cable Cars

Cable cars have been transporting people around San Francisco since the late 19th century. The cars run on tracks and are moved by an underground cable on three routes. Their familiar bells can be heard ringing from blocks away. Tickets may be purchased at the cable car turnarounds at the ends of each route. Each one-way ride will provide spectacular views of the city's celebrated hills as well as exhilarating transportation.



Alcatraz

Alcatraz, the notorious former prison, is located on an island of the same name in the middle of San Francisco Bay. Some of the United States' most notorious criminals were incarcerated there. Though several tried, no inmate ever made a successful escape from "The Rock." The prison was closed in the 1960's and stories about Alcatraz are legendary. A visit to Alcatraz today is fascinating. Recorded cell-house tours are available, allowing visitors to learn about the prison as they explore the buildings and grounds. To reach the island, take an Alcatraz Cruises ferry from Pier 33. Advance reservations are recommended: www.alcatrazcruises.com.

Union Square

Union Square is the place for serious shoppers. Major department stores and the most exclusive designer boutiques line streets like Post, Sutter, Geary, Grant, Stockton and Powell. The Westfield San Francisco Shopping Centre houses the largest Bloomingdale's outside of New York and the second largest Nordstrom in the U.S.

North Beach

North Beach, the city's Italian quarter, isn't a beach at all. It's a neighborhood of European-style sidewalk cafes, restaurants and shops near Washington Square along Columbus and Grant avenues. The beautiful Church of Saints Peter and Paul is a beloved landmark. Coit Tower atop Telegraph Hill offers a splendid vantage point for photos of the bridges and the Bay. Inside the tower, floor-to-ceiling murals painted in the 1930s depict early San Francisco.

Chinatown

The entrance to Chinatown at Grant Avenue and Bush Street is called the "Dragon's Gate." Inside are 24 blocks of hustle and bustle, most of it taking place along Grant Avenue, the oldest street in San Francisco. This city within a city is best explored on foot; exotic shops, renowned restaurants, food markets, temples and small museums comprise its boundaries. Visitors can buy ancient potions from herb shops, relax and enjoy a "dim sum" lunch or witness the making of fortune cookies.

Cultural Events

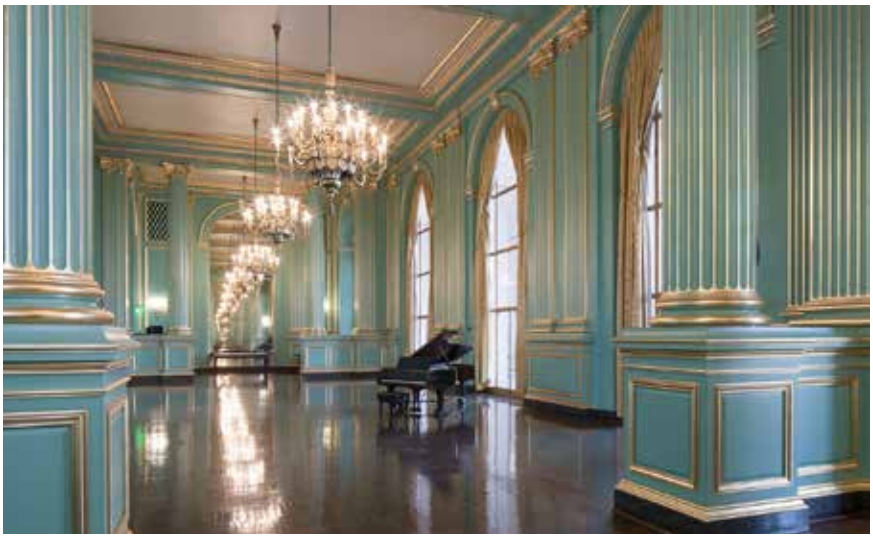
A visit to San Francisco would not be complete without a cultural experience. The city is home to internationally recognized symphony, opera and ballet companies. Many playwrights introduce their works in San Francisco and avant-garde theatre and dance companies dot the city. The San Francisco Museum of Modern Art, the Asian Art Museum, the de Young Museum, the Legion of Honor and other museums and galleries are devoted to the finest of classical and contemporary arts. San Francisco is also home to the California Academy of Sciences—the only place on the planet with an aquarium, a planetarium, a natural history museum, and a four-story rainforest all under one roof.

The content above was provided by the San Francisco Travel Association. The original article was written by Angela Jackson.

Social Activities

Welcome Reception

Tuesday, September 5, 2017
18:00 – 19:30
Immediately follows
Dr. Gene Yeo's keynote talk
San Francisco War Memorial
Green Room
401 Van Ness Ave.
San Francisco



Closing Dinner

Friday, September 8, 2017
19:30 – 21:30
Sens Restaurant
4 Embarcadero Center, 3rd floor
Located beside the Hyatt Regency San Francisco Hotel. You can access the restaurant at the entrance closest to Drumm and Sacramento Streets.

Conference Committees

Local Planning Committee

John W. DAY, M.D., Ph.D., Stanford University, USA
Katharine HAGERMAN, Ph.D., Stanford University, USA
John PORTER, Ph.D., Myotonic Dystrophy Foundation, San Francisco, USA
James VALENTINE, J.D., Hyman, Phelps & McNamara, P.C., Washington, D.C., USA
Molly WHITE, Myotonic Dystrophy Foundation, San Francisco, USA

Local Scientific Committee

Tetsuo ASHIZAWA, M.D., Houston Methodist Neurosciences Research Program, USA
John W. DAY, M.D, Ph.D., Stanford University, USA
Richard MOXLEY, III, M.D., University of Rochester, USA
John PORTER, Ph.D., Myotonic Dystrophy Foundation, San Francisco, USA
Maurice SWANSON, Ph.D., University of Florida, USA

International Scientific Committee

Tetsuo ASHIZAWA, M.D., Houston Methodist Neurosciences Research Program, USA
Guillaume BASSEZ, M.D., Ph.D., Henri Mondor University Hospital, France
David BROOK, Ph.D., University of Nottingham, Queen's Medical Centre, UK
John W. DAY, M.D, Ph.D., Stanford University, USA
Bruno EYMARD, M.D., Institut de Myologie, France
Geneviève GOURDON, Ph.D., Institut Imagine, France
Tiemo GRIMM, M.D., University of Würzburg, Germany
Peter HARPER, M.D., Cardiff University (emeritus), UK
Shoichi ISHIURA, Ph.D., University of Tokyo, Japan
Ralf KRAHE, Ph.D., University of Texas, Houston, USA
Adolfo LÓPEZ DE MUNAIN, M.D., Ph.D., University of Basque Country, Spain
Mani MAHADEVAN, M.D., University of Virginia, Charlottesville, USA
Giovanni MEOLA, M.D., University of Milan, Italy
Darren MONCKTON, Ph.D., University of Glasgow, UK
Richard MOXLEY, III, M.D., University of Rochester, USA
Nakaaki OHSAWA, M.D., University of Tokyo, Japan
Christopher PEARSON, Ph.D., University of Toronto, Canada
Jack PUYMIRAT, M.D., Ph.D., Université Laval, Quebec City, Canada
Laura RANUM, Ph.D., University of Florida, Gainesville, USA
Mark ROGERS, M.D., University of Wales, UK
Benedikt SCHOSER, Ph.D., University of Munich, Germany
Nicolas SERGEANT, Ph.D., Université de Lille, France
Maurice SWANSON, Ph.D., University of Florida, Gainesville, USA
Charles THORNTON, M.D., University of Rochester, USA
Bjarne UDD, M.D., Ph.D., University of Helsinki, Finland
Andoni URTIZBEREA, M.D., Hôpital Marin, France
Berend WIERINGA, Ph.D., University of Nijmegen, The Netherlands

Sponsors

Biogen

IONIS PHARMACEUTICALS

MYOTONIC DYSTROPHY FOUNDATION

Care and a Cure

The Marigold Foundation
Creating Hope & Opportunity

NIH

MDA

AgapeInitiative

Myotonic Dystrophy SUPPORT GROUP

We're proud to sponsor the IDMC Annual Conference

Through cutting-edge science, Biogen discovers, develops and delivers to patients worldwide therapies for the treatment of neurodegenerative and rare diseases.

WWW.BIOGEN.COM

Biogen

Chairs

Tetsuo Ashizawa
Houston Methodist Hospital

Guillaume Bassez
Centre de Référence Maladies Neuromusculaires, Henri Mondor Hospital

Andy Berglund
University of Florida

David Brook
University of Nottingham, United Kingdom

Thomas Cooper
Baylor College of Medicine

John Day
Stanford University

Bruno Eymard
APHP – La Pitie-Salpetriere Hospital, Service de Myologie

Cynthia Gagnon
Université de Sherbrooke/GRIMN

Mario Gomes-Pereira
Inserm

Chad Heatwole
University of Rochester

Ralf Krahe
University of Texas MD Anderson Cancer Center

Ami Mankodi
NINDS, NIH

Darren Monckton
University of Glasgow

Christopher Pearson
The Hospital for Sick Children, Toronto

John Porter
Myotonic Dystrophy Foundation

Giovanni Meola
IRCCS Policlinico San Donato

Jacinda Sampson
Stanford University

Benedikt Schoser
Friedrich-Baur-Institute, Ludwig-Maximilians-University

Nicolas Sergeant
Inserm

S. Subramony
University of Florida

Maurice Swanson
University of Florida

Derick Wansink
Radboud University

Bé Wieringa
Radboud University

Speakers

C. Frank Bennett
Ionis Pharmaceuticals
Carlsbad, United States

Beverly Davidson
University of Pennsylvania
Philadelphia, United States

Baziel Van Engelen
Radboud University
Njmegen, The Netherlands

Richard Finkel
Nemours Children's Hospital
Orlando, United States

Adrian Krainer
University of Texas
Houston, United States

Laurence Mignon
Ionis Pharmaceuticals
Carlsbad, United States

Emmanuel Mignot
Stanford University
Palo Alto, United States

Francesco Muntoni
University College London
London, United Kingdom

Christopher Pearson
Hospital for Sick Kids
Toronto, Canada

Stuart Peltz
PTC Therapeutics
Plainfield, United States

Laura Ranum
University of Florida
Gainesville, United States

Charles Thornton
University of Rochester
Rochester, United States

Gene Yeo
University of California, San Diego
San Diego, United States

Invited Speakers

Gene Yeo — Keynote Speaker

University of California San Diego

Visualization and Elimination of Toxic DNA and RNA

Invited Speakers

Mini Symposium – Therapeutic Targeting of RNA

Adrian Krainer

Cold Spring Harbor Laboratories

ASOs Modification of RNA Splicing in SMA

Nusinersen (Spinraza™): The First FDA/EMA-Approved Treatment for Spinal Muscular Atrophy

SMA is a motor-neuron disease, caused by mutations in *SMN1*. Patients retain one or more copies of the nearly identical *SMN2* gene, which mainly expresses mRNA lacking exon 7, coding for an unstable protein isoform. The small amount of full-length mRNA and protein expressed from *SMN2* only partially compensates for the loss of *SMN1*. Together with Ionis Pharmaceuticals, we developed nusinersen, an antisense oligonucleotide (ASO) that efficiently promotes exon 7 inclusion and restores SMN protein levels. Nusinersen hybridizes to intron 7 of the *SMN2* pre-mRNA, preventing binding of the splicing repressors hnRNPA1/A2 to a bipartite intronic splicing silencer; this in turn facilitates binding of U1 snRNP to the intron 7 5' splice site, resulting in enhanced exon 7 inclusion. Clinical trials of nusinersen in SMA patients, sponsored by Ionis and Biogen, began at the end of 2011. Nusinersen (Spinraza™) was approved by the FDA in December 2016, and by the EMA in June 2017.

We are continuing to explore aspects of SMA pathogenesis and treatment, using ASO therapy in SMA mouse models. We found that SMA is not motor-neuron cell-autonomous in the mouse models, such that correcting *SMN2* splicing in peripheral tissues exclusively is necessary and sufficient for full phenotypic rescue. We are also exploring prenatal ASO treatment, as it is likely that early intervention can have the greatest clinical benefit.

Hua Y, Sahashi K, Rigo F, Hung G, Horev G, Bennett CF, Krainer AR (2011) Nature 478: 123-6.

Hua Y, Liu YH, Sahashi K, Rigo F, Bennett CF, Krainer AR (2015) Genes Dev 29: 288-97.

Finkel RS, Chiriboga CA, Vajsar J, Day JW, Montes J, De Vivo DC, Yamashita M, Rigo F, Hung G, Schneider E, Norris DA, Xia S, Bennett CF, Bishop KM (2017) Lancet 388: 3017-26.

Invited Speakers

Mini Symposium – Therapeutic Targeting of RNA

Beverly Davidson

University of Pennsylvania

Mechanisms and Therapies in the CAG-Repeat Diseases

Invited Speakers

Mini Symposium – Therapeutic Targeting of RNA

Stuart Peltz

PTC Therapeutics

Small Molecule Compounds Modifying RNA

Invited Speakers

Mini Symposium – Therapeutic Targeting of RNA

C. Frank Bennett

Ionis Pharmaceuticals

Optimization of RNase H-Dependent Antisense Drugs for the Treatment of Myotonic Dystrophy

Invited Speakers

Christopher Pearson

The Hospital for Sick KIds

Epigenetic Mechanisms in Congenital Myotonic Dystrophy

Invited Speakers

Laura Ranum
University of Florida
Pathogenic Mechanisms of Repeat Expansions in Myotonic Dystrophy and ALS

Invited Speakers

Advances in Clinical Neuroscience

Emmanuel Mignot

Stanford University

Understanding Sleep Abnormalities in Narcolepsy and Myotonic Dystrophy

Invited Speakers

Mini Symposium – Neuromuscular Clinical Research Experience

Richard Finkel

Nemours Children's Hospital

Trial Design and Clinical Trial Results for SMA

Mini Symposium – Neuromuscular Clinical Research Experience

Francesco Muntoni

University College London

Infrastructure Considerations for DMD Clinical Trial Readiness

Infrastructure Considerations for DMD Clinical Trial Readiness

F. Muntoni[1]

1. Dubowitz Neuromuscular Centre, Great Ormond Street Institute of Child Health, University College London, London, UK

Introduction: The improved understanding of the roles of the various pathological processes which contribute to the progressive muscle loss of individuals with Duchenne muscular dystrophy (DMD) has led to the development of multiple experimental strategies, at various stage of development. Two broad approaches are being used: a. to restore structural integrity of the muscle fibres (i.e. dystrophin restoration); b. to deal with secondary consequences of the dystrophic pathology. The field has experienced some successes; however some studies have missed their efficacy endpoints, indicating the complexity of developing DMD therapies.

Objective: To analyse the outcome of recently completed clinical trials for which efficacy data are available.

Methods: Analysis of recently completed DMD phase II and phase III clinical trials in which clinical and / or biochemical efficacy were the primary outcome measures.

Results: Regarding biochemical outcome measures in dystrophin restoration strategies, there have been some partial success of a number of studies. Nevertheless in several of these studies the assays developed by the academic laboratories or industrial partners did not completely satisfy the stringent regulatory requirements and this led to confusion on the significance of findings.
Regarding the studies focused on clinical outcome measures; a number of studies failed to meet their primary endpoints, in some instances due to lack of efficacy of the drug under development; in others also due to the choice of the outcome measures, or the inclusion- exclusion criteria for the clinical trial. New knowledge has emerged on the impact of standard of care on disease progression that is necessary to take into account for powering appropriately the clinical trials.

Conclusions: The DMD experience gives us important lessons for study design and engagement of regulatory authorities that will hopefully facilitate future clinical trials.

Invited Speakers

Mini Symposium – Neuromuscular Clinical Research Experience

Charles Thornton

University of Rochester Medical Center

Myotonic Dystrophy Clinical Research Network and Natural History Studies

Invited Speakers

Mini Symposium – Neuromuscular Clinical Research Experience

Baziel Van Engelen

Radboud University Nijmegen Medical Centre

OPTIMISTIC: Observational Prolonged Trial In Myotonic Mystrophy Type 1 to Improve QoL-Standards, a Target Identification Collaboration

Invited Speakers

Mini Symposium – Neuromuscular Clinical Research Experience

Laurence Mignon

Ionis Pharmaceuticals

Lessons from DMPKRx Clinical Trials in Myotonic Dystrophy

Program at a Glance

Tuesday, September 5, 2017

15:00 - 17:00	Registration
17:00 - 19:30	Keynote Speaker, Performance and Opening Reception – San Francisco War Memorial
	Visualization and Elimination of Toxic DNA and RNA – Gene YEO, University of California San Diego
	Axis Dance Performance
	Opening Reception

Wednesday, September 6, 2017

8:30 - 10:30	Mini Symposium - Therapeutic Targeting of RNA Chairs: Maurice SWANSOn and Mario GOMES-PEREIRA
	ASOs Modification of RNA Splicing – Adrian KRAINER, Cold Spring Harbor Laboratories
	Mechanisms and Therapies in the CAG-repeat Diseases – Beverly DAVIDSON, University of Pennsylvania
	Small Molecule Compounds Modifying RNA – Stuart PELTZ, PTC Therapeutics
	Optimization of RNase H-Dependent Antisense Drugs for the Treatment of Myotonic Dystrophy – C Frank BENNETT, Ionis Pharmaceuticals
10:30 - 11:00	Coffee Break
11:00 - 11:30	Invited Lecture
	Epigenetic Mechanisms in Congenital Myotonic Dystrophy – Christopher PEARSON, Hospital for Sick Kids
11:30 - 12:30	DM Session 1 Microsatellite Instability and Epigenetics Chairs: Darren MONCKTON and John CLEARY
	Platform Presentations S1-01 to S1-04
12:30 - 13:00	Flash Poster Session Chairs: Christopher PEARSON and Derick WANSINK
13:00 - 14:30	Lunch and Poster Viewing
14:30 - 17:00	DM Session 2 Disease Mechanisms: DM1, DM2 and CDM Chair: Thomas COOPER
	Platform Presentations S2-01 to S2-10

Program at a Glance

Thursday, September 7, 2017

8:30 - 9:30	Learning from Other Disorders Chair: Benedikt SCHOSER
	Pathogenic Mechanisms of C9Orf72 Repeat Expansion – Laura RANUM, University of Florida
9:30 - 10:00	DM Session 3 Cancer and Aging in DM Chair: Nicolas SERGEANT
	Platform Presentations S3-01 to S3-02
10:00 - 10:30	Coffee Break
10:30 - 12:00	DM Session 4 Clinical, Ethical and Social Issues and Disease Burden Chair: Cynthia GAGNON
	Platform Presentations S4-01 to S4-06
12:00 - 12:30	Flash Poster Session Chairs: Bruno EYMARD, Guillaume BASSEZ and Giovanni MEOLA
12:30 - 14:00	Lunch and Poster Viewing
14:00 - 16:30	DM Session 5 Specific Disease Features – CNS, Cardiac, GI Chairs: Chad HEATWOLE, Ami MANKODI and Nicolas SERGEANT
	Platform Presentations S5-01 to S5-10
16:30 - 17:00	Organization and Outreach Update

Program at a Glance

Friday, September 8, 2017

8:30 - 9:30	Advances in Relevant Clinical Neuroscience Chair: John W. DAY
	CNS and Sleep – Emmanuel MIGNOT, Stanford University
9:30 - 10:30	DM Session 6 Cell models for DM Chair: David BROOK
	Platform Presentations S6-01 to S6-04
10:30 - 11:00	Coffee Break
11:00 - 12:00	DM Session 7 Animal Models and Tissue-specific Mechanisms Chairs: Tetsuo ASHIZAWA and Bé WIERINGA
	Platform Presentation S7-01 to S7-04
12:00 - 12:30	Flash Poster Session Chairs: Ralf KRAHE and Guillaume BASSEZ
12:30 - 13:30	Lunch and Poster Viewing
13:30 - 16:30	Mini Symposium - Neuromuscular Clinical Research Experience Chair: John PORTER
	SMA – Richard FINKEL, Nemours Medical Center
	DMD – Francesco MUNTONI, University College London
	DMCRN – Charles THORNTON, University of Rochester
	OPTIMISTIC – Baziel VAN ENGELEN, University of Nijmegen
	DMPKRx – Laurence MIGNON, Ionis Pharmaceuticals
16:30 - 17:30	DM Session 8 RNA Clinical Research Methods : Biomarkers, Outcome Measures, Registries, Trial Design and Therapeutics Chairs: S. SUBRAMONY and Jacinda SAMPSON
	Platform Presentations S8-01 to S8-04
19:30 - 21:30	Closing Dinner - Sens Restaurant

Program at a Glance

Saturday, September 9, 2017

All Saturday events are held at the Hyatt Regency Embarcadero

	Patients, Families, Investigators, Clinicians - Working Toward Common Goals for DM
6:00 - 7:00	Embarcadero Fun Run
7:00 - 9:00	MDF Drug Development Roundtable Breakfast provided
9:00 - 9:45	Presentation of Awards for IDMC Participants
9:45 - 10:00	Coffee Break
10:00 - 12:45	Bringing the Patient Voice to CNS-Targeting Drug Development in Myotonic Dystrophy Lunch provided
13:00 - 13:50	Grant Writing Training
15:30 - 17:00	Industry Updates

Detailed Program

Tuesday, September 5, 2017

15:00 - 17:00	Registration
17:00 - 19:30	Keynote Speaker, Performance and Opening Reception – San Francisco War Memorial
	Opening Remarks - John W. DAY, Stanford University Chair: Andrew BERGLUND, University of Florida Visualization and Elimination of Toxic DNA and RNA – Gene YEO, University of California San Diego
	Axis Dance Performance
	Opening Reception

Wednesday, September 6, 2017

8:30 - 10:30	Mini Symposium - Therapeutic Targeting of RNA Chairs: Maurice Swanson and Mario GOMES-PEREIRA
	ASOs Modification of RNA Splicing Adrian KRAINER, Cold Spring Harbor Laboratories
	Mechanisms and Therapies in the CAG-repeat Diseases Beverly DAVIDSON, University of Pennsylvania
	Small Molecule Compounds Modifying RNA – Stuart PELTZ, PTC Therapeutics
	Optimization of RNase H-Dependent Antisense Drugs for the Treatment of Myotonic Dystrophy C Frank BENNETT, Ionis Pharmaceuticals
10:10 - 10:30	Panel Q&A
10:30 - 11:00	Coffee Break
11:00 - 11:30	Invited Lecture
	Epigenetic Mechanisms in Congenital Myotonic Dystrophy Christopher PEARSON, Hospital for Sick Kids
11:30 - 12:30	DM Session 1 Microsatellite Instability and Epigenetics Chairs: Darren MONCKTON and John CLEARY
11:30 - 11:45	**S1-01** Progenitor Allele Length, Residual Variation in Somatic Instability and Variant Repeats Predict Age at Onset in the DM1 OPTIMISTIC Cohort Sarah CUMMING
11:45 - 12:00	**S1-02** DMPK Methylation Levels are Associated with Muscular and Respiratory Profiles in DM1 Independent of Repeat Length Cécilia LÉGARÉ
12:00 - 12:15	**S1-03** The Origin and Historical Route of Myotonic Dystrophy Type 2 Mutation Across Europe Jovan PEŠOVIĆ

Detailed Program

Wednesday, September 6, 2017

12:15 - 12:30	**S1-04** Shortening DM1 CTG Trinucleotide Repeat Tracts: A Possible Approach to Gene Therapy Lucie POGGI
12:30 - 13:00	Flash Poster Session Chairs: Christopher PEARSON and Derick WANSINK
13:00 - 14:30	Lunch and Poster Viewing
4:30 - 17:00	DM Session 2 Disease mechanisms: DM1, DM2 and CDM Chair: Thomas COOPER
14:30 - 14:45	**S2-01** Derepressing Muscleblind Expression by miRNA Sponges Ameliorates Myotonic Dystrophy-like Phenotypes in Drosophila Ruben ARTERO
14:45 - 15:00	**S2-02** Reducing Toxic RNA Repeats in Myotonic Dystrophy Models Jana JENQUIN
15:00 - 15:15	**S2-03** Identification of a Novel Kinase Target in DM Pathophysiology Ami KETLEY
15:15 - 15:30	**S2-04** Rbfox2 Fetal Isoform Overexpression Drives Arrhythmogenic Effects in Myotonic Dystrophy Type 1 Hearts Chaitali MISRA
15:30 - 15:45	**S2-05** Phenotype-genotype/Epigenotype Correlation and Aberrant Inflammatory Signaling in Congenital Myotonic Dystrophy Masayuki NAKAMORI
15:45 - 16:00	**S2-06** Mis-regulation and Mis-splicing of a Conserved Myogenic lncRNA in Human and Mouse DM1 Skeletal Muscle Emmanuelle QUERIDO
16:00 - 16:15	**S2-07** Disrupted Prenatal RNA Processing and Myogenesis in Congenital Myotonic Dystrophy James THOMAS
16:15 - 16:30	**S2-08** Loss of Zinc Finger Protein 9 Encoded by a Myotonic Dystrophy Type 2 Gene Causes Muscle Atrophy Lubov TIMCHENKO
16:30 - 16:45	**S2-09** Maturation and Nuclear Retention of Single DMPK Transcripts Derick WANSINK
16:45 - 17:00	**S2-10** RAN Translation Regulated by Muscleblind Proteins in Myotonic Dystrophy Type 2 Tao ZU

Detailed Program

Thursday, September 7, 2017

8:30 - 9:30	Learning From Other Disorders Chair: Benedikt SCHOSER
	Pathogenic Mechanisms of C9Orf72 Repeat Expansion Laura RANUM
9:30 - 10:00	DM Session 3 Cancer and Aging in DM Chair: Nicolas SERGEANT
9:30 - 9:45	**S3-01** Benign and Malignant Tumors in Patients Diagnosed with Myotonic Dystrophy Type 1 Rotana ALSAGGAF
9:45 - 10:00	**S3-02** Organ-specific Risk of Cancer in Patients with Myotonic Dystrophy: Results from a Meta-analysis Shahinaz GADALLA
10:00 - 10:30	Coffee Break
10:30 - 12:00	DM Session 4 Clinical, Ethical and Social Issues and Disease Burden Chair: Cynthia GAGNON
10:30 - 10:45	**S4-01** Factors associated with Health-related Quality of Life in Children with Congenital Myotonic Dystrophy Craig CAMPBELL
10:45 - 11:00	**S4-02** Do Research Publications Fit with DM1 Individuals Expectations? Céline DOGAN
11:00 - 11:15	**S4-03** Strength Evolution in Myotonic Dystrophy Type 1 Over a 9-year Period Cynthia GAGNON
11:15 - 11:30	**S4-04** Causes and Predictors of Early Mortality in a Large U.S. Myotonic Dystrophy Type 1 Adult Cohort William GROH
11:30 - 11:45	**S4-05** Development and Validation of a Disease-Specific Patient-Reported Outcome Measure for DM2: the Myotonic Dystrophy Type-2 Health Index (MD2HI) Chad HEATWOLE
11:45 - 12:00	**S4-06** Longitudinal Evaluation of Disease Progression in Congenital Myotonic Dystrophy Nicholas JOHNSON
12:00 - 12:30	Flash Poster Session Chairs: Bruno EYMARD, Guillaume BASSEZ and Giovanni MEOLA

Detailed Program

Thursday, September 7, 2017

12:30 - 14:00	Lunch and Poster Viewing
14:00 - 16:30	DM Session 5 Specific Disease Features – CNS, Cardiac, GI Chairs: Chad HEATWOLE, Ami MANKODI and Nicolas SERGEANT
14:00 - 14:15	**S5-01** Changes in Brain Structure and Function in Adults with Myotonic Dystrophy Type 1: A 1-Year Longitudinal Study Ian DEVOLDER
14:15 - 14:30	**S5-02** A Phenotypic Profile of Cognitive Function in Congenital Myotonic Dystrophy Melissa DIXON
14:30 - 14:45	**S5-03** A 34-year Prospective Study of Long-term Cardiac Outcome in DM1 Patients with Normal ECG at Baseline Laura FIONDA
14:45 - 15:00	**S5-04** Progression of Frontal Cognitive Impairment in DM1 Adult Phenotype Over a 13-year Follow-up Benjamin GALLAIS
15:00 - 15:15	**S5-05** Excessive Daytime Sleepiness, Executive Dysfunction and Structural Brain Changes in Myotonic Dystrophy Type 1: the DM1-Neuro Study Mark James HAMILTON
15:15 - 15:30	**S5-06** Natural History of the Heart in Myotonic Dystrophy Type 1: A Cardiac Magnetic Resonance Imaging Follow-up of 11 Patients Aura Cecilia JIMENEZ-MORENO
15:30 - 15:45	**S5-07** Correlation Between Delayed Gastric Emptying and Gastrointestinal Symptoms in Type-1 Myotonic Dystrophy (DM1) Paul JOSEPH
15:45 - 16:00	**S5-08** Macroscopic and Microscopic Diversity of Mis-splicing in the Central Nervous System of Myotonic Dystrophy Type 1 Takashi KIMURA
16:00 - 16:15	**S5-09** $GABA_A$ Receptor Antagonists to Treat Hypersomnia and Impaired Cognition in DM David RYE
16:15 - 16:30	**S5-10** Risk for Complications after Pacemaker or Cardioverter Defibrillator Implantations in Patients with DM1 Karim WAHBI
16:30 - 17:00	Organization and Outreach Update

Detailed Program

Friday, September 8, 2017

8:30 - 9:30	Advances in Relevant Clinical Neuroscience Chair: John W. DAY
	CNS and Sleep – Emmanuel MIGNOT, Stanford University
9:30 - 10:30	DM Session 6 Cell models for DM Chair: David BROOK
9:30 - 9:45	**S6-01** CUG Repeats and MBNL1: A Toxic Combination for the XRN2 Exonuclease Annie Junzhen BARGSTEN
9:45 - 10:00	**S6-02** CUG RNA Toxicity in Astrocytes is Associated with Adhesion and Migration Deficits and Affects Neuritogenesis Diana Mihaela DINCÃ
10:00 - 10:15	**S6-03** Immortalized Human Myotonic Dystrophy Muscle Cell Lines to Assess Therapeutic Compounds Denis FURLING
10:15 - 10:30	**S6-04** Generating Isogenic Myoblast Cell Models by CRISPR/Cas9-mediated Editing, Regulating and Targeting Genes in the Myotonic Dystrophy Type 1 (DM1) Locus Bé WIERINGA
10:30 - 11:00	Coffee Break
11:00 - 12:00	DM Session 7 Animal Models and Tissue-specific Mechanisms Chairs: Tetsuo ASHIZAWA and Bé WIERINGA
11:00 - 11:15	**S7-01** Antisense Oligonucleotides (ASOs) Preferentially Target Repeat-containing Transcripts in Skeletal Muscle Samuel CARRELL
11:15 - 11:30	**S7-02** Insights from Evaluations of Genetic Modifiers of RNA Toxicty Mani MAHADEVAN
11:30 - 11:45	**S7-03** Differential Effects of Intronic vs. Exonic Repeats in DM2 Transgenic Mice Zhenzhi TANG
11:45 - 12:00	**S7-04** Enhancement of Splicing Correction by Ligand Conjugated Antisense (LICA) Oligos in Live DM1 Mice Thurman WHEELER
12:00 - 12:30	Flash Poster Session Chairs: Ralf KRAHE and Guillaume BASSEZ
12:30 - 13:30	Lunch and Poster Viewing

Detailed Program

Friday, September 8, 2017

13:30 - 16:30	Mini Symposium - Neuromuscular Clinical Research Experience Chair: John PORTER
	SMA – Richard FINKEL, Nemours Medical Center
	DMD – Francesco MUNTONI, University College London
	DMCRN – Charles THORNTON, University of Rochester
	OPTIMISTIC – Baziel VAN ENGELEN, University of Nijmegen
	DMPKRx – Laurence MIGNON, Ionis Pharmaceuticals
16:00 - 16:30	Panel Q&A
16:30 - 17:30	DM Session 8 RNA Clinical Research Methods : Biomarkers, Outcome Measures, Registries, Trial Design and Therapeutics Chairs: S. SUBRAMONY and Jacinda SAMPSON
16:30 - 16:45	**S8-01** mRNA Splicing Biomarkers of DM1 in Human Urine Layal ANTOURY
16:45 - 17:00	**S8-02** DM-Scope, a French Nationwide Registry to Decipher Pediatric Myotonic Dystrophies' Clinical Complexity Guillaume BASSEZ
17:00 - 17:15	**S8-03** MicroRNA Functional Deregulation and Potential as Biomarkers in Myotonic Dystrophy Alessandra PERFETTI
17:15: - 17:30	**S8-04** The Pharmacological Small Molecule, AMO-02, Reduces Adult and Congenital Myotonic Dystrophy Type 1 in the Pre-clinical Studies Mei WANG
19:30 - 21:30	Closing Dinner – Sens Restaurant

Saturday, September 9, 2017 All Saturday events are held at the Hyatt Regency Embarcadero

Patients, Families, Investigators, Clinicians - Working Toward Common Goals for DM	
6:00 - 7:00	Embarcadero Fun Run
7:00 - 9:00	MDF Drug Development Roundtable – Breakfast provided
9:00 - 9:45	Presentation of Awards for IDMC participants
9:45 - 10:00	Coffee Break
10:00 - 12:45	Bringing the Patient Voice to CNS-Targeting Drug Development in Myotonic Dystrophy – Lunch provided
13:00 - 13:50	Grant Writing Training
15:30 - 17:00	Industry Updates
17:45-19:30	MDF Closing Dinner Party – Ticket Required

S1-01

Session 1: Microsatellite Instability and Epigenetics

Progenitor Allele Length, Residual Variation in Somatic Instability and Variant Repeats Predict Age at Onset in the DM1 OPTIMISTIC Cohort

CUMMING Sarah A[1], ADAM Berit A[1], MCGHIE Josephine[1], BAROSS Stephanie[1], BOYD Roxanna J[1], MARTIN Stuart S[1], OPTIMISTIC consortium[2], MONCKTON Darren G[1]

1. University of Glasgow, UK
2. European Community, Concentris Research Management GMBH, Germany

Introduction: Somatic instability in DM1 is expansion-biased, so age at sampling can confound estimation of repeats. By using small-pool PCR (SP-PCR), the estimated progenitor allele length (ePAL) may be determined from the lower edge of the distribution of bands. The ePAL is the main determinant of age at onset, modified by patient-specific differences in somatic instability. In ~5% of patients, the expanded allele is interrupted by variant repeats, which stabilise the allele, often leading to milder symptoms and delayed onset. Understanding the genetic variation underlying symptomatic differences might aid both stratification of participants, and interpretation of results, for clinical trials in DM1. We therefore conducted a genetic analysis of the CTG expansion in the OPTIMISTIC (the observational prolonged trial in myotonic dystrophy type 1 to improve quality of life – standards, a target identification collaboration) patient cohort.

Methods: SP-PCR was used to determine ePAL and mode in blood DNA from 250 patients. DM1 expansions were also screened for variant repeats by AciI digestion. Linear regression modelling was used to determine relationships between genetic and phenotypic data.

Results: For 247 participants, ePAL and mode were determined. 21 patients had variant repeats (8.4%). ePAL was a major modifier of age at onset, which was significantly delayed in patients with variant repeats. Somatic instability was modified by ePAL, age, and an interaction between the two. Variant repeats significantly reduced the degree of somatic instability. Lastly, age at onset was modified by residual variation in somatic instability.

Discussion: In this cohort, age at onset was modified by ePAL and residual variation in somatic instability. Variant repeats reduced somatic instability and delayed disease onset, emphasising the important contribution of somatic instability to the symptoms of DM1.

Grant Support: The European Community's Seventh Framework Programme (FP7/2007–2013) no. 305697. The Myotonic Dystrophy Support Group, UK.

S1-02

Session 1: Microsatellite Instability and Epigenetics

DMPK Methylation Levels are Associated with Muscular and Respiratory Profiles in DM1 Independent of Repeat Length

LÉGARÉ Cécilia[1,2,3],GAGNON Cynthia[3,4], OVEREND Gayle[5], BOUCHARD Michaël[1,2], GUAY Simon-Pierre[1,2], MONCKTON Darren G[5], MATHIEU Jean[3,4], HÉBERT Luc J[6], BOUCHARD Luigi[1,2,3]

1. Department of Biochemistry,Université de Sherbrooke
2. ECOGENE-21 Biocluster
3. GRIMN
4. École de réadaptation, FMSS, Université de Sherbrooke
5. Institute of Molecular,Cell and Systems Biology, University of Glasgow
6. Université Laval, Centre interdisciplinaire de recherche en réadaptation et intégration sociale

Introduction: DM1 shows high phenotypic variability only partially explained by CTG repeat length. Other factors such as epigenetic changes may explain some of this additional phenotypic variability. Thus, we aimed to evaluate how DNA methylation at the DMPK locus is associated with muscular and respiratory profiles in DM1 patients.

Methods: Pyrosequencing of bisulfite treated DNA was used to quantify DNA methylation levels at the DMPK locus in blood from 90 adult onset DM1 patients. Modal CTG repeat length was assessed using small pool PCR. Muscular and respiratory profiles were evaluated according to standard procedures.

Results: DNA methylation levels at three sites upstream of the CTG expansion were negatively correlated with modal CTG repeat length ($rs = 0.224$; $p = 0.040$, $rs = 0.317$; $p = 0.003$ and $rs = 0.241$; $p = 0.027$), whereas a positive correlation was observed with downstream epigenetic marks ($rs = 0.227$; $p = 0.037$). Multiple linear regression modeling showed that DNA methylation significantly and independently contributed to variability in ankle dorsiflexor ($ß = 0.340$; $p = 0.001$, $ß = -0.363$; $p = 0.013$ and $ß = 0.467$; $p = 0.001$), grip ($ß = 0.173$; $p = 0.089$) and pinch ($ß = 0.219$; $p = 0.028$) strengths as well as in forced vital capacity ($ß = 0.368$; $p = 0.002$ and $ß = 0.249$; $p = 0.021$) peak expiratory flow ($ß = -0.236$; $p = 0.039$) and maximal inspiratory ($ß = 0.266$; $p = 0.083$ and $ß = -0.399$; $p = 0.012$) and expiratory pressures ($ß = -0.211$; $p = 0.067$).

Discussion: DNA methylation at the DMPK locus is associated with muscular and respiratory phenotypes in DM1 independently of the CTG repeat length. Adding epigenetic markers to current molecular CTG repeat length measures could improve prognostic accuracy.

Grant Support: This project was supported by the Canadian Institutes of Health Research, the Fondation du Grand Défi Pierre Lavoie and Muscular Dystrophy UK.

S1-03

Session 1: Microsatellite Instability and Epigenetics

The Origin and Historical Route of Myotonic Dystrophy Type 2 Mutation Across Europe

PEŠOVIĆ Jovan1, BRKUŠANIN Miloš[1], PERIĆ Stojan[2], RADVANSZKY Jan[3], MAŠIREVIĆ Srdjan[1], KOVČIĆ Vlado[1], MUSOVA Zuzana[4], STEHLÍKOVÁ Kristýna [5], LEONARDIS Lea[6], KEKOU Kyriaki[7], JOVANOVIĆ Vladimir[8], MAZANEC Radim[4], BRAJUŠKOVIĆ Goran[1], RANUM Laura P[9], RAKOČEVIĆ STOJANOVIĆ Vidosava[2] and SAVIĆ-PAVIĆEVIĆ Dušanka[1]

1. University of Belgrade-Faculty of Biology, Belgrade, Serbia
2. University of Belgrade-School of Medicine, Neurological Clinic, Belgrade, Serbia
3. Inst. for Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences, Bratislava, Slovakia
4. Dept. of Biology and Medical Genetics, Dept of Neurology, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic
5. Centre of Mol Biology and Gene Therapy, University Hospital Brno, Czech Republic
6. Inst of Clinical Neurophysiology, University Medical Center Ljubljana, Ljubljana, Slovenia
7. Dept of Medical Genetics, University of Athens, Athens, Greece
8. Inst for Biological Research “Siniša Stanković”, Belgrade, Serbia
9. Center for NeuroGenetics, University of Florida, Gainesville, FL, USA

Introduction: Myotonic dystrophy type 2 (DM2) is predominantly a disease of the European Caucasians. Haplotype analyses have suggested a founder European mutation ~4000-11000 years old. This period coincides with the Neolithic Age, when the Near Eastern farmers and later herders from the Pontic- Caspian steppe had settled in Europe, shaping the genomic architecture of the Europeans. We aimed to reconstruct historical route of DM2 mutation across Europe.

Methods: CL3N122, CL3N99, CL3N59, CL3N119, CL3N19 and CL3N23 loci were genotyped in 413 individuals from Serbian, Greek, Slovenian, Slovakian and Czech DM2 families. 378 healthy and 70 DM2 unambiguously phased haplotypes by family segregation analysis, and additional 55 DM2 German haplotypes (Liquori et al. 2003) were used for the coalescent modeling of intra-allelic variability in the software DMLE+. Proportion of sampled DM2 chromosomes was 2.152e-3, assuming the reported DM2 mutation frequency in Finland (1/1830). The maximum likelihood estimation (MLE) of the mutation age with 95% CI was determined separately for the population growth rate set at 0.025, 0.03, 0.035, 0.04 and 0.045.

Results: The estimated DM2 mutation age, considered as the range of determined MLEs, is 200-280 generations (~4000–5600 years assuming 20 years/generation). It dates back in the Late Neolith and the early Bronze Age when massive migrations of herders from the Pontic-Caspian steppe to Europe occurred (Yamnaya culture, 3500–2200 BCE), accompanied by an expansion of the Corded Ware culture (2800–2200 BCE), whose individuals were genetically the most similar to the Yamnaya ones.

Discussion: DM2 mutation was probably spread across Europe by the Yamnaya migrations, which brings a novel insight in its origin and historical route. According to epidemiological and patient registry data, the distribution of DM2 mutation seems to reflect a decreasing Yamnaya ancestry from the north to the south in the present-day Europeans.

Grant Support: Grant No 173016, MESTD, R Serbia

S1-04

Session 1: Microsatellite Instability and Epigenetics

Shortening DM1 CTG Trinucleotide Repeat Tracts: A Possible Approach to Gene Therapy

POGGI Lucie[1], MOSBACH Valentine[1], VITERBO David[1], DUMAS Bruno[1], RICHARD Guy-Franck[1]

1. Département Génomes et Génétique, Institut Pasteur CNRS UMR 3525 / Biologics Research, Sanofi R&D

Introduction: Myotonic dystrophy type 1 is caused by an expanded CTG repeat tract located in the 3'UTR of the DMPK gene. Most of the time, repeats tend to expand from generation to generation. However, when a trinucleotide repeat contraction occurred during transmission from father to daughter of an expanded myotonic dystrophy allele, clinical examination of the daughter showed no sign of the disease (O'Hoy et al. 1993). Shortening the expanded array to non-pathological lengths could suppress symptoms of the pathology and could be used as a new gene therapy approach (Richard, 2015). Therefore, we are investigating how synthetic repeat contractions can be achieved in eukaryotic cells, using yeast and mammalian cells as models.

Methods: A CTG repeat expansion from the 3' UTR of the DMPK locus was integrated into a yeast chromosome. A TALEN was designed to recognize this specific region and cut within the repeats. S. pyogenes Cas9 was targeted to the same region, using an appropriate guide RNA. Both nucleases were expressed under inducible promoters. After nuclease induction we analyzed the survival rate of cells and sequenced survivors to check for repeat contractions and mutations. Double-strand break repair was analyzed during time courses by Southern blot and terminal transferase-mediated PCR.

Results: We showed that a TALEN designed to recognize and cut a CTG triplet repeat was very efficient at shortening the repeat (>99% cells showed contraction) and highly specific as no other mutation was detected in yeast cells (Richard et al. 2014). Similar experiments were performed with a S. pyogenes Cas9, but the repair was leading to large chromosomal rearrangements around the cut site as well as a 30-fold decrease in the survival rate (Mosbach et al, 2017). We also induced the TALEN into DM1 patient cells and mice cells containing DM1 CTG repeats; preliminary results show that repeats were contracted to non-pathological lengths in both cases.

Discussion: We demonstrated that it is possible to induce specific contractions of CTG repeat tracts in yeast cells. Surprisingly double-strand break repair outcome depends on the nuclease used, raising an important issue for future gene therapy applications. We are now investigating the effect of other highly specific nucleases through a GFP-based assay in yeast to quantify the cleavage efficiency and specificity on CTG repeat tracts. Six different nucleases (wild-type S. pyogenes Cas9 and mutant derivatives, S. aureus Cas9 and Cpf1) will be compared. The observed contractions in mammalian cells upon TALEN induction are also being confirmed. We hope that these results will give insights into which nuclease is the most suitable for contracting trinucleotide repeat tracts in vivo and pave the way towards new gene therapy approaches.

Grant Support: Institut Pasteur, CNRS, Sanofi

Abstract shortened due to length – see poster for more information

S2-01

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Derepressing Muscleblind Expression by miRNA Sponges Ameliorates Myotonic Dystrophy-like Phenotypes in Drosophila

ARTERO Ruben[1,2,3], CERRO-HERREROS Estefania[1,2,3], FERNANDEZ-COSTA Juan Manuel[1,2,3], SABATER-ARCIS Maria[1,2,3], CHAKRABORTY Mouli[1,2,3], LLAMUSI Beatriz[1,2,3]

1. Translational Genomics Group, Incliva Health Research Institute, Valencia, Spain
2. Department of Genetics and Interdisciplinary Research Structure for Biotechnology and Biomedicine (ERI BIOTECMED), University of Valencia, Valencia, Spain
3. CIPF-INCLIVA joint unit

Introduction: Myotonic Dystrophy type 1 (DM1) originates from alleles of the DMPK gene with hundreds of CTG repeats in the 3′UTR. CUG repeat RNAs accumulate in foci that sequester Muscleblind-like (MBNL) proteins away from their functional target transcripts. Thus endogenous upregulation of MBNL proteins is a potential therapeutic approach to DM1. Using Drosophila model, we have studied endogenous regulation of Mbl (fly orthologue) by microRNAs which regulate protein synthesis.

Methods: Based on unpublished data, orthology with human miRNAs, and TargetScan predictions we selected a group of candidate miRNAs for Mbl regulation. miRNA sponge constructs were expressed in muscle of DM1 model flies to test the effect of the specific miRNA inhibition on the Mbl expression and on DM1-like phenotypes.

Results: Here we identified two miRNAs, dme-miR-277 and dme-miR-304, that differentially regulate Mbl RNA isoforms in miRNA sensor constructs. We also showed their sequestration by sponge constructs derepressing endogenous Mbl not only in a wild type background but also in a DM1 Drosophila model expressing non-coding CUG repeats throughout the musculature. Enhanced Mbl expression resulted in significant rescue of pathological phenotypes, including reversal of several mis-splicing events and reduced muscle atrophy in DM1 adult flies. Flies had also improved muscle function in climbing and flight assays, and had longer lifespan compared to disease controls. Given the success with the Drosophila model, we are currently extending these observations to mammalian DM1 experimental systems.

Discussion: Our data support a relevant role of miRNAs in Mbl regulation and provide proof of concept for a similar potentially therapeutic approach to DM1 in humans.

Grant Support: Funded with grants SAF2012-36854 and SAF2015-64500-R, awarded to R.A. by the Ministerio de Economia y Competitividad (including funds from the ERDF). MC was the recipient of Santiago Grisolía award.

S2-02

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Reducing Toxic RNA Repeats in Myotonic Dystrophy Models

JENQUIN Jana[1], DANNEHOWER Kacie[1], COONROD Leslie[2], NAKAMORI Masayuki[3], BERGLUND J Andrew[1]

1. University of Florida Center for NeuroGenetics and Department of Biochemistry & Molecular Biology, Gainesville, FL
2. University of Oregon Institute of Molecular Biology, Eugene, OR
3. Osaka University, Osaka, Japan

Introduction: Myotonic Dystrophy type 1 (DM1) and type 2 (DM2) are neuromuscular diseases caused by microsatellite expansions in non-coding regions of the genome giving rise to RNAs having a toxic gain of function. The toxic RNAs formed aggregate into nuclear foci that sequester a class of RNA-binding proteins called muscleblind-like proteins (MBNLs). Sequestration prevent MBNLs from performing important roles in RNA processing, which ultimately cause DM disease symptoms. Our approach is to specifically reduce or eliminate transcription from these repeat expansions, thereby alleviating the downstream deleterious effects of the toxic RNA.

Methods: These data were generated using HeLa cell models, patient-derived myoblasts, and the HSALR DM1 mouse model. RT-PCR and RNAseq were used for splicing and transcript expression analyses, FISH for nuclear foci visualization, and western blots for protein expression.

Results: Our previous work demonstrated that ActD decreased CUG RNA levels in a dose-dependent manner in DM1 models. In a DM1 mouse model, ActD significantly reversed mis-splicing and did not globally inhibit transcription. We have also demonstrated that heptamidine, a pentamidine analog, was able to partially reverse mis-splicing in multiple DM1 models. Here we demonstrate that furamidine, another pentamidine analog, reverses mis-splicing with equal efficacy and reduced toxicity compared to ActD and heptamidine and RNAseq analyses shows that furamidine has the fewest off-target effects in a DM1 mouse model. Also, DB1242, another pentamidine analog, effectively reverses mis-splicing in a DM1 HeLa cell model. Both furamidine and DB1242 rescued mis-splicing in a DM2 HeLa cell model suggesting molecules can target both CUG and CCUG repeats.

Discussion: We will discuss multiple mechanisms through which we propose these molecules are working to rescue mis-splicing. Our results in DM1 and DM2 models indicate that transcription inhibition could be a viable treatment approach across many GC-rich expansion diseases.

Grant Support: NSF Graduate Research Fellowship (JJ) and Wyck Foundation/Myotonic Dystrophy Foundation grant.

S2-03

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Reducing Toxic RNA Repeats in Myotonic Dystrophy Models

JENQUIN Jana[1], DANNEHOWER Kacie[1], COONROD Leslie[2], NAKAMORI Masayuki[3], BERGLUND J Andrew[1]

1. University of Florida Center for NeuroGenetics and Department of Biochemistry & Molecular Biology, Gainesville, FL
2. University of Oregon Institute of Molecular Biology, Eugene, OR
3. Osaka University, Osaka, Japan

Introduction: Myotonic Dystrophy type 1 (DM1) and type 2 (DM2) are neuromuscular diseases caused by microsatellite expansions in non-coding regions of the genome giving rise to RNAs having a toxic gain of function. The toxic RNAs formed aggregate into nuclear foci that sequester a class of RNA-binding proteins called muscleblind-like proteins (MBNLs). Sequestration prevent MBNLs from performing important roles in RNA processing, which ultimately cause DM disease symptoms. Our approach is to specifically reduce or eliminate transcription from these repeat expansions, thereby alleviating the downstream deleterious effects of the toxic RNA.

Methods: These data were generated using HeLa cell models, patient-derived myoblasts, and the HSALR DM1 mouse model. RT-PCR and RNAseq were used for splicing and transcript expression analyses, FISH for nuclear foci visualization, and western blots for protein expression.

Results: Our previous work demonstrated that ActD decreased CUG RNA levels in a dose-dependent manner in DM1 models. In a DM1 mouse model, ActD significantly reversed mis-splicing and did not globally inhibit transcription. We have also demonstrated that heptamidine, a pentamidine analog, was able to partially reverse mis-splicing in multiple DM1 models. Here we demonstrate that furamidine, another pentamidine analog, reverses mis-splicing with equal efficacy and reduced toxicity compared to ActD and heptamidine and RNAseq analyses shows that furamidine has the fewest off-target effects in a DM1 mouse model. Also, DB1242, another pentamidine analog, effectively reverses mis-splicing in a DM1 HeLa cell model. Both furamidine and DB1242 rescued mis-splicing in a DM2 HeLa cell model suggesting molecules can target both CUG and CCUG repeats.

Discussion: We will discuss multiple mechanisms through which we propose these molecules are working to rescue mis-splicing. Our results in DM1 and DM2 models indicate that transcription inhibition could be a viable treatment approach across many GC-rich expansion diseases.

Grant Support: NSF Graduate Research Fellowship (JJ) and Wyck Foundation/Myotonic Dystrophy Foundation grant.

S2-03

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Identification of a Novel Kinase Target in DM Pathophysiology

KETLEY Ami[1], GHIDELLI-DISSE Sonja[2], BAMBOROUGH Paul[3], GHOSH Tushar[1], SEDEHIZADEH Saam[1], POWALOWSKA Paulina[1], RICHARDSON Linda[4], TANG Zhenzhi[4], LEISTMAN Sarah[4], BOSCHE Markus[2], BANTSCHEFF Marcus[2], RUDIGER Martin[6], MOSSAKOWSKA Danuta[7], DREWRY David[8], ZUERCHER William[8], DREWES Gerard[2], UINGS Iain[7], THORNTON Charles[4], HAYES Christopher[5], BROOK David[1]

1. School of Life Sciences, University of Nottingham,United Kingdom
2. Cellzome GmbH, Molecular Discovery Research,GlaxoSmithKline, Heidelberg, Germany
3. Computational and Modelling Sciences, GlaxoSmithKline, United Kingdom
4. University of Rochester Medical Center, Rochester, USA
5. School of Chemistry,University of Nottingham, United Kingdom
6. Screening Profiling and Mechanistic Biology, GlaxoSmithKline, United Kingdom
7. Discovery Partnerships with Academia, GlaxoSmithKline, United Kingdom
8. Department of Chemical Biology, GlaxoSmithKline, North Carolina, USA

Introduction: Myotonic Dystrophy type 1 (DM1), is an RNA-based disease caused by a transcribed CTG-repeat expansion within the 3' UTR of the DMPK gene. Mutant repeat expansion transcripts remain in the nucleus of patient's cells, forming foci that contribute substantially to the pathophysiology of the condition. Previously we developed a high throughput screening assay based around this feature and identified that kinase inhibitors were successful in removing nuclear foci and lead to improvements in key disease features. We now report the kinase target responsible.

Methods: In this work we utilised our high content imaging screen to assess a large, well characterised kinase inhibitor library for compounds that reduce nuclear foci to identify their common targets. A combination of chemoproteomics and mass spectrometry revealed a novel kinase target in DM pathophysiology. This kinase was analysed by western blot analysis and by detailed expression analysis in patient derived cells and biopsy samples. A compound inhibitor of this kinase was tested in the HSALR mouse model to assess phenotypic effects.

Results: Here we report a novel kinase as a key target in DM cell biology. This kinase protein is elevated in DM1 cell lines and patient muscle biopsies. Inhibition leads to the dissolution of nuclear foci and degradation of repeat expansion transcripts. Inhibitor treatment in the HSALR mouse led to beneficial phenotypic effects on myotonia and improvement of key splice isoforms.

Discussion: Our methods of target deconvolution have successfully led to the identification of a novel kinase associated with DM. The identification of this protein gives new insight into DM cell biology. Our methods validate the use of phenotypic screening in drug discovery and highlight a collection of molecules suitable for further development in the therapeutic effort in DM research. Some of the inhibitors identified are currently the subject of clinical trials for other indications and provide valuable starting points for a novel drug development programme in DM1.

Grant Support: University of Nottingham Hermes Fellowship; Myotonic Dystrophy Support Group; Marigold Foundation

S2-04

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Rbfox2 Fetal Isoform Overexpression Drives Arrhythmogenic Effects in Myotonic Dystrophy Type 1 Hearts

MISRA Chaitali[1], BANGRU Sushant[1], PARKER Darren J[1], HEDHLI Jamila[2], COOPER Thomas A[4], DOBRUCKI Wawrzyniec L[2], KALSOTRA Auinash[1,3]

1. Department of Biochemistry, University of Illinois, Urbana-Champaign, IL
2. Department of Bioengineering, University of Illinois, Urbana-Champaign, IL
3. Carl R. Woese Institute for Genomic Biology, University of Illinois, Urbana-Champaign, IL
4. Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX

Introduction: Myotonic Dystrophy type 1 (DM1) is a dominantly inherited disease and the second most common form of adult onset muscular dystrophy. Although skeletal muscle wasting are the major symptoms, cardiac dysfunctions are the second leading cause of death in DM1, which often include conduction defects and arrhythmias. DM1 is caused by mutation in Dystrophia myotonia protein kinase (DMPK) gene, with expanded CUG trinucleotides tandem repeat (CUGexp) at the 3' UTR. The accumulation of CUGexp RNA forms nuclear foci and causes trans-dominant toxic effects including i) disrupted functions of RNA binding proteins, MBNL1 and CELF1 (ii) interruption of Mef2 transcription network and aberrant levels of miRNA; and (iii) several other transcriptional and post-transcriptional perturbations. However, the exact mechanisms by which CUGexp RNA causes electrophysiological and contractility abnormalities in DM1 cardiomyocytes are still unknown.

Methods:

Results: We have discovered that steady-state protein levels of the RNA-binding protein Fox2 (RBFOX2), a critical splicing regulator for striated muscle, are drastically upregulated in DM1 heart tissues, which is accompanied by simultaneous skipping of a developmentally regulated 43bp muscle-specific exon in the Rbfox2 transcript. This results in selective upregulation of a fetal RBFOX2 splice isoform (RBFOX2′43) in adult cardiomyocytes. Remarkably, phenotypic studies with transgenic mice overexpressing RBFOX2′43 isoform specifically in cardiomyocytes identified conduction abnormalities and arrhythmias that are consistent with DM1 pathology. RNA Sequencing identifies RBFOX2Δ43 driven splicing and mRNA abundance defects in genes that are commonly misregulated in DM1. These sets of genes include the assembly of the ECC apparatus, ion channel intrinsic function, or key adaptor or cytoskeletal proteins thereby explaining the cardiac abnormalities seen in DM1.

Discussion: Collectively, we have uncovered a novel role of RBFOX2 in DM1 cardiac pathogenesis and identified the molecular mechanisms responsible for its splice isoform switching in DM1 heart.

Grant Support: NIH, March of Dimes, AHA, Myotonic Dystrophy Foundation.

S2-05

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Phenotype-genotype/Epigenotype Correlation and Aberrant Inflammatory Signaling in Congenital Myotonic Dystrophy

NAKAMORI Masayuki[1], HAMANAKA Kohei[2], THOMAS James[3], WANG Eric[3], TAKAHASHI Masanori[1], SWANSON Maurice[3], NISHINO Ichizo[2], MOCHIZUKI Hideki[1]

1. Department of Neurology, Osaka University Graduate School of Medicine
2. Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry
3. Department of Molecular Genetics and Microbiology, University of Florida, College of Medicine

Introduction: Myotonic dystrophy types 1 (DM1) and 2 (DM2) are dominantly-inherited neuromuscular disorders caused by expanded CTG and CCTG repeats in DMPK and CNBP genes, respectively. Both disorders are clinically similar, with toxic gain-of-function phenotype of expanded repeat RNA. Conversely, congenital myotonic dystrophy (CDM), a severe DM form, is only found in DM1. CDM is also characterized by large repeat expansion, highly methylated CpGs upstream of repeats, and muscle fiber immaturity not observed in adult DM, suggesting specific pathological mechanisms.

Methods: To investigate the specific mechanisms responsible for CDM, we studied muscles from 10 CDM (age less than 18 months) patients and four age-matched disease controls. We analyzed the size of the expanded repeats and evaluated pathological findings in muscles from CDM. We examined gene expression profiles and CpG methylation status by RNA-seq and methylation sequencing, respectively. We also studied splicing patterns of disease-associated transcripts and sense/antisense transcription at the DMPK locus by RT-PCR.

Results: We revealed a correlation of muscle immaturity with expanded repeat length and CpG methylation status in CDM muscles. Aberrant CpG methylation was associated with transcriptional dysregulation at the repeat locus in both directions, thereby, increasing toxic RNA burden. Additionally, enhanced RNA toxicity upregulated an inflammatory signaling pathway related to myocyte differentiation and the expression of the pathway genes was correlated with CDM muscle immaturity.

Discussion: We propose that aberrant CpG methylation, associated with highly expanded repeats, enhances RNA toxicity and upregulates the inflammatory signaling pathway, resulting in severe CDM phenotypes.

Grant Support: This work was supported by grants from Japan Society for the Promotion of Science (25713034, 15K15339, and 16H05321 to M.N.), and the NIH ((NS058901 and NS098819 to M.S.S.).

S2-06

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Mis-regulation and Mis-splicing of a Conserved Myogenic lncRNA in Human and Mouse DM1 Skeletal Muscle

QUERIDO Emmanuelle[1], BURGOCI Vasile[1], DANIK Marc[1], CHARTRAND Pascal[1]

1. Department of Biochemistry and Molecular Medicine, Université de Montréal, Montréal, QC, Canada

Introduction: Long noncoding RNAs (lncRNAs) are emerging as novel and important regulators during the development of metazoans. Some lncRNAs act as mediators of chromatin modifiers and control the epigenetic states of several loci. LncRNAs expressed from the imprinted Dlk1-Dio3 cluster are involved in the regulation of muscle development. The Meg3 lncRNA from this cluster interacts with the PRC2 complex and controls the epigenetic state of several genes, including the Dlk1-Dio3 cluster itself. While recent evidence suggests a deregulation of specific lncRNAs in Duchenne and Facioscapulohumeral muscular dystrophies, the role of lncRNAs in the pathogenesis of DM1 is still unclear.

Methods: We used RNA seq and quantitative RT-PCR to measure the expression level of non-coding RNAs from skeletal muscles of DM1 patients and HSALR model mice. Alternative splicing of Meg3 lncRNA was assessed by RT-PCR. Inhibition of HSA-CUG200 transgene expression in HSALR mice was performed by intraperitoneal injection of furamidine.

Results: RNA seq and RT-qPCR revealed that lncRNAs from the Dlk1-Dio3 cluster are overexpressed in the skeletal muscles of DM1 patients and HSALR mice. Analysis of the alternative splicing profile of Meg3 revealed a novel splicing pattern in HSALR mice compared to FVB. Interestingly, the same splicing profile is observed in Mbnl1 KO mouse, and Meg3 is overexpressed in Mbnl1 KO mice, suggesting a role for Mbnl1 in regulating Meg3 lncRNA. Treatment of HSALR mice with furamidine, an inhibitor of HSA-CUG200 transgene expression, restored the expression of Meg3 lncRNA to typical levels, suggesting that mis-expression of Meg3 is linked to the expression of the CUG-rich transgene and not an indirect consequence of the myopathy.

Discussion: Pro-myogenic lncRNAs from the imprinted Dlk1-Dio3 cluster are mis-regulated and mis-spliced in DM1.

Grant Support: This work was supported by grants from the Canadian Institutes of Health Research and Muscular Dystrophy Canada.

S2-07

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Disrupted Prenatal RNA Processing and Myogenesis in Congenital Myotonic Dystrophy

THOMAS James[1], SZNAJDER Łukasz[1], BARDHI Olgert[1], ASLAM Faaiq[1], ANASTASIADIS Zacharias[1], SCOTTI Marina[1], NISHINO Ichizo[2], NAKAMORI Masayuki[3], WANG Eric[1], SWANSON Maurice[1]

1. Molecular Genetics & Microbiology, University of Florida, Gainesville, FL
2. Department of Neuromuscular Research, Osaka University, Osaka, Japan
3. Department of Neurology, Osaka University, Osaka, Japan

Introduction: Myotonic dystrophy type 1 (DM1) is an adult-onset, CTG microsatellite expansion (CTG^{exp}) disorder where expressed CUG^{exp} RNAs functionally inactivate MBNL proteins and result in the re-emergence of developmentally immature RNA and protein isoforms in adult muscle. While this pathogenesis model accounts for adult-onset myopathy, the molecular basis of congenital DM (CDM) is unknown. Here, we test the hypothesis that disrupted RNA alternative processing results in pre/perinatal myopathy characteristic of CDM.

Methods: Using RNA- and PolyA-seq, we profile the CDM muscle transcriptome and generate a molecular signature of disease for use as a benchmark in animal model studies.

Results: We identify prominent RNA mis-processing in CDM, and through a comparison to DM1 muscle transcriptome data, we provide evidence that CDM is a severe, DM1-like RNA mis-processing disorder that mostly differs in age-of-onset. This model is supported by transcriptome analysis of muscle development where we find evidence of CDM-relevant, MBNL-regulated exons undergoing prenatal RNA isoform transitions that are largely completed by birth. Therefore, we expect these splicing events are susceptible to CUG^{exp}-associated mechanisms *in utero*. We test this possibility, and the contribution of MBNL proteins to CDM disease, using *Mbnl* double and triple muscle-specific knockout mice and find MBNL loss-of-function results in frequent perinatal lethality, respiratory distress, reduced body weight and failure to thrive as well as muscle histopathology, congenital spliceopathy, and gene expression abnormalities characteristic of CDM.

Discussion: Together, these findings support MBNL-responsive RNA mis-processing as a major contributor to CDM disease and establish testable mouse models to further explore the role of co/post-transcriptional gene regulation during development.

Grant Support: NS058901, NS98819 to M.S.S; OD017865 to E.T.W.; KAKENHI 15K15339 and 16H05321 to M.N; MDF and Wyck Foundation/Myontonic Dystrophy Foundation fellowship to Ł.J.S.

S2-08

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Loss of Zinc Finger Protein 9 Encoded by a Myotonic Dystrophy Type 2 Gene Causes Muscle atrophy

WEI Christina[1], TIMCHENKO Nikolai[2], **TIMCHENKO Lubov**[2]

1. Department of Surgery, Cincinnati Children's Hospital, Ohio
2. Department of Neurology Cincinnati Children's Hospital, Ohio

Introduction: Myotonic Dystrophy type 2 (DM2) is a neuromuscular disease caused by an expansion of intronic CCTG repeats in the ZNF9 gene. ZNF9 protein binds to DNA and RNA; however, its biological role is not well understood. Examination of ZNF9 in DM2 biopsied muscle showed that some patients have a reduction of ZNF9; however, some patients have no changes of ZNF9. To determine if a reduction of ZNF9 affects skeletal muscle, we examined the phenotype of the mouse model with deletion of Znf9.

Methods: Mouse model with disrupted Znf9 gene was generated and skeletal muscle phenotype was analyzed by a variety of assays including histochemical, physiological and molecular approaches. Immunohistochemical analysis was used to examine ZNF9 expression in human muscle sections from patients with DM2.

Results: We found that ZNF9 is critical for the maintenance of muscle sarcomeric organization during postnatal period. A loss of ZNF9 causes muscle atrophy, characterized by the reduced muscle weight, small-sized and thin myofibers and the grip weakness. Myofibers in heterozygous Znf9 KO mice vary in size and the myofiber size variability is worsening with age. Homozygous Znf9 KO mice develop muscle wasting at young age; whereas only old heterozygous Znf9 KO mice develop significant muscle atrophy. Proteomic analysis of skeletal muscle from Znf9 KO mice showed that the loss of ZNF9 causes a misregulation of muscle contractile proteins and proteins, maintaining global translation. We also confirmed our previous findings that ZNF9 is reduced in cytoplasm of human DM2 myofibers.

Discussion: The detailed study of Znf9 KO mice revealed that ZNF9 is required for the development and organization of myofibers. ZNF9 plays a role in the control of proteins of muscle contractile apparatus and proteins regulating global translation. A development of muscle atrophy in old heterozygous Znf9 KO mice shows that a cytoplasmic reduction of ZNF9 in patients with DM2 might lead to the age-dependent muscle wasting and weakness.

Grant Support: NIH, NIAMS to LTT

S2-9

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Maturation and Nuclear Retention of Single DMPK Transcripts

GUDDE Anke[1], GONZÁLEZ-BARRIGA Anchel[1], VAN KESSEL Ingeborg[1], ANDRÉ Laurène[1], WIERINGA Bé[1], **WANSINK Derick**[1]

1. Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

Introduction: Dominant in the complex pathological cascade in myotonic dystrophy type 1 (DM1) is the expression of expanded DMPK transcripts. Having an accurate picture of DMPK mRNA expression will contribute to understanding DM1 pathology and successful RNA-directed therapy. We therefore studied synthesis, processing and nuclear residence of DMPK transcripts in muscle.

Methods: We used myoblast cultures and skeletal muscle samples from DM1 patients and unaffected controls in our study. Through cell fractionation, RNA size separation and RNA FISH we were able to study RNA processing of cytoplasmic versus nuclear, and normal versus expanded DMPK transcripts.

Results: Similar splice modes were identified in normal and expanded DMPK transcripts indicating that triplet repeat length does not affect splicing in cis. We confirmed that 90% of expanded DMPK transcripts reside in the nucleus, but discovered that also 30% of normal DMPK transcripts are nuclear, a much higher proportion than for most mRNAs, like those from housekeeping genes ACTB and GAPDH. Despite the different distribution patterns, normal and expanded DMPK mRNAs contained equal and very long poly(A) tails of up to 500 nucleotides. Precise quantification of expression level pointed out that expanded DMPK mRNAs occur between one and at most a few dozen molecules per cell. Since this RNA copy number essentially equals the number of nuclear foci detected by RNA FISH, each focus typically reflects presence and location of a single expanded DMPK transcript.

Discussion: Our findings refine the RNA toxicity model: (i) Repeat expansion affects nuclear residence time of DMPK mRNAs, but not through effects on alternative splicing or poly(A) tail length. (ii) Foci are single RNA molecules, which implicates that each expanded DMPK transcript alone is able to sequester MBNL protein and trigger abnormal RNP complex formation.

Grant Support: This work was supported by the Prinses Beatrix Spierfonds in combination with the Stichting Spieren voor Spieren (grant number W.OR10–04).

S2-10

Session 2: Disease Mechanisms: DM1, DM2 and CDM

RAN Translation Regulated by Muscleblind Proteins in Myotonic Dystrophy Type 2

ZU Tao[1,2], CLEARY John D[1,2], LIU Yuanjing[1,2], BAÑEZ-CORONEL Monica[1,2], BUBENIK Jodi L[1,2], AYHAN Fatma[1,2], ASHIZAWA Tetsuo[7], XIA Guangbin[1,3,6], CLARK H. Brent[8], YACHNIS Anthony T[4], SWANSON Maurice S[1,2,5], RANUM Laura PW[1,2,3,5,6]

1. Center for NeuroGenetics, University of Florida, Gainesville, FL
2. Department of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
3. Department of Neurology, University of Florida, Gainesville, FL
4. Department of Pathology, Immunology and Laboratory Medicine, College of Medicine, University of Florida, Gainesville, FL
5. Genetics Institute, University of Florida, Gainesville, FL
6. McKnight Brain Institute, University of Florida, Gainesville, FL
7. Neurological Institute, Houston Methodist Hospital, Houston, TX 77030
8. Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN

Introduction: Myotonic dystrophy types 1 and 2 are caused by CTG and CCTG expansion mutations located in the non-coding regions of *DMPK* or *CNBP*, respectively, and are thought to be caused by RNA gain of function effects in which the CUG or CCUG expansion transcripts dysregulate RNA binding proteins.

Methods: RAN translation is examined in cell culture model and human autopsy brains.

Results: We show that the DM2 CCTG expansion mutation is bidirectionally transcribed and that both CUGG and CAGG expansion RNAs undergo repeat associated non-ATG (RAN) translation, resulting in the expression of tetra-peptide expansion proteins from all three reading frames with poly-(LPAC) or poly-(QAGR) repeat motifs, respectively. We demonstrate that these RAN proteins accumulate in human DM2 autopsy brains in distinct patterns. LPAC staining varies substantially between brain regions and is primarily found in neurons, astrocytes and glia in grey matter regions of the brain, including frontal cortex, hippocampus and basal ganglia. In contrast, antisense QAGR RAN proteins accumulate within white matter in DM2 brains and sites of QAGR RAN protein accumulation correlated with white matter abnormalities. Codon replacement experiments show that LPAC and QAGR proteins are toxic to cells independent of RNA gain of function effects. Finally, we show that nuclear sequestration of CCUG transcripts by MBNL proteins, prevents the expression of LPAC but not QAGR RAN proteins.

Discussion: These data demonstrate that novel LPAC and QAGR tetrapeptide RAN proteins accumulate in DM2 autopsy brains. Additionally, we show nuclear sequestration of CCUG RNAs by MBNL proteins decrease steady-state levels of LPAC suggesting a two-phase model of disease, initially involving nuclear retention of expansion RNAs and RBP depletion and a later phase in which cytoplasmic expansion RNAs undergo RAN translation and exacerbate disease.

Grant Support: NIH, MDA, Myotonic Dystrophy Foundation & Marigold Foundation

S3-01

Session 3: Cancer and Aging in DM

Benign and Malignant Tumors in Patients Diagnosed with Myotonic Dystrophy Type 1

ALSAGGAF Rotana[1,2], ST. GEORGE Diane Marie M[2], PFEIFFER Ruth M[1], WANG Youjin[1], ZHAN Min[2], WAGNER Kathryn R[3,4], GREENE Mark H[1], AMR Sania[2], GADALLA Shahinaz M[1]

1. Division of Cancer Epidemiology and Genetics, National Cancer Institute,Bethesda, MD, USA
2. Department of Epidemiology and Public Health, University of Maryland, Baltimore,MD, USA
3. Hugo W. Moser Research Institute at Kennedy Krieger Institute, Baltimore, MD 21205, USA
4. Departments of Neurology and Neuroscience, Johns Hopkins University School of Medicine, Baltimore, MD, USA

Introduction: Multiple case reports have suggested that Myotonic Dystrophy type 1 (DM1) patients may be at elevated risk of various benign and malignant tumors. Recent epidemiological studies provided evidence that brain, ovarian, endometrial, colorectal, and thyroid cancers may be part of the DM phenotype, but this literature remains scanty, and systematic evaluation of the risk of benign tumors has not been performed.

Methods: Utilizing the UK Clinical Practice Research Datalink (CPRD), a large primary care database, we identified 999 DM1-affected and 14,849 DM1-free individuals from 1988-2016, matched on age, gender, clinical practice, and registration year. We used Cox regression models to calculate organ-specific hazard ratios (HRs) for benign and malignant tumors. The baseline hazard was stratified on the matched sets and models were adjusted for average number of doctor visits per year. For tumors that only appeared in DM1 patients, we used Fisher's exact test to assess associations.

Results: Compared to DM-free individuals, DM1 patients were at significantly higher risk of thyroid cancer (HR=20, 95%CI=3.4-117.7), cutaneous melanoma (HR=3.6, 95%CI=1.0-13.1), uterine fibroids and polyps (HR=3.2, 95%CI=1.4-7.0), and colorectal polyps (HR=3.0, 95%CI=1.2-7.7). Pilomatricoma and benign tumors of the salivary glands were found only in DM1 patients, with corresponding Fisher's exact p-values <0.001. Our data also suggested, albeit not statistically significant, elevated risks of cancers arising in the brain (HR=2.7, 95%CI=0.6-12.9), pancreas (HR=2.3, 95%CI=0.5-10.5), and ovary (R=2.0, 95%CI=0.4-9.9).

Discussion: Our results suggest that DM1 tumorigenesis affects the skin, female genital organs, endocrine system, and possibly the digestive system. These findings may guide clinical management and scientific planning for investigating the molecular mechanisms underlying tumorigenesis in DM.

Grant Support: Intramural Research Program of the National Cancer Institute, USA.

S3-02

Session 3: Cancer and Aging in DM

Organ-specific Risk of Cancer in Patients with Myotonic Dystrophy: Results from a Meta-analysis

EMPARANZA Jose I[1], LÓPEZ DE MUNAIN Adolfo[2,3,4], GREENE Mark H[5], MATHEU Ander[6], FERNÁNDEZ-TORRÓN Roberto[2,3,4], **GADALLA Shahinaz M**[5]

1. Department of Clinical Epidemiology, University of the Basque Country, San Sebastian, Spain
2. Department of Neurology. Hospital Universitario Donostia
3. Neuroscience Area. Instituto Biodonostia, University of the Basque Country, San Sebastian, Spain
4. CIBERNED (Institute Carlos III, Ministry of Economy and Competitiveness, Madrid, Spain
5. Clinical Genetics Branch, National Cancer Institute, Bethesda, USA
6. Oncology Area. Institute Biodonostia, San Sebastián, Spain

Introduction: There is recent accumulating evidence that patients with myotonic dystrophy (DM) are at excess risk of developing cancers. However, inconsistencies existed regarding affected anatomic sites.

Methods: Between January 1, 2011 and December 31, 2016, we identified five cohort studies (3 population-based, and 2 hospital-based) evaluating the risk of cancer in patients with DM. We conducted a meta-analysis and calculated the pooled standardized incidence ratio (SIR) and 95% confidence intervals (CI) for cancer sites that were reported in at least 3 studies using a random effects model. The between-study heterogeneity was assessed by I2 statistic and was considered significant when I2 > 50%.

Results: Patients with DM were at approximately two-fold excess risk of all cancers combined (N=3 studies, SIR=1.89, 95% CI= 1.6 - 2.2, p<0.001). The pooled estimated risk was high for cancers of the endometrium (N =5 studies, SIR=7.5, 95% CI=4.7-11.8, p<0.001), thyroid (N=4, SIR=8.5, 95% CI=3.6-20.1, p<0.0001), ovary (N=4, SIR=5.6, 95% CI=3.0-10.3, p<0.001), brain (N=3, SIR=6.2, 95% CI=3.5-11.1, p<0.001), colorectum (N=4, SIR=2.2, 95% CI=1.4-3.5, p=0.001), testis (N=4, SIR=5.9, 95% CI=2.3-15.1, p<0.001), cutaneous melanoma (N=5, SIR=2.5, 95% CI=1.3-4.6, p=0.005), and non-hodgkin lymphoma (N=3, SIR=2.7, 95% CI=1.3-5.8, p=0.007). DM patients were not at excess risk of developing cancers of the breast (N=3, SIR=1.1, 95% CI=0.69-1.8, p=0.66), prostate (N=4, SIR=1.2, 95% CI=0.6-2.5, p=0.64), lung (N=4, SIR=1.7, 95% CI=0.9-3.4, p=0.11), kidney (N=3, SIR=2.1, 95% CI=0.8-5.3, p=0.1), or leukemia (N=3, SIR=2.4, 95% CI=0.7-8.0, p=0.16).

Discussion: This meta-analysis identified the cancer profile in patients with DM. Such information may guide patient clinical management and plans for molecular investigations toward understanding the mechanism behind DM-carcinogenesis.

Grant Support: Intramural research program, National Cancer Institute, US

S4-01

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Factors Associated with Health-related Quality of Life in Children with Congenital Myotonic Dystrophy

ROGERS Stephanie[1], ELALOUL Basmah[1], CEBALLOS Delia[1], HICKS Rhiannon[1], ZAPATA Eugenio[1], BERGGREN Kiera[2], BAX Karen[1], DIBELLA Deanna[2], PUCILLO Evan[2], JOHNSON Nicholas[2], **CAMPBELL Craig**[1]

1. Western University, London, Ontario
2. University of Utah, Salt Lake City, Utah

Introduction: Congenital myotonic dystrophy (CDM) is a genetic neuromuscular disease that significantly affects health-related quality of life (HRQoL). However, the relationship between disease manifestations in CDM and HRQoL has not been well-characterized. This study aims to evaluate the relationship between HRQoL and different disease factors in CDM.

Methods: Children with CDM aged 0-13 years were enrolled at two sites. PedsQL™ Generic Core Scales and Neuromuscular Module Parent Proxy-Reports were used to measure HRQoL. Neuropsychological function, physical capacity, and orofacial and grip strength were assessed. CTG repeats and comorbidities were used to measure disease severity. Correlations between disease factors and HRQoL were computed with the Spearman correlation coefficient or the Kruskal-Wallis test in Stata 13.0.

Results: 48 patients with CDM were enrolled (24 females, 24 males). Greater daytime sleepiness was associated with poor HRQoL determined by generic (′=-0.41, P=0.007) and neuromuscular (′=-0.40, P=0.01) measures. Higher adaptive functioning was associated with better HRQoL determined by the neuromuscular module: communication (′=0.39, P=0.03), daily living skills (′=0.54, P=0.002), and socialization (′=0.52, P=0.005). Higher physical capacity was associated with better physical (′=0.37, P=0.04) but not overall HRQoL. Stronger grip strength correlated with better HRQoL, determined by the neuromuscular measure (′ =0.44, P=0.02). An increased number of comorbidities was associated with poor overall HRQoL determined by generic (′=-0.40, P=0.009) and neuromuscular (′=-0.32, P=0.05) measures.

Discussion: Several factors associated with HRQoL have been identified in children with CDM. Daytime sleepiness, adaptive functioning, physical capacity, grip strength, and comorbidities had the most pronounced effect on HRQoL. These may be promising factors to target in treatment plans.

Grant Support: NINDS; The Muscular Dystrophy Association; Valerion Therapeutics; Biogen; Utah Neuromuscular Research Fund

S4-02

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Do Research Publications Fit with DM1 Individuals' Expectations?

DE ANTONIO Marie[1], **DOGAN Celine**[1], LABRO Mathilde[1], HAMROUN Dalil[2], GEILLE Alain[3], BASSEZ Guillaume[1]

1. Myology Institute, Paris, France
2. CHU de Montpellier, Montpellier, France
3. DM patient group, AFM-Telethon, Evry, France

Introduction: In the past years, the scientific knowledge on DM1 clinical issues and disease progression greatly improved. In parallel, patients self-reported data, like patient foundation-driven initiatives using questionnaires, helped to address patients' needs and expectations. To explore the relevance of current DM1 research areas, we designed a study comparing research areas of interest observed in literature with (i) objective symptoms prevalence and (ii) patients' expectations.

Methods: We first conducted a review of the literature to identify DM1 symptoms and disease clinical domains most frequently studied by researchers. Then, the results were compared with (1) analysis of standardized data collection of a large cohort of DM1 adult patients (n=2469) included in the DM-Scope registry observational study; (2) assessment of DM1 individual's quality of life (n=190) using the InQoL questionnaire, and finally (3) DM1 individuals self-reported data collected during a nationwide AFM-Telethon association survey (n=1100).

Results: We show that scientific publications do not fully cover the most frequent symptoms experienced by DM1 patients, either objectively measured by clinicians or self-reported. Researchers mainly focus on disabilities and life-threatening conditions like cardiac and respiratory defects, muscular disability, and cognitive impairment. Other domains, including dysphagia, digestive tract dysfunction, pain and fatigue are underrepresented in literature with regard to the frequency of patient complaints.

Discussion: This study emphasises that the scientific publications subjects do not fully overlap with patient expectations. The results may help researchers to plan studies fitting with patient expectations.

Grant Support: AFM-Telethon.

S4-03

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Strength Evolution in Myotonic Dystrophy Type 1 Over a 9-year Period

GAGNON Cynthia[1,2], PETITCLERC Émilie[1,2], KIERKEGAARD Marie[3], MATHIEU Jean[1,2], DUCHESNE Élise[2,4], HÉBERT J Luc[5]

1. Faculté de médecine et sciences de la santé
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires
3. Karolinska University Hospital
4. Université du Québec à Chicoutimi
5. Université Laval, CIRRIS

Introduction: Muscle impairments, which include muscle weakness, is the cardinal feature of DM1. The pattern of muscle strength loss progression goes from distal to proximal with the late-onset phenotype being less impaired than the adult phenotype. There are few data on the progression of muscle weakness using quantitative muscle testing (QMT). The objectives of this study were to document the progression of muscle strength impairment in nine muscle groups among adults with DM1 over a 9-year period, and to compare this progression between phenotypes (adult and late-onset) and genders.

Methods: DM1 patients (N=110) with the adult of late-onset phenotypes were recruited from the registry of the Neuromuscular Clinic of the Saguenay-Lac-St-Jean (Québec, Canada). The maximum isometric muscle strength of nine muscle groups was assessed using a standardised protocol of QMT: neck flexors, shoulder abductors, elbow flexors and extensors, wrist extensors, hip flexors, knee flexors and extensors, and ankle dorsiflexors. The assessment was performed twice over a 9-year period by two trained physiotherapists.

Results: For the whole group of patients, a significant decline was seen over the 9-year period for all muscle groups, excepted for hip flexors. The mean strength loss varies from 24.5 to 56.0%. This decline was also associated to phenotype and gender. The rate of decline was similar for adult and late-onset phenotypes, but was faster for men than women. Men were stronger than women at baseline, which may explain the faster loss of strength for this group.

Discussion: This unique study supports results from previous cross-sectional studies. Also, important results were the similar decline between phenotypes, illustrating the need for interventions for all these patients.

Grant Support: The study was funded by the Canadian Institutes of Health Research (CIHR) (grant no. JNM-108412) and Muscular Dystrophy Canada.

S4-04

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Causes and Predictors of Early Mortality in a Large U.S. Myotonic Dystrophy Type 1 Adult Cohort

GROH William J[1], GROH Miriam R[2], BHAKTA Deepak[2]

1. Medical University of South Carolina, RH Johnson VAMC, Charleston, SC
2. Indiana University, Indianapolis, IN

Introduction: There is limited data on the causes and predictors of early mortality in patients (pts) with myotonic dystrophy type 1 (DM1) surviving to adulthood. The objectives of the work is to determine the epidemiology of early mortality in U.S. patients with DM1.

Methods: Analysis from a U.S. registry with clinically- and genetically-verified adult DM1 pts (age at entry≥18 yrs) enrolled at MDA clinics (1997-2005) and prospectively followed (study entry–N=406; age: 42±12 yrs; male: 205 (50.5%); CTG repeats: 629±386; muscular impairment rating score (MIRS): 3.2±1.0). Causes of death were adjudicated by death certificate and medical records review.

Results: Follow-up of death was complete through 30-June-2014 (11.9±4.7 yrs). 172 (42.4%) of pts died with a median Kaplan-Meyer (K-M) survival of 63.1 yrs. Early mortality was defined as age at death in the lowest K-M quartile (≤54.4 yrs, 80 pts, median age at death: 47.2 yrs; 12 <40 yrs). Causes of early mortality were respiratory failure (41, 51.2%), sudden unexpected possibly cardiac (26, 32.5%), non-sudden cardiac (1, 1.3%), non-sudden other (11, 13.8%, 4 cancer), and uncertain cause (1, 1.3%). Study entry characteristics associated with early mortality, using the 188 pts with survival to at least 54.4 yrs as a comparison group (96 alive, 92 dead), included age at symptom onset (per decile increase, RR 0.45; 95% CI 0.35–0.58, p<0.001), congenital or childhood DM1 onset (RR 7.6; 95% CI 2.8–20.2, p<0.001), MIRS (per 1-level increase, RR 1.5; 95% CI 1.2–2.0, p=0.002), and CTG repeat length (per 1-log increase, RR 13.4; 95% CI 5.0–35.7, p<0.001). Gender, cardiac diagnosis, and an abnormal electrocardiogram did not associate with early mortality.

Discussion: DM1 pts who survive to adulthood are at risk of early mortality. The most common causes of death are respiratory failure followed by cardiac issues, similar to DM1 pts dying at an older age. Factors associated with early mortality are those indicative of earlier disease onset, more severe muscle weakness, and more prolonged CTG repeat expansion.

Grant Support: Research grant from Biogen, Inc.

S4-05

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Development and Validation of a Disease-Specific Patient-Reported Outcome Measure for DM2: the Myotonic Dystrophy Type-2 Health Index (MD2HI)

HEATWOLE Chad[1], JOHNSON Nicholas[2], DEKDEBRUN Jeanne[1], DILEK Nuran[1], LUEBBE Elizabeth[1], MARTENS William[1], MCDERMOTT Michael[1,3], MOXLEY Richard III[1]

1. The University of Rochester Medical Center, Department of Neurology, Rochester, NY
2. The University of Utah, Salt Lake City, UT
3. The University of Rochester Medical Center, Department of Biostatistics and Computational Biology, Rochester, NY

Introduction: In preparation for clinical trials and to bolster existing clinical trial infrastructure for DM2, we examine the development and validity testing of a novel clinical trial instrument to measure DM2 disease burden.

Methods: We conducted in depth interviews with 15 patients to identify the issues of greatest importance to DM2 patients. We subsequently performed a cross-sectional study of 74 DM2 participants to identify the prevalence and relative importance of 310 individual symptoms identified by participants as being important to DM2 (The PRISM-2 Study). Using predetermined methods we selected items to include in the MD2HI based on overall relevance, predicted responsiveness to therapeutic intervention, specificity of language, and application to the greater DM2 population. Exploratory factor analysis was conducted to create and determine the internal consistency of DM2 subscales.

Results: We evaluated all 310 symptomatic questions included in the PRISM-2 study. Suboptimal symptomatic questions were removed based on low relevance, poor predicted responsiveness, ambiguous wording, and a lack of generalizability to the greater DM2 population. Factor analysis was used to group questions into DM2 subscales. These DM2 subscales measure: myotonia, pain, cognition, sleep, fatigue, ambulation, gastrointestinal issues, proximal leg, core, shoulder, and hand weakness, emotional issues, changes in body image, reductions in social satisfaction and performance, vision, swallowing function, specific activity participation, communication, hearing, and neck strength. The MD2HI is being further tested in a longitudinal study of DM2 patients conducted at the University of Rochester.

Discussion: Initial evaluation of the MD2HI provides evidence that it covers the wide variety of issues and symptoms that are most important to patients with DM2 and that it can potentially be used to estimate patient-reported disease burden during future DM2 clinical trials.

Grant Support: NIH (NIAMS, NINDS), The Nathan and Ruth Goldberg Memorial Fund.

S4-06

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Longitudinal Evaluation of Disease Progression in Congenital Myotonic Dystrophy

JOHNSON Nicholas E[1], DIBELLA Deanna[1], MCINTYRE Melissa[1], DIXON Melissa M[1], CROCKETT Rebecca[1], BOUNSANGA Jerry[2], HUNG Man[2], CAMPBELL Craig[3]

1. Department of Neurology, University of Utah
2. Department of Orthopedics, University of Utah
3. Department of Pediatrics, University of Western Ontario

Introduction: Congenital myotonic dystrophy (CDM) is the severe, infantile-onset form of myotonic dystrophy. Prior cross-sectional studies identified variable improvement in symptoms with age, in contrast to adults. This study provides longitudinal evaluation of symptoms in children with CDM.

Methods: Participants with CDM and healthy controls between the ages of 0-13 were enrolled in the study cohorts, stratified by age to ensure an even distribution of ages. Visits occurred over a 2-day period at baseline, 12, and 24 months. Each cohort received an age appropriate clinical evaluation. This includes neuropsychological testing, oral facial strength testing, strength and functional testing, DEXA, ECG, and quality of life measurements.

Results: 49 participants with CDM and 29 age matched healthy controls were enrolled. The mean decline over 12 months in the IQ was -7.2%. The mean change in the lip force was 11%, with participants under 10 years of age improving 17.2% and those older than 10 improving 0.8%. On the six minute walk, the mean improvement up to age 10 was 48.9%, then after age 10 it was 4.9%. The mean right grip strength increased 0.42 kgs (SD 1.87), or 7.3%. The total body mass increased 33.7% over 12 months. The PDSS, a measure of daytime sleepiness, improved 3.8% for those participants under 10 years old and declined -44.5% for those participants over age 10.

Discussion: This work supports improvement in some functional measures during childhood, and identifies a potential plateau in strength after the age of 10. These data identify potential clinical outcome assessments for use in therapeutic trials.

Grant Support: Research supported by the Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapeutics, Utah Neuromuscular Fund.

S5-01

Session 5: Specific Disease Features - CNS, Cardiac and GI

Changes in Brain Structure and Function in Adults with Myotonic Dystrophy Type 1: A 1-Year Longitudinal Study

DEVOLDER Ian[1], CROSS Stephen[1], JOHNSON Claire[1], GUTMANN Laurie[2], MAGNOTTA Vince[3], NOPOULOS Peggy[1]

1. University of Iowa, Psychiatry
2. University of Iowa, Neurology
3. University of Iowa, Radiology

Introduction: Neuroimaging studies have identified significant disruptions in white matter structure and function in the brains of individuals with myotonic dystrophy type 1 (DM1). However, little is known about disease progression within the central nervous system (CNS) in DM1. Longitudinal studies of brain structure and function will be critical in identifying potential biomarkers of disease progression within the CNS.

Methods: Using a combination of structural MRI and diffusior tensor imaging (DTI), we examined brain structure and function in a group of 17 individuals with adult-onset DM1 and 15 healthy controls (males and females, ages 30-63) at two time points, approximately one year apart. A mixed-effect ANOVA was used to examine the effect of DM1 on 1-year change in brain volumes and structure. Age, gender and intracranial volume (ICV) were controlled for. We also examined the relationship of disease duration (DD; years since onset of symptoms) to one-year change in brain structure and function using linear regression analyses.

Results: It was found that patients with DM1 showed significantly more decline in total white matter volumes after one year, particularly within the frontal lobe. Controls showed no change in white matter volumes after one year, while subjects with DM1 showed a decrease in white matter volumes. A similar pattern was found for volumes of the thalamus and DTI measures of white matter health. Diseased duration (DD) was highly predictive of 1-year change in white matter volumes. Those who have experienced the disease the longest showed the largest 1-year change in white matter and thalamus volumes.

Discussion: Results point to a progressive deterioration of white matter structure and function, which is measurable even within a 1-year time frame. These neuroimaging measures may provide valuable biomarkers of disease progression within the CNS for DM1.

Grant Support: This research is supported by grant number 5R01NS094387-02 from the NINDS and a Wyck Foundation/Myotonic Dystrophy Foundation Fellowship grant.

S5-02

Session 5: Specific Disease Features - CNS, Cardiac and GI

A Phenotypic Profile of Cognitive Function in Congenital Myotonic Dystrophy

DIXON Melissa M[1], OTTERUD Brith[1], BERGGREN Kiera N[1], CROCKETT Rebecca[1], ADAMS Heather[2], BOUNSANGA Jerry[3], HUNG Man[3], BAX Karen[4], CAMPBELL Craig[5], ZIELINSKI Brandon[6], JOHNSON Nicholas E[1]

1. Department of Neurology, University of Utah
2. Department of Neurology, University of Rochester
3. Department of Orthopedics, University of Utah
4. Department of Education, University of Western Ontario
5. Department of Pediatrics, University of Western Ontario
6. Department of Pediatrics, University of Utah

Introduction: Congenital Myotonic Dystrophy (CDM) is associated with impaired cognitive function. Previous studies have associated CDM with intellectual disability, autism, and brain abnormalities. This study establishes a cognitive phenotype for CDM that includes global IQ, executive function (EF), adaptive behavior (AB), autism traits, and brain morphology and function.

Methods: Participants with CDM (ages 0-13) were enrolled. IQ, EF, AB, and autism traits were measured. A subset of participants (ages 7-13) received brain MRI to establish baseline measures of structure and function in CDM.

Results: 51 participants with CDM were enrolled, and 13 participants took part in the MRI sub-study. Mean global IQ was 66.1 (sd=18.1). EF was identified by teacher and parent report. Teachers reported significant global executive dysfunction (x′=73 sd=13.6). Parents identified working memory impairment in EF (x′=68.6 sd=10.7). AB was significantly lower than established norms (x′=70 sd=16.2). Repetitive behavior was clinically elevated (x′=16.4 sd=14.7). Participants scored within normal limits for social communication and on an autism screening measure. Strong correlations were identified between global IQ and AB (r=.71 p=.001), communication AB and plan/organization (r=.76 p=.001), and repetitive behavior and social communication (r=.71 p=.00001). Correlations were also identified between IQ and communication AB (r=.63 p=.001), and AB and EF (working memory: r=.61 p=.028; plan/organization: r=.68 p=.011). MR imaging identified ventricular enlargement and white matter abnormalities.

Discussion: Cognitive function in CDM is characterized by extremely low IQ, global executive dysfunction, low AB, and increased repetitive behaviors. Social communication remains intact. Structural brain differences in CDM may be associated with cognitive function, and may be potential biomarkers of CDM.

Grant Support: Myotonic Dystrophy Foundation, Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapeutics, Utah Neuromuscular Fund.

S5-03

Session 5: Specific Disease Features - CNS, Cardiac and GI

A 34-year Prospective Study of Long-term Cardiac Outcome in DM1 Patients with Normal ECG at Baseline

FIONDA Laura[1], DE SANTIS Tiziana[1], VANOLI Fiammetta[1], BUCCI Elisabetta[1], LICCHELLI Loretta[1], MORINO Stefania[1], Di PASQUALE Antonella[1], Garibaldi Matteo[1], BOTTA Annalisa[2], PIGNATELLI Giulia[3], FRATTARI Alessandra[3], ATTALLA EL ALABIEB Nadia[3], GABRIELE Erica[3], TESTA Marco[3], ANTONINI Giovanni[1]

1. Department of Neuroscience, Mental Health and Sensory Organs, University of Rome "Sapienza"
2. Genetic Institute, University of Tor Vergata, Rome
3. Cardiologic Clinic, Sant'Andrea Hospital, Rome

Introduction: Cardiac involvement is a major determinant of survival in DM1. The evaluation of incidence and predictors of cardiac conduction/rhythm abnormalities (CCRA) is crucial in planning preventing strategies for cardiac sudden death (CSD).

Methods: Longitudinal FU study of 103 DM1 patients (56 M, 47 F), consecutively recorded in our database between Jan 1st 1982 and Dec 31st 2015, yearly submitted to ECG and ECG-Holter monitoring, free from cardiac abnormalities at baseline and followed for at least one year at the date of Dec 31st 2016.

Results: 55/103 patients had a first CCRA after a 6-year median FU (range 1-23, IQR 4-12 yrs.). Risk and incidence were 53.4% and 6.83% respectively. Conduction changes occurred as first CCRA in 39 patients (risk 37.86%, incidence 4.84%); arrhythmia in 16 patients (risk 15.53%, incidence 1.98%). 75% of patients were free from CCRA after 5 years of FU; 50% after 11 years; 25% after 20 years. Males, MIRS>3 and MIRS change were significantly more frequent among patients with CCRA at FU end ($p=0.04$; $p=0.012$; $p=0.001$ respectively). The incidence of CCRA was independently predicted by age at database inclusion (HR=1.55; 95%CI: 1.30-1.84; $p<0.0001$) and by CTG expansion (HR=2.58; 95%CI: 1.27-5.23; $p=0.008$). Most of patients developed CCRAs during the fourth and the fifth decade. Age at onset of CCRA was lower in patients with MIRS progression ($p=0.003$) and in patients with higher CTG expansion ($p<0.0001$). CSD occurred in 9 patients (Risk 8.7%. Incidence 0.72%). It was associated with MIRS progression ($p=0.013$), CTG expansion ($p=0.039$) and atrial flutter ($p=0.014$).

Discussion: Though DM1 patients may have CCRA at any time, particularly those aged over 30 years, with higher CTG expansion and MIRS change should be carefully controlled with periodical ECG. Our findings demonstrate the importance of cardiac follow-up in DM1 and the necessity for patient stratification in therapeutic trials.

Grant Support: None.

S5-04

Session 5: Specific Disease Features - CNS, Cardiac and GI

Progression of Frontal Cognitive Impairment in DM1 Adult Phenotype Over a 13-year Follow-up

GALLAIS Benjamin[1,2,3], GAGNON Cynthia[1,2,3], MATHIEU Jean[1,2,3], RICHER Louis[2,4]

1. École de réadaptation, Faculté de médecine et des sciences de la santé, Université de Sherbrooke (Québec, Canada)
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (Québec, Canada)
3. Centre de recherche Charles-Le-Moyne (Québec, Canada)
4. Département des sciences de la santé, Université du Québec à Chicoutimi (Québec, Canada)

Introduction: Myotonic dystrophy Type 1 (DM1) is an inherited disease leading to multisystemic problems involving central nervous system. Few longitudinal studies on cognition in adult-onset DM1 patients were conducted, and none has provided data on multiple time points. The aim is to describe the progression of cognitive abilities, after a 13-year follow-up with three time points in DM1 patients with adult-onset phenotypes.

Methods: 50 DM1 patients with adult phenotypes (juvenile and classic) were assessed three times (T1, T2, T3) within a 13-year period on specific cognitive abilities (verbal memory, visual attention, processing speed, visuo-constructive abilities and executive functions). Demographic data, level of muscular impairment and number of CTG repeats were also measured.

Results: Participants' mean age goes from 39 (T1) to 51 (T3). Results showed a significant worsening of raw scores for verbal learning, short and long-term verbal memory, visual attention speed, visuo-constructive and executive functions between T1 and T2 as well as T1 and T3. After controlling for age and education level, visuo-constructive and executive functions significantly declined from T1 to T3 and from T2 to T3.

Discussion: Results confirmed our previous hypothesis that frontal lobe related cognitive functions (i.e., visuo-constructive and executive functions) decline earlier (before 50 y/o) than other functions in the adult phenotype of DM1. However, we need to consider that DM1 life expectancy is greatly reduced and a high attrition phenomenon is always present in such long-term longitudinal studies. Consequently, participants that are more vulnerable tend to die between each evaluation time. Results about the factors related to this specific decline will be discussed.

Grant Support: This study was supported by the Canadian Institutes of Health Research (CIHR). BG is currently a postdoctoral fellow of the Wyck Foundation/Myotonic Dystrophy Foundation.

S5-05

Session 5: Specific Disease Features - CNS, Cardiac and GI

Excessive Daytime Sleepiness, Executive Dysfunction and Structural Brain Changes in Myotonic Dystrophy Type 1: the DM1-Neuro Study

HAMILTON Mark J[1,2], CUMMING Sarah[2], MCGHIE Josephine[2], ADAM Berit[2], The Scottish Myotonic Dystrophy Consortium, MCLEAN John[3], JAMPANA Ravi[3], EVANS Jonathan[4], LIVINGSTON Eric[5], BALLANTYNE Bob[1], LONGMAN Cheryl[1], FARRUGIA Maria E[7], MONCKTON Darren G[2]

1. West of Scotland Clinical Genetics Service, Queen Elizabeth University Hospital, Glasgow, UK
2. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
3. Department of Neuroradiology, Institute of Neurological Sciences, Queen Elizabeth University Hospital, Glasgow, UK
4. Institute of Health and Wellbeing, Gartnavel Royal Hospital, Glasgow, UK
5. Department of Respiratory Medicine, Glasgow Royal Infirmary, Glasgow, UK
6. John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
7. Department of Neurology, Institute of Neurological Sciences, Queen Elizabeth University Hospital, Glasgow, UK

Introduction: Central symptoms of myotonic dystrophy type 1 (DM1) such as fatigue, excessive sleepiness and cognitive deficits frequently impact quality of life. With the advent of drug trials in DM1, there is a pressing need to better understand the pathophysiological basis of these symptoms, and to evaluate tools that might act as outcome measures for central symptoms in clinical trials.

Methods: Forty six participants with adult-onset DM1 were recruited. CTG expansion was measured by small-pool PCR. All participants completed a cognitive evaluation, and a range of outcome measure questionnaires. Forty participants also underwent brain MRI, analysed to quantify grey matter volume and white matter lesion (WML) load. Domiciliary polysomnography and modified maintenance of wakefulness test were performed on a subset of 17 participants. Twenty age-matched unaffected controls completed cognitive tests and imaging.

Results: Fatigue and daytime sleepiness were frequently reported by patients, and correlated positively with low mood and pain ($p < 0.001$). WMLs and grey matter atrophy were greater among DM1 participants than controls, and increased with age. Greater WML number predicted poorer performance in the WASI-II block design test and Edinburgh Cognitive ALS screen ($p = 0.002$, 0.016), and were associated with greater self-reported fatigue ($p = 0.021$). Greater grey matter atrophy, as well as higher WML volume were associated with more prevalent 'frontal' personality traits reported by a relative on the Dex questionnaire ($p = 0.005$, <0.001). Preliminary analysis of sleep study data suggests a trend towards more severe sleep disordered breathing among patients with higher WML load.

Discussion: Preliminary results from the DM1-Neuro study demonstrate promising correlations between imaging data and clinical measures. Future directions include completion of further sleep studies, and analysis of diffusion tensor and resting-state functional MRI sequences already obtained.

Grant Support: Muscular Dystrophy UK, Chief Scientist Office

S5-06

Session 5: Specific Disease Features - CNS, Cardiac and GI

Natural History of the Heart in Myotonic Dystrophy Type 1: A Cardiac Magnetic Resonance Imaging Follow-up of 11 Patients

JIMENEZ-MORENO Aura Cecilia[1], CASSIDY Sophie[2], HOLLINGSWORTH Kieren G[3], PARIKH Jehill[2], MCSTAY Ruth[3], BATES Matt[3], TRENELL Michael[2], MACGOWAN Guy[1], LOCHMULLER Hanns[1], GORMAN Grainne[3], The European OPTIMISTIC Consortium

1. Institute of Genetic Medicine, Newcastle University
2. Institute of Cellular Medicine, Newcastle University
3. Institute of Ageing and Health, Newcastle University

Introduction: It is well known that myotonic dystrophy type 1 (DM1) is associated with increase cardiac mobility and mortality. Cardiac magnetic resonance imaging (CMRI) is an objective and accurate method that allows assessment of cardiac structure and function. We aimed to utilise this method to analyse the natural progression of this cohort's cardiac phenotype and report a reproducible methodology useful for clinical trials.

Methods: 26 patients enrolled for the OPTIMISTIC trial participated in this sub-study and were scanned at baseline and 10 months (±2 months) after. For the purposes of this abstract, the whole cohort was analysed at baseline and compared to an aged-match healthy group and 11 patients with no intervention were analysed at follow-up as a potential representation of the natural progression of then heart disease. All examinations were performed using the 3.0 T Philips Achieva MRI scanner with a six channel cardiac array.

Results: Among other variables, stroke index differed significantly from the healthy controls (mean ±SD): 34±7 vs 39±6 ($p=0.02$), and showed a strong correlation to clinical reports of daily physical activity as reported by an activity monitor ($r=0.54$, $p<0.05$).After a period of 9.7 months (min 7.2 max 13) cardiac index reduced significantly from 2.2 (±0.5) to 1.9 (±0.3) ($p=0.04$) which may reflect already disease progression. Cardiac torsion was analyzed for the first time and it showed to be reduced in the DM1 cohort.

Discussion: This is the first study to report follow-up analysis of the heart of DM1 by using CMRI technology. The early findings of cardiac disease progression should not be taken for granted and a similar study with a larger sample should be considered. The torsion results suggest an epicardial dysfunction as a contributor of the reduced ejection fraction in DM1 patients.

Grant Support: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007– 2013) under grant agreement n° 305697

S5-07

Session 5: Specific Disease Features - CNS, Cardiac and GI

Correlation Between Delayed Gastric Emptying and Gastrointestinal Symptoms in Type-1 Myotonic Dystrophy (DM1)

JOSEPH Paul[1,2,3], KHOO Tien K[1,4], FLETCHER Linda[3,5], KOLOSKI Natasha[3,5], HANSEN Teresa[3], PATHMANATHAN Pavthrun[1], JACKSON Richard[1,2], HOLTMANN Gerald[3,5]

1. Griffith University, School of Medicine, Queensland, Australia
2. Dept. of Medicine, Logan Hospital, Queensland, Australia
3. Dept. of Gastroenterology and Hepatology, Princess Alexandra Hospital, Queensland, Australia
4. School of Medicine, University of Wollongong, New South Wales, Australia
5. Faculty of Medicine, University of Queensland, Australia

Introduction: Very little is known about the link between Gastrointestinal (GI) symptoms and gastric motor and sensory function. We aimed to quantitate GI symptoms and gastric motor and sensory function and their interrelationships.

Methods: This case-controlled, age-matched, prospective observational study involved 20 patients with DM1and 10 healthy controls(c). GI symptoms were collected using the Structured Assessment of the Gastro Intestinal Symptoms scale(SAGIS). Gastric function was assessed using the 13C-octanoic acid gastric emptying breath test, expressed as t1/2 minutes(min). Visceral function was assessed using a Nutrient Challenge Test (NCT) that comprised consumption of 600 ml of Nestle Resource™ Plus over 20 min, orally with self-reporting of GI symptoms.

Results: Twenty (10 male, 10 females, mean age 40 ±15.6 years) DM1 patients and ten healthy volunteers (6 male, 4 females, mean age 40 ± 14.6 years) were tested. GE was significantly delayed in all DM1 patients (mean t ½ 282.2 min, range 146-652 min) vs c (117 min, range 94-134 min $p<0.001$). GE was significantly delayed in DM1 without GI symptoms compared to controls (mean difference 140 min; $p<0.001$). There was no significant difference in GE rates in DM1 subjects with GI symptom (mean 306.8 min, range 157-652 min) compared to those without GI symptoms (mean 257.6 min, range 146-600 min; $p=0.226$). Fullness was the main symptom observed following the NCT. Participants who experienced GI symptoms after NCT had more delayed GE.

Discussion: DM1 patients, whether they had GI symptoms or not, experienced significantly delayed gastric emptying compared to healthy controls. This study confirms that DM1 patients have significantly delayed GE. An augmented visceral sensitivity in response to a standardised nutrient challenge may be linked to further delayed GE in a subset of DM1 subjects.

Grant Support: Done with in-kind support from the Gastroenterology Department, Princess Alexandra Hospital, Brisbane, Australia.

S5-08

Session 5: Specific Disease Features - CNS, Cardiac and GI

Macroscopic and Microscopic Diversity of Mis-splicing in the Central Nervous System of Myotonic Dystrophy Type 1

FURUTA Mitsuru[1,2], **KIMURA Takashi**[1], NAKAMORI Masayuki[2], MATSUMURA Tsuyoshi[3], TAKAHASHI Masanori[2], FUJIMURA Harutoshi[3], JINNAI Kenji[4], MOCHIZUKI Hideki[2], YOSHIKAWA Hiroo[1]

1. Division of Neurology, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
2. Department of Neurology, Osaka University Graduate School of Medicine, Suita, Osaka, Japan
3. Department of Neurology, National Hospital Organization Toneyama Hospital, Toyonaka, Osaka, Japan
4. Department of Neurology, National Hospital Organization Hyogo-Chuo Hospital, Ohara, Hyogo, Japan

Introduction: Myotonic dystrophy type I (DM1) is a multi-organ disease caused by CTG-repeat expansion in the DMPK gene. Sequestration of the splicing factor MBNL2 by abnormal DMPK mRNA leads to aberrant splicing in many genes in DM1 central nervous system (CNS). Interestingly, splicing misregulation of most MBNL2-regulated genes occurs in the temporal cortex but not in the cerebellum of autopsied DM1 patients. Difference of splicing misregulation among cell layers using autopsied DM1 brains has not been shown. By expanding the study into other regions of the CNS, we found differences in splicing abnormalities among tested regions of the CNS from DM1 patients.

Methods: Using autopsied samples from 15 DM1 and 11 disease controls, ratios of splicing variants of eight MBNL2-regulated genes were tested at several regions of CNS. Then, we performed laser capture microdissection (LCM) of the cerebellar cortex to clarify splicing difference among cell layers.

Results: In the frontal and temporal cortices and the hippocampus of DM1, many genes were aberrantly spliced, but severity differed among the brain regions. By contrast, there were no significant differences in the ratio of splicing variants for most of the genes in the cerebellar cortex and spinal cord between DM1 and control samples. LCM demonstrated splicing misregulation in the molecular layer of the cerebellum, but no significant mis-splicing was detected in the granular layer.

Discussion: This is the first study to reveal mis-splicing in a functional cell layer of DM1 and to compare splicing misregulation in a wide region of the CNS using statistical analysis.

Grant Support: none

S5-09

Session 5: Specific Disease Features - CNS, Cardiac and GI

$GABA_A$ Receptor Antagonists to Treat Hypersomnia and Impaired Cognition in DM

MCCONNELL Ona[1], WANG Eric[1], BASSELL Gary[2], MOODY Olivia A[3], JENKINS Andrew[3], **RYE David**[4]

1. Center for Neurogenetics, University of Florida, Gainesville, FL
2. Department of Cell Biology, Emory University School of Medicine, Atlanta, GA
3. Department of Anesthesiology, Emory University School of Medicine, Atlanta, GA
4. Emory Sleep Center, Department of Neurology, Emory University School of Medicine, Atlanta, GA

Introduction: Individuals with Myotonic Dystrophy have a wide range of CNS symptoms including hypersomnia and cognitive impairment. DM mouse models lacking MBNL proteins are hyper-sensitized to GABA agonists, suggesting altered inhibitory activity in the CNS in DM. The cerebrospinal fluid of patients with a related condition, idiopathic hypersomnia (IH), has been previously discovered to contain a benzodiazepine-like substance whose effects can be reversed by $GABA_A$ receptor antagonists. Motivated by our clinical studies reporting that IH patients are responsive to $GABA_A$R antagonists, we hypothesized there was a shared pathology, suggesting a new treatment for CNS symptoms in DM.

Methods: We assayed cerebrospinal fluid of several DM1 subjects for benzodiazepine activity using patch-clamp electrophysiological assays. We treated these patients with $GABA_A$R antagonists, clarithromycin or flumazenil, and administered subjective and objective tests of wakefulness, arousal, and cognitive function. We also studied the splicing status of the g subunit of the $GABA_A$R in post-mortem DM1 brain.

Results: The CSF of several DM1 patients was found to contain benzodiazepine-like activity. Furthermore, the g subunit of the $GABA_A$R is mis-spliced, suggesting DM patients would be hyper-sensitive to benzodiazepine activity. Treatment of DM1 subjects with $GABA_A$R antagonists dramatically increased wakefulness, arousal, and cognitive function as assessed by subjective and objective metrics. These data suggest that use of $GABA_A$R modulators may be an effective treatment for hypersomnia and impaired cognition in DM.

Discussion: The GABA axis is perturbed in DM. Not only are there endogenous benzodiazepines present, but mis-splicing of the g subunit of the $GABA_A$R subunit also yields $GABA_A$ receptors that are hypersensitive to benzodiazepines. Use of $GABA_A$R antagonists that specifically modulate binding of benzodiazepines may be a viable therapy for DM.

Grant Support: NIH DP5 OD017865 (ETW), NIH NS089719 (DBR, AJ).

S5-10

Session 5: Specific Disease Features - CNS, Cardiac and GI

Risk for Complications After Pacemaker or Cardioverter Defibrillator Implantations in Patients with DMI

WAHBI Karim[1], SOCHALA Maximilien[1], LAZARUS Arnaud[2], BECANE Henri Marc[3], STOJKOVIC Tanya[3], FAYSSOIL Abdallah[3], LAFORET Pascal[3], BEHIN Anthony[3], EYMARD Bruno[3], DUBOC Denis[1], MEUNE Christophe[1]

1. Cochin Hospital, Paris, France
2. InParys clinical research associates, Saint Cloud, Paris, France
3. Pitie Salpetriere hospital Myology Institute, Paris, France

Introduction: Pacemakers (PM) and implantable cardioverter defibrillators (ICD) may be indicated for sudden death prevention in myotonic dystrophy type 1 (DM1), however the risk of complications after the placement of these devices is unknown. Our objective was to compare the rate of device-related complications between PM and ICD implantations in patients with DM1.

Methods: Among 914 patients with DM1 included in the DM1 Heart Registry between January 2000 and January 2010, we retrospectively selected 23 patients who were implanted with an ICD and matched them to 46 controls with a PM on age, gender, and year of device placement.

Results: Over a 6 years follow-up period, we observed device-related complications in 9 ICD recipients (inappropriate shocks in 5, lead dysfunction in 5, infection in 2) and in 3 PM recipients (lead dysfunction in 3). Patients with an ICD had, compared to those with a PM, higher rates of complications (39.1% vs. 6.5%, p = 0.0006) and more frequent complications requiring hospitalisation and/or re-intervention (respectively 30.4% and 21.7% vs. 0%).

Discussion: Our study shows a higher risk of device-related complications after the implantation of an ICD than for a PM in patients presenting with DM1.

Grant Support: AFM-Téléthon.

S6-01

Session 6: Cell Models for DM

CUG Repeats and MBNL1: A Toxic Combination for the XRN2 Exonuclease

BARGSTEN Annie[1,2], SCHNEIDER Mary[2], MORISAKI Tatsuya[3], STASEVICH Tim[3], WILUSZ Jeffrey[1,2], WILUSZ Carol[1,2]

1. Cell and Molecular Biology Program Colorado State University, Fort Collins, Colorado, USA
2. Department of Microbiology, Immunology and Pathology Colorado State University, Fort Collins, Colorado, USA
3. Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, Colorado, USA

Introduction: Targeting CUG-repeat-containing mRNAs for degradation is a promising therapeutic avenue for myotonic dystrophy, but we know little about how and where these mutant mRNAs are naturally decayed. Characterizing the enzymes and pathways involved in decay of CUG-repeat transcripts may lead to a better understanding of how current pre-clinical therapies work and eventually to new therapeutic approaches.

Methods: We established a Tet-off C2C12 mouse myoblast model to study decay of DMPK reporter mRNAs containing 0 (CUG0) or ~700 (CUG700) CUG repeats in the 3'UTR. We utilized qRT-PCR and northern blotting to assess the pathway and rate of decay of DMPK reporter mRNAs following depletion of mRNA decay factors by RNAi.

Results: Both CUG0 and CUG700 transcripts degrade surprisingly rapidly with a half-life of ~1hr but they are targeted by different enzymes in different subcellular compartments. The CUG0 mRNA is turned over in the cytoplasm by XRN1 exonuclease, while the CUG700 mRNA is targeted by nuclear XRN2. The region of the mutant transcript upstream of the repeats is degraded significantly faster than the downstream region, implying that XRN2 is slowed by the repeats. Consistent with this, depletion of XRN2 stabilizes only the upstream region. Depletion of MBNL1 enhances decay of the downstream region and renders it dependent on XRN2. We are currently evaluating the rate of dispersion of MBNL1 in live cells following inhibition of transcription to estimate the rate of decay for the repeat region.

Discussion: Both normal and repeat containing mRNAs are rapidly targeted by 5'-3' pathways. However, binding of MBNL1 prevents nuclear XRN2 from efficiently processing the repeat region. Thus, decay of the repeats and the region downstream is significantly delayed. We believe that the downstream region and perhaps the repeats are eventually degraded by 3'-5' pathways. We will discuss how our findings influence perception of the toxic RNAs in myotonic dystrophy.

Grant Support: NIH-NIAMS R01AR059247 to CJW.

S6-02

Session 6: Cell Models for DM

CUG RNA Toxicity in Astrocytes is Associated with Adhesion and Migration Deficits and Affects Neuritogenesis

DINCA Diana[1,2], SICOT Géraldine[1,2], HUGUET-LACHON Aline[1,2], CHHUON Cerina[3], GUERRERA Chiara[3], GOURDON Geneviève[1,2], GOMES-PEREIRA Mário[1,2]

1. Inserm UMR 1163, Laboratory of CTGDM, Paris, France
2. Paris Descartes – Sorbonne Paris Cité University, Imagine Institute, Paris, France
3. Proteomics Platform 3P5-Necker, Université Paris Descartes-Structure Fédérative de Recherche Necker, Inserm US24,CNRS UMS3633, Paris, France

Introduction: In myotonic dystrophy type 1 (DM1), expanded DNA repeats are transcribed into toxic RNA. In the central nervous system (CNS), RNA foci accumulate in multiple cell types, leading to cognitive and behavioral changes. However, we do not know the extent to which each cell type is affected or how it drives CNS pathology.

Methods: To investigate CNS dysfunction, we are using the DMSXL transgenic mice. These mice exhibit relevant neurological phenotypes, in association with RNA foci in neurons and glia. They provide a renewable source of primary neurons and astrocytes to resolve cell type-specific phenotypes and associated molecular abnormalities.

Results: DMSXL primary astrocytes show greater RNA foci accumulation and missplicing, relative to neurons, in association with defective cell growth, adhesion, cell polarization and migration. In contrast, the growth profile of DMSXL primary neurons remains unaltered, but late neurite arborization is significantly impaired. Interestingly, defects in neuritogenesis are aggravated by the presence of DMSXL astrocytes in co-culture. Global proteomics of homogenous cultures revealed expression changes in key proteins of the Rho GTPases signaling cascade in DMSXL astrocytes, relative to wild-type controls. Interestingly, the RhoGTPase pathway regulates cell adhesion and spreading. Protein expression defects are currently being validated in mouse and human tissue samples.

Discussion: DM1 has a critical impact on glia cell biology, which may affect neuronal physiology through defective neuroglial crosstalk. The dysregulation of the Rho GTPase signaling in DMSXL astrocytes could mediate the glial phenotypes described. Our results provide new insight into the cellular and molecular mechanisms of DM1 brain disease and open new avenues for future research.

Grant Support: This work is supported by grants from AFM-Téléthon, Inserm, Paris Descartes University and the French Ministry of Higher Education and Research.

S6-03

Session 6: Cell Models for DM

Immortalized Human Myotonic Dystrophy Muscle Cell Lines to Assess Therapeutic Compounds

ARANDEL Ludovic[1], POLAY-ESPINOZA Micaela[1], MATLOKA Magdalena[1], BAZINET Audrey[1], DE DEA DINIA Damily[1], MOULY Vincent[1], KLEIN Arnaud[1], **FURLING Denis**[1]

1. Sorbonne Universités UPMC, Inserm Centre de Recherche en Myologie, Institut de Myologie, Paris, France

Introduction: Although DM animal models have been developed to investigate pathophysiologic mechanisms and evaluate the efficacy of therapeutic approaches, cellular models remain needed for prior compounds evaluation or screenings. Primary muscle cell cultures derived from biopsies of DM patients represent a valuable model since the C/CTG expansions are expressed within its natural genomic context. However several concern such as accessibility and availability of DM biopsies, limited proliferative capacity of human myoblasts support the development of immortalized human DM muscle cell lines in which replicative senescence is bypassed but that display robust and reliable disease-associated features.

Methods: Immortalized DM1 (2600CTG) and DM2 (4000CCTG) human muscle cell lines displaying nuclear RNA foci were generated by transduction of lentiviral vectors expressing the catalytic subunit of the human telomerase (hTERT) and the natural p16 ligand, Cdk4.

Results: Selected clones of DM immortalized myoblasts behave as parental primary myoblasts with a reduced fusion capacity of DM1 myoblasts when compared to control and DM2 cells. Alternative splicing defects were measured in differentiated DM1 but not in DM2 muscle cell lines. Splicing alterations did not result from differentiation delay because similar changes were found in immortalized DM1 transdifferentiated fibroblasts in which the myogenic differentiation has been forced by MyoD overexpression. As a proof-of-concept, we showed that antisense approaches alleviate disease-associated defects and a RNA-seq analysis confirmed that the vast majority of misspliced events in immortalized DM1 muscle cells were modulated by antisense treatment.

Discussion: We generated new immortalized DM1 muscle cell lines displaying characteristic disease-associated molecular features such as nuclear RNA-aggregates and splicing defects that can be used as robust readouts for the screening of therapeutic compounds.

Grant Support: AFMTelethon-Institut de Myologie, France Génomique.

S6-04

Session 6: Cell Models for DM

Generating Isogenic Myoblast Cell Models by CRISPR/Cas9-mediated Editing, Regulating and Targeting Genes in the Myotonic Dystrophy Type 1 (DM1) Locus

VAN AGTMAAL Ellen L[1], ANDRÉ Laurène M[1], WILLEMSE Marieke[1], VAN CRUCHTEN Remco[1], VAN KESSEL Ingeborg DG[1], VAN DEN BROEK Walther JAA[1], GOURDON Geneviève[2,3], FURLING Denis[4], MOULY Vincent[4], WANSINK Derick G[1], **WIERINGA Bé**[1]

1. Radboud Institute for Molecular Life Sciences, Department of Cell Biology, The Netherlands
2. Inserm UMR 1163, Paris, France
3. Paris Descartes -Sorbonne Paris Cité University, Imagine Institute, Paris, France
4. Sorbonne Universités, UPMC Univ Paris 06, Inserm UMRS974, CNRS FRE3617, Center for Research in Myology, Paris, France

Introduction: Recently, we have reported* promising first steps towards use of CRISPR/Cas9-therapy for DM1, aimed at removal of the (CTG•CAG)n-repeat. Still, further fundamental and therapeutic studies are needed to better understand the complex etiology of disease in tissues of DM1 patients.

Methods: By use of genome editing and promoter targeting in myoblasts from DM1 patients, unaffected individuals or transgenic DM1 mice we have generated different new DM1 cell models A summary of requirements for - and effects of - genome targeting with different Cas9-guide RNA combinations will be given.

Results: Complete precise excision of the repeat tract from normal and mutant DMPK alleles, with sizes between 0.1-8 kbp, could be achieved at high frequency by dual-simultaneous CRISPR/Cas9-cleavage at either side of the (CTG•CAG)n sequence. Introduction of only one dsDNA break in either the 5'- or 3' unique flanks of the expanded trinucleotide repeat is undesirable, as it promotes uncontrollable instability with deletion of large segments from the expanded region, rather than clean DNA repair. Importantly, aspects of impaired myogenic differentiation were normalized upon loss of large repeats. We have also used the CRISPR/Cas9 system as a tool to generate isogenic myoblast models with (i) a deleted G-quadruplex motif/CTCF site in the 3' UTR region of the DMPK gene, and (ii) altered expression of genes from the DM1 locus.

Discussion: Cas9/CRISPR-based genome editing and targeting is a versatile approach for study of how repeat expansion and structural elements in the 3'UTR of DMPK mRNA affect myogenic potential and RNA-processing or -nucleocytoplasmic partitioning in cell models for DM1 (see also abstracts by Wansink and van Cruchten).

Grant Support: This work was supported by ZonMw (TOP grant NL91212009) and by the Prinses Beatrix Spierfonds (grant number W.OR12-05; with contribution from the Stichting Spieren voor Spieren).

** van Agtmaal et al. (2017) Mol. Therapy 25, 1-20.*

S7-01

Session 7: Animal Models and Tissue-specific Mechanisms

Antisense Oligonucleotides (ASOs) Preferentially Target Repeat-containing Transcripts in Skeletal Muscle

CARRELL Samuel T[1,2], TANG Zhenzhi[2], BENNETT C Frank[3], THORNTON Charles A[2]

1. Department of Biomedical Genetics, Medical Scientist Training program, University of Rochester,
2. Department of Neurology, University of Rochester
3. Ionis Pharmaceuticals

Introduction: Evidence suggests that transcripts containing an expanded CUG repeat (CUGexp) are sensitized to RNase H mediated ASO knockdown, perhaps due to prolonged dwell time in the nucleus (Wheeler, 2012). We tested this possibility in myogenic cells and transgenic mice.

Methods: Immortalized myogenically-transformed fibroblasts from DM1 patients were cultured for 7 days in 1 – 10 uM Ionis 486178, a gapmer ASO targeting DMPK (Pandey, 2016). The cells were heterozygous for rs527221, a non-synonymous SNP in DMPK exon 10. DMPK knockdown was assessed by RT-qPCR. Allelic ratios of DMPK transcripts were determined by high throughput sequencing. Mouse studies were performed in HSAXLR transgenic mice expressing human skeletal actin having (CUG)440 in the 3' UTR, or an allelic variant expressing skeletal actin without the CUGexp tract (HSANR). Subcutaneous injections of Ionis 445232, an ASO targeting transgene mRNA (Wheeler, 2012), were given twice weekly for 4 weeks. Knockdown in hindlimb muscle was assessed by RT-qPCR.

Results: Free-uptake of ASO in cell culture led to 10-60% knockdown of DMPK mRNA, relative to vehicle-treated controls. However, ASO knockdown did not alter the allelic ratio of DMPK transcripts. In contrast, transgene mRNA knockdown in HSAXLR mice (28 ± 3% in vastus, 27 ± 10% in tibialis anterior, and 31 ± 7% in gastrocnemius) was significantly greater than in HSANR mice (12 ± 9% in vastus, 0 ± 10% in tibialis anterior, and 13 ± 11% in gastrocnemius).

Discussion: These studies are the first to show allelic selectivity of ASO knockdown for CUG expanded transcripts in vivo. However, selective knockdown may depend on cell type, cell cycling, sequence context, or route of administration.

Grant Support: National Institute of Health grants U54NS048843 and U01NS072323, University of Rochester Medical Scientist Training Program. DM1 cells were a kind gift from Profs. David Brook and John W. Day.

S7-02

Session 7: Animal Models and Tissue-specific Mechanisms

Insights from Evaluations of Genetic Modifiers of RNA Toxicity

MAHADEVAN Mani S[1], YADAVA Ramesh S[1], MANDAL Mahua[1], YU Qing[1], MAHADEVAN Karunasai[1]

1. University of Virginia, Department of Pathology

Introduction: We have generated a new mouse model of RNA toxicity called DM200. This model recapitulates classic DM1 phenotypes including RNA foci, splcing defects, myotonia, and cardiac conduction defects. Using a genetic approach, and the DM200 model, we have undertaken evaluations of modifiers of RNA toxicity.

Methods: Mouse models:

A. DM200 express a tetracycline inducible GFP-DMPK 3'UTR (CTG)200 transgene using a DMPK promoter
B. MBNL1 and MBNL2 knockout mice (courtesy of Dr. M. Swanson)
C. MBNL1 over-expressing mice (courtesy of Dr. L. Ranum)
D. DMPK knockout mice (courtesy of Dr. B. Weiringa)

Phenotypes: EMG, ECG, 30 minute treadmill runs, grip strength
Tissue Analyses: RNA foci, RNA splicing defects, H&E, fiber sizing, IF for RAN translation products, etc.

Results: The DM200 mice demonstrate robust and reversible phenotypes associated with RNA toxicity, including myotonia, cardiac conduction defects, and RNA splicing defects in skeletal muscle and heart, and abundant RNA foci in both tissues. Evaluation of DMPK deficient mice (up to 6-12 old) revealed results that were discrepant with some published data about cardiac conduction defects. In the context of RNA toxicity, the absence of DMPK had negligible additional deleterious effects. Results from ongoing experiments evaluating the effects of MBNL1 dosage in RNA toxicity using the MBNL1 over-expression and MBNL1 deficient mice will be presented. Preliminary results suggest differences between reported results from experiments using the HSA-LR and the ongoing experiments.

Discussion: The DM200 model presents an opportunity to evaluate genetic modifiers in an independent mouse model of RNA toxicity. Using this model, we have tested the effects of various potential modifiers of RNA toxicity in DM1. The implications of confirmatory and discrepant results with respect to pathogenesis and potential treatment options/approaches will be discussed.

Grant Support: NIAMS: R01AR062189 & R01AR071170, The Stone Circle of Friends, The Marigold Foundation.

S7-03

Session 7: Animal Models and Tissue-specific Mechanisms

Differential Effects of Intronic vs. Exonic Repeats in DM2 Transgenic Mice

TANG Zhenzhi[1], CARRELL Ellie[1], THORNTON Charles A[1]

1. University of Rochester Medical Center

Introduction: RNA dominance may occur with expanded repeats in introns or exons. Gain-of-function from intronic repeats is the more surprising, because introns have rapid turnover. We compared toxicity of expanded CCUG repeats (CCUGexp) in an intronic vs. exonic location in transgenic mice.

Methods: We used targeted transgenesis with PhiC31 integrase (Tasic, 2011) to generate DM2 mouse models with 1100 CCTG repeats in intron 3 or the 3' UTR of a human skeletal actin (HSA) transgene (HSA-DM2in or HSA-DM2ex, respectively). In the 3' UTR, expression of CCUGexp RNA was conditional upon excision of a floxed transcription terminator cassette (TTC).

Results: Prior to recombination, HSA-DM2ex mice did not express CCUGexp RNA or develop nuclear foci. After excision of TTC, HSA-DM2ex mice developed robust nuclear foci and large accumulation of CCUGexp RNA. Homozygous HSA-DM2ex mice displayed alternative splicing defects that are characteristic of human DM2. HSA-DM2in mice also developed nuclear foci of CCUGexp RNA, but they were less intense than HSA-DM2ex mice, and alternative splicing remained normal, despite higher levels of HSA transgene expression. Notably, probes for flanking sequence localized to foci by FISH in both models. Histologic studies and analyses of transgene RNA are ongoing.

Discussion: Exonic CCUG repeats are more deleterious. Despite location in a relatively short intron (~4.6 kb), the intronic CCUGexp RNA formed conspicuous nuclear foci, suggesting the possibility of slow decay. HSA-DM2in and HSA-DM2ex mice may prove useful for studying antisense oligonucleotide or small molecule treatments for DM2.

Grant Support: NIH NS094393 and NS048843.

S7-04

Session 7: Animal Models and Tissue-specific Mechanisms

Enhancement of Splicing Correction by Ligand Conjugated Antisense (LICA) Oligos in Live DM1 Mice

HU Ningyan[1], ANTOURY Layal[1], BARAN Timothy[2], RIGO Frank[3], BENNETT Frank[3], FOSTER Thomas[2], **WHEELER Thurman[1]**

1. Massachusetts General Hospital
2. University of Rochester
3. Ionis Pharmaceuticals

Introduction: LICA chemistry adds specific conjugates to antisense oligos (ASOs) that are designed to increase drug uptake into target tissues. In a recent clinical trial, a LICA oligo targeting APO(a) in the liver was several-fold more potent than the unconjugated parent ASO, enabling a >10-fold lower dose and improving tolerability (Viney, et al., 2016). Our goal was to determine the therapeutic efficacy and potency of a LICA oligo vs. unconjugated ASO in a transgenic mouse model of DM1.

Methods: We used TR;HSALR bi-transgenic mice. The TR transgene consists of DsRED and GFP in mutually exclusive reading frames (Orengo, 2006). In this system, inclusion of ATP2A1 exon 22 results in DsRED expression, while exclusion of exon 22, as in DM1, results in GFP expression. Quantitation of the DsRED/GFP ratio by in vivo fluorescence imaging enables non-invasive estimation of splicing outcomes. ASOs were an unconjugated MOE gapmer targeting ACTA1 and a LICA modified version of the same ASO. We delivered drugs by subcutaneous injection (2.5, 8.5, 12.5, or 25 mg/kg twice weekly for 4 weeks), monitored splicing outcomes in live mice by serial imaging and in muscle tissue by RT-PCR, measured transgene knockdown by qPCR, and determined tissue drug levels by capillary gel electrophoresis.

Results: At Day 7, DsRED/GFP ratios in LICA oligo-treated mice were greater than in mice treated with unconjugated ASO, and remained higher through Day 42. Splicing rescue was dose-dependent. After 6 weeks, tissue drug concentrations were significantly higher and transgene levels lower in mice treated with LICA oligo than with unconjugated ASO. Histology showed no evidence of toxicity.

Discussion: LICA gapmer oligos achieve efficient dose-dependent target knockdown in skeletal muscle, while the more robust splicing correction suggests greater target engagement of LICA oligos than unconjugated ASOs. These data support further development of LICA technology for treatment of DM1.

Grant Support: NINDS.

S8-01

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

mRNA Splicing Biomarkers Of DM1 In Human Urine

ANTOURY Layal[1], HU Ningyan[1], BALAJ Leonora[1], DAS Sudeshna[1], CLARK Tim[1], BREAKEFIELD Xandra[1], WHEELER Thurman[1]

1. Massachusetts General Hospital

Introduction: Extracellular RNA (exRNA) in serum and urine includes mRNA and non-coding RNA that can serve as genetic biomarkers of glioblastoma, prostate cancer, and other disease states. In a recent clinical trial for DM1, mRNA splicing outcomes in serial muscle biopsies were used to monitor therapeutic antisense oligonucleotide (ASO) drug effects. However, tissue biopsies are invasive, impractical for long term monitoring of therapeutic response, and require general anesthesia in children. We examined whether ex-mRNA splice events in human serum or urine could meet sensitivity and specificity as robust markers of DM1.

Methods: We isolated exRNA from biofluid samples of individuals with DM1 (N = 27), individuals with a muscular dystrophy besides DM1 (MD controls; "MDC"; N = 10), and unaffected (UA; N = 26) controls, examined RNA integrity by capillary gel electrophoresis, gene expression by qPCR, and quantitated mRNA splicing outcomes by RT-PCR. Using a training set (DM1 + UA; N = 34 total), principle component regression, and ROC analysis, we developed a predictive model of urine splicing outcomes in DM1. The remaining DM1 and UA samples (N = 19) formed an independent validation set.

Results: In our training set, we identified 10 transcripts that are spliced differently in urine from DM1 patients as compared to MDC or UA individuals. Our predictive model was 100% accurate in our independent validation set. By contrast, alternative splicing of all transcripts examined in serum was similar in DM1, MDC, and UA subjects.

Discussion: Urine provides a renewable source of mRNA splice events that can serve as a powerful composite biomarker of DM1, suggesting its potential to monitor therapeutic response. Non-invasive measurements of splicing outcomes may enable convenient titration of ASO dose during the course of treatment, and facilitate clinical trials to children with DM1 earlier.

Grant Support: The Elaine and Richard Slye Fund; Wyck Foundation/Myontonic Dystrophy Foundation; MDA; NIH.

S8-02

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

DM-Scope, a French Nationwide Registry to Decipher Pediatric Myotonic Dystrophies' Clinical Complexity

LAGRUE Emmanuelle[1], DOGAN Céline[2], DE ANTONIO Marie[3], HAMROUN Dalil[4], EYMARD Bruno[5], **BASSEZ Guillaume**[2]

1. CHRU de Tours, Université François Rabelais de Tours, INSERM U930, Tours
2. Centre de Référence Maladies Neuromusculaires, CHU Henri Mondor, Créteil, France
3. Centre de Référence Maladies Neuromusculaires, CHU Henri Mondor, Créteil, France, Inserm UMRS872, team22, Centre de recherché des cordeliers, Paris, France
4. Institut Universitaire de Recherche Clinique, CHU de Montpellier, France
5. Centre de Référence Maladies Neuromusculaires Paris-Est, CHU Pitié-Salpêtrière, Paris, France

Introduction: Myotonic dystrophy type 1 (DM1) is known to exhibit marked phenotypic disparities due to highly variable age of onset and heterogeneous multisystemic involvement. Despite recent clinical progress in adult DM1, pediatric descriptions remain scarce. We therefore aimed to phenotypically characterize a large DM1 pediatric cohort to provide a solid frame of data for future evidence-based health management.

Methods: Among the 2697 genetically confirmed DM1 patients included in the French DM-Scope registry, we enrolled all patients under 18 years-old, whose data were collected by 24 neuropediatric centers (January 10 - February 16). Comprehensive cross-sectional analysis of most relevant qualitative and quantitative variables was performed.

Results: We studied 314 children (52% females / 55% congenital, 31% infantile, 14% juvenile form). The age at first visit was inversely correlated with the CTG repeat length. The paternal transmission rate was higher than expected, especially in the congenital form (13%). A continuum of highly prevalent neurodevelopmental alterations was observed, including slowness (83%), attention deficit (64%), written language (64%) and spoken language (63%) disorders. 5% exhibited autism spectrum disorders. Overall musculo-skeletal impairment was mild. Despite low prevalence, cardio-respiratory impairment could be life-threatening, and frequently occurred early in the first decade (25.9%). Gastrointestinal symptoms (27%) and cataracts (7%) were more frequent than expected, while endocrine or metabolic disorders were scarce.

Discussion: The pedDM-scope study is the first to detail the main genotype and phenotype characteristics of the three DM1 pediatric subgroups. It highlights striking profiles which could be useful in health care management (including transition into adulthood) and health policy planning.

Grant Support: All phases of this study were supported by an AFM-Téléthon Grant.

S8-03

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

MicroRNA Functional Deregulation and Potential as Biomarkers in Myotonic Dystrophy

PERFETTI Alessandra[1], GRECO Simona[1], FUSCHI Paola[1], CARDANI Rosanna[1], CARDINALI Beatrice[2], CAPPELLA Marisa[2], GARCIA-MANTEIGA Jose M[3], MEOLA Giovanni[1], FALCONE Germana[2], MARTELLI Fabio[1]

1. IRCCS Policlinico S. Donato, San Donato Milanese, Milano
2. Institute of Cell Biology and Neurobiology, National Research Council, Monterotondo Scalo, Roma
3. Center for Translational Genomics and BioInformatics, San Raffaele Scientific Institute, Milano

Introduction: In Myotonic dystrophy type 1 (DM1) the mutated transcripts lead to RNA toxicity, causing dysfunctions of mRNA splicing, protein translation and microRNAs (miRNAs) expression. miRNA are short noncoding RNAs that, after maturation, are loaded onto the RISC effector complex that destabilizes target mRNAs and represses their translation. Additionally, miRNAs are also present in bodily fluids, representing attractive potential biomarkers. Here we present data implicating both tissue and plasma miRNAs in DM1. The aim was twofold: 1) identifying miRNAs functionally deregulated in DM1 skeletal muscles; 2) validating miRNAs as disease biomarkers.

Methods: The RNAs associated to RISC immunoprecipitates of muscle biopsies derived from 3 DM1 patients and 3 matched controls were analyzed by RNA-sequencing. For DM1 biomarker identification, RNA was extracted from plasma of more than 200 DM1 and matched controls, and the expression of miRNAs previously identified as differently expressed in the plasma/serum of small groups of DM1 patients was investigated.

Results: RNA-sequencing revealed 6 miRNA/ mRNA target couples enriched or depleted in the RISC complexes of DM1 patients. These data were also confirmed in 16 independent patients and controls. Using immortalized fibroblasts with inducible MyoD, some of these interactions were also confirmed. Then, we identified 8 miRNAs significantly deregulated in the plasma of DM1 patients. The levels of these miRNAs, alone or in combination, discriminated DM1 from controls significantly, and correlated with both skeletal muscle strength and creatine kinase values. Finally, the identified miRNAs were also found deregulated in the plasma of a small group of DM2 patients.

Discussion: In DM1 skeletal muscles, we found functionally relevant miRNA/mRNA interactions involved in fibrosis process, as well as mRNAs associated to muscle mass regulation and metabolism. Moreover, we showed the potential of plasma miRNAs as DM1 disease marker

Grant Support: Telethon-Italy (n. GGP14092), AFM-Telethon (n. 18477), Cariplo Foundation (n. 2013-0887), Ministero della Salute (Ricerca Corrente, RF-2011-02347907 and PE-2011-02348537).

S8-04

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

The Pharmacological Small Molecule, AMO-02, Reduces Adult and Congenital Myotonic Dystrophy type 1 in the Pre-clinical studies

WANG Mei[1], STOCK Lauren[1], KIEFER VALANEJAD Leila[2], MARTINEZ Anna[3], GOURDON Genevieve[4], TIMCHENKO Nikolai[2], SNAPE Mike[5], TIMCHENKO Lubov[1]

1. Department of Neurology, Cincinnati Children's Hospital, Cincinnati, Ohio
2. Department of Surgery, Cincinnati Children's Hospital, Cincinnati, Ohio
3. Centro de Investigaciones Biológicas (CIB, CSIC), Madrid, Spain
4. Inserm UMR 1163, Paris, France
5. AMO Pharma, London, UK

Introduction: Myotonic Dystrophy type 1 (DM1) is a neuromuscular disease caused by expanded CTG repeats in the 3' UTR of the DMPK gene. Very long CTG expansions affect infants at birth causing congenital DM1 (CDM1). Therapeutic approaches for DM1 and CDM1 based on the degradation of the mutant DMPK mRNA are under development. Here we propose an alternative therapeutic approach that targets GSK3-CDK4/CUGBP1 axis which is affected by the mutant CUG repeats in both DM1 and CDM1. It has been shown that CUG repeats alter CUGBP1 activity in biopsied muscle of patients with adult form of DM1 through a pathological increase of GSK3 beta kinase activity, which normally regulates CUGBP1 activity. Therefore, inhibitors of GSK3 can be proposed to treat DM1 pathology. Here we present the results of the study, testing benefits of a pharmacological small molecule inhibitor of GSK3, AMO-02, on DM1 and CDM1 pathologies in primary human myoblasts and in mouse models of DM1 (HSA mice) and CDM1 (DMSXL model).

Methods: CDM1 myoblasts were treated with AMO-2 and the efficiency of myoblasts fusion and mis-splicing were examined by molecular methods. The effect of AMO-02 on the neuromotor activities, muscle histopathology, GSK3beta-signaling and mis-splicing in HSA and DMSXL mice was examined by histological, physiological, behavioral and molecular tests.

Results: Delayed myogenesis and mis-splicing were corrected in CDM1 myotubes treated with AMO-02. AMO-02 also corrected grip weakness, reduced muscle histopathology and mis-splicing in HSA muscle. The treatment of DMSXL mice with AMO-02 corrected the GSK3beta-cyclin D3-CUGBP1 pathway and CUGBP1-dependent RNA targets in skeletal muscle and in brain. Overall growth and neuromotor activities were significantly improved in homozygous DMSXL mice that were produced by DMSXL females, treated with AMO-02 during gestation.

Discussion: A small molecule drug AMO-02, that easy enters all tissues including brain, is a promising candidate for the clinical studies of treatments of adult and congenital DM1.

Grant Support: CCHMC fund and SRA from AMO Pharma to LT.

P-001

Session 1: Microsatellite Instability and Epigenetics

Four Spanish Patients Carrying Interruptions in the DMPK Gene Expansion

BALLESTER-LOPEZ Alfonsina[1], KOEHORST Emma[1,2], ALMENDROTE Miriam[1,3], RAMOS FRANSI Alba[1,3], MARTÍNEZ-PIÑEIRO Alicia[1,3], LUCENTE Giuseppe[1,3], LINARES Ian[1], NUÑEZ Judith[1], GUANYABENS Nicolau[4], CANO Antoni[4], CASADEVALL Teresa[5], RODRÍGUEZ-PALMERO Agustí[1,6], MONLLEÓ-NEILA Laura[1,6], PINTOS-MORELL Guillem[1,6], COLL-CANTÍ Jaume[1,3], NOGALES-GADEA Gisela[1]

1. Neuromuscular and Neuropediatric Research Group, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Campus Can Ruti, Universitat Autònoma de Barcelona, Badalona, Spain
2. Maastricht University, Maastricht, The Netherlands
3. Neuromuscular Pathology Unit. Neurology Service. Neuroscience department, Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain
4. Neurology Unit. Neuroscience department, Hospital de Mataró, Barcelona, Spain
5. Neuromuscular Unit. Neurology Service. Neuroscience department, Hospital Comarcal Sant Jaume de Calella, Barcelona, Spain
6. Neuropediatric Unit. Pediatric Service. Hospital Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

Introduction: In the last years, some patients with Myotonic Dystrophy type I (DM1) carrying interruptions in the CTG expansion, have been described. These interruptions are associated with certain clinical presentations of DM1: some cases with mild affectation and absence of muscle atrophy and CNS involvement, while some others with Charcot-Marie-Tooth neuropathy, encephalopathy and deafness. However, the number of DM1 patients described for these interruptions is low, approximately 5% of the few reported series. In this study, a cohort of 38 DM1 Spanish patients has been analyzed with the aim of finding new patients carrying interruptions and determining their phenotypes.

Methods: Blood DNA was obtained from the participants and triplet-primed PCR (at the 5' and 3' sites) and long PCR was used to determine the presence of these interruptions. A patients' registry was elaborated containing clinical information

Results: Four patients of our registry carried interruptions at the 3' site of CTG expansion, among them 3 sisters affected with DM1 and another patient. One patient, the youngest, remained mostly asymptomatic and comes to the clinics from genetic counseling. The rest of the patients, older, have some traits of the classical DM1 phenotype: myotonia, ptosis, cataracts, but also cardiac problems. The older patient has myalgia, lung dysfunction and hyper somnolence. All of them have a previous familiar case of sudden death.

Discussion: In our series, 10% of the DM1 patients carried interruptions in the CTG expansion. Inheritability of the interruptions was shown in three sisters of our registry. As in previous reports, the phenotype associated to these defect was milder in the young case, but a wide affectation was observed in the others. As in the rest of DM1 cases, cardiological assessment should be performed regularly

Grant Support: This work is mainly covered by competitive national funding: AGAUR, Generalitat de Catalunya and Instituto Carlos III, Ministerio de Economía, Industria y Competitividad, Gobierno de España

P-002

Session 1: Microsatellite Instability and Epigenetics

CpG Methylation, a Parent-of-origin Effect for Maternal-biased Transmission of Congenital Myotonic Dystrophy

BARBE Lise[1], LANNI Stella[2], LOPEZ-CASTEL Arturo[2,3], FRANCK Silvie[1], SPITS Claudia[1], KEYMOLEN Kathelijn[4], SENECA Sara[4], TOME Stephanie[2,5], MIRON Ioana[6], LETOURNEAU Julie[7], LIANG Minggao[2,8], CHOUFANI Sanaa[2], WEKSBERG Rosanna[2,8,9], WILSON Michael D[2,8], SEDLACEK Zdenek[10], GAGNON Cynthia[7], MUSOVA Zuzana[10], CHITAYAT David[6,9], SHANON Patrick[11], MATHIEU Jean[7], SERMON Karen[1*], PEARSON Christopher E[2*]

1. Departement for Reproduction and Genetics, Vrije Universiteit Brussel
2. Genetics and Genome Biology, The hospital for Sick KIds, Toronto
3. Genera Biotech, Valencia
4. Center of Medical Genetics, Universitair Ziekenhuis Brussel
5. INSERM, Laboratory of CTG Repeat Instability and Myotonic Dystrophy, Paris
6. The Prenatal Diagnosis and Medical Genetics Program, Dept. of Obstetrics and Gynecology, Univ. of Toronto
7. Groupe de Recherche Interdisciplinaire sur les Maladies Neuromusculaires, Saguenay
8. Department of Molecular Genetics, University of Toronto
9. Division of Clinical and Metabolic Genetics, The Hospital of Sick Kids,Toronto
10. Departement of Biology and Medical Genetics, Charles University Prague
11. Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto

Introduction: CTG repeat expansions in DMPK cause myotonic dystrophy (DM1) with a continuum of severity and ages-of-onset. Congenital DM1 (CDM1), the most severe form, presents distinct clinical features, large expansions, and almost exclusive maternal transmission. The correlation between CDM1 and expansion size is not absolute, suggesting the contribution of other factors.

Methods: We determined CpG methylation flanking the CTG repeat in 79 blood samples from 20 CDM1 individuals, 21, 27, and 11 non-CDM1 individuals with maternal, paternal, and unknown inheritance, and collections of maternally- and paternally-derived chorionic villus samples (7 CVS) and 4 human embryonic stem cells (hESCs).
Results: All but two CDM1 individuals showed high levels of methylation upstream and downstream of the repeat, greater than non-CDM1 individuals (p=7.04958E-12). Most non-CDM1 individuals were devoid of methylation, where one in six showed downstream methylation. Only two maternally-derived non-CDM1 individuals showed upstream methylation, and these were childhood onset, suggesting a continuum of methylation with age-of-onset. Only maternally-derived hESCs and CVS showed upstream methylation. In contrast, paternally-derived samples (27 blood samples, 3 CVS, and 2 hESCs), never showed upstream methylation. CTG tract length did not strictly correlate with CDM1 or with methylation.

Discussion: Thus, CpG methylation patterns flanking the CTG repeat are stronger indicators of CDM1 than repeat size. Spermatogonia with upstream methylation may not survive due to methylation-induced reduced expression of the adjacent SIX5, thereby protecting DM1 fathers from having CDM1 children. Thus, DMPK methylation may account for the maternal bias for CDM1 transmission, larger maternal CTG expansions, age-of-onset, clinical continuum, and may serve as a diagnostic indicator.

Grant Support: This work was supported by CIHR, Muscular Dystrophy Canada, Tribute Communities, Kazman Family, Marigold Foundation, Petroff Family, FWO Vlaanderen, Leerstoel M Aerens, Methusalem and ABMM.

P-003

Session 1: Microsatellite Instability and Epigenetics

Expanded [CCTG]n Repetitions are Not Associated with Abnormal Methylation at the CNBP Locus in Myotonic Dystrophy Type 2 (DM2) Patients

SANTORO Massimo[1], MAIORCA Francesca[2], FONTANA Luana[2], CENTOFANTI Federica[2], MASSA Roberto[3], SILVESTRI Gabriella[4], NOVELLI Giuseppe[2], **BOTTA Annalisa**[2]

1. Don Gnocchi Foundation, Milan, Italy
2. Dept. of Biomedicine and Prevention, Medical Genetics Section, University of Rome Tor Vergata, Italy
3. Department of Systems Medicine (Neurology), University of Rome Tor Vergata, Italy
4. Policlinico Gemelli Foundation, Institute of Neurology, Rome, Italy

Introduction: Myotonic Dystrophy type 2 (DM2) is a multisystemic disorder associated with an expanded [CCTG]n repeat in intron 1 of CNBP gene. DM2 is characterized by myotonia and muscle dysfunction, and less commonly by cardiac conduction defects, cataract and diabetes mellitus. Epigenetic modifications have been reported in many repeat expansion disorders, either as a mechanism to explain somatic repeat instability or transcriptional alterations in the disease genes. The purpose of our work was to determine the effect of DM2 mutation on the methylation status of CpG islands flanking the [CCTG]n expansions.

Methods: By pyrosequencing, we characterized the methylation profile of six different CpG sites within the region downstream the [CCTG]n expansion in the whole blood and in the skeletal muscle of DM2 patients (n=72 and n=7, respectively) and healthy controls (n=50 and n=7, respectively). Finally, relative mRNA transcript levels of CNBP gene were evaluated in leukocytes and in skeletal muscle tissues from controls (n=10 and n=7, respectively) and DM2 patients (n=16 and n=7, respectively) by qRT-PCR analysis.

Results: In the region downstream of the [CCTG] repetitions, the pyrosequencing analysis showed a methylation percentage of CpG sites around 25-30%. Statistical analyses did not demonstrate any significant differences in the methylation profile between DM2 patients and controls either in blood samples and in skeletal muscle tissues. Accordingly, CNBP gene expression levels are not significantly altered in DM2 patients. Additional region of the CNBP gene are currently being investigated in order to exclude an in cis effect of the [CCTG] expansion on the DM2 locus.

Discussion: These results show that the DM2 mutation, differently from DM1, does not influence the methylation status of the region downstream to the [CCTG]n repeat expansions, suggesting that other molecular mechanisms are involved in the pathogenesis of DM2.

Grant Support: None

P-004

Session 1: Microsatellite Instability and Epigenetics

Contracting CAG/CTG Repeats Using the CRISPR-Cas9 Nickase

CINESI Cinzia[1], YANG Bin[1], AESCHBACH Lorène[1], DION Vincent[1]

1. Center for Integrative Genomics, University of Lausanne

Introduction: Myotonic Dystrophy type 1 (DM1) is a neuromuscular disorder that remains without a cure. It is caused by an expanded CTG repeat present in the DMPK 3'UTR. To date, treatment attempts have focused on the symptoms of the disease rather than its cause: the expanded CTG repeat. Because longer tracts cause more severe phenotypes, contracting the repeats may provide a therapeutic avenue. There is currently no efficient treatment for inducing contraction biases.

Methods: Here we used a GFP-based chromosomal reporter that can monitor expansions and contractions in the same cell population.

Results: We found that CRISPR-Cas9 nickase activity targeted to CAG/CTG repeat tract induced almost exclusively contractions; with no detectable effect at repeats of non-pathogenic lengths. Contractions most likely arose from DNA gap intermediates rather than via single-strand break repair. We are currently testing whether this approach is applicable to patient-derived samples, specifically for the counteracting the cellular phenotypes of Myotonic Dystrophy type 1 (DM1). In addition, we are designing tools for viral-based delivery of Cas9 nickase orthologues in vivo.

Discussion: Our results will shed light on whether the Cas9 nickase and a single guide RNA targeted to the expanded repeat is a promising gene-therapy-based treatment for DM1 as well as 13 other genetic disorders.

Grant Support: This work is supported by a Swiss National Foundation professorship ('144789) and a grant from the Gebert Rüf Stiftung (GRS-060/14) to V.D.

P-005

Session 1: Microsatellite Instability and Epigenetics

CpG Methylation, a Hallmark for Congenital Myotonic Dystrophy Type 1, is Allele-length Dependent

MORALES Fernando[1], ZHANG Baili[2], CORRALES Eyleen[1], VÁSQUEZ Melissa[1], SANTAMARÍA Carolina[1], SIRITO Mario[2], ESTECIO Marcos[3], KRAHE Ralf[2] & MONCKTON Darren G[4]

1. Instituto de Investigaciones en Salud/Universidad de Costa Rica, Costa Rica
2. Department of Genetics University of Texas MD Anderson Cancer Center, USA
3. Department of Epigenetics & Molecular Carcinogenesis, University of Texas MD Anderson Cancer Center, USA
4. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

Introduction: DNA methylation at the DMPK locus has been reported previously, but is understudied. Its relationship to clinical subtypes of DM1, CTG repeat expansion size and age, has just started to be elucidated.

Methods: Using blood DNA from 219 Costa Rican DM1 patients, we measured methylation levels of two CTCF sites (upstream/CTCF1 and downstream/CTCF2 of the DM1 mutation) previously implicated as differentially methylated in CDM1, by Pyrosequencing-based methylation analysis.

Results: We observed significant association between estimated progenitor allele length (ePAL) size and CDM1 phenotype ($p<0.0001$). ROC curve analysis showed that patients with ePAL>(CTG)566 have an increased probability of developing CDM1. The overlap between different clinical subtypes was less than 20% ($p<0.0001$). Methylation at both CTCF sites was different by DM1 clinical subtype ($p<0.0001$), being almost exclusively present in CDM1. Levels of methylation were dependent on the clinical subtype, age and ePAL size ($p<0.0001$), suggesting that methylation, a hallmark of CDM1, could be a consequence of ePAL size. Both ePAL size and intergenerational CTG increment were significantly associated with methylation status ($p<0.0001$). Based on ROC curve analysis, DM1 patients with ePAL>(CTG)608 and an intergenerational (CTG)n increase <562 repeats, had an increased probability for a methylated mutant allele. Finally, there was strong association between methylation and maternal inheritance ($p<0.0001$), with almost exclusively maternal transmission (20 of 22 cases, 91%).

Discussion: ePAL size was associated with the CDM1 phenotype. DNA methylation at the CTCF sites flanking the DM1 expansion could be a consequence of ePAL size and intergenerational CTG increase, and was almost exclusively transmitted through the maternal germline. What the underlying cause for this parent-of-origin effect is remains to be determined.

Grant Support: Universidad de Costa Rica & Muscular Dystrophy Association, USA

P-006

Session 1: Microsatellite Instability and Epigenetics

Tissue-specific CTG·CAG Instability in Muscle Modifies Age of Onset in Myotonic Dystrophy Type 1

MORALES Fernando[1], CORRALES Eyleen[1], VÁSQUEZ Melissa[1], ZHANG Baili[2], FERNÁNDEZ Huberth[3], ALVARADO Fernando[3], CORTÉS Sergio[3], SANTAMARÍA Carolina[1], Marigold Myotonic Dystrophy Biomarkers Discovery Initiative, KRAHE Ralf[2], MONCKTON Darren G[4]

1. Instituto de Investigaciones en Salud/Universidad de Costa Rica
2. Hospital Calderon Guardia, Escuela de Medicina, Universidad de Costa Rica
3. Department of Genetics, University of Texas MD Anderson Cancer Center
4. Institute of Molecular Cell and Systems Biology, College of Medical Veterinary and Life Sciences, University of Glasgow

Introduction: Previously, we demonstrated that individual-specific variation in somatic instability of CTG repeat length measured in blood DNA modifies the relative severity of symptoms in DM1. However, the haematopoietic system is not a major target for disease pathology and blood shows relatively low levels of somatic mosaicism compared to other tissues. In contrast, muscle is a primary target of pathology and most patients present with gains of thousands of repeats in muscle. We thus hypothesised that individual-specific expansion rates in muscle would also modify disease severity in DM1.

Methods: We estimated the progenitor allele length (ePAL) in blood and modal allele lengths in blood, buccal, skin and muscle cells of DM1 patients. We also analysed muscle strength and histopathology.

Results: We observed a significant correlation between muscle weakness and histopathology ($p < 0.0001$). Regarding somatic instability, we observed that patients with ePALs < 100 CTG repeats showed a similar modal allele length in all tissues. In contrast, in all patients with estimated progenitor allele lengths > 100 repeats we observed large expansions in skin (averaging ~ 2,000 repeats) and even larger expansions in muscle (averaging ~ 2,500 repeats)($p < 0.001$). As expected, there was positive correlation between ePAL and modal allele length in skin and muscle ($p < 0.001$). However, we did not observe the expected positive age effect on allele length, most likely as a function of the highly non-random sampling of older patients with small ePALs. Most importantly, we observed that patients with larger relative expansions in muscle presented at an earlier age of onset than predicted by ePAL alone ($p < 0.001$).

Discussion: Individual-specific somatic instability in muscle contributes directly toward variation in disease severity in DM1.

Grant Support: This work was funded by the University of Costa Rica, The Marigold Foundation and the Association Francaise contre les Myopathies.

P-007

Session 1: Microsatellite Instability and Epigenetics

Possibilities and Limitations of Massively Parallel Sequencing in Characterisation of Microsatellite Loci

BUDIS Jaroslav[1,2], KUCHARIK Marcel[3], DURIS Frantisek[1,4], KADASI Ludevit[5,6], ZRUBCOVA Michaela[6], FICEK Andrej[6],SZEMES Tomas[2,6], **RADVANSZKY Jan**[2,5]

1. Faculty of Mathematics, Physics and Informatics, Comenius University, Bratislava, Slovakia
2. Geneton s.r.o., Bratislava, Slovakia
3. Medirex a. s., Bratislava, Slovakia
4. Slovak Centre of Scientific and Technical Information, Bratislava, Slovakia
5. Institute for Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences Bratislava, Slovakia
6. Department of Molecular Biology, Faculty of Natural Sciences, Comenius University, Bratislava, Slovakia

Introduction: In repeat-expansion disorders (REDs) both normal and mutation range repeat numbers are highly variable, posing a challenge for molecular diagnostics. In the majority of REDs there is still no single method that would allow identification and sizing of the entire allele range. Therefore, at least two complementing methods are generally required, while sequencing has no special place among them. Using myotonic dystrophy (DM) as a model, we developed an STR genotyping algorithm that allows massively parallel sequencing (MPS) to be applied, as a first tier test, for the exclusion of the presence of REDs associated expansions.

Methods: Our STR typing algorithm was tested on MPS data from a commercial sequencing panel. Repeat number estimations for several REDs associated loci, including the DMPK gene, were performed from FASTQ data using our specialized in house algorithm. Our automated pipeline allows extraction of the most probable genotyping results and visualize them in a readable form.

Results: As second generation sequencing (SGS) has standard amplification derived limitations, expanded alleles remain generally un-amplified and un-detected, making SGS-based testing un-useful for DM1 diagnosis confirmation. Assessed genotypes of normal-range alleles were, however, highly concordant with those generated by conventional methods, suggesting that SGS allows diagnosis exclusion if two normal-range alleles are identified. Moreover a multiple sequence alignment viewer and a sequence logo module of our tool allows better visualization of the exact sequence of the motif of each allele, their phasing and also their possible interruptions.

Discussion: Reliable STR genotyping/annotation tools have the potential to extend the possibilities of MPS not only into the field of REDs molecular diagnostics but also into research settings by allowing sequence characterisation of the motif structures beyond simple estimation of their length

Grant Support: Supported by VEGA_2/0115/15

P-008

Session 1: Microsatellite Instability and Epigenetics

A Single CAG Interruption in 5' End of CTG Repeats is Associated with Contractions Across Generations and Stabilization of the Repeat in Blood

TOME Stéphanie[1,2], DANDELOT Elodie[1,2], DOGAN Céline[3*], GENEVIEVE David[4], PEREON Yann[5], BERTRAND Alexis[1,2], ARNAUD Pauline[6], CHASSERIAU Raphaele[5], CINTAS Pascal[7], COBO ESTEBAN Ana-Maria[8], CRUZ Marie-Carmen[7], HAMROUN Dalil[9], MAGOT Armelle[5], SIMON Marie[4], URTIZBEREA Andoni[8], BONNEFONT Jean-Paul[4], BASSEZ Guillaume[3] and GOURDON Geneviève[1,2]

1. Inserm UMR1163, Laboratory of CTG Repeat Instability and Myotonic Dystrophy, Paris, France
2. Paris Descartes – Sorbonne Paris Cité University, Paris, France
3. Neuromuscular Reference Center, INSERM U955, Henri Mondor Hospital, Créteil, France
4. Molecular genetic laboratory, Necker Hospital, Paris, France
5. Neuromuscular Reference Center, Hôtel-Dieu Hospital, Nantes, France
6. Department of genetic, Bichat Hospital, Paris, France
7. Neuromuscular Reference Center, Purpan Hospital, Toulouse, France
8. Neuromuscular Reference Center, Marin Hospital, Hendaye, France
9. Centre Hospitalo-Universitaire de Montpellier, Montpellier, France

Introduction: In myotonic dystrophy type 1 (DM1), CTG repeats are unstable in both germline and somatic cells, with a typical bias towards expansions in >90% of parental transmissions. However, some contractions are observed in 10% of the paternal transmissions and only 3% in the maternal transmissions. Our aim is to identify new factors involved in the formation of CTG contractions and to decipher the mechanisms promoting CTG repeat contractions in DM1 patients.

Methods: We analyzed DMPK and CTG repeat sequences in blood from two French DM1 families that show a striking decrease of the CTG repeat length over successive maternal transmissions using triplet-primed PCR and direct sequencing. Both 5' and 3' ends of the CTG expansions were analyzed. We further evaluated somatic instability in blood from these DM1 patients compared to DM1 patients carrying uninterrupted repeats matched in age and in CTG repeat lengths.

Results: We found a single CAG interruption in 5' of the CTG expansion associated with the apparent CTG repeat contraction observed through maternal transmissions in one DM1 family and three 5' CCG interruptions in the other. We also observed that 5' interrupted CTG repeats are stabilized in blood. Furthermore, our results showed that intergenerational instability occurs preferentially in 3' of the interruptions, which gives new information about the mechanism involved.

Discussion: Our data strongly suggest that the presence of a single CAG interruption at the 5'end might be sufficient to stabilize CTG repeats and/or generate CTG repeat contractions. Nonetheless, we cannot exclude the role of trans –acting genetic factor in this family but also in the family carrying the 5' CCG interruptions. Our future aim is to characterize the mechanisms by which the various interruptions promote contractions or stabilization of the repeats. The results should improve the molecular diagnostic and prognosis and should give clues on new possible therapeutic strategies.

Grant Support: This work is supported by grants from AFM-Téléthon 19757, Inserm, Paris Descartes University and the French Ministry of Higher Education and Research.

P-009

Session 2: Disease Mechanisms: DM1, DM2 and CDM

SCN4A as Modifier Gene in DM2 Patients with Early and Severe Myotonia

BINDA Anna[1], RENNA Laura Valentina[2], FOSSATI Barbara[3], BOSÈ Francesca[2], BOTTA Annalisa[4], VALAPERTA Rea[5], MEOLA Giovanni[2,3,6], RIVOLTA Ilaria[1], CARDANI Rosanna[2]

1. School of Medicine and Surgery, University of Milano Bicocca, Monza, Italy
2. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico San Donato, Milan, Italy
3. Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Milan, Italy
4. Department of Biomedicine and Prevention, Tor Vergata University of Rome, Rome, Italy
5. Research Laboratories, IRCCS Policlinico San Donato, Milan, Italy
6. Department of Biomedical Sciences for Health, IRCCS Policlinico San Donato, University of Milan, Italy

Introduction: Myotonia is mild and inconsistent in myotonic dystrophy type 2 (DM2) and it has been correlated with the disruption of the alternative splicing of CLCN1. Mutations in CLCN1 and SCN4A genes can act as modifiers in these patients leading to an amplification of their myotonic symptoms. A 32 years old DM2 patient with an early severe myotonia since he was 12 years old came to our Neuromuscular Center. Mexiletine treatment resulted ineffective. No mutation was found on CLCN1 gene, but SCN4A gene showed G2717C base exchange, a variant considered a benign polymorphism. In the proband mother, also affected by DM2 but without the SCN4A polymorphism, no clinical myotonia was observed. The aim of the study was to compare the sodium current properties in myoblasts and the action potential features in myotubes derived from the proband and his mother muscle biopsies.

Methods: Patch clamp in voltage and in current clamp mode was used for electrophysiological recordings.

Results: Preliminary results in myoblasts showed no change in the steady state activation properties, but a significant shift in the availability curve (V1/2 -73,9.2±0,2 mV n=8 and V1/2 -78,7±0,2 mV n=9 proband and mother respectively). No differences were found in the recovery from the fast inactivation. In myotubes, the minimum current necessary to elicit an action potential was lower in the proband than in his mother (272,6±6 pA n=9 and 462,1±130 pA, n=6), respectively.

Discussion: We suggest that SCN4A polymorphism induces a more excitable substrate potentially aggravating the effect of the DM2 mutations in our patient. This finding suggests that SCN4A gene screening should be performed in DM2 patients with early and severe myotonia without mutations in CLCN1 gene. Moreover, when clinical features are uncommon, additional genes and/or modifying factors need to be explored to account for the phenotype. The detection of modifying factors may have important clinical implication such as the identification of appropriate drug treatment.

Grant Support: FMM, FAR

P-010

Session 2: Disease Mechanisms: DM1, DM2 and CDM

A Role for the Receptor for Advanced Glycation End Products (RAGE) in the Development of Metabolic Abnormalities in Myotonic Dystrophy

DOZIO Elena[1,2], RENNA Laura Valentina[3], VIANELLO Elena[1], CORSI ROMANELLI Massimiliano Marco[1], MEOLA Giovanni[1,3,4], **CARDANI Rosanna**[3]

1. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
2. Laboratory of Molecular Pathology, IRCCS, Policlinico San Donato, Milan, Italy
3. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico San Donato, Milan, Italy
4. Department of Neurology, IRCCS-Policlinico San Donato, Milan, Italy

Introduction: Myotonic dystrophies (DM) are inherited neuromuscular disorders characterized by metabolic dysfunctions and cardiovascular diseases. Receptor for advanced glycation end products (receptor for AGE, RAGE) is a cell-surface protein initially identified as a receptor for AGE, but also able to bind other nonglycated molecules, like S100/calgranulin family of peptides, High Mobility Group Box 1 protein, amyloid-′ peptide and macrophage-1 antigen. Ligand engagement of RAGE induces oxidative stress and promotes inflammation, two mechanisms strongly related to cardio-metabolic disorders. RAGE also exists as a soluble circulating molecule (sRAGE), a decoy receptor able to prevent ligand binding at cellular level and therefore the induction of the inflammatory response. We aimed to study the RAGE axis and its association with oxidative stress in DM patients.

Methods: Plasma sRAGE and 8OHdG (8-hydroxy-2′-deoxyguanosine) levels, used as a marker of oxidative stress, were quantified by ELISA assays in 47 non diabetic DM (37 DM1, 10 DM2) patients and 32 age-matched healthy subjects (CTR). Western blot was used to analyse skeletal muscle RAGE expression in DM, CTR and type 2 diabetic patients (T2DM).

Results: In DM patients sRAGE plasma levels were significantly reduced compared to CTR ($p<0.05$). On the contrary, 8OHdG plasma levels were significantly increased ($p<0.05$). No differences were observed between DM1 and DM2. In DM patients displaying low plasma sRAGE levels, skeletal muscle expression of RAGE was similar to that observed in T2DM patients and higher than than observed in DM patients with high sRAGE levels.

Discussion: Reduced plasma sRAGE levels are associated to increased oxidative stress and RAGE hyper-expression in muscle samples. sRAGE could be a potential soluble marker which suggests the activation of RAGE-mediated oxidative stress in skeletal muscle of DM patients.

Grant Support: FMM-Fondazione Malattie Miotoniche.

P-011

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Detection of RAN Translation from Intronic CCUG Repeats in Muscle Tissue

CARRELL Ellie[1], TANG Zhenzhi[1], THORNTON Charles A[1]

1. Department of Neurology, University of Rochester, Rochester, NY

Introduction: Repeat-associated non-AUG (RAN) translation occurs in numerous nucleotide repeat diseases. RAN translation has been mainly studied in cell culture and the CNS, in the context of expanded repeats in 5' UTRs or first introns (Cleary, 2017). Expanded CCUG repeats (CCUGexp) in DM2 encode (LPAC)n peptide in all reading frames. We studied RAN translation of CCUGexp RNA in muscle fibers.

Methods: Rabbit polyclonal antibodies were raised against (LPAC)4 and affinity purified. Expanded CCTG repeats were inserted at different locations in UBA52 minigenes, a gene selected for compact size, strong basal expression, and tolerability for overexpression. Constructs were introduced into muscle fibers by in vivo electroporation. Muscle tissue was obtained from transgenic mice expressing (CCUG)1,100 in intron 3 or in the 3' UTR of human skeletal actin (HSA) transgenes.

Results: Affinity-purified anti-LPAC antibodies generated specific perinuclear/cytoplasmic staining on HEK293 cells or muscle sections expressing ATG-(CCTG)280. When monitored by reporter activity after electroporation of UBA52 constructs into hindlimb muscle, RAN translation was detected for (CCUG)280 in the 5' UTR or intron 2, but not for (CCUG)140. RAN translation was not detected for (CCUG)280 in the 3' UTR. In contrast, transgenic mice expressing (CCUG)1,100 in intron 3 or the 3' UTR of HSA failed to show RAN peptides in tissue sections, despite high levels of CCUGexp accumulation.

Discussion: RAN translation was detected for intronic CCUG repeats when electroporated into skeletal muscle, but not for genomic constructs expressing longer repeats at higher levels. Circumstances permissive for RAN translation of intronic CCUG repeats are being examined.

Grant Support: National Institutes of Health grants NS094393 and NS048843

P-012

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Electromechanical Delay During Goal-directed Movements in Myotonic Dystrophy Type 1

CHRISTOU Evangelos A[1], YACOUBI Basma[1], CASAMENTO-MORAN Agostina[1], LOTT Donovan[1], SUBRAMONY Subramony H[1]

1. University of Florida

Introduction: Myotonic dystrophy type 1 (DM1), the most common inherited muscular dystrophy, affects both the central nervous system (CNS) and muscle. Recent evidence suggests that the electromechanical delay (EMD), which is the transformation of the neural signal to muscle force and consequently movement is longer in DM1. However, it remains unknown whether a longer EMD in DM1 impairs walking and whether it is related to a central (neurochemical) or peripheral (mechanical) deficiency.

Methods: Eight individuals diagnosed with DM1 (38.6 ± 12.2 years, 3 women) and 8 age-matched controls (36.6 ± 16.8 years, 3 women) performed 50 trials of fast goal directed movements with ankle dorsiflexion aiming at a spatiotemporal target (9o at 180 ms). We recorded the electromyographic activity (EMG) of the primary agonist (tibialis anterior; TA) and antagonist (soleus; SOL) muscles and quantified the following: 1) EMD: latency from the onset of TA EMG to onset of movement; 2) time to peak TA EMG (ttpTA): latency from the onset to the peak of TA EMG; 3) time to peak movement (ttpM): latency from onset of movement to peak spatial displacement. We assessed function in DM1 with the 6-minute walk test.

Results: EMD was significantly longer in DM1 patients than healthy controls (158.7 ± 18.4 vs 82.6 ± 9.5 ms; $P<0.01$). Longer EMD was associated with slower walking in DM1 ($R2=0.5$). The ttpTA was significantly longer in DM1 than controls (148.6 ± 10.9 vs 119.2 ± 4.8; $P<0.01$) and was strongly associated with longer EMD ($R2=0.8$, $P<0.01$). In contrast, the ttpM was similar for DM1 and controls (186.5 ± 8.1 vs 180.1 ± 6.7; $P<0.01$) and was weakly associated with a longer EMD ($R2=0.24$, $P<0.01$).

Discussion: The longer EMD in DM1 is associated primarily with a neurochemical (assessed with ttpTA) than a mechanical (assessed with ttpM) deficiency and contributes to slower walking.

Grant Support: None.

P-013

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Elevated Antisense Transcript Levels in Myotonic Dystrophy Type 2 Patients

CLEARY John D[1], BUBENIK Jodi L[1], ZU Tao[1], LIU Yuanjing[1], BANEZ-CORONEL Monica[1], XIA Guangbin[1,2,3], ASHIZAWA Tetsuo[4], SWANSON Maurice S[1,5], RANUM Laura PW[1,2,5]

1. Center for NeuroGenetics and Department of Molecular Genetics and Microbiology College of Medicine, University of Florida
2. Department of Neurology, College of Medicine, University of Florida
3. McKnight Brain Institute, University of Florida
4. Neurological Institute Houston Methodist Hospital, Houston, Texas
5. Genetics Institute, University of Florida

Introduction: Myotonic dystrophy type 2 (DM2) is a multisystemic adult-onset disease caused by a CCTG•CAGG repeat expansion mutation in intron 1 of the cellular nucleic acid-binding protein gene. DM2 is thought to be caused by RNA gain-of-function effects in which expansions transcripts sequester and dysregulate RNA binding proteins. Multiple expansion mutations, including DM1, are bidirectionally transcribed and under repeat associated non-ATG (RAN) translation. To test if antisense CAGG RNAs play a role in DM2, we examined RNA from DM2 patient and control autopsy tissue.

Methods: We performed RT-PCR on frontal cortex samples from DM2 and control brain tissue. Strand-specific RT-PCR was performed using primers downstream of the antisense CAGG expansion by semi-quantitative- and qPCR. RNA FISH, UV crosslinking and cross-linking IP (CLIP) was used to characterize the DM2 CAGG antisense transcripts.

Results: We show that antisense transcript levels are dramatically increased (~ 5-20 fold) in frontal cortex from DM2 cases compared to controls. We performed RNA FISH on the same samples but found no convincing RNA foci. UV crosslinking, PAGE and CLIP analysis shows CAGG and CCUG transcripts bind distinct proteins: CLIP confirmed that CCUG transcripts bind MBNL1, while CAGG expansions did not crosslink with MBNL1. CLIP analysis identified hnRNPA1 as a novel CAGG RNA binding protein. We also show that CAGG antisense transcripts undergo RAN translation to produce RAN proteins with Gln-Ala-Gly-Arg repeat motifs (see Zu et al abstract).

Discussion: We show that both sense and antisense transcripts are expressed, with CAGG antisense transcripts upregulated in DM2 versus control frontal cortex. The elevated antisense transcripts level in affected brain tissue is also seen in C9orf72 ALS/FTD suggesting that repeat expansions may increase antisense transcription and/or the stability of the antisense transcript.

Grant Support: NIH NINDS PO1-NS058901 (LWR, MS, HBC), the Muscular Dystrophy Association (LWR) and a Wyck Foundation/Myotonic Dystrophy Foundation Postdoctoral Fellowship (JDC)

P-014

Session 2: Disease Mechanisms: DM1, DM2 and CDM

The Contribution of Nuclear and Cytoplasmic CELF1 Protein to Muscle Wasting in Myotonic Dystrophy Type 1

COX Diana[1,2], COOPER Thomas[1,2]

1. Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA
2. Department of Biochemistry and Molecular Biology, Baylor College of Medicine, Houston, TX, USA

Introduction: Myotonic dystrophy type I (DM1) is caused by expansion of a CTG repeat in the 3' untranslated region of the Dystrophia Myotonica-Protein Kinase (DMPK) gene. The primary cause of pathogenesis is the expanded repeat (CUGexp) RNA expressed from the expanded allele. The CUGexp RNA accumulates in nuclear foci and affects the functions of at least two families of RNA binding proteins: muscleblind-like (MBNL) and CUGBP Elav-like family (CELF). MBNL is sequestered on CUGexp RNA foci producing a loss of function. The contribution of MBNL to myotonic dystrophy has been well established through the generation of MBNL knockout mice. CUGexp RNA also induces post-transcriptional up regulation of CELF1, producing a gain of function.

Methods: Our lab used inducible transgenic mice that overexpress CELF1 in adult skeletal muscle to demonstrate that CELF1 up regulation is pathogenic and reproduces DM1-like muscle phenotypes, including muscle wasting and dystrophic muscle histology. CELF1 functions in the nucleus as a splicing regulator and in the cytoplasm as a regulator of mRNA stability and translation. I have recently generated and tested transgenic mice for skeletal muscle-specific and tetracycline-inducible expression of active CELF1 derivatives localized exclusively to either the nucleus or the cytoplasm.

Results: My preliminary results show that mice expressing the nuclear but not cytoplasmic derivative of CELF1 exhibit a strong histological phenotype suggesting an exclusive role for nuclear CELF1 functions in DM1 pathogenesis.

Discussion: Muscle from early time points following induction will be used for transcriptome analysis by RNA-seq that will be filtered against RNA-seq data from an in-house mouse model expressing CUGexp RNA that exhibits muscle wasting and from DM1 muscle samples to identify specific molecular targets of CELF1 the mediate skeletal muscle features of the disease.

Grant Support: This work is funded by NIH R01AR045653 and MDA 276796.

P-015

Session 2: Disease Mechanisms: DM1, DM2 and CDM

The Impact of Alternative Exon 7 of NFIX on Transcriptional Activity of its Protein: Consequences for DM

CYWONIUK Piotr[1], SOBCZAK Krzysztof[1]

1. Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, Poland

Introduction: Myotonic dystrophy is multisystemic disorder caused by expansion of CTG (DM1) or CCTG (DM2) repeats in specific genes. Mutated RNA having expanded repeats are localized in cell nuclei and sequester MBNL proteins what leads to abnormalities in alternative splicing pattern of many genes. One of the misregulated gene is NFIX which alternative exon 7 is negatively regulated by MBNLs. The aim of this project was to understand what are the consequences of exon 7 inclusion on activity of NFIX transcription factor.

Methods: We performed a series of experiments with luciferase constructs being under control of NFIX-sensitive promoter derived from IGFBP5. Cells were co-transfected with NFIX isoforms having or not exon 7 or with antisense oligonucleotide (AON) which induces alternative exon 7 skipping in endogeneous NFIX mRNA. We also studied the influence of abnormal splicing of NFIX on transcriptome changes in DM1 and DM2 muscles using the results of whole-transcriptome studies.

Results: IGFBP5 luciferase construct revealed higher transcriptional activity of exogenous NFIX isoform without exon 7. Silencing of MBNLs and AON-based skipping of exon 7 confirm this observation. We also found that expression level of several genes considered to be NFIX-dependent is correlated with NFIX exon 7 inclusion in skeletal muscles of DM patients and healthy controls.

Discussion: NFIX isoform without alternative exon 7 shows higher transcriptional activity. Abnormal splicing of NFIX may contribute to pathomechanism of DM by changes in expression of many NFIX-sensitive genes including ENO3, DES, DUSP26, etc.

Grant Support: Foundation for Polish Science TEAM program, the Polish National Science Centre 2014/15/B/NZ2/02453 and Ministry of Science and Higher Education of the Republic of Poland under the Leading National Research Centre (KNOW programme).

P-016

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Consequences of ATP2A1 Missplicing on Muscle Function

DE DEA DINIZ Damily[1], SUREAU Alain[1], ARANDEL Ludovic[1], RAU Frederique[1], MARIE Joelle[1], KLEIN Arnaud[1], FURLING Denis[1]

1. Sorbonne Universités UPMC, Inserm Centre de Recherche en Myologie, Institut de Myologie, Paris, France

Introduction: SERCA1 encoded by the ATP2A1 gene is involved in the excitation-contraction coupling and relaxation of the striated muscle by acting as one of the major Ca2+ ATPase responsible for the re-uptake of cytosolic Ca2+ into the sarcoplasmic reticulum. Developmental regulation of ATP2A1 exon 22 by alternative splicing leads to two distinct isoforms that are expressed either in adult (SERCA1a) or fetal (SERCA1b) tissues. This splicing switch modifies the C-terminus domain of SERCA1 but also ATPase and Ca2+ uptake activities, which are reduced for SERCA1b when compared to SERCA1a. ATP2A1 exon 22 is misspliced in affected muscles of DM1 patients however its consequence on skeletal muscle function is poorly understood.

Methods: To investigate the impact of fetal SERCA1b re-expression in adult muscles, we propose to mimic ATP2A1 exon 22 missplicing in wild-type animal models using antisense approaches.

Results: An in vitro analysis using antisense oligonucleotides and splicing regulator factors was first performed to determine the more potent region/antisense sequence(s) allowing significant skipping of exon 22. Next, selected antisense sequences were cloned into a modified U7-snRNA cassette and vectorized in adeno-associated viral vectors. Currently, adult skeletal muscles of wt mice were transduced with theses AAV-U7 antisense vectors to determine their efficacy. In parallel, we used the ZebraFish model (Danio rerio) in which corresponding exon 22 is conserved and fully included one day post-fertilization. Antisense morpholinos were microinjected in 1-2 cell stage eggs and 2-5 day-old morphants were analyzed. Preliminary results show an exclusion of the corresponding exon 22 from and consequences at both physiological and morphological levels are under evaluation

Discussion: This study should help us to better understand the consequences of abnormal SERCA1b re-expression on adult muscle function and its implication in DM1 pathophysiology

Grant Support: AFM / Institut de Myologie

P-017

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Does Gender Affect Proteomics and Histomorphology of Skeletal Muscle in Myotonic Dystrophy Ype 1

ROUSSEL Marie-Pier[1,2], HOULE Olivier[1], MORIN Marika[1], JORG Marylie[1], DUFRESNE Sébastien[1,2], GAGNON Cynthia[2,3] and **DUCHESNE Elise**[1,2]

1. Université du Québec à Chicoutimi
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires
3. Université de Sherbrooke

Introduction: Myotonic dystrophy type 1 (DM1), is the most frequent autosomal dominant myopathy in adults. The DM1 adult phenotype displays great heterogeneity in symptoms, namely muscle weakness, and gender has been reported as a factor influencing its clinical profile. However, it remains unknown if gender affects proteomics and histomorphology of skeletal muscle in DM1.

Methods: Muscle biopsies were taken in the Vastus lateralis of 7 women and 9 men (48.0 ± 4.0 and 49.5 ± 3.8 years old, respectively). Western blots were used to assess the level of expression of markers of muscle protein synthesis (p-AKT/AKT and p-mTOR/mTOR) and breakdown (MuRF1 and Atrogin-1). In another set of experiments, histological and immunohistological stainings have permitted to assess the proportion of centrally nucleated fiber (CNF) (hematoxylin and eosin), cross-sectional area (CSA) and proportion of type I and II myofibers (anti-skeletal fast isoform labelling) and percentage of collagen (masson's trichrome) and fat infiltration (red oil). Analyses were carried out using the ImageJ system.

Results: Women have presented a higher catabolic profile when compared with men as demonstrated by the increased level of expression of MuRF1 (m: 0.73 fold ± 0.05 vs w: 1.45 fold ± 0.08; p=0.00000046) and Atrogin-1 (m: 1.01 fold ± 0.09 vs w: 1.67 fold ± 0.39; p=0.13). No significant differences were reported between gender for the percentage of CNF (m: 7.27% vs w: 7.13%), fibrosis area (m: 0.90% vs w: 0.96%), fat area (m: 0.48% vs w: 0.65%) and the proportion of type I and II myofibers (m: 44% [type I] and 58% [type II] vs w: 32% [type I] and 68% [type II]). Myofiber type I CSA, but not type II CSA, was significantly smaller for women (0.0031 mm2 vs 0.0058 mm2 for men; p=0.0116).

Discussion: These results suggest that differences exist at histological and proteomics levels between men and women with DM1. Those must be taken into account in the development of clinical trials.

Grant Support: This research project has been supported by the Fondation de l'Université du Québec à Chicoutimi, Fonds de recherche du Québec-Santé, The Marigold Foundation, CORAMH and Fondation du Grand Défi Pierre Lavoie.

P-018

Session 2: Disease Mechanisms: DM1, DM2 and CDM

An Engineered RNA Binding Protein with Improved Splicing Regulation of Myotonic Dystrophy Specific Mis-splicing Events

HALE Melissa[1,2], RICHARDSON Jared[1,2], DAY Ryan[1,2], WANG Eric[2,2], BERGLUND[1,2] J Andrew

1. Department of Biochemistry and Molecular Biology, University of Florida
2. Center for NeuroGenetics, University of Florida.
3. Department of Molecular Genetics and Microbiology, University of Florida

Introduction: The muscleblind-like (MBNL) family of proteins are key developmental regulators of alternative splicing. MBNL1 contains four zinc finger (ZF) motifs that form two tandem RNA binding domains (ZF1-2 and ZF3-4) that each bind YGCY RNA motifs. To better understand the difference between these two domains and develop improved proteins as potential therapeutics for myotonic dystrophy (DM), we designed synthetic MBNL1 proteins with duplicate ZF1-2 or ZF3-4 domains, referred to as MBNL1(1-2,1-2) and MBNL1(3-4,3-4), respectively.

Methods: Cell-based splicing assays, western blots, EMSAs, and RNA Bind-n-seq (RBNS) analysis were used to determine the splicing activity, RNA binding affinity and specificity of wild-type (WT) and synthetic MBNL1s.

Results: Biochemical characterization of these engineered proteins revealed significant differences in the activities of each ZF domain. Analysis of splicing regulation by the two synthetic proteins compared to WT MBNL1 revealed that MBNL1(1-2,1-2) had 5-fold increased splicing activity while MBNL1(3-4,3-4) had 4-fold decreased activity. RBNS showed that the differences in splicing activity were due to differences in RNA binding specificities between the two ZF domains and was not dictated by binding affinity. ZF1-2 binds YGCY motifs with high specificity while ZF3-4 acts as a more general RNA binding domain.

Discussion: Our findings indicate that ZF1-2 drives alternative splicing via recognition of canonical YGCY RNA motifs and ZF3-4 allows for MBNL1 to bind a wider array of RNA substrates via its reduced requirements for specific sequence recognition. Our studies also serve as a proof of principle that MBNL1 can tolerate modifications and retain function. Further rational design strategies to modify MBNL1 are being utilized to create more stable and active synthetic MBNL1 proteins for use as protein therapeutics for DM and other microsatellite diseases.

Grant Support: This research was supported by UF start-up funds to the Berglund Lab.

P-019

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Novel Splicing Changes are Identified in the Muscle of Children with Congenital Onset Myotonic Dystrophy

JOHNSON Nicholas[1], OTTERUD BRITH[1], MCCORQUODALE Donald[1], MAYNE Katie[1], POLANSKY Caitlin[1], DUNN Diane[2], IMBURGIA Carina[1], WEISS Robert[2]

1. Department of Neurology, University of Utah
2. Department of Human Genetics, University of Utah

Introduction: Congenital myotonic dystrophy (CDM) is the most severe form of myotonic dystrophy type-1 (DM1). In adults with DM1, the toxic RNA repeat sequesters MBNL1 and results in aberrant RNA splicing of many transcripts. It is unclear if CDM is the result of mis-regulation of RNA splicing, and which transcripts are targeted.

Methods: Muscle biopsies were obtained from children with CDM, adults with DM1, healthy adult controls, and pathologically normal pediatric muscle. RNA was isolated and cDNA libraries were constructed. Paired-end RNA-Seq was performed. Analysis used MAJIQ to generate percent spliced in (PSI) values. Sample sets were analyzed with Weighted Gene Co-expression Network Analysis (WGCNA) to identify splicing patterns.

Results: 11 muscle biopsies from children with CDM (age 2 month-16 years), 9 pediatric controls (age 1 month-13 years), 16 adult DM1 patients (ages 29-57), and 6 adult healthy controls (ages 19-28) were used for analysis. MAJIQ identified 1900 splicing events with adequate read depth and a PSI>0.15. WGCNA identified 4 patterns of splicing. The majority of the splicing events (1214) were the same in cases of CDM and DM1. Previously reported splicing in events (e.g., INSR, CLCN1) were identified in children with CDM and did not vary with age. 111 splicing events varied with age. 140 events were unique to CDM, many (e.g., PALLD) specific to development.

Discussion: Children with CDM have the RNA splicing events previously identified in adults with DM1, despite a divergent phenotype. There are also a minority of splicing events that are specific to CDM, largely related to transcripts regulating development.

Grant Support: Research supported by the Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapeutics, Utah Neuromuscular Fund.

P-020

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Characterisation of Respiratory Dysfunction in a Cohort of Myotonic Dystrophy Type 1 Patients

HAWKINS Aaron M[1,2], ABDUL RAZAK Kavitha[1,2], KHOO Tien K[1,4], TRAN Khoa[1,3], JACKSON Richard V[1,2]

1. School of Medicine, Griffith University, Gold Coast, Queensland, Australia,
2. Department of Medicine, Logan Hospital, Meadowbrook, Queensland, Australia,
3. Department of Respiratory Medicine, Logan Hospital, Meadowbrook, Queensland, Australia
4. School of Medicine, University of Wollongong, New South Wales, Australia

Introduction: Respiratory manifestations of myotonic dystrophy type 1 (DM1) include restrictive lung disease, chronic alveolar hypoventilation and aspiration pneumonia. These have a significant impact on quality of life and mortality in DM1 patients. This prospective study aims to characterise the prevalence of respiratory dysfunction in a cohort of DM1 patients.

Methods: 26 DM1 patients were evaluated prospectively with history and examination, muscle weakness was assessed using Muscular Impairment Rating Scale (MIRS), SpO2 and venous blood gas both measured on room air, genetic analysis and Respiratory Function Tests including Maximal Inspiratory Pressure (MIP) and Maximal Expiratory Pressure (MEP) measurement. Base excess >=4 and elevated bicarbonate were used as markers for chronic hypercapnia.

Results: Mean age 43 ± 13.5 years, 65% male and 35% female. Forced Vital Capacity (FVC) was <80% predicted in 65% of patients, MIP and MEP were <50% of predicted in 58% and 88% of patients, respectively. 22% of patients had a base excess >=4.0. 9% had bicarbonate levels >33mmol/L. FVC was correlated with age (-0.398, p=0.044), MIRS Score (-0.592, p=0.001) and SpO2 (0.481, p=0.017). MIP and MEP correlated with each other (0.521, p=0.009). The group with base excess >=4.0 had a significantly higher age (Z=-2.051, p=0.040), MIRS score (Z=-2.605, p=0.009), and bicarbonate (Z=-3.080, p=0.002), plus a significantly lower FVC %predicted (Z=-2.834, p=0.005), MIP %predicted (Z=-1.749, p=0.080) and O2 Saturation (Z=-2.137, p=0.033). The group with bicarbonate >33mmol/L had significantly lower FVC % predicted (Z=-1.965, p=0.049). The group with MEP <50% had a significantly higher MIRS score (Z=-2.044, p=0.041). MIP <50% was not associated with total MIRS score (p=0.779). There was no significant correlation between CTG repeat length and FVC, MIP or MEP.

Discussion: Respiratory muscle dysfunction is common in DM1. Our findings suggest that more significant respiratory dysfunction is associated with increased age and muscle weakness but not with CTG repeat length.

Grant Support: None

P-021

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Post-Mortem Tissues of a Congenital Myotonic Dystrophy (CDM1) Infant Born Unto a CDM1 Mother

KEKA Islam Shamima[1], LANNI Stella[1], SHUEN Andrew Y[1], CHITAYAT David[2,3], MIRON Ioana[4], SHANNON Patrick[5], PEARSON Christopher E[1]

1. Genetics & Genome Biology Peter Gilgan Centre for Research & Learning The Hospital for Sick KIds
2. The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, University of Toronto
3. Division of Clinical and Metabolic Genetics, The Hospital for Sick Kids, Toronto
4. The Prenatal Diagnosis and Medical Genetics Program, Department of Obstetrics and Gynecology, University of Toronto
5. Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto

Introduction: Congenital myotonic dystrophy (CDM1), the most severe form of myotonic dystrophy type 1 (DM1), shows an extreme parent-of-origin effect with an almost complete bias for maternal transmission, often coinciding with transmissions of large CTG expansions in the DMPK gene. CDM1 symptoms appear in utero and are evident at birth. Many CDM1 children do not survive past their first birthday and have never been reported to procreate. The source of either the distinct clinical features of CDM1 or the cause of the maternal-bias have long remained unknown.

Methods: We received a rare three-generation CDM1 family, with the first known case of an affected CDM1 mother who gave birth to a CDM1 infant. The mother was diagnosed while in utero with CDM1, confirmed at birth, and presents >1000 CTG repeats. She became pregnant twice. The first child had CDM1 confirmed at birth, and had 2100 CTG repeats in the blood. The child passed two months after birth. The second pregnancy was terminated at 16-weeks.

Results: We harvested post-mortem tissues of the CDM1 infant, including various muscles, heart, various brain regions, liver, pancreas, and testis. We harvested CVS cells, fpetal muscle, brain, heart and testis from the second pregnancy. Samples are fresh frozen tissues, fixed tissues (sections) and tissue-derived cell lines. We also established lymphoblastoid and skin fibroblasts cell lines from the DM1-affected grandmother, the CDM1 mother and the non-affected father.

Discussion: Tissues and cell lines from clinically confirmed CDM1 individuals are extremely rare. This precious wide-spectrum collection presents a unique opportunity to gain insight into CDM1, by investigating RNA-toxicity, sequestered proteins, aberrant splicing, RAN-translation, transcriptome analysis, DNA methylation, epigenetics, tissue- and cell-type specificity, etc. Such findings should illuminate paths of CDM1 diagnosis, aetiology, and possibly treatment.

Grant Support: CIHR

P-022

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Genetic Analysis of Co-occurrence of Myotonic Dystrophy Type 1 (DM1) and Fuchs Endothelial Corneal Dystrophy in Patients

KUMARASWAMY Lakshmi Narayanan[1], TIWARI Anjana[1], ZHANG Nan[1], HASHEM Vera[1], SIMPSON Ericka[1,2,3], PANDIT Rahul T[3], ASHIZAWA Tetsuo[1,2,3]

1. Research Institute, The Methodist Hospital, Houston, Texas, USA
2. Neurological Institute, The Methodist Hospital, Houston, Texas, USA
3. Departments of Neurology and Ophthalmology, The Methodist Hospital, Houston, Texas, USA

Introduction: Expansions of CTG repeats in non-coding regions cause a handful of inherited diseases, including myotonic dystrophy type 1 (DM1), Fuchs endothelial corneal dystrophy (FECD) and spinocerebellar ataxia type 8. These disorders are characterized by a toxic RNA gain-of-function, where the expanded RNA sequesters a variety of RNA binding proteins to form insoluble nuclear foci. In DM1, the CTG expansion (5-4000 repeats) occurs in the 3'-UTR region of the DMPK gene, causing a multisystemic disorder. In FECD, the intronic CTG expansion (10-2600 repeats) in the TCF4 gene causes corneal endothelial cell degeneration, which may be found in up to 4% of the general population as a threat for vision loss. It's been known that mutation in the TCF4 gene leading to the 79% FECD. A clinical report has shown that some DM1 patients also exhibit FECD characteristics. We hypothesize that the two diseases could be pathologically connected at an RNA level by means of the shared CUG repeat expansion.

Methods: The study was approved by the Institutional Review Board of the Houston Methodist, all participants were enrolled after written consent. We have a father-daughter pair with the clinical diagnosis of FECD and known DM1 CTG repeat expansion.

Genotyping: Genomic DNA was extracted from leukocytes of the blood. Flanking PCR, Repeat primed PCR along with capillary electrophoresis were carried out for the analysis. Fluorescent in situ hybridization (FISH): Skin tissues of FECD patients were used to generate fibroblast cells and were used to study the RNA foci using Fluorescence-labeled CAG probe.

Results: Our preliminary study reveals that the CTG expansion in the TCF4 gene and that in the DMPK gene can coexist in the same individual and form RNA foci primarily in the nucleus.

Discussion: Our preliminary study reveals that expanded CUG repeat transcripts from the TFC4 gene and the DMPK gene can coexist in the same individual, altering phenotypes of neither DM1 nor FECD.

Grant Support: None

P-023

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Gene Expression Profiling Identifies Patterns of Muscle Specific Gene Musclin and Fiber Type Dysregulation in Adult and Congenital Myotonic Dystrophy

MCCORQUODALE Donald[1], MAYNE Katie[1], OTTERUD Brith[1], DUNN Diane[1], JOHNSON Nicholas[1], WEISS Robert[1]

1. Utah Program for Inhertited Neuromuscular Diseases (UPIN), University of Utah

Introduction: We employ RNA-Seq gene expression profiling to explore how gene expression may contribute the congenital (CDM) and adult myotonic dystrophy type 1 (DM1) phenotype.

Methods: Strand-specific cDNA libraries were prepared using rRNA-depleted total RNA isolated from skeletal muscle from CDM, DM1, healthy as well as muscle disease controls. RNA sequencing was performed using the Illumina HiSeq 2500 instrument. Gene expression analyses were performed with EdgeR/Bioconductor software.

Results: A number of disease-specific gene expression differences were observed in both CDM and DM1 as compared to age-matched controls. Here we focus on the observed up-regulation of musclin (OSTN), a muscle-specific gene that is regulated by the insulin receptor and is newly identified mediator of exercise endurance. This finding occurs in the setting of changes in genetic networks that include gene that contribute to muscle fiber type and mitochondrial regulation.

Discussion: Upregulation of musclin expression is a disease-specific change seen in both CDM and DM1. We describe how musclin may have a pathologic role in exercise intolerance in CDM/DM1, and how it may be a marker of more widespread abnormalities in expression levels of genes that determine fiber type in CMD/DM1.

Grant Support: K23 (Dr. Johnson, Health Endpoints and Longitudinal Progression in Congenital Myotonic Dystrophy, NINDS 1KSNS091511-01) and R21 (Dr. Weiss, NINDS 1R21NS100040-01).

P-024

Session 2: Disease Mechanisms: DM1, DM2 and CDM

A Longitudinal Study of Age Equivalent Receptive Communication and Performance on Functional Outcome Measures in Congenital Myotonic Dystrophy

MCINTYRE Melissa[1], DIXON Melissa M[1], PUCILLO Evan M[1], DIBELLA Deanna[1], CROCKETT Rebecca[1], HUNG Man[2], BOUNSANGA Jerry[2], BUTTERFIELD Russell J[2], CAMPBELL Craig[3], JOHNSON Nicholas E[1]

1. Department of Neurology, University of Utah
2. Department of Orthopedics, University of Utah
3. Department of Pediatrics, University of Western Ontario

Introduction: Children with congenital myotonic dystrophy (CDM) have intellectual, communication, and motor delays. It is not well studied how intellectual and communication delays affect performance on functional measures such as the four-stair climb (4SC), rise from floor (RFF) and 10-meter run (10MR) tests.

Methods: Participants with CDM and healthy controls (HC) were recruited in to three age-based cohorts. Motor performance was assessed in the two oldest cohorts using the 4SC, RFF, and 10MR. Each participant's Age Equivalent Receptive Communication was calculated using the Vineland Adaptive Behavior Scales-II. All assessments were performed at baseline, 12 months and 24 months.

Results: 49 children with CDM and 29 HC were enrolled. The 10MR time improved by 6.5% over 12 months. Participants with CDM performed a valid 4SC at 8.28 y/o, RFF at 8.03 y/o, and 10MR at 8.17 y/o. When adjusted for age equivalent receptive communication, participants performed a valid 4SC at 5.09 adjusted years, RFF at 5.23 adjusted years, and 10MR at 5.19 adjusted years. Additionally, each participant's chronological age suggested/showed significant weak-modest negative correlations for 4SC (r= -.39, p=.032) and 10MR (r= -.39, p=.034). There was not a significant correlation between participants age equivalent receptive communication and the 10MR, 4SC, or RFF.

Discussion: Chronological age shows a weak to modest correlation with performance on 4SC and 10MF. Age equivalent receptive communication does not appear to correlate with performance or improvement on these functional measures. This would suggest that performance on these functional measures is more closely associated with motor function and planning as opposed to receptive communication delays in this population.

Grant Support: Research funding supported by the Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapeutics, Utah Neuromuscular Fund.

P-025

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Mild Phenotype in DM1 Young Boy Due to Interrupted Repeat of the DMPK Expanded Tract

MEOLA Giovanni[1,2,3], VALAPERTA Rea[4], BOSÈ Francesca[3], FOSSATI Barbara[2], RENNA Laura Valentina[3], BRIGONZI Elisa[2], CARDANI Rosanna[3]

1. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
2. Department of Neurology, IRCCS-Policlinico San Donato, Milan, Italy
3. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico San Donato, Milan, Italy
4. Research Laboratories, IRCCS Policlinico San Donato, Milan, Italy

Introduction: Myotonic Dystrophy type 1 (DM1) is a multisystem autosomal dominant disorder caused by the expansion of (CTG)n in the 3'UTR of the DMPK gene. Several DM1 patients with different patterns of CCG/CTC/CGG interruptions at the 3' or 5'end of the DMPK expanded tract have been reported. However, the role of these interruptions in DM1 pathogenesis is still unclear. Recently, a clinically asymptomatic 14 years old boy came to our Neuromuscular Center with a positive genetic test for DM1 paternally transmitted. The aim of this study was to characterize the proband at clinical, histopathological and biomolecular level.

Methods: A global clinical evaluation was performed. The genetic test was performed using "Myotonic Dystrophy SB kit" followed by TP-PCR analysis. Histological analysis and FISH in combination with MBNL1 immunofluorescence were performed on tibialis anterior biopsy. Alternative splicing of several genes commonly involved in DM1 pathology was also analysed by RT-PCR.

Results: The proband was clinically asymptomatic and did not show delayed development nor history of intellectual or learning disabilities. EMG was also negative. The genetic test revealed the presence of expanded allele only in his father but neither in the proband nor in his mother. TP-PCR electropherogram results indicated the presence of an expanded allele in both directions (Forward and Reverse) in the father and of two normal alleles (12/15 CTG repeats) in the mother. A pathological allele with a 3' interruption in the TP-PCR Forward primer was evident in the proband. Proband's skeletal muscle analysis did not show histological alterations and FISH+MBNL1 immunofluorescence did not show nuclear accumulation of mutant RNA or MBNL1 protein. No alterations of splicing pattern of the genes analyzed were observed.

Discussion: Our results support the hypothesis that interrupted repeat within the DMPK expanded alleles can modulate the phenotype in DM1 patients.

Grant Support: FMM-Fondazione Malattie Miotoniche

P-026

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Towards Population-based Screening and Diagnosis in Myotonic Dystrophy

OYENIYI Tolulope[1], CUMMING Sarah[1], HAMILTON Graham[2], MONCKTON Darren[1]

1. Institute of Molecular, Cell and System Biology, University of Glasgow,
2. Glasgow Polyomics, Wolfson Wohl Cancer Research Center, University of Glasgow, UK

Introduction: Although the most common form of muscular dystrophy in adults, the true incidence of the myotonic dystrophies is unknown: DM1 is estimated as 1 in 8000, for DM2, no universal incidence estimate is known. DM is associated with a low reproductive fitness, expanded alleles are lost from the population thus a decreasing incidence is expected. However, this has been found to be untrue, suggesting that new cases must be arising from the premutation allele pool to replace lost mutant alleles. This research applies high throughput sequencing to initiate a faster cheaper, and accurate method for DM diagnosis on a population scale.

Methods: Traditional diagnostics employs conventional PCR-based fragment length analysis and capillary electrophoresis. Expansions beyond a certain threshold are not detectable using this method. Our method seeks to improve on this: we apply high throughput amplicon sequencing from conventional and repeat primed-PCR (RP-PCR) to detect DM expansions regardless of size.

Results: DM1: 250 known DM1-affected individuals previously genotyped by Southern hybridization were screened by RP-PCR: expansions were detected in 249 individuals (99.6% success). To further evaluate the sensitivity of the RP-PCR assay, 400 randomly sampled individuals of the general Scottish population, screened by conventional PCR and had no expansions were confirmed to truly have no expansions when screened by RP-PCR. This especially confirms the 67 homozygous individuals screened, to be true homozygotes. DM2: Conventional PCR was used to screen the same 400 individuals as in DM1. No expansions were detected. DM2 RP-PCR screening is ongoing for further confirmation of genotypes.

Discussion: NGS in combination with the PCR assays offers a platform for high throughput population screening. This would help to provide predictive-testing for pre-symptomatic individuals and to evaluate the distribution of alleles at the DM loci.

Grant Support: MDSG, Wellcome Trust, TETFund

P-027

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Receptor and Post-receptor Abnormalities Contribute to Insulin Resistance in Myotonic Dystrophy Type 1 and Type 2 Skeletal Muscle

RENNA Laura Valentina[1], BOSÈ Francesca[1], FOSSATI Barbara[2], CAVALLI Michele[3], MEOLA Giovanni[1,2,3], CARDANI Rosanna[1]

1. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico San Donato, Milan, Italy
2. Department of Neurology, IRCCS Policlinico San Donato, Milan, Italy
3. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Introduction: Myotonic dystrophies (DM) are neuromuscular multisystemic disorders that are characterized by metabolic dysfunctions such as insulin resistance, hyperinsulinemia and a fourfold higher risk of developing Diabetes mellitus type 2 (T2DM). Since it's known that insulin resistance is a risk factor for cardiovascular disease, neuropathy and loss of muscle mass, metabolic changes in DM patients might contribute to worsen some aspects of the disease at heart, skeletal muscle and brain level. Splicing alteration of insulin receptor (IR) gene is considered one of the causes of metabolic dysfunctions, however it cannot be excluded that post-receptor signalling abnormalities could also contribute to this feature of DM. The aim of this study is to investigate the mechanisms that contribute to the peripheral insulin resistance in DM patients.

Methods: The basal phosphorylation of AKT, p70S6K, GSK3′ and ERK1/2 have been analysed by western blot in proximal and distal muscles from 8 DM1, 5 DM2 and 7 healthy subjects. RT- PCR has been performed to evaluate IR alternative splicing. Insulin pathway activation has been investigated in insulin stimulated myotubes derived from muscle biopsies of 5 DM1, 5 DM2, 6 healthy and 2 T2DM subjects.

Results: Our results indicate that DM skeletal muscle exhibits high basal phosphorylation of AKT, GSK3′ and ERK1/2. Moreover, the in vitro analysis has shown that, despite the similar expression of fetal IR isoform, insulin action appears to be impaired in DM myotubes as compared to CTR in terms of protein activation and glucose uptake.

Discussion: In vivo and in vitro studies show an alteration in activation of several proteins of insulin pathway that might contribute to DM insulin resistance. Further investigations will be necessary to identify novel biomarkers that could be target for therapeutic intervention.

Grant Support: Wyck Foundation/Myotonic Dystrophy Foundation Fellowship award. FMM-Fondazione Malattie Miotoniche.

P-028

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Effect of Acute Eccentric Exercise on Skeletal Muscle Hypertrophy and Atrophy Signalling Pathways in Men with Myotonic Dystrophy Type 1

ROUSSEL Marie-Pier[1,2], HÉBERT Luc J[3,4], RIBEIRO Fernanda[1,2], SCHOSER Benedikt[5], GAGNON Cynthia[2,6] and DUCHESNE Elise[1,2]

1. Université du Québec à Chicoutimi
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires
3. Université Laval
4. Centre interdisciplinaire de recherche en réadaptation et intégration sociale
5. Ludwig-Maximilians-Universität München
6. Université de Sherbrooke

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic disease characterized by muscle weakness and atrophy. Strength training is safe and can induce strength increase in DM1, but it remains unclear if gains are due to neuronal adaptations or muscle hypertrophy.

Methods: Nine men with late onset or adult DM1 forms were recruited to perform an exercise-induced muscle injury protocol consisting of 12 series of 10 maximal eccentric contractions of the right knee extensors using an isokinetic dynamometer. Muscle biopsies were taken in the Vastus lateralis 7 days prior and 24 hours after the protocol. Western blots were used to assess the level of expression of markers of muscle protein synthesis (p-AKT/AKT and p-TOR/mTOR) and breakdown (MuRF1 and Atrogin-1), before and after exercise.

Results: There is a great heterogeneity in response to exercise within participants. Interestingly, subjects who have shown an increase in p-AKT/AKT ratio, have also shown an increase in p-mTOR/mTOR ratio. Almost all patients have shown a decrease in the level of expression of MuRF1 and the average value is significantly decreased post-exercise (0.73 fold ±0.08; p=0.01). The only patient who has presented an increase in MuRF1 expression level, has presented a concomitant decrease in protein synthesis markers. Overall, little variation has been reported in the level of expression of Atrogin-1 (0.97 fold ±0.35; p=0.33).

Discussion: These results suggest that DM1 patients can present various responses to exercise-induced muscle damage when considering simultaneously hypertrophy and atrophy signaling pathways. From a clinical standpoint, although very preliminary, these results are encouraging as they suggest that skeletal muscle in DM1 can undergo adaptations in some patients and physical exercise can trigger positive cellular and molecular responses.

Grant Support: This research project has been supported by the Réseau provincial de recherche en adaptation-réadaptation du Québec.

P-029

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Engineering Synthetic MBNL Proteins that Displace Wild Type MBNL from Toxic RNA

SHOTWELL Carl[1], HALE Melissa[1], BERGLUND Andy[1]

1. University of Florida

Introduction: RNA binding proteins (RBPs) have been shown to have essential functions in various aspects of RNA biology. Most RNPs are made up of modular RNA biding domains (RBD), where specific domains have activity independent of the entire protein. A protein family of interest in the myotonic dystrophy (DM) field is the muscleblind-like (MBNL) family of proteins due to its sequestration to expanded CUG and CCUG repeats. The MBNL proteins are key regulators of several RNA processing events. MBNL1 contains two tandem sets of zinc fingers (ZF1-2 and ZF3-4) that bind YGCY motifs in RNA. The CUG and CCUG repeats have been shown to form A-form RNA helices in vitro. Double stranded RNA binding domains (dsRBD) bind to double stranded RNA in a shape-dependent manner to A-form RNA. Taking advantage of the modular nature of RBPs, new synthetic proteins were created that contained ZF1-2 of MBNL1 and a dsRBD. The dsRBDs chosen were the second dsRBD of TRBP (Homo sapiens) and the second dsRBD of XLRBPA (Xenopus laevis). We hypothesize that the fusion of these domains to MBNL1 will generate a protein that will bind the toxic repeat RNA and displace the wild type (WT) MBNL proteins from the repeats.

Methods: Cell models of DM1 were used to determine the splicing rescue of the synthetic proteins compared to wild type MBNL1. In vitro binding assays will be used to determine the RNA binding activity of these synthetic MBNL1s to both CUG/CCUG repeats and substrates derived from MBNL regulated pre-mRNAs.

Results: Previous work has shown that synthetic MBNL proteins with duplicate domains can affect splicing. The work showed that MBNL1(1-2, 1-2) has a fivefold increased splicing activity compared to the WT MBNL1. The MBNL1(1-2, XLRBPA) protein has shown an increase in splicing activity compared to the MBNL1(1-2, 1-2) and WT MBNL1.

Discussion: The synthetic proteins rescue splicing in cell models of DM1 and have the potential to have improved affinity for expanded repeats.

Grant Support: UF startup fund

P-030

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Microsatellite Expansion Transcript Mis-processing as Diagnostic and Pathomechanism Biomarkers

SZNAJDER Łukasz[1], THOMAS James[1], REID Tammy[1], CLEARY John[1], OLIVEIRA Ruan[1], RANUM Laura[1], SWANSON Maurice[1]

1. Department of Molecular Genetics and Microbiology, Center for NeuroGenetics and the Genetics Institute, University of Florida, College of Medicine, Gainesville, Florida, USA

Introduction: While microsatellite expansions occur in both coding and non-coding regions, including 5' and 3' untranslated regions and introns, microsatellite diversity is a prominent feature of intronic expansions with seven different repeats causing hereditary diseases ranging from CCTG in myotonic dystrophy type 2 (DM2) to GGGGCC in C9orf72-linked amyotrophic lateral sclerosis with frontotemporal dementia (C9-ALS/FTD). In this study, we tested the hypothesis that microsatellite expansions lead to host transcript mis-processing that is useful as a disease biomarker.

Methods: Computational and molecular biology.

Results: Using DM2 and C9-ALS/FTD, which are caused by expansions in the first introns of two distinct genes, as examples we show that host gene misprocessing is readily detectable both by RNA-seq and RT-PCR in affected tissues. To pursue the possibility that this is useful as a diagnostic assay, we test and observe disease-associated RNA misprocessing in peripheral blood lymphoblasts and lymphoblastoid cell lines. Finally, we link these RNA-misprocessing to disease pathomechanisms.

Discussion: Expanded microsatellite repeats cause RNA mis-processing of resident transcripts in affected and unaffected tissues, and when assayed in readily accessible tissues such as peripheral blood, provide sensitive and disease-specific diagnostic biomarkers.

Grant Support: This study was supported by grants from the NIH (NS058901, NS98819 to M. Swanson) and Ł. Sznajder postdoctoral fellowship was awarded by the Wyck Foundation/Myotonic Dystrophy Foundation.

P-031

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Certified TP-PCR Assay to Detect Interrupted Sequences in Normal and Expanded DMPK Gene

VALAPERTA Rea[1,2], DE SIENA Claudia[1,2], CARDANI Rosanna[3], PERFETTI Alessandra[4], BOSÈ Francesca[4], FOSSATI Barbara[5], BRIGONZI Elisa[5], CAVALLI Michele[5], MEOLA Giovanni[5,6], COSTA Elena[1,2]

1. Research Laboratories, IRCCS Policlinico San Donato, San Donato Milanese, Italy/
2. Service of Laboratory Medicine, IRCCS Policlinico San Donato, San Donato Milanese, Italy
3. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
4. Research Laboratories, IRCCS Policlinico San Donato, San Donato Milanese, Italy
5. Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
6. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Introduction: Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease, caused by an expanded CTG repeat in the non-coding 3' UTR of the dystrophia myotonica-protein kinase (DMPK) gene. Here, we tested a new diagnostic assay able to identify large expansions and normal or expanded alleles containing interrupted sequences in DMPK alleles.

Methods: Blood samples were collected from patients enrolled in our Neuromuscular Center from 2014 to 2017. Ninety-five genomic DNAs previously analyzed by conventional PCR and Southern Blot were used to validate a new molecular assay based on the TP-PCR technology. The presence of the interruptions was confirmed by direct sequencing.

Results: After bidirectional TP-PCR screening, 65/95 (68.4%) resulted DM1-positive while 30/95 (31.6%) were DM1-negative. Moreover, among 20/95 (21%) samples showing a single allele by conventional methods, 12/95 (12.6%) resulted false-negative homozygous, showing the presence of the large expanded alleles by TP-PCR. In particular, 5/95 (5.3%) presented a sequence interruptions: 3/95 within the 3'end and 2/95 in the 5'end of the expanded tract. The remaining 8/95 (8.4%) were true normal homozygotes for 5 CTG repeats. No interruptions were found in all normal alleles. Clinical features and familiarity confirm the genetic characterization of all patients analyzed.

Discussion: In conclusion, TP-PCR method resulted a useful method to identify the presence of interrupted sequences in expanded DMPK alleles. We suggest the employment of this new certified molecular assay where all reagents are pre-packaged and ready to use.

Grant Support: This study was supported by IRCCS Policlinico San Donato and FMM Fondazione Malattie Miotoniche.

P-032

Session 2: Disease Mechanisms: DM1, DM2 and CDM

The Structure and Sequence Arrangement of RNA Regulatory Elements Modulates MBNLs' Splicing Response

TAYLOR Katarzyna[1], CYWONIUK Piotr[1], SOBCZAK Krzysztof[1]

1. Department of Gene Expression, Institute of Molecular Biology and Biotechnology, Adam Mickiewicz University, Poznan, PL

Introduction: Three tissue-specific paralogs, MBNL1, MBNL2 and MBNL3, selectively recognize and bind to an RNA consensus motif YGCY/A (Y stands for pyrimidines) within target pre-mRNAs or mRNAs. They all regulate the inclusion of many alternative exons with very often weaker effectiveness observed for MBNL3. There is not much known about structural and sequence features of RNA regulatory elements and their modulating impact on MBNLs activity. These determinants could constitute a differentiating factor of the paralogs' activity and help to better understand different myotonic dystrophy phenotype during disease progression.

Methods: We applied both in vitro RNA binding assays for recombinant MBNLs and in cellulo splicing assay using a modified minigene possessing an alternative exon and a removable RNA
regulatory region in a downstream intron. The RNA secondary structure of tested MBNL targets was determined experimentally.

Results: Both assays proved to be efficient and complementary tools showing MBNLs binding preferences towards specific secondary structures and sequences of several RNA regulatory elements. We showed that the structure of these elements can module MBNL activity perhaps via changes of protein conformation. We also noticed that the MBNL3 is either an inhibitor or a coactivator of MBNL1, depending on the RNA structure and sequence arrangement. These observations were confirmed for many endogenous transcripts in cells with a knock down of MBNL1 and MBNL3.

Discussion: Alternative splicing pattern is the result of different splicing activity of MBNL paralogs induced by an arrangement of RNA regulatory elements and their localization. The presence of MBNL3 in cells seems to attenuate a stronger splicing activity of MBNL1 for specific RNA targets.

Grant Support: Foundation for Polish Science TEAM program, the Polish National Science Centre 2014/15/B/NZ2/02453 and Ministry of Science and Higher Education of the Republic of Poland under the Leading National Research Centre (KNOW programme).

P-033

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Structure of CUG-repeat Expanded DMPK Transcripts

VAN CRUCHTEN Remco TP[1], WIERINGA Bé[1], WANSINK Derick G[1]

1. Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Radboud University Medical Centre, Nijmegen, The Netherlands

Introduction: The DM1 phenotype can only partially be explained by the effects of protein sequestration to the hairpin structure in the CUG-expanded DMPK mRNA. We wondered whether the structure of sequences flanking the repeat is altered by repeat expansion, and could contribute to abnormal protein binding and RNA fate specification. Of specific interest is a putative G-quadruplex, an RNA structure associated with protein binding and regulation of translation, predicted near the CUG repeat. We aim to determine the effect of repeat length on the structure of DMPK mRNA and learn more about its role as a pathogenic molecule and therapeutic target.

Methods: We applied Selective 2' Hydroxyl Acylation analyzed by Primer Extension (SHAPE) on in vitro transcribed DMPK RNAs containing various CUG repeat lengths. Transcripts were probed with NAI, a chemical that modifies flexible, non-base pairing nucleotides after which this RNA was used in a reverse transcription (RT) reaction. The RT halts at modified nucleotides and mapping these locations resulted in a base pairing profile, which was used in modeling of RNA structure. Besides, we measured the formation of a G-quadruplex by quantification of RT-stops induced by this structure.

Results: By probing exon 15 of DMPK mRNA containing either 5, 38, 69 or 147 CUG triplets, we found that the repeat has only limited effects on folding of flanking sequences. Pilot experiments indicated that transcripts with a pathogenic repeat feature unique structural elements. Interestingly, G-quadruplex formation appeared to be influenced by the repeat expansion.

Discussion: Our data suggests that DMPK transcripts fold largely independently of repeat length, which can be important for our understanding of the disease etiology of DM1. We are currently expanding our efforts towards probing DMPK RNA structure in DM1 patient-derived muscle cells and evaluate the physiological role of G-quadruplex formation in DMPK mRNA.

Grant Support: This work was funded by the Radboud Institute for Molecular Life Sciences.

P-034

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Abnormal Nuclear Aggregation and Myofibril Degeneration During Myotube Formation in Myotonic Dystrophy Type 1

WANG Yanlin[1], HAO Lei[2], LI Hui[2], GUO Xiuming[2,3], ZENG Desmond[2], XU Yumin[1], **XIA Guangbin**[4]

1. The First Affiliated Hospital of Zhengzhou University
2. University of Florida
3. The First Affiliated Hospital of Chongqing Medical University
4. University of New Mexico

Introduction: Myotonic Dystrophy Type 1 (DM1) is a fatal disease from progressive muscular wasting. Regenerative medicine using muscle cell transplantation has emerged as a promising therapeutic modality, especially with the advancement of iPS technology, which can potentially provide unlimited muscle precursor cells for cell transplantation. However, there is an unmet need to identify in vitro outcome measures to demonstrate the therapeutic effect before moving such therapies to in vivo clinical trials. In this study, we examined the muscle regeneration (myotube formation) in normal and DM1 myoblasts in vitro to establish outcome measures for therapeutic monitoring.

Methods: Early passage of myoblast cells from normal and DM1 were subjected to myotube formation. Myotube formation was evaluated either by myotube formation index (the percentage of nuclei in myosin-positive myotubes over total number of nuclei). Nuclear aggregation was calculated by nuclear aggregation index (the percentage of myotubes with nuclei aggregation (more than 3 nuclei at the end of myotube that are not aligned linearly)).

Results: We have found abnormal nuclear aggregation during early stage of myotube formation and myofibril degeneration at late stage. The two outcome measures were validated in the evaluation of rescue of abnormal DM 1 myotube formation by normal myoblasts.

Discussion: We have found two prominent abnormal phenomena in DM1 myotube formation: early nuclear aggregation and, later, myotube degeneration. The nuclear aggregation assay is easy to perform and could be a sensitive outcome measure for monitoring therapeutic effects.

Grant Support: NIH/NIAMS grant K08 AR064836 to Xia; Promise to Kate Conte Foundation; Marigold Foundation Ltd.; Muscular Dystrophy Fund from Fraternal Order of Eagles Charity Fund

P-035

Session 2: Disease Mechanisms: DM1, DM2 and CDM

Frequent Inversion of Expanded CTG Repeats in Myotonic Dystrophy Type 1 by Paired gRNA-CRISPR/Cas9

WANG Yanlin[1,2], HAO Lei[1], LI Hui[1], GUO Xiuming[1,3], ZENG Desmond[1], **XIA Guangbin**[4]

1. University of Florida
2. The First Affiliated Hospital of Zhengzhou University
3. The First Affiliated Hospital of Chongqing Medical University
4. University of New Mexico

Introduction: Therapeutic genome editing has been emerging as a fundamental cure for neurogenetic disorders. For disorders involving nucleotide repeat expansion in non-coding region, the targeted deletion of the whole pathogenic repeats is promising. However, its clinical safety issue has not been evaluated. Myotonic Dystrophy type 1 is a disease caused by expanded CTG repeats in 3 prime untranslated region (3'-UTR). In this study, we used DM1iPS cell derived neural stem cells (NSCs) as cellular model to specifically evaluate the rate of inversion of the expanded repeat by paired CRISPR/Cas9 flanking the CTG repeats.

Methods: gRNA-CRISPR/SpCas9 and gRNA-CRISPR/SaCas9 flanking the CTG repeats in the 3'-UTR were screened and the efficiency of targeted deletion and the rate of inversion were evaluated in DM1 iPS cell derived DM1 NSCs by PCR and double-FISH.

Results: Paired gRNA-CRISPR/Cas9 induced efficient deletion of expanded CTG repeat. However, this approach also incurred frequent inversion of CTG repeats in both mutant and normal allele.

Discussion: Paired gRNA-CRISPR/Cas9 flanking the CTG repeats caused frequent inversion of expanded CTG repeats in DM1. The biological effect of this inversion is undetermined. However, the main concern will be the production of polyglutamine, which is toxic and caused many neurodegenerative disorders. Caution is warranted in using this approach for therapeutic genome editing.

Grant Support: NIH/NIAMS grant K08 AR064836 to Xia, Promise to Kate Conte Foundation
Marigold Foundation Ltd., Muscular Dystrophy Fund from Fraternal Order of Eagles Charity Fund

P-036

Session 3: Cancer and Aging in DM

Cancer Cumulative Incidence and Cancer-free Mortality in First-degree Relatives of Patients with Myotonic Dystrophy Type I (DM1)

BEST Ana F[1], HILBERT James E[2], WOOD Libby[3], MARTENS William N[2], NIKOLENKO Nikoletta[3], MARINI-BETTOLO Chiara[3], LOCHMÜLLER Hanns[3], ROSENBERG Philip S[1], MOXLEY Richard T III[2], GREENE Mark H[1], GADALLA Shahinaz M[1]

1. Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD, USA
2. Department of Neurology, Neuromuscular Disease Center, University of Rochester Medical Center, Rochester, NY, USA
3. John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, UK.

Introduction: Patients with DM1 are at excess risk of specific cancers. Here, we evaluated cancer risk in DM1 first-degree relatives.

Methods: We collected personal and family information from 390 genetically- and/or clinically-confirmed DM1 patients participating in the US and the UK DM Registries. We used competing risk statistics to calculate the cumulative risk of cancer and cancer-free death, and evaluate the relationship between the cancer status of probands and relatives.

Results: 434 cancer cases and 304 cancer-free deaths were reported among 2,104 first-degree relatives of DM1 probands. Parents' cancer and non-cancer mortality risk diverged by DM-status after age 50 (Cancer: 16.1%, 13.4%, and 11.2%; Mortality: 9.7%, 7.5, and 3.7% by age 60 in DM-affected, unknown, and unaffected, respectively). Among siblings, cancer risk was highest in those reported as DM-unknown (27.2% by age 60, vs. 14.1% in DM-affected & 12.1% in unaffected). Offspring cancer incidence was similar across groups (~3.5% each by 30). Cancer-free mortality risk by age 60 was higher in siblings (DM-affected: 23.1%, unknown: 15.6%) than parents (9.8%, 7.5%, respectively). No deaths were reported among DM-unaffected offspring; observed non-cancer mortality risks by age 30 were 10.4% for affected and 4.7% for unknown. Cancer risk was higher in parents of probands with cancer (HR=2.1, $p<0.0001$).

Discussion: High incidence of cancer in siblings reported as DM-unknown suggests that cancer risk in DM patients is not associated with disease severity. The relatively low cancer incidence in offspring regardless of DM status suggests that the mechanism of DM-associated cancer is likely age-related. Observed higher mortality in successive generations reflects DM1 genetic anticipation. High mortality in DM-unknown relatives highlights the importance of genetic testing and clinical surveillance of family members.

Grant Support: Intramural research program, National Cancer Institute, US

P-037

Session 3: Cancer and Aging in DM

Premature Aging in DM1 Patient Derived Fibroblasts is Related to BMI-1 Pathway

GARCÍA-PUGA Mikel[1], FERNÁNDEZ-TORRÓN Roberto[2], AIASTUI Ana[3], ZULAICA Miren[2,4], MATHEU Ander[1,5], LÓPEZ DE MUNAIN Adolfo[2]

1. Oncology Area, Biodonostia Health Research institute, San Sebastian, Spain
2. Neuroscience Area, Biodonostia Health Research institute, San Sebastian, Spain
3. Cell Culture Platform, Biodonostia Health Research Institute, San Sebastián Spain
4. CIBERNED, Center for Biomedical Research in Network on Neurodegenerative Diseases, 28049 Madrid, Spain
5. IKERBASQUE, Basque Foundation for Science, Bilbao, Spain

Introduction: DM1 is a genetic multisystem disorder, clinically characterized by a progressive muscular weakness, atrophy and myotonia. DM1 patients present increased oncogenesis and signs of premature aging that might be a consequence of alterations in aging mechanisms. *BMI-1* is a regulator of stem cell self-renewal and cancer cell proliferation and could be an interesting target to analyze the oncologic risk in DM1. Our main purpose was to analyze the role of $p16^{INK4a}$ and the oxidative stress-induced senescence in DM1 culture.

Methods: We established 12 fibroblast lines from DM1 patients and 9 from healthy donors. We studied proliferation, senescence, DNA damage and mitochondrial oxidative stress.

Results: We identified that DM1 fibroblasts are less proliferative and undergo senescence at earlier passage than controls. DM1 fibroblasts express increased levels of $p16^{Ink4a}$ and accumulate elevated number of senescence ′-Galactosidase positive cells. DM1 derived fibroblasts are also more sensitive to DNA damage. We observed that DM1 derived cells present lower metabolic consumption. Altogether these data reinforce the idea that at cellular level DM1 cells experienced a premature aging. Conversely, PBMCs from DM1 patients present lower levels of miRNA200c-141 and $p16^{INK4a}$ and increased expression of BMI-1. These results could be related to the risks of several cancers detected in DM1 cohorts.

Discussion: Our data suggests that fibroblasts could be a model to characterize the aging-related events. In particular, DM1 fibroblasts exhibit a premature senescence phenotype compared to healthy donors linked to $p16^{INK4a}$. Given the role of $p16^{INK4a}$ in cell cycle regulation and the recent implication of oxidative stress in stem cell senescence, we hypothesize that in DM1 a dysbalance in the tight regulation of BMI-1 pathway could address the DM1 cells to neoplastic proliferation or to a premature senescence.

Grant Support: Ander Matheu was granted with an Ikerbasque Research Fellow position and Mikel García Puga was recipient of a predoctoral fellowship from UPV/EHU (PIF 15/245). This work was supported by CIBERNED and grants from Isabel Gemio Foundation, Diputacion Gipuzkoa (OF30/2014), Ministry of Economy and Competition and FEDER Funds to Adolfo López de Munain (FIS PS 09–00660 and PI147436). Ander Matheu was supported by Ministry of Economy and Competition (PI13/02277) and European Union (Marie Curie CIG 2012/712404).

P-038

Session 3: Cancer and Aging in DM

Myotonic Dystrophy: Links to the Nuclear Envelope

MEINKE Peter[1], HINTZE Stefan[1], LIMMER Sarah[1], MENSEL Raphaela Victoria[1], SCHOSER Benedikt[1]

1. Friedrich-Baur-Institute, Munich, Germany

Introduction: Myotonic dystrophies (DM) are slowly progressing multisystemic diseases with a predominant muscular dystrophy - making DM the most frequent muscular dystrophy in adulthood. DM is caused by heterozygous DNA-repeat expansions in the DMPK gene (DM1) or the CNBP gene (DM2). The repeat-containing RNA accumulates in ribonuclear foci and splicing factors are sequestered to these foci, resulting in abnormal regulation of alternative splicing. DM patients show overlapping phenotype presentations with progeroid laminopathies, which are caused by mutations in nuclear envelope proteins.

Methods: In search for molecular signatures of this overlap we did investigate nuclear envelope proteins in a set of human primary control and patient myoblasts using immunofluorescence staining and western blot.

Results: This revealed an enrichment of nucleoplasmic reticuli (NR) in DM1 and DM2 patient myoblasts. NR formation seems in DM1 seems to correlate with repeat length – which in turn correlates with disease severity. NR enrichment correlates with reduced proliferative capacity of myoblasts.

Discussion: Altered NR abundance has been shown in Hutchinson-Gilford Progeria Syndrome as well, implying a possible shared pathomechanism between DM and laminopathies.

Grant Support: None.

P-039

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Association of Peripheral Neuropathy with Sleep Related Breathing Disorders in Myotonic Dystrophies

BANACH Marta[1,2], ANTCZAK Jakub[2], ROLA Rafal[3]

1. Department of Neurology, Jagiellonian University Collegium Medicum, Cracow, Poland
2. Department of Clinical Neurophysiology, Institute of Psychiatry and Neurology, Warsaw, Poland
3. First Department of Neurology, Institute of Psychiatry and Neurology, Warsaw, Poland

Introduction: Myotonic dystrophies type 1 and 2 (DM1, DM2) are inherited diseases, characterized by myotonia and myopathy. Additional symptoms include peripheral neuropathy, sleep related breathing disorders (SRBD) and others. The mechanisms of their development in DM are not fully understood. On the other hand, both conditions are associated with each other in a complicated relation. This relation was described in patients with diabetes mellitus, Charcot Marie Tooth and in general population. In this preliminary study we investigated if the association between peripheral neuropathy and SRBD extends to the DM population.

Methods: 16 DM1 patients (8 women, 8 men, mean age 379 ±14.1; 20-69) and 8 DM2 patients (4 women, 4 men, mean age 47.6 ±14.1; 20-65) underwent the nerve conduction study (NCS) of four sensory and four motor nerves and the diagnostic screening for SRBD. In both subgroups, the parameters from NCS were correlated with apnea hypopnea index (AHI) and with mean arterial, nocturnal oxygen saturation (mean SaO2).

Results: In both groups, the amplitude of ulnar sensory nerve action potential (SNAP) correlated with the mean SaO2. In DM2 group also the median SNAP correlated with the mean SaO2. In DM1 group median SNAP correlated with AHI as did the distal motor latency of the ulnar nerve.

Discussion: Our results indicate there may be a complicated and reciprocal association between neuropathy and SRBD in DM1 and DM2. Nocturnal hypoxemia may contribute to axonal degeneration. The neuropathy may contribute to the muscle weakness, which in turn may cause respiratory events.

Grant Support: None.

P-040

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Myotonic Dystrophy Type 1: Neuropsychological and Psychological Functioning Aspects

BERTOLDO Enrico Giuseppe[1], FOSSATI Barbara[2], BRIGONZI Elisa[2], CARDANI Rosanna[3,4], QUADRI Emilia[1], PAGLIUCA Silvana[1], CALLUS Edward[1], MEOLA Giovanni[2,3,5]

1. Clinical Psychology Service, IRCCS Policlinico San Donato, San Donato Milanese, Italy
2. Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
3. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
4. Service of Laboratory Medicine, IRCCS Policlinico San Donato, San Donato Milanese, Italy
5. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Introduction: Myotonic Dystrophy type 1 (DM1) is characterized by a multi-systemic involvement including cognitive impairment. Particular personality traits have also been described in these patients. The purpose of the study is to evaluate the cognitive performances and the psychological aspects of DM1 patients and to correlate these results to genetic and clinical data.

Methods: To date, ten DM1 patients (6 females) undergoing neurological evaluation were screened for neuropsychological deficits (MMSE Mini-Mental State Examination, Frontal Assessment Battery (FAB), memory (STM, LTM, working), integration capacities, visual-spatial ability, attention (selective, divided, alternate) executive functions, praxis, discrimination and logic capabilities) and psychopathology Symptom Check List 90-R (SCL-90-R). Results obtained were correlated to CTG, age at onset, disease duration, MIRS, MRC and Epworth Sleepiness scales.

Results: In our group, 3 patients had a childhood-onset, 4 a juvenile-onset and 3 an adult-onset. The disease duration was ranging between 2 and 39 years. No correlation was found comparing the genetic and clinical variables with the cognitive performance and psychopathology data. Females had no cognitive impairment and showed a better resilience with no evidence of psychopathological traits. Unemployed women got results in the lower limits of the norm in the global neuropsychological assessment and in the FAB. Men showed signs of psychopathological traits in 7 out of 9 areas with levels in the high/moderate range.

Discussion: These preliminary results showed no correlation between genetic/clinical variables, cognitive performance and psychopathology symptoms. Psychopathological traits have been detected in our male patients while females showed a better psychological functioning. Occupation could be a protective factor for cognitive impairment. Evaluation is ongoing on a larger number of DM1 patients, results will be presented.

Grant Support: Fondazione Malattie Miotoniche.

P-041

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Cognitive Performance Using Computer Interface in a Multicenter Cohort of Ambulatory Adults with Non-congenital Myotonic Dystrophy Type 1 (DM1)

DEUTSCH Gayle K[1], SAMPSON Jacinda[1], HAGERMAN Katharine A[1], DENT Gersham[2], DAY John W.[1], and the Myotonic Dystrophy Clinical Research Network[1,3,4,5,6,7]

1. Stanford University School of Medicine
2. Biogen
3. University of Rochester
4. University of Florida McKnight Brain Institute
5. Kansas University Medical Center
6. Ohio State University Wexner Medical Center
7. National Institute of Neurological Disorders and Stroke

Introduction: Cognitive difficulties occur in individuals with DM1. CogState is a multitask computerized battery for testing cognition. We studied the feasibility of using CogState for longitudinal assessment of DM1.

Methods: 112 patients were studied at 6 centers. Study patients were 46 men and 66 women, from 19 to 67 years of age (mean = 43.05), having symptom onset after age 10 and ability to walk for 6 minutes. CogState was performed on laptop computers at baseline and repeated in 3 and 12 months. Only baseline and 3-month data were analyzed. Four CogState modules were administered: Detection (DET) assessed psychomotor function, Identification (IDN) assessed attention, One-Back (ONB) assessed working memory, and Groton Maze Learning Test (GML) assessed executive function. Z-scores were calculated based on age from historical healthy control data (CogState Ltd). Paired t-tests were conducted to determine change over time and intra-class correlation (ICC) estimates were calculated to determine test-retest reliability (single measure, absolute-agreement, 2 way mixed effects model).

Results: 98% of testing sessions were completed properly. Z-scores of group means ranged from -0.42 to -0.95. Lowest performance was on the ONB task. Three months later, there was a significant improvement on GML but no significant change on other measures. ICC = .48 for DET, ICC = .69 for IDN, ICC = .65 for ONB and ICC = .58 for GML.

Discussion: Brief computerized testing of cognitive function is feasible in non-congenital DM1. Preliminary analyses indicated that DM1 subjects scored .42 to .95 standard deviations below healthy controls on cognitive tests with most impairment in working memory, consistent with prior studies. Performance on 3 of 4 modules showed no change over a 3-month period, but the 4th module showed possible test learning effects. Test-retest reliability was poor for DET, but moderate for the other measures. CogState's usefulness for assessing baseline cognition and DM1 progression will be discussed.

Grant Support: NINDS, Muscular Dystrophy Association, Myotonic Dystrophy Foundation, The Marigold Foundation, NIH P01, Biogen.

P-042

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

A Study of the Capacity for Artistic Practice to Shape Research and Care in the Field of DM

DONACHIE Jacqueline[1,2]

1. Northumbria University
2. Glasgow City Museums

Introduction: Contemporary art can be an effective way of communicating complicated science to a range of lay audiences, particularly in the context of medical research. However, a rationale that seeks to only 'explain' can limit the cultural significance of artworks by overstating their illustrative capacity, an outcome that severely reduces impact.

Methods: Based on the first-hand experience of an artist whose career has engaged with the opportunities afforded by science-art collaboration, this study seeks to address the illustration problem by exploring new methods of working across art and science that challenge representations of myotonic dystrophy. *Hazel*, a film made by the artist with the participation of eleven women (IDMC-10), is placed at the centre of this as a case study, alongside *Pose Work for Sisters*, a new short film (2016) which will be screened as part of the presentation. Pioneering work with the UK DM Patient Registry facilitated participant recruitment, and it is this process that forms one of the unique contributions to knowledge of the research (IDMC-09, Poster).

Results: As the conclusion of this Doctorate there is also now a publication that presents the Hazel interviews in full, plus new commissioned writing, inspired by the women's experience, in both fiction and social anthropology. By illuminating the multiple loss experienced by women faced with physical and social decline, this research offers a practical and theoretical image of the capacity contemporary artists have to shape research in ways more ambitious than illustration, more extensive than the communication of family insights. Thus it can embrace a much-needed form of interdisciplinary leadership that is built upon an artist's scope to say powerful things by at times presenting information in innovative ways.

Discussion: By addressing new, creative ways to extend the DM story this research explores the impact possible through creative activity that directly addresses scientific research.

Grant Support: Arts and Humanities Research Council (UK), Wellcome Trust Northumbria University, Marigold Foundation (publication), Glasgow Museums (publication)

P-043

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

The Reliability and Validity of the Swedish Version of the Fatigue and Daytime Sleepiness Scale (FDSS)

EDOFSSON Ulrika[1], WINBLAD Stefan[2], LINDBERG Christopher[1]

1. Neuromuscular Centre, Sahlgrenska University Hospital, Gothenburg, Sweden.
2. Department of Psychology, University of Gothenburg, Sweden

Introduction: Fatigue and excessive daytime sleepiness are frequently experienced by patients with DM1 and these symptoms have a negative impact on daily activity, participation and quality of life. Although there are few successful treatment options, there exists a need to develop instruments with the aim to reliably measure symptoms and follow patients over time in clinical practice and research. The aim of this study was to translate and psychometrically evaluate the Fatigue and Daytime Sleepiness Scale (FDSS). Shortly, the FDSS has been devised with the aim to measure these symptoms as a single clinical entity, consisting of 12 self-assessment questions covering both fatigue and daytime sleepiness. The original version of FDSS has been found to be reliable and valid. The study was performed at the Neuromuscular Centre, Sahlgrenska University Hospital, Gothenburg, Sweden.

Methods: The FDSS was translated into Swedish using the forward-and backward procedure. The scale was administered to 43 patients on two occasions at a two week interval. At baseline assessment, the patients also received the question of whether they experienced fatigue and/or sleepiness (yes or no). The group of patients consisted of 24 women and 19 men with an average age of 46.9 years (four with the childhood form, 32 classical and seven with a late onset). The statistical analysis included an evaluation of intra-rater reliability, internal consistency and construct validity.

Results: The FDSS showed an excellent intra-rater reliability (PCC = .91) and acceptable internal consistency (Cronbach's alpha = .71). The scale successfully distinguished patients experiencing fatigue and sleepiness from those who did not (mean FDSS score of 10.8 vs 7.1, p = .002).

Discussion: The present study supports the use of the translated version of the FDSS for the measurement of fatigue and daytime sleepiness in patients with DM1.

Grant Support: West Sweden Muscle Foundation.

P-044

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Evaluation of Postural Control and Falls in Individuals with Myotonic Dystrophy Type 1

EICHINGER Katy[1,2], QUINN Jill R[2], PANDYA Shree[1]

1. Department of Neurology, University of Rochester, Rochester, NY
2. School of Nursing, University of Rochester, Rochester, NY

Introduction: Falls and instability are significant health concerns that impact quality of life. The purpose of this study was to assess postural control and examine its relationship with self-reported falls in individuals with myotonic dystrophy type 1 (DM1).

Methods: Postural control was assessed using the International Classification of Function at the body-structure function (BSF), activity, and patient reported level in individuals with DM1. BSF level assessment of postural control was performed using a body-worn inertial sensor to measure postural sway and lower extremity strength was measured using manual muscle testing. The Timed Up and Go, Five Times Sit to Stand, and Mini-BESTest were performed as activity level measures and the Activities-specific Balance Confidence Scale (ABC) as a patient reported measure of balance. Self-reported falls were collected using a fall history questionnaire at baseline as well as weekly for 12 weeks.

Results: Thirty-four individuals with DM1 between the ages of 18-60 completed the prospective study. Sixty-two percent reported a fall during the 12 weeks following their baseline visit. Postural sway parameters in individuals with DM1 were significantly different from normative data. The Mini-Bestest was moderately correlated with fall status (-0.43; 0.01), however, none of the measures of postural control in this small sample were found to be a significant predictor of fall status.

Discussion: Postural sway measured using a body-worn sensor was able to differentiate between individuals with DM1 and normative data suggesting that postural sway may be a feasible way to measure balance impairment in individuals with DM1. However, these measures, along with other measures of postural control, were not significant predictors of falls in this study. Future studies involving larger samples and longer durations are needed to determine predictive validity and sensitivity of postural control measures in individuals with DM1.

Grant Support: None.

P-045

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Stronger Focus on Physical Difficulties than on Mental Aspect in Health Related Quality of Life Among Japanese Myotonic Dystrophy Type 1 Patients

ENDO Makiko[1], ODAIRA Kaori[2], ONO Ryohei[2], KURAUCHI Go[2], KOSEKI Atsushi[3], GOTO Momoko[2], SATO Yumi[2], KON Seiko[2], WATANABE Norio[4], SUGAWARA Norio[1], TAKADA Hiroto[2], KIMURA En[1]

1. National Center of Neurology and Psychiatry
2. National Hospital Organization, Aomori Hospital
3. National Hospital Organization, Hanamaki Hospital
4. Graduate School of Medicine, Kyoto University

Introduction: Myotonic dystrophy type 1 (DM1) is a common form of muscular dystrophy which presents variety of symptoms that can affect patients' quality of life (QOL). Despite the importance of clarifying patients' subjective experience in both physical and psychosocial aspects for improved symptom management, there has not been enough evidence on QOL of DM1 patients in Japan.

Methods: DM1 patients completed a set of questionnaires which is comprised of Short Form-36 (SF-36) for measuring health related QOL, Barthel index, the center for epidemiologic studies depression scale (CES-D) and Epworth sleepiness scale (ESS). Correlation analysis of the variables has been performed. As the recruitment is still ongoing, this is a provisional presentation of the current data. 50 patients in total are expected to be included in the final analysis.

Results: 32 patients participated in the study. Mental component summary scores (MCS) of SF-36 were higher (mean = 54.1) compared to physical component summary scores (PCS, mean = 25.4) among these patients. At the same time, higher vitality and physical function were in association with lower CES-D scores ($P < 0.01$), and daytime sleepiness scores and depression scores were positively correlated ($P < 0.01$).

Discussion: Patients seem to focus more on their physical difficulties rather than on mental aspects. However, the extent of physical symptoms such as vitality, physical function and sleepiness do appear to affect their mood. These interim findings suggest that results of self-reported measures should be collected and analyzed from various dimensions, both disease-specific and general, in DM1 patients. This procedure will be taken into account at final data analysis.

Grant Support: This work is supported by JSPS Kakenhi grant number JP16K09735.

P-046

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

The Patient-reported Impact of Myotonic Dystrophies (DM) on Schooling and Employment status

FOSSATI Barbara[1], BRIGONZI Elisa[1], QUATTRI Enrico[1], CAVALLI Michele[1], MEOLA Giovanni[1,2,3]

1. Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
2. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
3. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, San Donato Milanese, Italy

Introduction: The value of employment has been worldwide pointed out to be an important factor for preservation of ′mental and physical health. This statement is even more relevant in people presenting chronic disabilities, such as Myotonic Dystrophies (DM). The purpose of this study is to evaluate the educational level and the employment status in DM Italian patients to better understand the impact of the disease on these items.

Methods: A self reported questionnaire with the aim to investigate the educational level, the employment status and the patient's perception about how being affected by DM may influence their working life has been developed. The information collected have been correlated with type of DM, age, gender and severity of the disease.

Results: To date we included in the study 91 DM 1 and DM2 patients, mean age 45.5 + 14.02, 59% males. Fifty-six % of them have an occupation while 15% are unemployed; the others are students, retirees, and housewives. The majority of both employed and unemployed patients (about 2/3) does not think that DM influenced their working life, but 27% of the entire population had to change the type of work because of the onset of physical limitation and 11% lost their jobs because of the DM. The 27% of the employed patients have a Tertiary level education, 39% have an Upper secondary education, 33% have a Lower secondary education while only 1% have a Primary education. All the illiterate patients are unemployed.

Discussion: Information collected provided a broad overview of the educational and employment status of DM population. A major proportion of patients are employed and less than half of these ones think that to be affected by the disease conditioned their working life. Undertaking work revealed statistically significant correlation with a higher level of education as well as higher level of education increased the chance of employment and it reduces the feeling of being biased by DM. Even if DM causes significant disability, it doesn't appear to preclude patients from having a job.

Grant Support: None.

P-047

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

The Italian National Registry For Myotonic Dystrophy Type 1 And 2

FOSSATI Barbara[1], BRIGONZI Elisa[1], CAVALLI Michele[1], COMI Giacomo Pietro[2], D'ANGELO Grazia[3], LIGUORI Rocco[4], LOGULLO Francesco[5], PEGORARO Elena[6], SANSONE Valeria[7], SANTORO Lucio[8], SCARLATO Marina[9], SICILIANO Gabriele[10], SILVESTRI Gabriella[11], VITA Giuseppe[12], MEOLA Giovanni[13,14,15]

1. Department of Neurology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
2. Ospedale Maggiore Policlinico Milano, Università degli Studi di Milano
3. Polo Scientifico di Bosisio Parini
4. Università di Bologna
5. Clinica Neurologica, Università Politecnica delle Marche, Ancona
6. Università degli Studi di Padova
7. NEuroMuscular Omnicenter (NEMO), Università degli Studi di Milano
8. Università Federico II Napoli
9. Ospedale San Raffaele, Università Vita-Salute, Milano
10. Università di Pisa
11. Policlinico Gemelli, Università Cattolica del Sacro Cuore, Roma
12. Università degli Studi di Messina
13. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
14. Department of Neurology, IRCCS-Policlinico San Donato, Milan, Italy
15. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS-Policlinico San Donato, Milan, Italy

Introduction: The Italian national Registry for Myotonic Dystrophies (DM) is a patient-entered and physicians-entered database that collects demographics, genetics, clinical and quality of life information. The data gathered through the Registry over three consecutive years from the beginning of the patients' enrolment, that started on November 2013, were analysed.

Methods: A network of 18 neuromuscular expert centers, spread across Italy, has been created. A reserved website was made available and a database, in which the data are collected, validated, and updated, was developed. Two different pathways were prepared: one is openly accessible by the patients while the other one is filled by neurologists.

Results: Over the three years, 655 DM patients actively took part in the Registry. Only eleven of the 18 network centers' managed to start collecting data; 522 properly filled forms have been submitted by clinicians. A major proportion (87.2%) of patients are affected by DM1, with a mean age of 39.8 years old. The percentage of male is 62,3%. The time-lag in diagnosis was 8.8+8.7 in DM1 and 12.9+14.3 in DM2. At onset, the majority of DM1 manifested myotonia (65.6%) or distal weakness, while DM2 presented mostly proximal weakness; moreover cramps and muscle pain resulted to be more frequent in DM2 early stage. Evaluating the current condition, in DM1 patients myotonia is the most frequent symptom (74.1%). Muscle weakness affects 68.9% of DM1 and the totality of DM2 patients, with the classical distribution. Both groups present high frequency of hypersomnia (55.7%). Cardiac involvement is more frequent in DM1. More than 50% of the DM patients has a job and 41% of them refers that the disease affects their working life.

Discussion: Information entered by patients and clinicians in the Italian DM Registry provided a broad overview of the Italian DM population. Register enrolment developed in a unique occasion to reach out for DM patients and get in touch with them, both by human and clinical way. It is a key opportunity to have information on natural history of the disease and to define a homogeneous cohort of patients for clinical trial.

Grant Support: None.

P-048

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Identifying Missing Links in the Rehabilitation of DM1 Patients Diagnosed in Adulthood

FROST, Asger[1]

1. Rehabilitation Center for Neuromuscular Diseases

Introduction: We have experienced that disease progression for a large part of DM1 patients with adult onset jeopardize attachment to the labor market as well as contact with different parts of the welfare treatment system, which, in turn, affects their social status considerable.

Methods: We included a group of adults with DM1. We followed their disease progression and rehabilitative interventions over a period of five to ten years. By describing their course of DM1 (MIRS-level/labor market affiliation/personal relations/leisure activities) in relation to the rehabilitative interventions made, we hoped to identify possible missing links in the rehabilitation of this group.

Results: We identified 52 adults between 30 and 60 years of age, diagnosed from January 2007 to December 2012. Out of these, 34 patients were included, because they were referred less than one year after their diagnosis. MIRS-level/labor market affiliation/personal relations/leisure activities were recorded at the time of referral and at June 1, 2017. Distribution of age for 34 patients (13 male/21 female) at the time of diagnosis was 21-53 years (mean 36.4 y). 23/34 had received their diagnosis based on family history while 11/34 were found by genetic testing (upon clinical work-up). Large differences in MIRS level at time of diagnosis (1-4). At the time of diagnosis, 30/34 had positive labor market attachment (LMA), of which 15 persons were in full time jobs. At time of follow-up only 4 had positive LMA, and 12 had unknown status.

Discussion: The progression of DM1 have many physical, social and job-related consequences over a 5-10 year period. Contact were lost with approx. 1/3 of the group, even with patients who we at entrance thought were able to continue the rehabilitative efforts initiated. (Participation in workshops/involvement of relatives/ labor market affiliation/social relations). The poster will indicate where the missing links can be found within the Danish health system.

Grant Support: None.

P-049

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Quality of Life is Affected by Subjective Symptom Burden in Patients with Myotonic Dystrophy

FUJINO Haruo[1,2], SAITO Toshio[3], TAKAHASHI Masanori[4,5], TAKADA Hiroto[6], NAKAYAMA Takahiro[7], IMURA Osamu[8], MATSUMURA Tsuyoshi[2]

1. Department of Special Needs Education, Oita University
2. Graduate School of Human Sciences, Osaka University
3. Department of Neurology, National Hospital Organization Toneyama National Hospital
4. Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine
5. Department of Neurology, Osaka University Graduate School of Medicine
6. Department of Neurology, National Hospital Organization Aomori National Hospital
7. Department of Neurology, Yokohama Rosai Hospital
8. Graduate School of Human Sciences, Osaka University

Introduction: Myotonic dystrophy is the most common muscular dystrophy in adults. Progressive muscular weakness, fatigue, myotonia, and daytime sleepiness are prevalent in patients with the disorder. Such features often lead to the restriction of activities and social functioning, which influence the daily life and quality of life of patients. The aim of this study was to examine the relative contributions of subjective symptom burden to the quality of life in patients with myotonic dystrophy.

Methods: Eighty-six patients with myotonic dystrophy type 1 (DM1) recruited from four hospitals in Japan were included in this study. Quality of life was measured by the Individualized Neuromuscular Quality of Life (INQoL). The severity and impact of common muscle symptoms and quality of life index (QoL index) were used in the analysis. The QoL index was calculated from the impact of the disease in specific areas of daily life. Multiple regression analysis was used to assess the relative contributions of the variables.

Results: Patients' quality of life was significantly explained by the severity of symptoms, employment status, and subjective symptom burden (muscular weakness, fatigue, and myotonia). Clinical variables and subjective symptom burden explained the variance of the QoL index (adjusted R-squared = 0.81; $p < 0.001$).

Discussion: Muscular weakness, fatigue, and myotonia were the symptoms that affected the quality of life in patients. Reducing the burden of these symptoms could be an important target for interventions to improve the quality of life in patients with myotonic dystrophy.

Grant Support: This work was supported in part by research grants from the Japan Agency for Medical Research and Development AMED (Practical Research Project for Rare/Intractable Diseases, 16ek0109172 and 17ek0109259), the Ministry of Health and Welfare of Japan (H28-Nachitou(Nan)-Ippan-030), and the Japan Society for the Promotion of Science KAKENHI (17K14067).

P-050

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Validation of the Japanese Version of the Myotonic Dystrophy Health Index (MDHI)

FUJINO Haruo[1,2], MATSUMURA Tsuyoshi[3], TAKADA Hiroto[4], OGATA Katsuhisa[5], SHINGAKI Honoka[2], IMURA Osamu[2], HEATWOLE Chad[6], TAKAHASHI Masanori[7,8]

1. Department of Special Needs Education, Oita University
2. Graduate School of Human Sciences, Osaka University
3. Department of Neurology, National Hospital Organization Toneyama National Hospital
4. Department of Neurology, National Hospital Organization Aomori National Hospital
5. Department of Neurology, National Hospital Organization Higashisaitama Hospital
6. Department of Neurology, University of Rochester Medical Center
7. Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine
8. Department of Neurology, Osaka University Graduate School of Medicine

Introduction: The Myotonic Dystrophy Health Index (MDHI) is a disease-specific, patient-reported outcome measure designed to measure therapeutic response and patient-reported disease burden during myotonic dystrophy. It is important to have outcome measures that have been properly validated across populations and cultures. The aim of this study is to confirm reliability and validity of the MDHI in Japanese patients.

Methods: Fifty-three patients with myotonic dystrophy type 1 (DM1) recruited from four hospitals participated in this study. The mean age of the patients was 47.8 (SD = 11.1) years. The patients completed the Japanese version of the MDHI. A subset of the patients completed the MDHI-J twice to examine test-retest reliability (time interval: 2 weeks ± 1 week). A higher score represents a greater patient-reported disease burden. Concurrent validity was also examined using other measures assessing domains of the MDHI.

Results: The mean of the MDHI total score was 25.8 (SD = 25.1) in the current sample. All subscales showed high internal consistency (0.79–0.97). Intraclass correlation coefficients ranged from adequate to high (0.63–0.95) for the subscales and total score. Generally, MDHI subscales were associated with relevant domains of other validated measures.

Discussion: We examined the reliability and validity of the Japanese version of the MDHI (MDHI-J) in Japanese patients with myotonic dystrophy. Consistent with the original version of the scale, the MDHI-J could be a reliable and valid measure to evaluate disease burden in Japanese patients.

Grant Support: This work was supported in part by research grants from the Japan Agency for Medical Research and Development AMED (Practical Research Project for Rare/Intractable Diseases, 16ek0109172 and 17ek0109259), the Ministry of Health and Welfare of Japan (H28-Nachitou(Nan)-Ippan-030).

P-051

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

The CREATION Biobank

GAGNON Cynthia[1,2,3], BOUCHARD Luigi[1,2], DUCHESNE Élise[2,4], MATHIEU Jean[1,2,3]

1. Université de Sherbrooke
2. Groupe de recherché interdisciplinaire sur les maladies neuromusculaires
3. Centre de recherche Charles-Le Moyne
4. Université du Québec à Chicoutimi

Introduction: Conducting research in rare diseases such as myotonic dystrophy type 1 (DM1) may present specific challenges such as recruitment issue and getting access to well-characterized biological samples. The Saguenay-Lac-St-Jean region (Québec, Canada) presents the highest worldwide prevalence reaching about 158 DM1 individuals per 100,000 inhabitants. Access to this large cohort facilitates studies on natural history of the disease. The objective is to introduce to the research community the biobank entitled “CREATION Biobank for Clinicians, Researchers and Expert patients Alliance for Therapeutic Innovation on Neuromuscular disorders.

Methods: DM1 patients with the juvenile, adult and late-onset phenotypes were recruited from the registry of the Neuromuscular Clinic of the Saguenay-Lac-St-Jean (Québec, Canada). Sample collection was part of a larger study documenting the progression of multisystemic impairments. This project had 3 phases: T1 in 2002, T2 in 2011, and T3 in 2015.

Results: A total of 200 patients at T1, 115 patients at T2, and 95 patients at T3 were assessed. Data on muscle strength, cognitive function, functional capacity, fatigue, cardiac and pulmonary systems were collected using standardized protocols. A total of 3257 biological samples were stored in CREATION. At T1 and T2, only blood samples were collected; epithelial cells and skin and muscle biopsies were also collected at T3. In addition, for biological samples, results for several analyses are available such as CTG repeat length. An electronic platform is available to the research and academic community to consult the catalogue and the procedures and to make requests to access CREATION.

Discussion: CREATION biobank will accelerate knowledge acquisition in DM1 by making available biological samples from phenotypically well-described patients.

Grant Support: The Marigold Foundation, Canadian Institute of Health Research and Muscular Dystrophy Canada.

P-052

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Independant Living Among Adults with the Childhood Phenotype of Myotonic Dystrophy Type 1 – An Exploratory Study

GAGNON Cynthia[1,2,3], TREMBLAY Marjolaine[1,2,3], CÔTÉ Isabelle[1,2,3], GALLAIS Benjamin[1,2,3]

1. Faculté de médecine et des sciences de la santé, Université de Sherbrooke
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires
3. Centre de recherché Charles-Le-Moyne

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic neuromuscular disorder categorized into five clinical phenotypes: 1) Congenital; 2) Infantile/childhood; 3) Juvenile; 4) Adult; and 5) Late-onset. Patients with the childhood phenotype have an age of onset between 1 and 10-year old and present often with a normal to limit intelligence. However, significant restrictions were clinically observed in social participation and recently documented in a retrospective study. They often require support from family or external services to be able to accomplish their daily activities. The objective of this study is to document their ability to live independently as they reach adulthood.

Methods: Case study design with data triangulation from several sources (medical file, questionnaires, tests, caregivers). Adults with the childhood phenotype were recruited from the registry of the Neuromuscular Clinic (Quebec, Canada), and their relatives were invited to participate thereafter. Medical files were reviewed using a standardized extraction grid and all participants completed the Independent Living Scale.

Results: A total of 10 DM1 patients and 9 relatives participated. Results show that people with the childhood phenotype have a semi-autonomous independence level. They show limitations regarding the accomplishment of several tasks such as housekeeping and meal preparation. They also present significant difficulties with money management.

Discussion: These preliminary results tend to confirm clinical observations that adults with the childhood phenotype need help from their relatives or external services to live at home. A more complete portrait of the central nervous involvement during adulthood is necessary as it could permit to explain the difficulties highlighted in this study.

Grant Support: Fondation de ma vie, Fonds de dotation Santé Jonquière.

P-053

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Reliability of the Test of Mastication and Swallowing Solids in Patients with Myotonic Dystrophy Type 1

SCHINDLER Antonio[1], **GINOCCHIO Daniela**[2], PIZZORNI Nicole[3], BIANCHI Federica[4], FEROLDI Sarah[4], GASPERONI Chiara[4], DAVIDE Cecchetto[1], MORETTI Michela[1], SANSONE Valeria[2]

1. Phoniatric Unit, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Milan, Italy
2. Fondazione Serena ONLUS, Centro Clinico NEMO, Milan, Italy
3. PhD course in Nutritional Science, Department of Biomedical and Clinical Sciences "L. Sacco", University of Milan, Milan, Italy
4. Department of Neurological Rehabilitation, ALS Centre, Istituti Clinici Scientifici Maugeri IRCCS, Milano, Italy

Introduction: Dysphagia is common in individuals with myotonic dystrophy type 1 (DM1) even in the early stage. Reduced tongue strength leading to piecemeal deglutition and pharyngeal pooling as well as impaired mastication have been reported, stressing the need of valid and reliable tools to assess oral phase of swallowing in subjects with DM1. The Test of Mastication and Swallowing Solids (TOMASS) is a validated tool to objectively assess the efficacy and the efficiency of solid bolus ingestion. Aim is to investigate the reliability of the TOMASS in DM1 population.

Methods: Ten participant with diagnosis of the DM1 with a median age of 52.5 (range 36-66) were recruited. The TOMASS was conducted both in an ecological situation and while performing fiberoptic endoscopic evaluation of swallowing (FEES). Both FEES and TOMASS were video-recorded and recordings were assessed by three blinded raters. The number of discrete bites, the number of masticatory cycles and the number of swallows for the TOMASS and the number of white-out in FEES were counted; total time needed to complete the TOMASS was recorded. The intraclass correlation coefficient (ICC) with a confidence interval at 95% was used to assess inter-rater reliability of TOMASS. The number of swallows in TOMASS and the number of white-out in FEES were correlated through the Kendall rank correlation coefficient.

Results: ICC of 0.982 (0.948-0.995) for discrete bites, of 0.982 (0.949-0.995) for masticatory cycles, of 0.864 (0.660-0.961) for swallows and of 0.993 (0.981-0.998) for total time were found. A correlation of 0.862 (p=0.002) was found between the number of swallows in TOMASS and the number of white-out in FEES.

Discussion: These preliminary data suggest that TOMASS is a reliable tool to assess oral phase of swallowing in individuals with DM1 and support its use beside instrumental assessment and mealtime observation in order to conduct a more comprehensive assessment of swallowing in this population.

Grant Support: None.

P-054

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Respiratory Muscle Function: Forced Vital Capacity & Cough Peak Expiratory Flow in Patients with Myotonic Dystrophy Type 1

HAMMARÉN Elisabet[1], LINDBERG Christopher[1]

1. Sahlgrenska University Hospital, Neuromuscular Centre

Introduction: Persons with myotonic dystrophy type 1 and MIRS grade 4 or 5 often show signs of respiratory insufficiency. This insufficiency increases over time and is life threatening, causing chronic hypercapnic respiratory failure and a reduced ability of airway clearance. The most important reason might be the reduced muscle force of the diaphragm and the abdominal muscles. Patients with myotonic dystrophy do often show symptoms as daytime sleepiness and excessive fatigue. Our experience is that this in some cases is reversible, when using mechanical ventilation. A way of determine the need for further examination is clinical assessment of the functional vital capacity (FVC) and the cough peak expiratory flow (CPEF). We have followed persons with DM1 since 1996 and have recently looked at collected respiratory function data.

Methods: Patient chart data of FVC and CPEF between 1996 to 2017 have been studied retrospectively.
The data will be analyzed in relation to sex, age and symptom debut (childhood, classical/adult, late onset). Data from persons with congenital form of DM1 were excluded.

Results: The assessment of FVC is biased if the lip muscles are weak. The use of a mask when assessing FVC and CPEF can be recommended. Persons with DM1 often feel uncomfortable with nightly mechanical ventilation and will need a longer time to accept breathing help.

Discussion: It is of great importance to follow symptoms and signs of respiratory failure in persons with myotonic dystrophy. The air leakage at the lips when assessing FVC has to be taken into account. The use of positive expiratory pressure exercise have been tried with positive results. Airway clearance by coughing and huffing after using positive expiratory pressure treatment might be a way to prevent pneumonia and should be recommended.

Grant Support: The Local Research and Development Board for Gothenburg and Södra Bohuslän.

P-055

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Investigations of Calcium Metabolism in DM1

HLAING Phyu[1], JACKSON Richard[2,3]

1. Princess Alexandra Hospital, Woolloongabba, Queensland, Australia
2. School of Medicine, Griffith University, Queensland, Australia
3. Department of Medicine, Logan Hospital, Meadowbrook, Queensland, Australia

Introduction: Patients with type 1 myotonic dystrophy (DM1) have a higher incidence of hypercalcaemia compared to the general population. The nature and effects of dysregulated calcium metabolism underpinning this phenomena have not been fully characterised.

Methods: Retrospective review of medical records of patients with DM1 attending a DM clinic at Logan Hospital, Brisbane, Queensland between October 2005 and January 2017 and who each had concurrent serum assays performed of corrected calcium (cCa), 25 hydroxyvitamin D (25 Vit D), parathyroid hormone (PTH), phosphate (PO4), and for whom results were available for estimated glomerular filtration rate (eGFR), and bone mineral densitometry (BMD) tests. 24 hour urine calcium level was done in some patients concurrently.

Results: Thirty two patients with DM1 (16 females, 16 males) were reviewed of whom eight (25%) had elevated cCa and elevated PTH consistent with primary hyperparathyroidism (PHP). Another ten patients (31.3%) had raised PTH with normocalcaemia consistent with early PHP. Seven of these eighteen (38.8%) patients collected 24 hour urine calcium level. Six of these seven patients had a low 24 hour urinary calcium level with one result within normal range. In all 32 cases, only three (9.4%) had borderline low 25 Vit D, two (6.3%) had low PO4. All patients had normal eGFR and none were osteoporotic (T-score <-2.5) on BMD tests.

Discussion: One in five patients with DM1 were hypercalcaemic with PTH levels consistent with PHP but no evidence of osteoporosis and their 24 hour urine calcium level was significantly low which mimics Familial Hypocalciuric Hypercalcemia (FHH). An insensitive calcium sensing receptor, due to aberrant splicing of it's messenger RNA, is a possible explanation.

Grant Support: None.

P-056

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Responsiveness of Performance-based Outcome Measures in Myotonic Dystrophy Type 1

KIERKEGAARD Marie[1,2], PETICLERC Émilie[3], HEBERT Luc J[4,5], MATHIEU Jean[3], GAGNON Cynthia[3]

1. Allied Health Professionals Function, Function area Occupational therapy and Physiotherapy, Karolinska University Hospital, 171 76 Stockholm, Sweden
2. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy, Karolinska Institutet, Stockholm, Sweden
3. Faculty of Medicine and Health Sciences, Centre de recherché Charles Le-Moyne, Université de Sherbrooke, Sherbrooke, Québec, Canada
4. Faculty of Medicine, Rehabilitation (Physiotherapy) and Radiology
5. Nuclear Medicine Department, Université Laval, Québec City, Québec, Canada.

Introduction: Outcome measures that reflect mobility, balance, muscle strength and manual dexterity are needed for endpoints in future trials in myotonic dystrophy type 1 (DM1). There is, however, a need to assess responsiveness of such measures.

Methods: A total of 113 adult DM1-patients were included in this nine-year longitudinal study. Responsiveness of timed up-and-go, Berg balance scale, quantitative muscle testing, grip and pinch-grip strength, and Purdue pegboard were assessed by criterion and construct approaches. Patient-reported perceived changes (worse/stable) in balance, walking, lower-limb weakness, stair-climbing and hand weakness were used as criteria. Predefined hypotheses about expected area under the receiver operating characteristic curves (criterion approach) and correlations between relative changes (construct approach) were explored.

Results: The direction and magnitude of median changes in outcome measures were in line with patient-reported changes. Median changes in timed-up-and-go, grip and pinch-grip strength, and Purdue pegboard did not exceed known measurement errors. Most criterion (72%) and construct (70%) approach hypotheses were confirmed. There was an overlap in change scores between stable/worse groups in all outcome measures except for quantitative muscle testing measures.

Discussion: The performance-based outcome measures were able to capture changes, but responsiveness was mostly inadequate except for relative changes in quantitative muscle testing measures. Knowledge on measurement errors is needed to interpret the meaningfulness of these longitudinal changes.

Grant Support: The Einar Belvén Foundation; the Réseau Provincial de recherche en adaptation-réadaptation (REPAR-FRQS), Canada; and the Fonds de recherche du Québec en Santé, Canada (grant number 22193).

P-057

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Pain and Pain Medication Use in Patients with Myotonic Dystrophy Type 1 (DM1) and Type 2 (DM2)

FITZGERALD Bryan P[1], CONN Kelly M[1], HILBERT James E[2], **LUEBBE, Elizabeth A**[2], PARKHILL Amy L[1], and MOXLEY III Richard T[2]

1. Wegmans School of Pharmacy, St. John Fisher College, Rochester, NY
2. Department of Neurology, University of Rochester Medical Center, Rochester, NY

Introduction: Pain is a common manifestation of patients with DM1 and DM2. Limited data are available to compare pain treatments and adherence to pain medication between these groups.

Methods: To address this need, we recruited adult patients from the National Registry at the University of Rochester to complete an online questionnaire about their pain. Questions covered demographics and pain information, non-pharmacological pain management, non-steroidal anti-inflammatory drug (NSAID) use, opioid use, other pain medication use, pain medication adherence factors, and beliefs about pain medication. Descriptive statistics, including measures of central tendency, are used to summarize the responses.

Results: 70 DM1 (57.1% female) and 38 DM2 (47.4% female) patients completed the survey. The frequency of pain at time of DM diagnosis was 39.5% in DM2 and 18.6% in DM1. The majority of patients reported pain in the prior week (67.5% of DM2 and 52.9% of DM1). Pain occurred for over 5 years in 42.1% of DM2 and 34.3% of DM1 patients. Approximately 60% of patients reported the use of non-drug treatments (most commonly physical therapy, massage, and heat). More DM1 patients reported use of NSAIDs (46.67% in DM1 and 37.5% in DM2), and about 20% of both groups reported NSAIDs as effective to manage pain. Current opioid use was reported by 34.6% of DM2 and 16.2% of DM1 patients. Nearly 70% of patients indicated the opioids were effective. The majority of DM2 (80.8%) patients believed that pain medications can be addictive compared to DM1 (42.1%). Approximately 33% of patients in both groups indicated the need for more effective pain medications.

Discussion: Pain frequency is high in DM1 and DM2, often with earlier onset of pain in DM2. Opioid use was high in DM patients (about 25%) relative to the general US population (7%). Pharmacists can facilitate pain management and reduce the risk of opioid addiction in DM patients via a multidisciplinary approach to care.

Grant Support: The National Registry is supported through the National Institute of Neurological Disorders and Stroke (NIH Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center grant #U54-NS048843).

P-058

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Genetic and Clinical Gender-Related Specificities Of Myotonic Dystrophy Type 1 in a Large Italian Database

RASTELLI Emanuele[1], LOZZI Flavia[1], ANTONINI Giovanni[2], BOTTA Annalisa[3], BUCCI Elisabetta[2], CASALI Carlo[4], COSTANZI-PORRINI Sandro[5,6], FREZZA Erica[1], GIACANELLI Manlio[6], GRECO Giulia[1], INGHILLERI Maurizio[7], NOVELLI Giuseppe[3], PENNISI Elena Maria[8], PETRUCCI Antonio[6], PIANTADOSI Carlo[9], ROSSI Salvatore[10], SILVESTRI Gabriella[10], TERRACCIANO Chiara[1], VANACORE Nicola[11], **MASSA Roberto**[1]

1. Center for Neuromuscular Disorders, University of Rome Tor Vergata, Rome, Italy
2. NESMOS Dept. at S. Andrea Hospital
3. Dept. of Biomedicine and Prevention, University of Rome Tor Vergata, Rome, Italy
4. Dept. of Medico-Surgical Sciences & Biotechnologies, Sapienza University of Rome, Rome, Italy
5. Medical Genetics Unit, Sapienza University of Rome, Rome, Italy
6. Neuromuscular and Neurological Rare Diseases Center, S. Camillo-Forlanini Hospital, Rome, Italy
7. Dept. of Neurology and Psychiatry, Sapienza University of Rome, Rome, Italy
8. Neurology Unit, S. Filippo Neri Hospital, Rome, Italy
9. Neurology Unit, S. Giovanni-Addolorata Hospital, Rome, Italy
10. Department of Geriatrics, Neuroscience and Orthopedics, UCSC, Rome, Italy
11. National Centre for Epidemiology, Surveillance and Health Promotion, National Institute of Health, Rome, Italy

Introduction: We have recently ascertained a different prevalence of myotonic dystrophy type 1 (DM1) among females and males (8.3 and 11/100,000, respectively). The aim of this work was to assess the prevalence of genetic and clinical features of DM1 in male and female patients in order to detect possible gender-related specificities.

Methods: Demographic, clinical, and genetic data of a large Italian DM1 population were retrieved from a multi-center database. Molecular characterization was performed using standard procedures. DM1 expansions were sized and classified according to CTG number into three genotypic classes. Gender differences were expressed as the risk ratio (RR) of men/women with 95% confidence interval (CI). RR was statistically significant at alpha=0.05 when 95% CI boundary values did not overlap value 1. P-values <0.05 were considered significant.

Results: In a sample of 943 patients, the male/female ratio was 1.2. Mean prevalent age, age at onset and age at death were similar for both sexes. Transmission of the mutation was paternal in 63% and maternal in 37% of patients. Females with a CTGn >500 were significantly more numerous. The prevalence of some major clinical features was significantly different, including: cardiac conduction defects and seborrheic dermatitis (M>F) and cataract, hypothyroidism and cutaneous xerosis (F>M). Other features, such as cardiac arrhythmias, sleepiness and overweight were equally prevalent.

Discussion: In DM1, both genotype and phenotype are influenced by gender-related factors, although the underlying mechanisms are still undetermined. Male patients are slightly more numerous and they have twofold probabilities of transmitting the disease than females. The latter more often have a larger CTGn and show a trend towards a higher transgenerational amplification as compared to males. Also, some clinical symptoms affect differently the two sexes. Therefore, gender should be considered as a variable for patients' stratification in the design of clinical studies.

Grant Support: None.

We are grateful to the Italian Myotonic Dystrophy Patients Association, DiMio, for constant encouragement of our efforts.

P-059

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Japanese Research Group for Myotonic Dystrophy: Collaboration to Facilitate Clinical Researches, Patient Registry and Patient Advocacy

MATSUMURA Tsuyoshi[1], TAKAHASHI Masanori P[2]., KIMURA En[3], The Japanese clinical research group for myotonic dystrophy

1. Department of Neurology, National Hospital Organization Toneyama National Hospital
2. Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine
3. Translational Medical Center, National Center for Neurology and Psychiatry, Japan

Introduction: Patient registries, clinical trial networks, outcome measures, best practice care, and patient support groups are essential for success of clinical trials in myotonic dystrophy (DM), which has multi-organ complications and poor medical compliance. We formed a research group in 2014 to build these clinical platforms in collaboration with related organizations.

Methods: We organized several projects to establish evidences in the managements of arrhythmia, respiratory failure, glucose intolerance, social cognitive impairments and patients with congenital form. In addition, two subjective outcome measures, the individualized neuromuscular quality of life (INQoL) and the myotonic dystrophy health index (MDHI), were translated into Japanese and validated in collaboration with the copyright holders (Mapi Research Trust, University of Rochester). Triggered with the establishment of national DM registry (http://www.remudy.jp/) in October 2014, we conducted outreach activities to facilitate its enrolment. A website (http://DM-CTG.jp/) was set up in December 2014 to provide clinical information and open seminars were held in 13 cities across Japan from January 2015 to January 2017.

Results: The results of several studies have been published and reported in several papers in this meeting. By the end of May 2017, over 620 patients participated the registry and the registry data provide epidemiological information. Moreover, participants of open seminars formed a patient advocacy group (DM family: http://www.dm-family.net/) in March 2016 and the number of member reached over 90.

Discussion: Our group could facilitate clinical researches, registry and patient advocacy. We hope the collaboration with specialists and patient group We are planning to develop a best practice guideline for DM to improve the care of DM patients.

Grant Support: This study was supported by the grant from the Japan Agency for Medical Research and Development (16ek0109172, 17ek0109259).

P-060

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Japanese Myotonic Dystrophy Type 2 Patients Carry a Haplotype Different from the European Founder Haplotype

MATSUURA Tohru[1], KIMURA Takashi[2], KITAO Ruriko[3], KOMORI Tetsuo[3], TODA Tatsushi[4], OHNO Kinji[5]

1. Division of Neurology, Department of Medicine, Jichi Medical University
2. Department of Neurology, Asahikawa Medical Center, National Hospital Organization
3. Department of Neurology, Hakone Hospital
4. Division of Neurology/Molecular Brain Science, Kobe University Graduate School of Medicine
5. Division of Neurogenetics, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine

Introduction: Myotonic dystrophy type 2 (DM2) is a subtype of the myotonic dystrophies, caused by expansion of a tetranucleotide CCTG repeat in intron 1 of the cellular nucleic acid-binding protein (CNBP) gene. The expansions are extremely unstable and variable, ranging from 75–11,000 CCTG repeats. To date, DM2 mutations have been identified predominantly in European Caucasians. Although a small number of DM2 mutations have been reported in non-European populations, DM2 patients had been considered to originate from a single common founder because they shared an identical haplotype. We previously identified the first Japanese DM2 patient carrying a disease haplotype distinct from that shared among Caucasians, indicating that DM2 exists in non-Caucasian populations and has separate founders. However, there is still some possibility of a short-130kb common haplotype (between CL3N59 and rs1871922) around the DM2 expansion among Caucasian and Japanese DM2 patients.

Methods: We investigated all available Japanese and Caucasian DM2 individuals for seven informative SNPs between CL3N59 and rs1871922 to assess the founder haplotype.

Results: Linkage Disequilibrium (LD) block, ~13 kb, surrounding the DM2 repeat was identified including the above seven SNPs. We have found a unique haplotype common to three Japanese DM2 pedigrees, distinct from that shared among Caucasians.

Discussion: Japanese DM2 patients have a unique founder different from that shared among Caucasians. The number of so far identified Japanese DM2 pedigrees have recently amounted to four. Comparison of the association between phenotype and ethnic/haplotype differences will be made possible in the future by the clinical and genetic assessment of DM2 subjects with different founders.

Grant Support: This study was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science, as well as Research Grants for Intractable Diseases from the Ministry of Health, Labour and Welfare of Japan.

P-061

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Assessing The Impact Of Gender On The Phenotype Of Myotonic Dystrophy Type 2: A Cohort Of 307 Patients

MONTAGNESE Federica[1], MONDELLO Stefania[2], WENNINGER Stephan[1], SCHOSER Benedikt[1]

1. Friedrich-Baur-Institute, Department of Neurology, Ludwig Maximilians University of Munich, Germany
2. Department of Clinical and Experimental Medicine, University of Messina, Italy

Introduction: Introduction: Myotonic dystrophies are autosomal dominant diseases characterized by a combination of muscular and multisystemic involvement. Gender has been recently found to influence the phenotype of myotonic dystrophy type 1. Our aim was to study the impact of gender on the phenotype of myotonic dystrophy type 2 (DM2).

Methods: We retrospectively studied 307 patients with DM2 analysing the following data: i) demographics (age, gender), ii) clinical features (first symptom, diagnostic delay, presence of myotonia, weakness and/or pain, comorbidities), and iii) diagnostic assessments (serological tests, electromyography, muscle biopsy). Statistical analyses were performed.

Results: Our cohort comprised 186 females (61%) and 121 males. Muscle weakness was more common in women than men (64.9% vs. 43.8%, p=0.0006), while pain was a more frequent presentation in men (49.5% vs. 29.9%, p=0.001). Patients with weakness at onset were older than those with pain and myotonia (median 49, vs. 39 and 30 years, p<0.0001). A multinomial regression model revealed that age at onset and sex were significantly and independently associated with specific types of symptoms. Cataract and thyroid diseases occurred more frequently in women (p=0,002 and p<0,001). CK and GGT were more frequently abnormal in men (p<0,001 and p=0,019) whereas no differences were found for electromyography and biopsy results.

Discussion: In conclusion it seems that, as in DM1, gender influences, independently from age, the phenotype of DM2. These gender-specific manifestations should be considered in the diagnosis and management of patients.

Grant Support: None.

P-062

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Higher Frequency of Myotonic Dystrophy Type 2 than Type 1 Among Patients with Presenile Cataracts

PEŠOVIĆ Jovan[1], RAKOČEVIĆ-STOJANOVIĆ Vidosava[2], PERIĆ Stojan[2], SENĆANIĆ Ivan[3], BOŽIĆ Marija[4], BRKUŠANIN Miloš[1], SAVIĆ-PAVIĆEVIĆ Dušanka[1]

1. University of Belgrade-Faculty of Biology, Center for Human Molecular Genetics, Belgrade, Serbia
2. Neurology Clinic, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia
3. Ophthalmology Department, Clinical Centre Zvezdara, Belgrade, Serbia
4. Ophthalmology Clinic, Clinical Centre of Serbia, Belgrade, Serbia

Introduction: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) have a variable clinical presentation, diverse initial symptoms, and a long diagnostic delay. We aimed to assess meaningfulness of genetic screening in cataract patients in order to identify new cases with myotonic dystrophy.

Methods: The study enrolled unrelated Serbian patients diagnosed with cataracts before the age of 55 with neither personal nor family history of myotonic dystrophies. The pilot sample included 53 patients, while the independent replication sample enrolled 97 patients. Genetic testing was performed by repeat-primed PCR. Newly diagnosed cases underwent detailed clinical examination by neurologists.

Results: In the pilot sample, two DM1 (3.8%) and six DM2 mutation carriers (11.3%) were identified. DM1 cases had small expansions (<100 repeats), while DM2 cases had full mutations (>75 repeats). Supportive evidence was obtained in the replication sample even though a lower percentage of positive cases was observed – no DM1 and five DM2 (5.2%) cases. Four of them had full mutations and one had a repeat expansion of size between premutation and full mutation. Considering the whole sample, the frequency of DM2 positive individuals was statistically significantly higher compared to DM1 (7.3% vs. 1.3%, p=0.021). Clinical examination revealed variable multisystem symptoms with recognizable DM2 presentations, although the patients were not aware of the disease.

Discussion: In this study, including the first DM2 genetic screening in cataract patients so far, we showed that genetic testing may be useful for identifying new DM2 cases, while new DM1 cases seem to be sporadically identified among cataract patients.

Grant Support: This study was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia (grants no. 173016 and 175083).

P-063

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

A Myotonic Dystrophy type 1 Clinical Practice Guideline for Community Occupational Therapists

RAYMOND Kateri[1,2,4], PLOURDE Annie[1,3], GAGNON Éric[3], GAGNON Cynthia[1,2,4]

1. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (GRIMN) (Jonquière, Canada)
2. Faculté de médecine et des sciences de la santé, Université de Sherbrooke (Sherbrooke, Canada)
3. Clinique des maladies neuromusculaires de Jonquière, Centre intégré universitaire de santé et de services sociaux du Saguenay-Lac-Saint-Jean (Jonquière, Canada)
4. Centre de recherche de l'Hôpital Charles-Le Moyne (CR-HCLM), Centre intégré de santé et de services sociaux de la Montérégie-Centre (Longueuil, Canada)

Introduction: Occupational therapists working with myotonic dystrophy type 1 (DM1) face significant challenges in adopting an evidence-based practice. The level of proof is often weak and the only known occupational therapy clinical practice guideline (CPG) is long (around 100 pages) and dense. CPG is also developed for overspecialized rehabilitation center, and may not meet needs of an occupational therapist working in primary care such as home-based or community services. To increase the evidence-based practice of occupational therapists working in primary care, it is necessary to adapt recommendations and format of an occupational therapy CPG for people with adult phenotype DM1 (French version).

Methods: Using the model of Rare Knowledge Mining, focus groups were performed to tailor the existing CPG to primary and community care practice, extract the appropriate recommendations, validate the selected content with DM1 expert occupational therapists, and validate, with the target audience (that is occupational therapists working in primary care setting), the final content and the format of the short version of the DM1 occupational therapy CPG.

Results: Prior clinical recommendations have been identified to assist decision-making in occupational therapists working with clients with DM1 in primary or community care. The original CPG (88 pages) with full paragraphs and general recommendations was adapted in a short version (10 pages) with bullet points and specific primary or community care recommendations. Occupational therapists who contributed to the project were enthusiastic about the short version.

Discussion: To foster an effective practice, adapting a CPG to a user-friendly tool is important. This project allows the implementation of strategies to make the content of an existing CPG for people with adult phenotype DM1 clinically accessible and usable for occupational therapists working in primary care setting. Next step will consist in translating the short CPG to other languages.

Grant Support: Ordre des ergothérapeutes du Québec (OEQ).

P-064

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Participation in Daily and Social Activities in Myotonic Dystrophy Type 1: Predictors of Changes Over a 9-year Period

RAYMOND Kateri[1,2,3], LEVASEUR Melanie[2,3], MATHIEU Jean[1,3,4], GAGNON Cynthia[1,2,3]

1. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (GRIMN), Clinique des Maladies Neuromusculaires de Jonquière, Centre intégré universitaire de Santé et de Services Sociaux (CIUSSS) du Saguenay-Lac-Saint-Jean, Jonquière, Québec (Canada)
2. The Research Centre on Aging (CDRV), CIUSSS de l'Estrie-CHUS, Sherbrooke, Québec (Canada) Centre de recherche de l'Hôpital Charles LeMoyne (CR-HCLM), Centre intégré de Santé et de Services Sociaux (CISSS) Longueuil, Québec (Canada)
3. Faculty of Medicine and Health Sciences, Université de Sherbrooke, Québec (Canada)

Introduction: Individuals with myotonic dystrophy type 1 (DM1) present important restrictions in several domains of participation, such as nutrition and mobility, leading to important personal and societal consequences. Defined as the accomplishment of daily and social activities, participation results from the interaction between personal and environmental factors. Since a decreased participation over time has been documented for several domains, a better understanding of factors influencing changes in participation is required to support management of the disease and planning of services. This study thus aimed to identify the predictors and associated factors of participation changes over a 9-year period in adults with DM1.

Methods: A descriptive longitudinal study compared baseline (2002-2004) and follow-up (2011-2013) of 115 adults with DM1 registered at a Neuromuscular Clinic, in Saguenay (Quebec), Canada. Participation was documented with the Assessment of Life Habits questionnaire. Based on a literature review and an expert consultation, theoretical models were built to guide multiple linear regressions that aim to explain participation changes with various personal and environmental factors.

Results: Controlling for disease duration, age, and CTG repetitions, preliminary results indicated that lower pinch strength, lower walking speed, and higher body mass index are significant predictors for the decrease in participation (final results will be presented at the IDMC-11 meeting).

Discussion: Identification of predictors and associated factors of participation changes is important to help health professionals in implementing more efficiently healthcare and community services to prevent restrictions of participation over time. Moreover, a qualitative understanding of how personal and environmental factors influenced changes in participation would be necessary to complement the results of the present study.

Grant Support: Canadian Institutes of Health Research (grant no. MOP-49556 and JNM-108412) and Muscular Dystrophy Canada.

P-065

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Patient Experience of Genetic Diagnosis: A Retrospective Study Investigating and Comparing Diagnoses of Myotonic Dystrophy to Huntington's Disease

RODRIGUES Miriam[1,2], BOURKE David[1], DYSART Jo[1], ROXBURGH Richard H[1,3]

1. Auckland District Health Board
2. Muscular Dystrophy Association of New Zealand
3. University of Auckland

Introduction: How do people experience receiving a diagnosis of myotonic dystrophy (DM)? Receiving a diagnosis can be traumatic, especially when the disease is incurable and can cause significant morbidity such as DM and Huntington's Disease (HD), both progressive autosomal dominantly inherited neurological diseases.

This study aims to improve clinician preparedness for future consultations through improving understanding of the patient experience in receiving such a diagnosis.

Our objective was to assess the person's experience of receiving their diagnosis elucidating a crucial interaction during what can be a difficult time. There are many aspects to delivering bad news that can make the experience better or worse such as setting, language, support available etc. Issues relating to neurogenetic diseases include prognosis, heritability and functional limitation. Quantifying patient experience allows us to assess areas of inadequacy, success, and ultimately improve practice in delivering such diagnoses.

Methods: We constructed a questionnaire including qualitative and quantitative measures of diagnosis delivery incorporating issues specific to a neurogenetic diagnosis. Patients with DM (69) or HD (70) were mailed the questionnaire. Completion was voluntary and anonymous.

Results: Response rates for each were similar: 34% in HD; 38% in DM. Overall, results encompassing experience of diagnosis were worse for DM with a mean 'unsatisfactory' results of 6.4 questions for HD compared with a mean of 10.7 for DM ($p=0.03$).

Discussion: People diagnosed with DM had a worse experience of diagnosis than those with HD. Issues identified included not having an option from whom to receive their diagnosis, inadequate support for family members at time of diagnosis and inadequate support for patients after diagnosis.

This study provides new information about the experience in receiving a neurogenetic diagnosis enabling us to better understand patient needs at the time of diagnosis, in particular that of myotonic dystrophy.

Grant Support: None.

P-066

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Pharmacological and Non-pharmacological Treatment in Myotonic Dystrophy

HANNA Miriam[1,2], **RODRIGUES Miriam J**[1,3], ROXBURGH Richard H[3,4] THEADOM Alice[2] on behalf of the MD Prev study group

1. Muscular Dystrophy Association of New Zealand
2. Auckland University of Technology, Auckland, New Zealand
3. Neurology, Auckland District Health Board, Auckland , New Zealand
4. University of Auckland, New Zealand

Introduction: This study aimed to investigate the type, dosage and funding of pharmacological and non-pharmacological treatments taken by people with myotonic dystrophy (DM) in New Zealand (NZ), and to compare use of treatments with current recommendations.

Methods: Data on 213 individuals with DM identified through a nationwide population-based prevalence study, MD-Prev, were extracted. Socio-demographic information, details of funded and non-funded prescription medications as well as use of herbal, vitamin and nutritional supplements were analysed and compared to recommendations.

Results: The study sample was predominantly adults (96%) of NZ European ethnicity (92%), diagnosed with DM1 (94%). Amongst the study participants, overall satisfaction with the standard of healthcare received was high (mean of 7.5/10). On average, participants reported 3-5 visits to their GP per year, and only 54% reported access to a neurologist. 57% of respondents were taking prescription medications, with 120 different prescription medicines identified and grouped according to the WHO ATC/DDD classification. Some participants reported taking up to 13 medications with the majority taking up to 4 different medications. About half of the participants reported having other medical conditions. One quarter had been hospitalised and 9% had had surgeries in the past year. 9% were paying for non-funded medications such as modafinil and melatonin. 35% paid for supplements.

Discussion: The findings revealed that medication use amongst individuals with DM was high, with a number of associated comorbidities and symptoms being treated simultaneously. Some medications, for example modafinil, recommended for managing symptoms common in myotonic dystrophy were not being taken due to difficulty in access to these medications. This study has the potential to inform clinical practice, improve individuals' symptom management and healthcare access.

Grant Support: This is a sub-study of a larger study, MD Prev, funded by the Health Research Council of New Zealand.

P-067

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Evaluation of Predictor Factors of Respiratory Impairment in a Cohort of Italian Myotonic Dystrophy Type 1 Patients

ROSSI Salvatore[1], DELLA MARCA Giacomo[1], SMARGIASSI Andrea[2], MELEO Emiliana[3], SCIARRA Federico[4], CALVELLO Mariarosaria[2], MODONI Anna[1], RICCI Martina[1], SANCRICCA Cristina[4], PETRUCCI Antonio[5], COSTANZI Sandro[6], SILVESTRI Gabriella[1]

1. Institute of Neurology - Catholic University of the Sacred Heart, Fondazione Policlinico A. Gemelli – Rome
2. Pulmonary Medicine Department - Catholic University of the Sacred Heart, Fondazione Policlinico A. Gemelli – Rome
3. Neurosciences department - Centro NEMO - Rome Center for Neuromuscular and Neurological Rare Diseases
4. Unione Italiana Lotta alla Distrofia Muscolare (UILDM) sez. Lazio
5. Center for Neuromuscular and Neurological Rare Diseases - San Camillo Forlanini Hospital – Rome
6. Unit of Medical Genetics, Center for Neuromuscular and Neurological Rare Diseases - San Camillo Forlanini Hospital - Rome

Introduction: In Myotonic Dystrophy type 1 (DM1) respiratory failure represents main cause of death.

Methods: We performed a retrospective cross-sectional study in a cohort of DM1 patients followed-up at our Institution and San Camillo Forlanini Hospital in Rome aiming to estimate the prevalence and to assess any predictor factor of respiratory impairment in DM1.

164 DM1 adult patients who attended at least one spirometry were included. Main spirometric parameters (FEV1, FVC, FEV1/FVC, TLC, VC, MIP and MEP), gender, age at the spirometric evaluation, n(CTG), Body Mass Index (BMI), smoking habits, disease onset, Muscular Impairment Rating Scale (MIRS), presence of Excessive Daytime Sleepiness (EDS) and other symptoms suggestive of respiratory impairment, dysphagia, structural cardiomyopathy or non restrictive lung involvement, Obstructive Sleep Apnea Syndrome (OSAS), indication to and use of Non Invasive Ventilation (NIV) were collected.

Patients were divided in 2 groups based on the presence or lack of pulmonary restriction (FVC<80% of predicted). Both univariate and multivariate analysis were carried out.

Results: 48.78% patients showed a restrictive syndrome (FVC<80% of predicted) related to the neuromuscular disease. By univariate analysis, significant differences ($p<0.05$) between the 2 groups were observed for n(CTG), MIRS, FEV1, TLC, FVC, VC, MIP, OSAS, indication and use of NIV. In the multivariate model, only MIRS significantly differed between the groups ($p=0.003$).

Discussion: In DM1 MIRS confirms to be the only independent predictor of restrictive syndrome. OSAS and n(CTG) are also associated with this feature, thus we recommend a strict respiratory follow-up in DM1 patients showing these characteristics.

Grant support: Grants Linea D.1 2016 M.I.U.R.

P-068

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Exercise-induced Oxygen Desaturation During 6-minute Walk Test in DM1 : Is it Associated with Functional Exercise Capacity?

ROUSSEL Marie-Pier[1,2], DUCHESNE Elise[1,2], GAGNON Cynthia[2,3], RIBEIRO Fernanda[1,2]

1. Université du Québec à Chicoutimi
2. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires
3. Université de Sherbrooke

Introduction: Functional exercise capacity, which can be measured by 6-min walk test (6MWT), is often reduced in patients with Myotonic Dystrophy type 1 (DM1). Exercise-induced desaturation is characterized by reductions in oxygen saturation (SpO2) during 6MWT and is reportedly associated with poor functional prognosis in several diseases. Therefore, our objectives were to determine if oxygen desaturation occurs during 6MWT in patients with DM1 whose SpO2 is normal at rest and to study the association between low levels of SpO2 and functional exercise capacity.

Methods: For 56 patients with adult DM1 phenotype, besides routine pulmonary function tests, functional exercise capacity was assessed by a 6MWT with continuous SpO2 recording. Oxygen desaturation was defined as a reduction of SpO2 below 90%. The lowest SpO2 values reached during 6MWT were also documented in order to compare reductions from resting SpO2 (′SpO2).

Results: Exercise-induced oxygen desaturation occurred in 33% of the 56 patients evaluated. Functional exercise capacity was similar for patients who presented oxygen desaturation and those whose SpO2 remained above 90% (470±129 vs 430±131m, p=0.33, respectively). No significant associations were found between walked distance and ′SpO2 during 6MWT (= 0.05, p = 0.72).

Discussion: Despite having normal SpO2 at rest, around 30% of the patients presented exercise-induced desaturation. However, reductions of SpO2 were not associated with functional exercise capacity measured by 6MWT. This study demonstrates that continuous monitoring of SpO2 during 6MWT would be a useful low-cost measure allowing efficient assessment of inadequate blood oxygenation levels during effort that are not directly associated with low walking distances or predictable from SpO2 levels at rest.

Grant Support: Canadian Institutes of Health Research, Fonds de recherche du Québec – Santé, The Marigold Foundation, CORAMH, Fondation du Grand Défi Pierre Lavoie.

P-069

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Psychosocial and Economic Burden in DM1: A Snapshot in Northern Italy

CASIRAGHI Jacopoa[1], GOTTI Elena[1], LIZIO Andrea[1], RIMOLDI Chiara[1], ZANOLINI Alice[1], **SANSONE Valeria A**[1,2]

1. NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus , Milano, Italy
2. Biomedical Sciences for Health, University of Milan, Italy

Introduction: Cognitive and behavioral issues, arrhythmias, secretion management, gastrointestinal problems and respiratory impairment have an impact on the functional, emotional and psychosocial well-being of DM1 patients and their families. We provide a snapshot of preliminary results from a comprehensive study of the functional, psychosocial and economic burden associated with DM1 in Northern Italy.

Methods: We analyzed semi-structured interviews on costs and burden in DM1 from an initial group of 23 consecutive patients (mean age 45 years ± 13.4) and 20 caregivers (mean age 52.1 years ± 12.3) from Northern Italy, attending the NEMO Clinical Center in Milan in 2017.

Results: Functional status: 50% walk unaided; 57.8% have NIV indication; 42% use secretion management devices; 10% have PMs implanted. Caregivers report comprehension (42%), speech (64%), short-term memory problems (37%) and mood fluctuations (58%). Emotional and psychological aspects: 61% of patients and 70% of caregivers describe that quality of life (QoL) is worsening. Only 30% of patients vs 80% of caregivers perceive that the disease has a severe impact on daily living. Social and economic burden: 19% overall reduced their daily workload or stopped working because of the disease. Only 40% of patients actually working declared they are affected. 41% of patients receive additional National Health support and 60% require Bank support. Additional costs: DM1 related drugs, architectural barriers, health-related travels, health-operators.

Discussion: Although it is difficult to compare costs with studies in DM1 outside Italy due to differences in healthcare and in methodology, this preliminary analysis confirms the psycho-social and economic burden of DM1 in Northern Italy. Ongoing analysis on the detailed economic analysis will quantify the costs of illness and may provide the background to support Patient Associations and Advocacies in Italy and potentially to target human and economic resources.

Grant Support: None.

P-070

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

The Myotonic Dystrophy Health Index: Italian Validation of a Disease-specific Outcome Measure

SANSONE Valeria A[1,2], LIZIO Andrea[1], GRECO Lucia[1], GRAGNANO Gaia[1], ZANOLINI Alice[1], GUALANDRIS Marco[1], IATOMASI Marino[1], HEATWOLE Chad[3]

1. NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milano, Italy
2. Dept. Biomedical Sciences for Health, University of Milan, Italy
3. The University of Rochester Medical Center, Department of Neurology, Rochester, New York USA

Introduction: The Myotonic Dystrophy Health Index (MDHI) is a disease-specific, self-reported outcome measure that assesses total disease burden and 17 areas of myotonic dystrophy type 1 (DM1) specific health. This study describes the validation process of the MDHI in an Italian cohort of adult DM1 patients (MDHI-IT) and correlated total scores and subscales with several functional measures reflecting major areas of disease impact.

Methods: 38 Italian DM1 patients were interviewed regarding the form and content of the instrument. 29 of these were recruited to test the instrument's reliability, evaluating internal consistency and test-retest reliability. All of the 38 patients subsequently underwent a battery of clinical tests that were then compared to the MDHI-IT's total and subscores in order to assess the validity, in terms of known-group and concurrent validity.

Results: The internal consistency was excellent in the total MDHI-IT score (Cronbach's ′ = 0.97) and acceptable in all of its subscales, and the test-retest reliability was high for the overall scale (Intraclass Correlation Coefficient = 0.95) and for its subscales. MDHI-IT total scores and subscales were significantly associated with neuromuscular function, cognitive and social health, respiratory function, and quality of life.

Discussion: Overall, the Italian Myotonic Dystrophy Health Index is valid and well suited to measure the multi-dimensional aspects of disease burden in myotonic dystrophy.

Grant Support: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

P-071

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

A Respiratory Symptom Checklist for Patients with Myotonic Dystrophy Type 1: A Potential Tool to Screen and Monitor Chronic Respiratory Impairment

DE MATTIA Elisa[1], LIZIO Andrea[1], FALCIER Elisa[1], SANNICOLO' Giulia[1], GATTI Valentina[1], ROSSI Gabriella[1], ZANOLINI Alice[1], POZZI Susanna[1], GRECO Lucia[1], MESSINA Sonia[1], SFRAMELI Maria[1], LUNETTA Christian[1], RAO Fabrizio[1], **SANSONE Valeria Ada**[1]

1. NEuroMuscular Omnicentre (NEMO), Fondazione Serena Onlus, Milano, Italy

Introduction: Patients with Myotonic Dystrophy (DM) have a reduced life expectancy and death usually occurs for pneumonia. In patients with adult-onset DM1 respiratory involvement more frequently presents with symptoms such as fatigue, excessive daytime sleepiness, sleep disorders and reduced cognitive performance, which only indirectly point toward pulmonary function abnormalities. The aim of this work was to determine whether the Respicheck questionnaire is a reliable, reproducible and clinically meaningful scale to detect symptoms of respiratory involvement even in apparently asymptomatic patients.

Methods: Respicheck is a 9-domains questionnaire. The first 8 domains evaluate symptoms associated with ventilatory failure and domain 9 investigates recent respiratory infections. 44 DM1 patients were recruited for test-retest reliability, 17 of these and 41 new patients (n=58) underwent respiratory, motor and quality of life (QoL) assessments for validation analysis, including construct and criterion, known group and concurrent validity. All patients were included for the internal consistency reliability (n=85).

Results: Internal consistency of the questionnaire was good (Cronbach's α=0.8). The intraclass correlation coefficient was 0.89 ($p<.0001$) for the overall scale and showed a range of consistency values in the subscales. Respicheck total score and subscales were significantly associated with global respiratory impairment and specific parameters, motor function evaluations and QoL assessments.

Discussion: Respiratory involvement negatively impacts QoL perception in DM1 patients even though respiratory symptoms are not referred. Respicheck is a reliable and consistent tool to capture symptoms of respiratory involvement even in apparently asymptomatic patients. Ongoing studies will prove whether Respicheck is able to detect sensitivity to change and can be used as a reliable respiratory outcome measure during clinical and research studies in DM1 patients.

Grant Support: Spontaneous clinical research work.

P-072

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Self-reported Sleep Features in Myotonic Dystrophy

SAVAGE Carolyn M[1], CRASTA Sheela[1], HAGERMAN Katharine A[1], PEREZ Jennifer P[1], LEARY Eileen B[2], MIGNOT Emmanuel[2], SAMPSON Jacinda[1,3], DAY John W[1,3]

1. Department of Neurology, Stanford University School of Medicine, Stanford, CA
2. Stanford University Center for Sleep Sciences and Medicine, Stanford, CA
3. Department of Neurology, Stanford Healthcare, Stanford, CA

Introduction: Myotonic Dystrophy (DM) affects many body systems including skeletal muscles, heart and brain. As many as 90% of the adults with DM report fatigue as one of their two most bothersome symptoms. Because of the strong relationship between sleep disorders and fatigue, and the complex sleep abnormalities in DM, we asked adults with DM to complete the Alliance Sleep Questionnaire (ASQ), which employs many different validated patient-reported outcome scales to investigate multiple aspects of sleep.

Methods: The ASQ combines many scales validated in adults with neurological and sleep conditions including: Functional Outcomes of Sleep Questionnaire (FOSQ), Insomnia Severity Index (ISI), Insomnia Symptom Questionnaire (ISQ), Generalized Anxiety Disorder 7-item (GAD-7) scale, and Patient Health Questionnaire (PHQ-9) for depression. It also contains the Epworth Sleepiness Scale (ESS) and Fatigue Severity Scale (FSS) previously used in DM1 studies. A total of 141 adult participants (90 DM1, 15 DM2, 4 DM and 32 healthy controls) completed the ASQ online. Student's t-test was used to determine significance.

Results: The ESS, ISI, FSS and FOSQ scales showed statistically significant differences between controls and DM. FSS and ISI also reached clinical significance of fatigue and subthreshold insomnia respectively. ESS was worse in DM, but the average score did not reach the clinical definition of "sleepy." No significant difference was found between DM1 and DM2 in preliminary analyses. Individual questions within the ASQ revealed specific issues of greatest clinical significance to people with DM.

Discussion: The ASQ encompasses several clinical scales that are relevant to and significantly different in people with DM. Differences between DM1 and DM2 were not discernable, likely due to DM2 sample size. Future studies must include increasing sample size, longitudinal tracking, and cross comparison of patient-reported symptoms with abnormalities detected by actigraphy, as well as overnight and daytime sleep studies.

Grant Support: MDA, Myotonic Dystrophy Foundation, Marigold Foundation, NIH-P01

P-073

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Sleep Abnormalities in Myotonic Dystrophy Patients

SHROFF Sheetal[1], ASLAM Hina[1], THONHOFF Jason[1], PLEITEZ Milvia[1], ROSENFIELD David[1], SIMPSON Ericka[1]

1. Houston Methodist Neurological Institute

Introduction: Myotonic Dystrophy (DM) is a multisystem disorder which affects cardiovascular, musculoskeletal, endocrine and respiratory system. Sleep is equally affected thus increasing the morbidity and mortality. Sleep impairment and/or daytime somnolence are the commonly seen in these patients. This led to our review of the sleep characteristics in DM patients. We currently follow about 100 DM patients every year in Houston Methodist Myotonic Dystrophy Multidisciplinary Clinic (DMMC).

Methods: We did retrospective analysis of overnight sleep studies in 8 randomly selected DM patients who were seen in Houston Methodist Myotonic Dystrophy Multidisciplinary Clinic (DMMC) during 2012-2017.

Results: We looked at sleep studies of 8 patients (6 DM1, CTG repeats 270-900 & DM2 CCTG 140->15,000). Mean age was 44.5 years (SD 16.5, range 20-70 years), body mass index 27.2 kg/m2 (7.4, 19.6-42 kg/m2), male to female ratio was 2:6, Epworth Sleepiness Score (ESS) 7 (4, 2-15), sleep latency 50.3 min (57.8, 8-164.5 min), total sleep time 261.6 min (149.4, 51.5-424 min), sleep efficiency 61.2% (29.1, 10.9-87.7%), total Non-Rapid Eye Movement (NREM) sleep 84.5% (9.7, 72.2-100%), stage N1 13.4% (7.9, 3.7-25.1%), stage N2 67.8% (6.3, 59.5-75.7%), stage N3 2.4% (4.18, 0-9.7%), total Rapid Eye Movement (REM) sleep 16.3% (9.7, 0-27.8%), lowest desaturation 84.5% (5.2, 77-92%, 2 mild, 5 moderate, 1 severe), snore arousal index 1.26 (1.6, 0-3.7) and heart rate in sleep 64.3 bpm (13.1, 49.7-92.2 bpm). All but 2 had sleep apnea (4 mild, 1 moderate and 1 severe). Periodic limb movement index was abnormal in 25% of patients.

Discussion: Our patients had low sleep efficiency and prolonged sleep latency. Sleep predominantly consisted of NREM stage N1 and N2 compared to stage N3. REM sleep was reduced. Sleep apnea was seen in 75% of our patients. All our patients had significant desaturation during sleep. Further correlation of number of repeats and sleep abnormalities needs to be studied. We plan to expand this study to include larger patient population.

Grant Support: None

P-074

Session 4: Clinical, Ethical and Social Issues, and Disease Burden

Respiratory Assessment in Patients with Myotonic Dystrophy Type I

WENNINGER Stephan[1], GOLDINA Olga[1], BABACIC Haris[1], MONTAGNESE Federica[1], SCHOSER Benedikt[1]

1. Klinikum der Universität München

Introduction: Respiratory function in patients with adult-onset DM1 usully declines with duration of disease and is usually assessed by measuring forced vital capacity (FVC) and forced expiratory flow in first second (FEV1). Although not yet widely used, the maximal inspiratory pressure (MIP) and the maximal expiratory pressure (MEP) are validated estimators of the function of respiratory muscles. The aim of this study was to evaluate clinical symptoms of respiratory involvement using the "Respiratory involvement symptom check-list" for myotonic dystrophies and assess correlation with measured respiratory function using FVC, FEV1, MIP and MEP in DM1 patients.

Methods: The pulmonary function was assessed through measuring FVC and FEV1, both in upright and supine position, with spirometry. MIP and MEP were measured with digital manometry. We used the reference range for differentiating abnormal values. Clinical Symptoms of respiratory involvement were assessed by the "Respiratory involvement symptom check-list" for myotonic dystrophies.

Results: 55 patients were included in the analysis: 31 male (56.36%) and 24 female (43.64%). A statistically-significant linear relationship between the four main diagnostic measures was observed in the whole sample of DM1 patients. Almost 90% of DM1 patients had abnormal MEP, but only 55% had abnormal FVC. There was no correlation between total score or subscores of the "Respiratory involvement symptom check-list" for myotonic dystrophies and measures FVC, FEV1, MIP or MEP. There was no significant decline in FVC and FEV1 when changing the position from upright to supine.

Discussion: "Respiratory involvement symptom check-list" for myotonic dystrophies is a good screening tool to detect symptoms related to respiratory involvement, but does not predict FVC, FEV1, MIP or MEP. Our data suggest that MEP might be a measurement that is the most sensitive to respiratory ventilation-impairment – about 90% of DM1 patients had abnormal MEP values (49 out of 55), but only 55% had abnormal FVC (30 out of 55).

Grant Support: None.

P-075

Session 5: Specific Disease Features - CNS, Cardiac and GI

Value of Screening Cardiac Abnormalities in Myotonic Dystrophy Type 1 Patients

ABDUL RAZAK Kavitha[1,2], HAWKINS Aaron M[1,2], KHOO Tien K[1,4], BURRAGE Matthew K[3,5], ATHERTON John J[5,6], CHALLA Veera RP[1,3], JACKSON Richard V[1,2], DAHIYA Arun[1,3]

1. School of Medicine, Griffith University, Queensland, Australia
2. Department of Medicine, Logan Hospital, Meadowbrook, Queensland, Australia
3. Department of Cardiology and Medicine, Logan Hospital, Queensland, Australia
4. School of Medicine, University of Wollongong, New South Wales, Australia
5. School of Medicine, University of Queensland, Queensland, Australia
6. Department of Cardiology, Royal Brisbane and Women's Hospital, Herston, Queensland, Australia

Introduction: Cardiac abnormalities including Electrocardiographic (ECG) abnormalities and left ventricular (LV) dysfunction are associated with mortality in Type 1 Myotonic Dystrophy (DM1). Early detection of cardiac involvement identifies those at risk of developing cardiac complications and therefore may assist in their management. We sought to evaluate baseline prevalence of cardiac abnormalities in DM1 patients.

Methods: 29 DM1 patients were prospectively evaluated with clinical history and examination, genetic testing, 12-lead ECG, 24-hour Holter monitoring, transthoracic echocardiogram and cardiac MRI (CMR). Muscle involvement was graded using the Muscular Impairment Rating Scale (MIRS). Patients with symptomatic cardiac failure and any implantable cardiac device were excluded from the study.

Results: The mean age of patients was 42 + 13.2 years (62% males). 15 patients (51.1%) had >200 CTG repeat lengths and mean CTG repeat length was 437.33+213.26. The MIRS score correlated significantly with patient age (0.440, p=0.017). ECG abnormalities include sinus bradycardia (41.4%), atrial flutter (3.4%), PR interval >200msecs (37%), QRS duration>120msecs (20.7%) and corrected QTc >450msecs (13.8%). Echo LV Ejection Fraction (LVEF) < 55% (10.%) and grade 1 diastolic dysfunction (10.3 %) were both present. Echo RV S' (Velocity of the tricuspid annular systolic motion) was <10cm/sec in 46.7%, suggestive of early Right Ventricular (RV) systolic dysfunction. CMR LVEF was <55% in 13 patients (44.8%) and CMR RVEF <45% in 1 (3.4%) patient.

Discussion: ECG, echocardiogram and CMR abnormalities were not significantly correlated with MIRS score, CTG repeat lengths or gender. There is a high prevalence of cardiac functional and conduction abnormalities in DM1 patients, highlighting the importance of routine cardiac surveillance of DM1 patients to potentially reduce subsequent cardiac morbidity and mortality.

Grant Support: Griffith University Research Funds.

P-076

Session 5: Specific Disease Features - CNS, Cardiac and GI

Social Cognition Impairment in the Childhood-onset Form of DM1

ANGEARD Nathalie[1/4], HUERTA Erika[1], JACQUETTE Aurélia[2], CHABY Laurence[3], JEAN Xavier[3], COHEN David[3], GARGIULO Marcela[4/5], SERVAIS Laurent[4], EYMARD Bruno[4], HERON Delphine[2]

1. Inserm U1129, Paris, France; Paris Descartes University, Sorbonne Paris Cité, France; CEA, Gif sur Yvette, France
2. Centre de Référence "Déficiences intellectuelles", Département de Génétique et INSERM U 975, Groupe Hospitalier Pitié-Salpêtrière, APHP, Paris, France
3. CNRS UMR 7222 Institut des Systèmes Intelligents et Robotiques, et Service de Psychiatrie de l'Enfant et de l'Adolescent, Groupe Hospitalier Pitié-Salpêtrière, APHP, Paris, France
4. Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, APHP, Paris, France
5. Laboratoire de Psychologie Clinique et Psychopathologie (EA 4056), Université Paris Descartes, Sorbonne Paris Cité, Institut de Psychologie, Boulogne, France

Introduction: Brain involvement is now well recognized as a common feature in a substantial proportion of patients with DM1. Depending on the phenotypic expression, the degree of cognitive impairment remains heterogeneous, ranging from mental retardation (in the congenital form) to executive, theory of mind and emotional processing deficits (in the adult-onset form). While somatic symptoms may be relatively discrete in the childhood form, deficits in visuo-spatial functions as well as verbal working memory weakness, poor attentional process and alexythimia have been reported. The objective of the current study is to explore the extent of potential impairments on the recognition of emotional expressions and theory of mind domain within the childhood phenotype.

Methods: 26 patients aged 6 to 20-year olds with the childhood DM1 (paternal or maternal transmission) and 14 typically developing children/adolescents matched to the DM1 group (according to age and IQ) were included. All subjects took a computerized experimental task were they had to select one label
from a list choice that best described the emotion that was being expressed (visual, auditory or bimodal stimuli). Theory of mind was also evaluated with Nepsy II Verbal and Contextual subtests.

Results: Preliminary results highlight different patterns of performances according to (1) modal/multimodal emotional processing and (2) the valence of emotional expression. Patients also performed better in tasks requiring understanding how emotion relates to social context rather than tasks involving manipulation of another individual's perspective (such as false belief, deception or pretending).

Discussion: These data could provide a source of evidence of a continuum between neurocognitive deficits emerging during childhood and those occurring in adults and emphasize the early vulnerability of social cognition in the childhood DM1.

Grant Support: This paper was supported by a grant (n°16716) of the AFM (Association Française contre les Myopathies).

P-077

Session 5: Specific Disease Features - CNS, Cardiac and GI

Fatigue in Children with Congenital Myotonic Dystrophy

SINGERIS Stephanie[1], CEBALLOS Delia[1], HICKS Rhiannon[1], ZAPATA Eugenio[1], BERGGREN Kiera[2], CROCKETT Becky[2], DIBELLA Deanna[2], DIXON Melissa M.[2], MCINTYRE Melissa[2], PUCILLO Evan[2], JOHNSON Nicholas[2], **CAMPBELL Craig**[1]

1. Western University, London, Ontario
2. University of Utah, Salt Lake City, Utah

Introduction: Congenital Myotonic Dystrophy (CDM) is the infant-onset presentation of Myotonic Dystrophy type 1 (DM1). Fatigue is an important contributor to health related quality of life (HRQOL) in pediatric neuromuscular diseases. The objective of our study is to determine the impact of fatigue and the main factors associated with it in children with CDM.

Methods: 45 children with CDM and 21 controls aged 0-13 years were enrolled. Pediatric Daytime Sleepiness Scale (PDSS) was used as the measure of fatigue. HRQOL measured Quality of Life (PedsQLTM 4.0 Generic Core Scale, PedsQLTM 3.0 Neuromuscular Module); demographics using Participant Age; physical capacity using the Six Minute Walk Test (6MWT) in children ages > or = to 6 years, Manual Muscle Testing (MMT), and Body Mass Index (BMI); disease severity by CTG Repeat Size (CTGrs) and Comorbidities; imaging biomarkers by Lean Body Mass (DEXA Scan); quality of sleep by the Pediatric Sleep Questionnaire (PSQ). Correlations were run using the Pearson correlation.

Results: Highest levels of fatigue were found in patients with CDM (μ 12.04 +/- 6.37) when comparing to controls (μ 8.53 +/- 5.04). Fatigue was associated with poor HRQOL (PedsQL Generic Core score r=-0.407, p=0.008; PedsQL Neuromuscular module r=-0.399, p=0.012). Factors correlated with fatigue included: older age (r=0.364, p=0.014), decreased physical capacity (r=-0.695, p=0.012) and inadequate sleep quality (r= 0.323, p=0.030). No statistical significance was found between the other variables.

Discussion: Fatigue is a common feature in patients with CDM. In this sample it was associated with three principal factors: age, physical capacity and quality of sleep. The recognition of fatigue and understanding these associated factors could improve the treatment of patients with CDM.

Grant Support: Research supported by the Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapeutics.

P-078

Session 5: Specific Disease Features - CNS, Cardiac and GI

Cross-sectional Study of Orofacial Strength and Functional Outcomes in Congenital Myotonic Dystrophy

BERGGREN, Kiera[1], CROCKETT Becky[1], HUNG Man[2,3], BOUNSANGA Jerry[3], BUTTERFIELD Russell[1], CAMPBELL Craig[4], JOHNSON Nick[1]

1. University of Utah, Dept of Neurology
2. University of Utah, Dept of Family and Preventative Medicine
3. University of Utah, Dept of Orthopaedics
4. University of Western of Ontario, Dept of Paediatrics

Introduction: Children with congenital myotonic dystrophy (CDM) have communication difficulties and dysphagia that impact their quality of life. There is minimal data on the appropriate manner in which to objectively measure these issues.

Methods: Participants with CDM between the ages of 0 and 13 were enrolled in the study into cohorts based on age (0-2, 3-6, and 7-13), along with age matched healthy control participants (HC). Visits occurred over a two-day period. Lip and tongue strength, as measured by force or pressure applied was assessed in the two older cohorts using a lip force meter (LFM, in Newtons, N) and the Iowa Oral Performance Instrument (IOPI, in kilopascals, kPA). Children were also qualitatively assessed by a speech therapist while reading and eating to document overall function.

Results: Forty children with CDM and 29 controls were recruited. Of these, all 29 healthy controls were able to complete all measures. A subset of affected participants completed the strength and function trials. LFM average data for the two cohorts was 2.1 N (SD 0.9 N, younger) and 3.1 N (SD 3.6 N, older) for children with CDM. HC data was 7.4 (SD 1.8 N) and 19.6 N (SD 6.7 N), respectively. For the IOPI, affected participants averaged 10.9 and 13.1 kPa (SD 8.4 and 7.7 kPa respectively) for the two cohorts. HC averaged 36.1 and 42.8 kPa (SD 14.8 and 12.3 kPa). Both the IOPI (ICC=0.75) and LFM (ICC=0.96) had good test retest reliability. Ingestion of solids was significantly negatively correlated with performance on both IOPI (-0.463, p=0.046) and LFM (-0.612, p=0.004). Dysarthria was negatively correlated with IOPI performance (-0.547, p=0.012).

Discussion: This work demonstrates the reliability of use of instruments measuring lip and tongue strength in children with CDM and healthy controls and identifies correlations with features of dysphagia and dysarthria. These measures may be of use in therapeutic trials as markers of disease burden in CDM patients.

Grant Support: Research funding supported by the Muscular Dystrophy Association, NINDS (1K23NS091511-01), Valerion Therapuetics, Utah Neuromuscular Fund.

P-079

Session 5: Specific Disease Features - CNS, Cardiac and GI

Retinal Changes in Patients with Myotonic Dystrophy Type 1 (DM1)

DE SANTIS Tiziana[1], FIONDA Laura[1], VANOLI Fiammetta[1], CALAFIORE Silvia[1], BUCCI Elisabetta[1], MANNINO Giuseppe[1], MARAONE Giorgia[2], LICHELLI Loretta[1], ANTONINI Giovanni[1]

1. Sant'Andrea Hospital, NESMOS Department, Faculty of Medicine and Psychology, "Sapienza" University of Rome
2. A. Fiorini Hospital, Department of Ophthalmology, Terracina (LT), Italy, "Sapienza" University of Rome

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic disorder. Ocular manifestations like early cataract, ptosis, alteration of intraocular pressure, hypotony and retinal pigmentary changes are common. The aim of this study was to detect retinal morphology changes in DM1 with optical coherence tomography (OCT).

Methods: Forty-seven DM1 consecutive patients and 22 healthy controls were recruited. Exclusion criteria were: age <15 and >70 years, refractive error/astigmatism of ≥5.00 diopters, presence of any retinal disease and optic neuropathy, glaucoma, mature cataract, previous eye surgery and amblyopia. Fifty-two eyes of 26 patients were studied. Fovea thickness, macular Ganglion Cell Complex (GCC) and peripapillary Retinal Nerve Fiber Layer (pRNFL) measurements were performed by SD-OCT (Optovue).

Results: Results were compared using T-Student, Anova and Mann-Whitney tests. pRNFL thicknesses were significantly decreased in DM1 eyes compared to control group (average p=0,0021, RNFL Superior p=0,0013, RNFL Inferior p= 0,0091) regardless patients' MIRS (muscular impairment rating scale) score. GCC and fovea thicknesses were not statistically significant. There was an apparent negative correlation between the GCC in the worst-affected eye and the DM1 patients' MIRS scores (p=0,0354), whereas pRNFL thickness was not significant in patients with a different score. There was no association between the number of CTG repeats and fovea, pRNFL and GCC thicknesses (p=.0,3665, p=0,1025, p=0,4554). One possible explanation is that RNFL may be due to premature ageing.

Discussion: The study shows that SD OCT and pRNFL protocol in particular could allow a more accurate view in DM1 research providing understanding of the pathophysiology, as well as the detection of occult neuro-degeneration.

Grant Support: None

P-080

Session 5: Specific Disease Features - CNS, Cardiac and GI

Pyomyositis in a Patient with Myotonic Dystrophy Type 1

HAMILTON Mark J[1,4], LONGMAN Cheryl[1], SWORD David2[2], CHARON JP[3], MONCKTON Darren G[4], FARRUGIA Maria E[5]

1. West of Scotland Clinical Genetics Service, Queen Elizabeth University Hospital, Glasgow, UK
2. Department of Respiratory Medicine, University Hospital Ayr
3. Department of Radiology, University Hospital Ayr
4. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK
5. Department of Neurology, Institute of Neurological Sciences, Queen Elizabeth University Hospital, Glasgow, UK

Introduction: Pyomyositis is a primary infection of skeletal muscle with abscess formation. It is a significant source of morbidity in tropical climates, but much rarer in temperate regions, typically occurring only in association with immune-suppressing conditions such as HIV or malignancy. While it could be postulated that altered muscle architecture due to inherited muscle disease might predispose to muscle infection, no cases have previously been reported in this group. In this context, we present the clinical and radiological features of a male with myotonic dystrophy type 1 (DM1) who developed pyomyositis following an episode of urinary sepsis.

Methods: Written informed consent was provided by patient for publication of medical imaging and data from clinical records.

Results: A 44 year old male with a history of adult-onset DM1 and recurrent urinary tract infections presented with abdominal pain and fever. E. coli were isolated from urine and blood, and the patient improved with intravenous antibiotics. Before discharge, he complained of left thigh pain, and deep vein thrombosis was excluded by ultrasound. Ten days later the patient presented again with fever and tachycardia. The left posterior thigh was now tender and indurated. MRI identified a large fluid collection replacing the lower two thirds of the left semitendinosus muscle, confirming a diagnosis of pyomyositis. Other muscles showed low-grade oedema and fibro-fatty infiltration consistent with DM1. The patient again improved with intravenous antibiotics followed by percutaneous aspiration of the collection.

Discussion: It seems probable that DM1-related dystrophic changes impairing normal defences contributed to seeding of E.coli bacteraemia to muscle in this case. Clinicians should consider pyomyositis in the differential diagnosis of DM1 patients presenting with systemic signs of infection, and maintain a low threshold for imaging where patients report focal tenderness or swelling.

Grant Support: Muscular Dystrophy UK, Chief Scientist Office

P-081

Session 5: Specific Disease Features - CNS, Cardiac and GI

Modified Maintenance of Wakefulness Test and Level Polysomnography as a Tool for Clinical Sleep Assessment of Patients with Myotonic Dystrophy Type I

HAMILTON Mark James[1*], NIKOLENKO Nikoletta[2*], JIMENEZ-MORENO Cecilia[2], WOOD Elizabeth[2], ANDERSON Kirstie N[3], WEST Sophie[3], LOCHMÜLLER Hanns[2], ATALAIA Antonio A[2]
** equal contribution*

1. Institute of Molecular, Cell and Systems Biology, University of Glasgow, UK
2. John Walton Muscular Dystrophy Research Centre, University of Newcastle, UK
3. Regional Sleep Service of Newcastle upon Tyne Hospitals Foundation Trust

Introduction: Maintenance of Wakefulness Test (MWT) and Multiple Sleep Latency Tests (MSLT), preceded by a Polysomnography (PSG), are traditional tools for evaluation of excessive daytime sleepiness (EDS), though are labour-intensive and costly. We aimed to investigate the use of Ambulatory Polysomnography (aPSG) and modified MWT (mMWT) as an alternative means to quantify EDS in clinical, non-research settings for myotonic dystrophy type 1 (DM1) patients.

Methods: Seventeen patients with genetically confirmed DM1 were serially recruited from specialist outpatient clinics in two UK centres (Glasgow and Newcastle) for assessment of sleep-disordered breathing (SDB) and EDS by aPSG (American Academy Sleep Medicine level 2 full-polysomnography), followed by a 40 minute mMWT, commenced two hours after the end of the aPSG.

Results: Seventy one per cent of PSG results were of adequate quality. Of these, 25% showed evidence of SDB, defined as Apnea/Hypopnea Index and/or Oxygen Desaturation Index >=10/hour. Most patients with SDB had normal mMWT. Seven out of 14 patients had abnormal mMWT results, having fallen asleep during the test with a mean sleep latency of 13.7 +/- 5.47 minutes.

Discussion: The objective of this pilot study was to establish feasibility of aPSG followed by mMWT for future implementation as a standard procedure for routine assessment of SDB and EDS in DM1 patients. A little over 25% of tests were deemed not valid due to poor quality of traces, but as a trade-off this procedure offers less disturbance for patients and reduces the workload and costs of running traditional in-lab tests for all patients.

Grant Support: MDUK Fellowship (Dr. Mark Hamilton), NIHR TRC project PhenoDM1 and Wyck Foundation/ Myotonic Dystrophy Foundation grant.

P-082

Session 5: Specific Disease Features - CNS, Cardiac and GI

Abnormal Liver Function Tests in a Scottish Cohort with Myotonic Dystrophy Type 1

HAMILTON Mark J[1,2], CUMMING Sarah A[2] , BALLANTYNE Bob[1], McWILLIAM Catherine[3], ROBB Yvonne[4], LONGMAN Cheryl[1], FARRUGIA Maria E[5], PETTY Richard[5], MONCKTON Darren G[2], The Scottish Myotonic Dystrophy Consortium, **GREGORY Helen**[6]

1. West of Scotland Clinical Genetics Service, Queen Elizabeth University Hospital, Glasgow G51 4TF, UK
2. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8QQ, UK
3. East of Scotland Regional Genetics Service, Tayside University Hospitals NHS Trust, Ninewells Hospital, Dundee DD1 9SY, UK
4. South East Scotland Clinical Genetics Service, Western General Hospital, Edinburgh EH4 2XU, UK
5. Department of Neurology, Institute of Neurological Sciences, Queen Elizabeth University Hospital, Glasgow G51 4TF, UK
6. North of Scotland Clinical Genetics Service, Aberdeen Royal Infirmary, Aberdeen AB25 2ZA, UK

Introduction: Abnormal liver function tests (LFTs) have been noted in myotonic dystrophy type 1 (DM1) for many years, underlying mechanisms still unclear. A cohort of patients with DM1 in Scotland were studied retrospectively to explore correlations between abnormal LFTs, CTG length and other clinical features.

Methods: 228 adults with DM1 were studied, mean age 47 years. In 89, CTG expansion was measured by small-pool PCR. Abnormalities in LFTs, specifically transaminases (ALT), were recorded and any further liver investigations were ascertained. Correlations with ALT were looked for with age, CTG repeat length, fatigue, Epworth Sleep score and muscle power.

Results: 41% of the cohort had raised transaminases (ALT>50) though few had undergone further liver investigations. Ultrasound scan was performed in 52(23%), of which fatty liver was found in 18%, gallstones 8%, and cirrhosis 2%. There was a trend towards increasing ALT among older patients that did not reach statistical significance (p=0.157). Transaminases did not correlate with progenitor or modal allele length in univariate or in multivariate analysis including age. Substantial elevations of ALT were more common with significant muscle impairment (measured as MIRS). In a subset of 35 patients a significant positive correlation between AST and fatigue scores was found (p=0.01).

Discussion: Liver function test abnormalities were common in our DM1 population. Extended liver investigations were under-utilised, but yield of secondary diagnoses were low where these have been done. Fatty liver was the most common finding. Non-alcoholic fatty liver disease (NAFLD) is a common liver problem in the western world affecting 20-30% of the population. Long term effects of fatty liver in DM1 are unknown, though our data suggest a possible link to fatigue symptoms. Neither age nor CTG repeat length were significant predictors of liver dysfunction. A prospective phenotyping study including ultrasound would be helpful to further explore these clinical features.

Grant Support: None.

P-083

Session 5: Specific Disease Features - CNS, Cardiac and GI

Deranged Liver Function in Myotonic Dystrophy–Type 1 (DM1) and the Role of Fibroscan

JOSEPH Paul[1,2], KHOO Tien K[1,5], BLACK Marrianne[3], KOLOSKI Natasha[3,4], PATHMANATHAN Pavthrun[1], JACKSON Richard[1,2], HOLTMANN Gerald[3,4]

1. Griffith University, School of Medicine, Queensland, Australia
2. Dept. of Medicine, Logan Hospital, Queensland, Australia
3. Dept. of Gastroenterology and Hepatology, Princess Alexandra Hospital, Queensland, Australia
4. Faculty of Medicine, University of Queensland, Australia
5. School of Medicine, University of Wollongong, New South Wales, Australia

Introduction: Insulin resistance (IR) is a feature of DM1 and is associated with non-alcoholic fatty liver disease (NAFLD). This study aimed to characterise liver function in DM1 biochemically and by imaging studies.

Methods: We measured Liver Function Tests (LFTs) in 29 DM1 patients (17 males). A retrospective audit of previous LFTs and liver ultrasound (US) was done. Prospective laboratory investigations assessed for liver function (LF) abnormality were done in all patients. All patients with abnormal LFTs had liver US. All patients with abnormal LFTs and a normal liver US had a Fibroscan (FS), a non-invasive assessment of liver stiffness. FS measurement of less than 7 kPa indicated minimal or no fibrosis and more than 13 kPa indicated likely cirrhosis.

Results: The most common abnormal LFTs were elevated transaminases [Asparate Transaminase (AST), Alanine Transaminase (ALT) and Gamma-Glutamyl Transaminase (GGT)]. Males (64%) and females (50%) had abnormal ALT. AST was also abnormal in males (64%) and females (33%). Both genders had abnormal GGT (60%). Thirteen participants with abnormal LF had an US and 10 of these had FS. No participants had cirrhosis on US. However, two patients (both females) showed values consistent with cirrhosis on FS. Mean liver stiffness measured by FS was higher in females (8.85 kPa, SD;5.6) than males (5.43 kPa, SD; 2.6). The majority of patients did not have cirrhosis despite having abnormal LFTs in preceding years.

Discussion: There is a high prevalence of abnormal LF in DM1. This is the first study to use FS to evaluate abnormal LF in DM1. Fibroscan indicated 2 patients (20%) with possible cirrhosis, not diagnosed by US. Extensive screening using biochemical and imaging studies to determine other causes of the abnormal LF did not yield any specific aetiology. This suggests that IR and NAFLD may be a cause of abnormal LFTs in DM1.

Grant Support: Done with in-kind support from the Gastroenterology Department, PA Hospital, Queensland, Australia

P-084

Session 5: Specific Disease Features - CNS, Cardiac and GI

Implementing a Graded Exercise Therapy to Severely Fatigued DM1 Patients: Experience from the OPTISTIMIC Study

JIMENEZ-MORENO Aura Cecilia[1], OKKERSEN Kees[2,3], MAAS Daphne[4], KNOOP Hans[5,6], LOCHMULLER Hanns[1], GORMAN Grainne[7], VAN ENGELEN Baziel GM[2,3] on behalf of the OPTIMISTIC consortium

1. Institute of Genetic Medicine, Newcastle University, Newcastle upon tyne, UK
2. Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands
3. Department of Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands
4. Department of Physical Therapy, Radboud University Medical Centre, Nijmegen, The Netherlands
5. Expert Centre for Chronic Fatigue, VU Medical Centre, Amsterdam, The Netherlands
6. Department of Medical Psychology, Academic Medical Centre (AMC), University of Amsterdam, the Netherlands
7. Institute of Aging and Health, Newcaslte University, Newcastle upon Tyne, UK

Introduction: Exercise on Myotonic Dystrophy type 1 (DM1) patients has been acknowledged as important for health in general. However, DM1 disease-specific references of safety and feasibility are important. Thus, this report presents the experience from the multinational OPTIMISTIC study when implementing a graded exercise therapy (GET) as part of the overall study intervention

Methods: GET was officially implemented in two out of the four centers involved in the trial. 33 participants previously randomized to the intervention group received a structure GET program aiming to further increase their activity and aerobic levels. GET included an initial one-to-one appointment with a physiotherapist to explain the program aims and to establish a commonly decided goal. Activities recommended will vary from walking to swimming or joining a gym. Follow-up visits allowed the program to be re-structured or increased when needed and these were possible by phone.

Results: So far, results support GET as feasible and safe when lead by a disease-expert physiotherapist. An initial face-to-face visit with the participant allowed the explanation of exercise concept and its distinction from physical activity. Within 3 to 10 months a total of 236 sessions were delivered. The increment of the activity levels was objectively confirmed by these patients' activity monitor reports. Other outcome measures such as reported fatigue and 6MWT also improved from baseline to 10 months follow-up visit. This protocol of intervention will be presented.

Discussion: The efficacy of this intervention needs to be confirmed by comparing this group against the control group that followed care as usual, however, its safety and feasibility have been shown once more and there is the possibility to consider this protocol when introducing a GET program to a patient with DM1 as part of their standard of care.

Grant Support: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007– 2013) under grant agreement n° 305697.

P-085

Session 5: Specific Disease Features - CNS, Cardiac and GI

Factors Associated with Daytime Sleepiness and Fatigue in a Longitudinal Cohort of Patients with Myotonic Dystrophy Type 1

LABERGE Luc[1], GALLAIS Benjamin[2], MATHIEU Jean[2], AUCLAIR Julie[1], GAGNON Cynthia[2]

1. ÉCOBES – Recherche et transfert, Cégep de Jonquière, Jonquière; Département des sciences de la santé, Université du Québec à Chicoutimi, Chicoutimi
2. École de réadaptation, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Québec, Canada

Introduction: Daytime sleepiness and fatigue are two prominent symptoms of DM1 but little is known about their progression over time when controlling for individual factors.

Methods: At a 9-year interval, 200 and 115 patients respectively participated to Time 1 and Time 2 (T1, T2). Mixed-effect models were used to take into account repeated measures within a single linear regression model to identify correlated factors of daytime sleepiness and fatigue. Gender, age, BMI, IQ, CTG repeat number, degree of muscular impairment, physical pain, psychological distress, depression, diabetes hypothyroidism, habitual bedtime, and habitual sleep duration were considered. Final models comprise 277 and 275 observations for Daytime Sleepiness Scales (DSS) and Fatigue Severity Scale (FSS) scores, respectively. SAS 9.4 Proc Mixed was used for statistical analyses.

Results: Mean (SD) DSS score increased from 4.5 (2.9) at T1 to 5.3 (3.4) at T2 ($p<.01$). Controlling for sex (n.s.) and CTG repeat number (n.s.), the final model revealed that mean daytime sleepiness increased between T1 and T2 ($p<.05$). Also, higher daytime sleepiness was found in patients who are younger ($p<.05$) and who have a higher BMI ($p<.05$), greater muscular impairment ($p<.05$), higher psychological distress ($p<.001$) and with a history of depression ($p<.01$). Mean (SD) FSS score increased from 4.4 (1.7) at T1 to 4.8 (1.7) at T2 ($p<.01$). Controlling for age (n.s.), CTG repeat number (n.s.), and habitual bedtime (n.s.), the final model revealed that mean fatigue increased between T1 and T2 ($p<.05$). Also, higher fatigue was found in women ($p<.05$) and in patients with a higher habitual sleep duration ($p<.01$), higher BMI ($p<.05$), higher muscular impairment ($p<.001$), and higher psychological distress ($p<.001$).

Discussion: These results indicate that such measures as BMI and degree of muscular impairment that are part of physicians' routine check-up are useful to monitor daytime sleepiness and fatigue levels of DM1 patients.

Grant Support: Canadian Institutes of Health Research: grant no. JNM-108412.

P-086

Session 5: Specific Disease Features - CNS, Cardiac and GI

Effects of Brain Abnormalities on Muscle Function in DM1

NOPOULOS Peg[1], GUTMANN Laurie[1], PHETTEPLACE Janel[1], THEDENS Dan[1], DEVOLDER Ian[1], SHIELDS Richard[1]

1. University of Iowa

Introduction: Neuromuscular dysfunction in DM1 is due to a primary abnormality in muscle function. However, the brain is also significantly affected in DM1. Most of the CNS findings have been geared towards linking the brain findings to cognitive and behavioral issues, there has been little consideration for brain abnormalities playing a contributory role in neuromuscular dysfunction in DM1

Methods: Brain MRI determined tissue volumes and Diffusion Tensor Imaging (DTI) measures (white matter integrity measure). Brain function was determined by a cognitive battery and motor function was measured by MIRS. Disease Duration (DD) was noted. Sample included 30 DM1 subjects, 32 controls. On a subset of 13 DM1 and 12 control subjects calf MRI was obtained. Measures included muscle volume, T2 relaxation (measure of microstructural integrity) and fat fraction. Also in this subset, a motor accuracy score (coherence) was obtained during a weight bearing task. Analysis of covariance was used to control for the effects of age and sex for group comparisons. Measures of brain structure and DTI were then correlated with cognition, DD, MIRS, muscle MRI measures and coherence scores.

Results: DM1 showed reduced volumes of cerebral white matter and regional decrement in volume of the thalamus and putamen. DTI measures (fractional anisotropy; FA) revealed a diffuse reduction in white matter structural integrity in DM1 subjects. Brain FA was directly correlated with IQ, DD, and MIRS. It is possible that the measure of Brain FA and measures of MIRS are both related to DM1 pathology but NOT directly related to each other. However when DD was controlled for, FA remained significantly correlated to MIRS. Muscle T2 and the coherence score was also directly correlated to FA and remained significant after controlling for DD.

Discussion: Correlations between global cerebral FA and muscle structure/function suggests a CNS role in DM1 neuromuscular dysfunction.

Grant Support: NINDS R01-NS094387

P-087

Session 5: Specific Disease Features - CNS, Cardiac and GI

Metabolic Syndrome and Brain White Matter Hyperintensities in Myotonic Dystrophies

VUJNIC Milorad[1], **PERIC Stojan**[2], DAMJANOVIC Dusan[3], PESOVIC Jovan[4], SAVIC-PAVICEVIC Dusanka[4], BUCMA Tatjana[5], RAKOCEVIC-STOJANOVIC Vidosava[2]

1. Department of Pathophysiology, Faculty of Medicine, University of Banja Luka, Banja Luka, Republic of Srpska, Bosnia and Herzegovina
2. Neurology Clinic, Clinical Center of Serbia, School of Medicine, University of Belgrade
3. Center for Magnetic Resonance Imaging, Clinical Center of Serbia, Belgrade, Serbia
4. Center for Human Genetics, Faculty of Biology, University of Belgrade, Belgrade, Serbia
5. Institute for Physical Medicine and Rehabilitation “Dr. Miroslav Zotovi”, Banja Luka, Republic of Srpska, Bosnia and Herzegovina

Introduction: Brain white matter hyperintense lesions (WMHLs) are present in majority of myotonic dystrophy type 1 and 2 (DM1 and DM2) patients and they may lead to the cognitive and behavioral impairments. Aim of this study was to analyze association between metabolic syndrome (MetS) and WMHLs in patients with DM1 and DM2.

Methods: Study comprised 51 DM1 and 25 DM2 genetically confirmed patients. MetS was diagnosed in accordance with the 2009 Joint Criteria. Brain magnetic resonance imaging (MRI) was performed on 1.5T equipment. WMHLs load was analyzed using the Fazekas scale and Age Related White Matter Changes scale (ARWMC).

Results: WMHLs were found in 84% of DM1 and 64% of DM2 patients. In DM1 subjects the most affected lobes were temporal and frontal, and in DM2 patients, frontal, parietal and temporal. DM1 patients with MetS had higher WMHLs load in the deep white matter measured by Fazekas scale (1.2 ± 0.7 vs. 0.7 ± 0.6, $p<0.05$). On the other hand, DM2 patients with MetS had higher ARWMC score in the right temporal lobe (0.9 ± 0.3 vs. 0.2 ± 0.4, $p<0.05$). Age was a significant predictor of the higher WMHLs load in both DM1 and DM2 patients.

Discussion: Our results suggest that WMHLs in the brain of DM1 and DM2 patients may be associated with MetS. We hypothesize that treatment of metabolic impairments might have protective effect on WMHLs and consequent cognitive and behavioral changes.

Grant Support: This study was supported by the Ministry of Education, Science and Technological Development of Serbia granted to V.R.S. (grant# 175083).

P-088

Session 5: Specific Disease Features - CNS, Cardiac and GI

PROMIS GI – A Patient-reported Outcome Measure Capable of Detecting Gastrointestinal Issues in Myotonic Dystrophy

PEREZ Jennifer P[1], SANGCO Monica[1], HAGERMAN Katharine A[1], CRASTA Sheela[1], NGUYEN Linda A[2], SAMPSON Jacinda B[1], and DAY John W[1]

1. Department of Neurology, Stanford University, Palo Alto, CA
2. Department of Gastroenterology & Hepatology, Stanford University, Palo Alto, CA

Introduction: Myotonic Dystrophy (DM) affects many body systems including the gastrointestinal tract (GI). The NIH Patient-Reported Outcomes Measurement Information Systems (PROMIS) questionnaires include scales for gastroesophageal reflux, disrupted swallowing, diarrhea, bowel incontinence/soilage, nausea and vomiting, constipation, belly pain, and bloating/flatulence. The PROMIS GI questionnaire has been validated in the general U.S. population as well as for various GI disorders, but not DM. Many symptoms explored in the PROMIS GI are commonly reported in DM, so we administered the PROMIS GI questionnaire to a population of DM and control individuals to see if this patient-reported outcome measure would be appropriate to identify GI issues in DM.

Methods: Adults with DM enrolled in the Stanford Neuromuscular Recruitment Database and their unaffected family and friends were invited to complete the PROMIS GI questionnaire. Responses were collected from 103 individuals (n= 30 controls, n=73 DM) and uploaded to the NIH Health Measures' website for analysis. The scores obtained were further analyzed using the Student's t-test to determine significance between groups.

Results: All 8 subscales of the PROMIS GI were significantly different in controls versus DM ($p<0.05$). Though the DM2 sample size was very small compared to DM1, there was a significantly more severe score for diarrhea and a trend towards more severe incontinence in DM1 than DM2.

Discussion: The PROMIS GI questionnaire is a valid tool to determine the degree of GI disturbance in individuals with DM. It can guide GI clinical care and direct research to particular areas of the highest concern for individuals with DM. Future studies will help determine the longitudinal variability of GI symptoms in patients, as well as the relationship to other clinical and genetic aspects of the disease. There is also a need to collect more information regarding DM2 to help define clinically significant differences in GI symptoms between the two genetic forms of DM.

Grant Support: Myotonic Dystrophy Foundation, Muscular Dystrophy Association, Marigold Foundation and NIH-P01.

P-089

Session 5: Specific Disease Features - CNS, Cardiac and GI

Altered Intestinal Permeability in Myotonic Dystrophy: A Possible Relationship with Nonalcoholic Fatty Liver Disease?

PERNA Alessia[1], MACCORA Daria[2], ROSSI Salvatore[1], PETRUCCI Antonio[3], VALENZA Venanzio[2], SILVESTRI Gabriella[1]

1. Neurology Catholic University of Sacred Heart, Rome
2. Nuclear Medicine Catholic University of Sacred Heart, Rome
3. Neurology S. Camillo Forlanini Hospital, Rome

Introduction: Gut-derived endotoxin may be relevant in nonalcoholic fatty liver disease (NAFLD): recent studies suggest that increased intestinal permeability (IP) caused by the disruption of intercellular tight junctions, would play a role in its pathogenesis Myotonic dystrophies (DM1 and DM2) are caused by abnormal expansions of polymorphic repeats in non-coding regions of respective genes (*DMPK* in DM1*, CNBP* in DM2). A moderate increase in serum liver function indexes is frequently detected. Aim of the study was to investigate IP in DM patients, and its possible association with NAFLD.

Methods: Twenty-two DM patients (20 DM1, 2 DM2) were evaluated. Control groups included 32 healthy volunteers and 20 non-DM patients with NAFLD. NAFLD was defined by abnormal liver chemistry tests with ultrasound evidence of steatosis. IP was assessed using urinary excretion of ^{51}Cr-EDTA. After an overnight fast, patients were given to drink 0.37 MBq of ^{51}Cr-EDTA in 10 ml of water; the standard sample (1/50 of administered dose) and a 3-ml sample of 24/hours urine were measured by gamma counter. Urine sample results were considered indicative of altered IP when > 3%.

Results: 20/22 (90.9 %) DM patients showed altered IP, and 14/22 (63.6 %) had evidence of NAFLD. IP resulted significantly higher in DM patients compared to healthy volunteers (M±SD: 6.63%±5.27 vs. 2.02%±0.66; $p<0.001$), with values similar to non-DM patients with NAFLD (M±SD: 5.79%±2.71 vs. 5.63%±2.36; $p=0.51$).

Discussion: This study indicates that an abnormal intestinal permeability is almost invariably detected in DM patients, and it is frequently associated to NAFLD. Defects in intestinal absorption could be considered responsible for vitamin D deficiency, that is common in DM1 patients. Assessment of intestinal permeability by ^{51}Cr-EDTA study is a non invasive and sensitive test to detect the presence of gut mucosal damage in DM, and it might represent a useful diagnostic tool in the follow-up of DM patient.

Grant Support: None.

P-090

Session 5: Specific Disease Features - CNS, Cardiac and GI

Brain Imaging in Myotonic Dystrophy Type 1 – A Systematic Review

OKKERSEN Kees[1], MONCKTON Darren G[2], LE Nhu[1], TULADHAR Anil M[1], **RAAPHORST Joost**[1*], van ENGELEN Baziel[1*].

1. Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands
2. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK

Introduction: Objective: To systematically review brain imaging studies in myotonic dystrophy type 1 (DM1).

Methods: We searched Embase (index period 1974 to 2016) and Medline (index period 1946 to 2016) for studies in DM1 patients using the following methods: MRI, MRS, fMRI, CT, ultrasound, PET or SPECT. From 81 studies, we extracted clinical characteristics, primary outcomes, data on clinical-genetic correlations and information on potential risk of bias. Results were summarized and we calculated pooled prevalences of imaging abnormalities, where possible.

Results: In DM1, various imaging changes are widely dispersed throughout the brain, with apparently little anatomical specificity. We found general atrophy and widespread grey matter volume reductions in all four cortical lobes, the basal ganglia and cerebellum. The pooled prevalence of white matter hyperintensities is 70% (95% CI 64 to 77), as compared to 6% (95% CI 3 to 12) in unaffected controls. DTI shows increased mean diffusivity in all four lobes and reduced fractional anisotropy in virtually all major association, projection and commissural white matter tracts. Functional studies demonstrate reduced glucose uptake and cerebral perfusion in frontal, parietal and temporal lobes, and abnormal fMRI connectivity patterns. There is significant between study heterogeneity in terms of imaging methods, which together with the established clinical variability of DM1 may explain divergent results. Longitudinal studies are remarkably scarce.

Discussion: DM1 brains show widespread white and grey matter involvement throughout the brain, which is supported by abnormal resting state network, PET/SPECT and MRS parameters. Longitudinal studies evaluating spatiotemporal imaging changes are highly needed.

Grants: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 305697 (OPTIMISTIC project) and the Marigold Foundation.

P-091

Session 5: Specific Disease Features - CNS, Cardiac and GI

The Cognitive Profile of Myotonic Dystrophy Type 1 - A Systematic Review and Meta-analysis

OKKERSEN Kees[1], BUSKES Melanie[1], GROENEWOUD Johannes[2], KESSELS Roy PC[3,4], KNOOP Hans[5], van ENGELEN Baziel[1], **RAAPHORST Joost**[1]

1. Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, Nijmegen
2. Department for Health Evidence, Radboud University Medical Center, Nijmegen
3. Department of Medical Psychology, Radboud University Medical Center, Nijmegen
4. Center for Cognition, Donders Institute for Brain, Cognition and Behaviour, Radboud University, Nijmegen
5. Department of Medical Psychology, Academic Medical Centre (AMC), University of Amsterdam, Amsterdam

Introduction: Objective: To examine the cognitive profile of patients with myotonic dystrophy type 1 (DM1) on the basis of a systematic review and meta-analysis of the literature.

Methods: Embase, Medline and PsycInfo were searched for studies reporting ≥1 neuropsychological test in both DM1 patients and healthy controls. Search, data extraction and risk of bias analysis were independently performed by two authors to minimize error. Neuropsychological tests were categorized into 12 cognitive domains and effect sizes (Hedges' g) were calculated for each domain and for tests administered in ≥5 studies.

Results: DM1 participants demonstrated a significantly worse performance compared to controls in all cognitive domains. Effect sizes ranged from -0.33 (small) for verbal memory to -1.01 (large) for visuospatial perception. Except for the domains global cognition, intelligence and social cognition, wide confidence intervals were associated with moderate to marked statistical heterogeneity that necessitates careful interpretation of results. Out of the individual tests, the Rey-Osterrieth complex figure-copy (both non-verbal memory and visuoconstruction) showed consistent impairment with acceptable heterogeneity.

Conclusions: In the individual DM1 patient, cognitive deficits may include a variable combination of global cognition and involvement across many different domains. Although DM1 is a heterogeneous disorder, our study shows that meta-analysis is feasible, contributes to the understanding of brain involvement and may direct bedside testing.

Grants: The research leading to these results has received funding from the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° 305697 (OPTIMISTIC) and the Marigold Foundation.

P-092

Session 5: Specific Disease Features - CNS, Cardiac and GI

Abnormal Expression of the Axonal Guidance Cue Ephrin- A5 Receptor in Myotonic Dystrophy Type 1

ROQUEVIÈRE Sylvain[1], GAUTHIER Morgane, GOMEZ-PEREIRA Mario, MAURY Yves, MARTEYN Antoine, GOURDON C Geneviève, NEDELEC Stéphane, PESCHANSKI Marc, MARTINAT Cécile

1. ISTEM

Introduction: Although Myotonic Dystrophy type 1 (DM1) has long been characterized as a primarily muscular disease, a growing number of studies point out the importance of the central nervous system involvement in the clinical features of DM1. Psychological dysfunction, excessive daytime sleepiness, and neuropathological abnormalities have been described in DM1 patients. Nevertheless, the molecular bases of these neuronal affections are still poorly understood. Eph receptor and their corresponding ligands, ephrins, are membrane-anchored proteins that are considered as a crucial system in the development and maturation of the central nervous system.

Methods: We identified a splice defect affecting the expression of Eph receptor A5 (EPHA5) both in DM1 patient biopsies as well as in DMSXL mouse model of DM1. By using HEK transfected cells with different splice variant of EPHA5 and neurons derived from human pluripotent stem cells we try to identify how this gene defect could be involved in DM1.

Results and Discussion: Our results demonstrated that this splice event is, at least partially, controlled by MBNL and CUGBP protein. At the functional level, this splicing seems to be involved in the regulation of activity of the receptor and the downstream signaling pathway of EPHA5, and then modify cellular process.

Grant Support: This work is supported by AFM Téléthon, DIM biothérapie, Université Evry Val d'essone, and INSERM.

P-093

Session 5: Specific Disease Features - CNS, Cardiac and GI

Increased EEG Theta Spectral Power in Sleep in Myotonic Dystrophy Type 1

CHEUNG Joseph[1], RUOFF Chad[1], MOORE Hyatt[1], HAGERMAN Katharine A[2], PEREZ Jennifer P[2], SAKAMURI Sarada[2], MIGNOT Emmanuel[1], DAY John W.[2], **SAMPSON Jacinda B**[2]

1. Stanford Center for Sleep Sciences & Medicine, Department of Psychiatry and Behavioral Sciences, Stanford University
2. Department of Neurology, Stanford University Hospital and Clinics

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic disorder that involves the central nervous system (CNS). Individuals with DM1 commonly present with sleep dysregulation, including excessive daytime sleepiness and sleep disordered breathing. We aim to characterize electroencephalogram (EEG) power spectra from nocturnal polysomnograms (PSG) in DM1 patients compared to matched controls in the search for a DM1 disease biomarker of the CNS.

Methods: A retrospective, case-control (1:2) chart review of DM1 (n=18) and matched control patients (n=36) referred for clinical PSG at the Stanford Sleep Center was performed. Controls were matched based on age, gender, apnea-hypopnea index (AHI), body mass index (BMI), and Epworth Sleepiness Scale (ESS). Sleep stage and respiratory metrics for the two groups were compared. Power spectral analysis of the EEG C3-M2 signal was performed using the fast Fourier transformation.

Results: DM1 patients had significantly increased theta percent power in stage 2 sleep compared to matched controls. Theta/beta and theta/alpha percent power spectral ratios were found to be significantly increased in stage 2, stage 3&4, all sleep stages combined, and all wake periods combined in DM1 compared to controls. A significantly lower nadir O2 saturation was also found in DM1 versus controls as well.

Discussion: Compared to matched controls, DM1 individuals had increased EEG theta spectral power. Specifically, our findings suggest increased theta/beta and theta/alpha power spectral ratios in nocturnal PSG could be useful DM1 disease biomarkers in the CNS.

Grant Support: CHEUNG Joseph is supported by NHLBI T32 Training Grant HL110952.

P-094

Session 5: Specific Disease Features - CNS, Cardiac and GI

Taking Care of Dysphagia in Myotonic Dystrophies: Need for a Customized Pathway of Care

ZANOLINI Alice[1], GINOCCHIO Daniela[1], GOTTI Elena[1], CATTANEO Marina[1], LIZIO Andrea[1], **SANSONE Valeria Ada**[1]

1. NEMO (NEuroMuscular Omnicomprehensive) Clinical Center, Neurorehabilitation Unit, University of Milan

Introduction: Data on the prevalence and progression of dysphagia in DM1 and DM2 are missing. Management is often based on guidelines for more rapidly progressive diseases like ALS, but patients are unaware of it and have limited compliance. The aim of our work is to describe the diagnostic and customized management pathway of care in a cohort of DM1/2 patients at the NEMO Clinical Center in Milan.

Methods: 69 pts (mean age 44±13 years, 4% DM2) were subjected to FEES (Fiberoptic Endoscopic Evaluation of Swallowing) and nutritional-metabolic assessments. Objective (Penetration Aspiration Scale, PAS; the Dysphagia Outcome and Severity Scale, DOSS) and subjective assessments (I-EAT-10 and I-SWAL-QOL) were obtained.

Results: FEES detected dysphagia in most patients tested, but 60% was asymptomatic. 79.7% had mild dysphagia, yet 18% were obese (43.5±13.9 yrs, DOSS 3-5); 5.8% had severe dysphagia (DOSS 1-2); of these 25% were obese while 25% were underweight. 3% had indication to feeding tube placement. In 11 patients FEES detected no changes over time (mean follow-up in months: 18.9±6.3) and weight was unchanged. Baseline metabolism was abnormal in 60% of pts, needing a tailored diet. All dysphagic patients and caregivers were trained to modify food/liquids consistency and to deal with feeding tube care at home. Awareness of dysphagia and impact on QoL were low; adherence to post-exam recommendations was limited.

Discussion: Dysphagia is frequent in DM1 and does not seem to progress over time. Although patients are unaware of symptoms and show limited compliance, ¼ of our cohort was underweight and 3% required gastrostomy. Food consistency change, nutritional support and caregiver training are mandatory to avoid clinical consequences, hospedalization and compliance failure in this population of patients.

Grant Support: None.

P-095

Session 5: Specific Disease Features - CNS, Cardiac and GI

Spinal Cord Excitability, Muscle Properties, and Neuromuscular Control Accuracy in Type I Myotonic Dystrophy

SHIELDS Richard[1], GUTMANN Laurie[1], PHETTEPLACE Janel[1], DEVOLDER Ian[1], THEDENS Dan[1], BUELOW Aaron[1], LEE Jinhyun[1], NOPOULOS Peg[1]

1. University of Iowa

Introduction: Myotonic dystrophy type 1 (DM1) is the most common inherited muscular dystrophy in adults. The clinical manifestations of myotonia, muscle weakness, and muscle wasting are characteristic symptoms of DM1. There is a need for reliable physiological measures in myotonic dystrophy Type 1 (Neuromuscul Disord 2013). We assessed spinal cord excitability, soleus muscle twitch properties, and a global weight bearing movement task in people with DM1.

Methods: Eleven DM1 and eleven age matched control subjects were included. Subject received a battery of 4 tests; 1) assessment of spinal cord excitability via suppression using paired H-reflexes (H2/H1), 2) soleus muscle single (S) and double pulse (D) twitches, 3) fatigue via a repetitive 3 Hz stimulation, and 4) a global motor accuracy score (coherence) during a novel weight bearing task. We used a split plot repeated measures analysis of variance to test for differences within and between DM1 and control. Reproducibility was assessed for all measures.

Results: H-reflex suppression was not different between DM1 and control (0.40 and 0.31; $p=0.52$). The single twitch amplitude was less for DM1 compared to control (0.59 and 0.72; $p=0.03$). The double pulse to single pulse (D/S) ratio trended higher for DM1 compared to control (1.96 and 1.8; $p=0.08$). The weight bearing task error (coherence) was less for DM1 compared to control (0.42 and 0.66; p=0004). Coherence was correlated to the MIRS score ($r=0.07$; $p<0.05$). The reproducibility of all within session measurements were high ($r>0.87$).

Discussion: The reduced twitch amplitudes for DM1 group is consistent with the extensive atrophy. The enhanced D/S ratio for the DM1 group is consistent with impaired excitation contraction coupling. The functional weight bearing task accuracy (coherence) was the most robust measurement and highly correlated to disease severity. This functional test battery will assist us in detection and monitoring of DM1 progression.

Grant Support: NINDS R01-NS094387.

P-096

Session 5: Specific Disease Features - CNS, Cardiac and GI

Congenital and Juvenile Myotonic Dystrophy

STOKES Matthew[1], IANNACCONE Susan[1], CASTRO Diana[1]

1. UT Southwestern Medical Center Dallas and Children's Health

Introduction: Myotonic dystrophy (DM) is an autosomal dominant disorder associated with two loci, DM1 and DM2. DM1 is caused by a CTG trinucleotide repeat expansion in the dystrophia myotonica protein kinase gene. DM1 can occur as congenital, juvenile onset, early adult onset and late adult onset. Congenital DM refers to the presence of symptoms in the first month of life. The symptoms may include facial weakness, hypotonia, respiratory problems, feeding difficulties and clubfoot. Individuals with juvenile-onset DM1 develop symptoms between early childhood and adolescence. This form presents with intellectual disability, myotonia, cataracts, cardiac arrhythmias and GI complaints.

Methods: We performed a retrospective chart review of patients with DM1 seen in the pediatric neuromuscular clinics at UT-Southwestern. We defined congenital myotonic dystrophy (CDM) as symptoms in the first month of life; juvenile as onset 1 month to 12 yrs of age. Data regarding symptoms, disease course, cognitive features and family history were extracted.

Results: 71 patients were included; of those 69% were white-non hispanic, 55% were female. CDM was diagnosed in 61% of cases, and of those 65% were genetically diagnosed in the first year of life. In our clinic, 72% of patients inherited the mutation from the mother. The CTG trinucleotide repeat number ranged from 100 to 2100. Cognitive delay was present in 82% of patients: 91% with CDM and 68% with juvenile-onset. Over half of the patients had GI disturbances. Other comorbidities included cardiac abnormalities, restrictive lung disease, ocular, and orthopedic problems.

Discussion: In DM1, presenting symptoms varied and correlated with size of repeat expansion. The existence of different types of myotonic dystrophy has created a need to develop a diagnostic classification, however there is no consensus about the cut off of ages. CDM has a high incidence of cognitive problems, which are often not addressed properly.

Grant Support: None

P-097

Session 5: Specific Disease Features - CNS, Cardiac and GI

Evaluation of Glycemic Variability in Myotonic Dystrophy by Continues Glucose Monitoring

TAKADA Hiroto[1], IWAHASHI Hiromi[2], MATSUMURA Tsuyoshi[3], TAKAHASHI Masanori P[4], KIMURA Takashi[5]

1. Department of Neurology, Aomori National Hospital
2. Department of Diabetes Care Medicine, Graduate School of Medicine, Osaka University
3. Department of Neurology, Toneyama National Hospital,
4. Department of Functional Diagnostic Science, Graduate School of Medicine, Osaka University
5. Department of Neurology, Asahikawa National Medical Center

Introduction: Glucose intolerance is a well-known complication of myotonic dystrophy type 1 (DM1). Dynamic fluctuation of glucose concentration is not still clear in detail. The aim of the study is to elucidate daily glucose profiles in DM1 minutely using continues glucose monitoring (CGM).

Methods: CGM was performed in genetically diagnosed patients with DM1 for 72 hours. Oral glucose tolerance test (OGTT) was carried out at the last part of the CGM recording.

Results: Thirty patients with DM1 (female 10, male 20) were participated. The mean age of patients (+/- SD) was 49 +/- 14 years, the number of CTG repeat was 1228 +/- 673, and body mass index was 22.1 +/- 4.6. Patients who were diagnosed as normal glucose tolerance (NGT) by OGTT were 7, impaired glucose tolerance (IGT) were 15, and diabetes mellitus were 8. The mean plasma insulin level at the 120 minutes during OGTT in DM1 patients was 55.2 μU/mL in NGT, 85.1μU/mL in IGT, and 117.2μU/mL in diabetes. These levels were higher than those in obese subjects in each glucose tolerance without DM1 in a previous report, respectively. Regarding CGM profiles, three patterns of glucose variability were observed; (A) the glucose value changed within reference range all day, (B) the glucose value increased gradually from morning to evening, (C) the glucose value increased after breakfast and continued in the high level. The glucose tolerance proved to be getting worse in this order. Hypoglycemia was recorded in 6 of 7 DM1 patients with NGT. Hypoglycemia was observed not only in nighttime but also after meals.

Discussion: Hyperinsulinemia induced by insulin resistance in patients with DM1 was confirmed. Characteristic features of glucose daily profile in each glucose intolerance in DM1 patients were revealed by means of CGM. Hypoglycemia might usually occur in DM1 patients with NGT.

Grant Support: This work was supported in part by research grants from the Japan Agency for Medical Research and Development AMED (Practical Research Project for Rare/Intractable Diseases, 16ek0109172 and 17ek0109259), the Ministry of Health and Welfare of Japan.

P-098

Session 5: Specific Disease Features - CNS, Cardiac and GI

High-sensitive Cardiac Troponin T Assay (hs-cTnT) as Serum Biomarker to Predict Cardiac Risk in Myotonic Dystrophy

VALAPERTA Rea[1], DE SIENA Claudia[1], CARDANI Rosanna[2], RAMPOLDI Benedetta[3], FOSSATI Barbara[4], GAIA Paola[3], PICCOLI Marco[5], CIRILLO Federica[5], MEOLA Giovanni[6], COSTA Elena[1]

1. Research Laboratories, IRCCS Policlinico San Donato, San Donato Milanese, Italy/Service of Laboratory Medicine, IRCCS Policlinico San Donato, San Donato Milanese, Italy
2. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, San Donato Milanese, Italy
3. Service of Laboratory Medicine, IRCCS Policlinico San Donato, San Donato Milanese, Italy
4. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy
5. Laboratory of Stem Cells for Tissue Engineering, IRCCS Policlinico San Donato, San Donato Milanese, Italy
6. Department of Biomedical Sciences for Health, University of Milan, Milan, Italy/Department of Neurology,
7. IRCCS Policlinico San Donato, San Donato Milanese, Italy

Introduction: Myotonic dystrophy (DM) is a genetic disorder caused by nucleotide repeat expansions. Sudden death represents the main cause of mortality in DM patients, caused by the onset of severe arrhythmias: ventricular tachycardia, ventricular asystole, ventricular fibrillation or electromechanical dissociation. The aims of this study were: (i) to analyze the relationship between serum cardiac biomarkers with clinical parameters in DM patients, and (ii) to investigate the TNNT2 gene exons in DM patients with alteration in serum cTnT levels.

Methods: Serum cardiac biomarkers were evaluated in 59 DM patients and 22 healthy controls. An additional group of 62 controls with cardiac defects similar to DM were enrolled. High Resolution Melting (HRM) technology was used to identify genetic variation in the TNNT2 gene.

Results: NT-proBNP, hs-cTnT and CK levels were significantly increased in DM patients compared to healthy subjects ($p=0.0008$, $p<0.0001$, $p<0.0001$). Hs-cTnT levels were significantly higher in DM compared to control group with cardiac defects. A positive correlation was found between hs-cTnT and hs-cTnI in both DM patients and controls ($p=0.019$, $p=0.002$). Independently from the age, the risk of DM disease was positively related to an increase in hs-cTnT, but not to hs-cTnI. However, cTnI levels were realated to PR interval ($p=0.03$). Western blot analysis, on muscle tissue samples suggests that there is not skeletal muscle involvement. Finally, in 21 scanned DM patients, HRM analysis did not show any variation on 9, 10 and 11exons in the TNNT2 gene.

Discussion: The levels of hs-cTnT were significantly higher in DM patients. Analysis with anti-cTnT shows that this increase might be linked to heart problems. This finding suggests that hs-cTnT might represent a helpful serum biomarker to “predict” cardiac risk. HRM gene-scanning technique could provide new insights for basic research and clinical medicine.

Grant Support: This study was supported by IRCCS Policlinico San Donato and FMM Fondazione Malattie Miotoniche.

P-099

Session 5: Specific Disease Features - CNS, Cardiac and GI

Association Between Mutation Size and Cardiac Involvement in Myotonic Dystrophy Type 1

WAHBI Karim1, CHONG-NGUYEN Caroline[1], ALGALARRONDO Vincent[2], BECANE Henri Marc[3], ARNAUD Pauline[4], FURLING Denis[3], LAZARUS Arnaud[5], BASSEZ Guillaume[3], BEHIN Anthony[3], FAYSSOIL Abdallah[3], LAFORET Pascal[3], STOJKOVIC Tanya[3], EYMARD Bruno[3], DUBOC Denis[1]

1. Cochin Hospital Paris France
2. Antoine Beclere hospital Paris France
3. Pitie Salpetriere hospital Myology Institute Paris France
4. Bichat hospital Paris France
5. InParys clinical research associates, Saint Cloud, Paris, France

Introduction: In myotonic dystrophy type 1, the association between mutation size (CTG expansion) and the severity of cardiac involvement is controversial.

Methods: We selected 855 patients with myotonic dystrophy type 1 (women, 51%; median age, 37 years), with genetic testing performed at the moment of their initial cardiac evaluation, out of 1014 patients included in the Myotonic Dystrophy Type 1-Heart Registry between January 2000 and December 2015. We studied the association between CTG expansion size and other baseline characteristics and (1) cardiac involvement at baseline and (2) the incidence of death, sudden death, and other cardiac adverse events.

Results: At initial presentation, the median CTG expansion size was 530 (interquartile range, 300–830). In multivariate analysis, larger expansions were associated with the presence at baseline of conduction defects on the ECG and left ventricular systolic dysfunction. In a median 11.5 years of followup period, 210 patients died (25%), including 32 suddenly (4%). Supraventricular arrhythmias developed over lifetime in 166 patients (19%), sustained ventricular tachyarrhythmias in 17 (2%), and permanent pacemakers were implanted in 181 (21%). In Cox regression analyses, larger CTG expansions were significantly associated with (1) total death, sudden death, and pacemaker implantation in a model, including CTG expansion size, age, sex, diabetes mellitus, and (2) all end points except sudden death in a model including all baseline characteristics.

Discussion: The size of the CTG expansion in the blood of myotonic dystrophy type 1 patients is associated with total and sudden deaths, conduction defects, left ventricular dysfunction, and supraventricular arrhythmias.

Grant Support: AFM-Téléthon

P-100

Session 5: Specific Disease Features - CNS, Cardiac and GI

Survival in Myotonic Dystrophy Type 1 Predicted by the New DM1 Survival Risk Score

WAHBI Karim[1], PORCHER Raphael[2], LAFORET Pascal[3], FAYSSOIL Abdallah[3], STOJKOVIC Tanya[3], LEONARD LOUIS Sarah[3], BEHIN Anthony[3], FURLING Denis[3], ARNAUD Pauline[4], SOCHALA Maximilien[1], PROBST Vincent[5], BABUTY Dominique[6], PELLIEUX Sebylle[6], BASSEZ Guillaume[3], PEREON Yann[5], EYMARD Bruno[3], DUBOC Denis[1]

1. Cochin Hospital Paris France
2. Hotel Dieu hospital Paris France
3. Pitie Salpetriere hospital Myology Institute Paris France
4. Bichat hospital Paris France
5. Nantes Hospital Nantes France
6. Trousseau H Tours France; Francois Rebelais University Tours France

Introduction: A reliable prediction of survival in myotonic dystrophy type 1 (DM1) is critically important to plan a personalized health supervision.

Methods: We retrospectively analyzed the survival of 1,066 patients with genetically proven DM1 included in the DM1-Heart-Registry between January 2000 and November 2014 in Cochin and Pitié-Salpêtrière hospitals, assessed its predictors with multivariable Cox modeling, developed and internally validated a score to predict survival. We validated this score in an separate sample of 230 patients referred to the University Hospitals of Tours and Nantes.

Results: In the derivation sample, 241 patients (22·6%) died over a median follow-up of 11·7 years (interquartile range 7·7-14·3). Predictors of death were age, diabetes, need for support when walking, heart rate, systolic blood pressure, first-degree atrioventricular block, bundle-branch block, and lung vital capacity. The 10-year survival was 96·6% in the group with 0-4 points, 92·2% in the group with 5-7 points, 80·7% in the group with 8-10 points, 57·9% in the group with 11-13 points, and 19·4% in the group with >14 points. In the validation sample, the respective 10-year survivals were 99·3%, 80·6%, 79·3%, 43·2% 11-13 points, and 21·6%. Calibration curves revealed no deviation from the reference line. The C-index was 0·756 in the derivation and 0·806 in the validation sample.

Discussion: The DM1 prognostic score was a reliable aid in the prediction of survival.

Grant Support: AFM-Téléthon.

P-101

Session 5: Specific Disease Features - CNS, Cardiac and GI

Venous Thromboembolism in Adult Patients with Inherited Myopathies: A High-risk in Myotonic Dystrophy

WAHBI Karim[1], SOCHALA Maximilien[1], PORCHER Raphael[2], STOJKOVIC Tanya[3], BEHIN Anthony[3], LEONARD LOUIS Sarah[3], LAFORET Pascal[3], BASSEZ Guillaume[3], EYMARD Bruno[3], DUBOC Denis[1]

1. Cochin Hospital Paris France
2. Hotel Dieu hospital Paris France
3. Pitie Salpetriere hospital Myology Institute Paris France

Introduction: The risk of venous thromboembolism (VTE) in inherited myopathies is unknown.

Methods: We identified 2,810 patients (mean age = 40.1±15.4 years, women = 45,0%) referred to our institutions between January 2000 and December 2014 for the management of the 10 following genetically proven myopathies: myotonic dystrophy (DM), FSH myopathy, dystrophinopathies, mitochondrial diseases, glycogen and lipid-storage diseases, LGMD, nucleopathies, collagen VI-related disorders, myofibrillar, and congenital myopathies. We determined the incidence of VTE in each group of disease, searched for independent predictors of VTE, compared the incidence of VTE to a community-based population.

Results: Over a median 8.5 years follow-up duration, 102 patients had ≥1 VTE event, representing a cumulative incidence of 6.2%. VTE was unprovoked in 54 (52.9%) patients and occurred in patients with prior loss of ambulation due to myopathy in 15 (14.7%). The risk for VTE was the highest in DM with a cumulative incidence of 10.3%, while it was ≤4.4% in other diseases and DM patients had, compared to others, VTE events at younger age, more frequently unprovoked and unrelated to loss of ambulation, with more frequent recurrences and higher 30-days mortality. We identified 4 predictors of VTE events: increasing age, personal history of VTE, loss of ambulation, and DM with the hazard of VTE during follow-up. Incidence rates of VTE were higher in patients with DM than in a French community-based population, with the highest rate ratios for women (HR, 17.0; 95% CI, 10.0-29.1) and men (HR, 10.6; 95% CI, 6.35-17.5) aged 40 to 59 years. In patients with other myopathies, incidence rates were also higher but only for men aged 40 to 58 years and women aged up to 60 years with lower rate ratios than for DM patients.

Discussion: Patients with inherited myopathies have a moderately increased risk of VTE related to loss of ambulation. Patients with DM have a very high risk of VTE compared to other inherited myopathies and to the general population, requiring probably a specific management.

Grant Support: AFM-Téléthon.

P-102

Session 5: Specific Disease Features - CNS, Cardiac and GI

Diagnostic Yield of Extended Cardiac Rhythm Monitoring in Patients with Myotonic Dystrophy type I

FUHLBRIGGE Rebecca[1], BOULIER Kristin[1], BEUTNER Katherine[1], PEREZ Marco[1], **WHEELER Matthew**[1]

1. Stanford University School of Medicine

Introduction: In patients with myotonic dystrophy (DM), cardiac disease causes significant morbidity. Recommendations for screening and management of cardiac involvement in DM include routine ECGs and periodic cardiac monitoring. In other patient groups, extended rhythm monitors, worn for up to 14 days, have been shown to increase the sensitivity of detection of atrial arrhythmias as compared to Holter monitors. Given that atrial arrhythmias have been shown to be a risk factor for sudden death in DM, we sought to determine the effectiveness of extended rhythm monitoring in ascertaining atrial arrhythmias in patients with DM.

Methods: We retrospectively reviewed findings from 12 lead ECG, 14-day extended rhythm monitors, and ICD interrogations from patients seen at our cardiology clinic with a primary diagnosis of DM type 1.

Results: Our cohort consists of 41 patients referred for cardiac evaluation with DM1 who underwent ECG and rhythm monitoring for assessment of cardiac risk. Rhythm monitors were abnormal in 11 patients, 19 were referred for ICD, and 14 had ICDs placed. Five patients were referred for ICD based on ECG and rhythm monitors, 7 met criteria for ICD placement on ECG criteria alone, one patient was referred based solely on the rhythm monitor, and one patient was referred based on family history. Five patients were referred for ICD based on findings of SVT on the rhythm monitor; two had atrial arrhythmias detected by ICDs during 2 years of follow up. Extended rhythm monitoring detected 3 additional patients with abnormalities that 48 hour monitoring would have missed. As previously shown, patients who had severe ECG abnormalities were at higher risk for requiring significant pacing.

Discussion: In conclusion, while extended cardiac monitoring detected additional atrial arrhythmias, in our cohort it did not improve upon ECG data in predicting ICD findings in patients with DM1, although the duration of follow up was short and the examined patient population was small.

Grant Support: None

P-103

Session 6: Cell Models for DM

Neurons-derived hiPSC: An In Vitro Model for the Development of a Gene Therapy for Myotonic Dystrophy Type 1

AIT-BENICHOU Siham[1], JAUVIN Dominic[1], BENNETT C. Frank[2], ARZOUMANOV Andrey[3], GAIT Mike[3], VARELA Miguel[4], WOOD Matthew[4], PUYMIRAT Jack[1]

1. CRCHU de Québec LOEX/CMDGT,1401 18e rue, Québec, G1J 1Z4, Canada.
2. Ionis Pharmaceuticals, Carlsbad, USA.
3. MRC Laboratory of Molecular Biology, Cambridge Medical Campus, Cambridge, UK.
4. Department of Physiology, Anatomy and Genetics, University of Oxford, UK.

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic disease with dominant transmission that affects not only muscle but also many organs such as the brain. Cerebral damage included psychomotor disorder, mental retardation, loss of visuospatial and memory functions. Antisense oligonucleotides (AOs) strategy for the development of a gene therapy for DM1 brain deficits is limited by the fact that AOs do not cross the blood-brain barrier following systemic administration indicating that other delivery methods will have to be considered. The objective of this proposal is to develop a gene therapy for brain deficits in DM1 patients based on intrathecal injection of AOs.

Methods: We have derived 3 human DM1 and 3 normal iPSC lines. iPS cells that express pluripotency markers will undergo neuronal differentiation to obtain mature neurons. Based on data obtained in skeletal muscle, neurons will be treated with cET-486178, MOE-455569, 3 LNA (#5, #8 and #14) gapmer AOs which produce the destruction of mutant RNAs and Pip6a-PMO-(CAG)7 AO which binds to the repeats and prevents binding of the protein MBNL1. The efficacy of the best OA will be evaluated in vivo by intraventricular injection.

Results: The DM1 neurons have the characteristics of the disease with the presence of nuclear foci, sequestration of MBNL1 and the existence of splicing defects. Among these AOs, the Pip6a-PMO-(CAG)7 and the cET-486178 AO neutralize or destroy nuclear foci. This neutralization / destruction allowed the redistribution of MBNL1 and the correction of certain aberrant splicing. Intraventricular injection of cET-486178 AO in DM1 mice decreased up to 60% the levels of mutant RNAs in different brain areas.

Discussion: This study indicates that cET-486178 and Pip6a-PMO-(CAG)7 AO target mutant RNAs not only in muscle cells but also in neurons and, strongly support the feasibility of a therapy based on intrathecal injection of AOs in DM1 patients.

Grant Support: MDA subvention and Ionis Pharmaceuticals

P-104

Session 6: Cell Models for DM

A Decoy-based Gene Therapy Targeting CUGexp-DMPK Transcripts in DM1 Muscle Cells

ARANDEL Ludovic[1], MATLOKA Madgalena[1], KLEIN Arnaud[1], MARIE Joelle[1], TRAN Hélène[2], CAILLET-BOUDIN Marie-Laure[2], SERGEANT Nicolas[2], FURLING Denis[1]

1. Sorbonne Universités UPMC, Inserm Centre de Recherche en Myologie, Institut de Myologie, Paris, France
2. Inserm UMR837-1, Univ. Lille Nord de France, J-P Aubert Research Center, Alzheimer & Tauopathies, Lille, France

Introduction: Deleterious interactions of RNA binding factors with expanded CUG repeats leads to functional loss of MBNL1 resulting in alternative splicing misregulations and ultimately to DM1 symptoms. Correction of disease-associated phenotypes such as splicing defects and myotonia by antisense oligonucleotides that either degrade mutant transcripts or interfere with CUGexp repeats act through the release of sequestered MBNL1 from CUGexp-RNA. Here we assessed in vitro a decoy-based gene therapy to restore functional levels of MBNL1 in DM1 cells and inhibit CUGexp-RNA toxicity

Methods: DM1 and CTL muscle cells transduced with lentiviral vectors expressing truncated MBNLΔ polypeptide lacking splicing activity but keeping RNA binding property.

Results: Preliminary co-transfection experiments in Hela cells have shown that either GFP-MBNL1 or GFP-MBNLΔ reverses altered splicing of Tau exon 2 minigene that is induced by the expression of CUGexp-RNA. In addition, in vitro cross-linked experiments demonstrated that MBNLΔ binds to CUGexp-RNA and displaces MBNL1 from CUGexp repeats in a dose dependent manner. Since MBNLΔ is devoid of splicing activity but colocalizes with nuclear CUGexp-RNA aggregates, it suggest that MBNLΔ promotes the displacement of endogenous MBNL1 from CUGexp-RNAs and the recovery of its activity. This hypothesis was tested in DM1 muscle cells using an inducible Tet-on MBNLΔ construct. We showed that DM1 splicing defects of several pre-mRNAs are normalized by MBNLΔ expression in differentiated DM1 myoblasts whereas MBNLΔ has no effect in differentiated CTL myoblasts. An RNAseq analysis confirmed that the vast majority of DM1-missplicing events are no more significantly altered in treated DM1 cells compared to CTL cells indicating an almost complete recovery of MBNL1 splicing activity after MBNL treatment.

Discussion: Our results showed that MBNLΔ is able to act as a decoy in order to release functional endogenous MBNL1 from CUGexp-RNA foci in DM1 muscle cells.

Grant Support: AFMTelethon-Institut de Myologie, The French National Research Agency.

P-105

Session 6: Cell Models for DM

The Use of Pericytes in a Novel Cell-based Strategy for Correcting the Muscular Phenotype in DM1

AUSEMS Rosanne[1,2,3], VAN DEN BROEK Walther[4], GOURDON Geneviève[5,6], WANSINK Derick[4], VAN ENGELEN Baziel[2,3], VAN BOKHOVEN Hans[1,3]

1. Department of Human Genetics, Radboud University, Nijmegen, The Netherlands
2. Department of Neurology, Radboud University, Nijmegen, The Netherlands
3. Donders Institute for Brain Cognition and Behavior at Radboud University, Nijmegen, The Netherlands
4. Department of Cell Biology, Radboud University, Nijmegen, The Netherlands
5. Inserm UMR 1163, Paris, France
6. Imagine Institute, Paris, France.

Introduction: Mesoangioblasts (MABs) are mesenchymal stem cells, originally identified in the mouse embryonic dorsal aorta. MABs exhibit many similarities to pericytes (PCs), which are wrapped around the smaller capillaries. The discovery that PCs isolated from postnatal small vessels of skeletal muscle tissue have myogenic potency led us to explore the use of genetically corrected PCs as cellular therapy against the muscular phenotype of myotonic dystrophy type 1 (DM1). Stimulating the limited process of myogenic regeneration in DM1 skeletal muscle with ex-vivo corrected PCs is a promising approach to combat the muscular characteristics generated by the (CTG)n repeat expansion mutation.

Methods: DMSXL mice are transgenic DM1 mice which express an expanded DMPK transgene and replicate several muscle symptoms of the disease. Skeletal muscles of the hind limbs of these mice and wild type litter mates were isolated and tissue explants were cultured. ALP+/CD31- PCs were sorted from the population of weakly adherent cells by flow cytometry. These PCs were cultured in vitro and used for characterization of gene expression, cell growth and myogenic fusion characteristics.

Results: ALP+/CD31- PCs were isolated from skeletal muscle of DMSXL mice. These PCs differentiated into multinucleated myotubes when co-cultured with an inducer cell line. Expanded DMPK RNA expression and nuclear RNA foci were determined. We are currently optimizing dual sgRNA-guided excision of the (CTG)n repeat, like we have performed earlier in human and mouse myoblasts.

Discussion: Research into the correction of the repeat expansion and the systemic application of PCs is ongoing. To test the ability of PCs to reconstitute muscle fibers in vivo, we aim to apply intra-arterial injections with genetically corrected and fluorescently labeled PCs in DM1 mouse models. Due to the discovery of PCs, progress in the field of regenerative medicine calls for cautious optimism.

Grant Support: This work was funded by the Donders Institute for Brain Cognition and Behavior, Radboud University

P-106

Session 6: Cell Models for DM

Developing an In Vitro Neuronal Culture Model System to Study Myotonic Dystrophy

MOSS Kathryn[1], McCONNELL Ona[2], SAXENA Tanvi[2], WANG Eric T[2], **BASSELL Gary J**[1]

1. Department of Cell Biology, Emory University School of Medicine, Atlanta GA
2. Center for Neurogenetics, University of Florida, Gainesville FL

Introduction: Individuals with Myotonic Dystrophy Type I (DM1) have a wide range of CNS symptoms including hypersomnia, anxiety, anhedonia and cognitive impairment. Functional inactivation of Muscleblind (MBNL) RNA binding proteins by expanded CUG repeats may lead to CNS symptoms.

Methods: We used primary cortical neurons from mouse embryos as a model system to gain further insight into MBNL dynamics and the effects of CUG repeat dependent sequestration on neuronal and synaptic development. To examine the distribution of MBNL in cultured cortical neurons, we used antibodies for immunofluorescence. Neurons were also transfected with constructs to express fluorescent fusion proteins of MBNL1 and MBNL2. MBNL localization and dynamics were analyzed by fluorescence imaging in fixed and live neurons, respectively. To develop an in vitro model of DM, cortical neurons were transfected with constructs to express 480 or 960 CTG repeats. Fluorescence in situ hybridization (FISH) and immunofluorescence was used to detect RNA foci and MBNL colocalization in the nucleus. Antibodies to dendritic and synaptic markers were used to characterize effects of CUG repeat expression on neuronal and synaptic development.

Results: Primary cortical neurons express cytoplasmic isoforms of MBNL1 and MBNL2 in punctate/granular patterns along dendrites and axons. The cytoplasmic pool of MBNL in live neurons is motile; imaging of GFP-tagged cytoplasmic isoforms of MBNL1 and 2 reveal dynamic and bi-directional transport. MBNL was shown to interact with a specific kinesin. Expression of CUG repeats resulted in formation of intranuclear RNA foci and sequestration of MBNL. Neurons expressing CTG repeats have shorter and less branched dendrites and impaired synapse development.

Discussion: Primary neuronal cultures provide a useful approach to investigate the function of MBNL proteins in neuronal development and how impairments in cytoplasmic mechanisms may contribute to CNS symptoms in DM.

Grant Support: None.

P-107

Session 6: Cell Models for DM

MSH2 Knock-out Human Pluripotent Stem Cells as Model for CTG Repeat Instability in Myotonic Dystrophy Type 1

FRANCK Silvie[1], BARBÉ Lise[2], SERMON Karen[1]

1. Vrije Universiteit Brussel
2. Gladstone Institute

Introduction: Human pluripotent stem cells (hPSC), either embryonic stem cells (hESC) or induced pluripotent stem cells (iPSC) are a powerful tool to model repeat instability in DM1. Mismatch repair genes, especially Msh2, play an important role in inducing repeat instability in DM1 as shown in knock out mouse models. Also, human cell models with a downregulation of MSH2 induced by shRNA indicate MSH2 as a possible instability modifier. However, remaining MSH2 protein levels in down regulation experiments in human cell models and methods to measure instability with a limited resolution probably only yielded partial answers.

Methods: To fully understand the role of MSH2 in repeat instability in human models, we knocked out MSH2 in three DM1 hESC lines and two hiPSC lines derived from two different DM1 patients using CRISPR/Cas9 systems. Repeat instability in hPSCs was measured by PacBio sequencing of long range PCR fragments spanning the repeat, allowing accurate and complete assessment of the TNR length.

Results: Our preliminary data shows that the first MSH2 wild type DM1 hESC line has a wide repeat size distribution compared to its MSH2 mutant line in which the repeat lengths are less heterogeneous and cluster around a particular CTG expansion. Our results suggest that MSH2 drives repeat instability in DM1 hPSCs and that a lack of MSH2 could stabilize the CTG repeat. However, our other MSH2 knock-out hPSC lines still need to be analysed and could probably strengthen this first observation.

Discussion: We show that hPSC are excellent models for DM1, as we have reiterated the previously observed role of MSH2 in TNR instability. Differentiation of our MSH2 knock-out hPSC lines into disease-relevant tissues or manipulation of other instability modifiers will expand the model.

Grant Support: FWO grant G02315N, Chair Mireille Aerens and Methusalem grant to K. Sermon, Association Belge contre les Myopathies neuroMusculaires (ABMM).

P-108

Session 6: Cell Models for DM

Establishment and Analysis of an Inducible Glial Cell Model for the Study of the Central Nervous System Alterations in the Myotonic Dystrophy Type 1

AZOTLA-VILCHIS Cuauhtli Nacxitl[1], SUÁREZ-SÁNCHEZ Rocío[2], JANO-ITO Joan[2], LEYVA-GARCÍA Norberto[2], MAGAÑA-AGUIRRE Jonathan[2], CISNEROS-VEGA Bulmaro[1], **HERNÁNDEZ-HERNÁNDEZ Oscar**[2]

1. Departamento de Genética y Biología Molecular, Centro de Investigación y de Estudios Avanzados CINVESTAV-IPN Mexico City, México
2. Laboratorio de Medicina Genómica, Servicio de Genética, Instituto Nacional de Rehabilitación (INR-LGII), Mexico City, México

Introduction: Myotonic dystrophy type 1 (DM1) is a multisystemic inherited disease with neurological manifestations including hypersomnia, attention deficits, intellectual disability, reduced initiative and apathy. DM1 is caused by the expansion of CTG repeats in the 3'UTR of the DMPK gene. Mutant RNA accumulates in nuclear foci modifying alternative splicing of many secondary genes. In the DM1 brain, nuclear foci have been detected both in neurons and glia, but the potential contribution of glial cells in the pathogenesis is still unknown. To investigate this, we have established a new DM1 model based on MIO-M1 cell line.

Methods: By using the Tet-On system, stable cell lines were generated by transfections with doxycycline responsive plasmids, carrying a fragment of the 3'UTR of DMPK human gene with 0 or 960 CTG repeats. Transgene genome insertion was evaluated by TP-PCR and SP-PCR, DMPK expression was measured by qRT-PCR. FISH coupled to immunofluorescence was used to assess mutant RNA foci and MBNL1/2 co-localization. In addition, alternative splicing and gene expression changes were assessed by a microarray approach.

Results: A normal CTG tract was detected in a control MIO-M1 CTG(0) clone in contrast with 648 CTG repeats detected in a mutant MIO-M1 CTG(648) clone. Both clones cultured under induced conditions showed expression of the exogenous RNA. Foci nuclear RNA was observed only under induced conditions in the MIO-M1 CTG(648) clone co-localized with MBNL proteins. Interestingly, the missplicing of MBNL2 exon 7 was also observed. Finally, a distinctive transcriptome profiles were determined in both MIO-M1 CTG(o) and MIO-M1 CTG(648) clones.

Discussion: We have created a glial MIO-M1 cell-based model that recreates key molecular aspects of the DM1. This model will allow the study of the glia contribution to the neuropathogenesis of the DM1 and represents an additional tool to evaluate therapeutic strategies.

Grant Support: CB-2012-01 183697 / CONACyT Mexico.

P-109

Session 6: Cell Models for DM

Tagging RNA-Binding Proteins in Synapses with a Promiscuous Biotin Ligase (BirA)

HILDEBRANDT Ryan[1], MOSS Kathryn, BASSELL Gary J[2], WANG Eric T[1]

1. University of Florida
2. Emory University

Introduction: DM can cause decline in cognitive function, memory impairment, and hypersomnia, indicating defects in the central nervous system. In the CNS, RNA localization from neuronal cell bodies to synapses is important for maintaining proper function. Previous work suggests MBNL plays a role in transporting target RNAs to neurites, potentially for local translation at the synapse. We hypothesize that MBNL sequestration in DM causes mis-localization of RNAs away from synapses. To assess this hypothesis, we are developing methods to tag RNA-binding proteins with the pre- and post-synaptic compartments of cultured primary neurons.

Methods: A promiscuous biotin ligase derived from E. coli will be fused to markers for the pre- and post-synapse, Synapsin1 and PSD95. When expressed in cells, these fusion proteins will biotinylate other proteins in their vicinity upon addition of exogenous biotin. These constructs will be introduced into primary neurons from wild type or MBNL1/2 knockout mice. Protein-RNA complexes will be recovered from synapses under stringent denaturing conditions, using streptavidin-coated beads, or analyzed by immunofluorescence to confirm biotinylation.

Results: Constructs containing synaptic markers fused to BirA were made and introduced first into N2A neuroblasts. Following exogenous biotin introduction and IF staining, fusion protein signal was present along with streptavidin signal, in a protein expression and biotin-dependent manner. Following this initial test, constructs were introduced into primary cortical neurons (7 DIV). Colocalization of fusion proteins and streptavidin puncta was observed in neurons expressing PSD95-BirA, indicating biotinylation around fusion proteins.

Discussion: Mis-localization of RNAs at synapses can result in impaired neuronal activity and function. This method will allow us to identify RNAs that may be mis-localized in DM-affected neurons. These RNAs will become targets for future studies, in which consequences for mis-localization will be further evaluated and connected to cellular and physiological phenotypes in DM.

Grant Support: This research is supported by a grant from the NIH (NIH DP5 OD017865).

P-110

Session 6: Cell Models for DM

Characterization of Primary Cells from Myotonic Dystrophy Patients

HINTZE Stefan[1], MENSEL Raphaela[1], LIMMER Sarah[1], SCHOSER Benedikt[1], MEINKE Peter[1]

1. Friedrich-Baur-Institut Klinikum der LMU

Introduction: Myotonic dystrophies (DM) are caused by heterozygous DNA-repeat expansions in the DMPK gene (DM1) or the CNBP gene (DM2). These repeats are instable and mosaics have been described. Repeat-containing RNA accumulates in ribonuclear foci and splicing factors are sequestered to these foci, resulting in abnormal regulation of alternative splicing. Human primary myoblasts are an excellent source to investigate effects on cell cycle and differentiation. Here we characterized several DM1 and control cell lines for repeat length, cell cycle and differentiation effects, mis-splicing of known targets and metabolic defects.

Methods: For this we used Southern blot, immunofluorescence staining, a NGS-based RNA sequencing library and a metabolic analyzer.

Results and Discussion: The characterization of these cell lines provides us with an important resource for the testing of therapeutic approaches.

Grant Support: None.

P-111

Session 6: Cell Models for DM

A Cell Model System to Study the Molecular Mechanism of Repeat Associated Non-ATG Translation in Myotonic Dystrophy type 2

KHORAMIAN TUSI Solaleh[1,2], ZU Tao[1,2], CLEARY John[1,2], RANUM Laura[1,2,3]

1. Center for NeuroGenetics, University of Florida, Gainesville, USA
2. Genetics Institute, University of Florida, Gainesville, USA
3. McKnight Brain Institute, University of Florida, Gainesville, USA

Introduction: Microsatellite expansion mutations cause more than 30 neurological diseases, including myotonic dystrophy. Repeat expansions are found within both protein coding and non-coding regions of genes and are bidirectionally expressed producing both sense and antisense expansion transcripts. Interestingly, expansion mutations can produce proteins in all three reading frames without an AUG initiation codon. This repeat associated non-ATG (RAN) translation depends on repeat tract length, RNA structure and can occur in the absence of close cognate initiation codons or frameshifting. RAN proteins have now been reported in a number of neurological diseases such as SCA8, DM1, *C9orf72* ALS/FTD and HD. Understanding how RAN translation works will be critical for understanding the role of RAN proteins in disease and may help in developing effective therapies.

Methods: To determine the molecular mechanisms of RAN translation, we are utilizing the CCTG•CAGG expansion of myotonic dystrophy type 2 (DM2). This expansion mutation produces RAN proteins in both sense CCUG and antisense CAGG directions with repeat motifs of Leu-Pro-Ala-Cys (LPAC) and Gln-Ala-Gly-Arg (QAGR), respectively. Both RAN proteins accumulate in DM2 autopsy brains.

Results: To identify the cellular factors required for RAN translation and to determine the conditions leading to RAN protein production we are generating stable cell lines that can inducibly express the DM2 RAN proteins. We show expression of sense LPAC or antisense QAGR in our stable cell line by IF. Additionally we are performing mass spectrometry experiments in cell culture and cell-free systems to determine where RAN translation begins for LPAC and GAQR proteins.

Discussion: This approach will allow us to better understand the conditions required for RAN translation and how this process contributes to myotonic dystrophy and other repeat disorders.

Grant Support: This abstract is supported by NIHP01 NS058901 and Muscular Dystrophy Association.

P-112

Session 6: Cell Models for DM

CRISPR/Cas9 Genome Editing Reverts Pathological Splicing of Myotonic Dystrophy Type 1

LO SCRUDATO Mirella[1], POULARD Karine[1], KLEIN Arnaud[2], TOMÉ Stéphanie[3], MARTIN Samia[1], GOURDON Geneviève[3], FURLING Denis[2], BUJ-BELLO Ana[1]

1. INTEGRARE, Genethon, Inserm, Univ Evry, Université Paris-Saclay, 91002, Evry, France
2. Sorbonne Universités UPMC Paris 06, Inserm, CNRS, Centre de Recherche en Myologie, Institut de Myologie, Paris, France
3. INSERM UMR 1163, Institut Imagine, Université Paris Descartes-Sorbonne Paris Cité Paris, France

Introduction: Myotonic dystrophy type 1 (DM1) is an autosomal form of muscular dystrophy caused by the expansion of a CTG trinucleotide repeat. Specifically, this repetition is located at the 3' untranslated region (3′-UTR) of the DMPK gene. Mutated DMPK mRNAs, which carry elongated CUG repeats, become sequestration sites for splicing factors. The aberrant mRNAs-proteins interaction results in the formation of stable ribonucleoprotein complexes which are visualized as foci within the nuclei. As a consequence, the alternative splicing of numerous transcripts is modified and triggers several pathological changes in various tissues which in the end determine the signs of the DM1 disease. CRISPR/Cas9 is a genome editing tool based on two molecules that work as a complex, a RNA (sgRNA) and a nuclease (Cas9). sgRNA makes a specific interaction with the targeted DNA, by base-pair complementarity, and Cas9 determines DNA double-strand breaks (DSBs) at the dedicated sites.

Methods: We decided to apply CRISPR/Cas9 technology as a gene therapy approach of DM1. In particular, our strategy aims at the excision of the CTG expansion maintaining the frame of the DMPK gene. As disease model for in vitro studies, we have chosen immortalized myoblasts from patients harboring a long CTG repeat expansion, characterized by nuclear foci and DM1 pathological splicing alterations. These cells have been transduced with Cas9-sgRNA lentiviral vectors and tested 1) for the genomic deletion of DMPK CTG repeats by PCR and Southern Blot, and 2) for the presence of nuclear foci by FISH.

Results: Results from genomic analysis clearly showed a DNA resection of the CTG expansion at the DMPK 3'-UTR. Exact position of the end-joining has been checked by sequencing. We also investigated, upon muscle cell differentiation, the splicing patterns of several transcripts that are dysregulated in DM1 patients, and observed a reversion of the pathological pattern to a normal one.

Discussion: Our data overall demonstrate that the CRISPR/Cas9-mediated deletion of the 3′-UTR DMPK CTG repeats reverts the DM1 phenotype at molecular and cellular levels.

Grant Support: None.

P-113

Session 6: Cell Models for DM

Effect of Two Kinase Inhibitors on Myotonic Dystrophy Type 1 and Type 2 Fibroblasts

LOPEZ-MORATO Marta[1], MALIK Naveed[1], KETLEY Ami[1], BROOK David[1]

1. The University of Nottingham

Introduction: Myotonic dystrophy (DM) is a dominantly inherited RNA-gain-of-function disease caused by a repeat expansion in the 3'UTR of the DMPK gene (DM1) or in intron 1 of the ZNF9 gene (DM2). When the genes are transcribed, the expanded repeats are retained in the nucleus where they sequester MBNL proteins, a family of splicing factors, and aggregate forming inclusions called foci. These foci are in close proximity to the SC35 nuclear speckles in DM1 but not in DM2. There is currently no treatment available for DM to date, but efforts based on the disruption of foci with the aim to release MBNL proteins and restore the aberrant alternative splicing, offer potential. Herein we present results from the treatment of DM1 and DM2 fibroblasts with two kinase inhibitors, MLM1 and MLM2.

Methods: DM fibroblasts were treated with MLM 1 and 2 for 24 hours and foci elimination was studied performing FISH experiments. The effect of the compounds on SC35 speckles and MBNL was assessed by immunohistochemistry (IHC). The alternative splicing of NFIX and ITGA6 was also examined to assess the restoration of mis-splicing.

Results: MLM 1 and 2 reduce the number of foci per cell in both DM1 and DM2, although the effect is more potent in DM2 cells. SC35 IHC showed a reduction in the number of SC35 speckles, and MBNL was redistributed from nuclear to mainly cytoplasmic localisation at the same concentrations of drugs that eliminate foci. Based on 24hr treatment with each inhibitor we did not observe a restoration of the alternative splicing pattern of NFIX and ITGA6.

Discussion: Both kinase inhibitors disrupt foci in DM cells, but further work is needed to fully understand their mechanism of action. However they could serve as starting points for the development of a therapeutic strategy for DM.

Grant Support: This research was funded by the Myotonic Dystrophy Support Group UK.

P-114

Session 6: Cell Models for DM

CRISPR/Cas9 Mediated Genome Editing in Myotonic Dystrophy Type 1 Fibroblast: Implication for Therapeutics and Inducible Disease Model Development

MALIK Naveed Altaf[1], LOPEZ-MORATO Marta[1], KETLEY Ami[1], BROOK David[1]

1. The University of Nottingham

Introduction: Myotonic dystrophy type 1 (DM1) is caused by CTG repeat expansion within the DMPK gene and involves toxic RNA gain of function. Transcription of the mutant DMPK gene causes the accumulation of expanded CUG transcripts into nuclear RNA foci. Currently no drug is available for DM1. The present project is based on CRISPR/Cas9-induced genome editing and efforts are being made to knock-in an inducible promoter to regulate the expression of the endogenous DMPK gene in DM1 fibroblasts. This approach could help us in understanding its potential role for therapeutic genome editing and identifying early pathogenic changes in DM1.

Methods: gRNAs targeting DMPK gene were tested by in vitro assay. Immortalized human DM fibroblasts line KB (CTG400) was transfected with the inducible pTetone vector, expression vector (CRISPR/CAS9 + gRNA) and the linear hygromycin selectable marker by nucleofection. The effect of the insertion of the exogenous DNA on the production of RNA foci was evaluated by RNA fluorescence in situ hybridization.

Results: Target specific PCR amplification followed by DNA sequencing of 180 colonies revealed CRISPR/CAS9 target cutting efficiency of >50% while gene insertion efficiency of 8% (24/180). Further analysis showed that in 18 colonies linear hygromycin, in 03 colonies partial pTetone vector and in one colony pTetone vector (in wrong orientation) was integrated into the DMPK target site. RNA fluorescence in situ hybridization demonstrated that in 14/22 colonies, RNA foci were significantly reduced.

Discussion: Our results shows that CRISPR/Cas9-induced non homology end joining knock in enables integration of exogenous DNA into target site but it allows integration in either orientation. Work is ongoing to insert pTetone inducible vector in the required orientation in the DMPK target site.

Grant Support: The research was funded by Islamic Development Bank PhD Scholarship Programme and Myotonic Dystrophy Support Group, UK.

P-115

Session 6: Cell Models for DM

Development of a High-throughput Assay to Identify Novel Compounds that Block Transcription from Expanded CTG Repeats in Myotonic Dystrophy

REDDY Kaalak[1,2], MCCONNELL Ona[1], WANG Eric T[1,3], BERGLUND J Andrew[1,2]

1. Center for NeuroGenetics, University of Florida – College of Medicine, Gainesville USA
2. Department of Biochemistry and Molecular Biology, University of Florida, Gainesville USA
3. Department of Molecular Genetics and Microbiology, University of Florida, Gainesville USA

Introduction: In DM1, transcription of expanded CTG repeats generates toxic RNA, causing disease through multiple gain of function mechanisms. Toxic RNAs 1) sequester muscleblind proteins into nuclear foci away from their splicing functions, 2) undergo repeat associated non-ATG (RAN) translation to generate aggregating proteins and 3) form R-loops that may drive instability of the DNA. We hypothesize that blocking transcription of expanded repeats will mitigate known and potentially any unknown toxic RNA effects.

Methods: To identify compounds that selectively block transcription of expanded CTG repeats, we are developing a cell-based high-throughput screening assay. We are utilizing constructs with ~3.5 Kb of DMPK sequence containing either 0 or 480 interrupted CTG repeats and a unique barcode to distinguish which template the RNA is generated from. Transcription can be initiated in vitro from a T7 RNA polymerase promoter or from an SV-40 promoter in human cells in the presence of compounds that potentially modulate transcription. Nascent RNA from each template is quantified using qPCR with probes of different fluorescence that distinguish the unique sequence tag.

Results: We validated our screening strategy in vitro and in HeLa cells using Actinomycin D, a natural compound that was shown by the Berglund lab to selectively impede transcription from expanded CTG repeats in a DM1 human cell model and DM1 mouse model. We are characterizing the cell lines we generated and are performing initial screening with targeted compound libraries. Progress from our screening efforts will be presented.

Discussion: This project provides an important step towards identifying novel compounds with therapeutic potential in treating myotonic dystrophy and other neuromuscular disorders.

Grant Support: This project is supported by start-up funds from the University of Florida and a research grant from the Wyck Foundation and Myotonic Dystrophy Foundation (MDF). Reddy K is supported by a postdoctoral fellowship from the Wyck Foundation/Myotonic Dystrophy Foundation.

P-116

Session 6: Cell Models for DM

Testing Therapeutic Potential of Splice-switching Antisense Oligomers Targeting DMPK in Cell Models for DM1.

STEPNIAK-KONIECZNA Ewa[1], KONIECZNY Patryk[1], ZIELINSKA Julia[1], SOBCZAK Krzysztof[1]

1. Adam Mickiewicz University, Faculty of Biology, Institute of Molecular Biology and Biotechnology,
2. Department of Gene Expression, address: Umultowska 89, 61-614 Poznan, Poland

Introduction: We investigated whether splice-switching antisense oligonucleotides (AONs) directed at DMPK transcript can promote its reduction (strategy 1; S1) or skipping of expanded CUG repeats (CUGexp) from pre-mRNA (strategy 2; S2). Using cellular DM1 models, we tested whether these approaches have the potential of reducing the overall burden of toxic RNA.

Methods: We rationally designed AONs to target either internal constitutive DMPK exons (S1), or the last coding DMPK exon 15 harboring 3'UTR with CUGexp (S2). In S1, exon skipping shifts the reading frame and generates a premature stop codon in mRNA, thus increasing the likelihood of transcript degradation via RNA surveillance pathways. In S2, AONs redirect the splicing towards a cryptic alternative 3'-splice site downstream of CUGexp, thus giving rise to splice isoforms without exon 15 and CUGexp but novel terminal exon instead. We presumed that both strategies could be therapeutic by inducing foci dispersal and amelioration of DM1-related spliceopathy.

Results: We identified several AONs that efficiently switch the splicing of DMPK pre-mRNA. In both strategies S1 and S2, the efficacy of exon skipping was higher in pre-mRNA originating from the non-expanded DMPK allele. S1 had minimal effect on DMPK mRNA and protein level, but affected foci dispersal. Shift from adult to embryonic splice isoform was augmented for several transcripts. This adverse effect was influenced by the chemical modification of AONs and correlated with deregulated expression of MBNLs and CELF1. S2 noticeably increased the abundance of mRNA isoforms lacking CUGexp and containing novel terminal exon, and we are currently investigating the molecular outcomes of this phenomenon in DM1 cell models.

Discussion: Therapeutic potential of DMPK-targeting AONs requires further molecular analyses. Also, AON chemical modifications are associated with certain toxicities, which need to be addressed as DM1 requires chronic treatment.

Grant Support: National Science Centre grant 2014/15/B/NZ5/00142 (to E.S-K.).

P-117

Session 6: Cell Models for DM

Turning Skin into Brain: an In Vitro Approach to Model the DM1 Central Nervous System

VAN DER BENT Leontien[1,2], BROCK Roland[2], WANSINK Derick[1]

1. Department of Cell Biology, Radboud Institute for Molecular Life Sciences, Nijmegen, NL
2. Department of Biochemistry, Radboud Institute for Molecular Life Sciences, Nijmegen, NL

Introduction: The pathogenesis of myotonic dystrophy type 1 (DM1) has been studied intensively over the past 25 years, especially regarding skeletal muscle. However, symptoms related to the central nervous system, such as intellectual disability, behavioral issues and daytime sleepiness, also have a large impact on patients' quality of life. Therefore, there is a pressing need for more insight into the brain pathology of DM1. Our goal is to generate an in vitro model of the DM1 central nervous system to further study the brain pathology.

Methods: Human primary fibroblasts of DM1 patients and unaffected individuals were transduced with a lentivirus that encodes the transcription factor OCT-4, then cultured under reprogramming conditions for several weeks. The resulting neural precursor cells (NPCs) were characterized by RT-(q)PCR and RNA FISH.

Results: We have successfully transdifferentiated fibroblasts from a DM1 patient and from a healthy subject, as confirmed by RT-qPCR. Both WT and DM1 NPCs expressed DMPK at a lower level compared to fibroblasts and myoblasts. Accordingly, less ribonuclear foci were observed in DM1 NPCs. RT-PCR analysis showed that splicing of several known markers is largely embryonic and, in contrast to their parent fibroblasts, no differences were observed between DM1 and WT NPCs.

Discussion: Our results indicate that NPCs show a relatively mild molecular phenotype. We are presently deriving additional NPC lines and are planning to perform RNA-seq to further characterize these lines. By studying functional aspects of DM1 neural precursor cells and the astrocytes, oligodendrocytes and neurons that can be derived from them, we will hopefully be able to further elucidate the DM1 brain pathology. Specifically, we might be able to narrow down which neural cell types contribute most to the brain pathology. Additionally, these neural cells could be used for the development of more disease-specific in vitro model systems.

Grant Support: This work was funded by the Prinses Beatrix Spierfonds (www.prinsesbeatrixspierfonds.nl; grant number W.OR14-19).

P-118

Session 6: Cell Models for DM

RbFox as a Potential Modifier of MBNL Splicing in DM

WAGNER Stacey[1], OLAFSON Hailey[2], STRUCK Adam[1], KETCHUM Sunny[1], MAHADY Amy[1], RICHARDSON Jared[2], HALE Melissa[2], GUPTA Riti[1], EICHINGER Katy[3], THORNTON Charles[3], WANG Eric[2], BERGLUND Andy[2]

1. University of Oregon, Eugene, Oregon, USA
2. University of Florida, Center for NeuroGenetics, Gainesville, FL, USA
3. University of Rochester, Department of Neurology, University of Rochester Medical Center, Rochester, NY, USA

Introduction: The concentration of functional MBNL is reduced in DM by sequestration to the CUG/CCUG repeat expansion. Splicing factors RbFox and MBNL co-regulate many splicing events and share overlapping RNA binding sites within some pre-mRNAs. The goals of this study are to understand how the concentrations of MBNL and RBFox impact regulated splicing in cell lines and in DM patients. This work is fundamental to understanding how changes in splicing factor concentration in DM, and the interplay of splicing factors, dictate splicing decisions.

Methods: We quantified alternative splicing resulting from titration of MBNL using an inducible MBNL1 cell line. Strategic mutagenesis was performed to alter the organization of putative MBNL1 and RBfox1 binding sites within pre-mRNAs. RNAseq was performed over an MBNL1 gradient in the presence and absence of RBFox1, and within tibialis anterior and additional tissues in a DM1 patient cohort. A double inducible MBNL1/RBFox1 cell line has been created and is being validated.

Results: Splicing events demonstrate unique sigmoidal shaped curves in response to MBNL1 dose. Hundreds of these splicing events were found to be modulated by RBFox1. In some cases, RBFox1 dampens the MBNL1 dose response by using a shared binding site. Additional modes of co-regulation were observed and the potential mechanisms will be discussed. RbFox1's modification of MBNL's dose dependent effect on mRNA levels will also be discussed.

Discussion: This work indicates RBFox1 functions as a modifier in DM through its ability to significantly alter MBNL1 regulation. To evaluate if RBFox is a modifier in patients, we will address the following questions: How do differential levels of RbFox1/2 modify MBNL in the context of DM1 and DM2? Can RbFox1/2 compensate for MBNL1 depletion and in what contexts/tissues?

Grant Support: NIH Awards: F32AR063565, AR059833, DP5 OD017865-01, RC2HG005624, U54 NS048843.

P-119

Session 6: Cell Models for DM

A CRISPR-C2c2 Based Therapy to Target Toxic RNA in Microsatellite Expansion Diseases

ZHANG Nan[1], ASHIZAWA Tetsuo[1]

1. Houston Methodist Research Institute

Introduction: Microsatellite expansion diseases encompass more than 30 neurological and neuromuscular disorders, including Huntington's disease, various spinocerebellar ataxias, myotonic dystrophy etc. When repeat expansion of 3-6 nucleotides occurs in non-coding regions, the expanded RNA adopts unusual secondary structures, sequesters RNA binding proteins of important cellular functions, and forms insoluble nuclear foci. This toxic RNA gain-of-function may deplete cellular RNA binding proteins and contribute to multi-systemic disease pathogenesis. Hence, elimination of toxic RNA and foci has become the main focus of many studies. One of the leading therapeutic approaches utilizes antisense oligonucleotides (ASOs) to elicit RNase H-dependent toxic RNA degradation. Several drawbacks have been observed with this approach, including dose-dependent toxicity and off-target effects, poor drug delivery and uptake, severe thrombocytopenia, and drug accumulation and rapid clearance in the liver and kidney. Here, we report the development of a CRISPR-C2c2 based strategy to target and eliminate toxic RNA and foci. The C2c2 protein from Leptotrichia Shahii is a crRNA-guided RNase.

Methods: In vitro transcribed RNA repeats (CUG30, CAG30, GGGGCC15, AUUCU15, and GAA30) were tested for degradation by mixing with recombinant C2c2 proteins and specific guide crRNAs. The C2c2-crRNA apparatus was cloned into an "all-in-one" vector and transiently transfected into human myotonic dystrophy type 1 (DM1) fibroblasts. Fluorescent in situ hybridization (FISH) assays were performed to monitor the RNA foci reduction in comparison to untransfected cells.

Results: The CUG and CAG RNA repeats were more efficiently cleaved in vitro by C2c2 than other repeats. Our preliminary data showed a significant RNA foci reduction in C2c2-crRNA treated DM1 fibroblasts than untreated cells.

Discussion: By introducing tissue-specific promoters and different guide crRNAs, a wide range of microsatellite expansion diseases could potentially be treated by this approach.

Grant Support: This work is supported by 1RO1-NS083564.

P-120

Session 6: Cell Models for DM

A Flow Cytometry-Based Screen Identifies MBNL1 Modulators that Rescue Splicing Defects in Myotonic Dystrophy Type I

ZHANG Fan[1], SUN Yumei[1]

1. Rare Disease Research Unit, Pfizer Inc.

Introduction: Myotonic Dystrophy Type 1 (DM1) is a rare genetic disease caused by expansion of CTG trinucleotide repeats ((CTG)exp) in the 3' untranslated region of the DMPK gene. The repeat transcripts sequester the RNA binding protein Muscleblind-like protein 1 (MBNL1) and hamper its normal function in pre-mRNA splicing. Overexpressing exogenous MBNL1 in the DM1 mouse model has been shown to rescue the splicing defects and reverse myotonia. Although a viable therapeutic strategy, pharmacological modulators of MBNL1 expression have not been identified.

Methods: Here, we engineered a ZsGreen tag into the endogenous MBNL1 locus in HeLa cells and established a flow cytometry-based screening system to identify compounds that increase MBNL1 level.

Results: The initial screen of small molecule compound libraries identified more than thirty hits that increased MBNL1 expression greater than double the baseline levels. Further characterization of two hits revealed that the small molecule HDAC inhibitors, ISOX and vorinostat, increased MBNL1 expression in DM1 patient-derived fibroblasts and partially rescued the splicing defect caused by (CUG)exp repeats in these cells.

Discussion: These findings demonstrate the feasibility of this flow-based cytometry screen to identify both small molecule compounds and druggable targets for MBNL1 upregulation. With the potential of expanding to larger compound library and/or knockdown screens, it enables future detailed mechanism studies of MBNL1 expression and possible new therapeutic avenues for DM.

Grant Support: This study was supported by a postdoctoral fellowship from The Marigold Foundation.

P-121

Session 7: Animal Models and Tissue-specific Mechanisms

CNS-associated Behavioral Dysfunction in a Novel AAVCUG Based Neuronal Model of Myotonic Dystrophy Type I

BANERJEE Anwesha[1], ATTEH George[1], MCCONNELL Ona[2], PRICE Philip[1], WANG T. Eric[2,3], BASSELL J. Gary[1,4]

1. Department of Cell Biology, Emory University School of Medicine, Atlanta GA
2. Center for Neurogenetics, University of Florida, Gainesville, FL
3. Dept. of Molecular Genetics and Microbiology, University of Florida, Gainesville, FL
4. Department of Neurology, Emory University School of Medicine, Atlanta GA

Introduction: Myotonic Dystrophy Type I (DM1) displays a range of debilitating central nervous system (CNS) associated behavioral symptoms including hypersomnia, anxiety, anhedonia, which remains significantly understudied. Evidence shows that an RNA toxic gain of function mechanism plays a major role in the pathophysiology of the disease. The expanded CUG repeats in 3' untranslated region of the gene encoding dystrophia myotonica protein kinase (DMPK) results in accumulation of RNA foci in the nucleus and subsequent sequestration of Muscleblind (MBNL) RNA-binding proteins. MBNL proteins are therefore prevented from carrying out its normal function, which may lead to CNS associated symptoms observed in DM1.

Methods: We have used the method of Somatic brain transgenesis (SBT) using AAV9 virus to selectively express either 480 or 960 expanded CUG repeats in neurons during postnatal development (neonates) and in the adult brain. Fluorescent in situ hybridization (FISH) combined with immunohistochemistry were used to validate molecular and cellular phenotypes. Further, the animals were characterized using a battery of behavioral tests relevant to DM1 phenotypes such as anxiety, anhedonia and learning and memory deficits.

Results: FISH combined with immunohistochemistry analysis revealed accumulation of CUG RNA foci colocalized with MBNL2 protein in nuclei of neurons. Mice expressing CUG repeats display altered locomotion in open field, reduced sucrose preference and increased immobility in the Forced Swim Test, consistent with behavioral despair, anhedonia and lack of motivation to explore a new environment without displaying any gross motor abnormalities as observed in a grip test.

Discussion: This neuronal AAV DM1 mouse model recapitulates some DM1 phenotypes at both behavioral and molecular level and can be further used to provide mechanistic insight in RNA toxicity associated with the disease as well as test various therapeutic intervention strategies.

Grant Support: Postdoctoral Fellowship - Wyck Foundation/Myotonic Dystrophy Foundation.

P-122

Session 7: Animal Models and Tissue-specific Mechanisms

ABP1 Hexapeptide Binds CCUG Hairpin Structure and Rescues Myotonic Dystrophy-like Phenotypes in a Drosophila Model of Sardiac Dysfunction in DM2

CHAKRABORTY Mouli[1,2,3], GARCIA-LÓPEZ Amparo[4], ESTRADA Roger[5], ARTERO Rubén[1,2,3], and LLAMUSÍ Beatriz[1,2,3]

1. Translational Genomics Group, Incliva Health Research Institute, Valencia, Spain
2. Department of Genetics and Interdisciplinary Research Structure for Biotechnology and Biomedicine (ERI BIOTECMED), University of Valencia, Valencia, Spain
3. CIPF-INCLIVA joint unit
4. Department of Pharmaceutical Biochemistry, University of Geneva, Geneva, Switzerland
5. Grup d'Enginyeria Molecular (GEM), InstitutQuímic de Sarrià (IQS) – UniversitatRamonLlull (URL), Barcelona, Catalonia, Spain

Introduction: Heart involvement is common in myotonic dystrophy being the second most frequent cause of death. Sequestration of Muscleblind like-1 (MBNL1) into ribonuclear foci is known as the main trigger of muscle atrophy in DM1, however, the molecular causes of cardiac dysfunction remain unclear. To shed light into this problem, we studied the role of Mbl (fly orthologue) in cardiac dysfunction induced by CCUG expansions in Drosophila. Biological activity of D-hexapeptide ABP1, which was previously described to modify the structure of the hairpin and to rescue toxicity in DM1, was also investigated.

Methods: Model flies expressing 1100 CCUG repeats in cardiomyocytes were fed with ABP1. Fly recombinants co-expressing CCUG repeats and MblC were also used to rescue the phenotypes. Fly hearts were dissected and recorded to measure cardiac parameters using SOHA software. Dissected hearts were processed for immunofluorescence, to detect foci or Mbl, and for RNA extraction. In-vitro interaction of ABP1 and CCUG repeats was checked by EMSA, tryptophan quenching and differential scanning fluorimetry.

Results: Expression of CCUG repeats in cardiomyocytes brings about cardiac dysfunction including increased heart period, arrhythmia, and reduced contractility. These phenotypes were rescued to normal values by MblC co-expression and also by ABP1 treatment. ABP1 also reduced foci formation and released Mbl from cardiomyocytes improving SERCA splicing and expression of Cp6W1. At in-vitro level ABP1 interacts with CCUG hairpins and modifies their structure.

Discussion: Our data support an important role of Mbl in cardiac dysfunction induced by CCUG-toxicity and highlight the potential of therapies addressed to release Mbl from foci.

Grant support: Co-funded by grants from the ISCIII (PI13/00386, including ERDF funds), ERA-Net E-Rare framework (PI12/03106, including ERDF funds) and RTVE telemaraton to RA and BL. MC was the recipient of Santiago Grisolía award.

P-123

Session 7: Animal Models and Tissue-specific Mechanisms

Experimental Update About the Dystrophin Dp71: A Clue to Understanding Cataracts and Retinal Lesions in DM1 Patients

BRIGNOL Tuy-Nga[1], URTIZBEREA J. Andoni[2], MORNET Dominique[3], RENDON Alvaro[4]

1. Medical Affairs Department, AFM-Telethon, Evry, France
2. Reference Center for Neuromuscular Diseases Filnemus/Hôpital Marin, Hendaye, France
3. University of Montpellier / INSERM / CNRS UMR. Montpellier, France
4. Institut de la Vision/Inserm/ UPMC Paris 6, Paris, France

Introduction: Myotonic dystrophy type 1 (DM1) is associated with ocular complications such as cataracts, ptosis, and far less recognized, retinal damage. Experimental studies showed that the loss of functional MBNL1 caused an abnormal splicing of exon 78 of the DMD gene, resulting in the expression of an embryonic dystrophin severely impairing the muscle architecture. The DMD gene comprises 79 exons and large introns. Dp71, the smallest but multifunctional product of the DMD gene, participates in different cellular processes such as water homeostasis, cell adhesion, nuclear architecture. We hypothesize that the Dp71 plays a role in the molecular mechanisms of cataracts and retinal lesions in DM1 patients.

Methods: A targeted search in PubMed using the key-words “retina”, “myotonic dystrophy” and “Dp71” identified key papers to support our hypothesis.

Results: Reproducing DMD exon 78 missplicing in mice induced characteristic features of dystrophic DM1 skeletal muscles (Rau et al, 2015). In brain and retina, it has been shown that different dystrophin Dp71 isoforms are produced by alternative splicing of exons 71 to 74 and 78, and intron 77. Dystrophin Dp71 isoforms are differentially expressed in mouse brain and retina (Aragon et al, 2017). In retina, specific Dp71-null mice showed that deletion of Dp71 was associated with retinal vascular inflammation and vascular lesions (El Mathari et al, 2015). While studying Dp71 role on the retina, it has been shown for the first time that Dp71 knockout (KO-Dp71) mice also developed a progressive opacification of the crystalline lens, due to a progressive disorganization of the lens fiber cells (Fort et al, 2014).

Discussion: We suggest researchers to reproduce DMD exon 78 misplicing switch in mice in order to study its impact on retina and crystalline lens. It may be a valuable tool for a better understanding about the role of Dp71 in ophthalmic findings in DM1 patients.

Grant Support: None.

P-124

Session 7: Animal Models and Tissue-specific Mechanisms

Expanded CCUG Repeat RNA Expression in Fly Heart and Muscle Trigger Myotonic Dystrophy Type 1-like Phenotypes and Activate Autophagocytosis Genes

CHAKRABORTY Mouli[1,2,3], CERRO-HERREROS Estefania[1,2,3], PÉREZ-ALONSO Manuel[1,2,3], ARTERO Rubén[1,2,3], LLAMUSÍ Beatriz[1,2,3]

1. Translational Genomics Group, Incliva Health Research Institute, Valencia, Spain
2. Department of Genetics and Interdisciplinary Research Structure for Biotechnology and Biomedicine (ERI BIOTECMED), University of Valencia, Valencia, Spain
3. CIPF-INCLIVA joint unit

Introduction: Autosomal dominant myotonic dystrophies (DM1-2) are neuromuscular disorders caused by the pathological expansion of untranslated microsatellites. DM1 and DM2 are caused by expanded CTG repeats in the 3'UTR of the DMPK gene and CCTG repeats in the first intron of the CNBP gene, respectively. The mechanism of disease is common in both diseases; mutant RNAs retained in foci sequester several nuclear factors and cause alterations in RNA metabolism. For unknown reasons, symptoms of DM1 are generally more severe than DM2.

Methods: We generated model flies by expressing pure expanded CUG ([250]×) or CCUG ([1100]×) repeats in fly heart and muscles to study similarities and differences between DM1 and DM2 and compared them with control flies expressing either 20 repeat units or GFP. Muscle and heart performance alterations induced by expanded repeat expression were analyzed at functional (locomotion, survival and cardiac parameters), histological (crossectional muscle area), and molecular levels (Mbl retention in foci, missplicing and autophagy-related gene expression).

Results: We observed surprisingly severe muscle reduction and cardiac dysfunction in CCUG-expressing model flies. The muscle and cardiac tissue of both model flies showed DM1-like phenotypes including overexpression of autophagy-related genes, RNA mis-splicing and repeat RNA aggregation in ribonuclear foci along with the Mbl protein.

Discussion: These data reveal, for the first time, that expanded non-coding CCUG repeats have in vivo toxicity potential similar to expanded CUGs in both tissues, which suggests that specific, unknown factors quench CCUG-repeat toxicity in DM2 patients.

Grant Support: This study was co-funded by grants from the Instituto de Salud Carlos III (PI13/00386; including funds from the European Regional Development Fund), ERA-Net E-Rare framework from ISCIII, and RTVE TELEMARATON (FEDER, FUNDACION ISABEL GEMIO and ASEM).

P-125

Session 7: Animal Models and Tissue-specific Mechanisms

Mechanisms of Skeletal Muscle Wasting in a Mouse Model of DM1

MORRISS Ginny R[1], **COOPER Thomas A**[1,2,3]

1. Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA
2. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA
3. Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA

Introduction: A CTG-repeat expansion in the 3'-UTR of DMPK results in the clinical phenotypes associated with DM1, however, mechanisms that directly underlie skeletal muscle wasting in DM1 remain unknown.

Methods: We developed a Tet-inducible, skeletal muscle specific mouse model for DM1 expressing 960 interrupted CUG repeats in the context of human DMPK exons 11-15.

Results: Our model shows clear muscle wasting, based on reduced muscle weight, following 10 weeks of induction. The muscle wasting phenotype can be partially rescued in gastrocnemius and completely rescued in quadraceps and tibialis anterior muscles by turning off transgene expression. Additionally, severe histopathology is observed in repeat-expressing mice, including increased percent of fibers containing centralized nuclei and decreased fiber cross sectional area. Histopathology and fiber size reduction is improved upon cessation of repeat expression. Repeat expressing animals contain RNA foci that colocalize with MBNL1 proteins both of which disperse when repeat RNA expression is ceased. RNA-seq analysis indicated that changes to alternative splicing primarily affects cassette exons and were enriched for genes involved in cytoskeletal dynamics associated with actin binding and calcium signaling, GTPase activity and PI3K signaling. Splicing defects are mild, yet significant and consistent with previously reported splicing alterations known to correlate with muscle weakness in human DM1 patients. Additionally, RPPA analysis suggested that the PI3K and p53-mediated apoptotic signaling pathways are affected in repeat expressing mice.

Discussion: This mouse model has provided us with an experimental tool to identify significant events and pathways that contribute to progressive skeletal muscle wasting in DM1 and test therapeutic approaches.

Grant Support: This work is supported by National Institutes of Health grants R01AR045653, R01HL045565, and R01AR060733 (to T.A.C.), the Muscular Dystrophy Association (T.A.C) and the Myotonic Dystrophy Foundation (G.R.M).

P-126

Session 7: Animal Models and Tissue-specific Mechanisms

Zebrafish mbnl Mutants Model Key Molecular Phenotypes of Myotonic Dystrophy

HINMAN Melissa N.[1], GUILLEMIN Karen J.[1], BERGLUND J. Andrew[2]

1. Institute of Molecular Biology, University of Oregon
2. Center for NeuroGenetics, University of Florida

Introduction: We established stable zebrafish genetic models to facilitate studies of myotonic dystrophy (DM). Optically transparent larvae will enable direct study of DM-associated changes in development and gut motility, and germ-free fish will enable study of the role of microbiota in DM phenotypes. In addition, high zebrafish fecundity will allow for future drug screening.

Methods: The CRISPR/Cas9 system was used to generate zebrafish mbnl mutants and alternative splicing was analyzed by RT-PCR.

Results: Zebrafish single mbnl mutants (mbnl1-/-, mbnl2-/-, and mbnl3-/-) and double mbnl mutants (mbnl1-/-;mbnl2-/-, mbnl1-/-;mbnl3-/-, and mbnl2-/-;mbnl3-/-) are viable to adulthood. Overlapping mbnl expression patterns in zebrafish tissues may account for the viability of double mbnl mutants. Key molecular changes found in DM patients and mouse models are also observed in zebrafish, including changes in the auto-regulation of mbnl1 and mbnl2 alternative splicing. As in mouse models, mbnl1 and mbnl2 mutants display more robust alternative splicing changes than mbnl3 mutants. Double mbnl mutants exhibit larger splicing changes than single mbnl mutants, suggesting that mbnl dosage is important in splicing regulation. Molecular changes are present in larval mbnl mutants, but are greater in adult tissues, particularly in heart and skeletal muscle. Studies of the physical and behavioral phenotypes of mbnl mutant fish are in progress, and complementary zebrafish DM models are being generated through stable transgenic overexpression of CUG repeats.

Discussion: Zebrafish mbnl mutants accurately model DM-associated molecular changes and will be invaluable tools for studying DM-related phenotypes.

Grant Support: This work was supported by the Myotonic Dystrophy Foundation, the National Institutes of Health, and the Muscular Dystrophy Association.

P-127

Session 7: Animal Models and Tissue-specific Mechanisms

Vector Gene Modification Increases the Efficacy of an In Vivo RNA Interference-based Therapy for DM1

KAROLAK Matthew[1], ZAVALJEVSKI Maja[1], BISSET Darren[2], HALL John1, CHAMBERLAIN, Joel[1]

1. Department of Neurology, University of Washington
2. Department of Medicine, University of Washington

Introduction: A variety of therapeutic approaches target the expanded repeat-containing DMPK mRNA (ER-DMPK mRNA) to block or reverse its associated DM1 disease manifestations. Our approach employs the RNA interference (RNAi) pathway to reduce the ER-HSA mRNA in the HSALR model of DM1. HSA RNAi/ alkaline phosphatase (AP) expression cassettes were incorporated into an adeno-associated virus vector (AAV). The AAV HSA RNAi vector was partially effective in mitigating RNA toxicity in the HSALR mouse with systemic injection. The current study evaluated modifications in expression for therapeutic efficacy in vivo.

Methods: Initial attempts to increase AAV HSA RNAi vector dose led to AP reporter gene toxicity. We deleted of the AP gene promoter to test if a higher dose would result in greater HSA gene silencing. AAV HSA RNAi vectors lacking the AP promoter were injected at either high (5e10) or low (1e10) vector genomes into the HSALR TA muscles. The new vectors were compared to vectors previously tested that included the AP promoter, where administration increasing doses resulted in AP gene toxicity.

Results: Comparison of the low and high doses of the effective AAV HSA RNAi with the AP promoter deletion showed an approximately 50 to 95% reduction, respectively, in exclusion of exon 22 in the Atp2a1 transcript. In contrast, there was limited effectiveness at these doses with the previous AP promoter-containing HSA RNAi vectors. The less active AAV HSA RNAi vector showed no reduction to a 10-20% reduction in exclusion of Atp2a1 exon 22 with the AP promoter-deleted at low and high doses, respectively.

Discussion: Elimination of reporter gene expression reduced a barrier to optimization of RNAi vector effectiveness. Refinement of the AAV RNAi approach in the HSALR mouse model of DM1 increases the potential for systemic RNAi therapy as a DM therapy.

Grant Support: None.

P-128

Session 7: Animal Models and Tissue-specific Mechanisms

Enhanced Systemic Delivery of Antisense Oligonucleotides Using Cell-penetrating Peptide to Reverse RNA Toxicity in Myotonic Dystrophy

KLEIN Arnaud[1], ARANDEL Ludovic[1], ARZUMANOV Andrey[2], REVILLOD Lucile[3], VARELA Miguel[4], JAUVIN Dominic[5], FERRY Arnaud[1], BASSEZ Guillaume[3], GOURDON Geneviève[6], GAIT Mike[2], PUYMIRAT Jack[5] , WOOD Matthew[4], FURLING Denis[1]

1. Sorbonne Universités UPMC, Inserm, Centre de Recherche en Myologie, Institut de Myologie, Paris, France
2. Laboratory of Molecular Biology, Medical Research Council, Francis Crick Avenue, Cambridge UK
3. Neuromuscular Reference Center, Henri Mondor University Hospital, INSERM U955, Créteil, France
4. Department of Physiology, Anatomy and Genetics, University of Oxford, South Parks Road, Oxford UK
5. Department of Human Genetics, Centre Hospitalier Universitaire de Quebec, Quebec, Canada
6. Inserm UMR 1163, Paris Descartes-Sorbonne Cité University, Imagine Institute, Paris, France

Introduction: Antisense oligonucleotides (AON) targeting expanded-CUG transcripts to either degrade the pathogenic RNAs or interfere with abnormal binding/sequestration of RNA-binding proteins like MBNL1 have shown very promising results in DM1 models. Thus, injection of steric blocking PMO-CAG AON in DM1 mice reverses molecular changes and myotonia induced by CUGexp-RNA. However efficient skeletal muscles delivery of AON following systemic administration is still an ongoing challenge for muscular diseases.

Methods: Here we described the use of a PMO-CAG AON conjugated to an arginine-rich cell-penetrating peptide (CPP) designated as Pip6a, to enhance its systemic delivery.

Results: As preliminary experiments, we confirmed in vitro using human DM1 muscle cells that Pip6a-PMO-CAG-treatment induces the release of MBNL1 from nuclear foci and its relocalization into the nucleus, which leads to the normalization of DM1 splicing defects including SOS1, DMD, MBNL1 and LDB3 genes. Next we assess Pip6a-PMO-CAG in vivo using the DM1 HSA-LR transgenic model expressing CUGexp-RNA in skeletal muscles. Intravenous (IV) injection of a single dose of Pip6a-PMO-CAG (12.5 mg/kg) results in a significant correction of alternative splicing defects in treated HSA-LR mice. Subsequent experiments revealed that a complete correction of DM1 splicing defects as well as myotonia in skeletal muscles of HSA-LR mice is reached after three IV injections. This correction is also accompanied with a significant decrease in the level of foci. The beneficial effect is maintained for several weeks after the last injection without adverse effects. Finally, analysis of the transcriptome by RNA-sequencing shows that the pip6a-CAG7 treatment induces a global correction of the diseased transcriptome of HSA-LR mice, at both gene expression and alternative splicing levels.

Discussion: This study demonstrates that Pip6a-CPP-conjugated PMO-CAG reverses CUGexp-RNA toxicity in DM1 mice and represents an efficient tool for AON delivery in skeletal muscles for DM1.

Grant Support: AFM / MD Canada.

P-129

Session 7: Animal Models and Tissue-specific Mechanisms

(CCUG)n RNA Toxicity in a Drosophila Model for Myotonic Dystrophy Type 2 (DM2) Activates Apoptosis

YENIGUN Vildan Betul[1,2,3,#], SIRITO Mario[1,#], AMCHESLAVKY Alla[6,#], CZERNUSZEWICZ Tomek[1], COLONQUES-BELLMUNTJordi[5], GARCÍA-ALCOVER Irma[5], WOJCIECHOWSKA Marzena[1], BOLDUC Clare[2], CHEN Zhihong[2], LÓPEZ CASTEL Arturo[5], **KRAHE Ralf**[1,3,4,*], BERGMANN Andreas[2,3,6,*]

1. Department of Genetics, University of Texas MD Anderson Cancer Center, Houston, Texas, USA
2. Department of Biochemistry & Molecular Biology, University of Texas MD Anderson Cancer Center, Houston, Texas, USA
3. Graduate Program in Genes & Development, University of Texas Graduate School in Biomedical Sciences at Houston, Houston, Texas, USA
4. Graduate Program in Human & Molecular Genetics, University of Texas Graduate School in Biomedical Sciences at Houston, Houston, Texas, USA
5. Valentia BioPharma, Paterna, Spain
6. Department of Molecular, Cell and Cancer Biology, University of Massachusetts Medical School, Worcester, Massachusetts, USA

Introduction: The myotonic dystrophies are prototypic toxic RNA gain-of-function diseases. Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are caused by different unstable, noncoding microsatellite repeat expansions -- (CTG)DM1 in DMPK and (CCTG)DM2 in CNBP. Although transcription of mutant repeats into (CUG)DM1 or (CCUG)DM2 appears to be necessary and sufficient to cause disease, their pathomechanisms remain incompletely understood. To study the mechanisms of (CCUG)DM2 toxicity and develop a convenient model for drug screening, we developed and characterized a DM2 fruit fly Drosophila melanogaster model.

Methods: We generated DM2 and normal transgenic fruit flies with (CCUG)n repeats of variable length (n = 16 and 106).

Results: Expression of noncoding (CCUG)106, but not (CCTG)16, in muscle and retinal cells led to formation of (CCUG) ribonuclear inclusions and mis-splicing of genes implicated in the DM pathology. Mis-splicing could be rescued by co-expression of human MBNL1, while CUGBP1/CELF1 complementation did not. Flies with (CCUG)106 displayed strong disruption of the external eye morphology and the underlying retina. Furthermore, expression of (CCUG)106 in developing retinae caused a strong apoptotic response. Inhibition of apoptosis rescued the retinal disruption in (CCUG)106 flies. Finally, we tested two chemical compounds that have shown therapeutic potential in DM1 models. While treatment of (CCUG)106 flies with pentamidine had no effect, treatment with a PKR inhibitor blocked both formation of RNA foci and apoptosis in retinae of (CCUG)106 flies.

Discussion: Our data indicate that expression of expanded (CCUG)DM2 repeats is toxic, causing inappropriate cell death in affected fly eyes. Our Drosophila DM2 model may provide a convenient tool for in vivo drug screening.

Grant Support: NIH NIAMS AR48171, MDA RG 4321, Kleberg Foundation for Genetics to RK; NIH NIGMS GM118330 to AB; NIH NCI P30 CA016672.

P-130

Session 7: Animal Models and Tissue-specific Mechanisms

Mechanistic Insights Into Ventricular Tachyarrhythmias in Myotonic Dystrophy: An Optical Mapping Approach on Muscleblind-Like Compound Knockout Mice

CHOU Chung-Chuan[1,3,*], CHANG Po-Cheng[1,3,*], WEI Yi-Chia[2], SEAH Carol[2] and **LEE Kuang-Yung**[2,3]

1. Department of Cardiology, Chang Gung Memorial Hospital, Linkou, Taiwan
2. Department of Neurology, Chang Gung Memorial Hospital, Keelung, Taiwan
3. College of Medicine, Chang Gung University, Taoyuan, Taiwan

Introduction: Cardiogenic sudden death due to ventricular tachyarrhythmias is one of the major causes of death in myotonic dystrophy (DM) patients. Previous studies on Muscleblind-like (Mbnl) compound knockout (KO) (Mbnl1_/_; Mbnl2+/_) mice revealed DM phenotypes including robust myotonia, conduction block, dilated cardiomyopathy and unexpected sudden death. However, direct evidences of ventricular tachyarrhythmias were lacking and the cationic dynamics underlying this phenotype were not known.

Methods: Mbnl1_/_; Mbnl2+/_ mice were generated by crossing two single Mbnl KO lines. In vivo electrophysiological and optical mapping studies were performed.

Results: These Mbnl1_/_; Mbnl2+/_ mice were more vulnerable to programmed electrical pacing and developed atrial tachycardia and/or atrioventricular block (9/10) that were not observed in wild-type (WT) mice (0/14). They also showed pacing-induced ventricular tachyarrhythmias (9/10), more frequent than WT mice (1/14). Prolonged action potential duration, slower conduction velocity, and steeper conduction velocity restitution curves were found in the KO mice. Spatially discordant alternans was also more easily inducible in the KOs, compared to the WT mice. However, calcium dynamics were not changed.

Discussion: We found prolonged action potential duration and slow conduction velocity that lead to vulnerability of spatially discordant alternans and ventricular arrhythmia induction to pacing. Since no alterations of calcium dynamics were observed in the mutants, further exploration of the dynamic nature of INa will be promising.

Grant Support: Chang Gung Medical Research Grant, Keelung (CMRPG2D0181 and CMRPG2F0281 to Lee) and Linkou (CMRPG3F035 to Chou; CMRPG3D034 to Chang).

P-131

Session 7: Animal Models and Tissue-specific Mechanisms

Assessment of a Decoy-based Gene Therapy in DM1 Mice

MATLOKA Magdalena[1], ARANDEL Ludovic[1], FERRY Arnaud[1], KLEIN Aranaud[1], RAU Frédérique[1], NEY Michel[1], SERGEANT Nicolas[2], FURLING Denis[1]

1. Sorbonne Universités UPMC, Inserm Centre de Recherche en Myologie, Institut de Myologie, Paris, France
2. Inserm UMR837-1, Univ. Lille Nord de France, J-P Aubert Research Center, Alzheimer & Tauopathies, Lille, France

Introduction: Deleterious interactions of RNA binding factors with expanded CUG repeats lead to functional loss of MBNL1 resulting in alternative splicing misregulations and ultimately, to DM1 symptoms. Correction of disease-associated phenotypes such as splicing defects and myotonia by antisense oligonucleotides that either degrade mutant transcripts or interfere with CUGexp repeats act through the release of sequestered MBNL1 from CUGexp-RNA. Here we assessed in vivo a decoy-based gene therapy to restore functional levels of MBNL1 in DM1 mice and inhibit CUGexp-RNA toxicity.

Methods: Intramuscular injection of adeno-associated viral (AAV) vectors expressing truncated MBNLΔ polypeptide in WT and HSALR mice expressing 250 CTG repeats in skeletal muscles.

Results: Previous in vitro experiments have shown that the MBNLΔ tool (lacking splicing activity but keeping RNA binding property) can act as a decoy to release sequestered endogenous MBNL1 from CUGexp-RNA and correct splicing defects in DM1 muscle cells. To assess its efficacy in vivo, MBNLΔ construct was cloned into AAV vectors allowing an efficient transduction of skeletal muscles along with a long-term expression of the transgene. Injection of AAV-GFP-MBNLΔ into muscles of WT mice has shown no sign of muscle degeneration. Moreover, the splicing profile of genes abnormally spliced in DM1 was not perturbed. Then, the Gastrocnemius muscles of HSALR mice displaying both splicing defects and myotonia were injected with the same dose of AAV-GFP-MBNLΔ as WT mice and contralateral muscles were injected with saline. Seven weeks after treatment, myotonia was abolished and splicing defects of Clcn1, Serca1 and Mbnl1 genes were corrected in treated muscles. One year after a single injection, GFP-MBNLΔ still colocalizes with nuclear CUGexp-RNA foci and the correction of disease-associated defects is maintained.

Discussion: We propose that our MBNLΔ gene therapy approach could represent an alternate or complementary therapeutic strategy for DM1.

Grant Support: AFMTelethon-Institut de Myologie, ANR.

P-132

Session 7: Animal Models and Tissue-specific Mechanisms

CRISPR/Cas9 Overcomes the Challenges of Dmpk Knockin Development

OLIVEIRA Ruan[1], THOMAS James[1], IVANKOVIC Franjo[1], SWANSON Maurice S[1]

1. Department of Molecular Genetics and Microbiology, Center for NeuroGenetics, University of Florida Gainesville, FL.

Introduction: DM1 is caused by DMPK 3' UTR CTG expansions (CTGexp). In unaffected individuals, this locus contains 5-37 CTG repeats but these repeats expand into the thousands in DM1. Transcription of the expanded DMPK allele produces toxic RNAs that sequester MBNL proteins, disrupting the RNA processing of developmentally regulated genes. DMPK transgenic mice and Mbnl knockout models present many key manifestations of DM1, but no model to date has recapitulated the entire spectrum of disease symptoms, hindering the development of multisystemic therapeutic strategies. Our hypothesis is that improved DM1 modeling can be achieved by inserting large CTGexp into the endogenous mouse Dmpk gene, leading to expression of toxic transcripts with the correct Dmpk spatiotemporal distribution. Until recently, development of such a model has been hindered by the instability of targeting vectors carrying large expansions in bacterial strains and the small repeat numbers capable of generating viable mouse ES cells for blastocyst injections.

Methods: CRISPR/Cas9 components, together with recombination templates generated by rolling circle amplification, were injected into C57BL/6J mouse zygotes. Founders were genotyped by Southern blot and Dmpk expression was analyzed by RT-PCR and qPCR. RNA-FISH was performed to detect RNA foci in several tissues.

Results: CRISPR/Cas9 was successful in generating Dmpk knockin models of several repeat sizes. Heterozygous animals expressed similar levels of mutant and wild-type Dmpk transcripts. RNA foci are present in several tissues and recapitulate the Dmpk spatiotemporal distribution.

Discussion: Our strategy overcame technical limitations underlying the development of Dmpk knockin models carrying large CTGexp and these mice will contribute to our understanding of DM1 and provide a platform for therapeutic development.

Grant Support: This study was supported by grants from the NIH (NS058901, NS98819) and the MDA (RG 480539) to M.S.S.

P-133

Session 7: Animal Models and Tissue-specific Mechanisms

CRISPR-mediated Mis-splicing of Scn5a in Mice Reproduces Conduction Abnormalities Consistent with Myotonic Dystrophy

PANG Paul[1], ALSINA Katherina[1], KOUSHIK Amrita[1], PALAU Roberta[1], WEHRENS Xander[1], COOPER Thomas[1]

1. Baylor College of Medicine

Introduction: Cardiac abnormalities including arrhythmia and conduction delays are the second leading cause of mortality in patients with myotonic dystrophy type 1 (DM1). Despite the significant role of cardiac defects in DM1 patients, its cause remains largely unknown. Recent studies have shown that the cardiac voltage-gated sodium channel gene SCN5A (sodium voltage-gated channel alpha subunit 5) undergoes a different alternative splicing pattern in DM1 patients compared to the general population – a shift from the expression of the adult exon 6B to fetal exon 6A. This shift changes the expression of its gene product from the adult voltage-gated sodium channel type 1.5 (Nav1.5) isoform to its fetal Nav1.5 isoform with different electrophysiological properties. We hypothesize that this change contributes to arrhythmias and conduction delays in DM1. With the aim of determining the effects of increased fetal Nav1.5 expression, we created a CRISPR knockout of the Scn5a adult exon 6B in FVB mice, thereby shifting the expression toward fetal exon 6A.

Methods: Surface electrocardiography (ECG), echocardiography, and intracardiac electrophysiology (EP) studies were performed on homozygous (n=6) and heterozygous (n=8) knockout mice in addition to wild type littermates (n=8) at 2-3 months old.

Results: ECG showed decreased heart rate (p=0.0006) with prolonged QRS (p=0.0078) and PR (p=0.0196) intervals in the knockout mice compared to wild type. EP studies revealed prolonged sinus node recovery time (SNRT, p=0.0017) and atrioventricular effective refractory period (AVERP, p=0.0186) in addition to arrhythmias including sinus pause and premature atrial contractions. No significant differences were found in the echocardiogram. At 6 months of age, homozygous and heterozygous knockouts demonstrated exacerbated phenotypes.

Discussion: Our findings suggest that the change in Scn5a splicing pattern significantly contributes to heart dysfunction and in an age-dependent manner.

Grant Support: #MDA276796, R01HL045565, R01AR060733.

P-134

Session 7: Animal Models and Tissue-specific Mechanisms

Investigating the Molecular Basis of Sleep Dysregulation in Myotonic Dystrophy

PINTO Belinda[1], XU Fangke[2], ALLADA Ravi[2], WANG Eric[1]

1. Molecular Genetics and Microbiology Department, University of Florida
2. Northwestern University

Introduction: Myotonic Dystrophy Type 1 (DM1) is a neuromuscular disorder caused by expansion of CTG repeats in the 3' UTR of the DMPK gene. In addition to muscle symptoms, DM1 patients show a range of CNS phenotypes including hypersomnolence and sleep dysregulation. As regulation of the sleep/wake rhythms is closely linked to the circadian clock, we are investigating whether the clock is disrupted in DM1.

Methods: To address this, we are developing a Drosophila model of DM1 where we specifically express expanded CTG repeats in a subset of circadian clock neurons, the small and large lateral ventral neurons (s and l LNvs).

Results and Discussion: We find that LNv-specific expression of 270 CTG repeats, but not 19 CTG repeats, results in < 24 hour rhythm of locomotor activity in adult flies as well as loss of these neurons. Interestingly, the LNvs appear to be particularly sensitive to the CTG expansion, as cell-specific expression of these repeats does not have adverse effects on other neurons that regulate sleep such as the Dorsal Neurons, DN1, and the Mushroom Body. These data suggest that the DM1 mutation specifically disrupts the circadian clock. We are extending these studies by examining the LNvs expressing CTG repeats for cellular hallmarks of DM1. We are using FISH to examine these neurons for the presence of nuclear RNA foci that contain the expanded CTG transcripts and immunostaining analyses to determine the localization of the Muscleblind (Mbl) splicing factor, which is sequestered by the RNA foci in DM1. We are complementing these studies by assessing whether the mammalian circadian clock is disrupted in DM1 mouse models. We are examining these mice for defects in circadian rhythms through locomotor activity analysis and analysis of gene expression and neuronal activity rhythms in the circadian pacemaker, the Suprachiasmatic Nucleus. Our studies will provide critical insights into the molecular basis of the sleep disorders that plague DM1 patients.

Grant Support: NIH grant DP5OD017865.

P-135

Session 7: Animal Models and Tissue-specific Mechanisms

Tissue Specific Expression of Expanded CUG Repeat RNA to Investigate the Cardiac Pathogenesis of Myotonic Dystrophy Type 1

RAO Ashish N[1], KOLONIN Arseniy[2], COOPER Thomas A[1,2,3]

1. Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX, USA
2. Department of Pathology and Immunology, Baylor College of Medicine, Houston, TX, USA
3. Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA

Introduction: Cardiac involvement is a prominent feature of myotonic dystrophy type 1 (DM1) and affects up to 80% of DM1 patients. DM1 associated cardiac presentations are complex and consist of conduction defects, arrhythmias, compromised contractile function, histological and structural abnormalities. The molecular mechanisms leading to the cardiac manifestations in DM1 have not yet been defined. Additionally, there is a lack of well characterized mouse models that reproduce most of the cardiac features of the disease. A well characterized model will provide an experimental system to determine the mechanisms that contribute to the cardiac phenotype in DM1, and for testing potential therapeutic avenues for reversal of disease features. The goal of this project is to establish a DM1 mouse model based on the expression of expanded CUG repeat (CUGexp) RNA in the heart.

Methods: We generated a model that utilizes a bitransgenic system for cardiomyocyte specific and tetracycline responsive expression of 960 CUG repeats in the context of the DMPK 3'UTR. Preliminary characterization of conduction parameters was performed at a 2-month time point using electrocardiography (ECG). Cardiac RNA was examined for alternative splicing events which are misregulated in DM1 by RT-PCR analysis.

Results: ECG analysis revealed conduction defects - prolongation of QRS and QT intervals in the CUGexp RNA expressing mice in comparison to controls. Defects in splicing patterns were observed for several of the tested events in the CUGexp RNA expressing mice.

Discussion: These results demonstrate that our model reproduces the conduction abnormalities and splicing defects observed in DM1 patients. Future studies will complete characterization of this model for DM1 associated phenotypic and molecular manifestations and identification of the molecular basis for the pathogenic effects of the CUGexp RNA in heart.

Grant Support: This work is funded by the Muscular Dystrophy Association (#MDA 276796) and NIH grants R01HL045565, R01AR45653 and R01AR060733.

P-136

Session 7: Animal Models and Tissue-specific Mechanisms

Regulation of Tau Exon 10 Alternative Splicing by MBNL and Temperature: Implication in Myotonic Dystrophy

SERGEANT Nicolas[1], PETRY Franck[2,3], PAPON Marie-Amélie[2,3], POITRAS Isabelle[2,3], MORIN Françoise[2,3], NOEL Anastasia[2,3], BUÉE Luc[1] and PLANEL Emmanuel[2,3]

1. Univ. Lille, Inserm, CHU Lille, UMR-S 1172 - JPArc - Centre de Recherche Jean-Pierre AUBERT Neurosciences et Cancer, F-59000 Lille, France
2. Centre de recherche du Centre Hospitalier de l'Université Laval, Axe Neurosciences, P-9800, Québec, QC, Canada
3. Université Laval, Faculté de Médecine, Département de Psychiatrie et Neurosciences, Québec, QC, Canada

Introduction: Tau is a neuronal microtubule-associated protein that stabilize, organize microtubules mediated by its microtubule-binding (MT) repeats consisting of 3 or 4 motifs. Alternative splicing of tau exon 10 leads the expression of tau with either four (4R-tau) or three (3R-tau) MT motifs, respectively. During human brain development, 3R-tau are expressed from embryonic stages and 4R-tau expression begins after birth. An equal ratio of 3R and 4R-tau is expressed in adult human brain. Mis-splicing of Tau with an increased 3R-Tau expression is observed in myotonic dystrophies. Interestingly, transition from 3R to 4R is observed in rodents and is coincident with the regulation of body temperature. Thus, our preliminary data on body pups temperature show that pups are hypothermic during the first developmental stages, correlating with the expression of 3R-tau isoforms expression and 4R-tau expression appear with thermoregulation.

Methods: The purpose of this study is to analyze the regulation of exon 10 splicing and the mechanisms involved in the differential expression of 3R and 4R-tau in mice post-natal development. Tau exon 10 splicing and regulatory factors were analyzed from birth to post-natal 30. Moreover, the influence of temperature on tau exon 10 splicing was analyzed in mouse primary neuronal culture cells (PNC) as well as N2a mouse neuroblastoma which express both 3R and 4R tau. To determine whether the temperature sensitive splicing mechanism is preserved in human, N2a cell were transfected with a human tau exon 10 minigenes. MBNL1 and MBNL2 which are major splicing regulators of tau were analyzed.

Results: Our results show that 3R-tau mRNA is reexpressed in hypothermic animals. Moreover, hypothermia promoted tau exon 10 exclusion whereas hyperthermia promoted its inclusion in in primary neuronal culture cells and N2a cells transfected the human tau exon 10 minigene.

Discussion: Together, our results show for the first time a post-natal physiological regulation of Tau splicing resulting in the expression of protein isoforms which differentially promote the dynamic stability and architecture of microtubules. Moreover, this process is associated with modification of MBNL expression.

Grant Support: This work was support by Inserm, ANR THERADM (to NS and LB) and Alzheimer Society of Canada (to M.G. and F.R.P.) and grants to E.P. from the Canadian Institute of Health Research (MOP-106423, PCN-102993), Fonds de Recherche en Santé du Québec (16205, 20048) and the Natural Sciences and Engineering Research Council of Canada (354722).

P-137

Session 7: Animal Models and Tissue-specific Mechanisms

Comparative Analysis of Alternative Splicing and Expanded CUG-repeats in HSALR Mice and DM1 Patients

TANNER Matthew[1,2], CARRELL Sam[1,2], THORNTON Charles[1]

1. University of Rochester Medical Center
2. University of Rochester Medical Scientist Training Program

Introduction: Overexpression is used to model dominantly-inherited diseases in mice, at the risk of causing extraneous phenotypes. Transgene expression in HSALR mice is reported as > 1000-fold higher than expression of endogenous Dmpk (Gudde, 2016).

Methods: RNAseq was performed on quadriceps muscle of HSALR and wild-type mice (4 per group). Differential expression and alternative splicing were analyzed using DESeq2 and rMATS, respectively. Mouse splicing was compared to targeted RNAseq in DM1 patients. Levels of expanded CUG repeat (CUGexp) RNA in mice and DM1 patients were determined by cDNA slot blots.

Results: HSALR mice exhibit alternative splicing changes in ~750 cassette exons (PSI difference > 10%, adjusted $p < 0.05$) and expression changes in ~850 genes (> 2-fold difference, adj. $p < 0.05$). As reported in DM1, mis-spliced exons in HSALR mice exhibit enrichment of sequence motifs characteristic of Mbnl regulation. HSALR transgene expression is about 40-fold higher than endogenous Dmpk by RNAseq, although cDNA slot blots show that steady-state CUGexp levels are only 5- to 8-fold higher than in DM1 patients. In 10 splice events that are highly conserved in Mbnl regulation between human and mouse muscle, HSALR mice exhibit 62% of maximal splicing derangement observed in DM. DM1 patients with similar overall splicing derangement have lost ~58% of predicted strength, whereas previous studies have shown that HSALR mice only lose 30-40% of hindlimb muscle strength (Moyer, 2010).

Discussion: HSALR mice have widespread disruption of muscle gene expression and alternative splicing. They express CUGexp RNA at higher levels than DM1 patients, but in our hands this overexpression is not as extreme as previously reported. Despite high expression of CUGexp RNA, the severity of splicing defects in HSALR mice corresponds to that of only moderately-affected DM1 patients, indicating relative preservation of Mbnl function in the face of higher CUGexp accumulation.

Grant Support: NIH T32 GM007356, U54NS048843, and R37NS094393.

P-138

Session 7: Animal Models and Tissue-specific Mechanisms

Natural Xanthines Rescue Myotonic Dystrophy-like Phenotypes in Drosophila and Increase MBNL Expression Levels

LLAMUSI Beatriz[1,2,3], PASCUAL-GILABERT Marta[4], CHAKRABORTY Mouli[1,2,3], MIGUEL David[4], FERNÁNDEZ Carme[4], MIRANDA Francisca[4], FUENTES Javier[4], ARTERO Rubén[1,2,3]

1. Translational Genomics Group, Incliva Health Research Institute, Valencia, Spain.
2. Department of Genetics and Interdisciplinary Research Structure for Biotechnology and Biomedicine (ERI BIOTECMED), University of Valencia, Valencia, Spain.
3. CIPF-INCLIVA joint unit, Valencia, Spain
4. IUCT (Inkemia IUCT group), Mollet del Vallès, Barcelona, Spain

Introduction: Muscle mass wasting is one of the most debilitating symptoms of myotonic dystrophy type 1 (DM1), ultimately leading to immobility, respiratory defects, dysarthria, dysphagia and death in advanced stages of the disease. Malignant heart arrhythmias constitute an additional medical concern. Since the majority of current drugs ultimately derive from natural compounds here we sought to discover natural products able to improve DM1-like phenotypes in a Drosophila model of the disease.

Methods: INSR:luc spliceosensor flies were used to screen natural compounds for an increase in the reporter activity. Initial hits were validated in additional Drosophila phenotypes and in patient-derived fibroblasts.

Results: Starting from the observation that caffeine, one of the original hits of the Drosophila screen, promoted disperse MBNL1 protein expression in the nuclei of DM1 fibroblasts, we tested in cells the ability of additional xanthines to similarly increase MBNL1 levels. Among other combinations, mixtures of caffeine and theobromine (C/T) increased significantly (around 1.5-2 fold) expression of MBNL1 at the transcription level. Fluorescence polarization assays suggested that caffeine was unable to directly bind to CUG repeats, which suggests that these compounds do not inhibit MBNL1 sequestration but act at additional levels of the pathogenesis pathway. In the Drosophila DM1 model expressing 480 CTG repeats, C/T mixtures rescued impaired climbing and flight ability, and strongly improved heart dysfunction phenotypes, when CTG expansions were targeted to Drosophila cardiomyocytes. Further phenotypic assessments such as fly survival and missplicing of MBNL-dependent events are ongoing and will be reported at the meeting.

Discussion: Our data support a desired effect of a C/T mixtures on DM1 phenotypes in disease models. These effects stem, at least partially, from enhancement of MBNL1 expression levels.

Grant Support: This project was supported by the Spanish MINECO through the CDTI grant IDI-20151100.

P-139

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Cardiac Troponin T Skeletal Muscle Expression in Myotonic Dystrophy Type 1 and Type 2 Patients: A Possible Biomarker of Cardiac Dysfunctions

BOSÈ Francesca[1], RENNA Laura Valentina[1], FERRARI Nicola[1], ARPA Giovanni[2], FOSSATI Barbara[2], MEOLA Giovanni[3], CARDANI Rosanna[1]

1. Laboratory of Muscle Histopathology and Molecular Biology, IRCCS Policlinico San Donato, Milan, Italy
2. Department of Neurology, IRCCS Policlinico San Donato, Milan, Italy
3. Department of Neurology, IRCCS Policlinico San Donato, Milan, Italy, Department of Biomedical Sciences for Health, University of Milan, Milan, Italy

Introduction: Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are characterized by aberrant alternative splicing of different genes that have been linked to the multiorgan involvement. Alteration of cardiac Troponin T (cTnT) splicing observed in DM patients leads to the co-expression of both adult (excluding-exon 5) and fetal (including-exon 5) isoform of the protein in cardiac tissue that is considered one cause of cardiac dysfunctions in DM. Recently, cTnT aberrant splicing pattern has been observed in skeletal muscle of DM patients. The aim of this work is to investigate if skeletal muscle cTnT fetal expression is related to cardiac dysfunctions in DM patients.

Methods: Bicheps Brachii (BB) and Tibialis Anterior (TA) muscle biopsies of DM1, DM2 and healthy subjects (CTR) were collected and RNA was extracted; RT-PCR analysis was performed using primers flanking cTNT exon 5. DM1 and DM2 patients were divided in two subgroups on the basis of cardiac evaluation performed by ECG, echocardiogram and ECG-Holter. Histopathological analysis of muscle damage was also performed.

Results: A significantly higher expression of cTnT fetal isoform was observed in DM1 and DM2 skeletal muscle compared to CTR. Moreover, cTnT fetal expression was higher in DM1 and DM2 BB muscles and in DM1 TA muscles from patients with cardiac involvement (presenting at least one out of four altered parameters: augmented PR, QRS, QTc, reduced %FE) than in muscles of patients without cardiac abnormalities and of healthy subjects. A significant correlation has been found between cTnT fetal isoform expression and clinical parameters of cardiac involvement, whereas no correlation was detected between fetal expression of cTnT and histopathological parameters of muscle damage.

Discussion: cTnT fetal isoform expression in skeletal muscle of DM patients seems to be related to cardiac dysfunctions and not to skeletal muscle histopathological alterations.

Grant Support: FMM-Fondazione Malattie Miotoniche.

P-140

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

A Cross Sectional Clinical Outcome Measures Validation Optimistic Study

DE ANTONIO Marie[1], WENNINGER Stephan[2], DAIDJ Ferroudja[1], TREWEEK Shaun[3], VAN ENGELEN Baziel[4], SCHOSER Benedikt[2], **BASSEZ Guillaume**[1], OPTIMISTIC Consortium

1. Myology Institute, Paris, France
2. Friedrich-Baur-Institut, Munich, Germany
3. University of Aberdeen, UK
4. Radboud University, Nijmegen, The Netherlands.

Introduction: The heterogeneity of symptoms experienced by Myotonic dystrophy type 1 (DM1) patients creates particular challenges in generating valid outcome measures. Previous studies mostly used generic measures of quality of life or restricted disease domains and recommendations stressed the need for methodologically appropriate measures. Recently disease-specific patient-reported outcome measures have been developed, including the DM1-Activ scale and the Myotonic Dystrophy Health Index (MDHI). However both instruments have been assessed separately and validation studies are still limited.

Methods: We have taken advantage of the Optimistic European trial to conduct a validation study of main outcomes used for DM1 in a large multicenter (n=255) cohort. Here we present baseline validation data focusing on the distribution of data and concurrent validity of DM1-Activ and MDHI measurements.

Results: Participants' demographic and medical characteristics were similar across the four clinical sites as well as social participation (DM1 Activ), disease burden (MDHI), walking capacities (6MWT), pain (McGill pain) and fatigue (FDSS) scores. The number of observations was sufficiently large to address the distribution of outcome measures. A normal distribution was observed for DM1 Activ values, MDHI-total scores, and 6MWT distances, while pain questionnaire values were not normally distributed. Interestingly, we observed that both DM1 Activ and MDHI-data correlated with most other independent self-reported and objective measures.

Discussion: We substantiated during the Optimistic international trial a validation study of outcome assessments used for DM1. The results support the validity of both DM1-Activ and MDHI self-reported measures for use in adult patients with DM1.

Grant Support: European Community's Seventh Framework Programme (FP7).

P-141

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Quantifying Mutant RNAs Aggregates In Myotonic Dystrophy Human And Mouse Tissues

REVILLOD Lucile[1], DOGAN Céline[1], M. DE ANTONIO Marie[1], GOURDON Genevieve[2], FURLING Denis[1], PUYMIRAT Jac[3], **BASSEZ Guillaume**[1]

1. Institute of Myology - UM76, Université Paris, Inserm, CNRS, AIM, Paris
2. Inserm U781, Institut Imagine, Paris, France
3. CHUQ-C.H.U.L. Department of Genetics, Quebec, Canada

Introduction: Nuclear retention of CUG and CCUG expanded transcripts, called “foci”, is a hallmark of myotonic dystrophy type 1 (DM1) and type 2 (DM2) respectively. While pathophysiological consequences of these aggregates are better understood, rare studies focused on tissues anatomical and temporal distribution of foci and their relationships with DNA repeats expansion size and phenotype severity.

Methods: We set up an automated fluorescent in situ hybridization (FISH) protocol to specifically assess DM1 and DM2 foci. The frequency of foci, number of foci per nucleus, and foci area, were quantified in various skeletal muscles and other tissues in the DM-SXL mouse model and human muscle biopsies. We studied the impact of (1) expanded DMPK gene level, by comparing heterozygous to homozygous mice, (2) CTG expansion length, in mice carrying either 500 CTG or >1000 CTG, and (3) age, in 3-month-old vs 6-month-old mice, on the formation of foci. In addition, the mutant RNA aggregation load in DM1 and DM2 biopsies was compared with patients age and muscular impairment.

Results: We show that the frequency of nuclei containing foci, the number of foci per nucleus and the foci area correlated with expanded DMPK gene expression level, the CTG repeat expansion size but did not differed at two age points. The level of correlation did vary, depending on tissue types, the highest correlation being observed in muscle derivatives i.e. skeletal, smooth, and cardiac muscles. In human, the frequency of foci, number of foci/nucleus and foci area were higher in DM2 than in DM1 samples. As in DMSXL mice, a correlation between foci and leukocytes CTG repeat expansion size was observed. Moreover, in DM1 patients, but not in DM2, we show that the frequency of foci correlates with age and muscle impairment scores, suggesting that such aggregates could exemplify part of the muscular weakness progression.

Discussion: Quantifying the foci load could represent a powerful approach (1) for tissue monitoring in both preclinical and clinical trials aiming at reducing expanded mutant transcripts. (2) to estimate the duration and severity of the disease in relevant tissue.

Grant Support: This study is supported by AFM-Telethon.

P- 142

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

The UK Myotonic Dystrophy Patient Registry: Facilitating and Accelerating Clinical Research

CAMMISH Phillip[1], WOOD Libby[1], CORDTS Isabell[1,2], ATALAIA Antonio[1], MARINI-BETTOLO Chiara[1], MADDISON Paul[3], PHILLIPS Margaret[4], ROBERTS Mark[5], ROGERS Mark[6], HAMMANS Simon[7], STRAUB Volker[1], PETTY Richard[8], ORRELL Richard[9], MONCKTON Darren G[10], NIKOLENKO Nikoletta[1], JIMENEZ-MORENO Aura Cecilia[1], THOMPSON Rachel[1], HILTON-JONES David[11], TURNER Chris[12], LOCHMÜLLER Hanns[1]

1. John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
2. Department of Neurology, RWTH Aachen University, Aachen, Germany
3. Department of Neurology, Queen's Medical Centre, Nottingham, UK
4. Department of Rehabilitation Medicine, Derby Teaching Hospitals NHS Foundation Trust, Derby, UK
5. Department of Neurology, Salford Royal NHS Foundation Trust, Salford, UK
6. Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK
7. Wessex Neurological Centre, University Hospital of Southampton, Southampton, UK
8. Department of Neurology, NHS Greater Glasgow and Clyde, Southern General Hospital, Glasgow, UK
9. Department of Neurology, Royal Free Hospital, London, UK
10. Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, Scotland, UK
11. Department of Clinical Neurology, John Radcliffe Hospital, Oxford, UK
12. UCL MRC Centre for Neuromuscular Diseases, Institute of Neurology, London, UK

Introduction: Myotonic dystrophy type 1 (DM1) is the most frequent muscular dystrophy worldwide with complex, multi-systemic, and progressively worsening symptoms. There is currently no treatment for this inherited disorder and research can be challenging due to the rarity and variability of the disease.

Methods: The UK Myotonic Dystrophy Patient Registry is a patient self-enrolling online database collecting clinical and genetic information. For this cross-sectional "snapshot" analysis, 556 patients with a confirmed diagnosis of DM1 registered between May 2012 and July 2016 were included. An almost even distribution was seen between genders and a broad range of ages was present from 8 months to 78 years, with the largest proportion between 30 and 59 years.

Results: The two most frequent symptoms were fatigue and myotonia, reported by 79 and 78% of patients, respectively. The severity of myotonia correlated with the severity of fatigue as well as mobility impairment, and dysphagia occurred mostly in patients also reporting myotonia. Men reported significantly more frequent severe myotonia, whereas severe fatigue was more frequently reported by women. Cardiac abnormalities were diagnosed in 48% of patients and more than one-third of them needed a cardiac implant. Fifteen percent of patients used a non-invasive ventilation and cataracts were removed in 26% of patients, 65% of which before the age of 50 years.

Discussion: The registry's primary aim was to facilitate and accelerate clinical research. However, these data also allow us to formulate questions for hypothesis-driven research that may lead to improvements in care and treatment.

Grant Support: The registry is funded by grants from Muscular Dystrophy UK and Myotonic Dystrophy Support Group. Additional funding has been received from the OPTIMISTIC under the European Commission's Seventh Framework Programme (FP7/2007-2013) Grant agreement No. 305697, the Marigold Foundation, the National Institute of Health Research (NIHR) Rare Disease Translational Research Collaboration (RD-TRC), and Wyck Foundation.

P-143

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

The International DM-Scope Registry: Harmonizing Myotonic Dystrophy Data Collection

DOGAN Celine[1], DE ANTONIO Marie[1], HAMROUN Dalil[2], GAGNON Cynthia[3], PUYMIRAT Jack[4], BASSEZ Guillaume[1]

1. Myology Institute, Paris, France
2. CHU de Montpellier, France
3. Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Jonquière Canada
4. Laboratoire de Génétique, CHU de Laval, Québec, Canada

Introduction: In myotonic dystrophy type 1 (DM1) and type 2 (DM2), the variability of disease onset and the multisystemic involvement pose challenges for both care management and the design of clinical trials. Registries have been demonstrated as powerful tools to promote translational research in rare diseases. While several DM registries have been created, the low level of harmonization of datasets limits the use of registries in comparative epidemiology and the enrolment of patients in multicentre international trial.

Methods: We planned to revise the dataset of the DM-Scope registry, a database specifically dedicated to myotonic dystrophies, aiming at demonstrating the feasibility of standardizing the data collection of DM patients, for international use.

Results: As part of the binational, Quebec and France, iDM-Scope registry consortium project, we revised and updated the dataset. The dataset contains clinical and demographic data collected in a standardized manner during medical visits of DM patients in expert neuromuscular centres in Quebec and France. The respective sections include: CTG expansion size, clinical history, clinical neuromuscular and systemic clinical evaluation, professional status and social consequences. Recent outcome assessments have been included, based on validity results for use in patients with DM1. Data are updated prospectively during annual follow-up visits.

Discussion: The transnational iDM-Scope registry revised dataset and standardized procedures show promising evidence that uniform data collection can take place in different countries. Data harmonization may greatly facilitate the comparison of diseased populations between countries, health care planning, and the recruitment of selected patients in trials.

Grant Support: AFM Telethon

P-144

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Test-retest Reliability of Strength and Function Measures Across Multiple Sites in Individuals with Myotonic Dystrophy Type 1 (DM1)

EICHINGER Katy[1], DEKDEBRUN Jeanne[1], DILEK Nuran[1], CURRENCE Melissa[1], CHING Willie[2], DUONG Tina[3,4], GEE Richard[4], HERBELIN Laura[4], JOE Galen[2], KING Wendy[3], LOTT Donovan[5], PANDYA Shree[6], Myotonic Dystrophy Clinical Research Network (DMCRN)[1]

1. Department of Neurology, University of Rochester Medical Center, Rochester, NY
2. Department of Neurology, University of Kansas Medical Center, Kansas City, KS
3. Rehabilitation Medicine Department, Clinical Center, National Institutes of Health Bethesda, MD
4. Department of Neurology, Stanford University, Palo Alto, CA
5. Department of Neurology, The Ohio State University Wexner Medical Center, Columbus, OH
6. Department of Physical Therapy, University of Florida, Gainesville, FL

Introduction: Development of potential treatments for DM1 has created a need to examine the feasibility and reliability of motor outcome measures in multicenter studies.

Methods: Clinical evaluators from 6 sites participated in training sessions to standardize procedures for strength and function assessments. Ambulatory individuals with DM1 onset after age 11 were recruited to participate in a 1 year natural history study. Evaluations included assessments of strength (quantitative muscle testing (QMT) and manual muscle testing (MMT)) and function (6 Minute Walk Test (6MWT), 30 foot go, and time to ascend 4 stairs) at baseline, 3 months and 12 months. Test-retest reliability of data from the baseline versus 3 month study visit was determined using the intraclass correlation coefficient (ICC).

Results: Six primary evaluators completed training prior to enrollment of patients. As needed, the primary evaluators were responsible for training additional evaluators at their site. Twelve evaluators tested patients throughout the entire study. 106 individuals with DM1 completed baseline and 3 month visits. The key measurements demonstrated excellent test-retest reliability. The ICCs (range across sites) were: overall QMT 0.93 (0.80 to 0.97), overall MMT (0.95), 6MWT 0.92 (0.82-.99), 30 foot go 0.92 (0.78-0.98), time to ascend 4 stairs 0.90 (0.85-0.97).

Discussion: This study demonstrates the feasibility of using a comprehensive panel of outcome measures to obtain consistent evaluation of motor impairment in DM1. With in-person training, practice prior to patient testing, and consistency of the evaluator, it is possible to achieve excellent test-retest reliability in a multicenter study.

Grant Support: Muscular Dystrophy Association, Myotonic Dystrophy Foundation, National Institutes of Health Grant Number NS048843, Marigold Foundation, and Biogen

P-145

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Development of a Disease Severity Index for Myotonic Dystrophy type 1 (DSI-DM1)

GAGNON Cynthia[1], CAMPBELL Craig, ROGERS Mark, BASSEZ Guillaume, VENANCE Shannon, TURNER Chris, PHILIPS Margaret, MATHIEU Jean

1. Faculté de médecine et des sciences de la santé, Université de Sherbrooke/Groupe de recherche interdisciplinaire sur les maladies neuromusculaires/Centre de recherché Charles-Le-Moyne

Introduction: Myotonic dystrophy type 1 (DM1) is a complex multisystemic neuromuscular disease affecting all major systems with large intra and inter-individual variability. To determine the overall disease severity is therefore a real challenge for both researchers and clinicians. This project aimed to develop a health care provider scored disease severity index specifically designed for DM1 (DSI-DM1).

Methods: The DSI-DM1 was developed through literature review and Delphi process among international DM1 experts as part of the OMMYD initiative. Before developing the index, experts determined that it should be able to: (1) quantify disease severity on a continuous scale; (2) quantify changes in severity over long periods of time; (3) discriminate clinically relevant disease subsets with unique characteristics; (4) provide prognostic information relative to morbidity and mortality; (5) be easily used in clinical practice.

Results: The final version of the DSI-DM1 includes 26 items divided into 9 domains (central nervous, visual, respiratory, cardiovascular, urinary, digestive, muscular, motor function, and metabolic/endocrine). The index can be administered to the adult population with the adult or late-onset phenotype by the treating neurologist/geneticist. A pilot implementation will be conducted in the fall 2018 among several clinics.

Discussion: This index will be useful for the longitudinal follow-up of patients, outcome measurement and categorizing patients for clinical trials. DM1 clinical presentation is highly variable and the index will serve to better characterize patients in relation to their multisystemic involvement.

Grant Support: Association Française contre les myopathies

P-146

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Muscle MRI Measures Are Abnormal Early in Disease and Track Disease Progression: Support for Use as Biomarker in Clinical Trials

GUTMANN Laurie[1], SHIELDS Richard[1], THEDENS Dan[1], DEVOLDER Ian[1], PHETTEPLACE Janel[1], NOPOULOS Peg[1]

1. University of Iowa

Introduction: Gene therapy holds the promise for major impact on DM1. Urgently needed for these trials is a biomarker that: 1) is disease-specific and clinically relevant, and 2) and tracks disease progression. The overall aim of this study is to evaluate measures of skeletal muscle structure using Magnetic Resonance Imaging (MRI).

Methods: In this pilot sample, we evaluated 13 male adult-onset DM1 subject and 12 male healthy controls, age equivalent. Disease duration (DD) and muscle impairment rating scale (MIRS) were obtained. Disease duration ranged widely from 3 subjects who were presymptomatic to a subject with over 20 years of disease progression, and the mean DD was 8.28 years. A 3T MRI was used to acquire standardized calf images. Muscle volume was derived using T1 images. T2 relaxometry was used to assess skeletal microstructural changes with higher values indicating greater abnormality. Fat fraction (FF) quantification was performed using 3 point Dixon acquisition with higher values indicating more atrophy.

Results: The DM1 subjects showed decreased volume, higher T2, and higher FF in all muscles in the calf compared to controls. However in this small sample, this only reached significance with the T2 in the gastrocnemius. Additionally, T2 in the gastrocnemius was very strongly associated with disease duration (Pearson $r = 0.66$, $p = 0.01$), supporting the notion that this measure tracks disease progression. Finally, gastrocnemius T2 was highly correlated with MIRS scores (Pearson $r = 0.64$, $p =<0.001$) indicating a strong relationship between T2 and muscle dysfunction.

Discussion: Muscle MRI measures show promise as biomarkers for clinical trials. The most sensitive measure appears to be T2 which shows abnormality early in the course of the disease, tracks with disease progression, and is directly related to muscle function.

Grant Support: NINDS R01-NS094387

P-147

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

A Randomized, Placebo Controlled, Clinical Efficacy Trial of Mexiletine for Myotonic Dystrophy Type 1 (DM1)

HEATWOLE Chad[1], LUEBBE Elizabeth[1], ROSERO Spencer[1], EICHINGER Katy[1], MARTENS William[1], HILBERT James[1], DEKDEBRUN Jeanne[1], DILEK Nuran[1], JOHNSON Nicholas[2], TAWIL Rabi[1], ZAREBA Wojciech[1], MCDERMOTT Michael[1], THORNTON Charles[1], MOXLEY Richard III[1]

1. The University of Rochester, Rochester, NY
2. The University of Utah, Salt Lake City, UT

Introduction: Mexiletine is FDA approved for cardiac arrhythmias. Previous short-term studies have shown that mexiletine can safely improve myotonia in DM1. However, mexiletine's longterm effects on DM1 myotonia, ambulation, cardiac arrhythmias, clinical function, pain, and disease-burden have not been studied.

Methods: We evaluated the efficacy, tolerability, and safety of mexiletine in DM1 by performing a randomized, double-blind, placebo-controlled trial of mexiletine (150 mg three times daily) in ambulatory DM1 patients. The primary outcome measure was change in six minute walk distance over 6 months. Secondary outcomes included: myotonia, muscle strength, clinical function, swallowing, forced vital capacity testing, blood tests, lean muscle mass, Myotonic Dystrophy Health Index scores, and 24-hour Holter and EKGs results at 3 and 6 months.

Results: Forty-two DM1 participants (age 21 to 64, 69% female) were randomized to placebo or mexiletine. Forty participants completed the 6 month trial; two participants discontinued the study prematurely due to personal reasons. Two participants discontinued study medication but completed the study: one due to a >4 fold increase in PVCs by Holter monitoring and one secondary to a transient episode of dysarthria, blurred vision, and muffled hearing. One participant's dosing was reduced due to abdominal pain. Two serious adverse events occurred: one stroke and one cervical fracture due to a fall from a zip line. The study is complete. The determination of the treatment arm of these participants and a full data analysis is forthcoming.

Discussion: Mexiletine is a promising therapy for DM1 patients. Prior to widespread use in DM1, this therapy should be shown to be both safe and beneficial. Full results from this study will provide additional scientific data regarding the clinical use of this agent in the DM1 population and will be presented at the meeting.

Grant Support: FDA (R01FD003716), NIH, the Saunders, Abrams, and Nathan and Ruth Goldberg Memorial Funds

P-148

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Getting Ready for Clinical Trials in Myotonic Dystrophy Type 1 with the Validation of Functional Capacity Outcome Measures

JIMENEZ-MORENO Aura Cecilia[1], NIKOLENKO Nikoletta[1], MASSEY Charlotte[2], MOAT Dionne[1,3], KIERKERGAARD Marie[4], NEWMAN Jane[5], WOOD Libby[1], ATALAIA Antonio[1,3], TURNER Chris[2], LOCHMULLER Hanns[1,3]

1. John Walton Muscular Dystrophy Research Centre, Institute of Genetic Medicine, Newcastle University, Newcastle upon Tyne, UK
2. University College London MRC Centre for Neuromuscular Diseases, Institute of Neurology, London, UK
3. Newcastle upon Tyne Hospitals Foundation Trust, Newcastle upon Tyne, UK
4. Department of Neurobiology, Care Sciences and Society, Division of Physiotherapy, Karolinska Institute, Stockholm, Sweden
5. Wellcome Trust Centre for Mitochondrial Research, Newcastle University, Newcastle upon Tyne, UK

Introduction: The international initiative Outcome Measures in Myotonic Dystrophy type 1 (OMMYD) proposed a set of Functional Capacity Outcome Measures (FCOM) identified as significant when assessing physical capacity in DM1.

Methods: A cohort of 212 DM1 adults (49% males) currently enrolled at the multicenter natural history study of PHENO-DM1 in the UK have been assessed at baseline performing all of the proposed FCOM: (1) Six-Minute Walk Test (6MWT), (2) 10-Meter Walk Test, (3) 10-Meter Walk/Run Test, and (4) 30-Second Sit Stand Test, 2 to 4 were tested by three trials when possible. Additional clinical information to complete phenotype characterization was collected.

Results: Preliminary results showed the following: 12% of participants were wheelchair users in daily life activities and 19% used an assistive device (i.e. cane, crutches or walker) when performing ambulatory assessments. 204 patients completed the 6MWT with an average distance of 415m (SD+149). All participants performed at least one trial of the 10-mWt with an average of 9.9 secs (SD+4.4). 201 patients performed the 10-mWRt at an average of 6.1 secs (SD+3.4). Between 60%-73% of participants were able to perform three trials of each test. All repeated tests showed a significant mean change ($p<0.05$) from the first trial to the second but not from the second to the third. All tests showed a strong correlation with lower limb strength (r 0.42-0.52) and with grip strength (r 0.33-0.54). Known risk factors in this population such as fatigue, falls and pain are possible hazards when performing any of these tests.

Discussion: This data supports the validity of the FCOM described and establishes references values for future clinical trials. Follow-up results will allow further analysis and conclusions of progression to be made.

Grant Support: This study has received funding from: (1) the NIHR Rare Disease Translational Research Collaboration and The Wyck Foundation and (2) OPTIMISTIC grant agreement (305697) from the EU Seventh Framework Programme (FP7/2007-2013).

P-149

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Patient Reported Outcome Measure Patient Preferences in Trials Studying Myotonic Dystrophy Type 1 (PROMPTS DM1)

HEATWOLE Chad[1], JOHNSON Nicholas[2], DEKDEBRUN Jeanne[1], DILEK Nuran[1], EICHINGER Kate[1], HILBERT James[1], LUEBBE Elizabeth[1], MARTENS William[1], MCDERMOTT Michael[1,3], THORNTON Charles[1], MOXLEY Richard III[1]

1. The University of Rochester Medical Center, Department of Neurology, Rochester, NY
2. The University of Utah Medical Center, Department of Neurology, Salt Lake City, UT
3. The University of Rochester Medical Center, Department of Biostatistics and Computational Biology, Rochester, NY

Introduction: In preparation for clinical trials in myotonic dystrophy type-1 (DM1), it is important to develop and identify patient-reported outcome measures that are clinically relevant, reliable, valid, responsive, and well tolerated by patients. The selection of optimal clinical trial outcome measures can potentially better enable researchers to identify beneficial therapeutics in DM1 while lessening burden on DM1 clinical study participants.

Methods: Fifty-two DM1 patients enrolled in two clinical studies completed the Myotonic Dystrophy Health Index (MDHI), SF-36v2, Individualized Neuromuscular Quality of Life questionnaire (INQoL), and a questionnaire comparing the relevance, usability, overall preference, and perceived responsiveness of each measure. The associations between instrument scores and physical function, genetic test results, and employment status were examined. We used Spearman correlation coefficients to quantify the associations between the SF-6D, MDHI total score, and INQoL percentage score and select strength/functional scores (MMT score, six minute walk distance, and MIRS score) as well as CTG repeat length.

Results: The MDHI was preferred over the INQoL in 13/13 areas ($p \leq 0.05$) and preferred over the SF-36v2 in 12/13 areas ($p \leq 0.05$) with similar preference in the remaining area. The MDHI score produced the only score that was associated with participant employment status, CTG repeat length, and the three measurements of clinical function.

Discussion: This study provides insight into how DM1 study participants view three patient reported outcome measures. The MDHI correlates well with physical function and is viewed favorably by participants in DM1 clinical studies.

Grant Support: Support for this research was provided by the Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (NINDS U54NS48843), The National Institute of Arthritis and Musculoskeletal and Skin Diseases (NIAMS K23 AR055947) (NIAMS U01AR065119), The Food and Drug Administration (R01 FD003716), The Goldberg Nathan Foundation for Myotonic Dystrophy Type-2 Research, The Saunders Family Fund, and the Abrams Family Fund for Myotonic Dystrophy Research. The project described in this publication was supported by the University of Rochester CTSA award number UL1 TR002001 from the National Center for Advancing Translational Sciences of the National Institutes of Health. Special acknowledgement goes to all of the patients and medical staff who made this study possible.

P-150

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Development of Standard Operating Procedures to Assess Physical Functioning in People with Myotonic Dystrophy Type 1

KIERKEGAARD Marie[1]

1. Karolinska University Hospital, Karolinska Institutet

Introduction: The international initiative OMMYD was initiated 2011 with the aim to select the best available outcome measures to be used in research and clinical trials. Members of the Functional Capacity Outcome Measures (FCOM) group had reached consensus on four key tests to assess physical functioning, i.e. the Six-Minute Walk Test, the 10-Meter Walk Test, the 30-Second Chair Stand Test and the Nine-Hole Peg Test. There was, however, a need to develop standard operating procedures (SOPs) for these tests.

Methods: The SOPs were developed using consensus-building methodology over a period of 18 months. Following a first discussion at the OMMYD-3 meeting in 2015, the SOPs were revised based on published administration manuals and guidelines, the literature, and suggestions from the FCOM group. After repeated web-survey consultations within the FCOM group, the SOPs were further reviewed during the MDF physiotherapy meeting in September 2016. The 10-Meter Walk/Run Test was added after this meeting. A larger international Delphi consultation among DM1 experts was then performed. Taking in consideration comments and suggestions from this Delphi survey, a final version of the SOPs was compiled.

Results: The final SOPs included detailed information on description, equipment, preparation, administration and scoring of each of the five physical functioning tests. To facilitate the use of the tests, scoring sheets and an appendix including a CR-10 rating scale were also provided. The SOPs have been added as a supplement to the workshop report of the OMMYD-3 which has been submitted for publication in the Neuromuscular Disorders.

Discussion: The provision of SOPs ensures standardization of administration of tests to ascertain valid and reliable results, and facilitates and improves realization of multi-center research. Thanks to a successful international collaboration, detailed SOPs are now available for five physical functioning tests.

Grant Support: REPAR-FRQS Canada; AFM France.

P-151

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Identification of Exosomal Muscle-Specific miRNAs in Serum of Myotonic Dystrophy Patients Relating to Muscle Disease Progress

KOUTSOULIDOU Andrie[1], PHOTIADES Marinos[1], KYRIAKIDES Tassos[2], GEORGIOU Kristia[1], PROKOPI Marianna[3,4], KAPNISIS Konstantinos[4], ŁUSAKOWSKA Anna[5], NEARCHOU Marianna[6], CHRISTOU Yiolanda[7], PAPADIMAS George[8], ANAYIOTOS Andreas[3], KYRIAKOU Kyriakos[6], KARARIZOU Evangelia[8], ZAMBA PAPANICOLAOU Eleni[7], PHYLACTOU Leonidas[1]

1. Dept. of Molecular Genetics, Function & Therapy, Cyprus Institute of Neurology & Genetics, 1683 Nicosia, Cyprus
2. Yale Center for Analytical Sciences, Yale School of Public Health, New Haven, CT, U.S.A.
3. Dept. of Mechanical Engineering and Materials Science and Engineering, Cyprus University of Technology, Limassol, Cyprus
4. Theramir Ltd, Limassol, Cyprus
5. Dept. of Neurology, Medical University of Warsaw, Poland
6. Dept. of Electron Microscopy/Molecular Pathology, The Cyprus Institute of Neurology and Genetics, Nicosia 1683, Cyprus
7. Neurology Clinic D, Cyprus Institute of Neurology & Genetics, 1683 Nicosia, Cyprus
8. Dept. of Neurology, Eginitio hospital, Medical School of Athens, Greece

Introduction: DM1 is characterised by progressive muscle wasting and the discovery of reliable blood-based biomarkers could be useful for the disease progress monitoring. There have been some reports showing that the presence of specific miRNAs in blood correlate with DM1. In one of these, our group identified four muscle-specific miRNAs, miR-1, miR-133a, miR-133b and miR-206, which correlated with the progression of muscle wasting in DM1 patients. The levels of the four muscle-specific miRNAs were elevated in the serum of DM1 patients compared to healthy participants and were also elevated in the serum of progressive muscle wasting DM1 patients compared to disease-stable patients. This work aimed to characterise the ontology of these four muscle-specific miRNAs in the blood of DM1 patients.

Methods: Exosomes were isolated from serum samples of DM1 patients and characterised using scanning and transmission electron microscopy and western blot analysis. Total RNA, including miRNAs, was extracted from exosomes and the levels of the four muscle-specific miRNAs were determined using Real-Time PCR.

Results: We show that the four muscle-specific miRNAs are encapsulated within exosomes isolated from DM1 patients. Our results show for the first time, the presence of miRNAs encapsulated within exosomes in blood circulation of DM1 patients. More interestingly, the levels of the four exosomal muscle-specific miRNAs are associated with the progression of muscle wasting in DM1 patients. Further experiments show that the four muscle-specific miRNAs are possibly bound to proteins within exosomes in DM1.

Discussion: We propose that exosomal muscle-specific miRNAs may be useful molecular biomarkers for monitoring the progress of muscle wasting in DM1 patients. There has been a growing interest regarding the clinical applications of exosomes and their role in prognosis and therapy of various diseases and the above results contribute towards this way.

Grant Support: AFM-Telethon [Research grant number 18484] and A.G. Leventis Foundation.

P-152

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Upper and Lower Extremity Magnetic Resonance Imaging Correlates with Function and Strength in People with Myotonic Dystrophy Type 1

LOTT Donovan J[1], COOKE Korey D[1], PARK Hyun J[1], FORBES Sean C[1], TRIPLETT William T[1], WALTER Glenn A[1], VANDENBORNE Krista[1], ASHIZAWA Tetsuo[2], SUBRAMONY Sankarasubramoney H[1]

1. University of Florida, Gainesville, FL
2. Houston Methodist, Houston, TX

Introduction: While magnetic resonance imaging (MRI) has proven to be an excellent means for assessing skeletal muscle pathology in several patient populations, only a few initial MRI studies have been conducted in skeletal muscle for Myotonic Dystrophy Type 1 (DM1). The purpose of this pilot study was to examine muscle pathology of both the lower leg and forearm in DM1 using MRI and to determine the relationship of these MRI measures with function and strength.

Methods: MRI of the lower leg (n=18) and forearm (n=6) musculature was performed on adults with DM1 using a 3T whole-body MRI scanner for both quantitative T2 and 3Point Dixon images. We calculated T2 and fat fraction (FF) from the tibialis anterior (TA), flexor digitorum profundus (FDP), and flexor digitorum superficialis muscles (FDS). Functional abilities were assessed with the 30 foot go, time to ascend 4 steps, the six-minute walk test (6MWT), video hand opening time, and the Upper Extremity Functional Index (UEFI) questionnaire. Quantitative muscle testing was performed for the dorsiflexors (DF), grip, long grip, and pinch to assess strength.

Results: We found considerable heterogeneity for both T2 and FF in the TA, FDP, and FDS muscles of those with DM1. Fair to excellent correlations were found for the lower leg MRI measures with strength and function [FF being most strongly correlated with time to ascend 4 steps (r=0.62) and T2 being most strongly related to DF strength (r=-0.75 to -0.80) and distance walked for 6MWT (r=-0.66)]. For the forearm, FF of the FDP demonstrated the highest overall correlations with strength and function (r=-0.77 to -0.94). An excellent correlation was also noted between FF of the FDS and the UEFI (r=-0.94).

Discussion: These initial findings demonstrate the clinical meaningfulness of MRI measures in DM1. Further work should demonstrate their potential as an endpoint/outcome for future clinical trials investigating new therapies for DM1.

Grant Support: Wyck Foundation/Myotonic Dystrophy Foundation grant, University of Florida Clinical Translational Science Institute

P-153

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Quantifying Ankle-Dorsiflexion Myotonia With A Portable Device In Ambulatory Individuals With Myotonic Dystrophy Type 1: A 1-Year Follow Up Study

SHIMELLIS Hirity[1], GUTH Amanda[1], NNAMDI-EMERATOM Chioma[1], WANG Wenli[2], BULEA Thomas[3], VIPPA Tarun[1], GRUNSEICH Christopher[1], CHING Willie[3], CAPRON Kieu[3], JOE Galen[3], THORNTON Charles A[2], **MANKODI Ami**[1]

1. Neurogenetics Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD
2. Department of Neurology-CNDD, University of Rochester, NY
3. Rehabilitation Medicine, Clinical Center, National Institutes of Health, Bethesda, MD

Introduction: Myotonia may function as a physiological biomarker to rapidly assess drug effects in clinical trials of myotonic dystrophy type 1 (DM1). However, the optimal method to assess myotonia in multicenter trials is unknown. Ankle dorsiflexors (ADF) are affected early and prominently in DM1.

Methods: ADF torque during maximum voluntary isometric contraction followed by relaxation was measured with a portable device (IntelliStretch, RehabTek). Subjects completed 3 trials per leg with at least 2 minutes of rest between trials. The peak torque (Nm) and the time required for isometric force to relax from 90% to 5% of peak force (s) (RT) were determined. Reliability between measurements was assessed by Bland Altman plots. Correlations between these measures, QMT strength and CLCN1 splicing were examined in tibialis anterior muscle, a primary effector of the ADF.

Results: Ambulatory 22 DM1 subjects participating in a multicenter biomarker study (DMCRN) completed evaluations at baseline, 3 months and 1 year. At baseline, the RT was > 1 s in 11 subjects (historic controls < 1 s). Two subjects who demonstrated toe and ankle extension myotonia had the RT > 5 s. Data from age and gender-matched healthy controls will be presented. Mean difference between the ADF torque and the RT at baseline and 3-month visits were 0.3 Nm (SD 1.9) and 0.12 s (SD 0.75), respectively. From available data on the same muscle (n=14), we found a weak correlation between delayed RT and CLCN1 splicing abnormality predicting the loss of functional protein (Pearson r=0.57, p=0.03).

Discussion: It is feasible to measure the physiological biomarker of abnormal relaxation in the ADF muscles of DM1 subjects. Preliminary analysis demonstrate that the ankle device may provide reliable motor clinical endpoints of muscle strength and RT in frequently affected ADF of DM1 subjects. Further studies in a larger cohort are needed to define the relation between RT and splicing abnormalities.

Grant Support: The Intramural Research Program of the National Institute of Neurological Disorders and Stroke and the National Institutes of Health Clinical Center NS048843 and NS093177 (CAT).

P-154

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Magnetic Resonance Imaging of Leg Muscles in Patients with Myotonic Dystrophies

PERIC Stojan[1], MAKSIMOVIC Ruzica[2], BANKO Bojan[2], DURDIC Milica[2], BJELICA Bogdan[1], BOZOVIC Ivo[1], BALCIK Yunus[1], PESOVIC Jovan[3], SAVIC-PAVICEVIC Dusanka[3], RAKOCEVIC-STOJANOVIC Vidosava[1]

1. Neurology Clinic, Clinical Centre of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia
2. Centre for Radiology and Magnetic Resonance Imaging, Clinical Centre of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia
3. Centre for Human Molecular Genetics, Faculty of Biology, University of Belgrade, Belgrade, Serbia

Introduction: Magnetic resonance imaging (MRI) of muscles has recently become a significant diagnostic procedure in neuromuscular disorders. There is a lack of musle MRI studies in patients with myotonic dystrophy type 1 (DM1), and especially type 2 (DM2). Aim was to analyze fatty infiltration of leg muscles using 3.0 T MRI in patients with genetically confirmed DM1 and DM2 with different disease duration.

Methods: The study comprised 21 DM1 and 10 DM2 adult patients. Muscle MRI was performed in axial and coronal planes of the lower limbs using the following sequences: T1-weighted (T1w), T2-weighted (T2w), proton-density weighted (PDw), and 3-point Dixon. Five-point scale by Mercuri et al. was used.

Results: Fatty infiltration registered in at least one muscle of lower extremities was found in 71% of DM1 and 40% of DM2 patients. In DM1 patients, early involvement of the medial head of gastrocnemius and tibialis anterior muscles was observed with later involvement of other lower leg muscles and of anterior and posterior thigh compartments with relative sparing of the rectus femoris. In DM2, majority of patients had normal MRI findings. Early involvement of lower legs and posterior thighs was found in some patients. Less severe involvement of the medial head of the gastrocnemius compared to other lower leg muscles and less severe involvement of the rectus femoris, gracillis and adductor longus compared to other thigh muscles was another peculiarity.

Discussion: We described characteristic pattern and way of progression of muscle involvement in DM1 and DM2.

Grant Support: This study was supported by the Ministry of Education, Science and Technological Development of Serbia granted to V.R.S. (grant# 175083).

P-155

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Prospective Measurement of Quality of Life in Myotonic Dystrophy Type 1

PERIC Stojan[1], HEATWOLE Chad[2], DUROVIC Ervin[1], KACAR Aleksandra[1], NIKOLIC Ana[1], BASTA Ivana[1], MARJANOVIC Ana[1], STEVIC Zorica[1], LAVRNIC Dragana[1], RAKOCEVIC STOJANOVIC Vidosava[1]

1. Neurology Clinic, Clinical Center of Serbia, School of Medicine, University of Belgrade, Belgrade, Serbia
2. University of Rochester Medical Center, Rochester, NY, USA

Introduction: Generic patient reported outcome measures have had varied success in tracking QoL in myotonic dystrophy type 1 (DM1). The aim was to analyze changes of Individualized Neuromuscular Quality of Life questionnaire (INQoL) scores in clinic patients with DM1 over a six-year period.

Methods: Patients completed the INQoL at baseline and after a six year period through their attendance in a neurology outpatient clinic. Severity of muscular involvement in DM1 was analyzed using the kMuscular Impairment Rating Scale (MIRS).

Results: Ninety-nine DM1 patients completed a baseline visit. Sixty-seven of these patients were retested at an interval time. The overall INQoL score improved in our sample of patients ($p<0.05$) as did the following subscales: myotonia ($p<0.05$), pain ($p<0.05$), activities ($p<0.01$), social relationships ($p<0.01$) and body image ($p<0.05$). No changes were observed for the independence and emotions scales. There were no differences in mean change of INQoL scores between patients with worsened MIRS and those with no change in MIRS scale after follow-up ($p>0.05$).

Discussion: INQoL scores improved in our cohort of DM1 patients during a six-year period despite progression of muscle weakness. This must be better understood before the selection of the instrument for use in trials to measure therapeutic benefit in DM1 patients.

Grant Support: This study was supported by the Ministry of Education, Science and Technological Development of Serbia granted to V.R.S. (grant# 175083).

P-156

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Impeding Transcription of Expanded Microsatellite Repeats by Deactivated Cas9

PINTO, Belinda[1], SAXENA Tanvi[2], MENDEZ-GOMEZ Héctor[2], XIA Guangbin[3], DENES Lance[1], OLIVEIRA Ruan[1], SWANSON Maurice[1], WANG Eric[1,3]

1. Department of Molecular Genetics & Microbiology University of Florida
2. Department of Neurology Univeristy of Florida
3. Center for NeuroGenetics University of Florida

Introduction: Myotonic dystrophy (DM) is a multi-systemic neuromuscular disorder in which expanded CTG or CCTG repeats are transcribed, leading to a number of downstream pathogenic events. In particular, the repeat-containing toxic RNAs sequester the Muscleblind (MBNL) family of proteins, forming RNA foci and disrupting global splicing and RNA localization. Multiple studies have shown that silencing toxic RNA successfully inhibits DM pathology. Here, we describe studies to develop the CRISPR/Cas9 system as tool to block transcription of microsatellite repeat expansions in DM.

Methods: Deactivated Cas9 (dCas9) has been used to impair transcription in prokaryotes via gene body targeting but a similar approach has been ineffective in eukaryotes. We hypothesized that expanded microsatellite repeats may be uniquely sensitive to transcriptional blockade by dCas9.

Results and Discussion: Using a HeLa cell culture, plasmid-based approach coupled with deep sequencing, we observed repeat length-, PAM-, and strand-dependent reduction in the abundance of CTG and CCTG repeat-containing RNAs upon targeting dCas9 directly to repeat sequences. Consequently, RNA foci abundance and production of Repeat Associated Non-ATG (RAN) peptides characteristic of DM cells were drastically decreased. To apply this approach in vivo, we generated adeno-associated virus (AAV) expressing dCas9 and repeat-targeting guide RNAs (gRNAs). Transduction of muscle fibers from the HSALR DM1 mouse model reduced RNA foci. Treatment of patient-derived DM1 myoblasts with this AAV rescued aberrant MBNL-dependent splicing patterns. Currently, we are testing this approach in HSALR mice by performing temporal vein injections in P0 mice and examining them for reduction in myotonia, reduction in RNA foci and restoration of splicing patterns in skeletal muscle. Our data suggest that transcriptional inhibition of microsatellite repeats by dCas9 is a potentially viable therapeutic strategy for DM and other repeat expansion diseases.

Grant Support: NIH DP5 OD017865.

P-157

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

The New Zealand Myotonic Dystrophy Registry - Introduction of the Recommended Expanded Dataset

RODRIGUES Miriam[1,2], ROXBURGH Richard[1,3]

1. Neurology, Auckland District Health Board, New Zealand
2. Muscular Dystrophy Association of New Zealand, Auckland, New Zealand
3. Centre for Brain Research, University of Auckland, New Zealand

Introduction: The New Zealand Neuromuscular Disease Registry has been recruiting for 5 years. It aims to enable people with neuromuscular disease, including myotonic dytsrophy (DM) to participate in research.

Methods: Referrals to the registry can come from any source; self-referral, doctor or patient support organisations. Written consent is sought from all participants. This year the Registry has moved to collecting an expanded range of recommended clinical items. Importantly, patient self-report is used for some of these. The IT solution, generously provided by the Government of Western Australia, is a platform shared with the Australian DM Registry, the Rare Disease Framework (RDRF).

Results: 1019 people with over 70 different diagnoses have enrolled in the NZ NMD Registry. 18% of these have myotonic dystrophy - the largest cohort of any disorder included in the Registry. 170 DM1 patients and 11 DM2 patients are enrolled. 80% of DM1 and 100% of DM2 patients have molecular confirmation of their diagnosis. 15 (9%) have completed the patient questionnaire to date. The Registry was the largest single source of ascertainment for a nationwide population-based prevalence study. This indicated that about half of all myotonic dystrophy patients are enrolled in the Registry. The Registry has also been used to recruit patients to the landmark OCT study which first discovered that epiretinal membranes are a feature of myotonic dystrophy and to a study comparing swallowing impairment in myotonic dystrophy to that in stroke.

Discussion: An effective registry vital for myotonic dystrophy patients to take part in research and the promise of clinical trials makes increased enrolment a key priority. Changing to the collection of an internationally agreed expanded dataset enhanced with self-reported data also ensures trial readiness.

Grant Support: The NZ NMD Registry is funded by MDANZ through its research trust.

P-158

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Non-invasive Magnetic Stimulation of the Primary Motor Cortex in Type 1 Myotonic Dystrophy with a New Wearable Device

SIMPSON Ericka[1], THONHOFF Jason[1], JOHN Blessy[1], ROSENFIELD David[1], HELEKAR Santosh[1]

1. Department of Neurology, Methodist Neurological Institute, Houston Methodist Hospital, Houston, Texas

Introduction: Symptomatic treatment for muscle impairment in type 1 Myotonic Dystrophy (DM1) focuses upon improving muscle strength and reducing hyper-excitability of muscles. Since repeated neuromuscular electrical stimulation (NMES) provides some benefit in this domain, we explore the utility of non-invasive repetitive transcranial magnetic stimulation of the primary motor cortex. In our ongoing study, we used a newly developed portable wearable multifocal magnetic brain stimulator [Transcranial Rotating Permanent Magnet Stimulator (TRPMS)] to ascertain whether we can improve muscle function in DM1 patients.

Methods: This study is a small pilot randomized within-patient placebo-controlled double blind clinical trial. Our stimulation protocol involved delivery of 100 ms oscillatory stimulus pulses every 5 s for 40 min to the primary motor cortical strip on one randomly chosen side, and similar but sham (placebo) treatment pulses on the opposite side, all for five days per week for two weeks. We employed anatomical and functional magnetic resonance imaging to determine three sites of stimulation, 3 cm apart along the primary motor cortex on each side. We clinically assessed muscle strength using the Medical Research Council (MRC) scale and hand grip dynamometer. Our electromyographic assessment included measuring the compound muscle action potential amplitude with repetitive nerve stimulation in the first dorsal interosseous and trapezius muscles, before and after a short exercise test. All clinical evaluations and tests were performed bilaterally immediately before and after treatment, and also at one week, one month and six months follow-up time points. Six adult DM1 patients (aged 45.8 ± 11.8 years, 1 male, 5 females) have completed this two-week treatment.

Results: All patients tolerated the treatment well without any adverse effects. We will discuss results obtained from evaluating the efficacy of actual TRPMS treatment compared to sham treatment within each patient.

Discussion: This pilot study provides valuable initial data on the feasibility, safety and potential usefulness of repetitive multifocal TRPMS therapy in DM1.

Grant Support: This study was supported by Shanna and Andrew Linbeck Family Charitable Trust.

P-159

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

A Wireless Motion Capture System in Myotonic Dystrophy Type-1

STATLAND Jeffrey M[1], HUISINGA Jessie[1], BRUETSCH Adam[1], JENCI Nicole[1], PAULTER Mary[2], TRUJILLO Carenina[2], JOHNSON Nicholas[2]

1. University of Kansas Medical Center
2. University of Utah

Introduction: The development of candidate molecularly-targeted therapies for myotonic dystrophy type 1 (DM1) creates a need for patient-relevant outcome measures. Measures of dynamic motion while performing functional motor tasks may be more sensitive than traditional timed functional measures. We used a commercially available portable wireless motion analysis system (APDM Mobility Lab) to instrument a timed up and go (iTUG) in patients with DM1.

Methods: We performed a cross-sectional study of 19 genetically confirmed ambulatory adult participants with DM1 at the University of Kansas Medical Center (KUMC) and the University of Utah. For the iTUG, participants wear 6 synchronized wireless sensors while performing a standard 7m timed up and go test. Manual muscle testing on 18 bilateral muscles was performed (MMT, KUMC only), and participants were separated based on the Muscular Impairment Rating Scale (MIRS). Temporal and spatial gait parameters from the iTUG were compared to a commercial normative data base (APDM) and across DM1 groups.

Results: Participants were independently ambulatory (26 – 69 years of age, mean 45.9) and moderately affected (mean MIRS 3.1). Compared to normative data, participants with DM1- performed the task more slowly, with shorter stride length and slower stride velocity, and demonstrated postural instability during turning and turn to sit portions of the iTUG. The postural component of changes in gait was most sensitive to overall muscle impairment, with subjects with moderate to severe impairment (MIRS 4-5) taking 1.7 more steps per turn ($p=0.01$). Decreasing stride velocity, increasing turn time, and number of steps when turning was associated with decreased combined MMT score ($r='0.75\text{-}0.78'$).

Discussion: Participants with DM1 showed abnormalities in temporal and spatial gait parameters and evidence of postural instability using iTUG.

Grant Support: NCATS # KL2TR000119, Biogen, and the National MS Society RG 4914A1/2.

P-160

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Current Status of the Myotonic Dystrophy Registry of Japan

TAKAHASHI Masanori[1,2], TAKADA Hiroto[3], KURU Satoshi[4], NAKAMORI Masayuki[2], MATSUMURA Tsuyoshi[5], KIMURA En[6]

1. Department of Functional Diagnostic Science, Osaka University Graduate School of Medicine
2. Department of Neurology, Osaka University Graduate School of Medicine
3. Department of Neurology, National Hospital Organization Aomori National Hospital
4. Department of Neurology, National Hospital Organization Suzuka National Hospital
5. Department of Neurology, National Hospital Organization Toneyama National Hospital
6. Translational Medical Center, National Center for Neurology and Psychiatry, Japan

Introduction: Patient registries provide epidemiological data and facilitate the recruitment of patients eligible for clinical trials. The myotonic dystrophy (DM) registry of Japan was launched in October 2014 as a division of Remudy (www.remudy.jp) by a nationwide collaboration. The collected data are in harmony with the core dataset for the international registry proposed at the TREAT-NMD/Marigold Workshop. Although this is a patient-initiated registry, genetic diagnosis is a prerequisite, and clinical information provided by the patients should be verified by their physicians for accuracy.

Methods: To identify clinical questions for future research and to develop standards of care for DM, the DM population registered with the national registry was analyzed in a cross-sectional study.

Results: Of 602 patients registered as of March 2017, adult-onset DM1 comprised 86% and congenital form 13%, and DM2 only one case. Males constituted 50% and the mean age of patients was 41.9 years. While 57% of patients were ambulatory, 17% were non-ambulatory. On ECG, either PR > 240 ms or QRS > 120 ms was observed in 27% of patients, but a pacemaker or an implantable cardioverter defibrillator was utilized only in 1.7% of patients. Mechanical ventilation was used for 17% of patients. Glucose intolerance was detected in 24% of patients, and the most commonly used class of medication was DPP-4 inhibitors.

Discussion: A broad range of patients have enrolled in the registry which provides valuable data for clinical research. A registry that accumulates accurate clinical data is a powerful tool for the development of standards of care and for the measurement of the clinical outcome. Furthermore, it can foster communication between researchers and patients by providing information about clinical trials, research, and health care.

Grant Support: Partly supported by grants from the Japan Agency for Medical Research and Development (17ek0109259), the Ministry of Health and Welfare of Japan (H28-Nachitou(Nan)-Ippan-030)

P-161

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

GABA Receptor Antagonists for Hypersomnia and Cognition in DM1 – Case Study

MCCONNELL Ona[1], **WANG Eric**[1], BASSELL Gary[2], RYE David[2]

1. University of Florida
2. Emory University

Introduction: Myotonic Dystrophy (DM) patients are affected by hypersomnia and impaired cognition. In previous studies, it has been observed that GABAA receptor antagonists have been effective to treat patients with a related condition, idiopathic hypersomnia (IH). Interestingly, in one of these studies, an IH patient responsive to a GABAA receptor antagonist was later diagnosed with DM1, suggesting a shared pathology. This abstract describes the first-hand experience of a DM1 patient receiving a GABAA receptor antagonist, and effects on CNS symptoms in DM1.

Methods: The subject received a referral from their neurologist to the Emory Sleep Clinic because of constant daytime sleepiness and “brain fog”. The subject participated in an NIH-funded sleep study to measure sleep latency, REM frequency, and other parameters to characterize hypersomnia. The data showed that the subject exhibited typical symptoms of hypersomnia without sleep apnea, restless leg syndrome, etc. A skin biopsy, CSF and blood were collected. The subject was prescribed a topical and sublingual GABAA receptor antagonist. A psychomotor vigilance test and neuropsychological testing were performed while on and off the treatment.

Results: Following administration of this treatment for a week, the subject improved on several objective and subjective metrics, stating increased wakefulness and alertness, and decreased “brain fog”. The subject perceived improved ability to process information, with objective measurements by the symbol digit modalities test. The subject still complained of having extreme difficulty waking up in the morning, and speculated that some lozenges were more effective than others.

Discussion: This treatment has dramatically changed quality of life for the subject, but there are improvements that could be made. The half-life of the drug is short, therefore it must be taken throughout the day, and does not help with waking up in the morning. The supply is limited, as only 2 pharmacies in the US compound it; in addition, its current high price and lack of insurance coverage may preclude it from being widely accessible in this patient population.

Grant Support: None.

P-162

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Stanford Neuromuscular Recruitment Database and Tissue Biobank

WELSH Lesly[1], HAGERMAN Katharine A[1], CRASTA Sheela[1], DAY John W[1]

1. Department of Neurology, Stanford University, Palo Alto, CA

Introduction: Myotonic dystrophy (DM) is a rare, multi-systemic neuromuscular condition that affects approximately 1 in 8000 people. The Stanford Neuromuscular Program created both a Recruitment Database and a Biobank to facilitate research opportunities for people with DM and expand the DM research field.

Methods: The database identifies people interested in participating in research by matching their demographics and clinical details with requirements of current studies. The Biobank allows people to donate biological tissues either through research procedures, scheduled surgeries or anatomic donations. Donations are stored and used locally and shared with other academic investigators.

Results: The recruitment database has approximately 1000 participants with various neuromuscular conditions, including 310 DM-affected individuals. Of those, 217 have participated in Stanford research studies such as the DMCRN natural history study, IONIS drug trial, CHRI study of childhood-onset DM1, adult sleep and metabolism studies as well as completing many general and disease-specific questionnaires. The Biobank has collected tissues from 159 individuals with neuromuscular conditions, including 61 with DM, since it began collecting samples in 2014.

Discussion: Over the last 3 years, the database and biobank have been able to support multiple research studies nationally and internationally, and mobilized both the DM community and researchers for DM studies and trials. The design and implementation of the Database and Biobank can be used as a model to help new academic or private institutions develop their research programs. We are eager to continue working with investigators so that the on-going collection of data regarding DM subjects and disease controls in the Stanford Neuromuscular Database, and the specimens collected in the Stanford Neuromuscular Biobank can be of greatest value to the International DM research community.

Grant Support: Myotonic Dystrophy Foundation, Muscular Dystrophy Association, and Marigold Foundation.

P-163

Session 8: Biomarkers, Outcome Measures, Registries and Trial Design

Dysmetria in Myotonic Dystrophy Type 1

YACOUBI Basma[1], CASAMENTO-MORAN Agostina[1], LOTT Donovan[1], SUBRAMONY Subramony H[1], CHRISTOU Evangelos A[1]

1. University of Florida

Introduction: Myotonic dystrophy type 1 (DM1), the most common muscular dystrophy, is an inherited, autosomal dominant disease. Although DM1 is treated as a muscle disease, it clearly affects the central nervous system (CNS) with evidence for cerebellar dysfunction. A consequence of cerebellar dysfunction is dysmetria (inaccuracy of goal-directed movements), but it remains unknown whether DM1 increases dysmetria. Therefore, the purpose of this study was to characterize dysmetria and its relation to central and peripheral impairments in DM1.

Methods: Ten individuals diagnosed with DM1 (38.8 ± 12.4 years, 4 women) and 10 age-matched controls (37.6 ± 16.9 years, 4 women) performed 50 trials of fast goal directed movements with ankle dorsiflexion aiming at a spatiotemporal target (9o at 180 ms). We recorded the electromyographic activity of the primary agonist (tibialis anterior; TA) and antagonist (soleus; SOL) muscles and quantified the following: 1) dysmetria: endpoint error in ankle dorsiflexion; 2) central impairments: movement variability and the modulation of the agonist muscle activity in the first 100 ms of the contraction; 3) peripheral impairments: time for muscle relaxation and muscle MRI.

Results: Ankle dysmetria was greater in DM1 patients than healthy controls (28.7 ± 10.4 vs 10.6 ± 2.5 %; $P<0.01$). Dysmetria in DM1 was associated with central impairments. Specifically, dysmetria was correlated with greater variability in the first 100 ms of the movement ($R2=0.8$, $P<0.01$) and greater modulation of the agonist EMG from 10-20 Hz ($R2=0.15$, $P<0.01$). In contrast, dysmetria was not associated with peripheral measurements, such as delayed muscle relaxation ($R2=0.01$, $P>0.4$) or tibialis anterior muscle T2 weight ($R2=0.001$, $P>0.4$).

Discussion: These findings provide novel evidence that DM1 patients exhibit dysmetria relative to healthy controls and that central but not peripheral impairments contribute to these impairments.

Grant Support: None.

Author Index

A

ABDUL RAZAK Kavitha	P-020, **P-075**
ADAM Berit A	S1-01, S5-05
ADAMS Heather	S5-02
AESCHBACH Lorène	P-004
AIASTUI Ana	P-037
AIT-BENICHOU Siham	**P-103**
ALGALARRONDO Vincent	P-099
ALLADA Ravi	P-134
ALMENDROTE Miriam	P-001
ALSAGGAF Rotana	**S3-01**
ALSINA Katherina	P-133
ALVARADO Fernando	P-006
AMCHESLAVKY Alla	P-129
AMR Sania	S3-01
ANASTASIADIS Zacharias	S2-07
ANAYIOTOS Andreas	P-151
ANDERSON Kirstie N	P-081
ANDRÉ Laurène M	S2-09, S6-04
ANGEARD Nathalie	**P-076**
ANTCZAK Jakub	P-039
ANTONINI Giovanni	P-058, P-079, S5-03
ANTOURY Layal	S7-04, **S8-01**
ARANDEL Ludovic	P-016, **P-104**, P-128, P-131, S6-03
ARNAUD Pauline	P-008, P-099, P-100
ARPA Giovanni	P-139
ARTERO Rubén	**S2-01**, P-122, P-124, P-138
ARZOUMANOV Andrey	P-103, P-128
ASHIZAWA Tetsuo	P-013, P-022, P-119, P-152, S2-10
ASLAM Faaiq	S2-07
ASLAM Hina	P-073

Bold indicates presenting author

Author Index

A

ATALAIA Antonio A	P-081, P-142, P-148
ATHERTON John J	P-075
ATTALLA EL ALABIEB Nadia	S5-03
ATTEH George	P-121
AUCLAIR Julie	P-085
AUSEMS Rosanne	**P-105**
AYHAN Fatma	S2-10
AZOTLA-VILCHIS Cuauhtli Nacxitl	P-108

B

BABACIC Haris	P-074
BABUTY Dominique	P-100
BALAJ Leonora	S8-01
BALCIK Yunus	P-154
BALLANTYNE Bob	P-082, S5-05
BALLESTER-LOPEZ Alfonsina	**P-001**
BAMBOROUGH Paul	S2-03
BANACH Marta	**P-039**
BANERJEE Anwesha	**P-121**
BAÑEZ-CORONEL Monica	P-013, S2-10
BANGRU Sushant	S2-04
BANKO Bojan	P-154
BANTSCHEFF Marcus	S2-03
BARAN Timothy	S7-04
BARBÉ Lise	**P-002**, P-107
BARDHI Olgert	S2-07
BARGSTEN Annie	**S6-01**
BAROSS Stephanie	S1-01
BASSELL Gary J	**P-106**, P-109, P-121, P-161, S5-09

Author Index

B

BASSEZ Guillaume	P-008, P-099, P-100, P-101, P-128, **P-140**, **P-141**, P-143, P-145, S4-02, **S8-02**
BASTA Ivana	P-155
BATES Matt	S5-06
BAX Karen	S4-01, S5-02
BAZINET Audrey	P-104, S6-03
BECANE Henri Marc	P-099, S5-10
BEHIN Anthony	P-099, P-100, P-101, S5-10
BENNETT C Frank	S7-01, S7-04, P-103
BERGGREN Kiera N	P-077, **P-078**, S4-01, S5-02
BERGLUND J Andrew	P-029, P-118, P-115, S2-02, P-018, P-126
BERGMANN Andreas	P-129
BERTOLDO Enrico Giuseppe	**P-040**
BERTRAND Alexis	P-008
BEST Ana F	**P-036**
BEUTNER Katherine	P-102
BHAKTA Deepak	S4-04
BIANCHI Federica	P-053
BINDA Anna	**P-009**
BISSET Darren	P-127
BJELICA Bogdan	P-154
BLACK Marrianne	P-083
BOLDUC Clare	P-129
BONNEFONT Jean-Paul	P-008
BOSCHE Markus	S2-03
BOSÈ Francesca	P-009, P-025, P-027, P-031, **P-139**
BOTTA Annalisa	**P-003**, P-009, P-058, S5-03
BOUCHARD Luigi	P-051, S1-02
BOUCHARD Michaël	S1-02

Author Index

B

BOULIER Kristin	P-102
BOUNSANGA Jerry	P-024, P-078, S4-06, S5-02
BOURKE David	P-065
BOYD Roxanna J	S1-01
BOŽIĆ Marija	P-062
BOZOVIC Ivo	P-154
BRAJUŠKOVIĆ Goran	S1-03
BREAKEFIELD Xandra	S8-01
BRIGNOL Tuy-Nga	**P-123**
BRIGONZI Elisa	P-025, P-031, P-040, P-046, P-047
BRKUŠANIN Miloš	P-062, S1-03
BROCK Roland	P-117
BROOK David	P-113, P-114, S2-03
BRUETSCH Adam	P-159
BUBENIK Jodi L	P-013, S2-10
BUCCI Elisabetta	P-058, P-079, S5-03
BUCMA Tatjana	P-087
BUDIS Jaroslav	P-007
BUÉE Luc	P-136
BUELOW Aaron	P-095
BUJ-BELLO Ana	P-112
BULEA Thomas	P-153
BURGOCI Vasile	S2-06
BURRAGE Matthew K	P-075
BUSKES Melanie	P-091
BUTTERFIELD Russell J	P-024, P-078

Author Index

C

CALAFIORE Silvia	P-079
CALLUS Edward	P-040
CALVELLO Mariarosaria	P-067
CAMMISH Phillip	**P-142**
CAMPBELL Craig	P-024, **P-077**, P-078, P-145, **S4-01**, S4-06, S5-02
CANO Antoni	P-001
CAPPELLA Marisa	S8-03
CAPRON Kieu	P-153
CARDANI Rosanna	P-009, **P-010**, P-025, P-027, P-031, P-040, P-098, P-139, S8-03
CARDINALI Beatrice	S8-03
CARRELL Ellie	**P-011**, S7-03
CARRELL Sam	P-137, **S7-01**
CASADEVALL Teresa	P-001
CASALI Carlo	P-058
CASAMENTO-MORAN Agostina	P-012, P-163
CASIRAGHI Jacopoa	P-069
CASSIDY Sophie	S5-06
CASTRO Diana	P-096
CATTANEO Marina	P-094
CAVALLI Michele	P-027, P-031, P-046, P-047
CEBALLOS Delia	P-077, S4-01
CENTOFANTI Federica	P-003
CERRO-HERREROS Estefania	P-124, S2-01
CHABY Laurence	P-076
CHAKRABORTY Mouli	**P-122**, **P-124**, P-138, S2-01
CHALLA Veera RP	P-075
CHAMBERLAIN Joel	P-127
CHANG Po-Cheng	P-130

Author Index

C

CHARON JP	P-080
CHARTRAND Pascal	S2-06
CHASSERIAU Raphaele	P-008
CHEN Zhihong	P-129
CHEUNG Joseph	P-093
CHHUON Cerina	S6-02
CHING Willie	P-144, P-153
CHITAYAT David	P-002, P-021
CHONG-NGUYEN Caroline	P-099
CHOU Chung-Chuan	P-130
CHOUFANI Sanaa	P-002
CHRISTOU Evangelos A	**P-012**, P-163
CHRISTOU Yiolanda	P-151
CINESI Cinzia	**P-004**
CINTAS Pascal	P-008
CIRILLO Federica	P-098
CISNEROS-VEGA Bulmaro	P-108
CLARK H. Brent	S2-10
CLARK Tim	S8-01
CLEARY John D	**P-013**, P-030, P-111, S2-10
COBO ESTEBAN Ana-Maria	P-008
COHEN David	P-076
COLL-CANTÍ Jaume	P-001
COLONQUES-BELLMUNT Jordi	P-129
COMI Giacomo Pietro	P-047
CONN Kelly M	P-057
COOKE Korey D	P-152
COONROD Leslie	S2-02
COOPER Thomas A	P-014, P-133, **P-125**, P-135, S2-04
CORDTS Isabell	P-142

Author Index

C

CORRALES Eyleen	P-005, P-006
CORSI ROMANELLI Massimiliano Marco	P-010
CORTÉS Sergio	P-006
COSTA Elena	P-031, P-098
COSTANZI-PORRINI Sandro	P-058, P-067
CÔTÉ Isabelle	P-052
COX Diana	**P-014**
CRASTA Sheela	P-072, P-088, P-162
CROCKETT Rebecca	P-024, P-077, P-078, S4-06, S5-02
CROSS Stephen	S5-01
CRUZ Marie-Carmen	P-008
CUMMING Sarah A	P-026, P-082, **S1-01**, S5-05
CURRENCE Melissa	P-144
CYWONIUK Piotr	**P-015**, P-032
CZERNUSZEWICZ Tomek	P-129

D

DAHIYA Arun	P-075
DAIDJ Ferroudja	P-140
DAMJANOVIC Dusan	P-087
DANDELOT Elodie	P-008
DANIK Marc	S2-06
DANNEHOWER Kacie	S2-02
DAS Sudeshna	S8-01
DAVIDE Cecchetto	P-053
DAY John W	P-041, P-072, P-088, P-093, P-162
DAY Ryan	P-018
DE ANTONIO Marie	P-140, P-141, P-143, S4-02, S8-02
DE DEA DINIZ Damily	**P-016**, P-104, S6-03
DE MATTIA Elisa	P-071

Author Index

D

DE SANTIS Tiziana	**P-079**, S5-03
DE SIENA Claudia	P-031, P-098
DEKDEBRUN Jeanne	P-144, P-147, P-149, S4-05
DELLA MARCA Giacomo	P-067
DENES Lance	P-156
DENT Gersham	P-041
DEUTSCH Gayle K	**P-041**
DEVOLDER Ian	P-086, P-095, P-146, **S5-01**
DI PASQUALE Antonella	S5-03
DIBELLA Deanna	P-024, P-077, S4-01, S4-06
DILEK Nuran	P-144, P-147, P-149, S4-05
DINCÃ Diana	**S6-02**
DION Vincent	P-004
DIXON Melissa M	P-024, P-077, S4-06, **S5-02**
DOBRUCKI Wawrzyniec L	S2-04
DOGAN Céline	P-008, P-141, **P-143**, **S4-02**, S8-02
DONACHIE Jacqueline	**P-042**
DOZIO Elena	P-010
DREWES Gerard	S2-03
DREWRY David	S2-03
DUBOC Denis	P-099, P-100, P-101, S5-10
DUCHESNE Élise	**P-017**, P-028, P-068, P-051, S4-03
DUFRESNE Sébastien	P-017
DUMAS Bruno	S1-04
DUNN Diane	P-019, P-023
DUONG Tina	P-144
DURDIC Milica	P-154
DURIS Frantisek	P-007
DUROVIC Ervin	P-155
DYSART Jo	P-065

Author Index

E

EDOFSSON Ulrika	**P-043**
EICHINGER Katy	**P-044**, P-118, **P-144**, P-147, P-149
ELALOUL Basmah	S4-01
EMPARANZA Jose I	S3-02
ENDO Makiko	**P-045**
ESTECIO Marcos	P-005
ESTRADA Roger	P-122
EVANS Jonathan	S5-05
EYMARD Bruno	P-076, P-099, P-100, P-101, S5-10, S8-02

F

FALCIER Elisa	P-071
FALCONE Germana	S8-03
FARRUGIA Maria E	P-080, P-082, S5-05
FAYSSOIL Abdallah	P-099, P-100, S5-10
FERNÁNDEZ Carme	P-138
FERNÁNDEZ Huberth	P-006
FERNANDEZ-COSTA Juan Manuel	S2-01
FERNÁNDEZ-TORRÓN Roberto	P-037, S3-02
FEROLDI Sarah	P-053
FERRARI Nicola	P-139
FERRY Arnaud	P-128, P-131
FICEK Andrej	P-007
FIONDA Laura	P-079, **S5-03**
FITZGERALD Bryan P	P-057
FLETCHER Linda	S5-07
FONTANA Luana	P-003
FORBES Sean C	P-152
FOSSATI Barbara	P-009, P-025, P-027, P-031, P-040, **P-046**, **P-047**, P-098, P-139

Author Index

F

FOSTER Thomas	S7-04
FRANCK Silvie	P-002, **P-107**
FRATTARI Alessandra	S5-03
FREZZA Erica	P-058
FROST Asger	**P-048**
FUENTES Javier	P-138
FUHLBRIGGE Rebecca	P-102
FUJIMURA Harutoshi	S5-08
FUJINO Haruo	**P-049**, **P-050**
FURLING Denis	P-016, P-099, P-100, P-104, P-112, P-128, P-131, P-141, **S6-03**, S6-04
FURUTA Mitsuru	S5-08
FUSCHI Paola	S8-03

G

GABRIELE Erica	S5-03
GADALLA Shahinaz M	P-036, S3-01, **S3-02**
GAGNON Cynthia	P-002, P-017, P-028, **P-051**, **P-052**, P-056, P-063, P-064, P-068, P-085, P-143, **P-145**, S1-02, **S4-03**, S5-04
GAGNON Éric	P-063
GAIA Paola	P-098
GAIT Mike	P-103, P-128
GALLAIS Benjamin	P-052, P-085, **S5-04**
GARCÍA-ALCOVER Irma	P-129
GARCIA-LÓPEZ Amparo	P-122
GARCIA-MANTEIGA Jose M	S8-03
GARCÍA-PUGA Mikel	**P-037**
GARGIULO Marcela	P-076
GARIBALDI Matteo	S5-03

Author Index

G

GASPERONI Chiara	P-053
GATTI Valentina	P-071
GAUTHIER Morgane	P-092
GEE Richard	P-144
GEILLE Alain	S4-02
GENEVIEVE David	P-008
GEORGIOU Kristia	P-151
GHIDELLI-DISSE Sonja	S2-03
GHOSH Tushar	S2-03
GIACANELLI Manlio	P-058
GINOCCHIO Daniela	**P-053**, P-094
GOLDINA Olga	P-074
GOMES-PEREIRA Mário	S6-02, P-092
GONZÁLEZ-BARRIGA Anchel	S2-09
GORMAN Grainne	P-084, S5-06
GOTO Momoko	P-045
GOTTI Elena	P-069, P-094
GOURDON Geneviève	P-008, P-092, P-105, P-112, P-128, P-141, S6-02, S6-04, S8-04
GRAGNANO Gaia	P-070
GRECO Giulia	P-058
GRECO Lucia	P-070, P-071
GRECO Simona	S8-03
GREENE Mark H	P-036, S3-01, S3-02
GREGORY Helen	**P-082**
GROENEWOUD Johannes	P-091
GROH Miriam R	S4-04
GROH William J	**S4-04**
GRUNSEICH Christopher	P-153
GUALANDRIS Marco	P-070
GUANYABENS Nicolau	P-001

Author Index

G

GUAY Simon-Pierre	S1-02
GUDDE Anke	S2-09
GUERRERA Chiara	S6-02
GUILLEMIN Karen J	P-126
GUO Xiuming	P-034, P-035
GUPTA Riti	P-118
GUTH Amanda	P-153
GUTMANN Laurie	S5-01, P-086, P-095, **P-146**

H

HAGERMAN Katharine A	P-041, P-072, P-088, P-093, P-162
HALE Melissa	**P-018**, P-029, P-118
HALL John	P-127
HAMANAKA Kohei	S2-05
HAMILTON Graham	P-026
HAMILTON Mark J	**P-080**, P-082, **S5-05**, **P-081**
HAMMANS Simon	P-142
HAMMARÉN Elisabet	**P-054**
HAMROUN Dalil	P-008, P-143, S4-02, S8-02
HANNA Miriam	P-066
HANSEN Teresa	S5-07
HAO Lei	P-034, P-035
HASHEM Vera	P-022
HAWKINS Aaron M	**P-020**, P-075
HAYES Christopher	S2-03
HEATWOLE Chad	P-050, P-070, **P-147**, **P-149**, P-155, **S4-05**
HÉBERT J. Luc	S4-03, P-056, P-028, S1-02
HEDHLI Jamila	S2-04

Author Index

H

HELEKAR Santosh	P-158
HERBELIN Laura	P-144
HERNÁNDEZ-HERNÁNDEZ Oscar	**P-108**
HERON Delphine	P-076
HICKS Rhiannon	P-077, S4-01
HILBERT James E	P-036, P-057, P-147, P-149
HILDEBRANDT Ryan	**P-109**
HILTON-JONES David	P-142
HINMAN Melissa N	**P-126**
HINTZE Stefan	P-038, **P-110**
HLAING Phyu	**P-055**
HOLLINGSWORTH Kieren G	S5-06
HOLTMANN Gerald	P-083, S5-07
HOULE Olivier	P-017
HU Ningyan	S7-04, S8-01
HUERTA Erika	P-076
HUGUET-LACHON Aline	S6-02
HUISINGA Jessie	P-159
HUNG Man	P-024, P-078, S4-06, S5-02

I

IANNACCONE Susan	P-096
IATOMASI Marino	P-070
IMBURGIA Carina	P-019
IMURA Osamu	P-049, P-050
INGHILLERI Maurizio	P-058
IVANKOVIC Franjo	P-132
IWAHASHI Hiromi	P-097

Author Index

J

JACKSON Richard	P-055, P-083, S5-07, P-020, P-075
JACQUETTE Aurélia	P-076
JAMPANA Ravi	S5-05
JANO-ITO Joan	P-108
JAUVIN Dominic	P-103, P-128
JEAN Xavier	P-076
JENCI Nicole	P-159
JENKINS Andrew	S5-09
JENQUIN Jana	**S2-02**
JIMENEZ-MORENO Aura Cecilia	P-081, **P-084**, P-142, **P-148, S5-06**
JINNAI Kenji	S5-08
JOE Galen	P-144, P-153
JOHN Blessy	P-158
JOHNSON Claire	S5-01
JOHNSON Nicholas E	**P-019**, P-023, P-024, P-077, P-078, P-147, P-149, P-159, S4-01, S4-05, S4-06, S5-02
JORG Marylie	P-017
JOSEPH Paul	**P-083, S5-07**
JOVANOVIĆ Vladimir	S1-03

K

KACAR Aleksandra	P-155
KADASI Ludevit	P-007
KALSOTRA Auinash	S2-04
KAPNISIS Konstantinos	P-151
KARARIZOU Evangelia	P-151
KAROLAK Matthew	**P-127**
KEKA Islam Shamima	**P-021**
KEKOU Kyriaki	S1-03

Author Index

K

KESSELS Roy PC	P-091
KETCHUM Sunny	P-118
KETLEY Ami	P-113, P-114, **S2-03**
KEYMOLEN Kathelijn	P-002
KHOO Tien K	P-020, P-075, P-083, S5-07
KHORAMIAN TUSI Solaleh	**P-111**
KIEFER VALANEJAD Leila	S8-04
KIERKEGAARD Marie	**P-056**, P-148, **P-150**, S4-03
KIMURA En	P-045, P-059, P-160
KIMURA Takashi	P-060, P-097, **S5-08**
KING Wendy	P-144
KITAO Ruriko	P-060
KLEIN Arnaud	P-016, P-104, P-112, **P-128**, P-131, S6-03
KNOOP Hans	P-084, P-091
KOEHORST Emma	P-001
KOLONIN Arseniy	P-135
KOLOSKI Natasha	P-083, S5-07
KOMORI Tetsuo	P-060
KON Seiko	P-045
KONIECZNY Patryk	P-116
KOSEKI Atsushi	P-045
KOUSHIK Amrita	P-133
KOUTSOULIDOU Andrie	**P-151**
KOVČIĆ Vlado	S1-03
KRAHE Ralf	P-005, P-006, **P-129**
KUCHARIK Marcel	P-007
KUMARASWAMY Lakshmi Narayanan	**P-022**
KURAUCHI Go	P-045
KURU Satoshi	P-160
KYRIAKIDES Tassos	P-151
KYRIAKOU Kyriakos	P-151

Author Index

L

LABERGE Luc	**P-085**
LABRO Mathilde	S4-02
LAFORET Pascal	P-099, P-100, P-101, S5-10
LAGRUE Emmanuelle	S8-02
LANNI Stella	P-002, P-021
LAVRNIC Dragana	P-155
LAZARUS Arnaud	P-099, S5-10
LE Nhu	P-090
LEARY Eileen B	P-072
LEE Jinhyun	P-095
LEE Kuang-Yung	**P-130**
LÉGARÉ Cécilia	**S1-02**
LEISTMAN Sarah	S2-03
LEONARD LOUIS Sarah	P-100, P-101
LEONARDIS Lea	S1-03
LETOURNEAU Julie	P-002
LEVASEUR Melanie	P-064
LEYVA-GARCÍA Norberto	P-108
LI Hui	P-034, P-035
LIANG Minggao	P-002
LICHELLI Loretta	P-079, S5-03
LIGUORI Rocco	P-047
LIMMER Sarah	P-038, P-110
LINARES Ian	P-001
LINDBERG Christopher	P-043, P-054
LIU Yuanjing	P-013, S2-10
LIVINGSTON Eric	S5-05
LIZIO Andrea	P-069, P-070, P-071, P-094
LLAMUSÍ Beatriz	P-122, P-124, P-138, S2-01
LO SCRUDATO Mirella	**P-112**

Author Index

L

LOCHMÜLLER Hanns	P-036, P-081, P-084, P-142, P-148, S5-06
LOGULLO Francesco	P-047
LONGMAN Cheryl	P-080, P-082, S5-05
LÓPEZ CASTEL Arturo	P-129
LÓPEZ DE MUNAIN Adolfo	P-037, S3-02
LOPEZ-CASTEL Arturo	P-002
LOPEZ-MORATO Marta	**P-113**, P-114
LOTT Donovan J	P-012, P-144, **P-152**, P-163
LOZZI Flavia	P-058
LUCENTE Giuseppe	P-001
LUEBBE Elizabeth A	**P-057**, P-147, P-149, S4-05
LUNETTA Christian	P-071
ŁUSAKOWSKA Anna	P-151

M

MAAS Daphne	P-084
MACCORA Daria	P-089
MacGowan Guy	S5-06
MADDISON Paul	P-142
MAGAÑA-AGUIRRE Jonathan	P-108
MAGNOTTA Vince	S5-01
MAGOT Armelle	P-008
MAHADEVAN Karunasai	S7-02
MAHADEVAN Mani S	**S7-02**
MAHADY Amy	P-118
MAIORCA Francesca	P-003
MAKSIMOVIC Ruzica	P-154
MALIK Naveed	P-113, **P-114**

Author Index

M

MANDAL Mahua	S7-02
MANKODI Ami	**P-153**
MANNINO Giuseppe	P-079
MARAONE Giorgia	P-079
MARIE Joelle	P-016
MARINI-BETTOLO Chiara	P-036, P-142
MARJANOVIC Ana	P-155
MARTELLI Fabio	S8-03
MARTENS William N	P-036, P-147, P-149, S4-05
MARTEYN Antoine	P-092
MARTIN Samia	P-112
MARTIN Stuart S	S1-01
MARTINAT Cécile	P-092
MARTINEZ Anna	S8-04
MARTÍNEZ-PIÑEIRO Alicia	P-001
MAŠIREVIĆ Srdjan	S1-03
MASSA Roberto	P-003, **P-058**
MASSEY Charlotte	P-148
MATHEU Ander	P-037
MATHEU Ander	S3-02
MATHIEU Jean	P-002, P-051, P-056, P-064, P-085, P-145, S1-02, S4-03, S5-04
MATLOKA Magdalena	P-104, **P-131**, S6-03
MATSUMURA Tsuyoshi	P-049, P-050, **P-059**, P-097, P-160, S5-08
MATSUURA Tohru	**P-060**
MAURY Yves	P-092
MAYNE Katie	P-019, **P-023**
MAZANEC Radim	S1-03
MCCONNELL Ona	P-106, P-115, P-121, P-161, S5-09
MCCORQUODALE Donald	P-019, **P-023**
MCDERMOTT Michael	S4-05, P-147, P-149
MCGHIE Josephine	S1-01, S5-05

Author Index

M

MCINTYRE Melissa	**P-024, P**-077, S4-06
MCLEAN John	S5-05
MCSTAY Ruth	S5-06
McWILLIAM Catherine	P-082
MEINKE Peter	**P-038**, P-110
MELEO Emiliana	P-067
MENDEZ-GOMEZ Héctor	P-156
MENSEL Raphaela Victoria	P-038, P-110
MEOLA Giovanni	P-009, P-010, **P-025**, P-027, P-031, P-040, P-046, P-047, P-098, P-139, S8-03
MESSINA Sonia	P-071
MEUNE Christophe	S5-10
MIGUEL David	P-138
MIGNOT Emmanuel	P-072, P-093
MIRANDA Francisca	P-138
MIRON Ioana	P-002, P-021
MISRA Chaitali	**S2-04**
MOAT Dionne	P-148
MOCHIZUKI Hideki	S2-05, S5-08
MODONI Anna	P-067
MONCKTON Darren G	P-005, P-006, P-026, P-080, P-082, P-090, P-142, S1-01, S1-02, S5-05
MONDELLO Stefania	P-061
MONLLEÓ-NEILA Laura	P-001
MONTAGNESE Federica	**P-061**, P-074
MOODY Olivia A	S5-09
MOORE Hyatt	P-093
MORALES Fernando	**P-005, P-006**
MORETTI Michela	P-053
MORIN Françoise	P-136
MORIN Marika	P-017

Author Index

M

MORINO Stefania	S5-03
MORISAKI Tatsuya	S6-01
MORNET Dominique	P-123
MORRISS Ginny R	P-125
MOSBACH Valentine	S1-04
MOSS Kathryn	P-106, P109
MOSSAKOWSKA Danuta	S2-03
MOULY Vincent	P-104, S6-03, S6-04
MOXLEY Richard T III	P-036, P-057, P-147, P-149, S4-05
MUSOVA Zuzana	P-002, S1-03

N

NAKAMORI Masayuki	P-160, S2-02, **S2-05**, S2-07, S5-08
NAKAYAMA Takahiro	P-049
NEARCHOU Marianna	P-151
NEDELEC Stéphane	P-092
NEWMAN Jane	P-148
NEY Michel	P-131
NGUYEN Linda A	P-088
NIKOLENKO Nikoletta	P-036, P-081, P-142, P-148
NIKOLIC Ana	P-155
NISHINO Ichizo	S2-05, S2-07
NNAMDI-EMERATOM Chioma	P-153
NOEL Anastasia	P-136
NOGALES-GADEA Gisela	P-001
NOPOULOS Peg	**P-086**, P-095, P-146, S5-01
NOVELLI Giuseppe	P-003, P-058
NUÑEZ Judith	P-001

Author Index

O

ODAIRA Kaori	P-045
OGATA Katsuhisa	P-050
OHNO Kinji	P-060
OKKERSEN Kees	P-084, P-090, P-091
OLAFSON Hailey	P-118
OLIVEIRA Ruan	P-030, **P-132**, P-156
ONO Ryohei	P-045
ORRELL Richard	P-142
OTTERUD Brith	P-019, P-023, S5-02
OVEREND Gayle	S1-02
OYENIYI Tolulope	**P-026**

P

PAGLIUCA Silvana	P-040
PALAU Roberta	P-133
PANDIT Rahul T	P-022
PANDYA Shree	P-044, P-144
PANG Paul	**P-133**
PAPADIMAS George	P-151
PAPON Marie-Amélie	P-136
PARIKH Jehill	S5-06
PARK Hyun J	P-152
PARKER Darren J	S2-04
PARKHILL Amy L	P-057
PASCUAL-GILABERT Marta	P-138
PATHMANATHAN Pavthrun	P-083, S5-07
PAULTER Mary	P-159
PEARSON Christopher E	P-002, P-021
PEGORARO Elena	P-047
PELLIEUX Sebylle	P-100

Author Index

P

PENNISI Elena Maria	P-058
PEREON Yann	P-008, P-100
PEREZ Jennifer P	P-072, **P-088**, P-093
PEREZ Marco	P-102
PÉREZ-ALONSO Manuel	P-124
PERFETTI Alessandra	P-031, **S8-03**
PERIĆ Stojan	P-062, **P-087**, **P-154**, **P-155**, S1-03
PERNA Alessia	**P-089**
PESCHANSKI Marc	P-092
PEŠOVIĆ Jovan	**P-062**, P-087, P-154, **S1-03**
PETITCLERC Émilie	P-056, S4-03
PETRUCCI Antonio	P-058, P-067, P-089
PETRY Franck	P-136
PETTY Richard	P-082, P-142
PFEIFFER Ruth M	S3-01
PHETTEPLACE Janel	P-086, P-095, P-146
PHILLIPS Margaret	P-142, P-145
PHOTIADES Marinos	P-151
PHYLACTOU Leonidas	P-151
PIANTADOSI Carlo	P-058
PICCOLI Marco	P-098
PIGNATELLI Giulia	S5-03
PINTO Belinda	**P-134**, **P-156**
PINTOS-MORELL Guillem	P-001
PIZZORNI Nicole	P-053
PLANEL Emmanuel	P-136
PLEITEZ Milvia	P-073
PLOURDE Annie	P-063
POGGI Lucie	**S1-04**
POITRAS Isabelle	P-136
POLANSKY Caitlin	P-019

Author Index

P

POLAY-ESPINOZA Micaela	P-104, S6-03
PORCHER Raphael	P-100, P-101
POULARD Karine	P-112
POWALOWSKA Paulina	S2-03
POZZI Susanna	P-071
PRICE Philip	P-121
PROBST Vincent	P-100
PROKOPI Marianna	P-151
PUCILLO Evan M	P-024, P-077, S4-01
PUYMIRAT Jack	P-103, P-128, P-141, P-143

Q

QUADRI Emilia	P-040
QUATTRI Enrico	P-046
QUERIDO Emmanuelle	**S2-06**
QUINN Jill R	P-044

R

RAAPHORST Joost	**P-090, P-091**
RADVANSZKY Jan	**P-007**, S1-03
RAKOČEVIĆ-STOJANOVIĆ Vidosava	P-062, P-087, P-154, P-155, S1-03
RAMOS FRANSI Alba	P-001
RAMPOLDI Benedetta	P-098
RANUM Laura	P-013, P-030, P-111, S1-03, S2-10
RAO Ashish N	**P-135**
RAO Fabrizio	P-071
RASTELLI Emanuele	P-058
RAU Frédérique	P-016, P-131
RAYMOND Kateri	**P-063, P-064**
REDDY Kaalak	**P-115**
REID Tammy	P-030

Author Index

R

RENDON Alvaro	P-123
RENNA Laura Valentina	P-009, P-010, P-025, **P-027**, P-139
REVILLOD Lucile	P-128, P-141
RIBEIRO Fernanda	P-028, P-068
RICCI Martina	P-067
RICHARD Guy-Franck	S1-04
RICHARDSON Jared	P-018, P-118
RICHARDSON Linda	S2-03
RICHER Louis	S5-04
RIGO Frank	S7-04
RIMOLDI Chiara	P-069
RIVOLTA Ilaria	P-009
ROBB Yvonne	P-082
ROBERTS Mark	P-142
RODRIGUES Miriam J	**P-065, P-066, P-157**
RODRÍGUEZ-PALMERO Agustí	P-001
ROGERS Mark	P-142, P-145
ROGERS Stephanie	S4-01
ROLA Rafal	P-039
ROQUEVIÈRE Sylvain	**P-092**
ROSENBERG Philip S	P-036
ROSENFIELD David	P-073, P-158
ROSERO Spencer	P-147
ROSSI Gabriella	P-071
ROSSI Salvatore	P-058, **P-067**, P-089
ROUSSEL Marie-Pier	P-017, **P-028, P-068**
ROXBURGH Richard H	P-065, P-066, P-157
RUDIGER Martin	S2-03
RUOFF Chad	P-093
RYE David	P-161, **S5-09**

Author Index

S

SABATER-ARCIS Maria	S2-01
SAITO Toshio	P-049
SAKAMURI Sarada	P-093
SAMPSON Jacinda	P-041, P-072, P-088, **P-093**
SANCRICCA Cristina	P-067
SANGCO Monica	P-088
SANNICOLO' Giulia	P-071
SANSONE Valeria Ada	P-047, P-053, **P-069, P-070, P-071, P-094**
SANTAMARÍA Carolina	P-005, P-006
SANTORO Lucio	P-047
SANTORO Massimo	P-003
SATO Yumi	P-045
SAVAGE Carolyn M	**P-072**
SAVIĆ-PAVIĆEVIĆ Dušanka	P-062, P-087, P-154, S1-03
SAXENA Tanvi	P-106, P-156
SCARLATO Marina	P-047
SCHINDLER Antonio	P-053
SCHNEIDER Mary	S6-01
SCHOSER Benedikt	P-028, P-038, P-061, P-074, P-110, P-140
SCIARRA Federico	P-067
SCOTTI Marina	S2-07
SEAH Carol	P-130
SEDEHIZADEH Saam	S2-03
SEDLACEK Zdenek	P-002
SENĆANIĆ Ivan	P-062
SENECA Sara	P-002
SERGEANT Nicolas	P-131, **P-136**
SERMON Karen	P-002, P-107
SERVAIS Laurent	P-076
SFRAMELI Maria	P-071
SHANNON Patrick	P-002, P-021

Author Index

S

SHIELDS Richard	P-086, **P-095**, P-146
SHIMELLIS Hirity	P-153
SHINGAKI Honoka	P-050
SHOTWELL Carl	**P-029**
SHROFF Sheetal	**P-073**
SHUEN Andrew Y	P-021
SICILIANO Gabriele	P-047
SICOT Géraldine	S6-02
SILVESTRI Gabriella	P-003, P-047, P-058, P-067, P-089
SIMON Marie	P-008
SIMPSON Ericka	P-022, P-073, **P-158**
SINGERIS Stephanie	P-077
SIRITO Mario	P-005, P-129
SMARGIASSI Andrea	P-067
SNAPE Mike	S8-04
SOBCZAK Krzysztof	P-015, P-032, P-116
SOCHALA Maximilien	P-100, P-101, S5-10
SPITS Claudia	P-002
ST. GEORGE Diane Marie M	S3-01
STASEVICH Tim	S6-01
STATLAND Jeffrey M	**P-159**
STEHLÍKOVÁ Kristýna	S1-03
STEPNIAK-KONIECZNA Ewa	P-116
STEVIC Zorica	P-155
STOCK Lauren	S8-04
STOJKOVIC Tanya	P-099, P-100, P-101, S5-10
STOKES Mathew	P-096
STRAUB Volker	P-142
STRUCK Adam	P-118
SUÁREZ-SÁNCHEZ Rocío	P-108
SUBRAMONY SH	P-012, P-152, P-163

Author Index

S

SUGAWARA Norio	P-045
SUN Yumei	P-120
SUREAU Alain	P-016
SWANSON Maurice S	P-013, P-030, P-132, P-156, S2-05, S2-07, S2-10
SWORD David	P-080
SZEMES Tomas	P-007
SZNAJDER Łukasz	**P-030**, S2-07

T

TAKADA Hiroto	P-045, P-049, P-050, **P-097**, P-160
TAKAHASHI Masanori P	P-049, P-050, P-059, P-097, **P-160**, S2-05, S5-08
TANG Zhenzhi	P-011, S7-01, **S7-03**
TANNER Matthew	**P-137**
TAWIL Rabi	P-147
TAYLOR Katarzyna	**P-032**
TERRACCIANO Chiara	P-058
TESTA Marco	S5-03
THEADOM Alice	P-066
THEDENS Dan	P-086, P-095, P-146
THOMAS James	P-030, P-132, S2-05, **S2-07**
THOMPSON Rachel	P-142
THONHOFF Jason	P-073, P-158
THORNTON Charles A	P-011, P-118, P-137, P-147, P-149, P-153, S2-03, S7-01, S7-03
TIMCHENKO Lubov	**S2-08**, S8-04
TIMCHENKO Nikolai	S2-08, S8-04
TIWARI Anjana	P-022
TODA Tatsushi	P-060
TOMÉ Stéphanie	P-002, **P-008**, P-112
TRAN Khoa	P-020
TREMBLAY Marjolaine	P-052

Author Index

T

TRENELL Michael	S5-06
TREWEEK Shaun	P-140
TRIPLETT William T	P-152
TRUJILLO Carenina	P-159
TULADHAR Anil M	P-090
TURNER Chris	P-142, P-145, P-148

U

UINGS Iain	S2-03
URTIZBEREA Andoni	P-008, P-123

V

VALAPERTA Rea	P-009, P-025, **P-031**, **P-098**
VALENZA Venanzio	P-089
VAN AGTMAAL Ellen L	S6-04
VAN BOKHOVEN Hans	P-105
VAN CRUCHTEN Remco TP	**P-033**, S6-04
VAN DEN BROEK Walther	P-105, S6-04
VAN DER BENT Leontien	**P-117**
VAN ENGELEN Baziel GM	P-084, P-090, P-091, P-105, P-140
VAN KESSEL Ingeborg DG	S2-09, S6-04
VANACORE Nicola	P-058
VANDENBORNE Krista	P-152
VANOLI Fiammetta	P-079, S5-03
VARELA Miguel	P-103, P-128
VÁSQUEZ Melissa	P-005, P-006
VENANCE Shannon	P-145
VIANELLO Elena	P-010
VIPPA Tarun	P-153
VITA Giuseppe	P-047
VITERBO David	S1-04
VUJNIC Milorad	P-087

Author Index

W

WAGNER Kathryn R	S3-01
WAGNER Stacey	**P-118**
WAHBI Karim	**P-099, P-100, P-101, S5-10**
WALTER Glenn A	P-152
WANG Eric T	P-018, P-106, P-109, P-115, P-118, P-121, P-134, P-156, P-161, S2-05, S2-07, S5-09
WANG Mei	**S8-04**
WANG Wenli	P-153
WANG Yanlin	P-034, P-035
WANG Youjin	S3-01
WANSINK Derick G	P-033, P-105, P-117, **S2-09**, S6-04
WATANABE Norio	P-045
WEHRENS Xander	P-133
WEI Christina	S2-08
WEI Yi-Chia	P-130
WEISS Robert	P-019, P-023
WEKSBERG Rosanna	P-002
WELSH Lesly	**P-162**
WENNINGER Stephan	P-061, **P-074**, P-140
WEST Sophie	P-081, **P-102, S7-04**, S8-01
WIERINGA Bé	P-033, S2-09, **S6-04**
WILLEMSE Marieke	S6-04
WILSON Michael D	P-002
WILUSZ Carol	S6-01
WILUSZ Jeffrey	S6-01
WINBLAD Stefan	P-043
WOJCIECHOWSKA Marzena	P-129
WOOD Elizabeth	P-036, P-142, P-148, P-081
WOOD Matthew	P-103, P-128

Author Index

X

XIA Guangbin	P-013, **P-034**, **P-035**, P-156, S2-10
XU Fangke	P-134
XU Yumin	P-034

Y

YACHNIS Anthony T	S2-10
YACOUBI Basma	P-012, **P-163**
YADAVA Ramesh S	S7-02
YANG Bin	P-004
YENIGUN Vildan Betul	P-129
YOSHIKAWA Hiroo	S5-08
YU Qing	S7-02

Z

ZAMBA PAPANICOLAOU Eleni	P-151
ZANOLINI Alice	P-069, P-070, P-071, P-094
ZAPATA Eugenio	P-077, S4-01
ZAREBA Wojciech	P-147
ZAVALJEVSKI Maja	P-127
ZENG Desmond	P-034, P-035
ZHAN Min	S3-01
ZHANG Baili	P-005, P-006
ZHANG Fan	**P-120**
ZHANG Nan	P-022, **P-119**
ZHENZHI Tang	S2-03
ZIELINSKA Julia	P-116
ZIELINSKI Brandon	S5-02
ZRUBCOVA Michaela	P-007
ZU Tao	P-013, P-111, S2-10
ZUERCHER William	S2-03
ZULAICA Miren	P-037

Attendee Contact Information

A

ABDUL RAZAK Kavitha	Queensland Health drkavitharazak@gmail.com
AIT BENICHOU Siham	LOEX/CMDGT, Hôpital de l'Enfant-Jésus siham.ait-benichou.1@ulaval.ca
AKECHI Yuji	akechiyujiactg@gmail.com
AKIKAWA Kayo	akikawa@sr.catv.ne.jp
ALINSKY RADEY Nava	avi_rad@zahav.net.il
ALSAGGAF Rotana	National Cancer Institute rotana.alsaggaf@nih.gov
ANGEARD Nathalie	Inserm U 1129, Paris Descartes University, Sorbonne Paris Cité nathalie.angeard@parisdescartes.fr
ANTOURY Layal	Massachusetts General Hospital layal.antoury@gmail.com
ARANDEL Ludovic	Institut de Myologie, research center, UPMC/Inserm ludovic.arandel@upmc.fr
ARJOMAND Jamshid	GeneaBiocells jamshid.arjomand@geneabiocells.com
ARTERO Ruben	University of Valencia ruben.artero@uv.es
ASHIZAWA Tetsuo	Houston Methodist Hospital tashizawa@houstonmethodist.org
ASHLEY Peter	emma@cmmd.uk
ASHLEY Emma-Jayne	emma@cmmd.uk
AUSEMS Rosanne	Radboudumc Rosanne.Ausems@radboudumc.nl

B

BALLESTER-LOPEZ Alfonsina	Germans Trias i Pujol Health Sciences Research Institute aballester@igtp.cat
BANACH Marta	Jagiellonian University Collegium Medicum martabanach@yahoo.com
BANERJEE Anwesha	Emory University anwesha1985@gmail.com
BARBE Lise	University of California San Francisco lise.barbe@gladstone.ucsf.edu

Attendee Contact Information

B

BARGSTEN Annie Junzhen Zhang	Colorado State University jzannie.zhang@gmail.com
BASSELL Gary	Emory University School of Medicine gbassel@emory.edu
BASSEZ Guillaume	Centre de Référence Maladies Neuromusculaires, CHU Henri Mondor, Créteil, France guillaume.bassez@aphp.fr
BENNETT Monica	GeneaBiocells monica.bennett@geneabiocells.com
BENNETT Frank	Ionis Pharmaceuticals fbennett@ionisph.com
BERGGREN Kiera	University of Utah kiera.berggren@hsc.utah.edu
BERGLUND Andy	University of Florida aberglund@ufl.edu
BERTOLLI Jeanne	Centers for Disease Control and Prevention JBertolli@cdc.gov
BERTOLDO Enrico Giuseppe	I.R.C.C.S. POLICLINICO SAN DONATO enricogiuseppe.bertoldo@grupposandonato.it
BESECKER Robert	Ramman2474@aol.com
BEST Ana	National Cancer Institute ana.best@nih.gov
BINDA Anna	University of Milano-Bicocca anna.binda@unimib.it
BOSÈ Francesca	IRCCS-Policlinico San Donato francesca.bose@grupposandonato.it
BOTTA Annalisa	University of Rome Tor Vergata botta@med.uniroma2.it
BOWLER Margaret	Myotonic Dystrophy Support Group mdsg@tesco.net
BOWLER Ian	Myotonic Dystrophy Support Group mdsg@tesco.net
BRIAND Jean-François	AFM-Téléthon jfbriand@afm-telethon.fr
BRIGNOL Tuy Nga	AFM-Telethon tnbrignol@afm-telethon.fr
BROOK David	University of Nottingham, United Kingdom david.brook@nottingham.ac.uk

Attendee Contact Information

C

CAMMISH Phillip	Newcastle University phillip.cammish@ncl.ac.uk
CAMPBELL Craig	Western University craig.campbell@lhsc.on.ca
CARDANI Rosanna	IRCCS Policlinico San Donato rosanna.cardani@grupposandonato.it
CARRELL Samuel	University of Rochester samuel_carrell@urmc.rochester.edu
CARRELL Ellie	University of Rochester ellie_carrell@urmc.rochester.edu
CHAKRABORTY Mouli	University of Valencia, Spain mouli.chakraborty@uv.es
CHAMBERLAIN Joel	University of Washington jrcham@uw.edu
CHRISTOU Evangelos	University of Florida eachristou@ufl.edu
CINESI Cinzia	University of Lausanne cinzia.cinesi@unil.ch
CLEARY John	University of Florida john.cleary@ufl.edu
COOPER Tom	Baylor College of Medicine tcooper@bcm.edu
COX Diana	Baylor College of Medicine diana.cox@bcm.edu
CRASTA Sheela	Stanford University sheelat@stanford.edu
CUMMING Sarah	University of Glasgow sarah.cumming@glasgow.ac.uk
CYWONIUK Piotr	Adam Mickiewicz University piotr.cywoniuk@gmail.com

D

D'AMATO Marco	Myogem Health Company marco.damato@myogemhealth.com
D'ANJOU Guy	guy.danjou@umontreal.ca
DAVIDSON Beverly	University of Pennsylvania davidsonbl@email.chop.edu

Attendee Contact Information

D

DAY John	Stanford University jwday@stanford.edu
DE DEA DINIZ Damily	Institut de Myologie, research center, UPMC/Inserm damilyddiniz@gmail.com
DE SANTIS Tiziana	Department of Neurology, Mental Health and Sensory Organs (NESMOS) Faculty of Medicine and Psychology, University of Rome "Sapienza" Unit of Neuromuscular Disorders Sant'Andrea Hospital tiziana.desantis@hotmail.com
DEUTSCH Gayle	Stanford Neurosciences Health Center gdeutsch@stanfordhealthcare.org
DEVOLDER Ian	University of Iowa ian-devolder@uiowa.edu
DINCÃ Diana Mihaela	Imagine Institute - INSERM UMR 1163 diana.m.dinca@gmail.com
DIXON Melissa	University of Utah missy.dixon@genetics.utah.edu
DOGAN Celine	Myology Institute celine.dogan@aphp.fr
DONACHIE Jacqueline	jacquelinedonachie@hotmail.com
DOR Talya	talyad@hadassah.org.il
DUCHESNE Elise	Université du Québec à Chicoutimi elise1_duchesne@uqac.ca

E

EDOFSSON Ulrika	Neuromuscular Centre Sahlgrenska University Hospital ulrika.edofsson@vgregion.se
EICHINGER Katy	University of Rochester katy_eichinger@urmc.rochester.edu
EIGES Rachel	rachela@szmc.org.il
EKSTRÖM Anne-Berit	Northern Alvsborg County Hospital, Trollhättan, Sweden anne-berit.ekstrom@vgregion.se
ENDO Makiko	National Center of Neurology and Psychiatry endo_makiko@hotmail.com
ESPARIS Belen	Mount Sinai Medical Center in Miami besparis@gmail.com
EYMARD, Bruno	Institut de Myologie anne-marie.maronne@psl.ap-hop-paris.fr

Attendee Contact Information

F

FIELDER Graeme	Audentes Therapeutics gfielder@audentestx.com
FINKEL Richard	Nemours Children's Hospital Richard.Finkel@nemours.org
FIONDA Laura	University of Rome laurafionda@libero.it
FORMAKER Paul	Myotonic Dystrophy Foundation paul.formaker@myotonic.org
FORREST Kevin	kforrest@expansionrx.com
FOSSATI Barbara	IRCCS Policlinico San Donato barbara.fossati@grupposandonato.it
FRANCK Sylvie	Vrije Universiteit Brussel silvie.franck@vub.ac
FROST Asger	Rehabilitationcenter for Neuromuscular Diseases asfr@rcfm.dk
FUJINO Haruo	Oita University fjinoh@oita-u.ac.jp
FURLING Denis	Institut de Myologie, research center, UPMC/Inserm denis.furling@upmc.fr

G

GADALLA Shahinaz	National Cancer Institute gadallas@mail.nih.gov
GAGNON Cynthia	Université de Sherbrooke/GRIMN cynthia.gagnon4@usherbrooke.ca
GALLAIS Benjamin	Centre de recherche Charles-Le-Moyne bgallais@hotmail.com
GARCÍA-PUGA Mikel	Biodonostia Health Research Institute mikel.garcia@biodonostia.org
GEILLE Alain	AFM ageille@afm-telethon.fr
GINOCCHIO Daniela	Fondazione Serena ONLUS, Centro Clinico NEMO nicole.pizzorni@virgilio.it
GOMES-PEREIRA Mario	Inserm mario.pereira@inserm.fr

Attendee Contact Information

G

GREGORY Helen	Clinical Genetics, Ashgrove House, Foresterhill Health Campus helen.gregory2@nhs.net
GROH William	Medical University of South Carolina wmjgroh@gmail.com
GUTH Amanda	NINDS, NIH Amanda.Guth@nih.gov
GUTMANN Laurie	University of Iowa laurie-gutmann@uiowa.edu

H

HAGERMAN Katharine	Stanford University khagerma@stanford.edu
HÅKANSSON Karin	Centre for Rare Diseases - Västra Götaland karin.hakansson@vgregion.se
HALE Melissa	University of Florida melissahale@ufl.edu
HAMILTON Mark	University of Glasgow markhamilton1@nhs.net
HAMMARÉN Elisabet	Sahlgrenska University Hospital elisabet.hammaren@vgregion.se
HARVEY-DUREN Lisa	lisa@global.thunderbird.edu
HAWKINS Aaron	Griffith University aaron_hawkins91@hotmail.com
HEATWOLE Chad	University of Rochester Chad_Heatwole@urmc.rochester.edu
HELLER Jacqueline	drjackieheller@gmail.com
HERNANDEZ-HERNANDEZ Oscar	Instituto Nacional de Rehabilitación heroscar@gmail.com
HILDEBRANDT Ryan	University of Florida ryhildeb@ufl.edu
HINMAN Melissa	University of Oregon mhinman2@uoregon.edu
HINTZE Stefan	Friedrich-Baur-Institut Klinikum der LMU stefan.hintze@med.uni-muenchen.de

Attendee Contact Information

H

HIRATO Junya	j-hirato@nn.iij4u.or.jp
HLAING Phyu	Princess Alexandra Hospital phlaing5@gmail.com
HOSHINO Aya	Astellas Pharma aya.hoshino@astellas.com
HOWE Sarah	Marigold Foundation sarah.howe@marigoldfoundation.org

I

IMURA Osamu	Osaka University osamui@hus.osaka-u.ac.jp
IVANKOVIC-MACGIBBON Franjo	njofrica@ufl.edu

J

JACKSON Richard	Griffith University rick.jackson@health.qld.gov.au
JAUVIN Dominic	Laval University dominic.jauvin@crchudequebec.ulaval.ca
JENQUIN Jana	University of Florida jjenquin@ufl.edu
JIMENEZ-MORENO Aura Cecilia	Newcastle University a.c.jimenez-moreno@ncl.ac.uk
JOHNSON Nicholas	University of Utah nicholas.johnson@hsc.utah.edu
JOSEPH Paul	Griffith University drpauljoseph@gmail.com

K

KAALUND Rikke	Muskelsvinfonden rika@musckelsvindfonden.dk
KAROLAK Matthew	University of Washington mkarolak@uw.edu
KEKA Shamima Islam	The Hospital for Sick Kids kekabmb@gmail.com
KETLEY Ami	University of Nottingham ami.ketley@nottingham.ac.uk

Attendee Contact Information

K

KHORAMIAN TUSI Solaleh	University of Florida s.tusi@ufl.edu
KIERKEGAARD Marie	Karolinska University Hospital marie.kierkegaard@sll.se
KIMURA Takashi	Hyogo College of Medicine kimura@hyo-med.ac.jp
KIMURA En	enkimura@ncnp.go.jp
KLEIN Arnaud	Institut de Myologie, Research Center, UPMC/Inserm arnaud.klein@upmc.fr
KOUTSOULIDOU Andrie	The Cyprus Institute of Neurology and Genetics andriek@cing.ac.cy
KRAHE Ralf	University of Texas MD Anderson Cancer Center rkrahe@mdanderson.org
KRAINER Adrian	Cold Spring Harbor Laboratories krainer@cshl.edu
KRISTENSEN Bente	Rehabilitation Center for Neuromuscular Diseases bekr@rcfm.dk
KROKSMARK Anna-Karin	The Queen Silvia Children's Hospital, Sahlgrenska University Hospital anna-karin.kroksmark@vgregion.se
KUMARASWAMY Lakshmi Narayanan	Houston Methodist Research Institute lkumaraswamy@houstonmethodist.org

L

LABERGE Luc	ÉCOBES - Recherche et Transfert, Cégep de Jonquière luclaberge@cegepjonquiere.ca
LABONTE Larry	larry@iphouse.com
LAVANO Paola	paolalavano@hotmail.com
LEE Kuang-Yung	Keelung and Chang Gung University kylee@cgmh.org.tw
LÉGARÉ Cécilia	Université de Sherbrooke Cecilia.Legare@USherbrooke.ca
LEVY Rebecca	Stanford University rjlevy@stanford.edu

Attendee Contact Information

L

LINDBERG Christopher	Neuromuscular Centre, Sahlgrenska University Hospital, Gothenburg, Sweden christopher.lindberg@vgregion.se
LO SCRUDATO Mirella	Genethon mloscrudato@genethon.fr
LÓPEZ DE MUNAIN Adolpho	Biodonostia Health Research Institute adolfo.lopezdemunainarregui@osakidetza.eus
LOPEZ-MORATO Marta	University of Nottingham mbxml3@nottingham.ac.uk
LORD Larry	larrylord@mindspring.com
LOTT Donovan	University of Florida djlottpt@phhp.ufl.edu

M

MACKENZIE Don	Marigold Foundation don.mackenzie@marigoldfoundation.org
MAHADEVAN Mani	University of Virginia mahadevan@virginia.edu
MALIK Naveed Altaf	University of Nottingham mbxnam@nottingham.ac.uk
MANKODI Ami	National Institute of Neuromuscular Disorders and Stroke, NIH Ami.Mankodi@nih.gov
MASSA Roberto	Università di Roma Tor Vergata massa@uniroma2.it
MATLOKA Magdalena	Sorbonne Universités UPMC, Inserm Centre de Recherche en Myologie, Institut de Myologie magdalena.matloka@upmc.fr
MATSUMURA Tsuyoshi	National Hospital Organization Toneyama National Hospital tmatsumura-toneyama@umin.org
MATSUURA Tohru	Jichi Medical University tohrum@jichi.ac.jp
MCCONNELL Ona	University of Florida ona@ufl.edu
MCCONNELL Karen	karengmcconnell3@gmail.com
MCCORQUODALE Donald	University of Utah donald.mccorquodale@hsc.utah.edu

Attendee Contact Information

M

MCINTYRE Melissa	University of Utah melissa.mcintyre@hsc.utah.edu
MEINKE Peter	Friedrich-Baur-Institute, Ludwig-Maximilians-University Peter.Meinke@med.uni-muenchen.de
MEOLA Giovanni	IRCCS Policlinico San Donato giovanni.meola@unimi.it
MIGNON Laurence	Ionis Pharmaceuticals LMignon@ionisph.com
MIGNOT Emmanuel	Stanford University mignot@stanford.edu
MISRA Chaitali	University of Illinois, Urbana-Champaign chaitali@illinois.edu
MONCKTON Darren	University of Glasgow darren.monckton@glasgow.ac.uk
MONDRAGON GONZALEZ Ricardo	University of Minnesota mondr028@umn.edu
MONTAGNESE Federica	Friedrich Baur Institut Ludwig Maximilian University federica.montagnese@med.uni-muenchen.de
MORALES Fernando	Universidad de Costa Rica fernando.moralesmontero@ucr.ac.cr
MORRISON Robert	rmorrison222@yahoo.com
MUNTONI Francesco	University College London f.muntoni@ucl.ac.uk

N

NAKAMORI Masayuki	Osaka University Graduate School of Medicine mnakamor@neurol.med.osaka-u.ac.jp
NISHI Masamitsu	tbz.hcm.mi2@gmail.com
NOGALES-GADEA Gisela	Neuromuscular and Neuropediatric Research Group, Institut d'Investigació en Ciències de la Salut Germans Trias i Pujol, Campus Can Ruti, Universitat Autònoma de Barcelona gnogales@igtp.cat
NOPOULOS Peggy	University of Iowa Peggy-nopoulos@uiowa.edu

Attendee Contact Information

O

OE Tomoya	Astellas Pharmaceutical tomoya.ooe@astellas.com
OLIVEIRA Ruan	Center for NeuroGenetics, University of Florida ruan@ufl.edu
ORLANDO Lianna	Muscular Dystrophy Association lorlando@mdausa.org
OYENIYI Tolulope	University of Glasgow t.oyeniyi.1@research.gla.ac.uk

P

PANDYA Shree	University of Rochester Shree_Pandya@urmc.rochester.edu
PANG Paul	Baylor College of Medicine pdpang@bcm.edu
PARAMSOTHY Pangaja	PParamsothy@cdc.gov
PAULOSE Shirley	Stanford University spaulose@stanford.edu
PEARSON Christopher	The Hospital for Sick Kids christopher.pearson@sickkids.ca
PELTZ Stuart	PTC Therapeutics speltz@ptcbio.com
PEREZ Jennifer	Stanford University jpperez@stanford.edu
PERFETTI Alessandra	IRCCS-Policlinico San Donato alessandra.perfetti@grupposandonato.it
PERIC Stojan	University of Belgrade stojanperic@gmail.com
PERNA Alessia	Catholic University of the Sacred Heart aleperna74@alice.it
PEŠOVIĆ Jovan	University of Belgrade jovan.pesovic@bio.bg.ac.rs
PINTO Belinda	University of Florida belinda.pinto@gmail.com
PLAMONDON Stephanie	stephanie.plamondon@ahs.ca
PLANCO Lori	lplanco@bellsouth.net

Attendee Contact Information

P

PLANCO Mark	lplanco@bellsouth.net
POGGI Lucie	Sanofi & Institut Pasteur lucie.poggi@pasteur.fr
PORTER John	Myotonic Dystrophy Foundation john.porter@myotonic.org

Q

QUERIDO Emmanuelle	Université de Montréal e.querido@umontreal.ca

R

RAAPHORST Joost	Radboud University Medical Centre joost.raaphorst@radboudumc.nl
RADVANSZKY Jan	Institute for Clinical and Translational Research, Biomedical Research Centre, Slovak Academy of Sciences jradvanszky@gmail.com
RANDALL Tawana	Emory University tnranda@emory.edu
RANUM Laura	University of Florida ranum@ufl.edu
RAO Ashish	Baylor College of Medicine ashish.rao@bcm.edu
RAYMOND Kateri	Université de Sherbrooke kateri.raymond@usherbrooke.ca
REDDY Kaalak	Univeristy of Florida kaalak.reddy@ufl.edu
REID Tammy	Univeristy of Florida tammy.reid@ufl.edu
RENNA Laura Valentina	IRCCS Policlinico San Donato lauravalentina.renna@grupposandonato.it
RICHARDSON Jared	University of Florida jared.richardson@ufl.edu
RODRIGUE Xavier	xav_rod@hotmail.com
RODRIGUES Miriam	Auckland City Hospital mrodrigues@adhb.govt.nz
ROQUEVIÈRE Sylvain	ISTEM sroqueviere@istem.fr

Attendee Contact Information

R

ROSSI Salvatore	Catholic University of the Sacred Heart salvatorerossi309@gmail.com
ROUSSEL Marie-Pier	Université du Québec à Chicoutimi marie-pier.roussel1@uqac.ca
ROXBURGH Richard	richardr@adhb.govt.nz
RYE David	catpagerye@gmail.com

S

SAHAY Kashika	vtd2@cdc.gov
SAMPSON Jacinda	Stanford University jacindas@stanford.edu
SANGCO Monica	Stanford University msangco@stanford.edu
SANNICOLO Giulia	giulia.sannicolo@gmail.com
SANSONE Valeria Ada	Fondazione Serena - Centro Clinico NEMO valeria.sansone@centrocliniconemo.it
SANTORO Massimo	masantoro@dongnocchi.it
SAUNDERS Conan	conansaunders@gmail.com
SAVAGE Carolyn	Stanford University cjmclaug@stanford.edu
SCHMIDT Uli	GeneaBiocells uli.schmidt@geneabiocells.com
SCHOSER Benedikt	Friedrich-Baur-Institute, Dep of Neurology Ludwig-Maximilians-University Munich, Germany bschoser@t-online.de
SENOO Midori	Japanese DM Patients Association midori.senoo@gmail.com
SERGEANT Nicolas	Inserm nicolas.sergeant@inserm.fr
SHAH Sapana	Carolinas HealthCare System sapana.shah@carolinashealthcare.org
SHAW Kathryn	artwatch@bitstream.net
SHIEH Perry	UCLA pshieh@mednet.ucla.edu

Attendee Contact Information

S

SHIELDS Richard	University of Iowa richard-shields@uiowa.edu
SHIMELLIS Hirity	National Institute of Neuromuscular Disorders and Stroke, NIH hirity.s@gmail.com
SHOTWELL Carl	University of Florida carl.shotwell@ufl.edu
SHROFF Sheetal	Houston Methodist Neurological Institute sshroff@houstonmethodist.org
SILVESTRI Silvestri	Institute of Neurology - Catholic University of the Sacred Heart, Fondazione Policlinico A. Gemelli - Rome gabriella.silvestri@policlinicogemelli.it
SIMPSON Ericka	Houston Methodist Hospital ESimpson@houstonmethodist.org
SOBCZAK Krzysztof	Adam Mickiewicz University ksobczak@amu.edu.pl
STATLAND Jeffrey	University of Kansas Medical Center JStatland@kumc.edu
STEPHENSON Kristin	kstephenson@mdausa.org
STEPNIAK-KONIECZNA Ewa	Adam Mickiewicz University esk@amu.edu.pl
STOJANOVIC Vidosava	marijad@abba.rs
STOKES Mathew	University of Texas Southwestern Mathew.Stokes@UTSouthwestern.edu
SUBRAMONY S	University of Florida s.subramony@neurology.ufl.edu
SWANSON Maurice	University of Florida mswanson@ufl.edu
SZNAJDER Lukasz	University of Florida ljsznajder@ufl.edu

T

TAKADA Hiroto	Aomori National Hospital takada@aomorihosp.jp
TAKAHASHI Masanori	Osaka University Graduate School of Medicine mtakahas@sahs.med.osaka-u.ac.jp
TANG Zhenzhi	University of Rochester tangzhenzhi@gmail.com

Attendee Contact Information

T

TANNER Matthew	University of Rochester matttnnr13@gmail.com
TAYLOR Katarzyna	Adam Mickiewicz University taylorka87@gmail.com
THOMAS James	University of Florida jamesdthomas@ufl.edu
THORNTON Charles	University of Rochester charles_thornton@urmc.rochester.edu
TIMCHENKO Lubov	Cincinnati Children's Hospital Lubov.Timchenko@cchmc.org
TIMCHENKO Nick	Cincinnati Children's Hospital Nikolai.Timchenko@cchmc.org
TOMÉ Stephanie	INSERM stephanie.tome@inserm.fr
TSUCHIDA Yuya	tutinoko45@gmail.com

V

VALAPERTA Rea	IRCCS Policlinico San Donato rea.valaperta@grupposandonato.it
VAN CRUCHTEN Remco	Radboud Institute for Molecular Life Sciences remco.vancruchten@radboudumc.nl
VAN DER BENT Leontien	Radboud Institute for Molecular Life Sciences leontien.vanderbent@radboudumc.nl
VAN ENGELEN Baziel	Radboud Institute for Molecular Life Sciences Baziel.vanEngelen@Radboudumc.nl
VANOLI Fiametta	Department of Neuroscience, Mental Health and Sensory Organs, University of Rome "Sapienza" fiammetta.vanoli@gmail.com
VERSNEL Jennifer	Muscular Dystrophy UK J.versnel@musculardystrophyuk.org
VIJAYAN Saji	sajivijayan@lupin.com
VISSING John	Neuromuscular Research Unit, Department of Neurology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark John.Vissing@regionh.dk

Attendee Contact Information

W

WAGNER Stacey	University of Oregon sdwagner@uoregon.edu
WAHBI Karim	Cardiology Department, Cochin hospital karim.wahbi@aphp.fr
WANG Eric	University of Florida eric.t.wang@ufl.edu
WANG Mei	Cincinnati Childrens' Hospital Mei.Wang@cchmc.org
WANSINK Derick	Radboud University rick.wansink@radboudumc.nl
WELSH Lesly	Stanford University lwelsh@stanford.edu
WENNINGER Stephan	Klinikum der Universität München stephan.wenninger@med.uni-muenchen.de
WESTON Richard	godoggo@protonmail.com
WHEELER Thurman	Massachusetts General Hospital twheeler1@mgh.harvard.edu
WHEELER Matthew	Stanford University wheelerm@stanford.edu
WHITE Molly	Myotonic Dystrophy Foundation molly.white@myotonic.org
WIERINGA Bé	Radboud University Be.Wieringa@radboudumc.nl
WINBLAD Stefen	University of Gothenburg, Sweden stefan.winblad@psy.gu.se

X

XIA Guangbin	University of New Mexico guangbin.xia@gmail.com

Attendee Contact Information

Y

YACOUBI-KEYHANI Basma	University of Florida basma@ufl.edu
YEO Gene	University of California San Diego geneyeo@ucsd.edu

Z

ZHANG Nan	Houston Methodist Research Institute nzhang@houstonmethodist.org
ZHANG Fan	Pfizer Inc fan.zhang5@pfizer.com
ZU Tao	University of Florida taozu@ufl.edu

Notes

Notes

Notes

Notes