IDMC 10

INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING

Programme & Abstracts

8 - 12 June 2015

Campus des Cordeliers
Paris, France

www.idmc10.org

Partners

AFMTELETHON
The Marigold Foundation
MYOTONIC DYSTROPHY
MDA
Myotonic Dystrophy
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Organisation :

Index

Bienvenue

IDMC-10

En 1997, les organisateurs et fondateurs des congrès IDMC stipulaient:
"*Cinq années déjà se sont écoulées depuis la découverte de cette étrange mutation instable responsable de la dystrophie myotonique de Steinert. La liste des maladies associées à ce type de mutation continue de s'allonger… Malgré une progression considérable dans le domaine de la connaissance, le mécanisme moléculaire physiopathologique de la dystrophie myotonique de Steinert demeure le plus énigmatique de tous…*"
Claudine Junien and Tee Ashizawa , IDMC1, Paris 1997.

En effet, peu de choses étaient connues sur les conséquences de la mutation à l'origine de cette pathologie. Dans le but d'accélérer la recherche fondamentale et clinique avec l'objectif de savoir comment développer de nouvelles approches thérapeutiques, le consortium international sur la Dystrophie myotonique (DM) a été créé afin de regrouper ensemble non seulement les scientifiques et les cliniciens mais aussi les associations, les malades et leurs familles. Le premier congrès IDMC a été organisé à Paris grâce au soutien capital de l'AFM (Association Française contre les Myopathies) et de la MDA (Muscular Dystrophy Association).

Si l'on considère les 18 ans écoulés depuis, des progrès énormes ont été atteints non seulement pour la DM1 mais aussi pour la DM2, appelée précédemment PROMM. Grâce aux divers congrès IDMC qui se sont déroulés tous les 2 ans dans différentes régions du monde (Paris, 1997, France; Raleigh,1999, USA; Kyoto, 2001, Japon; Glasgow, 2003, UK; Québec, 2005, Canada; Milan, 2007, Italie; Wurzburg, 2009, Allemagne; Clearwater, 2011, USA; San Sebastian, 2013, Espagne) de nombreuses collaborations internationales et fructueuses se sont mises en place. De nouvelles et très actives associations de malades ainsi que, plus récemment, différentes industries pharmaceutiques ont rejoint le consortium dans son effort pour combattre la DM. La « communauté » DM est unique en son genre et a considérablement grandit ses dernières années. Le temps est venu pour le développement de nouvelles thérapies innovatrices pendant que la connaissance sur différents aspects de la maladie (fondamentale, clinique, psychosociale) continue de progresser.

Pour sa 10ième édition, le congrès IDMC est de retour à Paris où il a commencé et si 80 participants étaient présents à l'IDMC1, environ 3 fois plus sont attendus pour la 10ième édition, démontrant encore une fois les nouveaux défis et les forces croissantes consacrées à l'identification des mécanismes impliqués, à l'amélioration de la qualité de vie des malades avec l'espoir de guérir et de vaincre la maladie.

Nous somme heureux de vous accueillir à Paris et espérons que le congrès IDMC10 répondra à toutes vos attentes,

Geneviève Gourdon & Guillaume Bassez
Co-Chairs, IDMC-10 Organizing Committee

Welcome to IDMC-10

In 1997, the founders and organizers of the first IDMC meeting stated:
" Five years have elapsed since the discovery of this strange mutation responsible for myotonic dystrophy. The list of new diseases associated with this type of mutation continues to increase...Despite of the tremendous accumulation of data, the molecular mechanisms underlying the physiopathogenesis of DM remain the most enigmatic…"
Claudine Junien and Tee Ashizawa , IDMC1, Paris 1997.

Indeed, not much was known about the consequences of the DM1 mutation. In order to accelerate clinical and fundamental research, towards the development of new therapeutic strategies, the international DM consortium meeting was created to bring together not only scientists and clinicians but also associations and patients themselves. The first IDMC meeting, IDMC1, was organized in Paris with the substantial help of AFM (Association Française contre les Myopathies) and MDA (Muscular Dystrophy Association).

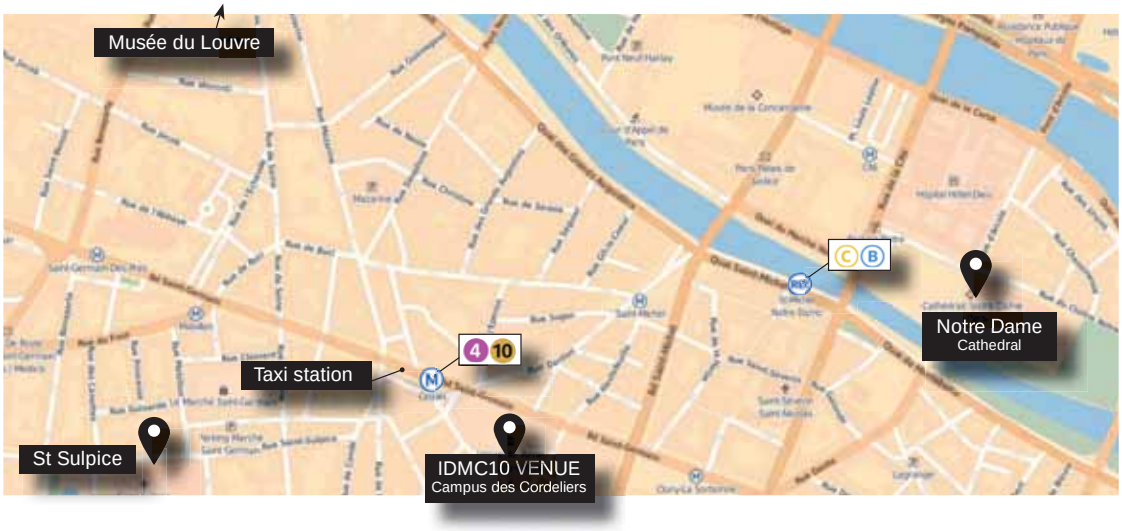
If we look back over the past 18 years, see IDMC1 program in this book, tremendous progress has been reached not only in DM1 but also in DM2 (previously known as PROMM). Through the regular IDMC meetings that were held in various parts of the world every 2 years (Paris, 1997, France; Raleigh, 1999, USA; Kyoto, 2001, Japan; Glasgow, 2003, UK; Québec, 2005, Canada; Milan, 2007, Italy; Wurzburg, 2009, Germany; Clearwater, 2011, USA; San Sebastian, 2013, Spain) fruitful international collaborations have been established. Furthermore, new active patient associations as well as, more recently, pharmaceutical companies have joined in the effort to fight against DM. The DM community and its spirit are unique and has considerably grown over the years. Time comes now for the development of innovative therapeutic strategies, while the knowledge on various aspects of DM (biological, clinical, and psycho-social) is still progressing.

For its 10th edition the IDMC meeting has returned to Paris where it started. While about 80 participants attended to IDMC-1, up to 3 times more attendees are participating in this new edition, demonstrating new challenges and the growing forces devoted to unravel disease mechanisms, to ameliorate DM patients' life and to finally cure the disease.

We are pleased to welcome you all in Paris and we hope that IDMC10 will fill your expectations,

Geneviève Gourdon & Guillaume Bassez
Co-Chairs, IDMC-10 Organizing Committee

Congress venue neigbourhood



How to move around the city

Paris public Transportation

The many **metro lines** and **regional trains** (RER) operate from 6:00am to 12:30am, with a train every 3 to 10 minutes. The public transport system also includes a comprehensive **bus network** and several **tramway routes**.

Within the metro stations, you can buy tickets at the ticket booths and from ticket dispenser machines. Never buy tickets offered by individuals: they might be unsuitable or faulty and cost you a fine.

Public Transportation around the Campus des Cordeliers

Metro	M 4 or 10	at Odéon station
Regional trains (RER)	RER C B	at Saint-Michel Notre-Dame station
Buses	86 87 96	at Saint-Germain Odéon bus stops

TAXI

Taxis do pick up passengers in the street.
Taxis are also available the Odéon taxi station (next to the métro station).
Alternatively, taxis can also be booked 24/7 for an immediate or a later journey through a radio taxi Company such as:

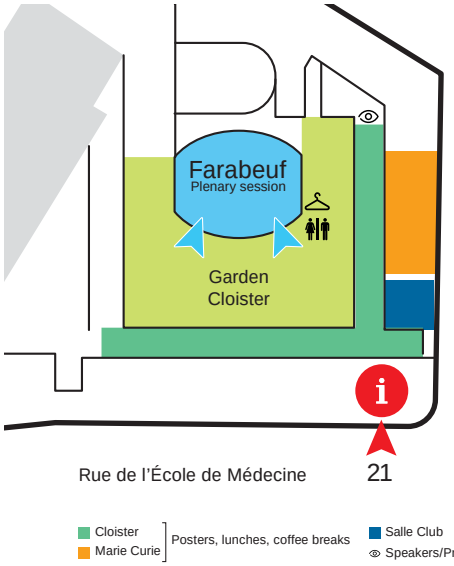
Taxi G7 : 01 47 39 47 39 | Taxis Bleus: 01 49 36 10 10 | Alpha Taxis: 01 45 85 85 85

BIKES

Take a bike, return it where you like, Vélib' is a self-service bike system available 24 hours a day, all year round. To access the service, buy a 1-day or a 7-day ticket online or at any Vélib' station.

Map of the main venue

IDMC-10 Venue : Campus des Cordeliers



The Cordeliers Campus is located at the very center of Paris, at the junction of "The Quartier Latin" and "Saint-Germain-des-Prés".

Built during the XIIIth century, "Les Cordeliers" was originally a Franciscan convent. This old historical site has always been dedicated to science and teaching with a College, part of the University of Paris. The medical tradition of the place was born – and is alive up to now – when a first anatomy lab for surgeons opened in the midst of the XVIth century. During the French Revolution, the monks were expelled and the place became famous for hosting one of the most famous think tank.

"Les Cordeliers" is now dedicated to medical research and teaching.

General informations

Meeting Venue:
Campus des Cordeliers
21 rue de l'Ecole de Médecine – 75006 Paris - France

Registration & Information
Starting on Tuesday June 9, at 7:30, a Registration & Information desk will be available, at the entrance of the Campus des Cordeliers, for the duration of the conference.

Meals:
Welcome cocktail, lunches and coffee-breaks are included in participants' fees.

Social events:
See specific pages in this brochure.

Language:
The official language of the IDMC-10 is English.

Internet:
Free Wi-Fi access is available within the Campus des Cordeliers. Upon request, each participant will be provided with individual access codes valid for the 4 meeting days.

Badge:
All participants must wear their badges visible at all times, within the Campus des Cordeliers as well as during the social events.

Oral Presentations:
Each speaker has a 10 minutes period for slides presentation with an additional five minutes for discussion. The session chairs will rigorously stick to the schedule. Speakers can review their presentation in the Speaker/Preview Room.

Flash Poster Presentations:
Authors selected for a Flash Poster presentation have a 3 minutes period to introduce and summarize orally their abstract in the plenary session room. A maximum of 3 slides is allowed to support this flash introduction.

Posters:
Each author is provided with a board and fixing material to display a poster summarizing his paper. Boards are allocated and identified using the poster code numbers from the book of abstracts. Posters should be attached on their boards on Tuesday June 9, from 730 to 10:00, and removed on Friday June 12, by 14:00.

Organizing Committee

Chairs

Geneviève GOURDON PhD
Institut Imagine, Inserm UMR1163, Paris

Guillaume BASSEZ MD PhD
Centre de Référence Maladies Neuromusculaires, AP-HP, Inserm U955 Créteil

Members

Jean-François BRIAND PhD
Direction scientifique, AFM-Téléthon, Evry

Michel BONNAIRE
Administrateur AFM-Téléthon, famille DM, Evry

Céline DOGAN PhD
Centre de Référence Maladies Neuromusculaires, AP-HP

Bruno EYMARD MD PhD
Institut de Myologie, Paris

Denis FURLING PhD
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Alain GEILLE
Groupe d'Intérêt Steinert, famille DM, AFM-Téléthon, Evry

Mario GOMES-PEREIRA PhD
Institut Imagine, Inserm UMR1163, Paris

Cécile MARTINAT PhD
I-STEM, Inserm UMR 861, Evry

Congress Organization

Agence eWenements
www.ewenements.com

Local Committees

Local Scientific Commitee

Chairs

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Cécile MARTINAT PhD
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Local Associations Committee

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Michel BONNAIRE
Administrateur AFM-Téléthon, famille DM, Evry

Alain GEILLE
Groupe d'Intérêt Steinert, famille DM, AFM-Téléthon, Evry

International Scientific Committee

International Scientific Commitee

Tetsuo ASHIZAWA
University of Florida, USA.

David BROOK
University of Nottingham. Queen's Medical Centre. Nottingham, UK.

John DAY
University of Stanford, USA.

Bruno EYMARD
Institut de Myologie Paris, France.

Geneviève GOURDON
Institut Imagine, Inserm UMR 1163, Paris, France.

Tiemo GRIMM
University of Würzburg, Germany.

Shoichi ISHIURA
University of Tokyo, Japan.

Ralf KRAHE
University of Texas, Houston, USA

Adolfo LÓPEZ DE MUNAIN
University of Basque Country, San Sebastián, Spain.

Mani MAHADEVAN
University of Virginia, USA.

Giovanni MEOLA
University of Milan, Italy.

Darren MONCKTON
University of Glasgow, UK.

Richard MOXLEY
University of Rochester, USA.

Nakaaki OHSAWA
University of Tokyo, Japan.

Christopher PEARSON
University of Toronto, Canada.

Jack PUYMIRAT
Université Laval, Quebec, Canada.

Laura RANUM
University of Florida, USA.

Mark ROGERS
University of Wales, UK.

Benedikt SCHOSER
University of Munich, Germany.

Nicolas SERGEANT
Inserm U837-1, Université de Lille, Lille, France.

Maurice SWANSON
University of Florida, USA.

Charles THORNTON
University of Rochester, USA.

Andoni URTIZBEREA
Hôpital Marin, Hendaye-Hôpitaux de Paris, France.

Bjarne UDD
University of Helsinki, Finland.

Be WIERINGA
University of Nijmegen, The Netherlands**.**

International Associations Commitee

Jean-François BRIAND
AFM-Téléthon, Evry, France

Michel BONNAIRE
Administrateur AFM, famille DM, Evry, France

Margaret BOWLER
Myotonic Dystrophy Support Group, UK

Valerie CWIK
Muscular Dystrophy Association, USA

Alain GEILLE
Groupe d'Intérêt Steinert, AFM-Téléthon, Evry, France

Don MACKENZIE
Marigold Foundation, Canada

Giovanni MEOLA
Fondazione Malattie Miotoniche – FMM, Italy

Molly WHITE
Myotonic Dystrophy Foundation, USA

Partners


AFMTÉLÉTHON
INNOVER POUR GUÉRIR


The Marigold Foundation
Creating Hope & Opportunity


MYOTONIC
DYSTROPHY
FOUNDATION


MDA
Fighting Muscle Disease


MD
Myotonic Dystrophy
SUPPORT GROUP


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Biogen


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PHARMACEUTICALS


genzyme
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Inserm


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aviesan
alliance nationale
pour les sciences de la vie et de la santé

Chairs

Tetsuo ASHIZAWA
University of Florida,
Gainesville, United States.

Guillaume BASSEZ
UPEC Paris Est University, Inserm U955,
Paris, France.

Andy BERGLUND
University of Oregon,
Eugene, United States.

David BROOK
University of Nottingham,
Nottingham, United Kingdom.

Nicolas CHARLET BERGUERAND
IGBMC, Translational Medicine and
Neurogenetics, Illkirch, France.

Thomas COOPER
Baylor College of Medicine,
Bellaire, United States.

John DAY
Stanford University,
Palo Alto, United States.

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Peter HARPER
Institute of Medical Genetics,
Cardiff, United Kingdom

Shoichi ISHIURA
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Tokyo, Japan.

Ralf KRAHE
University of Texas MD Anderson Cancer
Center, Houston, United States.

Mani MAHADEVAN
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Charlottesville, United States.

Cécile MARTINAT
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CECS, Evry, France.

Giovanni MEOLA
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San Donato, San Donato, Italy.

Darren MONCKTON
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Glasgow, United Kingdom.

Richard MOXLEY
University of Rochester, Medical Center,
Rochester, United States.

Christopher PEARSON
University of Toronto,
Toronto, Canada.

Jack PUYMIRAT
University Laval,
Quebec, Canada.

Laura RANUM
University of Florida, Center for Neurogene-
tics, Gainesville, United States.

Benedikt SCHOSER
Friedrich-Baur-Institute, LMU,
Munich, Germany.

Nicolas SERGEANT
INSERM, UMRS 1172 Alzheimer &
Tauopathies, Lille, France.

Lubov TIMCHENKO
CCHMC, University of Cincinnati,
Ohio, United States.

Eric WANG
Massachusetts Institute of Technology,
Boston, United States.

Derick WANSINK
RIMLS, Radboud University Medical Centre,
Nijmegen, The Netherlands.

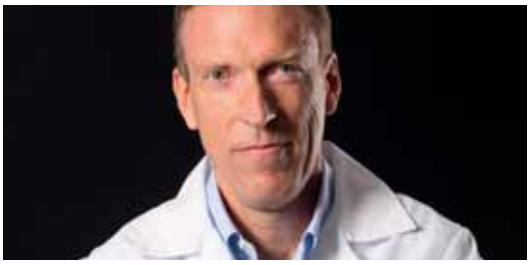
Bé WIERINGA
RIMLS, Radboud University Medical Centre,
Nijmegen, The Netherlands.

Invited Speakers

Tetsuo Ashizawa
University of Florida
Gainesville, Florida, United States

Claudine Junien
Université Versailles St Quentin
Versailles, France

Maurice Swanson
University of Florida College of Medicine
Florida, United States



Charles Thornton
University of Rochester
New York, United States

Friedrich Metzger
University in Freiburg
Germany

Social Activities

Welcome Party

Monday, June 8 - From 19:00
The Registration and Welcome Party are hosted at the Imagine Institute, a research and innovative healthcare institute, dedicated to a better understanding and treatment of genetic diseases.

The Welcome party will be held at Imagine's 7th floor venue and terraces, with an exceptional view on Paris roofs and monuments.

Besides the usual speeches and cocktail, we'll have a memorable live artistic performance by JonOne: the painting of a canvas dedicated to IDMC-10.

Imagine Institute: 24 boulevard du Montparnasse, 75015 Paris
Easy metro access through one of the 2 stations
• Duroc station (Line 10 or 13)
• Falguière station (Line 12)

Château de Versailles Visit & Diner in Versailles

Wednesday, June 10 - From 14:30 to 22:30-23:00

The Château de Versailles, which has been on UNESCO's World Heritage List for 30 years, is one of the most beautiful achievements of 18th-century French art. The site began as Louis XIII's hunting lodge before his son Louis XIV transformed and expanded it, moving the court and government of France from Paris to Versailles in 1682. Each of the three French kings who lived there until the French Revolution contributed to make it more beautiful. From one apartment to the other, from the opera to the chapel, discover an exceptional architecture and a unique interior decoration.

The visit is followed by a dinner in a «Versailles style» venue nearby the Château.

A return bus transfer is organised between Les Cordeliers and the Château de Versailles.
• **Departure from Les Cordelier at 14:30 (sharp!).**
• Return to Les Cordelier at 22:30-23:00

JonOne

“The future is in the canvas”

Born in 1963 in New York USA, based in Paris since 1987, works with alias JonOne.

Andrew John Perello also known as JonOne founded the group 156 All Starz in 1984 and began to work in the world of graffiti at the age of 17. In 1985 he began painting on canvas because it offerd him the opportunity to leave a mark that would stand the test of time. The same year, the gallery owner Rick Librizzi exposed his works in New York.

His first solo exhibition, entitled "Graffitism" took place in 1990 at the Gleditsch Gallery 45 in Berlin.

JonOne has exposed in a multitude of group and solo exhibitions around the world (Tokyo, Monaco, Paris, Geneva, New York, Hong Kong, Brussels ...). His paintings are an explosion of colors, as JonOne describes himself he is an "abstract expressionist graffiti painter."

"I am very grateful to be in a position where I can help to raise awareness and communicate on such an important cause.

I am honored to be invited to paint during the conference and hope that my small contribution can bring positive energy to all. "

For more information on the work Jonone, feel free to visit his website www.jonone.com

Social Activities

Gala Dinner – Cruise on a riverboat

Thursday, June 11
From 19:30 to 23:00

The gala dinner takes place on a riverboat.
The cruise along the Seine banks is one of the best opportunity to discover or rediscover most of the Paris prestigious monuments which have marked history.

Boarding from Escale de Grenelle
(48°51'09.9»N 2°17'07.0»E)
No transportation arranged.
Easy access through the two nearby (300m) metro an RER stations:
- **Metro, Bir Hakeim station (Line 6)**
- **RER, Champ de Mars / Tour Eiffel station (Line C)**

(see map)

Families' Day

IDMC10 meets Families' Day of AFM-Telethon on June 12

Plenary session, from 15:00 to 18:00

Within the beautiful Parc Floral de Paris, AFM-Telethon organizes every two years a unique event for those affected by neuromuscular disorders and their families. Over 2200 people from France and neighbouring countries are expected to come in order to get information, exchange experiences and, share warm and friendly moments.
IDMC10 will join Families' Day on June 12 from 15:00, with dedicated bus departure from the Cordeliers conference center at 13:30. Associations and families that were not participating to IDMC10 may join directly, FREE OF CHARGE, the Families' Day.

Families' Day Program

15:00 : Welcome by **Laurence Tiennot-Herment** (President of AFM-Telethon) + MDA representative (co-founders of the 1st Congress)

15:10 : Synthesis of IDMC10 Scientific Conference by **Jack Puymirat** and intervention by Frank Bennett (ISIS)

15:30 : Presentation of the Steinert Task Force set up by **AFM-Telethon**

15:40 : Synthesis of the Associations Workshop by **Jean-François Briand** and intervention of associations

15:55 : Questions / Answers session, chaired by **Alain Geille, Guillaume Bassez** and **Geneviève Gourdon**
The questions will have been collected upstream by the Associations and a selection of 20 + 2/3 additional questions from the audience will be discussed; 2/3 testimonials from families will be presented.

17:55 : Conclusion by **Laurence Tiennot-Herment** – Introduction to the remaining festive events (researchers/patients discussion area / buffet areas)

Information Village & Buffet after 18:00

At 18:00, the IDMC10 participants will discover the **Information Village of the Families' Day** in which more than 130 doctors and researchers, as well as experts of technical aids, care and day to day issues will answer families' questions.
Not to mention from 19:00, the exceptional **French Regions Buffet**: a fabulous tour of France's gastronomic specialties! A unique experience not to be missed!
Shuttles will be available to bring you back to the closest metro stations.

Programme at a glance

Programme at a glance

Monday, June 8

08:30 - 17:00	**OMMYD** Organized by Cynthia Gagnon
	Per Invitation
17:00 - 23:00	**REGISTRATION & WELCOME COCKTAIL** At Imagine Institute - 24 bd du Montparnasse - 75006 Paris
17:00 - 19:00	**Registration**
19:00 - 23:00	**Welcome Cocktail** • Welcome address • Live painting performance by JonOne

Programme at a glance

Tuesday, June 9

08:30 - 10:15	**INVITED LECTURE** Chairs : Bé WIERINGA, Tetsuo ASHIZAWA
08:30 - 09:20	**Sex-specific epigenetics in transgenerational responses to environmental impacts** Claudine JUNIEN
09:20 - 10:15	**The expanding future of myotonic dystrophy research** Maurice SWANSON - Charles THORNTON
10:15 - 10:45	Coffee break
10:45 - 11:45	**Session 1: Mutation, genetic and epigenetic** Chairs: Christopher PEARSON, Darren MONCKTON
	Oral presentations S1-O1 to S1-O4
11:45 - 12:30	**Session 2: Clinical and social issues** Chairs: Bruno EYMARD, Peter HARPER
	Oral presentations S2-O1 to S2-O3
12:30 - 13:00	**Flash Poster session 1 (FP1)** Chairs: Cécile MARTINAT, Mani MAHADEVAN
	Flash Poster presentations FP1-01 to FP1-06
13:00 - 14:00	Lunch and Posters viewing
14:00 - 15:00	**Poster session**
	Poster viewing and discussion with presenters
15:00 - 16:30	**Session 2: Clinical and social issues (cont.)** Chairs: Bruno EYMARD, Peter HARPER
	Oral presentations S2-O4 to S2-O9
16:30 - 17:00	Coffee break
17:00 - 18:45	**Session 2: Clinical and social issues (cont.)** Chairs: Giovanni MEOLA, John DAY
	Oral presentations S2-O10 to S2-O16
19:30 - 23:00	Chair/speaker Dinner

Programme at a glance

Wednesday, June 10

08:15 - 10:30	**Session 3: Disease mechanisms** Chairs: Nicolas CHARLET-BERGUERAND, Laura RANUM
	Oral presentations S3-O1 to S3-O9
10:30 - 11:00	Coffee break
11:00 - 13:00	**Session 3: Disease mechanisms** Chairs: Lubov TIMCHENKO, Shoichi ISHIURA
	Oral presentations S3-O10 to S3-O17
13:00 - 13:30	**Flash Poster session 2 (FP2)** Chairs: Eric WANG, Mario GOMES-PEREIRA
	Flash Poster presentations FP2-01 to FP2-06
13:30 - 14:30	Lunch and Posters viewing
14:30 - 19:30	**Château de Versailles Visit** Bus transfer from Les Cordeliers
19:30 - 23:00	**Dinner in Versailles** Return transfer to Center of Paris

Programme at a glance

Thursday, June 11

8:30 - 9:20	**INVITED LECTURE** Chairs : David BROOK
	Targeting selective SMN2 splicing modification for therapy in spinal muscular atrophy Friedrich METZGER
9:20 - 10:20	**Session 4: Therapeutic development** Chairs: Denis FURLING, Andy BERGLUND
	Oral presentations S4-O1 to S4-O4
10:20 - 11:00	Coffee break
11:00 - 12:30	**Session 4: Therapeutic development (cont.)** Chairs: Derick WANSINK, Thomas COOPER
	Oral presentations S4-O5 to S4-O10
12:30 - 13:00	**Flash Poster session 3 (FP3)** Chairs: Ralf KRAHE, Benedikt SCHOSER
	Flash Poster presentations FP3-01 to FP3-06
13:00 - 14:00	Lunch and Posters viewing
14:00 - 15:30	**Poster session**
	Poster viewing and discussion with presenters
15:30 - 16:00	Coffee break
16:00 - 18:00	**Associations Workshop and Posters viewing**
	Associations Workshop in Salle Club
	Poster viewing
19:30 - 23:00	**Gala Dinner** Meeting at Port de Grenelle

Programme at a glance
Friday, June 12

8:30 - 10:15	**Session 5: Biomarkers/Outcome measures/registry/therapeutic assays** Chairs: Richard MOXLEY, Arregui DEMUNAIN
	Oral presentations S5-O1 to S5-O7
10:15 - 10:45	Coffee break
10:45 - 11:45	**Session 5: Biomarkers/Outcome measures/registry/therapeutic assays (cont.)** Chairs: Jack PUYMIRAT, Nicolas SERGEANT
	Oral presentations S5-O8 to S5-O11
11:45 - 13:00	**Farewell session** Chairs: Geneviève GOURDON, Guillaume BASSEZ
11:45 - 12:00	**Anniversary & IDMC-10 Conclusion** Tetsuo ASHIZAWA
12:00 - 12:15	**Film Testimony** Jaqueline DONACHIE
12:15 - 13:00	**Prizes**
13:00 - 20:00	**IDMC-10 meets AFM-Téléthon Families' Day** in Parc Floral de Vincennes
13:30 - 15:00	Shuttle transfer to the Parc Floral de Vincennes & Lunch
15:00 - 18:00	• **Welcome words by the co-founders of the 1st IDMC Meeting** • **Synthesis of IDMC10 Scientific Conference** • **Presentation of the Steinert Task Force set up by AFM-Telethon** • **Synthesis of the Associations Workshop held during IDMC-10** • **International Questions / Answers session** • **Conclusion – Introduction to the remaining festive events of the evening**
From 18:00	**Opening of the Families' Day Information Village** More than 130 doctors and researchers, as well as experts of technical aids, care and day to day issues are available to answer families' questions
From 19:00	**Opening of AFM-Téléthon's French Regions Buffet** A fabulous tour of France's gastronomic specialties. A unique experience not to be missed!
	Shuttle transfer to the Porte de Vincennes métro station

Detailed Programme

Detailed Programme

Monday, June 8

08:30 - 17:00	**OMMYD** Organized by Cynthia Gagnon
	Per Invitation
17:00 - 23:00	**REGISTRATION & WELCOME COCKTAIL** At Imagine Institute - 24 bd du Montparnasse - 75006 Paris
17:00 - 19:00	**Registration**
19:00 - 23:00	**Welcome Cocktail** • Welcome address • Live painting performance by JonOne

Detailed Programme

Tuesday, June 9

08:30 - 10:15	**INVITED LECTURE** Chairs : Bé WIERINGA, Tetsuo ASHIZAWA
08:30 - 09:20	**Sex-specific epigenetics in transgenerational responses to environmental impacts** Claudine JUNIEN
09:20 - 10:15	**The expanding future of myotonic dystrophy research** Maurice SWANSON - Charles THORNTON
10:15 - 10:45	Coffee break
10:45 - 11:45	**Session 1: Mutation, genetic and epigenetic** Chairs: Christopher PEARSON, Darren MONCKTON
10:45 - 11:00	S1-O1: **Progression of the somatic mutational dynamics of the CTG repeat is not lineal or proportional to the estimated progenitor allele size in DM1 patients** Fernando MORALES et al. Instituto de Investigaciones en Salud (INISA)-Escuela de Medicina-Universidad de Costa Rica.
11:00 - 11:15	S1-O2: **Normal DM1 alleles contain no variant repeats or flanking mutations, suggesting these are secondary to repeat expansion in patients.** Sarah CUMMING et al. Molecular Cell and Systems Biology University of Glasgow
11:15 - 11:30	S1-O3: **MSH3 Overexpression Pancreas destabilizes CTG Triplet repeat in DM1 Mice Carrying 55 OR ~500 CTG Repeats** Stéphanie TOME et al. Inserm UMR 1163 - Paris Descartes – Sorbonne Paris Cité University-Imagine Institute.
11:30 - 11:45	S1-O4: **Population distribution of healthy range and premutation range (CCTG)n alleles of the myotonic dystrophy type 2 locus** Jan RADVANSZKY et al. Center for Molecular Medicine-Slovak Academy of Sciences-Bratislava-Slovakia
11:45 - 12:30	**Session 2: Clinical and social issues** Chairs: Bruno EYMARD, Peter HARPER
11:45 - 12:00	S2-O1: **Survival of Permanently Paced Patients with Myotonic Dystrophy Type 1 - Sudden Death Predictors and Role of Implantable Cardiac Defibrillators** Karim WAHBI et al. APHP - Cochin Hospital - Cardiology Department - Paris-Descartes - Sorbonne Paris Cité University - 75006 Paris - France - Myology Institute - Neurology Department - Pitié-Salpêtrière Hospital - Paris - France
12:00 - 12:15	S2-O2: **Cutaneous features of myotonic dystrophy type 1 and type 2** Roberto MASSA et al. Department of Systems Medicine Division of Neurology Tor Vergata University Rome Italy
12:15 - 12:30	S2-O3: **Dysregulation of Calcium Metabolism in Type 1 Myotonic Dystrophy** Phyu HLAING et al. Greenslopes Hospital
12:30 - 13:00	**Flash Poster session 1 (FP1)** Chairs: Cécile MARTINAT, Mani MAHADEVAN
12:30 - 12:35	FP1-1: **DMPK gene DNA methylation is correlated with cognitive and respiratory profiles in patients affected with myotonic dystrophy type 1** Cécilia LÉGARÉ et al. Département de biochimie - Faculté de médecine et des sciences de la santé-Université de Sherbrooke. ECOGENE-21 et Clinique des maladies lipidiques - CSSS de Chicoutimi

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12:35 - 12:40	FP1-2: **DM1 CpG methylation profile in DM1 patients: correlation with clinical and molecular features and effects on DMPK and SIX5 expression** Massimo SANTORO et al. Fondazione Don Carlo Gnocchi-Milan-Italy
12:40 - 12:45	FP1-3: **Development and validation of a new genetic assay for detection of myotonic dystrophy type 2** Rea VALAPERTA et al. Research Laboratories-IRCCS Policlinico San Donato-Milan-Italy
12:45 - 12:50	FP1-4: **Predictors of change in daytime sleepiness: preliminary findings of a 9-year longitudinal study in adults with DM1** Luc LABERGE et al. ÉCOBES – Recherche et transfert. Université du Québec à Chicoutimi
12:50 - 12:55	FP1-5: **A Longitudinal Study of Autism Spectrum Disorders in Children, Adolescents and Young Adults with Congenital and Childhood Myotonic Dystrophy Type 1** Anne-Berit EKSTRÖM et al. Department of Pediatrics - Institute of Clinical Sciences - The Queen Silvia Children´s Hospital - Sahlgrenska Academy at the University of Gothenburg - Sweden
12:55 - 13:00	FP1-6: **Childhood DM1 and Autism Spectrum Disorders: Is there a comorbidity?** Nathalie ANGEARD et al. Inserm U1129 - Université Paris Descartes
13:00 - 14:00	Lunch and Posters viewing
14:00 - 15:00	**Poster session**
	Poster viewing and discussion with presenters
15:00 - 16:30	**Session 2: Clinical and social issues (cont.)** Chairs: Bruno EYMARD, Peter HARPER
15:00 - 15:15	S2-O4: **Executive cognitive dysfunction in adult onset Myotonic Dystrophy type 1** Stefan WINBLAD et al. Department of Psychology-University of Gothenburg-Sweden
15:15 - 15:30	S2-O5: **Personality of DM1 Patients: Dimensional Approach from the Five Factor Model** Baptiste LIGNIER et al. Reference Center for Neuromuscular Diseases Hôpital Henri Mondor APHP Créteil France - Laboratory Cognition Santé Socialisation (C2S) EA 6291 Université Reims Champagne Ardennes Reims France
15:30 - 15:45	S2-O6: **Social participation in myotonic dystrophy type 1: a 9-years follow-up study** Kateri RAYMOND et al. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires (GRIMN) Jonquière Québec (Canada) / Faculty of Medicine and Health Sciences Sherbrooke University Québec (Canada)
15:45 - 16:00	S2-O7: **A 9-year longitudinal study of cognition in myotonic dystrophy adult phenotypes** Benjamin GALLAIS et al. Université de Sherbrooke
16:00 - 16:15	S2-O8: **A Longitudinal Assessment of Cognitive and Adaptive Functioning in the Congenital and Childhood Forms of Myotonic Dystrophy Type 1** Anne-Berit EKSTRÖM et al. Department of Pediatrics - Institute of Clinical Sciences - The Queen Silvia Children´s Hospital - Sahlgrenska Academy at the University of Gothenburg-Sweden
16:15 - 16:30	S2-O9: **Brain Tumors in Patients with Myotonic Dystrophy: A Population-based Study** Shahinaz GADALLA et al. National Cancer Institute, USA
16:30 - 17:00	Coffee break

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17:00 - 18:45	**Session 2: Clinical and social issues (cont.)** Chairs: Giovanni MEOLA, John DAY
17:00 - 17:15	**S2-O10: Results from the PRISM-2 Study: Patient Reported Impact of Symptoms in Myotonic Dystrophy Type 2** Chad HEATWOLE et al. University of Rochester
17:15 - 17:30	**S2-O11: Survey to Assess Fall History and Balance Confidence in Individuals with Myotonic Dystrophy (DM)** Katy EICHINGER et al. University of Rochester
17:30 - 17:45	**S2-O12: Association between lower limb muscle strength and functional impairments in adult and late onset phenotype of DM1** Emilie PETITCLERC et al. Faculty of Medecine and Health Sciences Sherbrooke University Sherbrooke Canada - Groupe de recherche interdisciplinaire sur les maladies neuromusculaires Neuromuscular Clinc Centre de Santé et de services Sociaux de Jonquière Jonquière Canada
17:45 - 18:00	**S2-O13: Diagnostic delay in myotonic dystrophy type 1 depends on the DM1 category** Christopher LINDBERG et al. Neuromuscular Center Sahlgrenska University Hospital Gothenburg Sweden
18:00 - 18:15	**S2-O14: Biographical disruption: shedding light on myotonic dystrophy type 1 and reproduction** Kori A LADONNA et al. Western University Schulich School of Medicine & Dentistry-Centre for Education Research & Innovation
18:15 - 18:30	**S2-O15: DM-Scope, a French nationwide registry to decipher pediatric myotonic dystrophies' clinical complexity** Emmanuelle LAGRUE et al. CHRU de Tours - Université François Rabelais de Tours - INSERM U930
18:30 - 18:45	**S2-O16: A cross-sectional evaluation of disease progression in Congenital Myotonic Dystrophy** Nicholas JOHNSON et al. University of Utah- Department of Neurology
19:30 - 23:00	Chairs/speakers Dinner

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Wednesday, June 10

08:15 - 10:30	**Session 3: Disease mechanisms** Chairs: Nicolas CHARLET-BERGUERAND, Laura RANUM
08:15 - 08:30	S3-O1: **The Human Myotonic Dystrophy Transcriptome** Eric T. WANG et al. Massachusetts Institute of Technology
08:30 - 08:45	S3-O2: **MBNLs regulate MBNL1 function by binding to the 5'UTR of MBNL1 mRNA and pre-mRNA** Patryk KONIECZNY et al. Department of Gene Expression; Institute of Molecular Biology and Biotechnology; Adam Mickiewicz University; Poznan; Poland
08:45 - 09:00	S3-O3: **Elucidating the relationship between higher order MBNL1 domain organization and splicing activity** Melissa A. HALE et al. Department of Chemistry and Biochemistry - Institute of Molecular Biology - University of Oregon
09:00 - 09:15	S3-O4: **MBNL1 regulates alternative splicing through finely tuned switch-like and rheostat mechanisms: implications for DM Biomarkers** Adam J. STRUCK et al. University of Oregon
09:15 - 09:30	S3-O5: **Muscleblind-like compound knockout mice recapitulate Tau mis-splicing in DM brain** Kuang-Yung LEE et al. Department of Molecular Genetics and Microbiology- Center for NeuroGenetics and the Genetics Institute- University of Florida- College of Medicine-Gainesville- Florida- USA. Department of Neurology- Chang Gung Memorial Hospital Keelung-Taiwan
09:30 - 09:45	S3-O6: **Compromised MBNL activity in myotonic dystrophy leads to disruption of developmentally regulated alternative polyadenylation** Ranjan BATRA et al. Department of Cellular and Molecular Medicine Institute for Genomic Medicine UCSD Stem Cell Program University of California San Diego CA USA
09:45 - 10:00	S3-O7: **Abnormal splicing switch of DMD exon 78 compromises muscle fiber maintenance in Myotonic Dystrophy** Frédérique RAU et al. Sorbonne Universités - UPMC Univ Paris 06 - INSERM UMRS974 - CNRS FRE3617 - Center for Research in Myology - Institut de Myologie - GH Pitié-Salpêtrière – Paris - France
10:00 - 10:15	S3-O8: **Implication of BIN1 in Myotonic dystrophy** Michel NEY et al. IGBMC, Illkirch - FRANCE
10:15 - 10:30	S3-O9: **Mutant Repeats vs. Resident Gene: Does CNBP Play a Role in Myotonic Dystrophy Type 2 (DM2)?** Ralf KRAHE et al. Department of Genetics - University of Texas MD Anderson Cancer Center - Houston - TX - USA
10:30 - 11:00	Coffee break
11:00 - 13:00	**Session 3: Disease mechanisms** Chairs: Lubov TIMCHENKO, Shoichi ISHIURA
11:00 - 11:15	S3-O10: **Bidirectional transcription and RAN translation from CCTG·CAGG expansions in DM2 patient brains** Tao ZU et al. Center for NeuroGenetics-Department of Molecular Genetics and Microbiology Genetics Institute-College of Medicine University of Florida Gainesville FL USA

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Wednesday, June 10

11:15 - 11:30	**S3-O11: RNA gain-of-function and RAN translation in mouse models of myotonic dystrophy type 2** John CLEARY et al. Center for NeuroGenetics. Department of Molecular Genetics and Microbiology
11:30 - 11:45	**S3-O12: Novel insight in antisense transcription in the DM1 locus: the DM1-AS gene and its products** Derick WANSINK et al. Department of Cell Biology-Radboud Institute for Molecular Life Sciences-Radboud University Medical Centre-Nijmegen-The Netherlands
11:45 - 12:00	**S3-O13: Use of CRISPR/Cas9-based genome editing for the generation of isogenic cell models to study repeat length effects in DM1** Ellen VAN AGTMAAL et al. Department of Cell Biology Radboud Institute for Molecular Life Sciences Radboud University Medical Center Nijmegen he Netherlands
12:00 - 12:15	**S3-O14: Cerebellum dysfunction in a mouse model of myotonic dystrophy is mediated by glutamate transport misregulation** Mario GOMES-PEREIRA et al. Inserm UMR1163 - Université Paris Descartes – Sorbonne Paris Cite. Imagine Institute. Paris. France.
12:15 - 12:30	**S3-O15: An inducible expanded CUG-repeat mouse model recapitulates skeletal and cardiac muscle phenotypes of DM1** Ginny MORRISS et al. Department of Pathology and Immunology - Baylor College of Medicine
12:30 - 12:45	**S3-O16: Innate Immunity and RNA Toxicity** Mani S. MAHADEVAN et al. University of Virginia, Department of Pathology, Charlottesville, VA, 22908, USA
12:45 - 13:00	**S3-O17: Misregulation of the alternative splicing of the cardiac sodium channel SCN5A is associated with cardiac conduction delay and heart arrhythmia in myot** Nicolas CHARLET-BERGUERAND et al. IGBMC, Translational Medicine and Neurogenetics, ILLKIRCH
13:00 - 13:30	**Flash Poster session 2 (FP2)** Chairs: Eric WANG, Mario GOMES-PEREIRA
13:00 - 13:05	**FP2-1: rbFox1 rescues CCUG, but not CUG toxicity, in Drosophila Myotonic Dystrophy models** Beatriz LLAMUSI et al. Incliva Health Research Institute & Department of Genetics, University of Valencia, Valencia, Spain
13:05 - 13:10	**FP2-2: RBFOX1 Modulation of MBNL1 Dependent Alternative Splicing Events in a DM1 Cell Model** Sunny C. KETCHUM et al. Department of Chemistry and Biochemistry - Institute of Molecular Biology - University of Oregon
13:10 - 13:15	**FP2-3: Differences in activity of three MBNL paralogs** Łukasz SZNAJDER et al. Department of Gene Expression - Institute of Molecular Biology and Biotechnology - Faculty of Biology - Adam Mickiewicz University - Umultowska 89 - 61-614 Poznań - Poland
13:15 - 13:20	**FP2-4: Aberrant methylation of the CTCF binding site is associated with antisense transcription and disease severity in congenital myotonic dystrophy** Masayuki NAKAMORI et al. Osaka University Graduate School of Medicine

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13:20 - 13:25	FP2-5: **Conditional HSAXLR transgenic mouse model with targeted single-copy integration** Zhenzhi TANG et al. Department of Neurology - University of Rochester Medical Center - NY USA
13:25 - 13:30	FP2-6: **Early hallmarks neurofibrillary degeneration and cognitive impairment in a double human Tau x DM1 mouse model** Cyril LAURENT et al. Inserm UMR S1172 - Alzheimer & Tauopathies - Jean-Pierre Aubert Research Centre - Faculty of Medicine - IMPRT - Lille - France
13:30 - 14:30	Lunch and Posters viewing
14:30 - 19:30	**Château de Versailles Visit** Bus transfer from Les Cordeliers
19:30 - 23:00	**Dinner in Versailles** Return transfer to Center of Paris / Les Cordeliers

Detailed Programme

Thursday, June 11

8:30 - 9:20	**INVITED LECTURE** Chairs : David BROOK
	Targeting selective SMN2 splicing modification for therapy in spinal muscular atrophy Friedrich METZGER
9:20 - 10:20	**Session 4: Therapeutic development** Chairs: Denis FURLING, Andy BERGLUND
09:20 - 09:35	S4-O1: **Therapeutic impact of systemic AAV-mediated RNA interference in the HSALR mouse model of myotonic dystrophy** Joel R. CHAMBERLAIN et al. Division of Medical Genetics- Department of Medicine- University of Washington- Seattle- WA 98195 USA
09:35 - 09:50	S4-O2: **Utilization of Toxic RNA Expansions to Synthesize Their Own Inhibitors In Cellulo** Suzanne RZUCZEK et al. The Scripps Research Institute
09:50 - 10:05	S4-O3: **Small Molecules that Control the Fate of CUG repeat RNA transcripts, the Causative Agent in Myotonic Dystrophy Type 1** Lien NGUYEN et al. Department of Chemistry-University of Illinois-Urbana-IL-USA-61801
10:05 - 10:20	S4-O4: **High Content Screening approach using DM1 mutated human Embryonic Stem cell derived cellular model** Yves MAURY et al. I-STEM. CECS
10:20 - 11:00	Coffee break
11:00 - 12:30	**Session 4: Therapeutic development (cont.)** Chairs: Derick WANSINK, Thomas COOPER
11:00 - 11:15	S4-O5: **A High-throughput Screening System for Up-regulation of Endogenous MBNL1 as a Potential Therapy for Type 1 Myotonic Dystrophy** Fan ZHANG et al. Pfizer Inc. Rare Disease Research Unit
11:15 - 11:30	S4-O6: **A novel genome editing-based strategy for Myotonic Dystrophy type 1** Mirella LO SCRUDATO et al. Genethon - INSERM UMR 951 - Evry France
11:30 - 11:45	S4-O7: **Highly specific contractions of CAG/CTG trinucleotide repeat by TALEN** Guy-Franck RICHARD et al. Unité de Génétique moléculaire des levures, Institut Pasteur, CNRS UMR 3525
11:45 - 12:00	S4-O8: **Congenital and Acquired Dmpk Reduction Does Not Affect Cardiac Conduction or Ejection Fraction in Mice.** Samuel CARRELL et al. Department of Biomedical Genetics University of Rochester
12:00 - 12:15	S4-O9: **Reduction of CUGexp RNA expression is insufficient to correct myopathy in DM1 mice** Thurman WHEELER et al. Department of Neurology - Massachusetts General Hospital - Boston MA
12:15 - 12:30	S4-O10: **Correction of the GSK-3beta-kinase - CUGBP1 signaling as therapeutic approach in adult and in congenital Myotonic Dystrophy type 1** Christina WEI et al. Division of Neurology Cincinnati Childrens
12:30 - 13:00	**Flash Poster session 3 (FP3)** Chairs: Ralf KRAHE, Benedikt SCHOSER
12:30 - 12:35	FP3-1: **Are Zinc Finger Nucleases Suitable Therapeutic Agents Against Myotonic Dystrophy Type 1?** Cinzia CINESI et al. Center for Integrative Genomics - University of Lausanne - 1015 Lausanne

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12:35 - 12:40	**FP3-2: Blockade of expanded microsatellite repeat transcription by CRISPR/dCas9** Tanvi SAXENA et al. Massachusetts Institute of Technology
12:40 - 12:45	**FP3-3: Oligonucleotides target mutant DMPK transcripts in human DM1 neuronal cells** Siham AIT BENICHOU et al. Unit of Human Genetics CRCHUQ Quebec
12:45 - 12:50	**FP3-4: Regional body composition and clinical outcome measures in patients with myotonic dystrophy type 1 (DM1)** Saam SEDEHIZADEH et al. University of Nottingham, UK
12:50 - 12:55	**FP3-5: Multiplex Alternative Splice Sequencing (MAS-Seq) for Analysis of Splicing Biomarkers of Myotonic Dystrophy Type 1 (DM1)** Wenli WANG et al. Department of Neurology-University of Rochester Medical Center
12:55 - 13:00	**FP3-6: Skeletal muscle and circulating micro RNA in myotonic dystrophy type 1.** Alessandra PERFETTI et al. Molecular Cardiology Laboratory-Policlinico San Donato-IRCCS-San Donato Milanese-Milan-Italy
13:00 - 14:00	Lunch and Posters viewing
14:00 - 15:30	**Poster session**
	Poster viewing and discussion with presenters
15:30 - 16:00	Coffee break
16:00 - 18:00	**Associations Workshop and Posters viewing**
	Associations Workshop (in the Club Room)
	Poster viewing
19:30 - 23:00	Gala Dinner Meeting at Port de Grenelle

Detailed Programme
Friday, June 12

8:30 - 10:15	**Session 5: Biomarkers/Outcome measures/registry/therapeutic assays** Chairs: Richard MOXLEY, Arregui DEMUNAIN
08:30 - 08:45	**S5-O1: DM-SCOPE, the international DM1 registry, sheds light on clinical classification** Céline DOGAN et al. Centre de Référence Maladies Neuromusculaires CHU Henri Mondor Créteil France.
08:45 - 09:00	**S5-O2: Natural history of motor impairment in myotonic dystrophy type 1 (DM1)** Katy EICHINGER et al. University of Rochester
09:00 - 09:15	**S5-O3: The Myotonic Dystrophy Health Index: Correlations with Clinical Tests and Patient Function** Chad HEATWOLE et al. University of Rochester
09:15 - 09:30	**S5-O4: Report from the Outcome Measure in Myotonic Dystrophy (OMMYD-3) meeting** Cynthia GAGNON et al. Université de Sherbrooke. Groupe de recherche interdisciplinaire sur les maladies neuromusculaires/CSSS de Jonquière
09:30 - 09:45	**S5-O5: Is one trial enough? - Assessments of walking, mobility, balance and fine hand use in people with myotonic dystrophy type 1** Marie KIERKEGAARD et al. Karolinska University Hospital - Karolinska Institutet
09:45 - 10:00	**S5-O6: Identification of outcome measures to quantify short-term skeletal muscle strength impairments in DM1** Jack PUYMIRAT et al. Unit of Human Genetics CRCHUQ Québec
10:00 - 10:15	**S5-O7: What are the Appropriate Cardiac Outcome Measures to Include in Phase 2, 3 Myotonic Dystrophy Type 1 Drug Trials?** William GROH et al. Univ. of South Carolina. WJB Dorn VAMC
10:15 - 10:45	Coffee break
10:45 - 11:45	**Session 5: Biomarkers/Outcome measures/registry/therapeutic assays (cont.)** Chairs: Jack PUYMIRAT, Nicolas SERGEANT
10:45 - 11:00	**S5-O8: OPTIMISTIC: Observational Prolonged Trial In Myotonic dystrophy type 1 to Improve Quality of Life- Standards, a Target Identification Collaboration.** Baziel VAN ENGELEN et al. Radboud University Medical Center The Netherlands
11:00 - 11:15	**S5-O9: Widespread networks' disconnection accounts for clinical symptoms in Myotonic dystrophy Type-1** Marco BOZZALI et al. Neuroimaging Laboratory IRCCS Santa Lucia Foundation Rome Italy
11:15 - 11:30	**S5-O10: Tracking the brain in myotonic dystrophy type 1 (DM1) and 2 (DM2): A 5-year longitudinal neuroimaging and neuropsychological follow-up study** Carla MERKEL et al. Department of Neurology - University Hospital of Bonn – Bonn - Germany
11:30 - 11:45	**S5-O11: Quantification of CUG-Repeat RNA Mass Using a Group II Intron Reverse Transcriptase** Samuel CARRELL et al. Department of Biomedical Genetics University of Rochester
11:45 - 13:00	**Farewell session** Chairs: Geneviève GOURDON, Guillaume BASSEZ
11:45 - 12:00	**Anniversary & IDMC-10 Conclusion** Tetsuo ASHIZAWA
12:00 - 12:15	**Film Testimony** Jaqueline DONACHIE

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Friday, June 12

12:15 - 13:00	**Prizes**
13:00 - 20:00	**IDMC-10 meets AFM-Téléthon at Families' Day** in Parc Floral de Vincennes
13:30 - 15:00	Shuttle transfer to the Parc Floral de Vincennes & Lunch
15:00 - 15:10	**Welcome words by the co-founders of the 1st IDMC Meeting** Laurence Tiennot-Herment (President of AFM-Telethon) & MDA representative
15:10 - 15:30	**Synthesis of IDMC10 Scientific Conference** Jack Puymirat & Frank Bennett (ISIS Pharmaceuticals)
15:30 - 15:40	**Presentation of the Steinert Task Force set up by AFM-Telethon**
15:40 - 15:55	**Synthesis of the Associations Workshop held during IDMC-10** Jean-François Briand & Associations
15:55 - 17:55	**International Questions / Answers session** Chaired by Alain Geille, Guillaume Bassez and Geneviève Gourdon
17:55 - 18:00	**Conclusion – Introduction to the remaining festive events of the evening** Laurence Tiennot-Herment
From 18:00	**Opening of the Families' Day Information Village** More than 130 doctors and researchers, as well as experts of technical aids, care and day to day issues are available to answer families' questions
From 19:00	**Opening of AFM-Téléthon's French Regions Buffet** A fabulous tour of France's gastronomic specialties. A unique experience not to be missed!
	Shuttle transfer to the Porte de Vincennes métro station

Oral Presentation Abstracts

S1-O1

1: Mutation, genetics and epigenetics

Progression of the somatic mutational dynamics of the CTG repeat is not lineal or proportional to the estimated progenitor allele size in DM1 patients

MORALES Fernando, Instituto de Investigaciones en Salud (INISA)-Escuela de Medicina-Universidad de Costa Rica San José Costa Rica
VÁSQUEZ Melissa, Instituto de Investigaciones en Salud (INISA)-Universidad de Costa Rica San José Costa Rica
SANTAMARÍA Carolina, Instituto de Investigaciones en Salud (INISA)-Escuela de Nutrición-Universidad de Costa Rica San José Costa Rica
VINDAS Rebeca, Instituto de Investigaciones en Salud (INISA)-Universidad de Costa Rica San José Costa Rica
CORRALES Eyleen, Instituto de Investigaciones en Salud (INISA)-Universidad de Costa Rica San José Costa Rica
ZHANG Baili, Department of Genetics-University of Texas MD Anderson Cancer Center Houston TX USA
SIRITO Mario, Department of Genetics-University of Texas MD Anderson Cancer Center Houston TX USA
CUENCA Patricia, Instituto de Investigaciones en Salud (INISA)-Escuela de Medicina-Universidad de Costa Rica San José Costa Rica
DEL VALLE Gerardo, Laboratorio de Neurofisiología (Neurolab) Curridabat San José Costa Rica
BRIAN Roberto, Servicio de Neurología-Hospital Nacional de Niños San José Costa Rica
SITTENFELD Mauricio, Servicio de Neurología-Hospital San Juan de Dios San José Costa Rica
KRAHE Ralf, Department of Genetics-University of Texas MD Anderson Cancer Center Houston TX USA
MONCKTON Darren, Institute of Molecular Cell and Systems Biology-College of Medical Veterinary and Life Sciences-University of Glasgow UK

Somatic mosaicism in myotonic dystrophy type 1 (DM1) is age-dependent, tissue-specific and expansion-biased, but there are no detailed data regarding the mutational dynamics during the lifespan in DM1 patients. By collecting a second and a third blood sample from the same individual, we investigated how the mutational dynamics progresses with time. Using SP-PCR we determined the lower boundary of the total allele distribution and measured the degree of somatic mosaicism in a cohort of DM1 patients sampled at three time points (TP) separated in total by 8-15 years. Interestingly, conservation of the lower boundary changes even in the same sample at different time points, when conserved. The detailed analysis of somatic instability indicated that the modal allele length and the degree of somatic instability increase with time ($p<0.05$), but their differences between time points are not ($p>0.05$). The increment in SV did not correlate with time ($p>0.05$) but it did with ePAL ($p<0.05$), and the increment in average correlated with time ($p>0.05$) but did not with ePAL ($p<0.05$), suggesting than in some samples the frequency of expansions and contractions are higher (or slower) than in other samples. Regarding the mutation rate, we observed that at some point, it became negative in some samples, probably due to the presence of contractions. We did not observed any differences on mutation rate between TP1-TP2 ($p>0.05$) but yes between TP2-TP3 ($p<0.05$). Finally, we calculated the expected average allele length at TP2 (using the MR at TP1) and TP3 (using the MR at TP2) and found that there are samples with larger or smaller averages allele lengths than the expected one. All these results indicate that the progression of the mutational dynamics on DM1 patients is no lineal, no proportional to the allele size and different with time, which suggests that in some patients and at some point, the mutational dynamics develop a faster or slower behavior during the lifespan of the patient.

Normal DM1 alleles contain no variant repeats or flanking mutations, suggesting these are secondary to repeat expansion in patients.

CUMMING Sarah, Molecular Cell and Systems Biology University of Glasgow
HAMILTON Graham, Glasgow Polyomics University of Glasgow
SYMEONIDI Efthymia, Molecular Cell and Systems Biology University of Glasgow
MARTIN Stuart, Molecular Cell and Systems Biology University of Glasgow
CONSORTIUM Scottish Myotonic dystrophy, Molecular Cell and Systems Biology University of Glasgow
MONCKTON Darren, Molecular Cell and Systems Biology University of Glasgow

The CTG repeat expansion in myotonic dystrophy type 1 (DM1) is usually pure, however in ≈5% of patients, the expanded alleles are interrupted by variant repeats, such as CCG, CGG or CTC. These stabilize the repeat-containing DNA, sometimes altering symptoms. Alleles in the premutation range (37-50 repeats) occasionally contain CCGCTG hexamers. We have also detected additional 5' or 3' flanking mutations in both pure and interrupted expanded alleles. It is not clear, however, whether variant repeats and mutations in the flanking sequences are present in normal alleles, or whether they are secondary to the expansion of CTG repeats into the disease size range. We therefore used the Illumina MiSeq platform to sequence normal alleles, both to detect any sequence variants and to look for any evidence of somatic instability.
We have sequenced and genotyped normal alleles from over 180 patients recruited to a large study of genetic and phenotypic variation in Scottish DM1 patients. Allele length frequencies were very similar to those previously seen in European populations. We also discovered two premutation alleles, one a pure 43 repeat allele and the other a "45 repeat" allele containing 16 CCGCTG hexamers. We found no variant repeats in normal alleles, and no additional mutations in the flanking sequences
We next examined the reads for somatic instability. We determined the proportion of reads that differ in length by one repeat from the genotype. The main determinant of the frequency of these reads is allele length. However, when the alleles were sorted by length, the age at sampling had no effect. This suggests the longer or shorter reads mostly result from PCR slippage, and that any low level somatic instability may be masked by this major source of length variation. Our sequence data show that normal alleles are very stable and vary little in sequence, therefore sequence variants in premutation and expanded alleles most likely arise after CTG repeat expansion.

S1-O3

1: Mutation, genetics and epigenetics

MSH3 Overexpression Pancreas destabilizes CTG Triplet repeat in DM1 Mice Carrying 55 OR ~500 CTG Repeats

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The expansions of trinucleotide repeat sequences (TNRs) cause numerous neurological and neuromuscular disorders, such as myotonic dystrophy type 1 (DM1), Huntington's disease (HD) and fragile X syndrome (FRAXA). While TNR tracts in the normal range are stable in somatic tissue and stably transmitted to offspring in the general population, triplet repeats expansions >40 usually become dramatically unstable with a strong tendency to expand across generations. Somatic mosaicism is age-dependent, biased towards expansions, highly tissue-specific, and is probably associated with symptom progression with age.
Several data have shown that mismatch repair proteins, such as MSH2 and MSH3 (required to maintain genomic integrity) are predominantly involved in the formation of CTG expansions in DM1 and HD mouse models. Interestingly, the absence of one functional *Msh3* allele was sufficient to decrease the frequency of expansions suggesting that MSH3 protein is a rate-limiting factor in this process.
To assess the impact of MSH3 overexpression on the dynamics of CTG repeat instability *in vivo*, we have created a new mouse model overexpressing MSH3 protein in some tissues (Tg(*Msh3*) mice). We have identified two Tg(*Msh3*) lines that highly overexpress MSH3 in pancreas and quadriceps. MSH3 overexpression increases MSH2 protein levels in these tissues. We have crossed Tg(*Msh3*) mice with DM1 transgenic mice carrying either 55 CTG or ~500 CTG repeats. The CTG repeat is strongly destabilized in pancreas from Tg(*Msh3*) mice carrying either 55 or ~500 CTG repeats and showing the highest MSH3-MSH2 overexpression levels.
Our data suggest that the dynamics of CTG repeat instability is sensitive to the quantity of MSH3 and that MSH3 overexpression stabilizes the MSH2 protein, *in vivo*.

S1-O4

1: Mutation, genetics and epigenetics

Population distribution of healthy range and premutation range (CCTG)n alleles of the myotonic dystrophy type 2 locus

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Myotonic dystrophy type 2 (DM2) is caused by an expansion of a $(CCTG)_n$ repeat in the *CNBP1* gene. This repeat is generally interrupted by one or more GCTG, TCTG or ACTG motifs. Uninterrupted tracts were, however, described in expanded alleles as well as in larger healthy-range alleles which are generally considered as DM2 premutations. The threshold for pathogenicity is still poorly described in the literature, while the smallest reported pathogenic allele had 75 CCTG repeats. We screened, using multiplex conventional PCR and bi-directional repeat-primed PCR, the DM1 and DM2 repeat motifs in more than four hundred individuals. We identified wider range and higher frequency of uninterrupted CCTG alleles than it was previously reported, with more than fifteen alleles characterised. These alleles spanned the whole spectrum of healthy-range alleles, from the smallest one up to the likely pathogenic range. We therefore investigated the intergenerational stability of those alleles in which it was possible. Moreover, in two unrelated patients with symptoms of neuromuscular disorder we identified two ambiguous alleles containing 31 and 34 uninterrupted CCTG repeats, respectively. Since further screening revealed a full range DM1 expansion in the *DMPK* gene in the first patient and a homozygous *CLCN1* stop mutation in the second patient we concluded that these “grey zone” alleles are most likely not pathogenic themselves, although, they represent unstable premutation alleles as both of them changed during intergenerational transmission. Our results support that the *CNBP* CCTG alleles can be divided basically to two groups, interrupted and uninterrupted alleles. Those with interruptions are most likely stable during intergenerational transmission. Uninterrupted alleles with up to ~30 CCTG repeats are likely stable during transmission, while instability gradually increases with increasing length of uninterrupted tracts above this threshold (financial support: VEGA_2/0115/15).

S2-O1

2: Clinical and social issues

Survival of Permanently Paced Patients with Myotonic Dystrophy Type 1 - Sudden Death Predictors and Role of Implantable Cardiac Defibrillators

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Aims: Sudden death (SD) occurring in permanently paced patients with myotonic dystrophy type 1 (DM1) has raised the issue of the indications for implantable cardiac defibrillators.

Methods and Results: Between January 2000 and October 2013, 1,388 patients >18 years of age were hospitalized at 6 French medical centres for management of DM1, of whom 328 underwent permanent pacing. We confirmed their vital status, classified all deaths, ascertained the implication of ventricular tachycardia (VT) as a cause of SD, searched for predictors of SD by Cox proportional hazards analysis, and calculated the hazard ratios (HR) and 95% confidence interval (CI). Over a median follow-up of 7 years and interquartile range (IQR) of 5 -10 years, 42 deaths occurred (overall survival = 82%), 13 of which (31%) were sudden, representing a 0.59% yearly and 8% overall incidence (95% CI: 4 - 13). A primary ventricular tachyarrhythmia (VTA) was the confirmed cause of SD in 5 patients, was excluded in 5, and was undetermined in 3. By multiple variable analysis, sustained VT (HR = 240.4; 95% CI 27.9 to 2074.2; $P < 0.0001$), non-sustained VT (HR = 12.1; 95% CI 2.54 to 58.1; $P = 0.002$) and atrial fibrillation (HR = 3.63; 95% CI 1.19 to 11.1; $P = 0.023$) were independent predictors of SD.

Conclusions: In permanently paced patients with DM1, a primary VTA was the cause of at least 50% of SD. Sustained and non-sustained VT were its main predictors. These observations suggest that cardiac defibrillators should be implanted instead of pacemakers in selected patients presenting with DM1.

S2-O2

2: Clinical and social issues

Cutaneous features of myotonic dystrophy type 1 and type 2

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Vitamin D deficiency in myotonic dystrophy type 1 and 2 (DM1 and DM2) has been recently demonstrated by us and others. We also showed that cutaneous synthesis of vitamin D, rather than malabsorption and liver dysfunction, may be responsible for vitamin D deficiency found in DM. Skin changes, such as baldness and epithelial tumors, have been described in DM1. The aim of this study was to explore in detail the cutaneous features of DM1 and DM2 patients. Clinical skin examination was performed in 60 DM1 (mean age 44.6 years) and 15 DM2 patients (mean age 51,4) and skin lesions were analyzed by means of dermoscopy.The number of nevi and other skin alterations were correlated to CTG expansion size and vitamin D levels. Compared to the general population, in DM1 and DM2 patients, a higher frequency of junctional nevi (52% and 50%, respectively), dysplastic nevi (30% and 17%), xerosis (33% and 27%), seborrheic keratosis (18% and 20%) and seborrheic dermatitis (28% and 25%) were found. Dermatofibromas were present in 9 DM1 and 1 DM2 patients, only 2 DM1 patients showed a pilomatrixoma, in 1 DM1 patient a basalioma was removed. In all, 22 nevi were excised and none showed melanoma features. In DM1 patients, the total number of nevi significantly correlated with CTG expansion size, whereas the number of junctional nevi and xerosis lesions inversely correlated with vitamin D levels. In conclusion, DM1 and DM2 patients display a high frequency of skin abnormalities, some of which correlate with genotype severity and vitamin D levels. Conversely, pilomatrixomas are not frequent in our population. Skin examination is higly informative, and should be performed in all DM1 and DM2 patients.

S2-O3

2: Clinical and social issues

Dysregulation of Calcium Metabolism in Type 1 Myotonic Dystrophy

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Background: Type 1 Myotonic Dystrophy(DM1) is characterised by a number of endocrine and metabolic disorders, including glucose dysregulation, primary hypogonadism, adrenocortical insufficiency and dyslipidaemia. One sizable previous report noted calcium metabolism disorders.

Aim: In an Australian cohort of DM1 patients, to perform a clinical audit of the relationship between blood levels of calcium, vitamin D, parathyroid hormone (PTH) and bone mineral density(BMD).

Methods: 67 DM1 patients of one of us(RVJ) are seen in clinic twice per year. Relevant pathology tests are done concurrently. BMD was performed in 36 patients.

Results: 14/67 (21%) had an increased corrected total calcium level(Cac++); 18/43 (42%) had an increased corrected ionised calcium level(Cai++), but only 12 of these showed an increased Cac++. 18/58(31%) had raised PTH done concurrently with blood Ca++ and vitaminD; of these, 13/18 had normal 25OHD and 5/18 had low 25OHD. 7/37(19%) had raised 1,25OHD with raised calcium and normal 25OHD; no patient had a raised 25OHD, whether calcium was raised or not. 10/37(27%) had increased 1,25OHD with normal PTH and calcium. 0/36 were osteoporotic (T-score <-2.5) on BMD, and only 2 were osteopenic (T-score from-1.5 to -2.5).

Conclusions: In an Australian cohort of DM1 patients, we found: 1) Calcium metabolism was abnormal in over 20%. 2) 21% had an increased total calcium, and 42% had an increased ionised calcium. 3) 22% had an increased PTH level, with a normal 25OHD level. 4) 27% had a raised 1,25OHD level with normal PTH, 25OHD and calcium levels. 5) In spite of having muscle wasting and, in some, hypercalcemia consistent with primary hyperparathyroidism, none of 36 patients tested had osteoporosis, and only 2 were osteopenic, on BMD.

Hypothesis: Disrupted mRNA metabolism in DM1 may lead to increased BMD and bone strength.

S2-O4

2: Clinical and social issues

Executive cognitive dysfunction in adult onset Myotonic Dystrophy type 1

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Background: executive cognitive dysfunction associated with apathy, reduced planning skills, flexibility and multitasking has been reported in adult onset Myotonic Dystrophy type 1 (DM1). Objectives: we aimed to assess executive functions using neuropsychological tests and to explore correlations with subjective ratings on frontal lobe related behavior, daytime sleepiness and fatigue. In total, 33 patients with DM1 (16 women and 17 men) participated, with 25 healthy controls. Self-perceived frontal lobe related behavior, daytime sleepiness and fatigue were assessed using the Frontal Systems Behavior Scale (FrSBe), Epworth Sleepiness Scale (ESS) and the Fatigue Impact Scale (FIS), respectively. The neuropsychological test battery included the Trail Making Test (TMT), Digit Symbol, Stroop Color Word Test, FAS and the Wisconsin Card Sorting Test (WCST). Results: patients with DM1 performed significantly worse than healthy control subjects ($p < .05$, with large effect sizes) on all tests measuring executive functions. This difference also included higher scores on the FrSBe self rating, indicating more apathy, executive dysfunctions and disinhibition. High ratings on apathy and fatigue were associated with lower scores on tests measuring mental flexibility and multitasking. Conclusions: results indicate that frontal lobe related behavioral- and executive dysfunctions is significantly more common in DM1 compared with healthy controls. This means that patients with adult onset DM1 perceive and acknowledge these cognitive impairments, which is also confirmed by neuropsychological test results. Furthermore, executive dysfunctions is associated with apathy and fatigue. These observations are indicative of possible treatment options for dysexecutive behavior in DM1 in future trials with the aim to reduce apathy and fatigue.

S2-O5

2: Clinical and social issues

Personality of DM1 Patients: Dimensional Approach from the Five Factor Model

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Background: In DM1, personality disorders' prevalence ranges from 30 to 40% (Delaporte, 1998). These disorders are homogeneous in Cluster C profile (i.e. avoidant, obsessive-compulsive and dependent disorders). During the last thirty years, a consensus emerged among personality psychologists. The inter-individual variability can be understood by the position of each on five dimensions: Extraversion (E), Agreeableness (A), Conscientiousness (C), Neuroticism (N) and Openness (O) (John & Srivastava, 2009). We did not find any study using this model with DM1 patients.
Objective: This study aimed to describe personality characteristics of a sample of DM1 patients with the Five Factor Model and to compare characteristics with a control group.
Method: French DM1 patients from French DM Scope registry filled in the Big Five Inventory during a neuromuscular consultation. All participants signed a consent form. The Big Five Inventory (Plaisant et al., 2010) has been developed to provide an effective and validated measure of the five dimensions.
Results: BFI results were compared with results of a matched group from a French database.
Conclusion: This study allowed describing normal and dimensional personality of DM1 patients. These characteristics could be compared with personality disorders found in previous studies and for optimization of healthcare.
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S2-O6

2: Clinical and social issues

Social participation in myotonic dystrophy type 1: a 9-years follow-up study

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Myotonic dystrophy type 1 (DM1) restricted individual's social participation, especially work, housing, mobility and leisure. As no cure of this disease is available, healthcare as well as community services have to plan for long-term management. Nevertheless, little is known on evolution of social participation. The objective of the study was to describe the evolution of social participation among individual with DM1 over a 9-years period. **Methods**: A longitudinal descriptive design was used to compare data from baseline (2002-04) with follow-up (2011-13; n = 115). Individual with congenital or childhood phenotypes of DM1 were excluded. The Assessment of Life Habits (LIFE-H) questionnaire was performed to assess social participation, except for work and education. A minimally clinically important difference (MCID) of 0.5 was used to detect significant clinical change. Paired T-test was done to compare baseline with follow-up and Wilcoxon's Rank-Sum Test was used to compare with normative values. **Results**: Statistically and clinically significant deterioration over nine years was found in six activities. Specifically, while nutrition, fitness, personal care, mobility, community life and recreation diminished ($p<.01$, MCID>.50), no difference was found in communication, housing, responsibilities and interpersonal relationships. Moreover, three activities were significantly under reference values at baseline, fitness, mobility and community life, while personal care and recreation were added at follow-up. **Conclusions**: As disease progress, new activities are restricted. Such description of the evolution of social participation may allow better planning of associated health related services. Further investigations of the personal and environment factors that might influence social participation evolution are required to support disease management and planning of services.

S2-O7

2: Clinical and social issues

A 9-year longitudinal study of cognition in myotonic dystrophy adult phenotypes

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Myotonic dystrophy Type 1 (DM1) is an inherited disease leading to multisystemic system's involvement including central nervous system. Few longitudinal studies on cognition in adult-onset DM1 patients were conducted. The evolution of the cognitive profile is still a matter of debate, and whether an eventual decline could be global or process-specific. The aims are to 1)describe the progression, after a 9-year follow-up, of cognitive abilities in DM1 patients with adult-onset phenotypes, and 2)compare the rate of cognitive evolution between adult and late-onset phenotypes. A total of 115 DM1 patients with the classic or the late-onset phenotype were assessed twice within a 9-year period on specific cognitive abilities (language, verbal, visual and working memory, visual attention, processing speed, visuoconstructive abilities and executive functions) as well as intellectual functioning. Demographic data, level of muscular impairment and number of CTG repeat were also measured. Results showed a significant worsening over time for memory, visual attention, and processing speed. The total means in executive functions and visuoconstructive abilities remained stable over time, but the percentage of patients with impaired scores increased. Intellectual functioning remained stable. The rate of decline was higher in the late-onset group than in the adult phenotype one on semantic fluency, verbal memory, and arithmetic. This longitudinal study on DM1 concerns the largest sample and the longest time period studied to date. While executive functions, language, and visual memory are impaired early in adult life, verbal memory, visual attention, and processing speed decline later. A global cognitive alteration appear at about 50 y/o and more. Results taken altogether are merely interpretable as an early and accelerated normal elderly process rather than dementia related characteristics. These findings are highly relevant in clinical practice, as well as for genetic counselling.

S2-O8

2: Clinical and social issues

A Longitudinal Assessment of Cognitive and Adaptive Functioning in the Congenital and Childhood Forms of Myotonic Dystrophy Type 1

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Intellectual disability (ID) of varying degrees is reported as being a key symptom in children and adolescents with Myotonic Dystrophy type 1 (DM1). The aim of the present study was to conduct a longitudinal follow up of the development of global cognitive abilities and adaptive skills in individuals with the congenital and childhood forms of DM1. Fifty-one of the 55 individuals from our previous study (Ekström et al 2009) participated and were divided into three subgroups according to age at onset and presenting symptoms: severe congenital (*n*=16), mild congenital (*n*=17) and childhood DM1 (*n*=18). The mean time between the first assessment (*T1*) and the follow-up (*T2*) was 7.7 years (range 6.7-9.3 years). Global cognitive functioning was assessed by the Wechsler scales of intelligence. The Vineland Adaptive Behaviour Scales (VABS) were used to assess adaptive functioning. A floor-effect was found regarding IQ-scores for individuals with moderate to severe intellectual disabilities. Ninety-two percent of the total group of participants fulfilled criteria of ID at *T2*, as compared to 90% at *T1.* In the severe congenital form, all individuals had ID at both occasions, 94% at a moderate to severe level at *T2* in comparison to 88% at *T1.* In the mild congenital form, 82% had ID at *T1* and 88% at *T2.* In childhood DM1 89% had ID at *T1* and 81% at *T2*. Eleven individuals functioned on a higher cognitive level with mean FSIQ 75.6 at *T1* as compared to 65.0 at *T2*. Change over time was not statistically significant. A comparison of raw scores between the two assessments was performed. A statistical significant increase in raw score was found in the domains Communication (p=0.001), Daily Living (p=0.016) and Socialization (p=0-011) showing an actual development in all domains. This development is however slower than what is to be expected in relation to cognitive level.

S2-O9

2: Clinical and social issues

Brain Tumors in Patients with Myotonic Dystrophy: A Population-based Study

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Background. Neuro-cognitive and CNS imaging studies have documented that the brain is a target organ in myotonic dystrophy (DM). Furthermore, patients with DM are at high risk of certain malignancies, including primary brain cancer, but detailed information on brain neoplasms in DM patients is lacking.
Methods. Data from 1,119 DM patients identified from the National Swedish Patient Register between 1987 and 2007 were linked to Sweden's National Cancer and Cause of Death Registers. Patient follow-up started at birth or age at the start of Swedish cancer registration (January 1, 1958), and ended at the age of brain neoplasm diagnosis, death, or in December 31, 2007. We calculated standardized incidence ratio (SIR) and cumulative incidence of brain neoplasms, and used the Kaplan-Meier calculator for survival statistics.
Results. Twenty patients developed brain neoplasms during follow-up (median age=53, range=2-76 years); SIR=5.4 (95%CI=3.4-8.1, $p=1x10^{-5}$), confirming prior results. Astrocytoma was the most common histological subtype (n=16, 80%), and almost all cases (n=19) developed after age 20. No statistically significant differences in gender-specific risks (SIR in: men= 6.3, 95%CI=3.2-11.3; women=3.8, 95%CI=1.8-6.9, p-heterogeneity=0.46) were observed. Our data found no association between the risk of brain tumor and congenital DM (co-diagnosis of mental retardation = zero in brain cancer patients *versus* 1.2% in non-cases). After accounting for competing mortality related to DM, the cumulative incidence of brain neoplasms reached 2.9% (95%CI=1.8-4.7%) by age 70. Five-year survival after brain cancer diagnosis was 52% (95%CI=29-75%) overall (number at risk=8), and 34% (95% CI=26-47%) for malignant neoplasms (number at risk=5).
Conclusion. Despite the high relative risk of DM-related brain tumors, the absolute risk is modest. Nonetheless, careful evaluation of DM patients with new central nervous system symptoms is warranted.

S2-O10

2: Clinical and social issues

Results from the PRISM-2 Study: Patient Reported Impact of Symptoms in Myotonic Dystrophy Type 2

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Objective:

To determine the frequency and relative importance of the most life-affecting symptoms in the myotonic dystrophy type-2 (DM2) population and to identify factors that are most highly associated with these symptoms.

Methods:

We conducted a cross-sectional study of adult DM2 patients from a National Registry of DM2 Patients to assess the frequency and relative importance of 310 symptoms and 21 symptomatic themes. Participant responses were compared by age categories, gender, educational attainment, employment status, and duration of symptoms. Responses from DM2 participants were compared to responses from DM1 participants obtained through a prior clinical study.

Results:

Seventy-four individuals with DM2 participated in this study. The symptomatic themes with the highest frequency in DM2 were the inability to do activities (94.4%), limitations with mobility or walking (89.2%); hip, thigh, or knee weakness (89.2%); fatigue (89.2%), and pain (79.7%). Participants identified the inability to do activities and fatigue as the symptomatic themes that have the greatest overall effect on their lives. Longer duration of symptoms, lower education level, and unemployment were associated with a higher average frequency of all symptomatic themes ($p<0.01$), but gender and age were not. Longer duration of symptoms, unemployment, female gender, and older age were associated with a higher average impact of all symptomatic themes among DM2 patients ($p<0.01$). Compared to DM1, DM2 participants reported higher rates of pain, activity limitation, and difficulty thinking.

Conclusions

DM2 patients' lives are affected by a variety of symptoms. The significance and frequency of these symptoms is distinct from DM1 and varies across DM2 subgroups categorized by different demographic features.

S2-O11

2: Clinical and social issues

Survey to Assess Fall History and Balance Confidence in Individuals with Myotonic Dystrophy (DM)

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Background: Individuals with DM report imbalance and falls that have a negative impact on quality of life. Individuals with myotonic dystrophy type 1 (DM1) fall more than healthy controls; however, little is known about the incidence of falls in this patient population. Additionally, decreased balance confidence has been found to be associated with risk of falls in other populations. The purpose of this study is to document the frequency of falls and balance confidence reported by individuals with DM. Methods: Patients attending the 2013 Myotonic Dystrophy Foundation Annual Conference in Houston, Texas were asked to participate in this study. Participants completed a survey that included a Demographic/Clinical profile, a Fall History Questionnaire, and the Activity-specific Balance Confidence Scale (ABC). The ABC is a 16 item questionnaire that assesses one's confidence in their balance ability while performing daily tasks. It is a reliable measure that has demonstrated the ability to predict falls in older adults. Descriptive statistics were used to summarize the demographic, fall history, and ABC data. Spearman's rank correlational analysis was used to determine the relationships between balance confidence and fall data. Results: 48 (60% female) participants with DM (69% DM1) completed the survey. 18 participants (38%) reported a fall in the past month and 33 (69 %) reported falls in the past 6 months. Of those that fell in the past 6 months, 12 (34%) reported an injury (5 with fractures). The mean score on the ABC was 73.7% (standard deviation=25.8; range 9.38-100). The ABC was moderately correlated with the 6 month fall status (r= -0.497; p=.001). Discussion: Falls are a significant concern in individuals with DM1. It is important to be able to identify individuals with DM who are at risk for falls in order to provide interventions aimed at improving safe mobility, reducing fear of falling and fall risk, and thereby improve quality of life.

S2-O12

2: Clinical and social issues

Association between lower limb muscle strength and functional impairments in adult and late onset phenotype of DM1

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INTRODUCTION: In DM1, lower limb muscle weaknesses can lead to significant limitations of physical activity. Although the profile of muscle impairments is believed to be different according to each DM1 phenotype, no study has described the muscle strength impairments and the functional activity limitations separately according to each phenotype and the identification of the respective influence of each lower limb muscle group weakness on physical function still needs to be done. **OBJECTIVES**: To 1) describe and compare the lower limb muscle strength impairments and functional limitations in DM1 adult and late onset phenotypes, and to 2) identify how the strength of each assessed muscle group explains the variations in functional scores. **METHODS**: Muscle strength of the hip flexors, knee extensors, knee flexors, and ankle dorsiflexors (MIRS, MMT, QMT) and functional capacities associated with mobility (Berg balance scale, Timed up & Go, 10 meter walking test, and Two minute walking test) of 107 participants were assessed. **RESULTS**: Overall, late onset showed less muscle weakness and activity limitations than the adult phenotype ($p < 0.001$-0.020), and when compared to reference values, the differences were significant for both phenotypes. In the adult phenotype, the muscle strength impairment was slightly more important distally ($p < 0.001$). A general progression of weakness and functional impairments was observed according to the MIRS. Proximal weakness (QMT) and limitations of physical activity were observed from the first MIRS grades. Ankle dorsiflexors was the only muscle group showing significant unique contribution to the variation observed in the functional tests. **CONCLUSION**: Adult and late onset phenotypes show different profiles of lower limb muscle impairments and limitations of activity, and should not be pooled for data analysis. To monitor the effectiveness of therapy in DM1, ankle dorsiflexors appear to be a good indicator of functional capacities.

S2-O13

2: Clinical and social issues

Diagnostic delay in myotonic dystrophy type 1 depends on the DM1 category

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Introduction The symptoms that are present at onset of DM1 are diverse, as are the clinical manifestations that the patients encounter over the years. We investigated a population-based cohort of adult DM1 patients in order to describe the diagnostic delay in all patients as well as in the different subtypes of DM1 and also for the different symptoms at onset.

Methods Adult DM1 patients alive in the western Sweden Region (n=230) were traced by outpatient registers and contacted by regular mail, and thereafter telephone interviewed regarding age at diagnosis and various organ manifestations and debut symptoms; muscle weakness, myotonia, cognitive impairment, cardiac conduction failure, cataract and gastrointestinal symptoms (n=177) in order to obtain the age at which each symptom had started. These data were used to describe the diagnostic delay.

Results The mean age for the first symptom was 20.5 years, and the mean time to diagnose was 11.1 years. When abdominal symptoms were the first symptom the delay was 16.6 years, while it was shorter if symptoms as muscle weakness (7.6 y), myotonia (9.2 y), cognitive (9.5 y), eyes 12.8 y) were the first. Patients with congenital DM1 (n=24) had a significantly shorter time span from symptom onset to correct diagnosis (5.1 y) than the childhood (15.7 y, n=37). Patients with the adult/classical form had a shorter delay (12.0 y, n=92), in particular the case in patients with the classical form had muscle weakness as onset symptom (7.5 y, n=34).

Discussion In this population based survey we found that there is a considerable delay to diagnosis especially in the childhood form. Gastrointestinal symptoms were those associated with the longest diagnostic delay. Awareness of the symptoms of DM1 may help many patients and families to a correct diagnosis.

S2-O14

2: Clinical and social issues

Biographical disruption: shedding light on myotonic dystrophy type 1 and reproduction

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Introduction: Care for individuals living with myotonic dystrophy (DM1) has been described as fragmented. This occurs - in part- as clinicians only see a snapshot of patients' and caregivers' lives during infrequent and time limited appointments. Multiple aspects of individuals' daily experiences are influenced by DM1, yet health care providers often use a biomedical approach to management. Patient and family centered care demands a medical sociological approach; biographical disruption (BD) posits that chronic illness causes a fundamental shift in identity and self-conceptualization. Better care provision requires a robust understanding of how individuals experience and manage DM1 at home.
Methods: Using BD as a framework, we conducted a secondary analysis of interview transcripts from three qualitative research studies (n = 13 patients; 7 caregivers) that explored aspects of living with DM1. We coded for DM1-related 'disruption', and the most frequent codes were consolidated into themes and categories.
Results: Three categories were identified: *disrupted narrative*, *disrupted chances*, and *writing a new biography*. Woven throughout were stories related to shifting life goals and reproductive health. Becoming a parent was disrupted by infertility, miscarriage, and considerations of genetic risk, and parenting was complicated by DM1-related fatigue and weakness. The birth of an affected child shifted a previously asymptomatic mother's identity from 'healthy' to 'patient', and parents felt guilty when their children were diagnosed. Reproductive experiences resulted in shock or blame; regardless, parents formed new identities as advocates for their children.
Discussion: DM1 shapes patients' and caregivers' identities and influences their decision-making. Is there a role for policy makers, health care teams and the DM1 community to provide tools and advocacy to support decision-making and self-management around reproductive health and parenting?

S2-O15

2: Clinical and social issues

DM-Scope, a French nationwide registry to decipher pediatric myotonic dystrophies' clinical complexity

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Background: Myotonic Dystrophy type 1 (MD1) is known to exhibit a highly variable phenotype regarding its age of onset and the changing systemic involvement. However, most clinical data arise from observational studies that focus on MD1 adult forms. Pediatric descriptions are scarce, rely on restricted cohort of patients and thus remain incomplete.
Objective: We aimed to phenotypically characterize a wide MD1 pediatric population especially concerning the overall disease history, the severity of cognitive impairment and the extent of systemic manifestations.
Method: Since 2010, the French DM-Scope registry includes applications that aim to optimize the annual clinical evaluation of adult MD1 patients, and to promote clinical research in this field. Throughout 2014, we focused on the pediatric population and developed specific tools (i.e. standardized form, synopsis) in order to better characterize the disease in children and improve their standard of care. Currently, 24 neuropediatric centers are involved in this observational study. Overall the French registry gather data from 2202 MD patients collected in 50 neuromuscular centers.
Results: DM-Scope has already enrolled 246 MD1 children (Conclusion: DM-Scope, the French nationwide clinical network on myotonic dystrophies constitutes a strong task force. Up to date, the registry describes the largest adult and pediatric MD cohorts worldwide. DM-Scope therefore provides a powerful platform designed to optimize routine clinical management and clinical research in the field of myotonic dystrophies.

S2-O16

2: Clinical and social issues

A cross-sectional evaluation of disease progression in Congenital Myotonic Dystrophy

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BACKGROUND: Congenital myotonic dystrophy (CDM) is a severe, infantile-onset form of myotonic dystrophy (DM1). There have been few clinical studies evaluating the progression of CDM through childhood. This study seeks to identify the progression of CDM through childhood and identify appropriate clinical endpoints for future therapeutic trials.
DESIGN/METHODS: Patients with CDM between 0-13 years of age at were enrolled in the study. Patients were divided into cohorts based on the following ages: 0-2, 3-6, and 7-13.. Control subjects in the same age groups were also recruited. Each cohort received an age appropriate clinical evaluation. This evaluation included neuropsychological testing, oral facial strength testing, strength and functional testing, DEXA, ECG, and measurements of quality-of-life. For this study, subjects were seen cross-sectionally over a two-day visit. All statistical analyses were pairwise comparisons with a p-value less than 0.001 reported as significant.
RESULTS: Thirty-seven subjects with CDM and 15 control subjects were recruited. In CDM patients, the mean IQ was reduced at 70.5 (SD 17.3). For oral facial strength, the mean lip force strength was significantly different in CDM patients (4.8 Newtons (SD 3.8)) compared to control subjects (21.0 Newtons (SD 5.7)). The six-minute walk was significantly different between CDM subjects (298.6 meters (SD 104.7)) and control subjects (577.3 meters (SD 65.6)). Measurements of grip strength, sleep quality, and quality of life were also significantly different.
CONCLUSIONS: This work comprehensively identifies the changes associated with CDM during childhood. Several measures identify significant differences between CDM and control subjects and may be useful during offer future therapeutic trials.

S3-O1

3: Disease mechanisms

The Human Myotonic Dystrophy Transcriptome

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Myotonic dystrophy (DM) is a highly multi-systemic disorder caused by expanded CTG/CCTG repeats. Muscle weakness, myotonia, cardiac arrhythmia, and profound fatigue are common, yet exhibit extreme variability across affected individuals. Many transcriptome changes occur in DM, in particular changes in alternative splicing. Some of these changes are dependent on Muscleblind-like (MBNL) and Cugbp- and ETR3-like factors (CELF); however, numerous additional cellular pathways are perturbed, including dysregulation of transcription factors, microRNA processing, and protein signaling. To comprehensively assess transcriptome changes occurring in DM, and to evaluate the extent to which each of these pathways may contribute to disease pathogenesis, we performed RNAseq on a diverse panel of ~150 DM and normal biopsies/autopsies. Samples included biopsies from tibialis anterior and quadriceps, and autopsies from the heart and additional skeletal muscles across the body.

Analyses of these data confirm previously observed correlations in mis-splicing and muscle weakness, and correlation in the extent of splicing dysregulation across multiple splicing events within a tissue, highlighting variability in molecular phenotypes across the patient population. These data suggest that DM1-mediated splicing dysregulation is more severe in distal relative to proximal muscles, and that distinct patterns of baseline alternative splicing patterns in these tissues may underlie these differences. Further analyses of these data should elucidate the contribution of MBNL depletion and CELF activation, among other pathways, to transcriptional dysregulation in DM. Additionally, this dataset provides a rich set of biomarkers that may be useful for monitoring therapeutic response.

These data will be available to the broader scientific community via a publicly accessible website and database. We thank all contributors to this study, particularly those who have donated biological material.

S3-O2

3: Disease mechanisms

MBNLs regulate MBNL1 function by binding to the 5'UTR of MBNL1 mRNA and pre-mRNA

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Muscleblind (MBNL) proteins belong to a family of RNA-binding proteins with crucial roles in alternative splicing regulation, mRNA stability and cellular localization. During tissue differentiation, expression level of MBNLs increases, while functional downregulation of their activity is a key contributor to the pathomechanism of a dominantly inherited muscle disorder - myotonic dystrophy (DM). MBNLs recognize their RNA targets using four zinc-finger (ZnF) domains arranged in two tandems. The RNA deep sequencing of UV cross-linking and immunoprecipitation (CLIP-seq) products revealed that MBNL1 binds to the 5'-most region of *MBNL1* exon 1 (e1) encoding both the major part of 5'UTR and N-terminal region of MBNL1 protein, which indicated a possible autoregulative function of MBNLs. We show that MBNLs, by binding to *MBNL1* e1, protect the cell from extreme MBNL1 protein concentrations in two distinct ways. Firstly, by generating an e1-deficient mRNA, which potentially encodes truncated, highly inoperative MBNL1 isoform lacking the first two ZnFs and secondly, by regulating proper localization of *MBNL1* mRNA containing e1 to the membrane fraction. Furthermore, we show that splicing of e1 is differently regulated depending on the transcription start of *MBNL1* pre-mRNA. Taken together, we reveal a novel autoregulative mechanism of MBNL1 function that fine-tunes MBNL1 amounts during cellular differentiation and in DM.

S3-O3

3: Disease mechanisms

Elucidating the relationship between higher order MBNL1 domain organization and splicing activity

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MBNL1 contains four zinc finger (ZF) RNA binding motifs that form two distinct binding domains: the first two ZFs fold into one domain (ZF1-2) and the second set of ZFs fold into another (ZF3-4). Combinatorial mutagenic analysis of the four ZFs revealed that the ZF pairs have differential affinity for RNA and hence differential splicing activity. Despite sequence and structural similarity between the two tandem ZF pairs, ZF1-2 bound with higher affinity to all tested RNA substrates and retained ~80% of WT-MBNL1 splicing activity. Little is known about the importance of the higher order domain organization of MBNL1. To evaluate the importance of MBNL1 ZF organization we designed several synthetic MBNL1 constructs including 1) an MBNL1(1-2,1-2) construct in which the ZF3-4 domain is replaced with the ZF1-2 domain to create a MBNL1 with two copies of ZF1-2 and 2) an MBNL1(3-4,3-4) with two copies of ZF3-4. Overexpression of these synthetic proteins in both HeLa and HEK-293 cells revealed that MBNL1(1-2,1-2) has splicing activity greater than WT-MBNL1 for several MBNL1 regulated events. MBNL1(3-4,3-4) retained minimal splicing activity as predicted. Data also indicates that lower concentrations of MBNL1(1-2,1-2) are required to regulate splicing. To further investigate the relationship between MBNL1 protein concentration and splicing activity stable inducible cell lines for both synthetic constructs and WT-MBNL1 have been created. Current data also suggests that rearrangement of the ZF pairs also alters both protein stability and RNA binding. Further experimentation is required to address these novel observations. A synthetic MBNL1 with increased RNA binding affinity could be used as a DM1 therapeutic to displace endogenous MBNL proteins from the toxic RNA.

S3-O4

3: Disease mechanisms

MBNL1 regulates alternative splicing through finely tuned switch-like and rheostat mechanisms: implications for DM Biomarkers

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The concentration of MBNL proteins has a central role in myotonic dystrophy. CUG and CCUG repeats sequester MBNL proteins and reduce the “free” or active concentration, which in turn leads to mis-splicing. We have created a stable cell line containing an inducible MBNL1 that allows one to vary the concentration (dose) of MBNL1 over a 15-fold range. Using this system we have found that splicing events respond in a non-uniform manner and show different sensitivities to MBNL1 protein. We found that dose-responsive behaviors can be “switch-like” (sharp transitions) or “rheostats” (gradual transitions). Studies of a splicing event that is highly conserved between species revealed the importance of MBNL cis-element organization in controlling dose-response behavior.

Analysis of muscle biopsies from DM1 patients revealed that splicing events are not perturbed uniformly. Events with comparable splicing behavior between DM1 muscle and the inducible system provided a mechanism to estimate the relative “free” MBNL concentration in each DM1 and control sample.. Using these estimates we categorized events based on their presumed sensitivity to MBNL activity and discovered that these events also display switch-like and rheostat behavior and require different concentrations of MBNL protein for splicing regulation. Together these studies demonstrate that simple models of binding site location and number are insufficient to predict MBNL dose responses. Furthermore, they highlight the utility of inducible systems to study relative binding site importance. This work has implications for using alternative splicing ratios as disease biomarkers, and also highlights the strengths and limitations of using cellular models to describe the perturbation of alternative splicing in DM and other spliceopathies.

S3-O5

3: Disease mechanisms

Muscleblind-like compound knockout mice recapitulate Tau mis-splicing in DM brain

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Central nervous system (CNS) dysfunction is one of the most challenging symptoms among myotonic dystrophy (DM) patients. Post-mortem studies reveal mis-splicing of microtubule-associated protein tau (MAPT) and neurofibrillary tangles (hyperphosphorylated tau protein aggregates) are the most common abnormalities. Retention of fetal isoforms of *MAPT* has been identified in the DM brain, and *in vitro* studies suggest loss of Muscleblind-like (MBNL) proteins directly regulate these splicing events. MBNL proteins colocalize with RNA foci containing expanded C(C)UG^{exp} mutant RNAs and sequestration of MBNL has been proposed to be the primary event for DM pathogenesis. However, single knockouts of the three *Mbnl* paralogs (*Mbnl1*, *Mbnl2*, *Mbnl3*) fails to show a comprehensive pattern of tau splicing misregulation and pathology consistent with the DM brain. To test if compound loss of MBNL proteins accounts for DM brain phenotypes, we generated *Mbnl1; Mbnl2* double KO (DKO) mice using a conditional knockout strategy (*Mbnl1*$^{\Delta E3/\Delta E3}$; *Mbnl2*$^{cond/cond}$; *Nestin-Cre*$^{+/-}$ (Nestin-Cre DKO). These mice are small, show impaired motor functions and adult mice are characterized by a nearly complete reversal to Mbnl-regulated fetal brain splicing patterns. Moreover, loss of Mapt exon 2,3 inclusion isoforms (0N), as well as the presence of the exon 10 exclusion isoform (3R), was observed. Specific anti-tau antibodies and 2D-GE coupled with immunoblot analysis revealed that tau protein expression was profoundly altered in the Nestin-Cre DKO brain with equivalent expression of the 0N4R (lacking exons 2 and 3 but including exon 10) and 0N3R (lacking exons 2, 3 and 10) tau isoforms. We conclude that both MBNL1 and MBNL2 loss of function account for tau misregulation in DM brain. Nestin-Cre DKO mice provide a novel animal model to characterize the molecular events leading to DM CNS disease.

S3-O6

3: Disease mechanisms

Compromised MBNL activity in myotonic dystrophy leads to disruption of developmentally regulated alternative polyadenylation

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MBNL proteins are sequestered by microsatellite expansion RNAs in the RNA-mediated disease myotonic dystrophy (DM). Although MBNL1 and MBNL2 regulate pre-mRNA alternative splicing during muscle and brain development, MBNL protein binding sites are most prevalent in the 3' UTRs of target RNAs. Here, we report that MBNL proteins bind near 3' end processing sites and regulate alternative polyadenylation (APA). Interestingly, loss of Mbnl activity in mouse embryo fibroblasts (MEFs) leads to misregulation of thousands of APA events. HITS-CLIP and minigene reporter analyses indicate that MBNL proteins directly regulate these polyadenylation switches. Consequently, APA is misregulated in a mouse polyCUG DM1 model and human DM1 autopsy skeletal muscles resulting in the persistence of fetal polyadenylation patterns. Moreover, this pattern of developmental dysregulation also occurs in the central nervous system. Frontal cortex from *Mbnl1*$^{-/-}$; *Mbnl2*$^{c/c}$; *Nestin-Cre*$^{+/-}$ (Nestin-Cre DKO) conditional mice and DM1 and DM2 autopsied frontal cortex and hippocampus display APA defects. Our results demonstrate that DM is characterized by misregulation of specific developmental RNA processing events at multiple levels.
Published material: Loss of MBNL leads to disruption of developmentally regulated alternative polyadenylation in RNA-mediated disease. *Molecular Cell 2014 Oct 23;56(2):311-22.*

S3-O7

3: Disease mechanisms

Abnormal splicing switch of DMD exon 78 compromises muscle fiber maintenance in Myotonic Dystrophy

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Dystrophin is part of a large dystrophin-associated glycoprotein complex (DGC) that stabilizes the membrane of muscle fibers, provides a strong mechanical link from the intracellular cytoskeleton to the extracellular matrix and mediates the transduction of extracellular signals to the muscle cytoskeleton. Moreover, muscle degeneration resulting from the expression of truncated dystrophin in Becker muscular dystrophy or its loss in Duchenne muscular dystrophy highlights the importance of this subsarcolemmal protein for muscle function. Dystrophin gene (*DMD*) is composed of 79 exons encoding a 427-kDa subsarcolemmal dystrophin protein in skeletal muscle. Nakamori et al. have reported an abnormal exclusion of dystrophin exon 78 in DM1 patients (Nakamori et al., 2007), however, the contribution of alternative splicing misregulation of dystrophin exon 78 on skeletal muscle dysfunction was not investigated yet. This abnormal splicing leads to the re-expression of an embryonic dystrophin isoform with an amphipathic α-helix C-terminus in place of a β-sheet C-terminus in the adult isoform. Using an exon–skipping approach, we forced expression of this embryonic dystrophin isoform in zebrafish resulting in a severe impairment of mobility and muscle architecture. Moreover, we reproduced *Dmd* exon 78 missplicing in skeletal muscles of adult wild type mice using an U7-snRNA antisense system. We observed that inappropriate *DMD* exon 78 exclusion leads to muscle fiber remodeling and ultrastructural abnormalities with the appearance of ringed fibers, sarcoplasmic masses or Z-band disorganization that are characteristic features of DM1 skeletal muscle. We propose that the abnormal splicing of *DMD* exon 78 compromises muscle fiber maintenance and contributes to the progressive dystrophic process in DM1.

S3-O8

3: Disease mechanisms

Implication of BIN1 in Myotonic dystrophy

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Myotonic dystrophy of type 1 (DM1), the most common dystrophy in adults, is an autosomal disorder characterized by muscular myotonia, muscle weakness, heart conduction defects and some others common features like ocular cataract and insulin resistance. DM1 is due to a large expansion of CTG repeats located in the 3'-UTR of the DMPK gene. Expanded CTG repeats are transcribed in pathogenic RNA, which sequester RNA-binding proteins such as the splicing regulator MBNL1, resulting in alternative splicing defects in DM1 patients.

We identified that the alternative splicing of the Bridging Integrator-1 (*BIN1*) mRNA is altered in DM1. *BIN1* has important functions in skeletal muscle, notably in the biogenesis of muscle T-tubules, which are key actors of the excitation-contraction coupling machinery. Moreover, mutations in *BIN1* or Dynamin 2 (*DNM2)* genes can leads to Centronuclear Myopathy, which shares some histopathological features with DM1.

We analyzed the splicing regulation of *BIN1* exon 7, and found that its inclusion is repressed by MBNL1, which binds to CUG motifs located upstream of *BIN1* exon 7. Consequently, exon 7 is aberrantly expressed in DM1 skeletal muscle. According to our experiments, the presence of exon 7 enhances the interaction of BIN1 protein with DNM2, confirming previous *in vitro* findings (Ellis et *al*., 2012).

Preliminary results using AAV-mediated expression of *BIN1* isoforms (-/+ ex.7) in skeletal muscle of wild type mice suggest that BIN1 containing exon 7 impacts on muscle structure and function, reproducing some DM1 features. We propose that forced interaction of BIN1 and DNM2 in skeletal muscle contributes to muscle atrophy and weakness, probably by displacing BIN1 from the triads.

S3-O9

3: Disease mechanisms

Mutant Repeats vs. Resident Gene: Does CNBP Play a Role in Myotonic Dystrophy Type 2 (DM2)?

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DM2 is caused by a $(CCTG)_n$ expansion in intron 1 of *CNBP* (*ZNF9*). We and others previously demonstrated reduced CNBP mRNA and protein levels in DM2 patients, primarily due to impaired processing of mutant pre-mRNAs. A previously published *Cnbp*$^{+/-}$ mouse suggested that many DM2 features can be elicited by CNBP haploinsufficiency without (CCTG)/$(CCUG)_{DM2}$ expansion or global missplicing. We have generated a second independent *Cnbp* knockout mouse model and confirmed this: our *Cnbp*-null mice show myotonia, myopathy and multi-system abnormalities consistent with DM2. Thus, CNBP insufficiency is directly implicated in the pathogenesis of DM2, but its role remains unknown. CNBP (cellular nucleic acid binding protein) is an RNA binding protein. As a first step towards determining the role of CNBP deficiency in the pathogenesis of DM2, we performed RIP-Seq on myoblasts from DM2 patients and normal controls to identify CNBP RNA targets. Using qRT-PCR, we demonstrated strong enrichment of known targets EEF1A, EEF2 and ODC1 in the immuno-precipitated RNA used for sequencing. Through our filtering algorithm, we identified over 350 potential RNA targets of CNBP. Pathway analysis of putative targets showed enrichment for genes involved in stress response and protein synthesis. MEME analysis of targets identified an enriched 8-bp motif in the target RNAs. Validation of selected novel targets is under way and consists of qRT-PCR to confirm enrichment in the RIP fraction and Western blotting to determine whether protein levels of putative targets are altered in myoblasts from DM2 patients or tissues from our *Cnbp*-null mice. This presentation will provide an update on the characterization of our *Cnbp* knockout mouse model and our efforts to identify CNBP targets contributing to the DM2 phenotype.

S3-O10

3: Disease mechanisms

Bidirectional transcription and RAN translation from CCTG•CAGG expansions in DM2 patient brains

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The myotonic dystrophies are multisystemic neuromuscular disorders characterized by progressive muscle weakness, myotonia, and central nervous system abnormalities. DM1 and DM2 are generally considered RNA gain-of-function diseases in which CUG and CCUG expansion transcripts dysregulate MBNL and CELF proteins resulting in RNA processing abnormalities. The discoveries of bidirectional transcription and repeat associated non-AUG (RAN) translation raise the possibility that up to six mutant proteins, three sense and three antisense can be expressed from a single expansion mutation. We now demonstrate that the DM2 CCTG repeat expansion is bidirectionally transcribed and RAN translation occurs across transcripts containing the DM2 CCUG or CAGG expansion mutation in transfected cells and human DM2 brains, producing tetra-repeat expansion proteins with a repeating Leu-Pro-Ala-Cys (LPAC) or Gln-Ala-Gly-Arg (QAGR) motifs, respectively. We observed RAN LPAC protein accumulation in neurons, astrocytes and glia in frontal cortex, hippocampus and basal ganglia in DM2 patients but not control brains. In contrast, RAN QAGR proteins show distinct patterns of accumulation in DM2 brains, suggesting that LPAC and QAGR proteins play different roles in the CNS features of DM2. Additionally, we show that overexpression of the MBNL protein sequesters CCUG RNA in the nucleus and blocks RAN protein accumulation. Conversely, RAN translation is elevated in *Mbnl1*$^{-/-}$; *Mbnl2*$^{-/-}$ knockout MEFs.
In summary, bidirectional RAN translation in DM2 suggests LPAC and QAGR RAN proteins contribute to the CNS features of DM2 and that MBNL overexpression may mitigate both RNA and RAN effects. Additionally, these data suggest a two phase model of disease which initially involves nuclear retention of expansion RNAs by MBNL and a later phase in which expansion RNAs exceed MBNL sequestration capacity and are exported to the cytoplasm where they undergo RAN translation which exacerbates disease.

S3-O11

3: Disease mechanisms

RNA gain-of-function and RAN translation in mouse models of myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is a multisystemic adult-onset disease caused by a large CCTG repeat expansion mutation in intron 1 of the *cellular nucleic acid-binding protein (CNBP)* gene. To separately examine the skeletal muscle and CNS features of DM2, we developed two transgenic mouse models. First we developed a tetracycline-inducible murine model to test the hypotheses that (CCUG)300 expansion transcripts lacking disease-specific flanking sequence replicate the skeletal muscle features of DM. Skeletal muscle from transgenic animals expressing (CCUG)300 repeats shows: 1) variation in fiber size and central nuclei indicative of myopathy; 2) electrical myotonia; 3) RNA foci; and 4) aberrant mRNA splicing patterns. Turning off the expression of the (CCTG)300 transgene resulted in reversal of the muscle histology, central nuclei and RNA foci formation. To investigate the CNS features of DM2, we crossed our TRE(CCTG)300 mice to mice expressing the tTA under the *calcium/calmodulin-dependent protein kinase II alpha (Camk2a)* promoter. CCUG expansion transcripts are expressed and form robust RNA foci throughout the forebrain in doubly transgenic animals. We are currently characterizing these animals for RNA processing abnormalities. Recently, several types of expansion transcripts have been shown to express proteins without an AUG-initiation codon by a novel process called repeat associated non-ATG (RAN) translation. We tested if RAN translation occurs in our mice and show the accumulation of a novel tetrapeptide DM2 RAN expansion protein containing Leucine-Proline-Alanine-Cysteine (LPAC) repeats. We are currently analyzing this CNS mouse model of DM2 to determine the distribution and time course of RAN protein accumulation and relative contributions of RNA gain of function and RAN translation to CNS phenotypes seen in these mice.

S3-O12

3: Disease mechanisms

Novel insight in antisense transcription in the DM1 locus: the DM1-AS gene and its products

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The unstable (CTG•CAG)n trinucleotide repeat in the DM1 locus is bidirectionally transcribed from genes with terminal overlap. The (CTG)n repeat ends up as a (CUG)n segment in the 3' UTR in *DMPK* transcripts encoding various DMPK protein isoforms. Transcription in the opposite direction produces antisense transcripts (*DM1-AS*) carrying a (CAG)n repeat. While detailed information is available on messenger RNAs and protein products from the *DMPK* gene, information on the *DM1-AS* gene and its products is still scarce. Understanding *DM1-AS* RNA production will help shape theories on the disease mechanism of DM1.

We combined bioinformatic analyses of publicly available RNA-sequencing data and genome analysis projects (e.g., ENCODE, FANTOM, Merck Research Laboratories) to predict *DM1-AS* transcription start sites, polyadenylation sites and primary and processed RNA products. *DM1-AS* RNAs mapped to a 6 kb area overlapping exon 15 of *DMPK*. Predicted transcription start sites were located in the region corresponding to intron 1-exon 1 of the *SIX5* gene. *DM1-AS* RNA is polyadenylated and potential transcription termination sites were found both downstream and upstream of the (CAG•CTG)n repeat, indicating that the repeat may not always be included in the primary transcript. *DM1-AS* RNAs occur at very low levels, are predominantly nuclear and are present in all cell lines investigated.

Preliminary RT-PCR experiments, using primer sets with appropriate spacing and orientation across inter- and intragenic areas of *SIX5* and *DMPK*, confirmed our main bioinformatic findings. Importantly, these experiments also revealed that *DM1-AS* transcripts are subject to extensive alternative splicing. All data will be combined in a new model for the *DM1-AS* gene and its products in DM1 pathobiology, which will be discussed during the meeting.

S3-O13

3: Disease mechanisms

Use of CRISPR/Cas9-based genome editing for the generation of isogenic cell models to study repeat length effects in DM1

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Current models for study of DM1 pathobiology include (i) cultured cells (myoblasts/fibroblasts/iPSCs) from patients and (ii) mice or cell lines that carry transgene insertions derived from the DM1 locus from patients or from artificial recombinant plasmids with a $(CTG.CAG)_n$ expansion. There are differences in genomic context and background of the $(CTG.CAG)_n$ repeat and a large variation in transgenic expression levels in these models. Hence, comparison of findings regarding the correlation between repeat length and severity of DM1-associated cell stress is problematic, even between cells of similar tissue type and differentiation state. Extrapolation of findings to the situation in DM1 patients is therefore difficult. New near-natural cell and animal models are therefore urgently needed.
Here we report on the use of CRISPR/Cas9-based genome editing technology for the generation of isogenic myogenic cell models that differ only in the presence of $(CTG.CAG)_n$ tracts with different lengths. Using standard prediction software we designed CRISPR/Cas9 nucleases capable of sequence-specific dsDNA cleavage immediately up- and downstream of the $(CTG.CAG)_n$ repeat tract in the expanded human DMPK gene. Data on cleavage efficiency, off-target effects and editing outcome with these nucleases, either used singly or in combination, in mouse DM500 myoblasts (Mulders et al., PNAS, 2009), immortalized 13/800 $(CTG.CAG)_n$ or wt/2600 $(CTG.CAG)_n$ human myoblasts will be presented. We demonstrate that the repeat tract can be efficiently and precisely excised from the 3' UTR of the *DMPK* gene by use of CRISPR/Cas9 nuclease combinations. More heterogeneous genomic alterations, resulting in repeat contraction were relatively frequently observed upon induction of a single dsDNA break at unique sequences in the vicinity of the $(CTG.CAG)_n$ tract. Additional data, pointing at effects of 3' UTR structure alterations for *DMPK* mRNA fate and intracellular distribution will be presented at the meeting.

S3-O14

3: Disease mechanisms

Cerebellum dysfunction in a mouse model of myotonic dystrophy is mediated by glutamate transport misregulation

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Brain function is compromised in myotonic dystrophy, leading to debilitating symptoms in children and adult patients. DM type 1 (DM1) is mediated by the abnormal accumulation of toxic RNAs carrying expanded CUG repeats, which disrupt alternative splicing, transcription and translation of downstream targets. In the central nervous system we do not know the extent of these molecular abnormalities, nor the cell populations, neuronal circuits and pathways primarily affected. To address this question, we have been using the DMSXL transgenic mouse model of DM1 developed in our laboratory, which express a human *DMPK* transgene carrying more than 1000 CTG repeats. Mouse phenotyping revealed deficits in cerebellum-dependent motor coordination, while electrophysiological profiling in alert mice demonstrated abnormalities in Purkinje cell firing. These results are intriguing, since cerebellum is not typically associated with DM1 neuropathology. To investigate the mechanisms behind Purkinje dysfunction, we studied RNA foci distribution and found extensive foci accumulation in astroglial Bergmann cells surrounding the Purkinje layer, in both mouse and human cerebellum. Interestingly, abundant RNA foci were associated with more severe spliceopathy in laser microdissected Bergmann glia from DMSXL mice, relative to neighbouring Purkinje neurons. Pharmacological strategies to increase glial glutamate recapture in DMSXL mice corrected Purkinje cell electrophysiology, as well as motor discoordination, indicating that mouse cerebellum dysfunction is mediated by defective glutamate uptake by the supporting Bergmann glia. Our results demonstrate for the first time the critical role of astrocyte function and neuroglial communication in disease biology, and open new perspectives to the development of pharmacological approaches based on the manipulation of glutamate signalling in DM1 brains.

S3-O15

3: Disease mechanisms

An inducible expanded CUG-repeat mouse model recapitulates skeletal and cardiac muscle phenotypes of DM1

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In myotonic dystrophy type 1 (DM1), expanded CUG-repeat containing RNA from the DMPK 3'-UTR results in clinical manifestations of the disease. Various mouse models have been developed to study mechanisms of disease progression and to test therapeutic approaches; however, the mechanisms directly leading to skeletal and cardiac muscle features remain poorly understood. We have developed Tet-inducible mouse models containing 960 interrupted-CUG repeats in the context of a genomic segment containing exons 11-15 of human DMPK, inducible in either skeletal or cardiac muscle, to express DMPK-CUGexp RNA in a dose-dependent manner. The skeletal muscle model shows alternative splicing changes consistent with those typically observed in DM1 that reverse upon removal of doxycycline. The extent of splicing changes is mild relative to DM1 muscle, however we observe muscle wasting based on decreased muscle weight and histopathology after four months of doxycycline induction. This includes increased number of fibers containing centralized nuclei (17.5%, compared with 0.54% in control animals) without overt signs of regeneration. Histopathology worsens with 21.37% of fibers containing centralized nuclei after nine months of induction. The heart model exhibits robust splicing transitions that reverse upon removal of doxycycline. Animals develop extensive contractility abnormalities detected by Doppler echocardiography. ECG abnormalities are apparent at high levels of induction but are complicated by similar abnormalities observed in rtTA-expressing control animals on doxycycline. These models will be used for detailed mechanistic studies in skeletal and cardiac muscle as well as to test therapeutic approaches for prevention and reversal of molecular and physiological features of DM1.

S3-O16

3: Disease mechanisms

Innate Immunity and RNA Toxicity

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The expanded (CTG) repeats in the *DMPK* 3'UTR cause myotonic dystrophy (DM1) via the formation of a toxic RNA. The resulting mRNA is capable of forming complex secondary structures and interacts with RNA-binding proteins, namely MBNL (Muscleblind-like) and CELF (CUGBP-Elav-like) family members. We have been investigating if this RNA engages the innate immune system. Members of the innate immune system use pathogen-associated molecular patterns (PAMPs), such as dsRNA structures, to identify threats and activate downstream pathways, such as NFKB and interferon stimulated genes. Our recent work has identified altered expression and activity of the canonical and non-canonical NFKB pathways. This has prompted us to focus on the role of a group of innate immune system receptors that recognize single and double strand RNA. Using both RT-PCR and western blotting, we detected changes in expression of mRNAs and proteins in the skeletal muscles from our inducible/reversible DMPK 3\'UTR (CTG)200 mouse models of RNA toxicity, as well as in tissues from DM1 patients. In addition, we are developing RNA immunoprecipitation (RIP) assays to investigate the interaction of these receptors with the 3\'UTR of the *DMPK* gene. Further biochemical experiments have demonstrated that these interactions can be disrupted through competition with synthetically generated double stranded RNA and by the *DMPK* 3\'UTR mRNA. Ongoing studies are now focused on identifying and mapping the minimal sequences required for these interactions between these RNA sensors and the *DMPK* 3'UTR. Furthermore, we are using genetic approaches to determine if the observed changes in the expression of these receptors have functional and molecular consequences in mouse models of RNA toxicity expressing the expanded *DPMK* 3\'UTR.

S3-O17

3: Disease mechanisms

Misregulation of the alternative splicing of the cardiac sodium channel SCN5A is associated with cardiac conduction delay and heart arrhythmia in myot

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Myotonic dystrophy (DM) is the most common muscular dystrophy in adults, and the first recognized example of a RNA-mediated disease. DM is caused by the expression of mutant RNAs containing expanded CUG or CCUG repeats. These mutant RNAs sequester muscleblind-like (MBNL) splicing regulators, leading to specific alternative splicing changes that ultimately result in the symptoms of myotonic dystrophy. Cardiac alterations, characterized by conduction delays and arrhythmia, are the second cause of death in DM. Using massive parallel RNA sequencing, we identified novel splicing alterations in heart samples of DM patients, among which, a switch from inclusion of the adult exon 6B toward usage of the embryonic exon 6A of the cardiac sodium channel, *SCN5A*. Mutations in *SCN5A* cause a variety of arrhythmic disorders, which present similar features to the cardiac alterations observed in myotonic dystrophy. We found that MBNL1 regulates alternative splicing of *SCN5A* and that depletion of MBNL activities reproduces the misregulation of *SCN5A* splicing observed in DM. Next, we determined that the splicing variant of *SCN5A* produced in DM, namely hNa$_v$1.5e, presents a reduced excitability compared to hNa$_v$1.5, which is encoded by the normal adult isoform of *SCN5A*. Importantly, reproducing this specific splicing alteration of *Scn5a* in wild-type mice is sufficient to promote heart arrhythmia and cardiac conduction delay, two predominant features of myotonic dystrophy. A result confirmed by computer prediction. In conclusion, misregulation of the alternative splicing of *SCN5A* in DM results in expression of an embryonic isoform of the cardiac sodium channel, which has altered electrophysiological properties and leads to cardiac arrhythmia and conduction delay in mice. We propose that these findings explain cardinal cardiac dysfunctions observed in DM patients.

S4-O1

4: Therapeutic development

Therapeutic impact of systemic AAV-mediated RNA interference in the HSALR mouse model of myotonic dystrophy

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RNA interference (RNAi) is a promising therapeutic approach for dominant genetic disorders that involve gain-of-function mechanisms. Myotonic dystrophy (DM) provides an opportunity to test the potential for therapeutic RNAi to reduce the burden of expanded microsatellite repeat mRNA that is responsible for the toxic RNA-mediated disease. A major challenge for the application of therapies for muscle disease is the need for efficient delivery to the wide distribution of muscles in the body. To overcome this hurdle we used recombinant adeno-associated virus (rAAV) to delivery of RNAi expression cassettes to the musculature in the HSALR mouse model of DM type I (DM1). We designed microRNA (miRNA)-based artificial RNAi hairpins to target HSALR mRNA for silencing of the CUG expanded repeats (CUG-EXP). Intravenous injection of a rAAV-HS--RNAi vector led to a 60-95% reduction in the CUG-EXP mRNA in HSALR mice that was accompanied by reduction of myotonic discharges, repeat-containing nuclear foci, aberrant pre-mRNA splicing events, and the frequency of hypertrophic myofibers. Immunoflourescent antibody staining of muscle cryosections showed partial redistribution of Mbnl1 protein in the nuclei of rAAV-HSA-RNAi treated mice. Significant reversal of hallmarks of DM1 in the rAAV RNAi-treated HSALR mice indicate that defects characteristic of DM1 can be mitigated with a systemic RNAi approach targeting a primarily nuclear transcript in terminally differentiated myofibers. These data demonstrate that rAAV-mediated delivery of RNAi has the potential to provide therapeutic benefit for DM patients and for other individuals with dominant muscular dystrophies.

S4-O2

4: Therapeutic development

Utilization of Toxic RNA Expansions to Synthesize Their Own Inhibitors In Cellulo

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Expanded RNA repeats have been identified as the causative agent in an increasing number of microsatellite disorders. It has been shown previously that such RNA expansions can be targeted with high affinity and selectivity by using modularly assembled small molecules. These molecules have been shown to modulate disease associated defects in both cellular and animal models. While this approach has been successful, targeting repeats with small molecule therapeutics is challenging as they require both the cellular permeability of low molecular weight RNA binders and the enhanced potency of high molecular weight oligomers. The advantages of both types of molecules could be synergized if low molecular weight binders could be transformed into potent, multivalent oligomers by a reaction that is catalyzed by binding to the RNA repeat within diseased cells. We have developed this approach and applied it *in cellulo* to target the r(CCUG)^{exp} RNA hairpin that causes in Myotonic Dystrophy 2 (DM2). Small molecule modules with strategically positioned alkyne and azide moieties bind adjacent internal loop motifs in the r(CCUG)^{exp} RNA hairpin and are transformed into oligomeric, potent modulators of DM2 RNA's cellular function. Herein we demonstrate that potent modulators of RNA function can be assembled *in cellulo* by using the cell as a reaction vessel and a disease-causing RNA as a catalyst to improve disease associated defects.

S4-O3

4: Therapeutic development

Small Molecules that Control the Fate of CUG repeat RNA transcripts, the Causative Agent in Myotonic Dystrophy Type 1

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Myotonic dystrophy type 1 (DM1) is caused by an abnormal expansion of CTG repeats in the *DMPK* gene. The transcribed CUG repeats (CUG^{exp}) sequester several proteins including MBNL1, an important alternative-splicing regulator. The reduction in MBNL1 levels leads to the disease phenotype. DM1 therapeutic agents have been developed to inhibit the deleterious complex formed between MBNL1 and CUG^{exp}. A recent study on DM1 pathogenesis, including the discovery of repeat associated non-ATG (RAN) translation and microRNA dysregulation, suggests that a more powerful approach for DM1 treatment is to degrade or terminate the formation of the toxic CUG^{exp}. Herein, we report a series of small molecules that control CUG^{exp} levels in cells. These small molecules are the conjugates of reported CUG^{exp} binders and cleaving units containing amino and imidazole groups. *In vitro* experiments, lead ligands selectively cleave CUG^{exp}, but not other RNAs. Further investigation shows that ligands are able to inhibit the *in vitro* transcription of CTG×CAG repeats. Treatment of DM1 model cells with ligands results in a decrease in CUG^{exp} mRNA levels. The lead ligand suppresses the neurodegeneration in DM1 *Drosophila*. The combined data demonstrates that small molecules regulate the CUG levels in cells through both cleavage and transcription pathways.

S4-O4

4: Therapeutic development

High Content Screening approach using DM1 mutated human Embryonic Stem cell derived cellular model

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Human embryonic stem cells (hES) and their progenies are powerful models for drug discovery and *in vitro* toxicology studies. In the purpose of monogenic disease, hES harvested from pre implantation genetic diagnosis allow to work on naive cells, directly on human mutations, without any cell engineering. Amplification and differentiation toward a specific cellular identity allow to reach a large amount of well characterized cells in cell types of interest, improving the test relevance.

We developed a High Content Screening (HCS) approach in order to explore the therapeutic potential of small molecules against the Myotonic Dystrophy type1 (DM1). This autosomal monogenic disease is characterized by an abnormal CTG tri-nucleotide expansion in the 3' untranslated region of the Dystrophia Myotonica Protein Kinase (DMPK) gene. Mesenchimal Stem cell (MSC) derived from DM1 mutated hES displays nuclear *foci* as observed in patients' myoblasts, sequestering RNA binding proteins. We thus developed automated methods to quantify intranuclear aggregates after fluorescent *in situ* hybridization and explored the ability of 12000 small molecules to destroy or modify these *foci*.

The screening campaign had been run in an automated manner in 384-wells plates from cell treatment to *foci* labelling. Hits selection was performed using two main parameters, the number of detected *foci* per cell and their average area, highlighting the ability of cardiac glycosides to increase the *foci* number. Subsequent analysis revealed the interest of these FDA approved drugs to rescue some of DM1 related splicing defects in several cellular models, through calcium signalling and functional experiments showed that digoxin was also able to reduce the myotube formation defect observed in DM1 myoblasts *in vitro*.

S4-O5

4: Therapeutic development

A High-throughput Screening System for Up-regulation of Endogenous MBNL1 as a Potential Therapy for Type 1 Myotonic Dystrophy

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Type 1 Myotonic Dystrophy (DM1) is caused by a microsatellite expansion in the 3'UTR of the *DMPK* gene. The transcripts with expanded $(CUG)_n$ repeats sequester the RNA binding protein Muscleblind-like 1 (MBNL1) and hamper its normal function in pre-mRNA splicing. The mis-splicing observed in DM1 due to loss of MBNL1 function presumably leads to the multi-systemic defects found in patients. It has been shown that overexpressing exogenous MBNL1 in the DM1 mouse model partially rescues the splicing defects and reverses myotonia. Although it is a viable therapeutic strategy, the mechanism of MBNL1 expression regulation is unclear and no small molecule or drug target for increasing *MBNL1* expression has been identified. Here, we used the CRISPR/Cas9 system to successfully engineer a ZsGreen tag into the endogenous *MBNL1* locus in Hela cells and established a flow cytometry-based high-throughput screening (HTS) system for the idenitification of MBNL1 up-regulators. . Utilizing a set of small molecules targeting epigenetic proteins, we identified compounds that increased *MBNL1* expression up to 2 fold. The majority of the hits targeted Histone deacetylase (HDAC) family members. An HDAC inhibitor partially rescues the splicing defect caused by $(CUG)_n$ repeats. These results suggest a novel epigenetic regulation mechanism for *MBNL1*. The detailed study of this molecular mechanism could provide potential avenues for therapeutic approaches in DM1.

S4-O6

4: Therapeutic development

A novel genome editing-based strategy for Myotonic Dystrophy type 1

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Genome editing is a mechanism of DNA modification dependent on the activity of nucleases that determines DNA double-strand breaks (DSBs) at specific sites. DSBs stimulate two major cellular mechanisms of DNA repair, non-homologous end joining (NHEJ) and homology-directed repair (HDR). Both mechanisms of DNA repair can be exploited to modify the genome. For example NHEJ can introduce insertions or deletions (indels), and HDR can lead to the exchange of a specific sequence by homologous recombination (HR) in presence of a DNA donor template.

CRISPR/Cas (clustered, regularly interspaced, short palindromic repeat / CRISPR-associated proteins) system represents last generation genome editing tool. This system is based on RNA-DNA interactions and on the activity of the nuclease Cas9: Cas9 is addressed to a specific sequence by a small guide RNA (sgRNA) that contains a string complementary to the DNA target site thus leading to targeted DSBs.

We aim at applying CRISPR/Cas9 technology for the gene therapy of myotonic dystrophy type 1 (DM1). DM1 is caused by an expansion of the CTG triplet repeat in the 3' untranslated region (3'-UTR) of the *DMPK* gene. We specifically focus on the development of a strategy to excise the *DMPK* CTG expansion. For that purpose we have generated several constructs that express a small size Cas9 nuclease under either an ubiquitous or a muscle-specific promoter and various expression cassettes for sgRNAs targeting 3'-UTR of the *DMPK* gene.

Transfection studies show that Cas9 is well expressed in the nucleus of various cell lines, including myoblasts. Moreover, upon concomitantly expression of Cas9 and sgRNAs we were able to excise the CTG expansion in primary cells derived from DM1 patients. We are currently producing adeno-associated vectors for *in vivo* delivery of these constructs in a DM1 mouse model.

S4-O7

4: Therapeutic development

Highly specific contractions of CAG/CTG trinucleotide repeat by TALEN

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Trinucleotide repeats are a specific class of microsatellites whose large expansions are responsible for many human neurological disorders, such as Huntington's disease, fragile X syndrome and myotonic dystrophy type 1 (DM1). DM1 is due to an expansion of CTG/CAG triplets in the 5'UTR of *DMPK* gene, which can reach thousands of repeats. Molecular mechanisms leading to these large expansions are poorly understood but *in vitro* studies have shown the capacity of these repeats to form secondary structures such as intramolecular hairpins. These structures probably interfere with mechanisms involving *de novo* DNA synthesis of such repeat. We want to shorten long CTG/CAG repeats to a non-pathological range with the help of artificial nucleases. We have already shown that a TALEN used to induce double-strand breaks (DSB) in a CTG/CAG repeat integrated in the yeast *Saccharomyces cerevisiae* leads to highly efficient repeat contraction. After TALEN induction, repeat contractions were observed in 99% of heterozygous cells and 100% in homozygous cells (Richard et al., *PLoS One*, 2014). The TALEN is now expressed in yeast mutants for DSB repair genes, in order to identify which repair mechanism(s) is (are) involved in repeat contraction. Preliminary data suggest that *RAD50* plays an important role in repairing such breaks. The same TALEN is also expressed in fibroblasts obtained from transgenic mice that carry the DM1 mutant allele, and preliminary data will be discussed.

Richard G. F., Viterbo D., Khanna V., Mosbach V., Castelain L., Dujon B., Highly specific contractions of a single CAG/CTG trinucleotide repeat by TALEN in yeast. *PLoS One* **9(4):e95611.** (Apr, 2014).

S4-O8

4: Therapeutic development

Congenital and Acquired Dmpk Reduction Does Not Affect Cardiac Conduction or Ejection Fraction in Mice.

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DM1-associated defects of cardiac conduction are attributed to DMPK loss-of-function, RNA gain-of-function, or both. Antisense oligonucleotides (ASOs) targeting toxic RNA have been shown to improve skeletal muscle phenotypes in mouse models of DM1. While ASO drugs may have similar potential to mitigate RNA toxicity in the heart, there is risk of aggravating the DMPK deficiency. To reexamine the role of DMPK in the conduction system and define cardiac risks of *DMPK*-targeting ASOs, we studied mice with congenital or acquired DMPK reduction. We obtained ECGs and echocardiograms on homozygous (-/-) and heterozygous *Dmpk* knockout (+/-) mice (Jansen et al, Nat Genet, 1996), compared to wild-type (WT) littermates. The +/- mice were treated with *Dmpk*-targeting ASOs or vehicle alone (saline). Administration of ASO was started at 2 months by subcutaneous injection of 50mg/kg weekly for 6 weeks, and then shifted to biweekly injections. Basal *Dmpk* expression in +/- mice was reduced to ~50% of WT, and was further reduced by ASOs (84 ± 3% decrease of mRNA, 93 ± 2% decrease of protein, relative to WT). Surface ECGs and echocardiography at 6 months and 10 months demonstrated no difference of heart rate, cardiac conduction, or ejection fraction in WT, saline-treated +/-, ASO-treated +/-, or -/- mice. Conscious, unrestrained ECGs were obtained at 11-12 months by radiotelemetry with signal averaging. Once again, heart rate and cardiac conduction showed no differences among WT, saline-treated +/-, ASO-treated +/-, or -/- mice. These results show that ASOs can induce posttranscriptional silencing of *Dmpk* in the heart. We found that constitutive absence of DMPK did not impact cardiac conduction or contractility, and the same was true for acquired DMPK reduction to levels that are <15% of WT. Our data support the idea that cardiac dysfunction in DM1 results mainly from RNA toxicity, which potentially could be prevented or alleviated by ASOs.

S4-O9

4: Therapeutic development

Reduction of CUGexp RNA expression is insufficient to correct myopathy in DM1 mice

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A common criticism of the HSA^{LR} mouse model is it has \"only a mild myopathy.\" For example, in young HSA^{LR} mice, expression of CUG^{exp} RNA, myotonia, and mis-splicing precede onset of severe pathology. However, a recent report showed that by 14 months of age, HSA^{LR} mice develop a marked increase in the frequency of internal nuclei and the number of myonuclei per fiber, abundant atrophic and hypertrophic fibers, more pronounced fiber size variability, and some focal regions of fibrosis, indicating progression from a mild to severe DM1-like myopathy (Wheeler, 2012). A notable difference from human DM1 was the absence of nuclear bags in HSA^{LR} muscle, possibly due to absence of CUG^{exp} RNA expression at the neuromuscular junction (Wheeler, 2007). Similar to HSA^{LR} mice, every human with classical adult onset DM1 is, by definition, mildly affected, or even unaffected, during childhood and early adulthood, but eventually develops progressive symptoms, presumably due to chronic life-long exposure to toxic CUG^{exp} transcripts. To test the therapeutic window for DM1 myopathy after chronic, long-standing CUG^{exp} RNA toxicity, we treated older HSA^{LR} mice with saline or gapmer antisense oligos (ASOs). Splicing outcomes in young and older HSA^{LR} ASO-treated mice were similar to wild-type. Yet, a blinded examiner found no difference in muscle histology from age-matched saline- or ASO-treated mice. Mitotic activity of myonuclei was inversely related to myopathy severity and age, and showed no change after ASO treatment, suggesting impaired muscle repair despite knockdown of CUG^{exp} RNA and splicing correction for several months. Quantitative analysis of muscle histology and functional outcomes in additional cohorts of treated mice are ongoing. Our preliminary results suggest DM1 myopathy may be difficult to correct with ASO monotherapy. Optimal outcome in DM1 patients may require complementary therapeutic approaches. Support: Muscular Dystrophy Association USA (234905).

S4-O10

4: Therapeutic development

Correction of the GSK-3beta-kinase - CUGBP1 signaling as therapeutic approach in adult and in congenital Myotonic Dystrophy type 1

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Myotonic Dystrophy type 1 is a neuro-muscular disease caused by unstable CTG triplet repeat expansions. DM1 exists in several clinical forms including adult form and the most severe congenital DM1 which affects patients at birth. The mutant CUG repeats cause the pathology through misregulation of RNA metabolism mediated by several key RNA-binding proteins. One of these proteins is CUGBP1. We found that, in normal muscle, CUGBP1 translational activity is regulated by the GSK3 beta kinase/cyclin D3 signaling pathway. In skeletal muscle of patients with adult form of DM1 the levels of GSK3 are increased which switches the CUGBP1 translational function from activation to repression. Our data show that the correction of this pathway is important for the DM1 therapy since short-term treatment of DM1 mice (HSA model) with the inhibitors of GSK3 reduces myotonia, increases body strength and reduces DM1 muscle histopathology. The longitudinal study examining the long-term effect of the inhibitors of GSK3 on DM1 pathology in HSA mice showed that the treatment of young HSA mice with the inhibitors of GSK3 dramatically improves muscle health in HSA mice up to 1 year of age. The beneficial effect of the inhibitors of GSK3 at young age suggests that these inhibitors might be important for the treatment of congenital DM1. We began analysis of the GSK3-CUGBP1 pathway in myoblasts and muscle biopsies from patients with congenital DM1. These studies showed that the levels of GSK3 beta are much higher in myoblasts from congenital DM1 relatively those in myoblasts from patients with adult form of DM1. We suggest that expansions containing >1,000 CTG repeats might strongly increase the levels of GSK3 beta at birth. This increase of GSK3 beta in the actively grown tissues might cause severe symptoms of congenital DM1. This hypothesis is being tested in a large number of muscle biopsies from patients with congenital DM1 of different age and in a mouse model for congenital DM1.

S5-O1

5: Biomarkers / outcome measures / registrie / therapeutic assays

DM-SCOPE, the international DM1 registry, sheds light on clinical classification

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Background: The wide phenotypic variability of myotonic dystrophies (DM) poses particular challenges for both clinical management and design of therapeutic trial. Currently, OMMYD consortium aims to define a consensual clinical classification for harmonization of international DM research.
Objective: We investigated the robustness of a DM1 classification in five clinical forms based on age of onset.
Methods: Uni/multivariate analyses of DM1 manifestations segmented into 153 items were performed in the widest collection of standardized data from DM population, the DM-Scope international Registry. We compared characteristics of 1962 French DM1 adult patients *vs.* 1100 Quebec one.
Results: Analyses strengthen robustness of the conventional age of onset-based classification of DM1 with regard to the triplet expansion size in the two populations ($p < 0.0001$), i.e. congenital (5%), infantile (19%), juvenile (26%), adult (39%) and late onset forms (11%). The five clinical forms distinguish from each others by the prevalence of the main symptoms as their apparition profiles.
Conclusion: In the context of emerging therapeutic approaches and harmonization of international DM network activities, our work provides a robust clinical classification confirmed in two independent large cohorts. The differences observed in the phenotypes' severity and evolution suggested that clinical forms should be included as one of primary criteria for the constitution of homogenous cohort in clinical trials.

S5-O2

5: Biomarkers / outcome measures / registrie / therapeutic assays

Natural history of motor impairment in myotonic dystrophy type 1 (DM1)

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Objective: Assess DM1 progression over 3 years using quantitative measures of motor function.
Background: Few studies have examined DM1 progression in large cohorts over long intervals.
Design: We performed a single-center prospective study of ambulatory adult patients with genetically proven non-congenital DM1. Timed functional tests (TFTs) were performed by standard methods. Strength was assessed by manual muscle testing (MMT, 15 muscle groups) and quantitative muscle testing (QMT, 7 muscle groups). Subjects were assessed at baseline, 1 year, and 3 years (3 year data are described here).
Results: 66 subjects (mean age 48 yrs, range 18 to 69 years, 46 females) completed 3 years of follow up. Scores on the Muscle Impairment Rating Scale ranged from 1 to 5. TFTs showed loss of motor performance but the magnitude of reduction was small. For example, 6 minute walk test (6MWT) distance was 421 m at entry and decreased by 17 meters at year 3 ($P < 0.005$). Similar percentagewise changes were noted in 30 Foot Go (23% slowing, $P < 0.001$) and ascending 4 stairs (27% slowing, $P < 0.001$). The overall combined MMT score showed significant decrease ($p=0.005$), with distal muscles demonstrating the most change. QMT showed significant decrease in the strength of distal muscles. The mean reductions of ankle dorsiflexion and hand grip were 9% ($P < 0.05$) and 6% ($P < 0.01$), respectively.
Conclusion: In accordance with clinical experience, the progression of muscle weakness and motor impairment over 3 years was modest. However, the extent of progression was quite variable between subjects, ranging from 98 m gain to 236 m loss in the case of 6MWT. Further studies are needed to identify genetic factors or baseline characteristics that predict future progression. Many of the quantitative measures showed good reliability from baseline to 1 year, and may prove to be sensitive for detecting improvement in response to therapy.

S5-O3

5: Biomarkers / outcome measures / registrie / therapeutic assays

The Myotonic Dystrophy Health Index: Correlations with Clinical Tests and Patient Function

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Introduction: The Myotonic Dystrophy Health Index (MDHI) is a disease-specific patient-reported outcome measure designed for use as a clinical trial outcome measure. The MDHI includes 17 subscales that measure overall patient disease burden and each of the most important symptomatic themes in this population. Here we examine the associations between the MDHI and over 60 other measures of disease burden in a cohort of individuals with myotonic dystrophy type-1.

Methods: We conducted a cross-sectional study of 70 patients with DM1. Participants were enrollees in the Study of Pathogenesis and Progression in Dystrophia Myotonica (STOPP DM) study at the University of Rochester. All enrolled participants completed the MDHI, strength and function testing, myotonia testing, respiratory function testing, gait testing, body composition testing, laboratory testing, additional patient-reported outcome measures, and underwent a clinical assessment by a board certified neurologist. We examined the associations between MDHI total and subscale scores and scores from other clinical tests.

Results: MDHI total and subscale scores were strongly associated with muscle strength, myotonia, motor function, and other clinical measures. The MDHI total score was associated with patient-reported disability status ($p=0.0003$) as were 14/17 of the MDHI subscales. Manual muscle testing utilizing 26 muscles was associated with MDHI total score ($r:-0.72$; $p<0.0001$) and all of the MDHI subscale scores. Quantitative muscle testing, pinch grip, time to go 30 feet, time to ascend 4 steps, time to descend 4 steps, forced vital capacity while sitting, six minute walk distance, and MIRS were associated with MDHI total score and all but one of the MDHI subscales.

Conclusion: Patient-reported health status, as measured by the MDHI, is associated with a wide range of clinical health measures. These results support the use of the MDHI as a valid tool to measure disease burden in DM1 patients.

S5-O4

5: Biomarkers / outcome measures / registrie / therapeutic assays

Report from the Outcome Measure in Myotonic Dystrophy (OMMYD-3) meeting

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Since the discovery of the gene of DM1, many research groups have worked on development of therapeutic approaches. The international scientific community has raised concerns over the lack of a consensus about clinical endpoints, absence of metrological sound outcome measures and the necessity of more studies on the natural history of the disease. All these points highlight the need to adopt a systematic multidisciplinary approach to choose clinical outcomes and their related outcome measures. To support this process of selection and validation of outcome measures for DM1, an international initiative has emerged. The Outcome Measure in Myotonic Dystrophy meeting, OMMYD-1 was launched in 2011. The third meeting will be held prior to IDMC-10 meeting in Paris, France in 2015. This meeting will convene researchers from around the world to discuss specific topics within seven special interest groups: 1) Patient-reported outcomes, 2) Functional capacity, 3) Cognitive functions, 4) Muscle testing and training; 5) Disease severity index, and 6) Hypersomnolence, fatigue and apathy; 7) Respiratory functions. The long-term objectives are: 1) To determine relevant clinical and patient outcomes in DM1; 2) to select related outcome measures to be used in clinical trials and natural history studies (among existing ones or by developing new outcome measures), and 3) to define metrological properties and establish normative data for each selected outcome measure.

The main conclusion following this third meeting for each special interest group will be presented.

S5-O5

5: Biomarkers / outcome measures / registrie / therapeutic assays

Is one trial enough? - Assessments of walking, mobility, balance and fine hand use in people with myotonic dystrophy type 1

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Assessments of walking, mobility, balance and fine hand use are essential in people with myotonic dystrophy type 1 (DM1), both to monitor progress and for evaluation of interventions. Commonly used functional capacity outcome measures are the six-minute-walk test (6MWT), the 10m-walk, the timed-up-and-go test, the timed stands-test, time taken to rise from supine or sitting to standing, one-leg stand test, walk in figure-of eight test, grip strength tests and the nine-hole peg test. There is, however, no consensus on number of trials to be performed on each test occasion or on how to report the result. Thus, the aims were to describe and evaluate differences between trials in these functional capacity measures. Seventy persons, (mean age 45 years, range 19-70), with DM1 participated in this cross-sectional study. The tests above were performed over two days, and three trials were if possible performed for each test except for the 6MWT where two trials were allowed. Between five and 16 participants with severe muscle weakness could not perform the tests of walking, mobility and balance, i.e. there were floor effects. Others with severe weakness had difficulties in performing all three trials. There were statistical significant differences over the three trials in all tests except for the 10m-walk test and grip strength tests. Pairwise comparisons showed that the second and third trial was in general better than the first. The best trial was significantly better in comparison to the mean of trails for each test ($p<0.001$). At which trial, i.e. the first, second or third, the individuals performed their best differed between individuals and tests. The conclusion and clinical implication of this study is that multiple trials should be performed for each test, i.e. two trials for the 6MWT and three trials for the others, and that the best trial should be used as a test result. This information is relevant and important for both clinical and research work.

S5-O6

5: Biomarkers / outcome measures / registrie / therapeutic assays

Identification of outcome measures to quantify short-term skeletal muscle strength impairments in DM1

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The aim of this study was to investigate the skeletal muscle weakness in 60 adult DM1 patients in a 5-year longitudinal retrospective study. The highest rate of decline in muscle strength among 11 muscle groups tested, assessed by Manual Muscle Testing, were the digit flexors (DF) (-6.2%/year) and the ankle dorsi-flexors (ADF) (-3.1%/year). The decrease became significant at 2- (DF;0.001) or 3-years (ADF, 0.04). The rate of decline of these 2 muscle groups was significantly associated with the duration of the disease (0.00006 for DF, 0.04 for ADF) but not with the gender (DF, 0.889; ADF, 0.236), age at evaluation (DF, 0.06, ADF, 0.5), age at onset (DF, 0.733, ADF, 0.06) or number of CTG repeat in the blood (DF, 0.09 and ADF, 0.0857). In a 5-year prospective study, the decrease in ADF muscle strength assessed by QMT was shown to be of 6,56%/year and it became significant at 18-months (0.004). The handgrip values were lower in DM1 patients compared to reference values. There was a significant difference between male and female handgrip force (0.021) and a highly negative association between the handgrip force and the number of CTG repeats in the blood (0.001), duration of the disease (0.0001) and age at evaluation (0.0097). The rate of decline in handgrip force was 6.2% / year and became significant at year 2 (0.0001). The rate of decline was significantly correlated with the age of patients (0.028) and the ADF strength (0.0001). These findings are suggesting that QMT of the ADF and handgrip are two key outcome measures that could be used in 2-year period clinical trials.

S5-O7

5: Biomarkers / outcome measures / registrie / therapeutic assays

What are the Appropriate Cardiac Outcome Measures to Include in Phase 2, 3 Myotonic Dystrophy Type 1 Drug Trials?

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Background: As antisense oligonucleotide myotonic dystrophy type 1 (DM1) therapy is tested in human clinical trials, it will be crucial to assess outcome measures in all affected organ systems. Cardiac abnormalities occur in DM1 as part of a multisystem involvement and lead to adverse events including premature mortality. **Objectives:** To assess cardiac outcomes and their frequency in DM1 in order to understand a natural history baseline necessary for the selection of measures and the determination of benefit / risk in therapeutic trials. **Methods:** Analysis from a U.S. registry with clinically- and genetically-verified DM1 enrolled at MDA clinics and prospectively followed (study entry–N=406; age: 42±12 yrs; male: 205 (50.5%); CTG repeats: 629±386; muscular impairment rating score: 3.2±1.0). **Results:** By last follow-up (11.2±4.2 yrs), 146 (36.0%) of the 406 pts had a total of 323 cardiac outcomes. The outcomes and findings observed (No. of cases [N], frequency [%], incidence [cases per 1000 pt-yrs], median age at outcome [yrs]) were: atrial fibrillation / flutter (72, 17.7, 11.2, 50.8); pacemaker implant (51, 12.6, 9.2, 51.1); defibrillator implant (26, 6.4, 5.7, 51.8); ejection fraction<0.50 (44, 10.8, 7.5, 51.7); heart failure (29, 7.1, 5.0, 55.2); sinus node dysfunction (14, 3.4, 2.4, 54.9); high-degree atrioventricular block (23, 5.7, 4.0, 51.1); ventricular tachycardia (14, 3.4, 3.1, 47.5), non-sudden cardiac death (8, 2.0, 1.8, 67.8), and sudden cardiac death (42, 10.3, 9.2, 52.4). All-cause death was (170, 41.9, 37.3, 55.4) with the majority due to respiratory failure (90, 22.2, 19.8, 55.4). Exclusion of pts with one or more of the characteristics at study entry of: age> 55.0, cardiac diagnosis, or severe ECG abnormality (non-sinus, PR≥240 ms, QRS≥120 ms) would decrease cardiac outcomes to 47 (18.1%) of 259 eligible participants during follow-up. **Conclusions:** Cardiac outcomes are common in DM1 and will require assessment in therapeutic clinical trials.

S5-O8

5: Biomarkers / outcome measures / registrie / therapeutic assays

OPTIMISTIC: Observational Prolonged Trial In Myotonic dystrophy type 1 to Improve Quality of Life- Standards, a Target Identification Collaboration.

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THE OPTIMITSTIC CONSORTIUM, www.optimisitc-dm.eu

OPTIMISTIC is a randomised controlled trial for patients with myotonic dystrophy type 1 (DM1). This trial looks beyond the traditional pharmacological solutions to address the current lack of therapies that can reduce, slow down or maintain the symptoms of the condition. Here we assess the impact of a unique intervention combining Cognitive Behavioural Therapy and exercise therapy on levels of fatigue, inactivity and quality of life. The main rationale for this intervention is based on our previously published DM1-specific model. Recruitment for this study began in April 2014 and in the first 11 months 195 participants have been successfully randomised between the four clinical sites of Munich, Newcastle, Nijmegen and Paris. A recruitment rate of over 17 participants a month, recruitment will continue through April 2015.
The study consists of two arms, “standard care” versus “intervention”, the intervention lasts a total of 10 months. Each participant, irrespective of randomisation is expected to attend four assessment visits consisting of clinical and functional outcomes (e.g. 6MWT and MIRS) along with assessments of fatigue, quality of life and cognitive function (e.g. CIS fatigue, DM-Activ, MDHI and Stroop test). In addition at three time points RNA, DNA and serum samples are collected in order to carry out genetic and molecular biomarker analysis. A small cohort of participants is being included in one of two MRI sub studies. The first assessing cardiac safety in Newcastle (involving 40 patients) and the second looking at skeletal muscle imaging biomarkers of the leg is being carried out in Nijmegen and Paris (involving 50 patients).
As the first European trial with a model based intervention for DM1, OPTIMISTIC marks an important step forward. The OPTIMISTIC consortium is developing the tools and know how to carry out large scale clinical trials for DM1 in Europe. This infrastructure is now in place for the benefit of future therapeutic trials.

S5-O9

5: Biomarkers / outcome measures / registrie / therapeutic assays

Widespread networks' disconnection accounts for clinical symptoms in Myotonic dystrophy Type-1

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Introduction and aims: Cognitive impairment and behavioral symptoms play a relevant role in determining disabilities in patients with Myotonic dystrophy type-1 (DM1). It has been recently shown that abnormalities in functional brain connectivity account for patients' personality traits. However, nowadays, there are no validated biomarkers available for assessing higher level dysfunctions in DM1. We used here resting state-functional magnetic resonance imaging (fMRI) to assess changes in brain connectomics in DM1 patients and potential relationships with clinical and cognitive features. Methods: Thirty-one patients with DM1 and 26 healthy controls underwent MRI at 3T, by collecting anatomical and functional imaging data at rest. Connectomics' measures were extracted from fMRI data based on the graph theory. Results: The global properties of connectomics in DM1 brains were strictly associated with MIRS scores. Interestingly, local measures of networks' efficiency (i.e., Nodal degree, Betweeness Centrality, Nodal Efficiency) were all reduced in patients compared to controls. When looking at single networks, DM1 patients showed a pattern of reduced connectivity in the cingulum and orbitofrontal cortex bilaterally. These areas resulted to include critical nodes for networks implicated in high level cognition and complex behaviour. The former aspect was confirmed by associations between strength of brain connectivity and measure of logical reasoning in DM1 patients. Conclusions: This preliminary study highlights specific pathophysiological mechanisms for higher level dysfunctions in DM1. The association with MIRS score confirms higher level dysfunctions as a core feature for DM1. Finally, this study opens new perspectives for patients' clinical prognosis and monitoring, and for monitoring potential pharmacological and non-pharmacological interventions.

S5-O10

5: Biomarkers / outcome measures / registrie / therapeutic assays

Tracking the brain in myotonic dystrophy type 1 (DM1) and 2 (DM2): A 5-year longitudinal neuroimaging and neuropsychological follow-up study

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It is unknown whether brain affection in DM1 and DM2 is due to neurodevelopmental defects, neurodegeneration, or both. We performed a prospective longitudinal study to compare changes in cognition and brain morphology including 16 DM1 (m/f: 6/10, age at baseline 42.5±6.5 years/y, disease duration/DD at baseline 13.4±7.5 y), 16 DM2 patients (m/f: 9/7, age 48.5±8.4 y, DD 11.4±9.1 y), and 17 healthy controls (m/f: 9/8, age 50.5±9.8 y). All subjects underwent neurological, neuropsychological (NP) and 3T-brain MRI examinations at baseline and 5.5±0.4 y follow-up using the identical hard- and software. Diffusion tensor imaging (DTI) was performed to analyse white matter (WM) affection (i.a. fractional anisotropy, FA). Statistical analyses included (i) group comparisons at baseline and follow-up using two-sample t-tests between DM1/DM2 and controls, and (ii) two-sample t-tests between DM1/DM2 groups and controls using pair wise skeletonised difference maps for longitudinal analyses ($p<0.05$, corrected for multiple comparisons). DTI group comparisons showed widespread WM affection in DM1 more than DM2 with similar patterns at baseline and follow-up. At follow-up, the number of voxels showing a significant FA reduction was increased in DM1 and DM2 when compared to baseline indicating slightly progressive WM changes. However, we did not find significant differences in FA changes over time between patient groups and controls by difference image analyses. Our findings were accompanied by a decline in motor tasks in DM1, and a mild deterioration in specific NP tasks in DM1 and DM2 compared to controls. We found only minor brain structural and NP changes over time in our series of patients. In conclusion, morphological changes as seen on MRI might be primarily neurodevelopmental. Nevertheless there are signs of a neurodegenerative component. Analyses of longitudinal voxel-based morphometry data are currently underway, and first results are fully in line with our DTI findings.

S5-O11

5: Biomarkers / outcome measures / registrie / therapeutic assays

Quantification of CUG-Repeat RNA Mass Using a Group II Intron Reverse Transcriptase

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Myotonic dystrophy type 1 (DM1) is caused by an expanded CTG-repeat in the 3`- untranslated region of the *DMPK* gene. Pathogenesis primarily involves accumulation of *DMPK* transcripts that contain the expanded CUG-repeat, and multiple therapeutic approaches are designed to reduce levels of the CUG-repeat RNA. To develop a method to directly assay the amount of CUG-repeat RNA in tissues, we have utilized a group II intron reverse transcriptase (GsI-IIC) that has high processivity and strong strand-displacement activity. First we generated $(CUG)_{100}$ RNA by in vitro transcription. Next we compared reverse transcription (RT) activity of two enzymes, using $(CUG)_{100}$ template that was tiled with $(CAG)_6$ primers. The GsI-IIC RT was capable of producing multiple complementary DNA (cDNA) products across the full length of $(CUG)_{100}$. In contrast, only short extension products were generated by Superscript III RT. Targeted reverse transcription was then performed in post-mortem samples of cardiac RNA using $(CAG)_6$-primer and three dNTPs (dATP + dGTP + dCTP but not dTTP). A DM1-specific band was detected by a DIG-labeled $(CTG)_7$-LNA probe on slot blot, which was absent from disease control heart tissues. Lastly, transgenic mice (HSA^{LR}) that express CUG-repeat RNA in skeletal muscle were given twice weekly 25 mg/kg injections of antisense oligonucleotide (ASO) or saline for 4 weeks. Measurements of the (CUG)-repeat RNA showed a 48% reduction in band intensity in ASO-treated animals, compared to saline-treated controls. Our results indicate that GsI-IIC RT is capable of reverse transcribing the highly-structured CUG-repeat RNA. Furthermore, it exhibits multiple strand displacement activity for tiled CAG-repeat primers. This method has potential application to quantify therapeutic response to ASOs or other putative treatments for DM1. A similar approach may be useful for other disorders caused by expanded tandem repeats.

Poster Presentation Abstracts

P-001

1: Mutation, genetics and epigenetics

Clinical spectrum and pathomolecular findings in Italian myotonic dystrophy type 2 premutated patients

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Myotonic dystrophy type 2 (DM2) is caused by an unstable [CCTG] tetranucleotide part of a polymorphic complex repeated motif [TG]n[TCTG]n[CCTG]n in the *CNBP* gene. The [CCTG] tract is generally interrupted by one or more GCTG, TCTG or ACTG motifs in the normal allele, whereas in premutation and expanded alleles is typically uninterrupted. DM2 patients show a pathological number of repeats starting from 75 to 11.000. However, in the last years few patients with DM2-linked myopathy and a $[CCTG]_{55}$ expansion have been reported. In accordance with these observations, here we describe two Italian patients with clinical and histological muscular abnormalities associated with DM2 premutated uninterrupted alleles. Patient A, a 51-year-old woman, showed progressive proximal leg weakness and pains leading to difficulties in walking and actually she uses a wheelchair. No myotonia was evident. Muscle biopsies performed at the age of 46 and 51 showed an increase of central nuclei and of fiber size variation, type I and II fiber atrophy and nuclear clumps. Patient B, a 26-year-old man, was a paucisymptomatic patient presenting iperCKemia, severe myalgia and no myotonia. Muscle histopathology showed a mild fiber size variation. Two CNBP alleles have been amplified by LR-PCR on DNA extracted from blood. Cloning and direct sequencing of the larger CNBP alleles indicate a CCTG repetition number of 36 and 53 in patients A and B respectively. FISH in combination with MBNL1-immunofluorescence performed on muscle sections did not show nuclear accumulation of mutant RNA or of MBNL1 and alternative splicing of CLCN1, MBNL1 and INSR were not altered. Haplotype analysis is currently in progress to assess whether these CNBP premutated alleles derive from the same founder origin as the European DM2 mutation. Further studies are necessary to understand if the myopatic phenotype described in these two Italian patients is linked to the DM2 locus or to other still undefined genetic mutations.

P-002

1: Mutation, genetics and epigenetics

DMPK gene DNA methylation is correlated with cognitive and respiratory profiles in patients affected with myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is caused by a CTG repeat expansion within the 3'-untranslated region of the *DMPK (dystrophia myotonica protein kinase*) gene. Although DM1 is an autosomal dominant disorder, the phonotype shows large phenotypic variability. Respiratory and cognitive functions are impaired in DM1 and the severity of symptoms is only partially explained by CTG expansion length. Therefore, other mechanisms such as epigenetics could explain the remaining phenotypic variability. **Objective**: To assess the impacts of DNA methylation profile at *DMPK* gene locus on variability of respiratory and cognitive functions in DM1. **Methods**: DNA methylation levels at *DMPK* gene locus were quantified using sodium bisulfite treated DNA and pyrosequencing. **Results:** DNA methylation levels at *DMPK* gene locus were associated with total pulmonary capacity (r_s=0.237; p=0.048), Stroop-word test results (r_s =0.267; p=0.015) and verbal fluidity (r_s=0.259; p=0.017). These associations were specific to the DM1 adult phenotype (n=89)**.** In patients with the late phenotype (n=26), DNA methylation levels were correlated with maximum expiratory pressure (r_s=-0.624; p=0.007), maximal median expiratory flow (r_s =0.513; p=0.018) and Stroop-word test results (r_s=0.427; p=0.047)**. Conclusion:** *DMPK* gene DNA methylation profile is associated with respiratory and cognitive clinical phenotypes in DM1. These results suggest that epivariations at *DMPK* gene locus are biomarkers of phenotypic variability and might predict the progression of the disease.

P-003

1: Mutation, genetics and epigenetics

Role of unexpanded CTG repeats at the DMPK gene in Mexican and Amerindian populations: Evidence of high frequency of DM1 in Mexico

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Myotonic dystrophy type 1 (DM1) is caused by abnormal expansion of CTG repeats in the 3'UTR of the *DMPK* gene. In unaffected individuals CTG repeats present a length of 5-34 CTG, this range is stably inherited because present a low mutation rate. However, recently it has been proposed that premutation and later-expanding alleles could be originated from normal alleles with >18 repeats, also called "Large Normal Alleles" (LNAs). To date, no epidemiological data are available about DM1 prevalence in Mexico, therefore we analyzed the variability of CTG repeats and the presence of LNAs in Mexican Population.

We performed a genotyping of CTG repeats in 644 Mexican-Mestizo subjects from different geographic regions. In addition, we evaluated 561 individuals from ten Mexican-Amerindian-groups (MAG). In addition we identified an extensive group of DM1 patients. Finally, we analyzed 17 STR's markers in the Y chromosome to demonstrate the ancestral influence of native-American subjects.

We identified 240 DM1 patients with classical DM1 and 20 with congenital DM1. Interestingly, we found at least 14 cases of premutation. In the general population, CTG repeats distribution showed a total of 27 different alleles and we found one case of premutation. The most common allele in all regions of Mexico was that of 13 CTG repeats followed by 11 and 5 repeats. With respect to the MAG, 11 and 13 repeats were the most prevalent alleles, representing between 71.11 and 97.82% of all repeats. We found two premutation alleles in MAG. In addition, we also compared the genetic architecture of Mexican populations with various world populations. Y-STR´s analysis suggested that particular distribution of CTG repeat alleles might be attributable from both European and Amerindian influence.

We have shown evidence of an increase of DM1 cases could be correlated with a high frequency of LANs in the Mexican population and the DM1 mutation presented European and Amerindian genetic influence.

P-004

1: Mutation, genetics and epigenetics

Defining a high resolution DM1 haplotype: absence of any disease specific variants

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All Eurasian DM1 patients share a common haplotype around the CTG repeat expansion reflecting an ancient founder event. Previous analyses revealed that the DM1 mutant chromosome shares the same haplotype as the common 5 repeat allele, but distinct from that associated with alleles in the range 10-15 repeats. The DM1 mutant chromosome also shares the same haplotype as the less frequent pool of alleles > 20 repeats, the frequency of which correlates with disease incidence. These data support the hypothesis that new DM1 expansions arise from normal alleles > 20 repeats. Whether the pool of potential DM1 alleles is further refined within this range or if there are any sequence variants that specifically tag the DM1 mutant chromosome and/or pool of predisposed alleles remains unknown. To address this issue we have used a target capture approach to sequence 2 Mb of unique sequence flanking the *DMPK* locus DNA in 20 Scottish DM1 patients. These data were used to genotype the individuals for all known SNPs in the region. Comparison of these data with the Great Britain dataset from the 1,000 genomes project allowed us to define the common DM1 haplotype in the Scottish population. In addition, we also searched for novel variants and identified >50 rare variants. None of these were located in the coding regions of *DMPK*, *SIX5* or *DMWD* suggesting that coding variation in these genes is unlikely to be a major modifier of disease severity in DM1. None of the rare variants were found in all individuals, revealing that there are no disease specific genetic markers of DM1 other than the CTG repeat expansion. However, a number of rare variants were shared between subsets of DM1 patients, suggesting the presence of some more recently derived sub-haplotypes within the Scottish DM1 population. These novel markers will provide a route to understand the relatedness of extant DM1 families and shed light on the origin of expanded DM1 alleles within the Scottish population.

P-005

1: Mutation, genetics and epigenetics

Genotype-phenotype correlations in the Saguenay-Lac-Saint-Jean myotonic dystrophy type 1 population

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Because of a recent founder effect the Saguenay-Lac-Saint-Jean region has a very high incidence of myotonic dystrophy type 1 with an estimated prevalence of ~1/500. This population was critical in efforts to locate the myotonic dystrophy type 1 mutation and has been the subject of many detailed clinical, psychological, epidemiological, genealogical and sociological investigations. Indeed, this cohort is one, if not the, most well characterised DM1 population. Of particular note, in 2002 200 patients were recruited to a longitudinal analysis of the factors modifying symptom severity and disease course. Ten years later, 115 of these patients were retested. The comprehensively phenotyped Saguenay-Lac-Saint-Jean cohort is also genetically homogenous, increasing the likelihood of identifying the genetic variants modifying disease severity. Although it is clear that longer CTG repeat tracts cause greater disease severity and earlier age at onset, genotype-phenotype correlations in myotonic dystrophy type 1 are notoriously imprecise. This is due at least in part to the highly unstable nature of the expanded CTG repeat and the failure to take into account the effects of age at sampling on measured allele length. Indeed, we showed previously that using small pool PCR to estimate the inherited or progenitor allele length we could dramatically improve genotype-phenotype correlations. Moreover, we also showed that disease severity is further modified by individual-specific rates of expansion. As a prelude to further defining the role of additional cis-and trans-acting genetic modifiers of disease severity, we have used small pool PCR to estimate the progenitor allele and rate of expansion in each member of the cohort and correlate these with age at onset and other measures of disease severity.

P-006

1: Mutation, genetics and epigenetics

Variant repeats in DM1 patients might be associated with milder clinical presentation

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Myotonic dystrophy type 1 (DM1) is one of the most variable inherited disorders. The number of CTG repeats in the *DMPK* gene may account for 45–60% of the phenotypic variance. Several studies reported variant repeats within the $(CTG)_n$ tract as potential disease modifiers.
A group of 190 DM1 patients was screened for variant repeats from both 3' and 5' ends of the $(CTG)_n$ tract using TP-PCR, and products showing aberrant profiles were sequenced in order to identify the type and location of variant repeats.
Variant repeats were detected at the 3' end of expansion in 3.7% of patients. No interruptions were identified at the 5' end. An interruption pattern $(CTG)_n(CCGCTG)_3(CTG)_4CCGCTGCCG(CTG)_2CCG(CTG)_{17}$ was observed in DF1 family (mother - DF1-1, 400-1200 repeats, and two sons - DF1-2, 300-700 repeats and DF1-3, 400-1100 repeats). The second interrupted allele $(CTG)_nCCG(CTG)_7CCG(CTG)_{12}$ was identified in DF2 family (father – DF2-1, and daughter – DF2-2), while the third one $(CTG)_nCCGCTGCCG(CTG)_2CCG(CTG)_{17}$ was detected in a female proband (DF3-1, 200-600 repeats). The age of onset in patients with CCG repeats was higher than expected from the number of CTG repeats and they showed little or no signs of percussion myotonia. Additionally, DF1-2 and DF1-3 had calf hypertrophy, a symptom typical for DM2 but absent in DM1, while DF3-1 had mild phenotype with more prominent proximal than distal weakness, also typical for DM2. Patient DF4-2 (250-350 repeats) was the only affected member in DF4 family carrying a variant repeat within the $(CTG)_nCTC(CTG)_{26}$ allele. She had several peculiarities not observed in her sister with similar expansion size and without variant repeats: completely normal strength of sternocleoidomastoid muscle and very mild hand myotonia.
Obtained frequency of 3' variant repeats (<5%) is in accordance with previous studies and our results support the observation that these DM1 patients may have a milder phenotype than expected only from the expansion size.

P-007

1: Mutation, genetics and epigenetics

Mutation screening of the MBNL and CELF genes reveals very low level of sequence heterogeneity without changes on protein level

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Although molecular pathogenesis of myotonic dystrophy is extremely complex the connecting factors between the two genetic types, DM1 and DM2, seems to be the expanded CUG and CCUG containing transcripts. The mechanisms of the dominant negative effect through the MBNL and CELF proteins driven spliceopahty is relatively well described and is supported by several studies on animal models. These observations led us to pose a question, whether mutations of the *MBNL* and *CELF* genes may lead to symptoms at least partially similar to those shared by DM1 and DM2. We screened therefore, using massively parallel sequencing, the protein coding parts of the *MBNL1*, *MBNL2*, *MBNL3*, *CELF1* and *CELF2* genes in 40 patients clinically suspected to have myotonic dystrophy but having no *DMPK*-CTG or *CNBP*-CCTG expansions identified. Except of two synonymous substitutions, c.120G>A (p.S40S) in *MBNL1* and c.69C>T (p.P23P) in *CELF1* - each in one individual, we did not identify any change in the protein coding parts of these genes. Literature search did not reveal studies aimed to mutational screening of these genes, except of one where *MBNL1* was screened in a connection to another disorder but without any variant identified. The Human Gene Mutation Database lists two regulatory *MBNL1* polymorphisms but without any variant in the coding region. The other four genes are not listed in this database. Although dbSNP, based on 1000 Genomes Project and Exome Sequencing Project data, contains a number of missense variations in these genes, they have extremely low frequency which is in the majority of cases lower than 0.1% (one or two variant alleles identified). Although we are still working on the extension of our data set, our preliminary findings suggest that the MBNL and CELF proteins are highly conserved with extremely low variability. Whether rare mutations of these genes can be DM causing or are lethal during early development should be further studied (financial support: VEGA_2/0115/15).

P-008

1: Mutation, genetics and epigenetics

DM1 CpG methylation profile in DM1 patients: correlation with clinical and molecular features and effects on DMPK and SIX5 expression

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Myotonic dystrophy type I (DM1) is a multisystem disorder caused by an abnormally expanded CTG-repeat in the 3\' UTR of *DMPK* showing phenotypic variability and age-dependent progression. A differential CpG methylation profile upstream of the CTG array was documented either in DM1 patients and tissues, suggesting that hypermethylation might modulate DM1 phenotype, possibly affecting expression levels of *DMPK* and/or its flanking genes. To clarify this issue, we characterized by methylation sensitive high resolution melting (MS-HRM), DM1 CpG methylation profile in leukocytes DNA of a cohort of molecularly characterized DM1 patients (13 childhood-onset, 37 juvenile/adult-onset and 7 congenital patients carrying conventional expansions and 9 adult-onset patients carrying variant expansions with CCG/CTC/CGG sequence interruptions at the 3'-end of the CTG array) and in 30 age-matched controls. *DMPK* and *SIX5* expression levels were assessed in methylated *vs* unmethylated DM1 patients by qReal-Time PCR. Hypermethylation (25-50%) involving DM1 locus upstream sequences including CTCF-1 site was found only in DM1 patients with $(CTG)_n$ >1000, whereas downstream sequences were not methylated in any case. On the other hand, DM1 variant expansions showed high methylation levels (25-50%) at the 3' of the CTG array, including CTCF-2 site. Methylation-sensitive enzymes analysis of PCR fragments confirmed that the abnormal methylation was specific to the expanded allele. Hypermethylation did not correlate with any clinical parameter analyzed (age, sex, MIRS, disease duration etc). Finally both *DMPK* and *SIX5* expression did not differ in methylated vs unmethylated DM1 patients. Our results suggest that either the CTG expansion size and the presence of CCG/CTC/CGG interruptions at the 3\' end would maintain a highly polarized pattern of CpG methylation at the DM1 locus, whereas, at least in leukocytes, DM1 locus hypermethylation would not influence *DMPK* or *SIX5* expression.

P-009

1: Mutation, genetics and epigenetics

European founder haplotypes in Serbian patients with myotonic dystrophy type 2

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Myotonic dystrophy type 2 (DM2) is a disease primarily found in populations with European ancestry with a common founder mutation, estimated to have originated 4000 to 11000 years ago. A significant number of genetically confirmed DM2 patients were identified in Serbia, and we examined whether they carry the European founder haplotypes described by Liquori et al. (2003).

Seven microsatellites surrounding the DM2 mutation (*CL3N122*, *CL3N99*, *CL3N59*, *CL3N117*, *CL3N119*, *CL3N19* and *CL3N23*) were genotyped in 27 unrelated Serbian DM2 families (64 patients and 39 healthy relatives). DM2 haplotypes were unambiguously constructed by determining the alleles cosegregated with affected family members. Additionally, our sample included 16 DM2 unrelated Serbian probands and one Albanian and one Hungarian. Their DM2 putative haplotypes were constructed by assigning the alleles present in DM2 haplotypes obtained from family analysis.

In 9 DM2 families and 5 DM2 probands, including the one of Hungarian origin, we observed haplotype A, referred to as consensus one. Haplotype B, originated by microsatellite mutations and one recombination event from haplotype A, was observed in 17 DM2 families and 13 DM2 probands, including the one of Albanian origin. Only one DM2 family harboured a haplotype similar to haplotype C, derived by two recombination events from haplotype A. In comparison to the German and Dutch DM2 haplotypes, haplotype B was more represented in Serbian patients, while haplotype C was rare.

Our results show that Serbian patients share the common European founder haplotypes, supporting that DM2 mutation in South Eastern Europe has the same origin as in Northern Europe. Considering that DM2 is very frequent in some European countries but very rare or even absent in others, as well as a different distribution of founder haplotypes, detailed epidemiology of DM2 in Europe together with haplotype analysis may facilitate tracing the historical route of the DM2 mutation in Europe.

P-010

1: Mutation, genetics and epigenetics

Modeling genetic instability of the CTG repeats in Myotonic Dystrophy type 1 (DM1) patient-derived iPS cell differentiation

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Myotonic Dystrophy type 1 (DM1) is caused by alterations in the DMPK gene. Expansion, anticipation and instability are the three main characteristics of the DMPK gene. DM1 is a chronic, slowly progressing, highly variable, inherited multisystemic disease. Progressive myotonia and muscle weakness, cataracts, cardiac conduction defects and endocrine dysfunction, including diabetes in younger patients, are the typical clinical features of DM1. According to previous papers, the increasing number of CTG repeats worsens the symptoms and differs depending on the generations, the ages and the tissues of the patients, such as skeletal muscles, cardiac muscles and neurons. Our aim is to reveal the instability in the size of the CTG repeats in DM1 by means of patient-derived iPS cells, which were generated from three patients, passed on several times, and induced into skeletal muscles, cardiac muscles and neurons. The distribution of the size of the CTG repeats of the undifferentiated iPS cells at passage numbers 15, 25 and 35, and of the skeletal muscles, cardiac muscles and neurons, which were induced from the undifferentiated iPS cells at passage numbers 15, 25 and 35 was analyzed by a small pool PCR, DIG southern and poisson distribution in a more precise way than in a previous study. Here we demonstrate that the dominant number of the CTG repeats increases during the propagation of the undifferentiated iPS cells. We conducted PCR analysis of the alternative splicing factors such as NFIX exon 7 in the induced skeletal muscles and Sorbs1 exon 25 in the induced neurons. We are studying epigenetic modifications of the iPS cells by targeting regions flanking the CTG repeats.

P-011

2: Clinical and social issues

Childhood DM1 and Autism Spectrum Disorders: Is there a comorbidity?

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Studies exploring the cognitive or psychiatric impairments in the childhood DM1 are scarce and show conflicting results in regards to a comorbid diagnosis of Autism Spectrum Disorders (ASD). The objective of this paper is to examine, on the basis of previous clinical studies, the plausible co-occurrence of childhood DM1 and ASD by (1) highlighting the specific cognitive and psychiatric profiles reported in both populations and 2) comparing the neuroanatomical and neuro-functional features of these two pathologies.
For cognitive ability, the mean Full-scale IQ of individuals with the childhood form of DM1 was globally assessed in the borderline range but a significant discrepancy was found with Performance IQ being lower than Verbal IQ. In ASD subjects with no mental retardation, a reverse dissociation was predominantly reported with lower VIQ than PIQ. Concerning psychiatric disorders in childhood DM1, most of the studies found that internalizing disorders were the most frequent whereas one study reported high prevalence of ASD. However, the DM1 patients with ASD were described as correlating with severity of DM1 and intellectual disability. Executive functions (predominantly working memory deficit in childhood DM1) and alexithymia or social cognition impairments were observed in both pathologies. Brain imaging studies in the childhood phenotype of DM1 revealed white matter abnormalities with no evidence of regional variation while a disrupted cortical connectivity pattern was suggested in ASD population.
In conclusion, it could be hypothesized that different forms of DM1 illustrate a continuum of dysfunctions in the area of socialization (isolation, lack of initiative in social interactions, social anxiety and autism) as suggested by Douniol et al. (2012). Recognition of specific cognitive and psychiatric features in the childhood-onset form of DM1 should be fundamental for detecting early symptoms and implementing optimal individual support.

P-012

2: Clinical and social issues

Emotional recognition in the childhood form of DM1

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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 selective executive and/or emotional deficits (in the adult-onset form). While somatic symptoms may be relatively discrete in the childhood form, deficits in visuo-constructive and visuo-spatial functions as well as verbal working memory weakness, poor attentional process and alexythimia have been reported. Scarce brain imaging studies suggest, in one hand, significant white matter abnormalities throughout the brain whereas other reported specific abnormalities in the bilateral temporal regions. In the adult phenotype, recent studies have shown significant correlation between lesions of the limbic system and dysfunction in the facial expression processing. The objective of the current study is to explore the nature and the extent of potential impairments on the recognition of emotional expressions in the childhood phenotype.

Subjects and Methods: 14 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, gender and IQ) were included. All subjects took a computerized task were they had to select one label from a list choice that best described the emotion that was being expressed (visual, auditory or multimodal stimuli). Preliminary results highlight different patterns of performance according to (1) unimodal/multimodal emotional processing and (2) the valence of emotional expression (positive, negative and neutral).

Conclusion: 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

P-013

2: Clinical and social issues

Incidence and predictors for cardiac conduction abnormalities in DM1. A prospective study in a cohort of DM1 patients with normal ECG at baseline.

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Cardiac conduction abnormalities (CCA) in Myotonic Dystrophy type 1 (DM1) range between 23 and 77% and increase the risk for arrhythmias. The identification of the predictors of CCA is a main point in the clinical setting of DM1 patients.
Of 151 patients recorded in our 33-year database, we prospectively evaluate the incidence and any predictors of CCA in 110 patients with a normal ECG at baseline and a follow-up (FU) longer than one year (median 8 years, IQR 4-17).
Forty-eight patients (43.6%) developed some type of CCA (incidence rate of 5.5% patients/year). After ten years of FU 75% of patients were free from CCA; 50% after 18 years; 25% after 23 years. PMK or IVD were implanted in 11.5% of patients (incidence rate of 1.5% patients/year); 14 patients had a cardiac death (incidence rate of 1.3% patients/year). Time to the onset of CCA in the group of patients who developed cardiac changes and time to the end of follow-up in the group who did not, were similar (p=0.38). 70.4% of patients with CCA underwent MIRS progression compared to 48.3% without CCA (p=0.02). CTG expansion in patients with and without CCA was not different (p=0.90). Cardiac death occurred in 22.7% of patients with CCA and in 6.6% without CCA (p=0.02).
Logistic regression analysis showed that, after correction for FU duration, male sex and MIRS progression were independent factors associated with the occurrence of CCA (p=0.004 and p=0.032 respectively). Linear regression analysis showed that the age at onset of CCA was inversely correlated with CTG expansion (p<0.0001). Cox regression analysis showed that, after correction for FU duration and CTG expansion, patients with MIRS progression had a significantly younger age at CCA onset compared to patients without it (p=0.001). Sex did not influence the age at onset of CCA.
Our study allowed us to obtain useful data about the occurrence of CCA in DM1 patients, stressing the importance of lifelong cardiac FU for these patients.

P-014

2: Clinical and social issues

Retinal changes in patients with Myotonic Dystrophy type 1 (DM1).

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Myotonic dystrophy type 1 (DM1) is the most common form of autosomal dominant adult muscular dystrophy. It is a multisystemic disorder, including several ocular manifestations like early cataract, ptosis, hypotony and retinal pigmentary changes. Spectral-domain Optical coherence tomography (SD-OCT) is a non-invasive, non contact, method to study retina and the optic nerve in vivo and quantify neurodegeneration.
The aim of this preliminary study was to detect retinal morphology changes in DM1 with SD-OCT. Of 47 consecutive DM1 patients, regularly attending our unit, 26 patients 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 including glaucoma, mature cataract, previous eye surgery and amblyopia. Fifty-two eyes of 26 patients and 44 eyes of 22 healthy controls were studied. Fovea thickness, macular Ganglion Cell Complex (GCC) and peripapillary Retinal Nerve Fiber Layer (pRNFL) measurements were performed by SD-OCT (Optovue). Results were compared using T-Student, Anova and Mann-Whitney tests. pRNFL thicknesses were significantly decreased in DM1 eyes compared to control group (p=0,0021, RNFL Superior p=0,0013, RNFL Inferior p=0,0091). GCC and fovea thicknesses were not statistically different from controls. 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, respectively). RNFL thickness reduction may be due to premature ageing.
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.

P-015

2: Clinical and social issues

Neural correlates of behaviour in DM1: cortical gray matter changes and functional hypoactivation of frontal and midline brain structures.

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INTRODUCTION
The adult-onset form of myotonic dystrophy (DM1), has a wide phenotypic spectrum and potentially may affect any organ including CNS with mild to severe involvement.
METHODS
We enrolled 63 patients with established clinical-genetic diagnosis of DM1; they underwent neurological assessment, psychological/neuropsychological evaluation and quality of life interview. A subgroup of 20 patients underwent 3T-MRI protocol with morphologic and functional investigation. Brain atrophy was measured with Voxel-based morphometry (VBM) and calculating Parenchymal Brain Fraction (PBF). fMRI examination investigated cortical BOLD response during a self-awareness task.
RESULTS
Neurological examination showed mild to severe muscle involvement (MIRS mean 2.98±0,92). Patients had frontal and visuo-spatial dysfunctions respectively in 47,3% and 42,6%; illness-unawareness was found in 52.1% and was smoothly associated to executive impairment (p=0.075).
VBM showed several clusters of reduced cortical gray matter (GM) in DM1 *vs* controls in fronto-temporal areas. PBF correlates with cognitive impairment in visuo-spatial and executive domains (TMT-A, TMT-B, REY-figure, Wisconsin Card Sorting Test). Within-group activation maps showed in patients higher activation in frontal and midline regions during self-awareness task. fMRI revealed frontal and midline brain hypoactive regions in patients with reduced illness-awareness.
CONCLUSIONS
Our data indicate the existence of a correlation between brain atrophy, expressed with PBF, and impairment in typical cognitive domains for adult DM1 patients; the relationship between MRI data and cognitive impairment should be supported by future findings from clinical and translational studies Finally, the association between illness-unawareness and specific brain regions hypoactivation may be a distinctive feature of this multisystemic disease.

P-016

2: Clinical and social issues

Cardiac and neurological abnormalities in patients with myotonic dystrophy

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Introduction. Myotonic dystrophy (DM) is an inherited multisystem disorder associated with myotonia, progressive skeletal muscle weakness and atrophy. Aim of the study was to assess the type and degree of neurological and cardiac involvement in patients (pts) with DM. Materials and methods: The material consists of 10 pts (6 male), in mean age of 35±13 years, treated for DM type I (DM1) - 7 pts and type II (DM2) - 3 pts. All pts underwent a comprehensive neurological examination including muscle strength assessment and neurophysiological studies as well as cardiac diagnostics including: standard electrocardiogram, 48-hour ambulatory electrocardiogram, echocardiographic examination, magnetic resonance imaging (MRI), late potentials assessment. Results: Muscle strength was moderately diminished (46-48 points in MRC scale) in 3 pts with DM1. Cardiovascular examinations revealed: QRS duration above 110 ms in 5 pts, clinically significant supraventricular arrhythmia or atrioventricualr block in 3 pts, focal myocardial fibrosis detected with MRI in 3 pts, asymmetric hypertrophy of inter-ventricular septum in 1 pt, presence of late potentials in 5 pts. We have not observed correlation between impaired muscle strength or nerve function and cardiac abnormalities. However, most pronounced cardiac abnormalities were observed in 2 male DM1 patients with diagnosed peripheral polyneuropathy and lowest MRC score. At a mean follow up of 3.2 ± 1.4 years none of the patients died. Conclusions: Cardiac and neurological involvement in patients with myotonic dystrophy is frequent and is characterized by phenotypic heterogeneity. Detection of cardiac and neurological abnormalities may require extensive diagnostics. We did not observe association between neurological and cardiac abnormalities.

P-017

2: Clinical and social issues

Prevalence of tumors among patients with Myotonic Dystrophy type 1 and lifestyle and clinical factors associated

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Background Recent studies documented an increased risk of neoplasm in patients with Myotonic Dystrophies, suggesting a pathogenic correlation to the genetic defect associated with such neurological disorders. However, none of these studies included either molecular characterization in DM patients or evaluation of their exposure to common cancer risk factors. Our objectives were to contribute to clarify if the genetic background specific for DM is specifically related to an increased risk of tumors. Methods We studied a cohort of consecutive patients with an established molecular diagnosis of DM1 to estimate the prevalence of benign and malignant tumors and assess the influence of lifestyle factors mainly correlated to occurrence of malignancies. The proportion of benign and malignant tumors was computed, and, concerning malignancies, the distribution of demographic, medical history, lifestyle habits and specific clinical features of DM1 was compared between patients with and without malignancies. We calculated the O/E ratio of cancers and their 95% CI. Results 59 benign tumors in 54 patients and 19 malignant tumors in 17 patients were diagnosed among 255 DM1 patients. Uterine fibroids were diagnosed in 37.6% female patients. Female gender and history of thyroid disorders were more common among patients with malignancies than cancer-free DM1 patients. No significant association with common lifestyle cancer risk factors was documented. Conclusion Overall, we report a higher prevalence of malignancies in DM1 patients than the general reference population, although it did not reach statistical significance. However, we documented a significant prevalence of cancers in DM1 females younger than 60 years of age.DM1 females also showed a high prevalence of uterine fibroids. The lack of association with common lifestyle cancer risk factors support a pathogenic link between increased risk of tumors and DM1, emphasizing the need for a systematic surveillance of such patients.

P-018

2: Clinical and social issues

PIMS a new french information portal on DM1 /DM2

BOURLIER, APIMS
RIVIERE, APIMS

PIMS is a new information portal on DM1 and DM2. PIMS is operated by APIMS organization created by parents. The aim is to provide useful information in comprehensive language to patients and families.
PIMS = Portail d\'information sur la Maladie de Steinert.
www.maladiedesteinert.info
APIMS = les AMIS du Portail d\'information sur la Maladie de Steinert.
This is a non-profit organization registered in 2014.

REQUIREMENTS

. Free access
. Understandable language by all (minimum of technical words)
. “Vital informations” on the homepage
. No more than one “Click” from homepage to find essential information
. Address easy to remember
. Information coming from authorized literature and validate by a doctor
. No online diagnosis, no forum
. No advertising

Structure :

- Home (Welcome)
- Steinert disease o / DM1 - the disease Forms
- What is new?
- Advanced Search
- Sources of Information
- Associations & Organizations
- Links Facebook
- DM2 / PROMM
- Emergency (Medical care of emergencies)
- Definition of DM1 (DM1 definition)
- DM1 - Symptoms & Support (Short description of the symptom and usual medical care When Existing)
- Muscle problems
- Heart Problems
- Respiratory Problems
- Gastrointestinal Disorders
- Nervous System Disorders & brain damage
- Sleep Disorders
- Eye Problems
- Genetic counseling and reproductive
- Hormonal disorders
- Hair loss
- Other Disorders
- Support for Speech Therapy
- Support for physiotherapy
- Education & Employment
- Essential Precautions (To Be Essential precautions taken by DM1 patients)
- Frequently Asked Questions (FAQ on practical issues)
- About us
- Warning
- Contact (Contact us)

P-019

2: Clinical and social issues

What can we learn about cataracts in myotonic dystrophy type 1 ? – A cross sectional observational study

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Objective
Primary cataract is often the first symptom of the adult-onset form of myotonic dystrophy type 1 (DM1). Although visual acuity is not dramatically impaired, the unusual nature of the lens opacities during an ophthalmologic exam likely calls for a neurologic consultation, and genetic testing to confirm the diagnosis of DM1. The aim of this study is to accurately describe the frequency of cataract and to document diagnostic delay in a large DM1 population.
Methods
The data were collected from DM-scope, a longitudinal observational DM1 registry. To date, it collects information from more than 2200 patients. It is supplemented by confidential and anonymous files, supplemented by physicians from 50 expert neuromuscular centers in France during annual patient follow-up visits. Analyses for this study have been performed on 1848 patients aged >18 years.
Results
The prevalence of cataract in the DM1 adult population was 63.1% and the mean age of onset was 40.3 years old. Interestingly, in the vast majority of cases cataract preceded the diagnosis of DM1 since cataract has been diagnosed and surgically treated prior to DM1 diagnosis in 65.4% and 63.9% of patients respectively. The mean diagnostic delay between cataract and DM1 confirmation was 6.6 years. The data confirmed a negative correlation between cataract's age of onset and CTG repeats number. In some patients, complications were reported following cataract surgery, including secondary cataract, secondary capsulorhexis contractions, and epiretinal membrane.
Conclusions
This study confirms the high prevalence of cataract in adult patients with DM1. However, early primary cataract do not frequently prompt to DM1 diagnosis. Since ophthalmologists can play a major role in early diagnosis of DM1, the results emphasize the need to increase ophthalmologists awareness. Furthermore, some complications may occur after cataract surgery in myotonic patients, who need to be closely followed-up. A better collaboration between ophthalmologists and neurologists from neuromuscular centers could optimize the eye care management of patients with DM1.

P-020

2: Clinical and social issues

DM-Scope registry as a powerful tool to assess therapies' efficiency in DM1: Impact on clinical features and quality of life

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Background
The multi organ involvement in myotonic dystrophy of type 1 is associated with progressive disagreements in daily life, most of these problems are caused by muscular weakness. Several studies described main complains reported by DM1 patients but any one made links with evolution of phenotype severity. In a context of therapeutics development, it becomes necessary to assess sensitive tools to evaluate impact of disease on quality of life and validate therapies' efficacy not only on clinical manifestations.
Objective
We aimed to test the relevance of a daily life's survey in DM1 patients clinically well characterized and enrolled in French DM-Scope registry.
Method
French DM1 patients filled in the translated Quality of life form, INQoL, (Vincent et al., 2007), first developed for neuromuscular disease in order to help researchers and clinicians to better understand patient's problems. The survey addresses symptoms' severity, physical abilities, self-sufficiency, relationships, emotional conditions, treatments' efficiency and compliances' difficulties. The responses were compared to the clinical features collected during the patient's annual medical visit and managed in the French DM-Scope registry (50 neuromuscular centers).
Results
Our work validated in DM1 patients an individualized muscle disease specific measure of quality of life for adults suitable for both clinical and research use. We will present the results of a correlations' study between INQoL responses and clinical data from Dm-Scope registry.
Discussion
An overview on quality of life in muscle diseases (Burns et al., 2012) revealed the lack of specific measure scales. Then, the assessment of a consensual specific survey in DM1, which can be linked with phenotype's severity, should be a powerful tool to use in clinical trials and medical care.

P-021

2: Clinical and social issues

Strength-Training Induces Skeletal Muscle Adaptations in Patients With Myotonic Dystrophy Type I: A Case Series Study

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INTRODUCTION: Myotonic dystrophy type 1 (DM1) is the most prevalent inherited neuromuscular disease in adults. This multisystemic disease is characterized by skeletal muscle impairment including muscle wasting. Slowing muscle wasting in this population using strength training seems a promising strategy, but it remains unknown if it would trigger cellular and molecular responses similar to the ones seen in healthy subject. **OBJECTIVE:** The objective of this case series study is to evaluate the effect of a strength-training program on skeletal muscle adaptations in patients with DM1. **METHODS:** Three males with DM1 (age = 36, 56, 47, respectively) underwent a 12-week strength-training program consisting of 2 sets of 6 exercises at 6 RM supplemented by functional tasks. *Vastus lateralis* muscle biopsy sample were obtained pre- and post-training program. The cross sectional area (CSA) of myofibers and the percentage of centrally nucleated fibers (CNF) were obtained following staining with hematoxyline/eosine. The proportion of fiber types (I and II) was determined by immunohistochemistry. **RESULTS:** Patient A showed: increase in CSA of both muscle fiber types (46% for type I and 24% for type II); muscle fiber-type switching in favor of type II; 2.26-fold increase in the percentage of CNF. Patient B showed: increase in CSA of muscle fiber type I (23%) and decrease of muscle fiber type II (26%). Patient C showed: decrease in CSA of muscle fiber type II (19%) and muscle fiber-type switching in favor of type I. **CONCLUSION:** Our results suggest that skeletal muscle of patients with DM1 can undergo adaptations. Individual differences, compliance to the training program and physical activities performed outside the research protocol limit the interpretation of our observations. Further studies comprising a higher number of participants are needed to validate our findings and determine to which extent and how skeletal muscles of patients with DM1 adapt to strength training stimulus.

P-022

2: Clinical and social issues

The usefulness of the instrument Occupational Self-Assessment in Myotonic Dystrophy Type 1

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Patients with DM1 have decline in cognitive capacities which can lead to difficulties, decreased occupational performance and level of participation. Further they tend to avoid expressing their problems and compliance to rehabilitation may be poor and these problems can be difficult to capture in an assessment situation. If possible we want to identify and capture statements that are of importance for reduced compliance in DM1 patients.

This pilot study was performed on 10 patients with DM1 and a control group, each were assessed with Occupational Self-Assessment (OSA). That is a two-part self-report that elicits patients perceptions and values concerning their own occupational performance and how important each task is, graded 1-4, in a series of 21 statements. The intention was, on a group level, to capture occupational performance, participation and the perceived importance of each task, to see any differences in estimation, and also to provide a picture of whether there is any statement that is more prominent than others.

At the group level a lower estimate was seen in the DM1 group with more of “lot of problem” in the estimation of occupational performance, mean 1.01 was found ($P<0.005$).

When comparing each statement in occupational performance there is a difference ($p<0.05$) in 11 of the 21 statements for example “to identify and solve problems in everyday life”.

DM1 patients scored the importance of each task lower than the controls, mean difference 0.48 ($P<0.05$).

The OSA instrument is easy to administrate and gives a visual picture of the patients’ estimation and the gap between occupational performance and valuation of how important each task is. The DM1 patients’ results show more dissatisfaction for example in “physically doing what I need to do” and “to get done what I need to do” and this has led to a deeper discussion about satisfaction with how life works and why a change may be justified.

P-023

2: Clinical and social issues

A Longitudinal Study of Autism Spectrum Disorders in Children, Adolescents and Young Adults with Congenital and Childhood Myotonic Dystrophy Type 1

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Cerebral involvement in Myotonic Dystrophy Type 1 (DM1) is well documented. Autism spectrum disorder (ASD) in addition to intellectual disability is reported in congenital and childhood DM1. The aim of the present study was to assess stability and change over time in diagnosis of ASD in a cohort of children, adolescents and young adults with congenital and childhood forms of DM1. Fifty-one of the 57 individuals from our previous study (Ekström et al 2008) participated and were divided into three subgroups according to age at onset and presenting symptoms: severe congenital (*n*=16), mild congenital (*n*=17) and childhood DM1 (*n*=18). The mean time between the first assessment (*T1*) and the follow-up (*T2*) was 7.7 years (range 6.7-9.3 years). At both times a structured interview was performed, at *T*1 the Autism Diagnostic Interview-Revised (ADI-R) and at *T*2 the Diagnostic Interview for Social and Communication Disorders (DISCO-10). In addition, the Autism Diagnostic Observation Scale-Generic (ADOS-G) (Lord et al., 2000) was added to the diagnostic procedure at *T*2. The Vineland Adaptive Behaviour Scales (VABS) were used to assess adaptive functioning. On the basis of all available information, clinical diagnoses of neurodevelopmental disorders were assigned according to the DSM-IV criteria at both *T1* and *T2*. Forty-nine percent (n=25) of the individuals with DM1 had ASD at *T*1 as compared to fifty-seven percent (n=29) at *T*2. The change was however not statistically significant. In the severe congenital form 68.8% had ASD at *T1* and 75% at *T2*. In mild congenital the prevalence of ASD was the same (64.7%) at both *T1* and *T2*. In the childhood form 16.7 % had ASD at *T1* and 33.3 % at *T2*. The mean standard scores of adaptive behavior as well as for the subdomain socialization standard score increased statistically significantly more between *T1* and *T2* in individuals without ASD than in those with ASD.

P-024

2: Clinical and social issues

Oropharyngeal dysphagia in myotonic dystrophy type 1 (DM1): role of coordination between respiratory and swallowing functions

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Introduction: although dysphagia is seldom a complaint in DM1, oropharyngeal and oesophageal abnormalities are present early in the disease and may be, at least in part, responsible for pneumonias, the most common cause of death in adult DM1. The aims of our study were: (i) to determine the existence and frequency of subclinical physiological abnormalities in oropharyngeal swallowing and (ii) to explore the role of coordination between respiratory and swallowing pattern abnormalities in the process.
Methods: fifteen patients with adult-onset DM1 (mean age 48±11.31) were subjected to manual muscle strength testing using the modified MRC scale and **c**ombined Fiberoptic Endoscopic Evaluation of Swallowing (FEES), respiratory phase and submental S-EMG recordings. The severity of swallowing dysfunction was determined using the Penetration Aspiration Scale (PAS) and the Dysphagia Outcome and Severity Scale (DOSS). For each patient respiratory parameters were collected. Data were compared to 15 age- and sex-matched controls.
Results: FEES detected mild to moderate dysphagia in 10 apparently asymptomatic patients (66.6%). None required non-oral feeding. In 74% of swallows, deglutition was followed by an expiration phase. Percentage of inspiration-deglutitio--inspiration patterns correlated to viscosity and bolus consistency. Mean swallowing apnea duration was 2.66 sec depending on bolus viscosity and size. Mean number of swallows/bolus was 2.8. A strong correlation between FVC/FEV1 and swallowing apnea duration was found (r=0.881/r=0.952).
Conclusions: we recommend to evaluate oropharyngeal motility early in the disease process so that adequate nutritional counselling and management can occur. Our data suggest that factors other than muscle weakness and myotonia may be involved in oropharyngeal dysphagia in DM1, including abnormal coordination between swallowing and respiratory functions creating the physiological preliminary background for additional treatment strategies.

P-025

2: Clinical and social issues

Assisted ventilation and respiratory outcomes in congenital myotonic dystrophy (CDM): a retrospective observational study in an italian cohort

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Mortality rates in CDM vary from 17-40% and the cause of death is generally respiratory insufficiency. However, cohorts are small and natural history data on respiratory involvement and outcomes still limited.
The aims of our study are (i) To describe pulmonary involvement and respiratory outcomes in a cohort of Italian patients with CDM; (ii) to clarify the relationship between initial assisted ventilation duration and respiratory outcomes; (iii) to correlate respiratory morbidity to developmental outcomes.
A retrospective chart review was performed in 39 CDM patients from 4 neonatal intensive care units and 5 neuromuscular units. Antenatal information, gestational age, birth weight, CTG expansion size, Apgar scores, oxygen saturimetry, timing and duration of assisted ventilation were obtained. Mortality at birth, number of children requiring tracheostomy, non-invasive ventilation or autonomous breathing at follow-up were recorded (mean follow-up of 17 years, range 1-31). Motor function, developmental and nutritional assessments were also included.
35 patients showed respiratory distress at birth (90%). Nineteen (54%) were weaned off ventilation; 6 patients required tracheostomy (17%); 12 patients (34%) required non-invasive ventilation for 10-14 hours/day while two 18-months old children (6%) are still on IV. Delayed motor milestones were found in all patients. Only 1 child required gastrostomy tube at birth. All children were discharged to their homes.. Two patients < 12 months were readmitted for acute respiratory failure.
Our data confirm and strengthen previous reports demonstrating that respiratory failure is a major concern in children with CDM. Prognosis is variable. How ventilation affects respiratory outcomes is still to be clarified. Our natural history data may provide physicians, caregivers and families with preliminary information on critical care management, family planning and disease burden.

P-026

2: Clinical and social issues

Causes of death in a cohort of patients with Myotonic Dystrophy (DM1) in Scotland.

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Causes of death were ascertained for adult patients from a population of all patients known to have a molecular diagnosis of DM1 in three areas of Scotland (North of Scotland, West of Scotland and South-east Scotland), from hospital records or from death certificates over a four year period. In some cases more than one cause of death was recorded. A total of fifty-nine patients with DM1 were known to have died between 01/01/2010 and 31/12//2014, of whom 32 (54%) were male and 27 (46%) were female. The main cause of death was pulmonary 47%, consisting of 19 (32%) pneumonia/aspiration pneumonia and 9 (15%) respiratory failure. Cardiac causes of death were given for 17 (29%). Of these, two were attributed to cardiomyopathy and two to arrhythmias. Other causes of death included sepsis in 6 (10%), malignancy in 6 (10%) and cerebrovascular accident in 3 (5%). Alzheimers, drug/alcohol related and liver failure secondary to chronic hepatitis B accounted for three further cases. Three cases had myotonic dystrophy as the primary cause of death. The mean age at death was 57.6y with no gender difference. The range was 26-85y, with a peak in the 6th decade (age 50-59y). This supports the findings of previous studies which have shown the major causes of death in myotonic dystrophy to be respiratory and cardiac, with cancer contributing to a small proportion of deaths.

P-027

2: Clinical and social issues

Prevalence of Myotonic Dystrophy type 1 in western Sweden

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Background The prevalence of DM1 in Sweden is not known in detail. The only previous Swedish study is from the Örebro County with 270.000 inhabitants published in 1993 and reported a prevalence of DM1 of 18 $x10^{-5}$ inhabitants.

Objectives The primary aim was to estimate the prevalence of DM1 in adults in western Sweden (1.6 million inhabitants), and compare figures in the city of Gothenburg and also in the area outside Gothenburg.

Methods The Neuromuscular Center at Sahlgrenska hospital has since more than 15 years been a reference center for adult patients with neuromuscular disorders in western Sweden. Diagnosed patients with DM1 were traced by outpatient registers, contacted by regular mail, and thereafter telephone interviewed. We asked about the possibility of other relatives, who may have DM1. Every patient's diagnose were verified by a DNA test, except in a few cases. The prevalence date was set to 2014-07-01.

Results We identified 230 DM1 cases (113 males/117 females) giving a prevalence of DM1 in adults of $17.8x10^{-5}$. In Gothenburg city we found 61 patients, and in the remaining area of the region 169 cases. The prevalence of DM1 in Gothenburg city was $14.1x10^{-5}$ which was significantly lower than in the remaining region which was 19.7 $x10^{-5}$ ($p = 0.027$). There were no statistically significant differences between the city of Gothenburg and the area outside Gothenburg in the prevalence of the four subgroups of DM1.

Discussion The observed prevalence of DM1 in adults in western Sweden seems to be somewhat higher than estimated elsewhere in the western world (12 $x10^{-5}$).

One reason for why DM1 is more prevalent in rural than in the urban areas could be the lack of initiative, which may keep them staying at their family home. Further, cognitive impairment and weak school performance may hinder them to take part in university studies which is another reason for young people to move into cities with universities such as Gothenburg.

P-028

2: Clinical and social issues

Is there a gender difference in adult muscle weakness progression in myotonic dystrophy type 1?

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We followed prospectively 43 patients (m/f, 18/25, mean (SD) age 41(9.4) years) with DM1 for leg muscle force, balance and falls during 5 years. All patients had muscular impairment corresponding to muscular impairment rating scale (MIRS) grade 3 or more.

After 5 years the distribution of patients in each MIRS impairment grade was significantly changed ($p<0.001$); no men or women had become better but 39% men and 24% women had become worse. When evaluated individually with respect to each patients baseline muscle force the median decrease of the ankle dorsiflexors was 18% in 5 years, but only 6-8% in the other leg muscles. The Mann-Whitney U analysis of the relative force change with respect to baseline force showed a statistically significant difference between genders.

Males had a more pronounced reduction in all leg muscle groups except for the hip flexors. For the whole group, time to walk 10 meters, timed up & go (TUG) and the step test showed a statistically significant ($p<0.001$) deterioration compared to baseline. In males the deterioration in time to walk 10 meters was twice as large as in females, mean (SD) +2.5(3.2) seconds vs. +1.2 (1.7). The number of patients experiencing at least one fall had increased from 58% to 77% after 5 years. All men had fallen at least once, n=18 (100%) at year five vs. n=12 (67%) at baseline, a significant change. The women showed less increase. Nearly half of the whole group had fallen ≥ 3 times, at baseline this was the case for a third. Injuries following falls had become worse, the patients had a higher degree of medical services previous year after 5 years than they had at baseline.

Both males and females showed significant changes in time to walk 10 meters, TUG and step test but the deterioration in muscle force showed to be more severe in males. We find it imperative to follow this patient group continuously, ask them about falls and refer them to physiotherapy treatment and follow-up.

P-029

2: Clinical and social issues

New signs and symptoms of Myotonic Dystrophy MD1 affected members in a Hungarian family

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Myotonic dystrophy type 1 (DM1) is a multisystem disorder that affects skeletal and smooth muscle as well as the eye, heart, endocrine system, and central nervous system. DM1 has an autosomal dominant inheritance, and affects males and females equally. There is also a considerable risk of anticipation. In our poster presentation the case of four members in a Hungarian family is processed. The gender of all affected family members is male. Among the three children of the father both of his sons was diagnosed with myotonia dystrophica type 1 (MD-1, expansion of the trinucleotide (CTG) repeat in the DMPK gene (chromosomal locus 19q13.2-q13.3)). The father died at the age of 58 in 2007. His symptoms was retrospectively ascertained. All four family members show varying degrees of muscle symptoms (relaxation difficulty of the limbs, muscle weakness and muscle atrophy etc.), but cognitive deficits only occur in two members of the family, while the other two members are characterized by a high intellectual level. Results of some comparative examinations are presented. Similarities and differences of laboratory blood test results (CK, liver enzymes etc.), neuropsychological tests, sleep diagnostics, social behavioral involvement etc. are demonstrated. In this presentation we focus on the changes of clinical symptoms occurred during the past 2 years.
It was also examined the effect of the disease on the central nervous system and personality. Last but not least we would like to raise the awareness of the need for logopaedic and special physioterapic treatment of patients suffering from myotonia dystrophica type DM1. Nordic Walking exercises were especially effective.

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P-030

2: Clinical and social issues

Statin and other antihyperlipidemic medication use and side effects in myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2)

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Purpose: Statins and other antihyperlipidemic medications are often associated with muscle side effects (*e.g.,* muscle pain). Limited data exist on the interaction between statin side effects and multi-system manifestations of DM. We evaluated the use of antihyperlipidemic medications in DM to assess the risks/benefits of statin use and to facilitate future research.
Methods: We analyzed data including self-reported medications within the US based National Registry of DM Patients and Family Members. Medications were categorized by primary indication. Antihyperlipidemic medications were further categorized by class (statin, fibrate, etc). To further assess medical history, comorbidities, adherence to medications, and factors influencing statin use, we also developed and mailed a survey to patients 18 years and older in the National Registry. The survey was completed online and contained 54 questions.
Results: The National Registry provided data on 952 DM patients (816 DM1 and 136 DM2). Their mean age at enrollment was 42.6 ± 16.1 years. Over 5,000 medications were analyzed. Antihyperlipidemic medications were used in 18.7% of DM patients (n=178/952). The majority of these medications were statins (n=114).
112 DM1 and 49 DM2 patients completed the survey. Current and previous statin use was reported by 32 DM1 and 20 DM2 of survey responders. Approximately half of these patients stopped taking statins (15 DM1 and 11 DM2 patients). The most prevalent reason for stopping statin use was side effects (10 in DM1 and 9 in DM2), nearly all of which were muscle pain and increased weakness.
Conclusions: Antihyperlipidemic medication use was higher among DM patients (18.7%) compared to the US population in general (11.4%). The interaction between potential statin-induced muscle symptoms and DM are still not fully understood, warranting careful monitoring of the risks/benefits of using these medications in DM.

P-031

2: Clinical and social issues

The Christopher Project: A Comprehensive Survey of Patients and Families Living with Myotonic Dystrophy in Canada and the United States

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Background: The Christopher Project is a collaborative, multi-phase research study of myotonic dystrophy (DM) patients and family members. It explores the unique perspective they have developed from personal experience in order to understand the challenges of managing this complex condition. The project is a partnership between the Muscular Dystrophy Association, Muscular Dystrophy Canada, Myotonic Dystrophy Foundation, University of Rochester Medical Center, Stanford University School of Medicine, Groupe de recherché interdisciplinaire sur les maladies neuromusculaires (Jonquiere, QC), and Marigold Foundation.

Objective: The project aims to identify areas of unmet need and determine how we can work together to improve health outcomes. The findings will:

- provide information to advocacy groups focused on developing programs and services to support patients and families

- add context to expert, clinical research aimed at providing better access and care

- give patients and families a greater understanding of their needs and how to better manage DM

Methods: 4,000 surveys were mailed out in Canada and the USA with quantitative and qualitative questions on current health, diagnostic experience, information and resources, symptomology, healthcare experience, treatments and interventions, challenges of daily life, and insurance.

Results: 1,180 completed surveys were returned (236 Canada/944 USA). The average age of respondents is 45, average age of disease onset is 26, and average age at diagnosis is 30. All types of DM are represented: 14% congenital DM type 1, 40% DM type 1, and 17% DM type 2. Almost 30% of respondents report that they do not know what type of DM they have.

Conclusions: Findings provide insight into the patient experience and suggest that there is much to be learned directly from patients and families about the challenges of living with DM. Analysis is ongoing and significant findings on symptomology, healthcare experience, and daily life will be presented.

P-032

2: Clinical and social issues

Cough impairment in myotonic dystrophy type 1: incidence and management in a neuromuscular tertiary care center.

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Death occurs in DM1 patients usually between the 5th and 6th decade, mainly because of respiratory failure, pneumonia being the most frequent cause (40%). There are however limited data on secretion management in DM1 and on how this may affect respiratory outcome and impact survival.
Accurate secretion management reduces pneumonia and lowers the risk of acute pulmonary complications and ultimately death. Peak Cough Expiratory Flow (PCEF) ≥ 270 l/min is considered the threshold value for efficient cough and adequate secretion management. When PCEF ≤ 270 l/min secretion management should be discussed.
The purpose of this study is to investigate cough impairment and describe secretion management in a cohort of adult DM1 patients attending a neuromuscular tertiary Centre in Northern Italy.
Demographic data, Manual Muscle Testing (modified MRC scale), Forced Vital capacity (FVC), Peak Expiratory Cough Flow (PCEF), Maximal Inspiratory and Expiratory Pressure (MIP-MEP), cough management's techniques and QoL (SF36v2 and INQoL) were collected from 67 adult DM1 patients (mean age 45.5 ± 13; mean CTG range 600-800, 37 females) from January 2013 to February 2014.
Mean PCEF was 295 L/min ±79 and mean FVC was 79% ± 0.2 of predicted values. Cough was ineffective in 19% of our patients (mean PCEF 211 L/min ±36) with mean FVC 53%±0.1 of predicted. Patients treated with mechanical inspiratory-expiratory machines, MI-E (54%) were older (mean age 55 years 9.8) than patients (46%) managed with air-stacking techniques (mean age 37 years ±9.4).
Cough may be impaired in DM1 patients irrespective of age and disease duration. Complete respiratory assessments, including PCEF measurements should be performed in all patients with DM1 at screening and early management of secretions should be recommended when PCEF ≤ 270 l/min. How this impacts quality of life perception and reduces acute respiratory infections, ultimately affecting outcome, will need to be determined over time.

P-033

2: Clinical and social issues

Cardiac Magnetic Resonance Imagining (MRI) cross-sectional study of Myotonic Dystrophy type-1

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Background: Cardiac MRI has shown to be a promising screening method to reveal early cardiac involvement in different neuromuscular diseases, including concentric hypertrophic remodelling and subendocardial dysfunction, even when standard cardiac evaluation (ECG and echocardiography)have shown normal results. A better understanding of any clinical variability in the myocardium of patients with myotonic dystrophy type 1 (DM1), requires a correlation of cardiac anthropometric results with any possible modifying clinical characteristics of the patient. This understanding would also benefit cardiac screening strategies, that are, to date, unclear.

Aims and Methods: To elucidate whether myocardial abnormalities could be identified in Myotonic Dystrophy type 1 (DM1) patients without overt clinical cardiac involvement. A cohort of 20 patients with genetically determined DM1 with high levels of perceived fatigue but still ambulant, were recruited from UK's OPTIMISTIC main-study sample population of 44 patients (27 men and 17 women).

Results: Twenty DM1 patients were screened for levels of fatigue and daytime sleepiness using CIS-fatigue score Checklist Individual Strength. The sample has a 3:1 men-to women ratio, a mean age (SD) of 55 (11) for women and 48 (10.5) for men and a mean time of active disease recalled of 17 (14) years. All patients have had an ECG within 12 months of study commencement and all demonstrated normal sinus rhythm; only one male patient reports to be taking cardiac medication. Currently 65% of the patients have an accelerometer reported activity status of Pervasively-Passive but three women and eight men will start exercising in the following months and at the end of the study a comparative MRI will be obtained.

Conclusions: Cardiac screening in DM1 patients has proven logistically difficult in the past; however, cardiac MRI appears to be a promising tool to measure cardiac functionality performance and disease progression over time.

P-034

2: Clinical and social issues

Habitual physical activity in patients with Myotonic Dystrophy type 1: an OPTIMISTIC sub study.

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Background: The association between habitual physical activity (HPA) levels and clinical phenotype in other neuromuscular disorders has been shown. However, the level of HPA in patients with Myotonic Dystrophy type 1 (DM1) and its relationship with disease burden is unknown.
Methods: HPA measured for 15 consecutive days using an ankle worn triaxial accelerometer (GENEActiv, Activinsights Ltd, UK), fatigue (Checklist Individual Strength; CIS), disease burden (Muscular Impairment Rating Scale; MIRS) and cardiopulmonary fitness (six minute walk test 6MWD) were assessed in 29 patients with genetically proven DM1.
Results: Patients with DM1 had significantly lower levels of HPA than age matched healthy population references. CIS scores, 6MWD or age were not predictors of HPA levels (Regression-Squared values <20%).
58.6% of the patients were classified as least active, in relation to activity levels, out of three possible categories.
Differences in gender were observed, a mean HPA for women 16.2% higher than men but not statistical significant (p= 0.14), however with statistical significant (p=0.29) higher records of time per day spent in vigorous locomotion, 27.7% more than men.
There were no significant differences in the mean levels of HPA amongst patients with MIRS score of 2 (n=7) or 3 (n=8), but patients with MIRS scores of 4 (n=13) showed a lower mean level of activity ($p<0.05$) and the single patient with a MIRS of 1 was dramatically more active than the rest.
Patients still capable of running showed over 32% higher activity levels.
Conclusions: These results demonstrate objectively for the first time, low levels of HPA in DM1. This may constitute a potential modifiable risk factor in DM1 patients.

P-035

2: Clinical and social issues

Myotonic dystrophy type 1 (DM1) patients and caregivers as educators: Nursing lessons learned

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Introduction: Health care providers have managed DM1 patients for decades, generally with a focus on on symptom control, surveillance and anticipation of life-threatening consequences. While important, this approach may miss opportunities to support patients and families as they navigate DM1 symptoms during every day activities. Nurse led clinics have been proposed as one model for ensuring holistic patient and family centered care; however, patients' and families' perspectives need to be integrated into current approaches to inform best practices.
Methods: Biographical disruption (BD) hypothesizes a fundamental shift in identity and self-conceptualization in the context of chronic illness. Using a BD framework, we conducted secondary content analysis of data from three qualitative research studies (n = 13 patients; n = 7 caregivers) that explored living with DM1. Verbatim transcripts of semi-structured interviews were independently coded for DM1-related 'disruption'. Content identified as 'new information' by an experienced neuromuscular nurse practitioner was separately analyzed.
Results: Preliminary coding identified patient and caregiver insights into living with chronic illness that were unanticipated yet informative. Findings suggest that patients have awareness of cognitive challenges, and may experience anxiety when providers discussed information inappropriately or insensitively. Also, patients and families manage DM1 in ways independent of clinician recommendations. There was a remarkable degree of intergenerational caregiving uncovered, including personal and financial support.
Discussion: Interdisciplinary care of DM1 families typically focuses on prevention of morbidity and mortality. Time constraints and biomedical approaches may deter patients and families from addressing their issues and needs. Research exploring patients' and families' stories may inform the DM1 health care community of how to best support those "living" with DM1.

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2: Clinical and social issues

Patient workshops as a method to identify, monitor and teach about symptoms in patients with juvenile onset DM1

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Background
Since 2006, The Danish Rehabilitation Centre for Neuromuscular Disorders (RCFM) has organized annual one week workshops for patients with juvenile onset DM1 with the aim to set up social networks for the youths and collect data on their physical function and everyday problems. The data collected are used to educate local providers in the disease and treatment/exercise options.
The purpose is to describe the disease progression and to make the patient accept and act on his/her functional impairment thus promoting self-care.
Methods
First time attendants are subject to a thorough physical examination consisting of a 6 min. walk test, functional tests, a manual muscle test of 11 muscle groups (recorded as MRC % score), range of motion, and forced vital capacity (FVC) measured by a calibrated spirometer. Muscular impairment is rated as MIRS score using the five-point muscular impairment rating scale. Attendants are also asked about oral motor problems, prevalence and identification of myotonia, sleep patterns and GI problems. At the following workshops, attendants get a more basic examination consisting of spirometry and evaluation of gait; further assessments are implemented according to the progression of the disease.
During a seminar, the patients' 24h rhythm and problems are registered. The observations are turned into personal rehabilitation plans and experience from one workshop is carried on to the following year's workshop.
Results
Fifty-two patients have attended the seminars with an average attendance of 3 (1-6) times. Examples of workshop themes are: respiration and usage of respiratory aids, oral stimulation/speech therapy, symptoms of cardiac problems.
The poster will present the symptoms and functional level of the attendants, workshop themes and usage.

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2: Clinical and social issues

Predictors of change in daytime sleepiness: preliminary findings of a 9-year longitudinal study in adults with DM1

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Background: Daytime sleepiness is a prominent and debilitating symptom of myotonic dystrophy type 1 (DM1). To date, no prospective cohort study of DM1 patients has examined clinical predictors of change in daytime sleepiness.

Methods: Two-hundred patients were evaluated in 2002-2004 (mean age (SD)=47.0 (11.8)) (Time 1). Nine years later, in 2011-2013 (mean age (SD)=52.1 (10.6)), 115 of these were re-evaluated using the Daytime Sleepiness Scale (DSS) (Time 2). The Wilcoxon signed-rank test and a stepwise multiple linear regression analysis were used to respectively estimate change in daytime sleepiness scores and to identify clinical predictors of daytime sleepiness score changes over the 9-year study period. Age, sex, BMI, CTG repeats, muscular impairment, diabetes, depression, pain, difficulty in climbing stairs, forced vital capacity, mean habitual sleep duration, mean habitual bedtime, psychological distress, and intellectual quotient at Time 1 were used as independent variables in the regression.

Results: Daytime sleepiness scores increased over the 9-year period (mean DSS(SD) varied from 4.5(2.9) to 5.3(3.4); $p<0.05$). Stepwise multiple regression results for change in daytime sleepiness scores between Time 1 and Time 2 (adjusted $R^2=0.2$, $p<0.001$) revealed that diabetes ($p<0.01$), higher CTG repeat number ($p<0.05$) and shorter habitual mean sleep duration ($p<0.05$) are associated with a greater increase in daytime sleepiness scores.

Conclusions: These preliminary results show that CTG repeat length has a predictive value for daytime sleepiness change in DM1 patients. Results also suggest that habitual longer sleep duration may act as a protective factor against increases in daytime sleepiness. Moreover, it is acknowledged that daytime sleepiness is associated with the metabolic syndrome but further research must document the mechanisms by which diabetes increases the likelihood to present higher daytime sleepiness levels.

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2: Clinical and social issues

Exploring the understanding of Anticipation in Myotonic Dystrophy Type 1 patients and families: A pilot study

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Myotonic Dystrophy Type 1 (DM1) is a dominantly inherited muscle condition predominantly characterised by muscle weakness and the phenomenon of anticipation, whereby there is increased severity of symptoms and earlier age of onset in successive generations. Anticipation is variable, highly unpredictable and may result in the birth of children severely affected with congenital myotonic dystrophy. This adds complexity to genetic counselling which may be further complicated as some patients with DM1 also have mild learning difficulties. The aim of this study was to explore communication between health professionals and patients with DM1, the understanding of Anticipation and how this may affect decisions about reproductive options. Using qualitative research methods, five interviews were conducted and analysed with six participants who had previous genetic counselling regarding anticipation with a genetics health professional. In addition thematic analysis was used to analyse clinical letters. This study confirmed important factors within understanding such as the importance of the clinician relationship and understanding from personal experience. This study also showed that though Anticipation as a concept appears well understood in this small group, patients did not understand the terminology. In addition other concepts such as chance are largely misunderstood in patients with learning difficulties. Therefore this study identifies important areas for further research into DM1 patient understanding of genetic concepts, particularly focusing on how this may affect reproductive decisions, on a much larger scale. In addition this study highlights that there is a significant lack of research within the genetic counselling of individuals with learning difficulties. Grant References: FfWG & YLCE

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2: Clinical and social issues

Towards a better evaluation of fatigue in DM1: French validation of three new assessment tools

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Background: Fatigue and sleepiness are the main symptoms of DM1 patients (Kalkman et al., 2005). Professional healthcare needs tools to precisely estimate these troubles and their consequences.
Objective: The aim of this study is to validate the French translation of three new tools in the field of fatigue.
Method: DM1 patients from French DM Scope registry and French students filled in forms: *Checklist Individual Strenght* assesses fatigue in a multidimensional approach (Vercoulen et al., 1994); *Fatigue and Daytime Sleepiness Scale* measures the sleepiness tendency and behaviors about fatigue (Hermans et al., 2013); *Self-Efficacy Scale* evaluates the self-efficacy perceived to involve in a healthy behavior (Hoffman et al., 2011).
Results: Internal consistency, test-retest, correlations and factorial analyses allowed us to compare the French versions with the English version.
Conclusion: Our study matches with the international network's activities to establish consensus in tools to use for clinical studies and practice.
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P-040

2: Clinical and social issues

Hypothyroid medication use in patients with myotonic dystrophy (DM)

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Purpose: There is an increased prevalence of thyroid nodules in patients with myotonic dystrophy (DM), and limited data suggest a higher prevalence of hypothyroidism. Under and over treatment of thyroid conditions remains a problem in the general public. Our goal was to evaluate the use of thyroid medications in DM patients to facilitate knowledge of endocrine dysfunction and help guide clinical care and pharmaceutical management.

Methods: We evaluated self-reported medication use for members enrolled in the US based and NIH sponsored National Registry of DM & Facioscapulohumeral Muscular Dystrophy (FSHD) Patients and Family Members. Medications were categorized by primary indication. We categorized medications used to treat hyperthyroidism and hypothyroidism. Thyroid medication use for DM patients was compared to FSHD patients and information from the US Centers for Disease Control and Prevention (CDC).

Results: The National Registry provided data on 952 DM patients (816 DM1 and 136 DM2) and 576 FSHD patients. Mean age of enrollment was 42.6 ± 16.1 years in DM and 49.9 ± 15.8 years in FSHD. Medications to treat hypothyroidism were used in 11.7% of DM patients (n=111/952) and in 8.7% of FSHD patients (n=50/576). The most common thyroid medication used was levothyroxine (11.1% in DM and 7.8% in FSHD). Only 1 DM and 2 FSHD patients were on medications to treat hyperthyroidism. The CDC estimates 5.1% of the population use a medication to treat hypothyroidism.

Conclusions: Medications to treat hypothyroidism are more prevalent in patients with DM compared to patients with FSHD and the US population. Higher use of thyroid medications in DM may be confounded by other endocrine dysfunction in DM (*e.g.,* insulin resistance). Future research is needed to analyze long-term treatment of hypothyroidism in DM, study its pathophysiology, and analyze its interactions with other comorbidities to facilitate care of DM.

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2: Clinical and social issues

Myotonic dystrophy type 2 and inflammatory myopathy : an incidental association?

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Introduction: Myotonic dystrophy type 2(DM2), is autosomal dominant disorder characterized by progressive muscle wasting and multi-systemic complications. Strong association between DM2 and autoimmune diseases was recently suggested and eosinophilic myositis was reported as a first manifestation in a patient with DM2.
Our aim is to present a case of a patient with inflammatory myopathy and DM2.
Case report:a 34-years old woman was admitted to our Department with a preliminary diagnosis of polymyositis (PM). Three months earlier she complained of arthralgia, myalgia, proximal muscle weakness and muscle stiffness. At that time CK, ANA and Jo-1 antibodies serum levels and eosinophils in peripheral blood were significantly elevated. EMG revealed acute myopathic changes. A family history of muscular diseases was negative. On admission, the patient presented proximal muscle weakness. The CK level was 28 times elevated, the level of eosinophils was increased. The EMG revealed myotonic discharges. Muscle biopsy revealed myopathic changes and mild inflammatory infiltration. In electron microscopy discrete degenerative changes with autophagic vacuoles as well as infiltrates containing eosinophils were found. Corticotherapy was started. Some clinical findings and data from the family history justified molecular test for DM2 which was positive . After six months of treatment arthralgia, myalgia, proximal muscle weakness and muscle stiffness disappeared , CK level normalized and EMG showed neither myopathic changes nor myotonic discharges.
Conclusions
1.The coexistence of DM2 and PM should be considered in atypical presentation of either disorder.
2.Due to significant overlap of clinicopathological symptoms in inflammatory myopathies, the diagnosis of the specific type of the disease may be difficult.
3. Whether there is an unusual coexistence of DM2 and PM or true association between those two diseases, remains to be elucidated.

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2: Clinical and social issues

Unexplained neutropenia in myotonic dystrophy type 2 female patients

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Introduction: Myotonic dystrophy type 2 (DM2) is a dominantly inherited muscle disorder with multiple organ involvement. Analyzing results of laboratory tests in 73 DM2 patients we unexpectedly found high percentage of neutropenia in female DM2 patients in our group. Therefore we decided to present detailed results of the performed tests.
Patients and Methods.
We analyzed selected laboratory tests results of 73 DM2 patients (44 females) admitted to the Department of Neurology in 2004-2014. Analyzed tests, amouth others, included complete blood count with differential , immunoglobulin (IgA, IgG, IgM) and CRP serum levels. A history of current infections, drugs and associated disorders was taken.
Results
A mean age of patients was 47 ±13 y (females: 51 ±12 y, males: 42±12 y). A mean age of onset was 37±12 (females: 39.6 ±13y, males: 33 ±13y).
IgG serum level was decreased in 36% of patients (in 28% of females and 48% of males). CRP was elevated in 5% of patients. Both RBC and HGB levels were decreased in 9 % of patients , only in females .
In 22 (30%) patients (17 females , 5 males) , the total WBC number was lower than 4 g/l (reference range: 4-10 g/l).
Complete blood count with differential was available in 17 patients with leukopenia (12 females, 5 males). Among them in 15 patients (10 females, 5 males) leukopenia was due to neutropenia (number of absolute neutrophils below 1.9 103/ μL , reference range: 1.9–8 103/ μL). Of these, 15 patients the absolute number of neutrophils was decreased below 1.5 103/ μL in 5 female and 1 male patients.
Conclusions :
1. Our study indicates that neutropenia is frequent in female DM2 patients.
2. Further studies are needed to determine the relationship between
neutropenia and DM2

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2: Clinical and social issues

Immune system abnormalities in italian patients with myotonic dystrophy type 1 and type 2

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In myotonic dystrophy, serological alterations related to the immune system are known, including low levels of IgG. Noteworthy, an association between myotonic dystrophy type 2 and autoimmune diseases has been recently described in the Dutch population. The aim of this study was to investigate the presence of serological immune system abnormalities in Italian patients with myotonic dystrophy type 1 and 2 (DM1 and DM2). Twenty-five DM1 and sixteen DM2 patients were tested for the presence of a wide panel of autoantibodies (anti nuclear (ANA), anti parietal gastric cells (APCA), anti intrinsic factor (FI), anti smooth muscle (ASMA), anti mitochondria (AMA), anti pancreatic insula (ICA), rheumatoid factor (FR), anti citrullinated peptide (CCP), anti endomysium (EMA), anti Saccaromyces cerevisiae (ASCA), anti neutrophil cytoplasmic (ANCA), anti cardiolipin). Serum levels of Immunoglobulins A, G and M, C-reactive-protein and circulating immune complexes were also measured in all patients. Twelve DM1 (48%) and eleven DM2 patients (69%) showed at least one positive antibody. DM1 showed a higher frequency of CCP and ASCA antibodies, as opposed to DM2 that displayed a higher frequency of ANA, ASMA, APCA, FI and FR antibodies. Around 40% of patients in both groups presented low levels of IgG, three patients in both groups showed low IgM, and two DM2 patients had low IgA levels. Among DM1, the majority of 'autoantibody positive' patients had a low CTG expansion (E1 subclass) and normal IgG values. In conclusion, Italian DM1 and DM2 patients seem to have an enhanced predisposition to develop autoimmune diseases and this tendency might be underestimated due to the low levels of serum immunoglobulins found in many patients. The underlying mechanisms of the immune system abnormalities found in DM need to be investigated.

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2: Clinical and social issues

Renal dysfunction is common in myotonic dystrophy

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We formerly reported that renal dysfunction is common in patients with advanced stage of Duchenne muscular dystrophy (DMD). It seemed that several factors such as long term low cardiac output, dehydration, recurrent urinary infection can be related to renal dysfunction of DMD patients. In this study, we made a cross-sectional assessment of renal function in 465 patients with neuromuscular disorders (DMD: 188, congenital muscular dystrophy (CMD): 32, myotonic dystrophy (DM): 156, facioscapulohumeral muscular dystrophy (FSHD): 50, motor neuron diseases (NMD): 39) retrospectively. The average ages were 23.8±10.7 in DMD, 19.1±9.3 in CMD, 43.3±15.2 in DM, 40.6±18.6 in FSHD and 55.7±19.0 in MND. Cystatin C was elevated (> 0.9mg/L) in 20 DMD, 0 CMD, 43 DM, 3 FSHD and 9 MND patients, respectively. Logarithm cystatin C (LgCys) was correlated to age. Although there were no significant differences in age between DMD and CMD, and among DM, FSHD and MND, LgCys was significantly higher in DMD compared to CMD, DM compared to FSHD. After adjustment of age, LgCys was higher in DMD and DM compared to CMD, FSHD and MND. In DM, LgCys was correlated to age, blood urea nitrogen, uric acid, creatine kinase, cardiac troponin T (cTnT), fractional shortening (FS), CTG repeats numbers. While cTnT and FS was associated with LgCys, cardiac functions were relatively preserved in DM patients. Only five patients presented low FS (<20%) and none showed high cTnT (>0.1ng/ml). Chronic kidney disease should be considered as a common complication in DM. Although further studies are needed to elucidate the pathomechanism, DM itself and subclinical cardiac dysfunction might cause renal dysfunction.

P-045

2: Clinical and social issues

Tell me what you are feeling: Facial emotions and DM1

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Background: Research to date have studied DM1 patient's abilities to identify facial emotions. Authors highlighted a deficit in the recognition of facial emotions (Winblad et al., 2006) or less sensibility to faces expressing fear, disgust and angry (Takeda et al., 2009) or angry and disgust (Kobayakawa et al., 2010). Yet one can question interlocutor\'s ability to identify the DM1 patient\'s emotion, which could also partly explain the difficulties of social adjustment in these patients.
Objective: We aim to study healthy interlocutor's ability to identify emotion on DM1 patient's face.
Method: We proceeded in two stages.
1. Creating the test tool

Faces of DM1 patients and unaffected individuals (controls) were filmed as they were watching video clips that may generate various emotions. At the end of each excerpt, they were asking what emotion(s) they experienced (joy, surprise, sadness, disgust, fear, or neutral emotion). They had to precise the emotion intensity (from very low to very strong). Only faces of respondents who felt one type of emotion, strong or very strong, have been selected, what gave 36 faces (18 from DM1 patients and 18 from controls) to be used as tests for our study.
1. The actual test

The 36 faces were presented randomly to 57 students (mean age: 32.7 years), mostly women. They were asked to identify the emotion expressed by each face.
Results: Preliminary results highlighted greater difficulties for students to attribute an emotion to DM1 patients' faces:
- A largest number of errors on attribution of emotions was observed for patients' faces.
When patients' faces were shown, subjects found themselves sometimes unable to choose an emotion and gave the answer "I don't know", which was never the case with a control face.

Conclusion: In DM1, we observed difficulties of recognition of facial emotions both by the DM1 patient and by his healthy interlocutors.

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2: Clinical and social issues

DM1: Better coping strategies for a better life

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Background: Emotion-focused coping strategies are used twice more often than problem-focused coping strategies in DM1 population. Yet, this coping style seems to be correlated with a poor quality of life (Ahlström and Sjöden, 1996). Besides, a study highlighted the fact that DM1 patients have a tendency to deny their problems (Nätterlund et al., 2001).
Objective: We aimed to explore their coping style in relation with the recognition of the disease.
Method: All participants signed a consent form. DM1 patients from French DM Scope registry filled in questionnaires: Ways of Coping Checklist-Revised (WCC-R) in its French version (Graziani et al., 1998); Illness Cognition Questionnaire (ICQ) of Evers and Kraaimaat (1998).
Results: Study of the correlations between coping style and illness acceptance.
Conclusion: Few studies focus on coping in DM1 population despite of its potential influence on illness' acceptance and patients' quality of life. This investigation could be essential for a better comprehension of DM1 patients' functioning and therapeutic strategies.

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P-047

2: Clinical and social issues

Dysphagia in Myotonic Dystrophy type 1: preliminary results of an integrated neurophysiological and swallowing protocol

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Dysphagia is under-diagnosed in Myotonic Dystrophy (DM1) patients, which are unaware about it, and may cause nutritional derangement and respiratory failure, secondary to ab ingestis pneumonia. Early diagnosis and management would be relevant to reduce morbility and improve quality of life of patients. We propose an integrated protocol to evaluate swallowing function in adult DM1 patients with the aim to define the real prevalence of dysphagia and to understand underlying pathogenetic mechanisms. Our protocol includes: laryngeal accelerometric and submental and cricopharyngeal (CP) muscles EMG recordings to evaluate Dysphagia Limit (DL) and swallowing jitter; needle EMG of genioglossus muscle (G-EMG); fiberoptic endoscopic evaluation of swallowing (FEES), quantitatively scored through the PAS and DOSS, and Water Swallow test (WST), Eating Assessment Tool (EAT-10) and Mini-Nutritional Assessment (MNA) surveys. Our preliminary results in 10 DM1 patients (6M, 4F, mean age 46 ±10), compared to 6 healthy subjects (HS) (3M, 3F, mean age 30 ±3), showed normal MUAPs mean duration of G-EMG, with steady presence of myotonic discharges (9/10 patients). All DM1 patients had reduction in DL compared to HS. 6/10 patients displayed abnormal swallowing jitter, 5 of them showed anomalies in other parameters of the swallowing reflex. Myotonia in CP muscle was present in 3 patients, it didn't seem to be correlated to other swallowing dysfunctions. None of the patient was at risk for malnutrition using the MNA; 5 patients failed the WST and 4 presented an EAT-10 score>3; 2 patients did not show signs of dysphagia at FEES; 4 and 5 patients presented signs of penetration respectively with liquids and semisolids. Our preliminary data confirm that swallowing problems are very common in DM1 patients and show that DL and swallowing jitter seem to be the most sensitive altered parameters. We are extending this protocol to a larger court of Italian patients.

P-048

2: Clinical and social issues

Thyroid abnormalities and myotonic dystrophy: A review of the literature and mechanistic considerations.

PAYNE Martin, Unaffiliated Personal Presentation

An increase in the prevalence of thyroid function abnormalities associated with myotonic dystrophy has been described in some recent publications and conference presentations, while others have described the thyroid as being relatively unaffected. A review of the literature of endocrine abnormalities associated with myotonic dystrophy has been conducted to clarify the situation. Clear evidence for clinically significant hypothyroidism or hyperthyroidism directly related to myotonic dystrophy appears to be lacking, although subtle changes in the hypothalamic-pituitary-thyroid axis appear better supported. In contrast abnormalities in other endocrine functions such as hypogonadism (particularly in males), insulin resistance and probably hyperparathyroidism are less controversial. However since some symptoms of hypothyroidism may be confused with clinical features of myotonic dystrophy and this treatable condition is common in the general population it is important to consider such a possibility in DM1 patients.

P-049

2: Clinical and social issues

Does attendance at a DM1 specialist management clinic reduce the risk of emergency admission to intensive care/high dependancy facility

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Respiratory and cardiac disease are the most common cause of death in DM1. Anaesthesia (GA) poses great risk. We hypothesise that attendance at a specialist management clinic (SMC) with triggers for referral to cardiac and respiratory services and provision of information about GA reduces risk of emergency admission to ITU. From 2009, all patients with DM1 in Scotland have been offered appointments at a SMC. We cross-referenced these patients to admissions to Scottish ITU beds for the years 2010 to 2013 inclusive. We accessed data for all 411 patients within the geographical areas covered by authors RP and CL. Of 411 patients 317 had been seen in a SMC. There were 39 clinical episodes in 33 patients. On 19 occasions patients had been seen in a SMC and on 20 occasions not. There were 15 planned admissions following anaesthesia, 5 in patients not seen in a SMC and 10 seen in a SMC - all patients survived. There were 24 emergency admission; 9 in patients known to the SMCs and 15 in patients not known. The causes of emergency admission included 8 with respiratory infection, 6 with gut or biliary tract sepsis and 5 with infection. There were 6 deaths; 4 in patients never seen in the clinic (cardiorespiratory deaths in 3). Two patients who had been seen in a SMC also died - one from aspiration pneumonia (patient had defaulted from review two years prior) and one patient from pneumonia who had been seen 9 months prior. Eight of these 24 admissions related to patients who developed respiratory compromise during the course of an admission. Four followed surgery and 3 of these had never been seen in an SMC. Four further admissions occurred during the treatment on a medical unit and 3 of these patients had never been seen in an SMC. Overall four patients out of 94 non-clinic attenders died compared to 2 patients out of 317 sometime clinic attenders. While there are many caveats and the numbers are small the data may suggest improved outcomes in patients attending an SMC

P-050

2: Clinical and social issues

CANADIAN NEUROMUSCULAR DISEASES NETWORK (CAN-NMD) – Collaborating to enhance clinical care, education and research

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INTRODUCTION: To date in Canada despite a highly collaborative neuromuscular diseases (NMD) community there has been no formalized effort to promote collaboration between neuromuscular diseases stakeholders nation-wide. Improving such collaboration will create new opportunities to share clinical best practices; collaborate to translate new hypotheses into therapeutics; and to improve the overall availability and sharing of knowledge on individual diseases such as myotonic dystrophy (DM). Funded in 2014, the CAN-NMD Network aims to facilitate enhanced clinical care for Canadian NMD patients including DM patients through knowledge and best practice sharing amongst healthcare professionals across the country.

METHODS: The CAN-NMD features several task forces each devoted to a specific aim of the Network's activities. The Clinical Care Task Force is focused on enhancing clinical care delivery. The Knowledge Translation Task Force is focused on improving the access to and availability of needed knowledge assets across NMD stakeholders. In October 2014 over 75 NMD clinicians, scientists and other stakeholders met to devise the specific activities on which the Network will focus its efforts over the next three years.

RESULTS: In the area of clinical care for NMD patients in Canada the CAN-NMD Network will focus its activities in three thematic areas: creating pathways; building community; and striving for excellence. Activities in these areas will include the creation of a national Wiki resource for NMD stakeholders building on the MNMWiki initiative in Quebec; an assessment of clinical care across the country to identify gaps and needs including patient focus groups; and enhancing the sharing of knowledge amongst healthcare professionals through national NMD rounds.

CONCLUSION: The creation of the CAN-NMD Network represents an important step forward to improve the consistency of clinical care for DM patients across Canada.

P-051

2: Clinical and social issues

Neuromyelitis optica in family with both myotonic dystrophy type 1 and type 2 mutations

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Background: Here we report the first family with both DM1 and DM2. One of the members also has neuromyelitis optica (NMO).
Cases report: Proband case (DS) was positive for both DM1 (65-70 CTG) and DM2 (100-1010 CCTG). When she was 53, pain and visual loss appeared in the left eye, and ten months later in the right eye. High dose steroid therapy was of no benefit. At age 54 she had mild weakness in proximal lower extremities (LE), calf hypertrophy, mild myotonia, cataract, bilateral partial atrophy of papilla, retrobulbar neuritis and insulin resistance. Brain MRI showed supratentorial white matter hyperintense lesions. Aquaporin 4 antibodies were positive in serum and cerebrospinal fluid, confirming diagnosis of NMO spectrum. Her sister SM underwent cataract surgery at age 39. Genetic analyses revealed DM2 (70-990 CCTG) and DM1 (59-64 CTG). At 43 she was diagnosed with hyperthyroidism, and at 47 she noticed difficulties while climbing stairs. She had mild weakness in upper extremities and proximal LE, calf hypertrophy, mild myotonia, prolonged relaxation of the left ventricle and polyneuropathy. Sister GI was negative for DM2 and had 59-64 CTG repeats. She refused to be examined. Sister DD was negative for DM1 and positive for DM2 (130-1150 CCTG). She had difficulties while climbing stairs at age 47. At age 51 examination revealed: mild proximal weakness in LE, calf hypertrophy, mild myotonia, cataract, single ventricular extrasystoles (VES) and diabetes. Mother of our patients only had eye cataract at age 79. Genetic analysis revealed 56-61 CTG repeats with negative DM2 mutation. Father of patients passed away and probably suffered from DM2 – he had walking difficulties and cataract before age of 60.
Conclusion: Simultaneous analysis for both DM1 and DM2 seems meaningful because presence of these two mutations is possible. Careful examination should be performed since association of autoimmune diseases and DM is not random and their symptoms may overlap.

P-052

2: Clinical and social issues

Gait pattern differences in DM1 and DM2 patients during dual task walking

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Background: Several previous studies investigated gait in patients with DM1. Main findings were slower gait velocity, shorter stride length (SL) and increased stance phase, mostly due to the ankle plantar and dorsal flexors weakness. Gait impairments have never been investigated in DM2 patients.

The aim of the study was to analyze gait characteristics in DM1 and DM2 patients, compared to healthy controls (HCs).

Method: Gait characteristics were measured in 24 DM1 (38.6 yrs) and 24 DM2 (50.7 yrs) patients and compared with two gender- and age-matched healthy control groups (HCs). Measurements were performed using GAITRite electronic walkway system.

Results: Both DM1 and DM2 patients differed in almost all gait parameters compared to HCs. SL was affected in both patients groups and in all walking conditions. However, there were no differences in variation of the cycle time (CVCT) in base and motor tasks. Only the mental task affected gait in both groups of DM patients. Lack of differences in swing time (ST) during mental task suggested that stability in DM is not more affected than in healthy subjects.
Regarding between-group comparison, SL was shorter in DM1 than in DM2. Cycle time (CT) and ST were similar in both groups in all conditions. However, variation of CT (CVCT) showed strong increase during mental tasks, particularly in DM2, while variation of ST (CVST) was increased during mental task in DM1.

Conclusion: Although gait is affected in both DM1 and DM2 patients, we observed different pattern of gait impairment. These differences might help to design separate symptomatic treatment strategies.

P-053

2: Clinical and social issues

Clinical and electrophysiological study of peripheral nerve involvement in myotonic dystrophy type 2 (MD2)

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INTRODUCTION: MD2 has been recognized as a polyorganic AD disease with predominant muscle involvement and an overall milder course than MD1. While peripheral nerve involvement (neuropathy) has been documented in MD1, it could also be a feature of MD2, regarding its pathophysiology and/or disturbed glucose or thyroid metabolism. AIM: To determine the frequency and type of neuropathy in MD2 patients, particularly in relation to the presence of diabetes mellitus/glucose intolerance (DM) and thyroid disease (TD). MATERIALS AND METHODS: Medical records including history, neurological and EMG examination, as well as laboratory tests for endocrinopathy have been reviewed in 30 Croatian patients (25F + 5M, 22 families) with symptomatic heterozygote CCTG expansion in *ZNF9*. Physical examination included semiquantitative estimate of limb force, tendon reflexes and sensory testing. EMG reports of MUPs sampling, nerve conduction velocities (NCV), the latency and amplitude of motor and neural potentials from 2-6 peripheral nerves were matched with 30 paired controls. MAIN RESULTS: Proximal muscle weakness and/or intermittent myotonic symptoms were present in 26/30 patients. In 12/30 patients the vibration sense was altered or absent in feet, and in 17/30, the ankle reflexes were reduced or absent without distal sensory complaints. EMG results were supportive of axonal polyneuropathy in 19/30 patients and carpal tunnel neuropathy in 10/30. Both conditions overlapped in 8/30 patients. Endocrinopathy was detected in 14/30 (7 DM, 7 TD). Out of 21 patients with either neuropathy, DM or TD signs were present in 6 patients each. CONCLUSION: We found physical and/or electrophysiological signs of neuropathy in up to two thirds of MD2 patients. Less than one half of all patients demonstrated the laboratory signs of endocrinopathy. We conclude that peripheral nerve involvement is likely a common inherent feature of MD2, irrespective of featured metabolic abnormalities.

P-054

2: Clinical and social issues

Development and validation of a new genetic assay for detection of myotonic dystrophy type 2

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Myotonic dystrophy (DM) is the most common adult form of muscular dystrophy, characterized by autosomal dominant progressive myopathy, myotonia and multiorgan involvement. Two distinct forms have been identified. Myotonic dystrophy type 1 (DM1) is caused by a [CTG] expansion in *DMPK* gene and myotonic dystrophy type 2 (DM2) is caused by a [CCTG] expansion in *ZNF9/CNBP* gene. The aim of this work was the development and validation of the new genetic test “Myotonic Dystrophy type 2 kit-FL” , based on the combination of Long-PCR and Southern Blot Analysis (SBA), to identify the DM2 disease.

A cohort of 106 individuals was analyzed. The results shown that 106/106 patients were correctly identified using the new molecular assay. In particular, 54 (51%) were DM2-positive, 39 (37%) were DM2/DM1-negative and 13 (12%) DM2-negative/DM1-positive. The inclusion of DM1-positive has allowed a better evaluation of the specificity. In our population 54/106 DM2-positive patients showed expanded alleles ranging from 75 and >5000 [CCTG] repeats (median: 1000-5000 range). SBA showed that the 15% (8/54) of DM2 positive samples had single sizeable expansion and 85% (46/54) showed multiple bands or smears. Comparative FISH analysis on muscle biopsies, revealed that sensitivity and specificity of new molecular assay were very high (>99%). Equivalent analytical performances were obtained using different DNA extraction methods. Clinical characterization was available in 32/54 DM2 subjects. Among affected individuals 87.5% (28/32) had electrical myotonia, 69% (22/32) proximal weakness, 41% (13/32) cataracts and about 37.5% (12/32) cardiac conduction defects. FISH analysis and clinical data were used to support the genetic analysis. Our data demonstrate that this assay increases the molecular detection rate to 99% and it is sufficient to correctly establish the presence or absence of [CCTG] alleles in DM2 disease.

P-055

2: Clinical and social issues

Delayed diagnosis in women with DM1: implications for care and cure

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Background. Diagnostic delay in DM1 hinders appropriate care especially regarding prevention of major cardiac arrhythmias and sudden death, general anaesthesia risks, perioperative potential respiratory insufficiency and does not allow adequate family planning. This applies especially to women in child-bearing age, who may give rise to the severe congenital form of the disease (CDM), known to be associated with a relatively high mortality rate from respiratory failure.
Objectives. The aim of our study is to quantify the delay between symptoms at onset and diagnosis of DM1 in a cohort of child-bearing age women to describe the consequences on the mothers and their CDM newborns.
Methods: We retrospectively reviewed charts from 37 women (mean age 43 ± 14) to quantify and detect reasons for the diagnostic delay using an arbitrarily designed questionnaire. Descriptive data on gestation, delivery, anesthesia and CDM critical care needs were recorded.
Results: The mean age at diagnosis was 28,8 ± 12,7 years. The mean time to diagnosis was 9,8 ± 8,6, longer than for men (8,3 ± 8,6 yrs). Reasons for diagnostic delay were (i) underestimation of symptoms (n = 67%); (ii) misdiagnosis (n = 32%). 4% of CDM in our cohort were born from undiagnosed mothers. Of these, polydramnios was detected in 87%. 2% of mothers had 1 or 2 spontaneous abortions prior to the diagnosis of DM1 when giving birth to CDM.
Conclusions: Delays in diagnosis in women with DM1 in child-bearing age hinder appropriate preventive care and cure both for mothers and children with CDM. Earlier detection of DM1 could provide more awareness of treatment for gestation, anesthesia risks and for early critical care of CDM newborns. There is a need to lower the threshold of genetic testing in non-index women even though apparently asymptomatic to help family planning and reduce the burdens of the disease.

P-056

3: Disease mechanisms

Generation of induced pluripotent stem cells as cellular model to study the pathogenesis of myotonic dystrophy type 2 (DM2)

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Human induced pluripotent stem cells (hiPSCs) are an innovative tool to study multisystemic disorders because this cell type can be derived from patients as a renewable source for unlimited cells that are difficult to acquire. Myotonic dystrophy type 2 (DM2) is caused by an unstable (CCTG)n expansion in intron 1 of the CNBP gene leading to a progressive disease involving muscle, heart and brain. To date, no cellular or animal models fully recapitulate the complex molecular and clinical phenotype of DM2 patients. In this study, we generated two DM2 and one normal hiPSC lines from dermal fibroblasts by lentiviral transduction using the hSTEMCCA-loxP polycistronic vector encoding the four Yamanaka\'s factors (hOct4, hSox2, hKlf4, and hc-Myc). The successful reprogramming of DM2 and control-derived hiPSCs has been confirmed by specific morphological, molecular, immunocytochemical markers and by their teratogenic potential when inoculated in vivo. We further demonstrate the stability of reprogrammed cells over 10 and more passages and their ability to differentiate into the three embryonic germ layers. CCTG expansions and intranuclear CCUG-containing RNA foci, hallmarks of DM2 in patients-derived fibroblasts, were conserved in DM2-hiPSCs. Further expression studies are currently in progress to determine if the DM2-hiPSCs show the spliceopathy typical of DM2 adult cells and tissues. The development of an experimental system based on hiPSCs technology could aid in the identification of molecular pathogenic mechanisms and personalized therapeutic treatments for DM2.

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3: Disease mechanisms

SCN4A as modifier gene in patients with myotonic dystrophy type 2

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Myotonia is generally mild and inconsistent in myotonic dystrophy type 2 (DM2) and it has been correlated with the disruption of the alternative splicing of the muscle chloride channel CLCN1. It is noteworthy that mutations in *CLCN1* gene can act as modifiers in patients with myotonic dystrophy type 2 leading to an amplification of their myotonia. However, more recently we have described a DM2 patient with a concomitant mutation of *SCN4A* genes showing a severe myotonia. We report a 32 years old DM2 patient who presented an atypical phenotype characterized by an early severe myotonia since he was 12 years old. *Mexiletine* treatment resulted ineffective in reducing myotonia. While no mutation on *CLCN1* gene was found, the genetic analysis of *SCN4A* gene showed a G2717C base exchange in exon 14 predicting an S906T substitution. This variant is considered a benign polymorphism however electrophysiological studies revealed that it affects the fast and slow gating processes. It is possible that the additive effect of the DM2 mutations and the S906T polymorphism may create the atypical severe phenotype observed in our patient. Indeed his mother, also affected by DM2, did not show the polymorphism and no clinical myotonia was observed at the neurological examination at the age of 65. This finding suggests that *SCN4A* gene screening should be performed in DM2 patients with early and severe myotonia without mutations in *CLCN1* gene. Moreover it suggests that 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.

P-058

3: Disease mechanisms

Heart dysfunction in a new Drosophila model of DM1 is rescued by pentamidine

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Up to 90% of Myotonic Dystrophy (DM) patients will develop cardiac abnormalities at some point of disease progression. The most common of them is varying degrees of heart block characterized by conduction defects and supraventricular and ventricular tachycardia, resulting in an important risk of sudden cardiac death. Despite its importance few animal model studies have focused on the heart dysfunction symptom. Here, we describe the characterization of the heart phenotype of a *Drosophila* model expressing pure expanded CUG repeats in adult heart. Morphologically, expression of 250 CTG repeats under the control of the heart-specific GMH5-Gal4 driver caused a narrowing of the dorsal vessel (homologous to human heart) compared to control expressing flies (20 repeats) and wild type animals. Combined immunofluorescence and *in situ* hybridization of Muscleblind and CUG repeats, respectively, in dissected fly hearts confirmed detectable ribonuclear foci and Muscleblind sequestration exclusively in flies expressing CTG expansions. Heart-specific expression of toxic RNA reduced the mean life of adult flies compared to controls expressing short repeats (20 CUG repeats) and kymograph analyses showed decreased contractility in the model fly hearts that resembled cardiac dysfunction in humans. Importantly, the heart dysfunction phenotype was rescued by pentamidine, a compound previously described to improve DM phenotypes, thus supporting the usefulness of the *Drosophila* model in the *in vivo* testing of promising therapeutic compounds against this critical aspect of the disease.

P-060

3: Disease mechanisms

Conserved miRNAs mis-regulated in the skeletal muscles of human and mouse DM1

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MicroRNA (miRNAs) are small non-coding RNAs derived from hairpin precursors. miRNAs mediate degradation or repress translation of their target mRNAs by partially pairing with specific sequences in their 3'-UTR. There is now conclusive evidence that miRNAs are key regulators of embryonic development and skeletal myogenesis in metazoans. While recent evidence suggests a deregulation of specific miRNA in skeletal muscle and heart of DM1 patients, the regulation and role of miRNAs in the pathogenesis of myotonic dystrophy are still unknown.

In order to identify miRNAs misregulated in DM1, we used microarrays to compare the expression of 890 miRNAs from skeletal muscle tissue samples from DM1 patients versus normal muscular tissue. We identified 31 miRNAs that are mis-regulated in DM1 muscle tissues (fold change > 1.5x; $p < 0.05$), with the majority (29) being overexpressed. To validate these results, we analyzed miRNAs expression from skeletal muscle tissue samples from DM1 mouse model (HSA-LR) versus normal (FVB) muscular tissue. Using the same parameters (fold change > 1.5x; $p < 0.05$), only 14 miRNAs were mis-regulated in skeletal muscle tissues of HSA-LR, all of them being up-regulated.

Of these miRNAs, only three were found to be overexpressed in both human and mouse DM1 muscles. Interestingly, all three miRNAs are expressed from the same large miRNA cluster, which is present in an imprinted genomic locus and is conserved between mouse and human genomes. A more detailed analysis of the mis-regulated miRNAs in both mouse and human revealed that 12 of the 29 up-regulated miRNAs in human DM1 muscle tissues are expressed from this cluster, while 6 of the 14 up-regulated miRNAs in HSA-LR mouse are also from this cluster. Altogether, these data suggest that a disproportionate number of miRNAs mis-regulated in DM1 are expressed from an evolutionary conserved miRNA cluster.

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3: Disease mechanisms

Antisense oligomers as a tool for identification of functional RNA targets for MBNL proteins

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Immunoprecipitation techniques combined with deep sequencing (i. e. CLIP-seq) enables identification of multiple RNA sequences potentially recognized by RNA-binding proteins e. g. Muscleblind-like protein 1 (MBNL1) which is a known regulator of alternative splicing. In these studies we propose an efficient method for verification of CLIP-seq signals both in *in vitro* and *in vivo* tests via inhibition of RNA/protein interaction by chemically modified short antisense oligomers (AOs). *In vitro* studies showed that all tested AOs complementary to functional MBNL1-binding motifs within a fragment of *Atp2a1* pre-mRNA blocked interaction with MBNL1. However, only 2'OMePS but not DNA, LNA and 2'OMe oligomers complementary to these intronic sequences significantly enhances the exclusion of alternative exon 22 of *Atp2a1* from both minigene and endogene. Then, based on CLIP-seq results we selected three potential MBNL-binding regions from intron 6 of *Pphln1*, exon 7 of *Nfix* and exon 10 of *Ldb3* to test activity of AOs. *In vitro* application of several AOs blocking different YGCY motifs allows us to identify one or two MBNL1-binding sites in ~200-nucleotides long *Pphln1, Nfix* and *Ldb3* pre-mRNA fragments. Unexpectedly, in cell models the 2'OMePS version of selected AOs blocked only functional MBNL1-binding motif for endogenous *Pphln1*, but not for exonic sequences of *Ldb3* and *Nfix*, for which opposite direction of splicing changes were observed. However, substitution of MBNL-sensitive elements in intron 22 of *Atp2a1* minigene with studied sequences of *Pphln1*, *Nfix* or *Ldb3* reconstitutes alternative splicing of exon 22 of *Atp2a1*. In these chimeric constructs exon 22 inclusion responds significantly on both MBNL1 overexpression and the presence of gene-specific AOs complementary to MBNL1-binding sites.

P-062

3: Disease mechanisms

Investigating the brain cell specificity of DM1 neuropathogenesis

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Myotonic dystrophy type 1 (DM1) is a complex, multisystemic disorder, in which the neurological manifestations severely affect the quality of life of the DM1 patients and their families. The abnormal expansion of the CTG repeat in the *DMPK* gene, leads to the toxic accumulation of expanded RNA in the nucleus and disrupts cell and tissue functions.
Numerous clinical, neurophysiological and imaging studies have confirmed the involvement of the central nervous system (CNS) in DM1, but the cellular and molecular pathways affected in the brain are not yet fully understood.
DMSXL mice express expanded *DMPK* transcripts in multiple tissues, including the CNS, and have provided a useful tool to investigate the molecular mechanisms of DM1 neuropathogenesis. They show foci accumulation and splicing defects in multiple tissues, notably in the CNS. As a result, DMSXL mice exhibit behavioral and cognitive phenotypes, electrophysiological defects, neurochemical changes and synaptic protein deregulation, indicative of neurological dysfunction.
RNA foci are more abundant in glial cells in vivo, in both DMSXL mice and DM1 brains, suggesting different cell type susceptibility to the accumulation of CUG repeats. We have used primary neuronal and glial cultures derived from DMSXL brains to investigate the cell type-specific disease mechanisms. Interestingly, DMSXL primary astrocytes have shown higher expression of expanded *DMPK* transcript, more abundant foci accumulation and more severe spliceopathy relative to primary neurons. Moreover, preliminary results suggest deregulated growth dynamics of primary astrocytes. All together these results indicate a deleterious effect of the expanded transcripts on astroglial biology.
We are currently confirming and extending the study of the impact of the DM1 mutation on glial cell lineages. In parallel, we will investigate DMSXL primary neurons, to provide insight into the neuronal cellular phenotypes associated with DM1 CNS dysfunction.

P-063

3: Disease mechanisms

An investigation of the cellular and microbial etiologies of gastrointestinal pathologies in myotonic dystrophy using zebrafish

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Myotonic dystrophy (DM), most well known for its muscle-related phenotypes, affects many different parts of the body, including the gastrointestinal (GI) tract. Common GI symptoms in DM patients include swallowing problems, slowed gastric emptying, decreased intestinal motility, abdominal pain, diarrhea, constipation, anal incontinence, and small intestinal bacterial overgrowth. A quarter of DM patients consider GI symptoms to be the most disabling aspect of their disease, yet few studies have focused on understanding the mechanisms underlying these symptoms.

We are using the zebrafish (*Danio rerio*) as a model system to study the digestive phenotypes of DM and how microbiota contribute to them. To model DM, we have used CRISPR technology to mutate *Mbnl1, Mbnl2,* and *Mbnl3* in zebrafish, and are generating transgenic fish that overexpress CUG repeats globally and in specific tissues such as visceral muscle and enteric neurons.

We will take advantage of the optical transparence of zebrafish larvae to directly compare gut motility between wild type and DM model fish, and will investigate the specific cell types that contribute to gut motility phenotypes. We will assay whether intestinal inflammation is increased in zebrafish DM models. In addition, we will ask how bacteria are altered in zebrafish DM models compared to wild type fish. Finally, we will use germ-free fish to ask whether altered microbiota are necessary or sufficient to cause DM-related digestive phenotypes.

Overall, these studies will provide important insight into the mechanisms behind the digestive symptoms of DM and the role, if any, that microbes play in them.

P-064

3: Disease mechanisms

CAG repeat RNA recapitulates some toxic features of CUG repeats

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Expanded CAG repeats within coding regions trigger disorders for which a protein gain-of-function mechanism has been proposed to explain neurodegeneration by polyglutamine-rich (polyQ) proteins. However, the emerging body of evidence indicates that RNA-mediated gain-of-function mechanism has also a profound role in neurotoxicity in polyQ diseases. Analysis of exogenous expression of untranslated CAG repeats in various cell lines, as well as human SCA3 and HD fibroblasts, and transgenic fly, worm and mouse models showed that transcripts containing mutant CAG repeats form intranuclear foci which sequester MBNL1 protein, what results, in some cases, in alternative splicing aberrations typical for CUG repeats. These results suggest that there may be common pathogenic mechanisms in the translated and untranslated repeat diseases. In the present study, to better understand the deleterious role of RNA in the pathogenesis of CAG repeat expansion diseases, we characterized in fibroblast cell lines derived from patients with various polyQ disorders the known RNA toxicity markers including RNA foci formation, MBNL1 sequestration and splicing misregulation. Additionally, we analyzed the unconventional translation called RAN translation in cellular model of SCA3 expressing different length of translated or non-translated CAG repeats. Using FISH analysis, we showed formation of intranuclear CAG RNA foci in fibroblasts of various polyQ diseases. Additionally, MBNL1 was trapped in the CAG RNA foci, however, observed MBNL1 co-localization with the mutant CAG repeats was not always accompanied by aberrant splicing of selected MBNL1-regulated genes. Furthermore, our preliminary results suggest that RAN translation on CAG repeats localized in translated exons is not abundant as described for the expanded repeats which appear in non-coding sequences. All together, expanded CAG repeat transcripts from different polyQ disorders, recapitulate some of the toxic features of CUG repeat RNA.

P-065

3: Disease mechanisms

Disruption of alternative splicing of CELF1 untranslated regions

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Gain of function of CELF1 protein plays important role in pathogenesis of myotonic dystrophy. Increased activity of CELF1 would be caused by posttranscriptional mechanisms including hyperphosphorylation by PKC which stabilized CELF1 protein in nucleus. Data obtained from Affymetrix all exon array hybridization experiments suggests that sequences of both 5' and 3' untranslated regions (UTRs) of CELF1 mRNA molecules undergo an abnormal alternative splicing in DM skeletal muscles. Using splicing-sensitive RT-PCR assays we confirmed these observations. The alternative splicing of both 5'- and 3'-UTR of CELF1 mRNAs is also efficiently regulated during differentiation of skeletal muscles, brain and heart. These splicing events at least partially depend on MBNL1 activity. Several alternative CELF1 5'-UTRs which vary in length and sequence were cloned into luciferase expression vectors to test their impact on translation. We did not, however, observed significant differences in translation efficacy of tested 5'-UTRs. Alternative splicing of different CELF1 5'-UTRs may also impact the sequence of encoded CELF1 protein by adding 27 amino acids to the N-terminus of the muscle-specific isoform. Two different CELF1 open reading frames were cloned into EGFP expression vectors. Longer, muscle specific CELF1 protein isoform which is significantly diminished in DM muscles showed weaker splicing activity on some minigenes. Our results suggest an existence of different posttranscriptional mechanisms, which can influence CELF1 activity in developmental and disease-specific processes by alternative splicing regulation of CELF1 UTR sequences.

P-066

3: Disease mechanisms

RBFOX1 Modulation of MBNL1 Dependent Alternative Splicing Events in a DM1 Cell Model

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Like MBNL, RBFOX1 is both an activator and a repressor of alternative exons. Several alternative splicing events are co-regulated by MBNL1 and RBFOX1, and many of these are mis-spliced in DM1. To gain insight into the role of RBFOX1 on MBNL regulated splicing, we used a stable inducible MBNL1 cell line to control the levels MBNL1 and compared MBNL1 splicing responses in the presence of absence of RBFOX1. This MBNL1 dosing cell line allows us to have exquisite control of protein levels to determine the range at which an event is regulated (slope) and the amount of protein required for regulation (sensitivity). We found that RBFOX1 decreased the slope and the range of percent spliced in (PSI) values of the MBNL1 splicing responses of the INSR and NFIX minigenes. Mutational analysis showed that the RBFOX binding site in each minigene was involved in this “dampening” effect, suggesting that the cause was RBFOX1 binding. In addition, we found that mutating the RBFOX1 binding sites altered the MBNL1 dose-responses when RBFOX1 was not present. This result may be due to MBNL and RBFOX binding sites overlapping in these co-regulated events. Our results therefore indicate that RBFOX1 may alleviate mis-splicing caused by MBNL sequestration for events where RBFOX1 and MBNL1 promote splicing in the same direction, and it may amplify mis-splicing when RBFOX1 and MBNL1 oppose each other. The extent of this modulation of MBNL dependent splicing events should differ between tissues where RBFOX1 expression levels vary as well as differences in sequestration of MBNL to the DM toxic RNA.

P-067

3: Disease mechanisms

Splicing patterns in various parts of the myotonic dystrophy type1 brain

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Background
In myotonic dystrophy type 1 (DM1), the degree of CTG repeat expansion and pathologic change vary among the parts of the central nervous system (CNS). It is known that the length of CTG repeat in the cerebellum is shorter than that in the cerebral cortices. We showed that in the brain with DM1, many aberrantly spliced genes were related to MBNL2 and few were related to MBNL1. We showed that the degree of aberrant splicing in DM1 was smaller in the cerebellum than in the temporal cortex.

Objective
We aimed to examine splicing variants ratio, DMPK mRNA level, and CTG repeat length.

Results
We used samples from the frontal cortex, temporal cortex, hippocampus, cerebellar cortex, and spinal cord.
By RT-PCR, we confirmed that a few MBNL2-related genes were aberrantly spliced in the cerebellum with DM1. Such genes were also aberrantly spliced in the spinal cord and other parts of the brain. In the frontal cortex, temporal cortex, and hippocampus, several MBNL2-related genes showed a similar aberrant splicing pattern; one gene showed abnormal splicing pattern only in the temporal cortex.
We measured DMPK mRNA level using real-time PCR. The cerebellum and temporal cortex expressed more DMPK mRNA than other parts of the CNS.
We measured CTG repeat length for some samples by small pool PCR. The CTG repeat length was not significantly different between the frontal and temporal cortices.

Conclusion
The patterns of MBNL2-related splicing were different among the parts of the central nervous system, although there were similarities in splicing between the cerebellum and spinal cord and among the cerebral cortices. These differences and similarities cannot be explained by CTG repeat length or DMPK mRNA level.

P-068

3: Disease mechanisms

Cooperation meets competition in microRNA-mediated DMPK transcript regulation

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The fundamental role of microRNAs (miRNAs) in the regulation of gene expression has been well-established, but many miRNA-driven regulatory mechanisms remain elusive. In the present study, we demonstrate that miRNAs regulate the expression of *DMPK*, the gene mutated in myotonic dystrophy type 1 (DM1), and we provide insight regarding the concerted effect of the miRNAs on the DMPK target. Specifically, we examined the binding of several miRNAs to the DMPK 3' UTR using luciferase assays. We validated the interactions between the DMPK transcript and the conserved miR-206 and miR-148a. We suggest a possible cooperativity between these two miRNAs and discuss gene targeting by miRNA pairs that vary in distance between their binding sites and expression profiles. In the same luciferase reporter system, we showed that miR-15b/16 binds to the non-conserved CUG repeat tract present in the DMPK transcript and that CUG-repeat-binding miRNAs might also act cooperatively. Moreover, we detected miR-16 in cytoplasmic foci formed by exogenously expressed RNAs with expanded CUG repeats. Therefore, we propose that the expanded CUGs may serve as a target for concerted regulation by miRNAs and may also act as molecular sponges for natural miRNAs with CAG repeats in their seed regions, thereby affecting their physiological functions. In addition, we found a number of long non-coding RNAs (lncRNAs) that also contain CUG repeats and may potentially participate in miRNA-regulated crosstalk between transcripts with CUG repeats, leading to changes in the regulatory network that might contribute to disease. Together, our findings support the role of miRNAs in DM1 pathogenesis and demonstrate their therapeutic potential.

P-069

3: Disease mechanisms

Early hallmarks neurofibrillary degeneration and cognitive impairment in a double human Tau x DM1 mouse model

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Myotonic dystrophy type 1 (DM1) is a multisystemic disease that affects several organs, including brain. Affected patients present cognitive impairments including attentional disability difficulties to recognize facial emotion and to perform visuoconstructive tasks. In the brain of patients with DM1, neurofibrillary degeneration (NFD) is reported in the temporo-insular brain region and it is associated with the mis-splicing of Tau exons 2/3 and 10. In order to determine the contribution of Tau to NFD development and cognitive disorders in DM1, we took advantage of the DMSXL transgenic mouse model of DM1, expressing the human locus of DM1 with long unstable CTG repeats (over a thousand) in the *DMPK* gene. This DMSXL model was crossed with a mouse transgenic for the human *MAPT/Tau* gene (hTau) but invalidated for the endogenous expression of the murine *Mapt* gene (mTauKO). Our results reveal spatial memory impairment using Morris Water Maze (MWM) task in transgenic DM1 mice expressing the hTau gene and mTauKO (DM+/- hTau mTauKO) compared to mTauKO, DM+/- mTau KO or mTauKO hTau mice. In addition, an increase of Tau pathology was observed by combined biochemical and immunohistochemical studies, associated to mis-splicing of tau transcript. Finally, a transcriptomic approach displayed a gene expression profile modified only in DM+/- hTau mTauKO mice, suggesting a gene interaction between the human locus of DM1 and hTau gene expression. In that context, the 14.3.3 gene, which the resulting protein may be implicated in tau phosphorylation, was found overexpressed in these mice and confirmed in a cell model. Overall this study highlights the relationships between a defective splicing of tau, neurofibrillary degeneration and memory impairments in transgenic DM1 mouse model.

P-070

3: Disease mechanisms

Increased autophagy and apoptosis contribute to muscle atrophy in a myotonic dystrophy type 1 Drosophila model.

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Muscle mass wasting is one of the most debilitating symptoms of myotonic dystrophy type 1 (DM1) disease, ultimately leading to immobility, respiratory defects, dysarthria, dysphagia and death in advanced stages of the disease. In order to study the molecular mechanisms leading to the degenerative loss of adult muscle tissue in DM1, we generated an inducible fly model of expanded CTG trinucleotide repeat toxicity resembling an adult onset form of the disease. Heat-shock induced expression of 480 CUG repeats in adult flies resulted in indirect flight muscle area reduction. In these model flies, reduction of muscle area was concomitant with increased apoptosis and autophagy. Inhibition of apoptosis or autophagy by the overexpression of *DIAP*, *mTOR,* or *muscleblind* or by iRNA silencing of autophagy regulatory genes, achieved a rescue of the muscle loss phenotype. These results were validated in skeletal muscle biopsies from DM1 patients in which we found downregulated autophagy and apoptosis repressor genes, and also in DM1 myoblasts were we found increased autophagy measured as increased LysoTracker signal. These findings provide new insight into the signalling pathways involved in DM1 disease pathogenesis.

P-071

3: Disease mechanisms

rbFox1 rescues CCUG, but not CUG toxicity, in Drosophila Myotonic Dystrophy models

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Myotonic dystrophy (DM) encompasses two genetically distinct entities, DM1 and DM2, which are caused by expanded CTG and CCTG repeats, respectively. Importantly, DM2 follows a more favorable clinical course than DM1, which is a puzzling observation since DM2 is characterized by higher number of expanded repeats compared to DM1. This paradox suggests that a mechanism, yet to be discovered, modulates the severity of DM pathologies. Searching for novel factors involved in DM, we identified that the RNA binding proteins, rbFOX1 and rbFOX2, bind to expanded CCUG repeats that cause DM2, but not to expanded CUG repeats that cause DM1. rbFOX binding to CCUG induced release of Muscleblind (Mbl) suggesting that the overexpression of these proteins could rescue phenotypes caused by expression of expanded CCUG repeats. To test this hypothesis we developed new Drosophila models of DM1 and DM2 that over-express rbFOX1. New DM fly models expressing 250 CUG or 1200 CCUG pure repeats in muscle showed muscle atrophy measured in indirect flight muscles (IFMs) in comparison to control flies expressing short versions of the repeats. Importantly, simultaneous overexpression of rbFOX1 and expanded CCTG repeats in Drosophila IFMs achieved complete rescue of muscle atrophy. In contrast, over-expression of rbFOX1 had no rescue effect in the fly model of DM1. FISH coupled to immunofluorescence assays demonstrated that overexpressed rbFOX1 co-localized with RNA aggregates of expanded CCUG repeats, but not with foci of expanded CUG repeats. On the contrary, endogenous Mbl co-localized with RNA aggregates of both expanded CUG and CCUG repeats. Overall, these results demonstrate that the sole expression of rbFOX1 can alleviate the toxic effect of expressing expanded CCUG repeats.

P-072

3: Disease mechanisms

Characterization of an Inducible/Reversible DMPK 3\'UTR (CTG)200 RNA Toxicity Mouse Model

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Previously (almost 10 years ago) we reported on the first inducible/reversible mouse model of RNA toxicity. Surprisingly, this model developed myotonic dystrophy with multi-systemic features while over-expressing a normal *DMPK* 3\'UTR with (CTG)5. Over the past decade, we have continued to pursue the development of an inducible/reversible mouse model that expresses a mutant *DMPK* 3\'UTR. Similar to the existing model, this mouse model uses the human *DMPK* promoter to drive the expression of a Tet-inducible transgene (Tet-On), except that it expresses eGFP-DMPK 3\'UTR (CTG)>200. Here we report on the ongoing development and characterization of these mice. Multiple, independent, inducible lines have been made. Though individually, they are asymptomatic, combinations of these lines yield mice that develop key aspects of DM1 including myotonia, cardiac conduction defects, RNA splicing defects and RNA foci. The translational utility of this model is dependent on the potential for phenotypic reversal. Results from ongoing studies determining the potential reversibility of various phenotypes will be presented. The potential for functional readouts such as treadmill running, grip strength and lifespan will be presented. The pre-clinical utility of this model will be discussed in the context of target identification and testing therapeutics. In addition, data from ongoing studies aimed at mechanisms of RNA toxicity including the role of putative antisense and RAN translation products will be presented.

P-073

3: Disease mechanisms

A high throughput fluorescence microscopy screen to identify proteins associated with RNA foci in myotonic dystrophy

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Myotonic dystrophy type I (DM1) is caused by an unstable CTG repeat expansion in the 3' UTR of the muscle kinase gene *DMPK*. The expanded CTG repeat is transcribed into CUG expanded repeat (CUGexp) RNA transcripts, which are retained in the nucleus and aggregate into distinct foci. CUGexp RNA foci sequester Muscleblind-like (MBNL) family splicing factors and alter the activity of other RNA binding proteins, leading to misregulated post-transcriptional gene expression for multiple genes. These CUGexp RNA foci are a characteristic feature of DM1, yet the full composition of CUGexp RNA foci is unknown. We are utilizing a high throughput immunofluorescence-based assay to objectively screen for proteins associated with CUGexp RNA foci. A library of monoclonal antibodies (mAbs), generated against nuclear extracts from DM1 patient immortalized fibroblasts, will be robotically screened in high throughput fluorescence microscopy to identify mAbs which localize in characteristic nuclear foci in DM1 cells but not in control cells. These positive hits will be validated and targets identified by mass spectrometry. A comprehensive understanding of the protein-protein and protein-RNA interactions associated with CUGexp RNA foci will improve therapeutic targeting and may reveal novel mechanisms of pathogenesis in DM1. This project will also provide a framework for future studies of aggregate composition in other disease models.

P-074

3: Disease mechanisms

A mature human skeletal muscle model to detect hypertrophic compounds by high content screening

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Myotonic dystrophy is characterized by multiple symptoms and particularly by a loss of skeletal muscle mass. This decrease of muscle mass causes weakness and frailty, which follows muscle atrophy and decrease of activity. Research on compounds able to renew muscle mass is therefore crucial to compensate tissue wasting. In this context, we have developed a physiological muscle model allowing the detection of compounds inducing atrophy and hypertrophy. When cultured on micropatterns, primary human myoblasts faster differentiate into myotubes displaying a higher level of sarcomere striation and nuclei alignment compared to standard culture conditions. Moreover, the use of micropatterns greatly standardized myotube formation and morphogenesis, enabling robust high throughput and high content screening. Thanks to the development of new image analysis algorithms and the reduced variability of myotube morphology, the achieved cellular model enabled accessing new parameters for myotube characterization upon drug treatment. This model can be used to develop disease models for muscular diseases including myopathies and other rare genetic diseases and neuromuscular disorders. To demonstrate the benefit of this model we tested IGF-1, a known compound inducing hypertrophy, to rescue the effects of muscle atrophy. The results showed increased Z' factors for fusion index and myosin area read-outs. Such model opens up new avenues for screening compounds that either induce hypertrophy or reverse atrophy which in turn will help identifying new compounds for muscle related disease treatment.

P-075

3: Disease mechanisms

Genome editing approaches for creating new mouse models of DM1

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Myotonic dystrophy (DM) is a genetic, multi-systemic disease. Type 1 DM is caused by a CTG repeat expansion in the DMPK gene, which results in a number of downstream pathogenic events, including sequestration of MBNL proteins by RNA, activation of CELF proteins, and translation of potentially toxic peptides. While a number of mouse models for DM1 have been valuable for understanding DM, none exhibit all of the symptoms experienced by DM1 patients, and none contain the CTG repeat expansion within the endogenous DMPK locus. The CRISPR/Cas9 system has been used to efficiently create double strand breaks at specific genomic loci. This has enabled rapid generation of a number of mouse models by embryonic stem cell culture or direct injection into mouse embryos.

Using CRISPR/Cas9, we aim to develop a mouse model in which expanded CTG repeats are located within the DMPK locus, with the goal of effectively modeling all symptoms experienced by DM1 patients. We are developing this model using a two-step process. First, we are humanizing the 3\' end of the DMPK locus, by replacing ~2 kilobases of mouse genomic DNA with human DNA. Humanization of the locus will allow us to subsequently insert expanded repeats from human DNA without requiring mouse homology arms. Donor DNA that contains expanded CTG repeats will be obtained by isolating the locus from DNA of affected individuals or cell lines, using Cas9 and a pair of guide RNAs *in vitro*. To maximize incorporation efficiency of this donor DNA, we have introduced a point mutation to create a new protospacer-adjacent motif in the humanized DMPK sequence, such that donor DNA cannot be cleaved.

This procedure should enable us to create mouse models with differing repeat lengths, and aid in further research and development of therapeutics for DM.

P-076

3: Disease mechanisms

Aberrant methylation of the CTCF binding site is associated with antisense transcription and disease severity in congenital myotonic dystrophy

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Background: Congenital myotonic dystrophy (CDM) is a severe form of DM with a highly expanded CTG repeat surrounded by hypermethylated CpG. CDM shares some features with adult DM, such as misregulated splicing; however, it has certain characteristic phenotypic and genotypic differences, one of the most striking difference being muscle fiber immaturity that contributes to a severe phenotypic manifestation. To elucidate the specific mechanism of this muscle immaturity, we investigated the phenotype–genotype correlation and profiles of splicing, expression, and methylation status in CDM muscles.
Methods: We used muscles from 10 CDM (age less than 18 months) patients and four age-matched controls. We analyzed the size of the expanded repeat by Southern blot and evaluated pathological findings in muscles from CDM. We examined gene expression profiles by using Agilent microarrays and qPCR. We also studied splicing patterns of disease-associated transcripts and antisense transcription at the DMPK locus by RNA-seq and RT-PCR. We analyzed the methylation profiles in the flanking region of the CTG tract by using the Ion Torrent PGM next-generation sequencer.
Results: The size of the expanded repeat in the muscles from CDM varied from 1350 to 2500 triplets. The length of the expanded repeat correlated with the pathological grade of immaturity and percentage of undifferentiated type 2C fibers. In contrast to adult DM, splicing abnormalities of most events were not related to the muscle immaturity. However, a characteristic methylation pattern at the CTCF binding site upstream of the repeat tract was correlated with the repeat size and muscle immaturity in CDM. Hypermethylation of the CTCF binding site suppressed antisense transcription at the repeat tract and reduced production of short CAG repeat RNA.
Discussion: Our results demonstrate a phenotype–genotype correlation and suggest a specific epigenetic disease mechanism in CDM.

P-077

3: Disease mechanisms

Analysis of mRNA decay by MBNL1

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RNA binding protein MBNL1 is known to regulate alte
ative splicing. MBNL1 has been reported to regulate many splicing events so far. In DM patient, dysfunction of MBNL1 by expanded CUG/CCUG repeat RNA causes aberrant alte
ative splicing in many genes.
Recently, several studies reported that MBNL1 binds preferentially 3'UTR region of RNA rather than intron and CDS (coding DNA sequence) of RNA by CLIP-seq analysis. Intron and CDS region of RNA are known to involve in splicing regulation whereas 3'UTR region of RNA is shown to be involved in stability and translation efficiency of RNA. These results suggest that MBNL1 binds 3'UTR of RNA and has another function besides alte
ative splicing regulation. Recent studies indicated that MBNL1 regulates mRNA degradation, localization and alte
ative polyadenylation via binding to 3'UTR of mRNA.
In this study, we paid attention to the regulation of mRNA decay by MBNL1. We investigated how many MBNL1-binding motifs are needed in 3'UTR to cause mRNA decay and which MBNL1 isoform contributes to mRNA decay. We overexpressed luciferase reporter constructs with MBNL1 to HEK 293 cells and conducted luciferase assay. Because MBNL1 binds directly to CCUG repeat RNA, we introduced different numbers of CCUG repeats in 3'UTR region of luciferase constructs. We also used three MBNL1 isoforms, $MBNL1_{40}$, $MBNL1_{40s}$ and $MBNL1_{42}$. $MBNL1_{40}$ is one of the major isoforms of MBNL1 and localizes in both cytosol and nuclei. $MBNL1_{40s}$ predominantly localizes in cytosol whereas $MBNL1_{42}$ predominantly localizes in nuclei.
From our study, all three MBNL1 isoforms showed reduced luciferase activity when reporter contains more than 24 CCUG sequences in 3'UTR. Among three MBNL1 isoforms, $MBNL1_{40s}$ tended to show most strong effect. By contrast, $MBNL1_{42}$ showed most weak effect. This tendency is consistent with strong cytosol localization of $MBNL1_{40s}$ and weak cytosol localization of $MBNL1_{42}$.

P-078

3: Disease mechanisms

Exploring the Functional Conservation of Muscleblind (Mbl) Proteins

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Muscleblind and Muscleblind-like (MBNL) are an evolutionarily conserved family of proteins that regulate alternative splicing and RNA localization. Disruption of MBNL in various species leads to a variety of defects and disease, including myotonic dystrophy (DM). Though a role for MBNL in DM has been firmly established, mechanisms by which MBNL performs its cellular functions remain to be fully elucidated. We approach this problem by exploring functional conservation of a diverse subset of MBNL homologs.

We identified homologs to human MBNL1 in four organisms including a basal metazoan; Trichoplax adhaerens, a primitive chordate; Ciona intestinalis, and the model organisms; Drosophila melanogaster and Caenorhabditis elegans. Multispecies protein alignment showed the zinc finger RNA-binding domains to be the most highly conserved region, suggesting conservation in RNA binding, and potential conservation of splicing functions. For all homologs, minigene splicing assays in HeLa cells demonstrated splicing functions similar to human MBNL1, for both exons repressed and activated by MBNL1. To gain a more comprehensive view of each homolog's splicing activities, we generated RNAseq libraries from MBNL1/2 knockout mouse embryonic fibroblasts stably expressing each homolog. All homologs showed nuclear and cytoplasmic localization, suggesting nuclear and extra-nuclear functions. To examine a role for each homolog in RNA localization, we also generated RNAseq libraries from cytoplasmic, membrane, and insoluble compartments of each cell line.

Our initial results demonstrate conserved splicing and subcellular localization of all MBNL homologs. These studies may provide insights into ancestral functions conserved through human MBNLs, and reveal specific protein domains required for these activities, aiding in our efforts to modulate MBNL activities for purposes of DM therapeutics.

P-079

3: Disease mechanisms

Deregulation of microRNA expression in skeletal muscles of myotonic dystrophy

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Myotonic dystrophy (DM) is the inherited, autosomal dominant, multisystemic disease that is caused by accumulation of toxic RNA containing either CUG or CCUG repeat expansion in the nucleus. The expression of expanded CUG or CCUG repeats results in sequestration of proteins involved in maturation of mRNA precursors. To date, misregulation of alternative splicing and alternative polyadenylation of hundreds of transcripts were described. Nevertheless, it seems that microRNA deregulation may also contribute to skeletal muscle pathology of DM1 and DM2 patients.
We used the Illumina next-generation sequencing platform to identify near a hundred of miRNAs that are deregulated in DM1 skeletal muscles. Among them there are microRNAs implicated in skeletal muscle differentiation, e.g. miR-181, miR-24, miR-27b, miR-125b, miR-30, miR-222. In DM2 the observed changes are not so numerous, however almost half of the miRNAs differentially expressed in DM2 was also deregulated in DM1 muscles. To eliminate biological heterogeneity that influences results of deep sequencing of microRNAs from DM patients, we also investigated disruption of miRNAs expression in DM1 mouse model (*HSA*^{LR}). We have found that ten out of 33 miRNAs deregulated in *HSA*^{LR} were affected also in DM1 patients. Deregulation of selected miRNA expression was confirmed by northern blot and RT-PCR. To check whether miRNAs misregulation is a result of changes in transcription or rather disrupted miRNA biogenesis we performed RT-PCR analysis for several miRNA precursors (pri-miRNA). We have found that whereas in mouse model the miRNA expression alternation arise predominantly from disrupted transcription, deregulation of miRNA in DM muscles must have also other cause. There are numerous mature microRNAs which expression is affected in DM1 skeletal muscles and which pri-miRNA expression level is not changed or even changed in opposite direction.

P-080

3: Disease mechanisms

Assessment of cardiac defects in a DM1 drosophila model and identification of associated candidate genes by a cell-specific approach: TU-tagging

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Myotonic Dystrophy Type 1 is a multisystemic disease, affecting particularly **skeletal muscles** and the **heart**. **Cardiac symptoms** occur in **80%** of DM1 patients, ranging from conduction defects to arrhythmia and are the second cause of death, mainly due to heart block. Moreover, a **positive correlation** between **CTG number repeats** and **cardiac involvement** has been reported [**1**].

To better understand the causes of cardiac symptoms, we took advantage of our DM1 *Drosophila* model [**2**], performing **phenotypic analyses** on **adult heart of DM1 flies** which express: i) 960 interrupted CTG, ii) a RNAi construct for Mbl (MBNL1 orthologue), iii) a gain-of-function for bru3 (CUGBP1 orthologue). These heart analyses showed similar symptoms, as observed in patients, such as **dilated cardiomyopathy or fibrillation**.

To identify new molecular actors responsible for the DM1 associated heart defects, we will perform **cardiac cell-specific transcriptional analyses**, using **TU-TAGGING** technique [**3**]. Disposing of **cardiac-specific coding and non-coding RNAs**, we will use RNA-sequencing to identify deregulated genes, differently spliced transcripts but also modified non-coding RNAs. The identified candidates, ranked depending on their conservation and deregulation level will be validated, paying particular attention to genes/transcripts whose expression is **sensitive** to the **number of CTG repeats**. We also plan to test whether identified candidates are deregulated in DM1 patients displaying cardiac abnormalities.

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P-081

3: Disease mechanisms

Activation of the Innate Immune Response Causes Reduced MBNL1 Expression and Alternative Splicing in Human Lens Cells.

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Recently we have shown that Type 1 Interferon (IFN) plays an important role in regulating gene expression changes observed in lens epithelial cells from DM1 and DM2 cataract patients (Rhodes et al., *Human Molecular Genetics*, 2012). To further investigate the potential role of IFN in DM we have studied alternative splicing in non-DM lens cells exposed to the immunostimulant and synthetic analogue of double-stranded RNA, polyinosinic:polycytidylic acid (pI:C) (Sigma). Lens epithelial cells were transfected with pI:C using Lipofectamine LTX reagent (Invitrogen). Gene expression and alternative splicing changes were measured using Real-Time PCR. Protein analysis was by Western blot and siRNA was used to inhibit expression of target genes. To confirm the effectiveness of pIC at inducing IFN in our cells we used Real-Time PCR to show a large time dependent increase in IFNb expression within the first 4 h after exposure. The increase in IFNb expression was followed by increased expression of the IFN regulated genes (IRGs) TLR3 and STAT1. In cells transfected with pI:C there was a marked decrease in exon 11 inclusion in the insulin receptor transcripts compared to the untreated controls within 8 h. In the same cells we detected a major decrease in the transcripts for both MBNL1 and MBNL2 which was confirmed at the protein level. Knock down of TLR3 or STAT1 transcripts using siRNA inhibited the effect of pIC on IFN and MBNL expression. Interestingly MBNL1 knock down caused an increase in the level of the MBNL2 transcript. In this study we show that the expression of MBNL1 and alternative splicing are regulated by innate immune pathways activated by dsRNA. This is further evidence for the potential role of dsRNA and IFN signalling in the pathogenic mechanism downstream of triplet repeat expression in DM.

P-082

3: Disease mechanisms

Abnormal expression of the axonal guidance cue Ephrin-A5 receptor in Myotonic Dystrophy Type1

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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. Alterations of personality that are associated with DM1 include avoidant personality, obsessive-compulsive, passive-aggressive, and schizotypic traits, whose occurrence is not attributable to the disabling condition of patients. Other studies have found severe impairment in all measures of general intelligence and verbal fluency and particularly frontal, executive, visuospatial, arithmetic, and attention ability deficits. The congenital forms of DM1 have been also associated with mental retardation. Nevertheless, the molecular bases of these neuronal affections are still understood.

Eph receptor tyrosin kinase and theirs corresponding ligands, ephrins, are membrane-anchored proteins that are considered as a crucial system in the development and maturation of the central nervous system. We identified a splice defect affecting the expression of EphA5 receptor both in DM1patient biopsies as well as in neurons derived from human embryonic stem cells carrying more than 1000 CTG. Interestingly, this defect appears to be specifically regulated by CUG-BP2. The potential contribution of these findings will be discussed.

P-083

3: Disease mechanisms

Chronic Endurance Exercise Training in a Mouse Model of Type I Myotonic Dystrophy

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Progressive muscle loss is a prominent feature of myotonic dystrophy that negatively impacts patient's quality of life. The molecular mechanisms of muscle wasting are unknown, and currently no therapies are available to combat muscle loss. Several smaller studies have evaluated the effects of exercise on muscle function in myotonic dystrophy. Results have been varied, and range from no effect to improvement in muscle strength. However, there is insufficient data available to make definitive recommendations to patients, and conce
remains that some forms of exercise could lead to increased muscle damage and muscle loss.
The purpose of this study is to evaluate the effects of chronic endurance exercise in a mouse model of myotonic dystrophy. Body weight, body composition, oxygen consumption, time to exhaustion, muscle force, muscle histology, and mitochondrial number will be monitored before and after 2 months of treadmill training in HSA-LR versus wild type mice using a protocol developed from protocols which have previously been reported to improve muscle function in wild type mice. These results will be compared to age matched sedentary mice of each genotype. Using a mouse model of myotonic dystrophy reduces confounding variables seen in human subjects, such as comorbid conditions and varying repeat lengths.
The results of this study will improve understanding of the impact of chronic exercise on muscle function in myotonic dystrophy.

P-084

3: Disease mechanisms

Differences in activity of three MBNL paralogs

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Muscleblind-like (MBNL) are conserved RNA-binding proteins that regulate cytoplasmic and nuclear RNA metabolism. There are three MBNL paralogs in human and mouse which contain at N-termini two conserved zinc finger tandem domains which allow interaction with specific RNA structures. Additionally, *MBNL* genes contain few partially self-regulated alternative exons that might modulate its cellular localization, affinity to RNA targets, splicing activity and protein-protein interaction capacity. Each MBNL might exist in numerous protein isoforms at different expression level across tissues and developmental stages. The understanding of differences between MBNL1, MBNL2 and MBNL3 and their splicing isoforms might shed more light on their impact on RNA metabolism in normal and pathological stages.
We developed cellular models expressing 12 different MBNL isoforms. Firstly, we compared ability of these MBNL paralogs to interact with transcripts containing expanded CUG repeats in nuclear foci. Using microscopic technics we characterized the impact of MBNL isoforms on foci formation and the mobility of trapped MBNL proteins. The results of FLIM and FRAP experiments suggest that MBNL isoforms have similar impact on foci formation, but their mobility in these structures are significantly different.
We also compared the activity of three MBNL paralogs and their splicing isoforms on alternative splicing activity of several endogenously expressed pre-mRNAs and few exogenous constructs. We noticed that MBNL paralogs regulate the same splicing events but they differ significantly in the strength of splicing activity. We tested whether these differences relied on various affinity to RNA targets or subcellular distribution of MBNL isoforms. Finally, we noticed that the sequences encoded by alternative exons of MBNLs influence on splicing activity but only for some splicing events.

P-085

3: Disease mechanisms

Conditional HSAXLR transgenic mouse model with targeted single-copy integration

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Previously we used a genomic fragment containing the human skeletal actin (HSA) gene to drive expression of RNA with expanded CUG repeats (CUG^{exp}) in skeletal muscle of transgenic mice (Mankodi et al, 2000). However, the resulting human skeletal actin – long repeat (HSALR) transgenic mice had certain limitations for studying DM1. The repeat expansion is relatively short, around 220 repeats, which may lead to incomplete nuclear retention. The transgene integration is multicopy, making it difficult to precisely monitor the repeat size of individual copies. Finally, the expression of CUG^{exp} RNA in muscle fibers is constitutive. In an effort to overcome these limitations we used targeted transgenesis with PhiC31 integrase (Tasic et al, 2011) to generate a new model, called HSAXLR. We generated an HSAXLR minicircle containing uninterrupted $(CTG)_{440}$ in the 3' UTR. In addition, a floxed transcription terminator cassette (TTC) was placed in the 3' UTR, upstream of the expanded repeat, to permit conditional expression of $(CUG)_{440}$. The HSAXLR minicircle and mRNA encoding integrase were microinjected in zygotes having a PhiC31 docking site at *ROSA26*. We obtained founder lines having single-copy genomic integration of *HSA*-$(CTG)_{440}$. Prior to recombination, the HSAXLR mice did not express CUG^{exp} RNA and did not develop nuclear foci or DM1-like phenotypes. After Cre-mediated excision of the TTC, HSAXLR mice developed robust nuclear foci and large accumulation of CUG^{exp} RNA. Homozygous recombined HSAXLR mice displayed myotonia and splicing defects that are characteristic of human DM1. Histologic studies are at an early stage, but younger homozygous HSAXLR mice have central nuclei, ring fibers, and variable atrophy of muscle fibers. HSAXLR mice may prove useful for studying the allelic selectivity of antisense knockdown, the effects of transcription on CTG^{exp} instability in muscle, and post-development effects of expressing CUG^{exp} RNA.

P-086

3: Disease mechanisms

Antisense oligomers as a tool for identification of functional RNA targets for MBNL proteins

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Immunoprecipitation techniques combined with deep sequencing (i. e. CLIP-seq) enables identification of multiple RNA sequences potentially recognized by RNA-binding e.g. Muscleblind-like protein 1 (MBNL1) which is a known regulator of alternative splicing. In these studies we propose an efficient method for verification of CLIP-seq signals both in *in vitro* and *in vivo* tests via inhibition of RNA/protein interaction by chemically modified short antisense oligomers (AOs). *In vitro* studies showed that all tested AOs complementary to functional MBNL1-binding motifs within a fragment of *Atp2a1* pre-mRNA blocked interaction with MBNL1. However, only 2'OMePS but not DNA, LNA and 2'OMe oligomers complementary to these intronic sequences significantly enhances the exclusion of alternative exon 22 of *Atp2a1* from both minigene and endogene. Then, based on CLIP-seq results we selected three potential MBNL-binding regions from intron 6 of *Pphln1*, exon 7 of *Nfix* and exon 10 of *Ldb3* to test activity of AOs. *In vitro* application of several AOs blocking different YGCY motifs allows us to identify one or two MBNL1-binding sites in ~200-nucleotides long *Pphln1, Nfix* and *Ldb3* pre-mRNA fragments. Unexpectedly, in cell models the 2'OMePS version of selected AOs blocked only functional MBNL1-binding motif for endogenous *Pphln1*, but not for exonic sequences of *Ldb3* and *Nfix*, for which opposite direction of splicing changes were observed. However, substitution of MBNL-sensitive elements in intron 22 of *Atp2a1* minigene with studied sequences of *Pphln1*, *Nfix* or *Ldb3* reconstitutes alternative splicing of exon 22 of *Atp2a1*. In these chimeric constructs exon 22 inclusion responds significantly on both MBNL1 overexpression and the presence of gene-specific AOs complementary to MBNL1-binding sites.

P-087

3: Disease mechanisms

RNA-binding proteins, CUGBP1, ZNF9 and DDX5/p68 in Myotonic Dystrophies type 1 and type 2

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Myotonic Dystrophies type 1 and type 2 are complex neuro-muscular diseases caused by CTG and CCTG expansions in the 3' UTR of the DMPK gene and in the intron 1 of ZNF9 gene respectively. The mutant CUG and CCUG repeats aggregate in toxic RNA foci. To improve degradation of these RNAs, we searched for RNA-binding proteins which are reduced during degradation of the mutant RNAs. One of these proteins was purified to homogeneity and shown to be RNA helicase DDX5/p68. We found that the levels of p68 are reduced in muscle biopsies from patients with DM1 and DM2. Correction of p68 in DM1/2 reduces the number of CUG and CCUG foci and degrades these mutant RNAs. The intramuscular injection of p68 in HSA mice causes significant reduction of DM1 muscle histopathology. These data suggest that the maintenance of the physiological levels of p68 in DM1/2 cells might remove toxic RNAs by their degradation which will lead to the reduction of DM pathology.

One of the key RNA-binding proteins, involved in DM, is CUGBP1. We are investigating the role of CUGBP1 in muscle function using CUGBP1 knock out mice. Detailed immuno-histochemical analysis showed that deletion of CUGBP1 alters the contractile apparatus in skeletal muscle.

It has been suggested that the reduction of ZNF9 activity in DM2 might be associated with muscle atrophy and weakness. Therefore, we used ZNF9 KO mouse model to study the role of ZNF9 in muscle. Similar to CUGBP1, complete deletion of ZNF9 affects muscle structure at the level of contractile proteins. Since ZNF9 is reduced, but not completely deleted in some patients with DM2, we have analyzed heterozygous ZNF9 KO mice. This analysis showed that myofibers in old ZNF9+/- KO mice are reduced in size and contain central nuclei. These data suggest that a reduction of ZNF9 without full deletion is sufficient to cause late-onset muscle atrophy.

P-088

3: Disease mechanisms

Evaluating the effects of MBNL1overexpression in a mouse model of RNA toxicity

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Myotonic dystrophy (DM1) is caused by an expanded (CTG) n tract in the 3' UTR of the DM protein kinase (DMPK) gene. The RNA transcripts produced from the expanded allele alter the function of RNA-binding proteins (MBNL1, CUGBP1 etc.). The loss of MBNL1 results in RNA splicing defects that contribute to disease. Overexpression of MBNL1 is proposed to be a therapeutic strategy to treat DM1 patients. The aim of this study is to see if overexpression of MBNL1 rescues the phenotypes in a mouse model of RNA toxicity. We have developed an inducible mouse model of RNA toxicity in which expression of the toxic RNAs result in the RNA foci formation, MBNL1 sequestration, splicing defects, myotonia, and cardiac conduction defects. To test the effects of MBNL1 overexpression, we generated RNA toxicity mice overexpressing MBNL1 in skeletal muscle. These mice were analyzed for muscle functions. We did not observe the rescue of myotonia in the RNA toxicity mice overexpressing MBNL1. We also analyzed skeletal muscle samples of RNA toxicity mice with or without MBNL1. Preliminary data onto RNA foci, splicing defects and muscle functions in these mice will be presented.

P-089

4: Therapeutic development

Oligonucleotides target mutant DMPK transcripts in human DM1 neuronal cells

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Therapeutic strategies for the impaired cognitive function found in DM1 has yet to be sufficiently addressed. Delivery of antisense oligonucleotides (ASOs) to the CNS has recently been clinically validated and thus ASOs is a promising therapeutic approache to impact this aspect of DM1. The objective of this study was to evaluate ASOs in human DM1 neuronal cells, to understand their potential to target the cognitive deficits in DM1 patients. We have derived iPSC lines from skin biopsies obtained from 4 human DM1 patients and 5 normal volunteers. All iPSC lines express the stem cell markers: SSEA-4, NANOG, TRA-1-60, TRA-1-81 and OCT4 and are pluripotent and differentiate into endodermal (positive for AFP, GATA-4), mesodermal (positive for GATA-2, FLK-1, VECAD, PECAM) and ectodermal (positive for PAX-6, bIIITubulin) layers. iPSC lines underwent induced neural differentiation and more than 90% of cells expressed neuronal markers such as TAU, MAP2, and beta III-tubulin whereas few cells were GFAP positive indicating that there are very few astrocytes. Intranuclear foci of DMPK-CUGexp-RNA were detected in DM1 neurons. Based on data previously obtained in skeletal muscle, we evaluated cET gapmer, MOE-gapmer, 3 LNA-gapmer (AO#5, #8 and #14) and Pip6a-PMO-(CAG)25 ASOs. The cET gapmer, MOE gapmer and LNA-gapmer ASOs were transfected using lipofectamin 3000, whereas pip6a-PMO ASO was added to the culture medium in the absence of transfectant. Among these ASOs, the Pip6a-PMO-(CAG)25 and the cET gapmer completely abolished CUGexp-RNA nuclear foci. Muscle blind-like (MBNL) protein 1 and 2 aberrantly spliced genes were completely normalized. Analysis of gene expression and additional mis-spliced RNAs are in progress. This study indicates that Pip6a-PMO-(CAG)25 and cET ASOs target CUGexp-RNA not only in muscle cells but also in neurons and, support the feasibility of this therapeutic strategy to treat brain deficit in DM1 patients.

P-090

4: Therapeutic development

Development of a humanized antibody platform, VAL-1205, capable of enhanced delivery of intracellular-targeted therapeutics

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A common need for a variety of potential protein replacement and oligonucleotide therapeutics is more effective intracellular delivery to the target tissue. If selectively delivered, these biologics have the ability to replace protein function, effect transcription, and disrupt RNA-protein interactions such as those found in the muscle and nerve tissue of myotonic dystrophy patients following accumulation of expanded CUG/CCUG repeats.

Here we describe the development of a clinically compatible humanized antibody, VAL-1205, engineered as an intracellular-delivery platform. Previous research identified that an anti-DNA antibody, 3E10MAb, discovered in a mouse model of systemic lupus erythematosis (SLE), had cell-penetrating properties. Investigators further demonstrated that murine 3E10 could be engineered as a single chain variant (scFv) and recombinantly expressed as a fusion to full-length proteins. These fusions were capable of cell internalization and retention of functional activity.

Robust screening for improved activity, handling, and preferred manufacturing suitability has culminated in the development of the VAL-1205 platform. This humanized MAb can be engineered into FAb or F(ab')2 fragments and expressed in several cell lines alone or as a recombinant fusion. Directed fluorescein- and radio-conjugates have demonstrated live cell uptake and in vivo biodistribution studies have confirmed significant tissue localization in mice, rats, and non-human primates.

Multiple product candidates utilizing VAL-1205 have demonstrated efficacy in cell-free, in vitro, and in vivo models of disease and are in the late stages of preclinical development. In cell models of myotonic dystrophy, both chemical antibody-oligo conjugates and muscleblind-like protein fusions correct splicing. Research is underway to validate the efficacy of such biologics in DM1 animal models and develop a novel therapeutic with enhanced function afforded via the VAL-1205 delivery platform.

P-091

4: Therapeutic development

In vitro and in vivo modulation of alternative splicing by the biguanide metformin

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Major physiological changes are governed by RNA alternative splicing, and its misregulation may lead to specific diseases. By the use of a genome wide approach, we show here that this splicing step is druggable by demonstrating the effects of the biguanide, metformin, on alternative splicing. The mechanism of action involves AMPK activation and the down-regulation of two RNA binding proteins. Impact of metformin treatment was tested on Myotonic dystrophy type I (DM1), chosen as a model of spliceopathy given that metformin is used to treat type 2 diabete in this multisystemic disease. We show that the drug promotes a corrective effect on several splicing defects associated with (DM1) in derivatives of human embryonic stem cell carrying the causal mutation of DM1 and primary myoblasts derived from patients. Biological effects of metformin were shown to be compatible with typical therapeutic dosages in a clinical investigation involving diabetic patients. The drug appears altogether to be a modifier of alternative splicing of a subset of genes and, as a consequence, may have novel therapeutic potentials for many more diseases besides those directly linked to defective alternative splicing.

P-092

4: Therapeutic development

A protocol to exacerbate the DMSXL mouse model skeletal muscle phenotype

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The DMSXL mouse model has been created with a large genomic fragment containing the human *DMPK* gene carrying >1000 CTG. This model shows molecular, physiological and histological defects that recapitulate most of the DM1 phenotype multisystemic manifestations such as brain, heart and skeletal muscle features. Nevertheless, reminiscent to DM1 patients background, the mouse phenotype is variable and sometimes moderate. In particular, reduced myopathic alterations in young animals can limit the assessment of therapeutic interventions. Here, we aimed at worsening the muscular phenotype in 2 month-old DMSXL mice using a forced eccentric exercise protocol to optimize the evaluation of biotherapies. Our results suggest that eccentric exercise can worsen the muscular weakness observed in DMSXL *vs.* WT, with a significant decrease of their specific maximal force (sP0) in gastrocnemius muscle. This acts independently to body weight gain, muscle weight changes, DMPK mRNA nuclear foci or HE staining histological abnormalities suggesting molecular deregulation pathways. Preliminary isoform quantification for candidate genes in WT gastrocnemius revealed that the splicing profile depend on state of development and can be affected for Ldb3 and Mbnl2 mRNA in non exercised DMSXL vs. WT opening to further molecular investigations in exercised DMSXL. We suggest that an eccentric exercise protocol could optimize biotherapy preclinical evaluation in the DMSXL model.

P-093

4: Therapeutic development

Are Zinc Finger Nucleases Suitable Therapeutic Agents Against Myotonic Dystrophy Type 1?

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Myotonic Dystrophy type 1 (DM1), a neuromuscular disorder that remains without a cure, is caused by an expanded CTG repeat in the 3'UTR of *DMPK*. This mutant mRNA interacts with proteins forming aggregates in the nucleus, resulting in the splicing of embryonic mRNA isoforms in adult tissues. To date, treatment attempts have focused on the symptoms of the disease rather than its cause: the expanded CTG repeat. Since the size of the repeat largely determines the age of onset and the severity of the phenotype, it has been proposed that contracting long repeats to a normal length could alleviate the DM1 symptoms. This hypothesis, however, has not been tested perhaps because there is currently no known way of specifically inducing contractions. Here we attempt to resolve this issue. We tested whether zinc finger nucleases (ZFNs) can induce contractions using a GFP-based assay for repeat instability in human cell lines. Contrary to what has been published, we found that transient transfection of ZFNs in this system could induce a 4- to 5-fold increase in both contractions and expansion frequencies. These results from short term treatment with the ZFNs cast doubt on their usefulness as therapeutics. However, previous studies have shown that longer repeats are better targets for these repeats and it is possible that longer term expression may lead to a biased accumulation of contractions. As an alternative, we are exploring the effect of other engineered nucleases in the same assay. Should we be successful in inducing contractions, we will also investigate whether DM1-derived lymphoblastoid cell lines can serve as model systems to study the impact of repeat instability on cellular phenotypes of the disease. Our results will shed light on whether the DM1 symptoms are reversible and whether engineered nucleases directed against TNRs could be used as therapeutic agents.

P-094

4: Therapeutic development

A multidisciplinary hit-to-lead approach with compounds that prevent CUG toxicity.

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The goal of this work is to advance in the development of five synthetic compounds and a hexapeptide identified in previous research projects as potential drug candidates for myotonic dystrophy type 1 (DM1). The DM1 is a dominant genetic disease in which a CUG triplet expansion in *DMPK* transcripts generates CUG hairpins that are toxic to neurons, myocytes and cardiomyocytes, leading to a series of symptoms such as cognitive dysfunction, cardiac arrhythmias and myotonia. There are no effective treatments for DM1 and the most advanced ones, based on antisense oligonucleotides that prevent the folding of ds(CUG), do not biodistribute well. Therefore, there is an unmet medical need to develop therapies for DM1. In collaboration with molecular design chemists and biopharmaceutical companies, we propose: (1) to boost the activity of the initial hits based on information from a computational model of binding to CUG hairpins; (2) to obtain measures of toxicity and biological activity of these derivatives in both cell and *Drosophila* models of the disorder, which are already available, to establish structure-activity relationships; (3) to validate results in murine models. Further details will be presented at the meeting.

P-095

4: Therapeutic development

Antisense oligonucleotide based strategy for targeting mutant Myotonic Dystrophy type 1 transcripts and their use as potential gene therapy tools

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Myotonic dystrophy type 1 (DM1) is a common form of muscular dystrophy. DM1 is an autosomal dominant genetic disorder, with the mutation found only on one of the two alleles. DM1 belongs to the group of defective RNA export diseases, since a major part of the pathogenic mechanism of the disease is the retention of the mutant transcripts in the nucleus. The presence of an expanded CUG trinucleotide repeat sequence in the 3\'-untranslated region (3\'-UTR) of the myotonic dystrophy protein kinase (DMPK) gene causes the attraction of RNA-binding proteins by these transcripts. Due to the occupation of the RNA-binding proteins, there is defective mis-splicing of several cellular transcripts. This is believed to be a major pathogenic mechanism of the disease and any attempt to repair the activities of the RNA-binding proteins or target the mutant transcripts should be beneficial. Antisense oligonucleotides (ASOs) are short strand of deoxyribonucleotide analogues that hybridize with the complementary RNA transcript in a sequence-specific manner. Formation of the ASO with the target transcript creates a heteroduplex which triggers RNase H activity, leading to transcript degradation. Recent studies using ASOs targeting all DM1 transcripts have produced promising results and have evolved to clinical trials. In this project we created a screening platform, based on transcript secondary structure that distinguishes ASOs that preferentially target various areas of the mutant 3' UTR. Furthermore, we designed ASOs of various sizes and chemical modifications in order to efficiently target the CUG repeat region of the mutant DMPK 3' UTR. The potential of these approaches is to have a more efficient and targeted approach for the degradation of the mutant transcripts and the potential therapy of DM1.

P-096

4: Therapeutic development

The treatment of myotonic dystrophy type 1 via the chemical based transfection of 2'OMe oligonucleotides

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Antisense oligonucleotide therapy is one of the most promising strategies for treatment of myotonic dystrophy type 1 (DM1). The delivery of oligonucleotides to their desired target has long been an obstacle in antisense therapy with a large number of delivery reagents or methods having adverse side effects. Promising work published detailing the successful delivery of various chemically modified oligonucleotides (CMOs) naked, via gymnosis, led to us investigating a number of these CMOs targeted at the repeat expansion of DM1. We initially discovered that several CMOs could enter the cell via gymnosis, however could not penetrate the nuclear membrane. As the target mRNA is trapped within the nuclear compartment we investigated several transfection reagents for their ability to deliver 2'OMe oligonucleotides to the nucleus using live cell fluorescent imaging and a modified northern blot based method. It was established that one reagent could successfully deliver 2'OMe oligonucleotides to the cell, with a high abundance of the oligonucleotide residing within the nuclear compartment. Further investigations were carried out to identify the downstream effects of treatment on the toxic repeat expansion. Using a PCR based method we found that the transfection reagent alone could significantly lower the abundance of the DMPK transcript. Further experimentation suggests that the mutant transcript could be selectively degraded at certain concentrations without the presence of oligonucleotide, whilst leaving the wild-type transcript functional. Toxicity studies also found a therapeutic window within which the adverse effects of these reagents were not present, even after prolonged exposure (10 days). The data suggests that care needs to be taken to ensure appropriate controls are examined when using transfection methods as these reagents themselves have the potential to disrupt the target transcript.

P-097

4: Therapeutic development

Blockade of expanded microsatellite repeat transcription by CRISPR/dCas9

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Myotonic dystrophy (DM), a multi-systemic neuromuscular disorder, is a microsatellite repeat expansion disease in which expanded CTG or CCTG repeats are transcribed, leading to a number of downstream pathogenic events. In particular, the RNA repeats sequester the MBNL family of proteins, leading to downstream splicing and mRNA localization changes. It has been previously observed that silencing of the toxic RNA can successfully reverse many of these splicing changes and downstream phenotypic effects. Recently, it has been demonstrated that a deactivated version of the CRISPR/Cas9 protein (dCas9) can block RNA Polymerase II initiation, leading to decreased production of specific transcripts. While these studies have shown that successful blockade requires use of guide RNAs (gRNAs) targeting the transcription start site, we hypothesized that the numerous CRISPR complexes that be targeted to expanded repeats using a single guide RNA may be sufficient to inhibit production of full-length transcripts. In Hela cells transfected with CTG or CCTG repeats, we observed significant rescue of MBNL-dependent splicing changes when introducing dCas9 and $(CAG)_7$ or $(CAGG)_5$ gRNAs, respectively. The extent of rescue increases as the number of repeats increases, and consistent with previous studies, splicing rescue is dependent on the strength of the CRISPR protospacer-adjacent motif. Preliminary results in DM myoblasts suggest a reduction in foci in the presence of dCas9 and $(CAG)_7$ gRNA. Injection and electroporation of plasmids encoding dCas9 and $(CAG)_7$ gRNA into the HSA^{lr} mouse model of DM led to slight rescue of MBNL-dependent splicing events. Further optimization of dCas9/gRNA delivery may improve splicing rescue. Currently, we are conducting assays to determine whether the efficiency of transcriptional blockade depends on the strand that is targeted by the gRNA, and are applying this system to other microsatellite repeat diseases, such as ALS.

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4: Therapeutic development

Genome Modification of Human DM1 iPS Cells leads to elimination of mutant transcripts and reversal of phenotypes

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Myotonic dystrophy type 1 (DM1) is caused by expanded CTG repeats in the 3'-untranslated region (3' UTR) of the *DMPK* gene. The advancement of induced pluripotent stem (iPS) cell technology has introduced new possibilities for developing cell-based therapies and correcting the mutation in DM1 iPS cells would be an important step towards autologous stem cell therapy. The objective of this study is to demonstrate *in vitro* genome editing to prevent production of mutant transcripts and reverse the phenotype of cells and tissues derived from human DM1 iPS cells. Genome editing was performed in human DM1 iPS cells. Integration of an editing cassette, which contained SV40/bGH polyA signals, upstream of DMPK 3'UTR CTG repeats was mediated by TALEN-induced double-strand break (DSB) and homologous recombination (HR). The expression of mutant CUG repeats was monitored by nuclear RNA foci, the molecular hallmark of DM1, using RNA fluorescence in situ hybridization (RNA-FISH) in neural stem cells, cardiomyocytes, embryoid bodies, and teratoma tissues derived from genome-modified DM1 iPS cells. We found genome-modified DM1 iPS cells still maintain pluripotency. The integration of the polyA signals led to elimination of mutant transcripts and complete disappearance of nuclear RNA foci and reversal of aberrant splicing in linear-differentiated neural stem cells, cardiomyocytes, embryoid bodies and teratoma tissue from genome-modified DM1 iPS cells. In conclusion, genome modification by integration of exogenous polyA signals upstream of the DMPK CTG repeat expansion prevents the production of toxic RNA and reversal of phenotypes in DM1 iPS cell and its progeny. Our data provide proof-of-principle evidence that genome modification may be used to generate genetically modified iPS cells as a first step toward autologous cell transfer therapy for DM1.

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4: Therapeutic development

Laminin promotes muscle development/regeneration by regulating pericyte differentiation

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Pericytes are perivascular mesenchymal stem cells. Previous studies show that they can either promote muscle regeneration by myogenesis or compromise muscle regeneration via adipogenesis. What regulates pericyte differentiation and fate determination, however, remains unknown. Here, by using mice with laminin deficiency specifically in pericytes, we report that laminin negatively regulates pericyte proliferation, inhibits pericyte adipogenesis, but is indispensable for pericyte myogenesis. Furthermore, we demonstrate that exogenous laminin alone can reverse the muscle dystrophic phenotype in these mice at the molecular, structural, and functional levels. In addition, we also performed RNAseq analysis using freshly isolated wild-type and laminin-deficient pericytes, and identified gpihbp1 as a key signaling molecule in pericyte differentiation/fate determination. Altogether, our data indicate a crucial role of laminin in pericyte stemness, identify an effective treatment for muscular dystrophy, and provide an innovative target for future drug development.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Physical disability in DM1 patients : A 5-years longitudinal retrospective study

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The aim of this study was to evaluate the ability to walk and run in 58 DM1 patients in a 5-year retrospective follow-up study, using a modified functional disability scale (FDS): 0 = normal; 1 = normal but with cramps and fatigability; 2 = unable to run; 3 = walking difficult but still possible to walk unaided; 4 = walking with a cane; 5 = walking with a walking frame and 6 = wheelchair bound. The mean age of patients was 47.4 ± 13 year, the mean number of CTG in the blood: 767 ± 503, the mean duration: 22.3 ± 14 years. Patients were evaluated every year during a 5 yrs period by the same investigator (JP). The handicap score at baseline was independent of the gender (p=0.367), the form of the disease (p=0.269) but was highly correlated with the age of the patients at first evaluation (p<0.0001), the duration of the disease (p<0.0001) and the number of CTG repeats in the blood (p=0.0015). The rate of increase in physical disability was 9% / yr and the FD score become significant at year 2 (p=0.009). The rate of increase in disability was independent of the gender (p=0.982), the form of the disease, the age of patients at first evaluation, the duration of the disease and the number of CTG repeats in the blood (p=0.78). Physical disability at 5-yr correlated with that at baseline, while higher physical disability at 5-yrs was associated with less strength at baseline determined by the MIRS (p= <0.0001) and with older age (p=0.037). There was no significant association with the gender (p=0.396), the form of the disease (p=0.069), the duration (p=0.502) and the number of CTG in the blood (p=0.315). A MIRS value at 4 at baseline predicted 83% of the patients in whom there was a change from ambulatory to non-ambulatory stage. This study demonstrates that the FDS is a useful method to quantify physical disability in follow-up studies, in DM1 patients.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

EMD, MMG and force combined approach: does it relate with muscular involvement in DM1 patients?

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Electromechanical delay (EMD) during muscular contraction and relaxation can give new insights on both the electrochemical and the mechanical components that characterize DM1 and could represent an important support for clinical evaluation. EMD, mechanomyogram (MMG), and force signals were recorded in eleven DM1 male patients *(age:38±15 yrs; body mass:75±14 kg; stature:1.78±0.07 m; onset:28±11 yrs; CTG expansion: 428±305; mean±sd)* and eleven age and body-matched healthy controls from the *tibialis anterior (TA)* and *vastus lateralis* (VL) muscles during maximum torque output under voluntary and electrically-evoked isometric contractions; furthermore we investigated the correlation between EMD parameters and most widely-used scores for clinical evaluation in DM1 patients such as MRC, MIRS and Rivermead score. Torque output resulted to be significantly lower in DM1 than in HC *(-39%, -29%, -52%, and -48% in TA and VL muscles, under electrically-evoked and voluntary contractions, respectively; p<0.05)*. $Delay^{TOT}$ and $R\text{-}Delay^{TOT}$ components were significantly longer in DM1 compared to HC in both muscles and under both contraction regimes. During relaxation the mechanical components were mainly affected compared to HC meanwhile electrochemical and mechanical components were similarly impaired in DM1 during the contraction phase.The reliability of the measurements was very high in both DM1 and HC *(ICC from 0.81 to 0.99)*.The statistically significant differences between patients with DM1 and HC in $Delay^{TOT}$ and $R\text{-}Delay^{TOT}$ components suggest that an EMG, MMG and force combined approach may be used as a valid tool to assess neuromuscular involvement and impairment degree. This approach could be used also to follow the efficacy of pharmacological or non-pharmacological interventions. Our purpose is to further extend this study by evaluating the correlation between *Tibialis anterioris* EMD and the atrophy degree shown in needle biopsies from the same muscle.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Italian national registry for myotonic dystrophies: the IRCCS policlinico san donato experience

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After a year-long experience in the management of DM1 and DM2 patients' enrolment at coordinator center IRCCS Policlinico San Donato, we made some considerations about the fully functionality and user-friendliness of the Registry site, the rightness and usefulness of the collected data and patients' compliance. Our population counts 182 patients, 87.2% DM1 *(mean age 39.8±13.3 yrs)* and 12.8% DM2 *(mean age 60.8±8.6 yrs).* In DM1 group the percentage of males is 62,3%, maternal transmission results to be 50.8%, with three congenital forms; 16.7% patients achieved an university degree, 52.5% has an occupation and 41% refers work life to be affected by the disease. In DM2 group the percentage of males is 44.4%, transmission is maternal in all cases; none of them achieved an university degree, 44% has a job and 33.3% refers that the disease had an impact on work life. 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 of disease (22.2%). No patients had cardiac involvement as initial symptom. At an evaluation of 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. Data collected so far are in line with the literature. The creation of a reserved website, accessible online, has made easier the compilation of the forms by the patients and it's been a precious instrument to record information for the clinicians. The national Registry is a unique database to have information on natural history of the disease and to define an homogeneous cohort of patients for clinical trial.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Review of the phenotypic classification in myotonic dystrophy type 1 – An international initiative from the OMMYD group

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OMMYD is a group of international experts that aims to select and validate common outcome measures in myotonic dystrophy type 1 (DM1). During the last conference in 2013, the lack of uniformity in documenting phenotypes across studies have been discussed and pointed out as an important issue among the members of the Cognitive Special Interest Group. It was proposed to conduct a Delphi consultation within the OMMYD community in order to review the name and criteria of each phenotype and then to propose a new phenotypic classification for DM1. This classification will be for research purpose only and will aim to standardize patient classification among DM1 studies to facilitate comparisons between them. A scoping review of the literature was made to document classification criteria used across studies. We consulted OMMYD members about all criteria that have been found to obtain their opinion and comments. Based on this process, a new phenotypic classification will be developed and presented at the OMMYD-3 in June 2015 (before IDMC-10) where participants will be invited to comment and suggest modifications to improve it. This presentation aims to expose the context that lead to this project, the result of the scoping review, what has emerged from the Delphi process and from the OMMDY meeting. The final version of the new phenotypic classification will also be presented.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Myotonic Dystrophy Family Registry: a patient self-report registry collecting demographic, symptomatic and disease burden measures.

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Background: The Myotonic Dystrophy Family Registry (MDFR) is a patient report registry that was established in March of 2013 to collect the core DM data set identified at the 2009 meeting in Naarden on Patient Registries. The registry now contains completed survey data from 1113 individuals. The self-report registry allows patients to enter and update their own data directly, allowing for fast accrual, but with the caveat that the data has not been reviewed by a clinician.
Objectives: Describe the demographics of registrants as well as selected self-report data about symptoms and burden of disease.
Methods: Participants are recruited through the MDF Web site, email communication from the MDF, referrals from health professionals and word-of-mouth. For this report, the frequency of selected data elements reported to the Registry as of March 1, 2015 was compiled.
Results: Diagnoses represented in the registry include 546 people with adult-onset DM1, 190 with adult-onset DM2 and 187 with congenital myotonic dystrophy and 190 with other diagnoses. The mean age of participant is 41.8 years and the number of male registrants is slightly more than half. The aggregate data show that 69% of respondents have difficulty walking, 75% have myotonia, 73% have daytime sleepiness and 77% report some degree of fatigue. Data on additional symptoms as well as burden of disease parameters are also collected.
Conclusion: Fatigue, myotonia, daytime sleepiness and difficulty walking were the most frequently reported symptoms by registrants. The prevalence of these symptoms in the DMFR is slightly lower than those reported by Heatwole and colleagues using the MDHI QOL instrument[1] , but the difference may be explained by variations in the questions and the fact that the MDHI study was limited to adults with DM1.
[1]Heatwole C, et al. Myotonic Dystrophy Health Index: initial evaluation of a disease-specific outcome measure. Muscle Nerve. 2014 June;49(6): 906-14.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

18-months follow-up in DM1 patients by means of various outcome measures

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease affecting the muscular function for which new treatments are being developed. To assess their effect, new outcome measures can be used to better characterize the neuromuscular function longitudinally and prospectively.
The main aim of our study consists in a natural history of DM1 patients over 3 years. Assessment included 6-minute walk distance (6MWD), muscle strength of hand grip, ankle flexion and extension, neck flexion, finger extension, adductor digiti minimi abduction, shoulder abduction, knee extension, big toe extension, reaction time, myotonia, nine hole peg test (9HPT), MoviPlate and balance measured in different testing conditions on a force plate. Baseline values of DM1 patients were compared to age- and sex-matched healthy controls. The changes of these variables were tested after 18 months.
24 DM1 patients and 24 healthy controls aged between 24 and 50 years were recruited in this study. Results show that the patients were significantly less capable for all variables compared to controls. After 18 months, patient deterioration was moderate. The reaction time was significantly worsened (p=0.006). Strength varied generally in small proportions (less than 14% at the highest) and presented a high between-individual variability. Statistical significance was however reached for ankle extension (p=0.039), ankle flexion (p=0.042), finger extension (p=0.012), shoulder abduction (p=0.045) and knee extension (p=0.002). All the other variables were not significantly changed.
This study confirms that DM1 is a very slowly progressive disease with large between-individual variations. Most of the endpoints used were only able to detect moderate changes over 18 months.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Tibialis Anterior muscle needle biopsy and sensitive biomolecular methods: a useful tool in Myotonic Dystrophy type 1

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Myotonic Dystrophy type 1 (DM1) is the most frequent of the adult-onset muscular dystrophies. DM1 results from expansion of CTG repeat in 3'UTR of DM protein kinase (DMPK) gene that gives rise to the accumulation of toxic RNA in nuclear foci inducing changes of alternative splicing of several genes. The therapeutic strategy for DM1 is targeting toxic RNA through systemic administration of Antisense Oligonucleotides (ASOs). Therefore it is necessary to develop new methods for monitoring the success of a clinical trial.
A Tibialis Anterior (TA) muscle needle biopsy is a minimally-invasive procedure that allows to obtain small tissues samples and that can be repeated on the same patient and on the same muscle over the time. Here we show that the very small muscle samples obtained by TA biopsies are enough to investigate several DM1 muscular biomarkers. Two pieces of TA muscle taken from 6 DM1 patients and 5 healthy subjects were immediately frozen in liquid nitrogen. Serial sections obtained from one piece (40 mg) were used for routine histological stainings and for fast and slow myosin immunostaining and then analysed for histopathological evaluation of skeletal muscle. FISH in combination with MBNL1-immunofluorescence was performed in order to evaluate the number of ribonuclear inclusions and MBNL1 foci. The other muscle piece (20 mg) was used for RNA extraction to assess: i) alternative splicing of 8 genes involved in the multi-systemic phenotype of DM1 (INSR, CAMK2G, CACNA1S, CLCN1, NFIX, PDLIM3, LDB3 and cTNT) by reverse transcriptase (RT)-PCR analysis and ii) DMPK expression levels by real time PCR analysis.
Our work underlines the feasibility and the test-retest reliability of TA muscle needle biopsy in combination with sensitive biomolecular methods as a useful tool for investigating the patho-molecular mechanisms in DM1 and also for monitoring the efficacy of a therapeutic intervention in a clinical trial.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Splicing Changes in the Blood and Muscle in Congenital Myotonic Dystrophy

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BACKGROUND: Congenital myotonic dystrophy (CDM) is the severe, infantile-onset form of myotonic dystrophy (DM1). Splicing changes leading to disruptions in gene regulation have been demonstrated in DM1, but it is unknown if patients with CDM experience the same changes in splicing. Furthermore, the splicing changes associated with DM1 have not been examined in the peripheral blood. Here we evaluate RNA splicing changes in the peripheral blood and skeletal muscle in CDM patients.
DESIGN/METHODS: Peripheral blood lymphocytes were isolated from two patients with CDM (ages 3 and 8) and two healthy controls (ages 5 and 10) using PAXgene tubes. Skeletal muscle was collected from the 3 year-old CDM patient and compared with skeletal muscle obtained from a Duchenne muscular dystrophy patient. cDNA libraries were prepared with an RNase H protocol (blood) or ribosomal rRNA depletion (muscle). RNA sequencing was performed using the Illumina HiSeq 2500 sequencer. Reads were aligned using TopHat. Splicing differences were analyzed with Miso and SJ count software.
RESULTS: Expression of *MBNL1* and *DMPK* are prerequisites for splicing changes in DM1. Both are expressed in the peripheral blood of CDM and control patients. Comparing CDM to control blood samples we identified 80 transcripts with differential inclusion of exons. These transcripts include *NDRG1*, whose splicing has been shown to be *MBNL1* dependent. Other *MBNL1* dependent transcripts were also identified. In skeletal muscle, known splicing changes associated with DM1, such as in *MBNL1* (exon 5), were identified.
CONCLUSIONS: This work demonstrates the presence of known myotonic dystrophy associated RNA splicing changes in the skeletal muscle of a CDM patient. Furthermore, there is a suggestion of splicing changes in the peripheral blood that may serve as potential biomarkers. Additional samples obtained longitudinally will be required to confirm these findings.

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5: Biomarkers / outcome measures / registrie / therapeutic assays

Comorbid Diagnostic Categories in Myotonic Dystrophy within an Administrative Database

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Comorbid illnesses among myotonic dystrophy (DM) patients are known to vary considerably from patient to patient. This spectrum has not been studied in large, nationally representative samples of DM patients, and frequencies as compared to the general population are unknown. The objective of this study was to estimate the frequency of comorbid diagnoses among DM patients in comparison to non-DM patients within Clinformatics™ Datamart Multiplan from Optum™, (Eden Prairie) and to determine commonly used medications. DM patients (N = 3,204) were identified as those with ≥2 medical claims for DM (ICD-9 Code: 359.2, 359.21, or 359.22) between 1 January 2004 to 8 May 2012. Two non-DM patients were matched to every DM case based on several criteria. Diagnoses were grouped using the Clinical Classification System. At least 40% of DM patients had at least one claim among the 13 most numerous comorbid diagnoses, most frequently other connective tissue disease (71.4%), diseases of the heart (69.8%), respiratory infections (68.5%), other lower respiratory disease (62.2%), and eye disorders (61.9%). DM patients were more likely to experience all of these diagnoses than non-DM patients: other connective tissue disease OR: 5.43, 95% CI: 4.85- 6.07; diseases of the heart OR: 7.21, 95% CI: 6.42-8.09; respiratory infections OR: 2.11, 95% CI: 1.91-2.33; other lower respiratory disease OR: 4.21, 95% CI: 3.80-4.66; and eye disorders OR: 3.12, 95% CI: 2.83-3.45. Anti-infectives (71.6%), analgesic narcotics (50.8%), hormones and corticoids (37.5%), antiarthritics (30.8%), and antidepressants (29.4%) were the most frequently used medications. The odds of the most frequent comorbid diagnoses among DM patients are at least double those of non-DM patients. This study demonstrates the significant breadth of comorbid diagnoses among the DM population, more frequent occurrences than those observed in the general population, and DM's multi-systemic nature.
All authors are employed by Biogen.

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Elevated muscle-specific miRNAs in serum of myotonic dystrophy patients relate to muscle disease progress

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The discovery of reliable and sensitive blood biomarkers is useful for the diagnosis, monitoring and potential future therapy of diseases. Recently, microRNAs (miRNAs) have been identified in blood circulation and might have the potential to be used as biomarkers for several diseases and clinical conditions. Myotonic Dystrophy type 1 (DM1) is the most common form of adult-onset muscular dystrophy primarily characterized by muscle myotonia, weakness and atrophy. Previous studies have shown an association between miRNAs and DM1 in muscle tissue and, recently, in plasma. The aim of this study was to detect and assess muscle-specific miRNAs as potential biomarkers of DM1 muscle wasting, an important parameter in the disease's natural history. Disease stable or progressive DM1 patients with muscle weakness and wasting were recruited and enrolled in the study. RNA isolated from participants' serum was used to assess miRNA levels. Results suggest that the levels of muscle-specific miRNAs are correlated with the progression of muscle wasting and weakness observed in the DM1 patients. Specifically, miR-1, miR-133a, miR133b and miR-206 serum levels were found elevated in DM1 patients with progressive muscle wasting compared to disease stable DM1 patients. Based on these results, we propose that muscle-specific miRNAs might be useful molecular biomarkers for monitoring the progress of muscle atrophy in DM1 patients.

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Skeletal muscle and circulating micro RNA in myotonic dystrophy type 1.

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miRNA are short noncoding RNAs that, after maturation, are loaded onto Ago2 protein to form the RISC effector complex that destabilizes target mRNAs and represses their translation. We and others established that both accumulation and localization of specific miRNAs are altered in DM1 patients. However, the functional implications of these miRNA aberrations are still largely unknown. In order to discover the miRNAs that are functionally deregulated in DM1, we analyzed the RNAs associated to Ago2 immunoprecipitates of muscle biopsies derived from DM1 patients and matched controls. Using RNA-Sequencing, we identified a combination of miRNA/target mRNA couples that interact on the RISC complex and are enriched in DM1 patients. Specifically, we found miRNAs known to be involved in muscle damage and disease, as well as a number of deregulated mRNAs that could be targets thereof. A number of potential miRNA/mRNA pairs that could be involved in the dystrophic phenotype have been highlighted.
Additionally, miRNAs are also present in bodily fluids, representing attractive potential biomarkers. In a recent work, we found a DM1-signature of 9 miRNAs deregulated in a small group of DM1 patients, finding a direct correlation with the stage of disease and an inverse correlation with muscle strength. To validate these findings, an independent group of 100 DM1 and 100 controls were recruited and a subset of DM1-signature miRNAs, defined by random forest statistical analysis, was assayed. We found that miR-133a,-27b and -454 levels discriminated DM1 from controls significantly and ROC curves displayed an area under the curve of 0.8, 0.6 and 0.7, respectively.
In conclusion, both skeletal muscle and plasma microRNAs are altered in DM1. Understanding miRNA regulation and role in DM1 is instrumental for unveiling DM1 molecular pathogenetic mechanisms and the development of new therapies.

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Regional body composition and clinical outcome measures in patients with myotonic dystrophy type 1 (DM1)

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POSTER PRESENTATION PLEASE

Background: The development and validation of primary and secondary outcome measures in DM1 is needed to inform clinical trial design of therapeutic drugs

Objective: A pilot study to compare regional and whole body composition of DM1 patients with matched healthy controls determined by dual-energy X-ray absorptiometry (DEXA). Establish any relationships between body composition and clinical outcome measures in DM1 patients.

Methods: 38 DM1 patients were recruited for DEXA scan and 31 matched to age-, gender-, weight- and height able bodied control volunteers were retrospectively screened from a large database of DEXA scans. The DM1 patients had the following clinical outcome measures: bilateral grip dynamometry, bilateral ankle dorsiflexion (AD) strength (hand-held myometry) and 6 minute walk test.

Results: The case-control analysis (n=31) demonstrates significant reduction of fat free mass index (FFMI (kg/m^2)) in the legs (left leg $p=0.004$, right leg $p=0.017$) and trunk ($p<0.0001$) and increased fat mass index (FMI (kg/m^2)) localised to the trunk ($p<0.0001$). In DM1 patients (n=38) average modal lymphocyte CTG repeat size was 857 (range 50-2947) and was correlated with whole body FMI ($p=0.01$, $r^2=0.17$) but not whole body FFMI ($p=0.32$). Linear regression of relevant regional FFMI and FMI composition correlated with 6MWT ($p=0.037$, $r^2=0.11$; $p=0.0001$, $r^2=0.33$), bilateral grip strength (FFMI and FMI $p<0.05$) and right AD ($p<0.05$).

Conclusion: Regional composition parameters determined by DEXA are correlated with relevant clinical outcome measures in DM1 and could therefore provide a useful secondary outcome measurement of disease progression in addition to muscle strength and timed functional tasks in clinical trials.

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Rationale for establishing a Myotonic Dystrophy Multidisciplinary Clinic

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Introduction: Myotonic Dystrophy (DM) is a multisystem disorder caused by expansion of CTG (type-1) or CCTG (type-2) repeats. Involvement of cardiovascular, pulmonary, skeletal muscles and endocrine system increases the risk of morbidity and mortality in these patients. This led to the establishment of Houston Methodist Myotonic Dystrophy Multidisciplinary Clinic (DMMC) in 2006. With the emergence of antisense oligonucleotides, DM has the potential to become a treatable genetic disorder and thus an established DMMC is critical for the care and research of this patient population.

Objective: To assess the utility of DMMC and its benefit upon a cohort of 37 patients over 8-years.

Methods: We retrospectively reviewed clinical notes of 37 DMMC patients seen during 2006-2014. The DMMC involves two neuromuscular specialists, pulmonologist, cardiologist, physical and occupational therapist, respiratory therapist, speech therapist, nutritionist, social worker, MDA representative and DME vendors. All team members evaluate patients during a single visit. We have 4 clinics each year. Patients get EKG every 6-months and annual ECHO, thyroid and diabetes screen.

Results: There is 35% increase in total number of patients over 8-years. We currently follow nearly 100 patients every year. Among our cohort of 37 patients (22-female; 15-male, average age 48.8 years and 94% have type-1) 64% have cardiac conduction defects and arrhythmias on EKG. 16% required pacemaker placement. 84% had a sleep study with sleep disordered breathing and use BiPAP/AVAPS. 16% developed diabetes but none had thyroid dysfunction. There was 1 death.

Conclusion: DMMC is beneficial for DM patients in the evaluation and management of systemic disease manifestations. The DMMC provides a great opportunity to the clinicians and researchers to study the natural course of the disease, develop measurable objective outcome measures and participate in clinical drug trials.

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What is our current knowledge of CTG repeat length and myotonic dystrophy type 1

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The translation of CTG repeat length into clinical disease aspects of the most frequent muscular disease in adulthood remains enigmatic. Presently, CTG repeat length is still discussed as a major predictor of disease onset and severity, esp. in congenital DM1. We re-addressed this aspect to our German DM registry, evaluating the correspondence between datas and long-term course of our clinical reevaluation. Here we were confronted, that this above mentioned CTG repeat length thesis is hampered by a considerably non-correspondence to observed clinical courses of muscular and non-muscular aspects in our DM1 patients. Therefore, data of more than 290 patients were reviewed in more depth. About 150 DM1 patients were fully analyzable regarding disease-specific data like CTG repeat length, age of onset, duration of illness, and MIRS on the one hand, and BMI, as a new indirect aspect for physical activity on the other hand. Interrelating those different variables was part of our researches. Main results show that correlation was found –as expected- between age of onset and repeat length ($p<0,01$) and, in addition, also MIRS and BMI correlated significantly ($p<0,01$). Interestingly, no correlation was found between length of repeats and MIRS ($p=0,21$). These results and further analytics lead to the assumption that age of onset is mainly determined by length of repeat, but the clinical course of this chronic-progressive disorder might be more influenced by life style factors like physical activity (BMI) than previously assumed. With further investigations more detailed knowledge and experience on this multisystemic disease will help to translate scientific acquaintance to a better management of this multifaceted disorder. Furthermore, this will help to improve the patients´ competence to cope with their daily life and increase their quality of life. Finally, we might depict additional avenue for a better adherence of DM1 patients to future clinical trials.

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A questionable survey for patient registry via internet in patients with myotonic dystrophy type 1

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Backgound
Patient registries have developed as helpful resources for clinical trials worldwide, especially in rare diseases. In Japan, Remudy is established as the national patient registry system for dystrophinopathy etc., which is planned to convert a registering method from documents to the internet. A characteristic feature of Remudy is patients register their own information. However, it could be difficult that patients register themselves via internet according to disorders with central nervous system involvements or care environmental problems. The aim of the study was to clear internet environment and possibility of operation on the web by a proxy in patients with mytonic dystrophy type 1 (DM1) as such problematic disorders representative.
Method
Thirty four patients with DM1 were participated. A questionable survey for patient registry was performed by means of hearing or interviewing.
Results
Seventy six percent of DM1 patients wished to register, and 24% responded “not sure”. Twelve percent of patients were possible to register via internet by themselves, and 18% answered “family can do on the web”. Fifty percent of patients could register by themselves using documents processing system. Forty six percent of patients replied to register if a proxy could operate an internet tool instead of them. Fifty nine percent of patients approved the presence of a proxy for operation on the web, but 21% did not accept. Regarding approval of a proxy, an attending doctor or hospital personnel of ambulatory was most common, and then a care manager or a legal guardian followed. On the other hand, answers of “anyone” or the anxiety about abuse of personal information were observed. Sixty eight percent of patients replied that neither they nor family can use the interet, or “not sure”.
Conclusion
A lot of patinets with DM1 have not been wired to the internet. For a proxy in web-operation, more than half of DM1 patients agreed but some took an opposite standpoint.

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The Japanese registry for myotonic dystrophy: collaboration between a national center, a national hospital network, and an academic institute

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Patient registries provide epidemiological data and facilitate the recruitment of patients eligible for clinical trials. In Japan, patient registries of muscular diseases, including dystrophinopathy and GNE myopathy, have been organized and operated by the National Center for Neurology and Psychiatry (NCNP) (Remudy, www.remudy.jp) and are internationally harmonized with the TREAT-NMD global registry.

The development of a national registry for myotonic dystrophy (DM) was initiated in 2012, and after successive meetings, a framework of the registry was proposed. The Japanese DM registry is funded by a national collaboration between the NCNP, Osaka University, and hospitals of the National Hospital Organization (NHO) specialized in neuromuscular diseases for facilitating clinical research and reducing the administrative work of a centralized office. The collected data are in accordance with the core dataset of the international DM registry proposed at the TREAT-NMD/Marigold International Workshop. Although this is a patient-reported registry, genetic diagnosis is a prerequisite for registration, and clinical information should be verified by physicians.

The registry was launched in October 2014, and it has drawn nationwide attention. Within 4 months, more than 100 patients registered, and this number is steadily increasing. All the registrants were DM1 and 14 % were congenital DM. Mean age was 41 years. Half the patients were ambulant, a quarter required assistance, and a quarter were non-ambulant.

A registry that collects and renews accurate clinical data nationwide is a powerful tool to accelerate development of new drug and clinical research for standards of care and outcome measure. Further, it can help the communication between researchers and patients by providing information about clinical trials, research and health care. It should also provide crucial data for the policy decision for this rare condition.

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Increase of cardiac troponin T (cTnT) serum levels in patients affected by Myotonic Dystrophy type 1 and type 2

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Myotonic dystrophy type 1 (DM1) and 2 (DM2) are autosomal dominant multisystemic disorders characterized by skeletal muscle and cardiac involvement. Recently, an elevation of circulating cardiac troponin T (cTnT) in patients with neuromuscular diseases without myocardial injury was reported. The aim of this study was to determine the clinical and biological significance of elevated cTnT in DM patients.
60 DM patients (46 DM1 and 14 DM2) were enrolled. Patients underwent cardiac assessment (ECG, 24h ECG-Holter and echocardiography) and routine blood tests (serum cTnT, cTnI and other cardiac biomarkers). cTnT protein expression was analyzed by western blot (WB) on skeletal muscle biopsies using the antibodies from hs-cTnT assay. Laboratory data were compared with healthy subjects.
53 (88.3%) DM patients showed elevated serum levels of cTnT not accompanied by an increase of cTnI values. Median concentrations of cTnT and cTnI were 28.5 pg/mL [IQR 18.75-41.25] and 4 pg/mL [IQR 1.9-7.5] respectively. Levels of cTnI in DM patients were above the 99th percentile (26.2 pg/mL), but 7 (11.7%) of these patients presented cTnT between the 75th and 99th percentile of references (5.7-14 pg/mL). The differences regarding cTnI between DM and cardiac patients, and cTnT between DM and healthy group, were statistically significant (p=0.002, p Cardiac evaluation was available in 43 DM patients; abnormal ECG was recorded in 15 (34.9%) patients with PR≥200ms and 22 (51.2%) with prolonged QRSD≥100ms. ECG-Holter did not identify any significant cardiac breaks. Low ejection fraction was present in 2 patients. WB revealed a positive immunoreaction by one antibody in DM and healthy skeletal muscle.
No correlation was found between increased levels of cTnT and cardiac manifestations. Serum increases of cTnT seem to be heart-related. Further validation of the current results and definition of appropriate cut-off values for cTnT for referral to cardiac investigations are required.

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Myotonic Dystrophy and the Canadian Neuromuscular Disease Registry: The Experience to Date

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INTRODUCTION: Patient registries in myotonic dystrophy (DM) represent a critical opportunity to compare national cohorts of patients internationally; to further elucidate disease characteristics; and to facilitate national and international research in the disease. Barriers to performing multicenter DM research exist in Canada that may be overcome by a comprehensive and collaborative registry.
METHODS: The Canadian Neuromuscular Disease Registry (CNDR) features multi-modal enrollment of eligible patients via participating neuromuscular clinics across Canada and "self-registration" through the CNDR National Office. The registry collects prospective medical data on patients with DM including all data elements required by the TREAT-NMD mandatory dataset. Data are collected at a minimum interval of once per twelve months through chart abstraction by trained personnel following routine clinical visits. The registry collects identified data allowing for the notification of subjects eligible for research studies and de-identified data are made available to researchers and other groups following submission of a data request to the CNDR National Office.
RESULTS: At the end of 2014 the CNDR was enrolling DM patients at 16 pediatric and adult specialty neuromuscular care clinics in British Columbia, Alberta, Ontario, Quebec, and Nova Scotia. The CNDR launched in June of 2011 and as of December 2014 had recruited 2,300 patients including 251 with myotonic dystrophy (3.5% of registry overall). Of these 251 participants, 85 have longitudinal data over the past 3 years and 242 are living. 172 have Type 1 (68%), 37 have Type 2 (15%), 42 (17%) have Congenital DM and the remaining do not have available genetics.
CONCLUSION: The CNDR represents the first national cohort of Canadian DM patients and an important step in facilitating new research for Canadians.

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Czech National Registry of Myotonic Disorders

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The patient registries belong to the core activities which can help us in planning of the effective health care, assessing standards of diagnosis and care, and answer the questions concerning on epidemiologic data.
The Czech National Registry of Myotonic Disorders was established in 2011 under the supervision of Czech Neuromuscular Society. The technology, the data collection, storage and backup and their analyses are provided by the Institute of Biostatistics and Analyses, Masaryk University, Brno. On-line data collection is based on a TRIALDB system developed on Yale University. For each patient is generated a unique ID; all data transfer is encrypted and the system is designed to prevent their unauthorized use during data transfer. Laws and regulations in Czech Republic require having an informed consent from all patients whose data are used in the registry. All claims for personal data protection were met. Data are stored on the central server in Oracle 9i database.
Up to January 2015 422 patients form 8 centres has been included. The majority (84%) of all records are from centres in Prague and Brno. The average annual recruitment during total 3.5 years period are 121 patients. The mean follow-up time in the registry is 16 months.
The majority consists from patients with DM2 (n=207, 49.1%) and DM1 including congenital form (n=157, 37.2%). Non dystrophic myotonias (chloride and natrium channelopathies) are represented with 26 persons (6.1%). The rest are asymptomatic mutation carriers and files with poor defined or missing data (32 items).
Among patients with DM (1 and 2) there are 219 females (63%) and 129 males (37%). Mean age in the time of the registry entering is 45 years, approximately 10 years after disease manifestation which was in patient with MD1 25 (10- 54) years and in persons with MD2 40 (17-62) years. Nearly all patients with MD 1 and MD2 are ambulatory (assisted or unassisted). Only 4 patients are wheelchair bound.

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Multiplex Alternative Splice Sequencing (MAS-Seq) for Analysis of Splicing Biomarkers of Myotonic Dystrophy Type 1 (DM1)

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Alternative splice events may serve as biomarkers of disease severity and therapeutic response in DM1 (Nakamori, 2013). We performed studies to validate splicing biomarkers and optimize assay precision and throughput. Candidate splice events were discovered through splicing-sensitive microarrays (Nakamori, 2013) and RNA-seq (courtesy of E. Wang, C. Burge, A. Struck, and A. Berglund, unpublished), with a focus on alternative cassette exons. 22 events were selected based on effect size (difference from healthy), suspected connection to DM1 biology, and size of the alternative exon (small exons preferred). We employed next generation sequencing on the MiSeq platform to analyze multiplex RT-PCR reactions generated from tibialis anterior muscle biopsy samples. We designed primer sets spanning each splice event and then used Nextera indexing (Illumina) to insert barcodes. A single MAS-seq run examined 22 splice events from up to 12 individuals. In each of 20 sequencing runs to date, at least 99% of reads passing quality controls were informative about one of the 22 events. Around 1 million reads were mapped per subject per run, producing an average ~45,000 reads per splice event per subject, thus generating 95% confidence intervals less than ±0.5% for “percent spliced in” (PSI). Across 13 samples analyzed on the same and different days, the within- and between-run variance was less than 2% for all splice events. For external validation against “gold standard” we compared MAS-seq to RNA-seq data (n = 25 samples). MAS-seq showed excellent agreement with RNA-seq, except for 3 events having the largest alternative exons. Finally, we examined test-retest reliability using 32 pairs of DM1 biopsy samples that were taken 2 to 3 months apart. 18 of 22 splice events showed “excellent” test-retest reliability (ICC>0.8). We conclude that MAS-seq analysis of splicing biomarkers may provide a precise and reliable indicator of therapeutic response in DM1.

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New ligation-based multiplex method for analysis of alternatively spliced changes in Myotonic Dystrophy

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Pathomechanism of myotonic dystrophy type 1 (DM1) and type 2 (DM2) is associated with nuclear accumulation of expanded CUG or CCUG repeat transcripts resulting in sequestration of proteins which regulate RNA metabolism. Among the best described molecular phenotypes of DM are alternative splicing abnormalities, which are the useful biomarkers of DM. Traditional RT-PCR assays used to determine splicing of individual exons are time consuming and obtained results are relatively hard to compare between laboratories. Therefore, we designed much faster and standardized Multiplex Ligation-dependent Probe Amplification (MLPA) method. Our new MLPA assay is a PCR-based method dedicated to analyze simultaneously several splicing events with use of one universal primer pair. We optimized this method and designed an assay for evaluation of ten splicing events differentially expressed in skeletal muscles of DM patients. Our assay includes also probes for six transcripts which are abnormally expressed in DM1. The expression changes of selected biomarkers correlate with severity of muscle weakness. We used our assay to compare alternative splicing pattern in several muscle samples from DM1, DM2 and non-DM patients. It turned out, that both sensitivity and reproducibility of MPLA assay is very high.

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UK Myotonic Dystrophy Patient Registry.

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The UK Myotonic Dystrophy Patient Registry (www.dm-registry.org/uk) was established in May 2012 with support from Muscular Dystrophy UK and the Myotonic Dystrophy Support Group, assisted by TREAT-NMD. The Registry is coordinated from the John Walton Muscular Dystrophy Research Centre (Newcastle) and collects clinical and genetic information about both DM1 and myotonic dystrophy type 2 (DM2) through a online portal. The data collected includes all items agreed at the 2009 TREAT-NMD and Marigold Foundation ENMC workshop.
Of the 410 DM1 patients enrolled in the first two years reporting 326 (79.5 %) report adult onset DM1 with 33 (8%) reporting childhood onset with symptoms beginning between the ages of 3 and 15, and 51 (12.4%) reporting infantile onset (symptoms at the age of 3 or younger). An even distribution is seen between genders (Female: 214, Male: 196) and a broad range of ages is present from 1 to 81 years old (mean 43.41 +/- 16.91), with the largest proportion, 62.7% between 30 and 59 years old**.**
The two most commonly reported symptoms in the Registry are fatigue and myotonia, reported to some severity by 323 and 302 patients respectively. They both occur across all ages and are present in the congenital, childhood and adult onset forms of the condition. Dysphagia occurs mostly in patients also reporting myotonia and a statistically significant association can be seen between the two with more severe myotonic occurring more often in those with dysphagia ($p = < 0.0001$).
The UK Myotonic Dystrophy Patient registry is an example of a novel patient driven registry. Its success can be measured by its continuous growth and utilisation. The registry has successfully assisted the recruitment and planning of a number of commercial and academic studies, including EU funded project OPTIMISTIC (www.optimistic-dm.eu). In addition to this purpose the registry also provides interesting and important data characterising the DM1 community in the United Kingdom.

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3: Disease mechanisms

Abnormal T Cell Development in a Myotonic Dystrophy Mouse Model

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Recognition of foreign antigens and tolerance to self is the hallmark of a healthy, functional immune system. T cells, a critical component of the adaptive immune system, begin their education of self and non-self proteins within the thymus where highly regulated events direct their maturation and development. Transcriptional regulation has been shown to play a critical role in thymocyte development, however co-/post-transcriptional modifications are also important regulatory mechanisms for T cell development that are less clearly understood. Here, we show the loss of Mbnl1, a developmentally regulated RNA processing factor, leads to dysregulation of thymocyte development in a myotonic dystrophy (DM) mouse model. Peripheral blood characterizations of *Mbnl1*$^{-/-}$ mice demonstrate a decrease in total circulating T cells consistent with previous literature reports in DM patients. *Mbnl1*$^{-/-}$ mice develop an enlarged thymus attributed to accumulation of $CD4^{+}CD8^{+}$ double positive thymocytes. *Mbnl1*$^{-/-}$ thymocytes exhibit impaired apoptotic pathways and proliferation defects *in vitro*. Thymic transcriptome comparisons between *Mbnl1*$^{-/-}$ and wild type mice display 474 perturbed splicing events in > 300 unique genes, including genes involved in T cell receptor assembly and signaling. Furthermore, 688 differentially expressed genes ($p_{adj} < 0.01$) are enriched for immune processes, such as lymphocyte activation and stimulus response. Since Mbnl1 regulates alternative splicing during the postnatal period, this study suggests fetal to adult RNA processing switches are necessary for normal thymocyte development. Decreased Mbnl1 function may compromise immune system health in DM patients.

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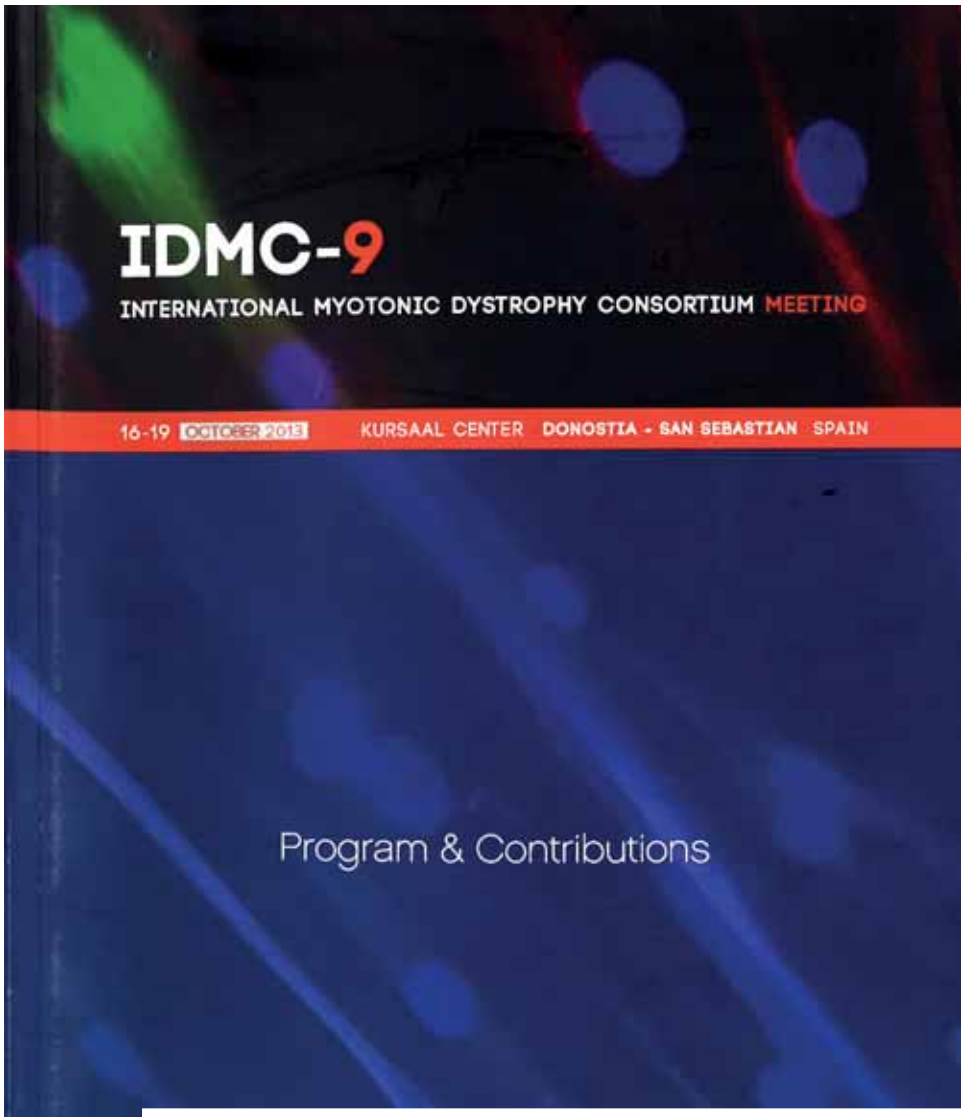
IDMC-10

IDMC-10



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INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING
8 – 12 JUNE 2015 | CAMPUS DES CORDELIERS | PARIS | FRANCE

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IDMC-10
INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING
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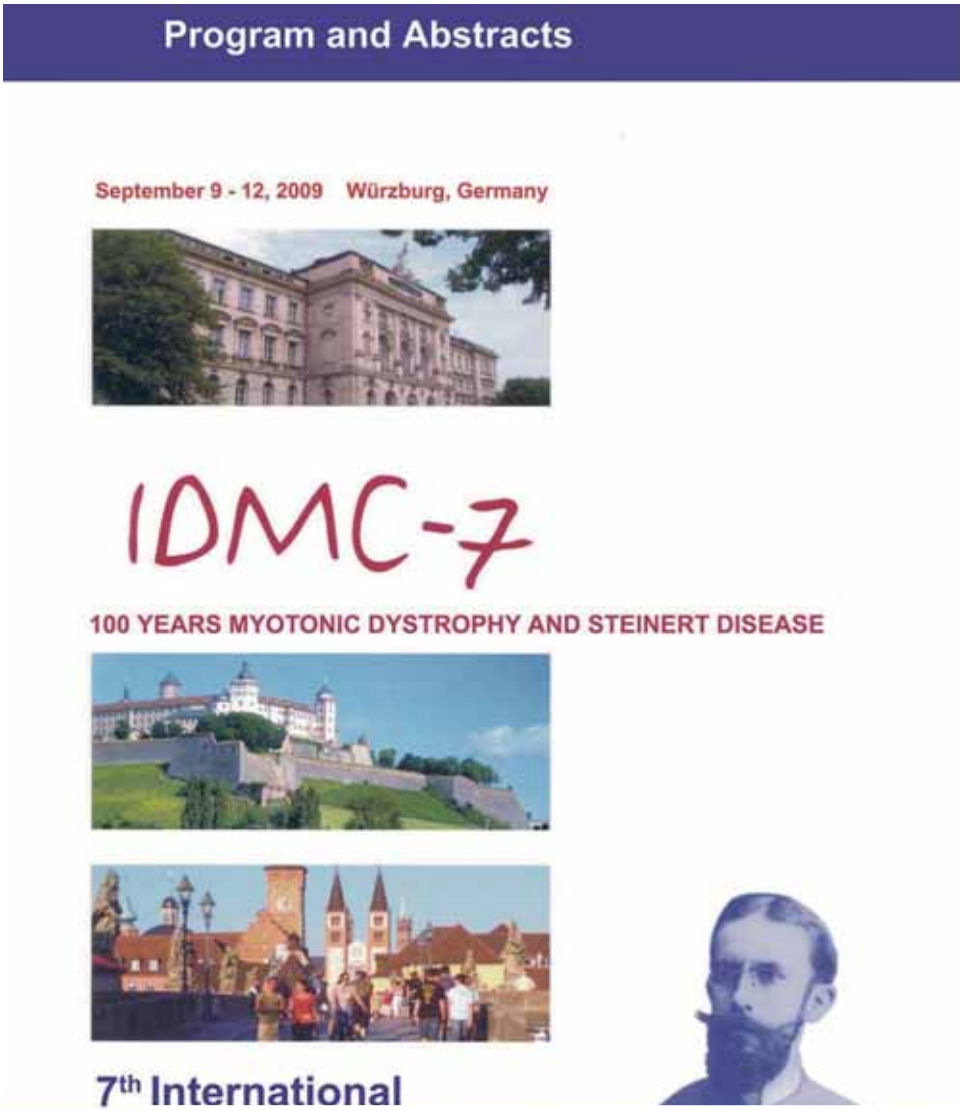
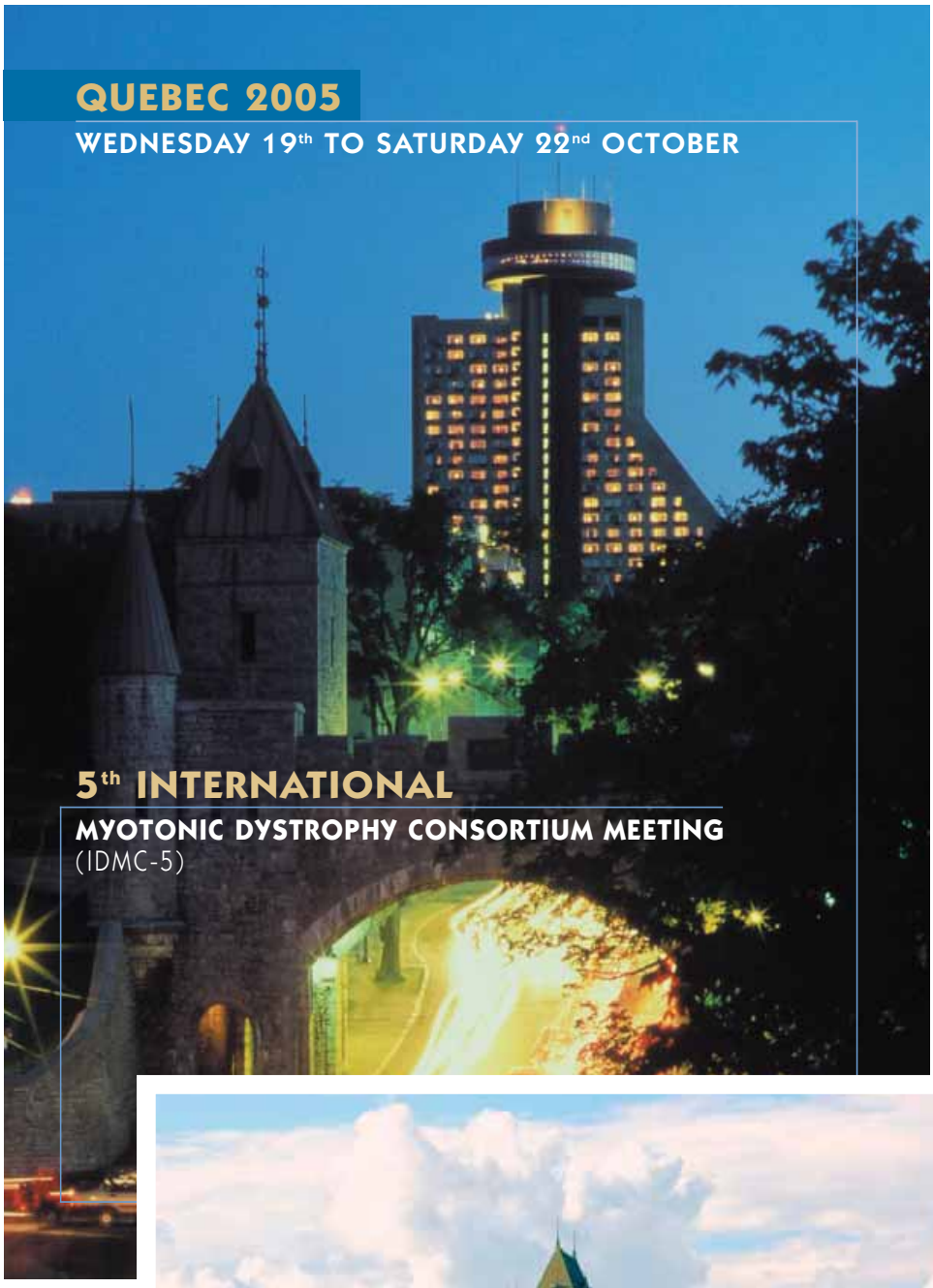

Program and Abstracts
September 9 - 12, 2009 Würzburg, Germany
IDMC-7
100 YEARS MYOTONIC DYSTROPHY AND STEINERT DISEASE
7th International


IDMC-6
6th International
Myotonic Dystrophy
Consortium Meeting
September, 12-15, 2007
University of Milan
Milan – Italy
Milano
Comune



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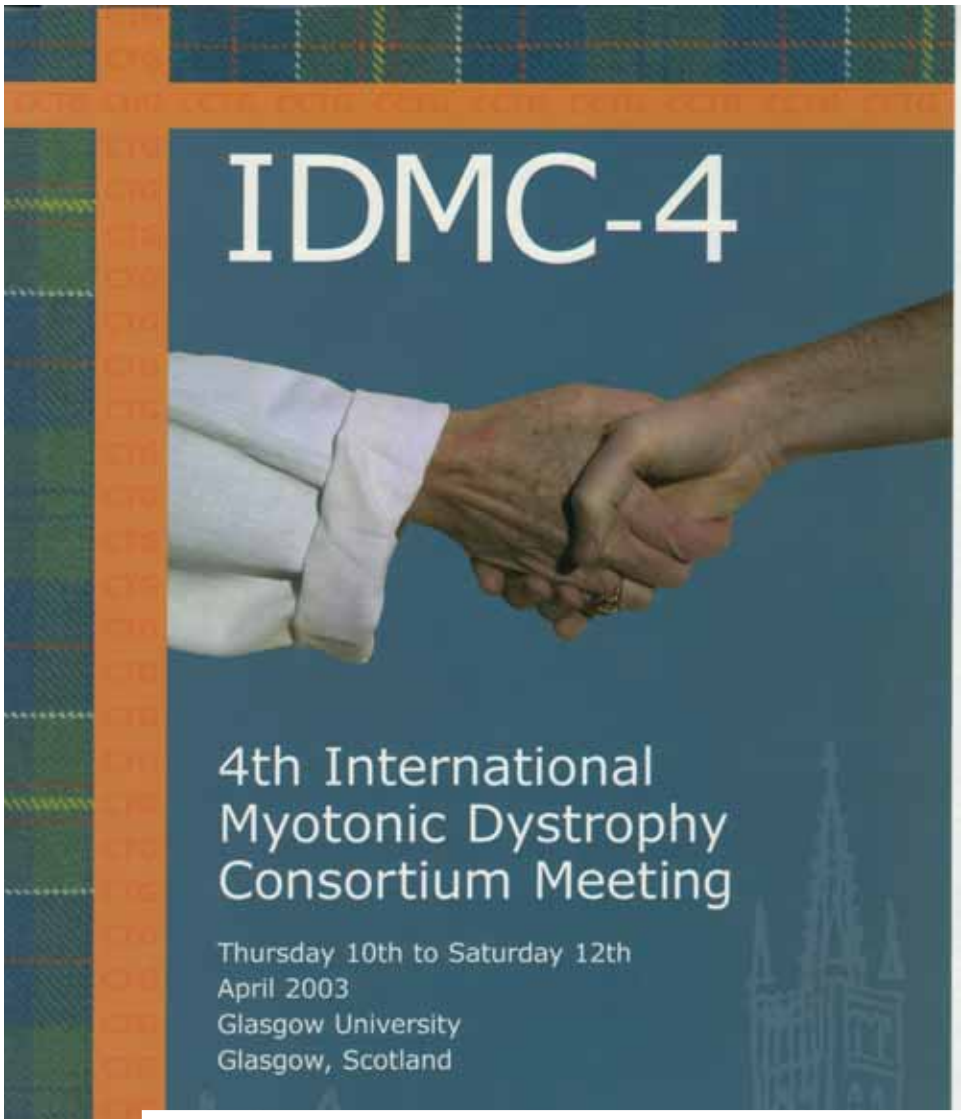
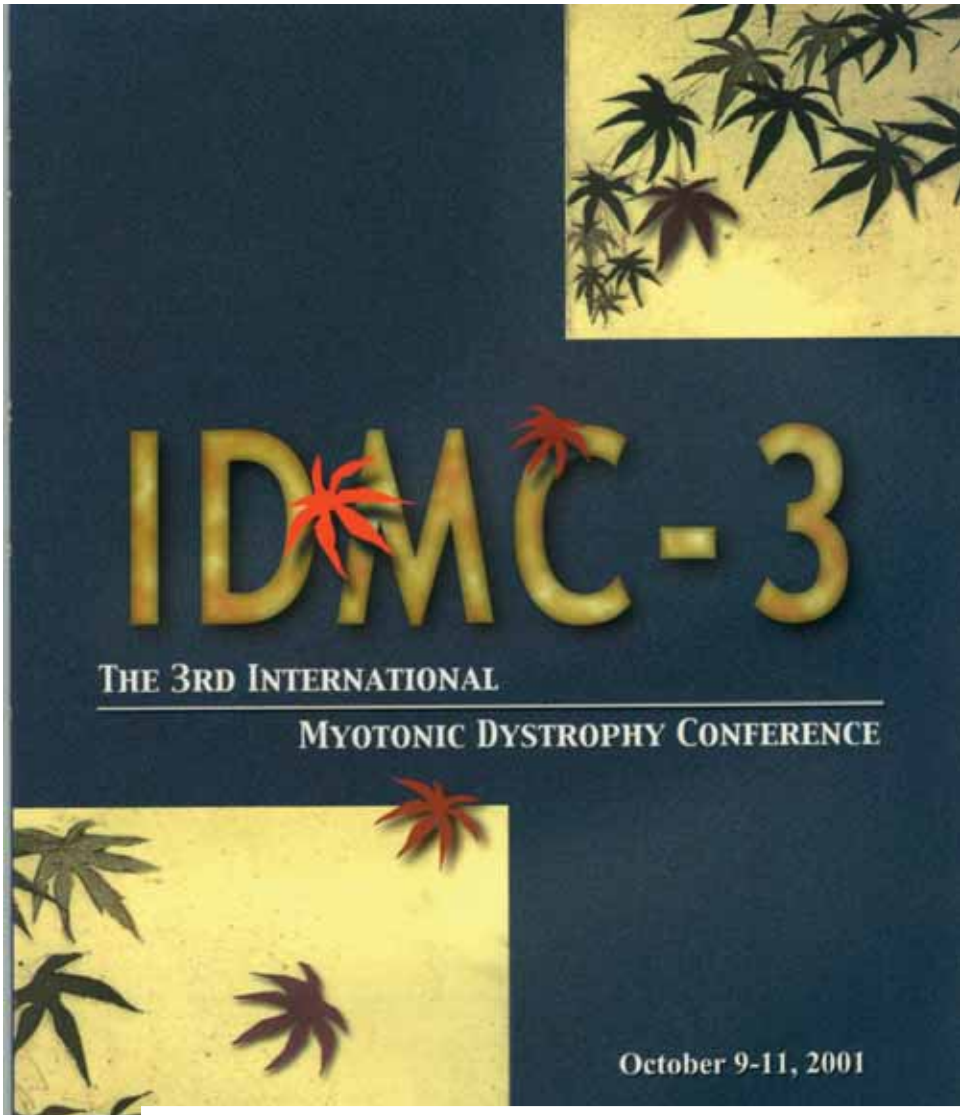

IDMC-4
4th International
Myotonic Dystrophy
Consortium Meeting
Thursday 10th to Saturday 12th
April 2003
Glasgow University
Glasgow, Scotland


IDMC-4
4th International
Myotonic Dystrophy
Consortium Meeting



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INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM MEETING
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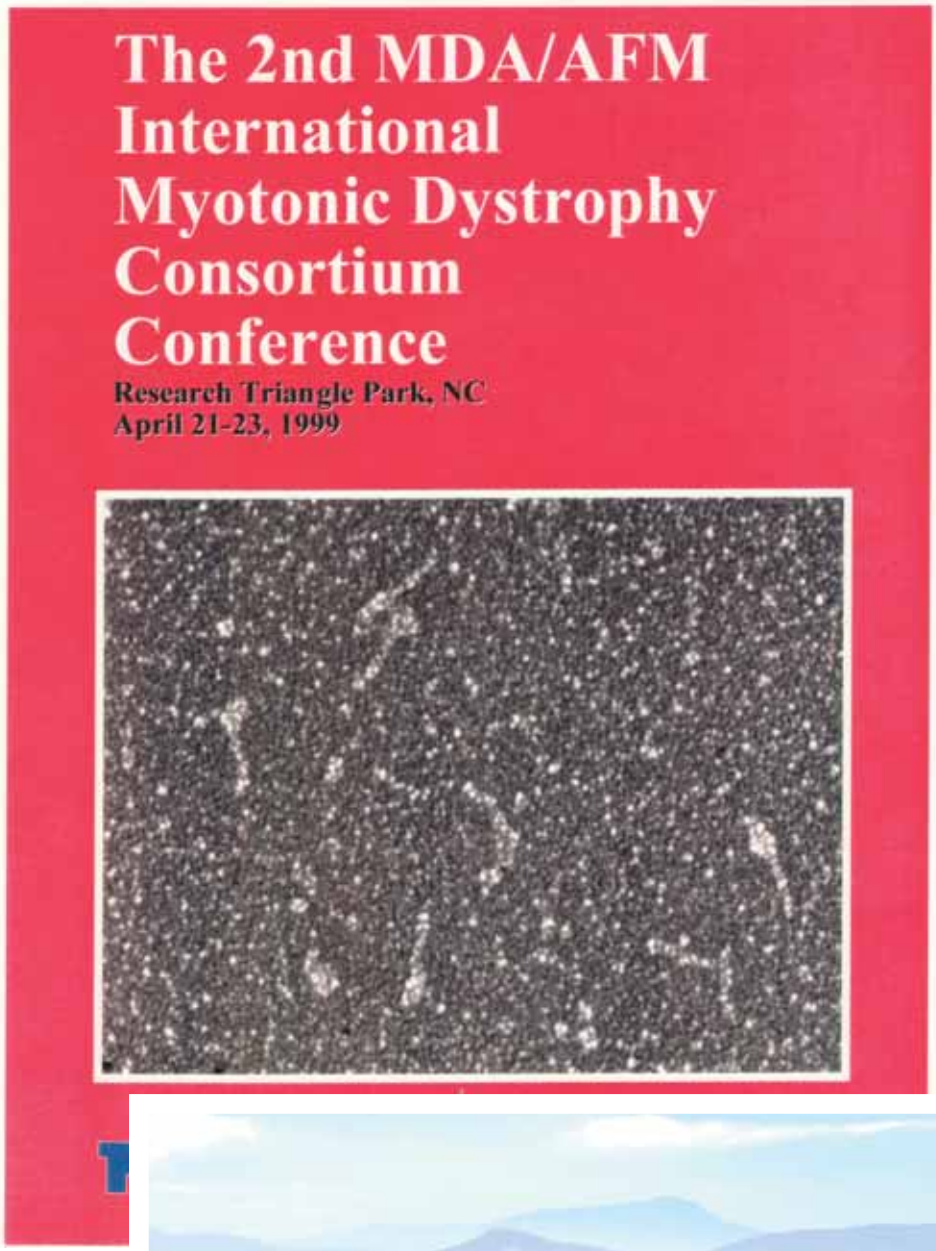
The 2nd MDA/AFM
International
Myotonic Dystrophy
Consortium
Conference
Research Triangle Park, NC
April 21-23, 1999

A glimpse of previous IDMC Meetings

July, Tuesday 1st, 1997
AFTERNOON

Scientific Conclusions; Need for Present and Future Collaborations
Chairperson: R. Korneluk

14.00 **R. Korneluk, K. Johnson, H. Epstein, C. Junien, B. Wieringa Discussion**

14.20 **J. Puymirat** - Data Bank

DIAGNOSIS AND TREATMENT

1- Treatment
Chairpersons: R.T. Moxley / T. Ashizawa

14.30 **R.T. Moxley / T. Ashizawa** - Review

14.40 **Investigator's Presentations**

R.T. Moxley, M. Rogers N. Ohsawa - A possible new approach to treatment in myotonic dystrophy : description of preliminary findings in a double blind randomized trial of troglitazone

15.00 **Round Table Discussion: Treatment prospects for triplet-associated diseases:**

R.T. Moxley, M. Rogers, T. Ashizawa, D. Duboc, B. Eymard, H. Epstein - Research-deduced treatment strategies, antisense, antioxidants, free radical scavengers, trophic factors, anabolic steroids, etc.

2- Diagnosis, Genetic Counselling and Psychosocial Problems
Chairperson: M. Baiget

15.30 **Investigator's Presentations**

G. Novelli - Prenatal prediction using long PCR

M. Baiget - Needs for Diagnosis Guidelines for DM

15.50 **C. Junien/ T. Ashizawa: Concluding remarks and Next Meeting**

	B. Eymard	- A new case of paternally inherited congenital myotonic dystrophy
	M. Giacanelli	- Magnetic resonance imaging (MRI) and genetic correlation in DM patients
	D. Duboc	- Correlation between decreased myocardial glucose phosphorylation and the DNA mutation size in myotonic dystrophy
	M.A. Cordoliani	- Overexpression of the tau 55 isoform in the brain of patients with myotonic dystrophy
12.20	**Discussion**	

6 – PROMM
Chairpersons : F. Lehmann-Horn / M.C Krahe

12.35	**F. Lehmann-Horn / M.C Krahe**	- Review
12.45	**Investigator's Presentations**	
12.50	**M.C. Koch**	- PROMM- the extended German experience
12.50	**Discussion**	

July, Tuesday 1st, 1997
MORNING

DISEASE MECHANISMS (CONTINUATION)

3- DMAHP / 59 (N9)
Chairpersons: K. Johnson / D. Brook

8.30	**K. Johnson / D. Brook**	- Review
8.40	**Investigator's Presentations**	
	K. Johnson	- Identification of an expression pattern for murine DMAHP
	S. Harris	- Identification of the DNA binding target of DMAHP
	C. Thornton	- DMAHP expression in human muscle
	M. Gennareli	- Reduced expression of DMAHP in DM brain
	S. Tapscott	- Transcription of DMAHP in normal and expanded alleles
	V. Funanage	- CTG repeat expansion does not interfere with expression of either DMAHP or 59 mRNA
	B. Wieringa	- The role of DMR-N9
9.30	**Discussion**	

4 – Transgenic animal models
Chairpersons : D. Housman / B. Wieringa

10.10	**D. Housman / B. Wieringa**	- Review
	Investigator's Presentations	
	P. Groenen	- Toward an animal model for DM
	S. Reddy	- Animal models for myotonic dystrophy
	D. Monckton	- Mouse models of DM
	G. Gourdon	- Transgenic models to study repeat amplification consequences
	M. Narang	- Transgenic Mouse Models of Myotonic Dystrophy
	J. Puymirat	- Transplantation of myotonic dystrophy myoblasts as a potential muscular model for myotonic dystrophy
11.10	**Discussion**	

5 – Clinical studies of the Disease Mechanisms
Chairperson : C. Thornton

11.30	**C. Thornton**	- Review
11.40	**Investigator's Presentations**	
	G. Butler-Browne	- An introduction to the development of skeletal muscle
	J.P. Barbet	- Immaturity of skeletal muscle in congenital myotonic dystrophy

	C. Milcarek	- Evidence for an RNA disorder
	C. Thornton	- Long CUG repeat tracks in RNA form structures that interact with dsRNA binding domain of PKR
16.05	**Discussion**	

2- DMPK Protein
Chairpersons: H. Epstein / B. Wieringa

16.45	**H. Epstein / B. Wieringa**	- Review
16.55	**Investigator's Presentations**	
	J. Puymirat	- Characterisation of a 54-kDA human protein kinase recognized by an antiserum raised against the myotonin kinase
	H. Epstein	- The DM protein kinase : Characterization and use of antibodies in evaluating DM in human muscle and lens
	H. Epstein	- Studies of DM kinase action in cultured lens and muscle cells
	S. Salvatori	- Myotonic dystrophy protein kinase is localized to the terminal cisternae of the sarcoplasmic reticulum
	J.R. Moorman	- Phosphorylation of the Na channel inactivation gate by DM kinase
	P. Groenen	- DMPK and Ca2+ homeostasis
	F. Lehmann-Horn	- Electrophysiology of myotonic dystrophy
17.40	**Discussion**	
19.30	**Dinner and Visit of the Medieval Louvre**	

June, Monday 30th, 1997
AFTERNOON

4. Population Genetics
Chairpersons: R. Chakraborty / K. Johnson

13.30 **R. Chakraborty / K. Johnson** - Review

13.40 **Investigator's Presentations**

- **A. Goldman** - Myotonic dystrophy haplotype analysis in African populations
- **R. Krahe** - Origin and evolution of the CTG repeat expansion associated with DM in primates
- **T. Miki** - Population genetics of CTG repeat
- **M.R. Passos-Bueno** - Segregation distortion of the CTG repeat at the myotonic dystrophy locus
- **R. Chakraborty** - Global haplotype diversity of normal CTG polymorphism at the DM locus: Implication for evolution and maintenance of myotonic dystrophy

14.15 **Discussion**

DISEASE MECHANISMS

1- DMPK DNA/RNA
Chairpersons : B. Wieringa / R. Korneluk

14.30 **Investigator's Presentations**

14.40
- **S. Tapscott** - Repeat expansion and local chromatin structure
- **V. Funanage** - Effect of CTG repeat expansion on chromatin structure and processing of DMPK mRNA in hybrid cell lines derived from DM and CDM patient
- **P. Steinbach** - The DMPK gene of severely affected patients is hypermethylated in a CpG island proximal to the expanded CTG repeat
- **R. Korneluk** - Transcription of the myotonic dystrophy kinase (DMPK) gene is controlled by a housekeeping type upstream promoter and a muscle specific enhancer located in the first intron
- **D. Brook** - Analysis of DMPK, DMAHP and 59 expression in DM cell lines
- **A.S. Lia** - Expression of DMPK and DMAHP during development
- **B. Davis/R. Singer** - Expansion of a CTG trinucleotide repeat in the 3'UTR of DMPK transcripts results in nuclear retention of transcripts
- **M.S. Swanson** - Role of triplet repeat RNA-binding proteins in myotonic dystrophy
- **L. Timchenko** - Altered phosphorylation and intracellular distribution of (CUG)n triplet repeat RNA binding protein in myotonic dystrophy and myotonin protein kinase knock out mice
- **R. Sinden** - Transcription by RNA polymerase II through triplet repeat-containing DNA from human myotonic dystrophy and fragile X locus

IDMC-1

June, Monday 30th, 1997 MORNING		
8.45	**C. Junien, T. Ashizawa, M.C. Lagrange Welcoming Introduction**	
9.05	**A. Roses**	Clinical Overview
9.15	**B. Wieringa**	Key Questions

INSTABILITY MECHANISMS		
	1- DNA Structure, Chromatin Structure, DNA Repair Chairpersons: R. Wells / R. Sinden	
9.25	**R. Wells / R. Sinden**	- Review
9.35	**Investigator's Presentations**	
	R. Wells	- DNA structure and molecular mechanisms of instability
	R. Sinden	- Unusual flexible helix structure of CTG repeat
	R. Sinden	- Role of mismatch repair genes in CTG stability: in vivo and in vitro studies
	J. Griffith	- Chromatin structure of expanded triplet DNAs
10.05	**Discussion**	
	2- Instability in Humans Chairpersons: T. Ashizawa / C. Junien	
10.40	**T. Ashizawa / C. Junien**	- Review
10.50	**Investigator's Presentations**	
	L. Martorell	- Somatic instability of (CTG)n repeat during human fetal development
	M. Anvret	- Variation of CTG repeat number of the DMPK gene in muscle tissue
	T. Ashizawa	- Instability of DM CTG repeats in cultured human cell lines
	T. Ashizawa	- Postzygotic instability of the CTG repeat in congenital myotonic dystrophy
	D. Monckton	- Male germline transmission of normal allele
11.25	**Discussion**	
	3- Instability in Animal Models Chairpersons: D. Monckton / R. Korneluck	
11.40	**D. Monckton / R. Korneluck**	- Review
11.50	**Investigator's Presentations**	
	D. Monckton	- Hypermutable myotonic dystrophy CTG repeats in transgenic mice
	G. Gourdon	- Intergenerational somatic and germinal CTG repeat instability in mice
	M. Narang	- Transgenic Mouse Models of Myotonic Dystrophy

1ère conférence internationale AFM/MDA du consortium sur la dystrophie myotonique de Steinert

AFM/MDA first international myotonic dystrophy consortium conference

PARIS
INSTITUT DE MYOLOGIE - AFM
30 juin - 1er juillet 1997
June 30 - July 1st 1997

The Denver Fund For Health & Medical Research

AFM
Association Française contre les Myopathies

MDA