Program and Abstracts

September 9 - 12, 2009 Würzburg, Germany

IDMC-7

100 YEARS MYOTONIC DYSTROPHY AND STEINERT DISEASE

Hans Steinert

7th International Myotonic Dystrophy Consortium Meeting

Welcome to the 7th International Myotonic Dystrophy Consortium Meeting (IDMC-7) in Würzburg, Germany

September, 9–12, 2009

The **International Myotonic Dystrophy Consortium (IDMC)** is a group of scientists and clinicians with a shared interest in the understanding of myotonic dystrophy (DM), both type 1 (DM1) and type 2 (DM2), and the principal aim of providing improved understanding and treatment for patients. 2009 marks the 100th anniversary of the first description of *dystrophica myotonica* (DM) in the clinical literature by Hans Steinert (1875–1911, Leipzig, Germany). In Würzburg Kenneth Ricker (1934–2004) was one of the first clinicians to recognize DM2 as a disease distinct from DM1. In memory of both scientists, it is therefore with great pleasure that we welcome you all to Würzburg for this special anniversary meeting.

Since the first IDMC meeting in Paris in 1997, remarkable progress has been made in DM research. Still much remains to be done to provide patients and their care providers with effective therapies for the many symptoms of this disease. The bi-annual IDMC meeting provides a unique forum to discuss the latest findings in the DM field. In keeping with previous IDMC meetings, IDMC-7 will cover a broad range of topics, including historic perspectives on DM1 and DM2, mechanisms of disease pathogenesis and repeat instability of the underlying DNA mutations, model systems for DM, and advances in treatment of these multisystem disorders. We hope IDMC-7 will provide a setting and atmosphere for open and stimulating exchange of the newest findings and ideas between basic researchers, clinicians (including neurologists, geneticists; pediatricians, internists, physical therapists, ophthalmologists, anesthesiologists, gynecologists), and translational scientists, as well as patients and their care providers.

Finally, we would like to thank the IDMC membership for electing us to organize IDMC-7 and all those who have helped with the organization of this meeting: our generous sponsors, who have made this congress possible, the organizing agency Carlo Praetorius (Munich), and last but not least all of you – scientists, patients and caregivers – who have come from 16 countries and four continents to Würzburg to make this another successful meeting. We hope the meeting will be memorable and you will enjoy the scientific and patient sessions and the social programs.

Thank you – Danke!

Ralf Krahe
Houston, USA

Tiemo Grimm
Würzburg, Germany

Benedikt Schoser
Munich, Germany

The IDMC-7 Chairmen

Hans Gustav Wilhelm Steinert

German Internist, 1875-1911

▲ **H.G.W. Steinert** (modified from the original photograph of Leipzig University Archives)

After attending the Gymnasium in Freiberg, Hans Gustav Wilhelm Steinert attended the Universities of Leipzig, Freiberg, Berlin and Kiel. He graduated in 1898 and subsequently held junior appointments in Halle an der Saale and Berlin. He settled in Leipzig, where he worked in the pathological institute and the medical clinic before being appointed professor in 1910. Most of his written works are in the field of neurological and muscular conditions.

Literature

Steinert H. Myopathologische Beitraege 1. Ueber das klinische und anatomische Bild des Muskelschwunds der Myotoniker. Dtsch Z Nervenheilkd, 1909,37:58–10

Der Bayerische Ministerpräsident

Scientific Committee

Welcoming Address

100 Years of Myotonic Dystrophy and Steinert Disease

7th International Myotonic Dystrophy Consortium Meeting in Würzburg, September 9-12, 2009

▲ Horst Seehofer

I cordially welcome all participants of the 7th International Myotonic Dystrophy Consortium Meeting to Bavaria.

Fortunately, in both of its forms Myotonic Dystrophy is a relatively rare disease. But whoever is affected with it – especially type 1 – has a heavy burden to carry. Modern genetics research has been able to identify the underlying cause of the disease. Thus, there is hope that one day it will be possible to sufficiently understand the disease mechanisms and to substantially help the patients. So far, medical practice has been restricted to treating the varied symptoms.

The neurologist Hans Steinert provided the first description of the classic type of the disease in Leipzig 100 years ago. This anniversary is a good opportunity to take stock where research has gotten us and to look ahead into the future. The patient day provides a unique opportunity for all participants to closely interact, including the patients, their care-givers and advocacy groups. I dearly thank the participating patient support-groups.

This is the first time the IDMC congress convenes in Germany, and Würzburg provides an excellent setting for it. With its expertise, the University has acquired an outstanding reputation in the field of medicine. Case in point is the work by the late neurologist Kenneth Ricker who here in 1994 described Myotonic Dystrophy type 2.

Over the last decade Bavaria has developed itself into a "health-land." In terms of quality of our healthcare institutions, our basic and translational biomedical research, and biotechnology, Bavaria has taken a leading position in the international comparison. The close ties between research and clinical practice guarantee outstanding healthcare to our people. In addition, the healthcare sector has become an important industry for the future and increasingly impacts our economy. The Bavarian State Government will continue to invest into it.

I sincerely wish all of the participants of the congress an interesting and stimulating discussions as well as a pleasant stay in Würzburg.

Horst Seehofer
Prime Minister of Bavaria

Congress Chairmen

Ralf Krahe
University of Texas,
M. D. Anderson Cancer Center,
Houston, USA

Tiemo Grimm
University of Würzburg,
Germany

Benedikt Schoser
University of Munich,
Germany

Local Chairmen

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Germany

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University of Würzburg,
Germany

Karlheinz Reiners
University of Würzburg,
Germany

Klaus Victor Toyka
University of Würzburg,
Germany

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Gainesville, USA

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Congress Organisation

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Congress Venue

Julius-Maximilians-Universität Würzburg
Neue Universität, Lecture halls,
Sanderring 2
97070 Würzburg, Germany

General information

Badges

Participants should collect name badges from the conference registration desks.

Coffee Breaks and Lunch

During the session breaks refreshments (coffee, tea, water) and lunch will be served free of charge to participants wearing name badges.

Currency

The official currency of Germany is the Euro (€).

Electricity

220V– 50Hz AC. Connectors can be obtained from your hotel reception or electronic shops.

Language

The official language of the congress will be English. On Saturday, September 12, will be simultaneous translation to German.

Smoking Policy

The IDMC-7 is a non-smoking conference. Please note that smoking is banned from all public buildings and restaurants.

Social Events

Wednesday, September 9, 2009, 7 pm

Welcome Reception

Foyer of the Lecture Hall

Bassiona Amorosa – an international contrabass sextet

Friday, September 11, 2009, 5 pm

Visiting the Residence

Admission by ticket only, you should pre-book at moment of your registration.

Friday, September 11, 2009, 6:45 pm

Winetasting Reception at the Cellar of the Residence

Meeting point at the fountain (Frankonia-Brunnen) in front of the Residence.

You will spend the evening in a historical Wine Cellar, the temperature will be max. 20 °C. Please consider adequate clothing.

Admission by ticket only, you should pre-book at moment of your registration.

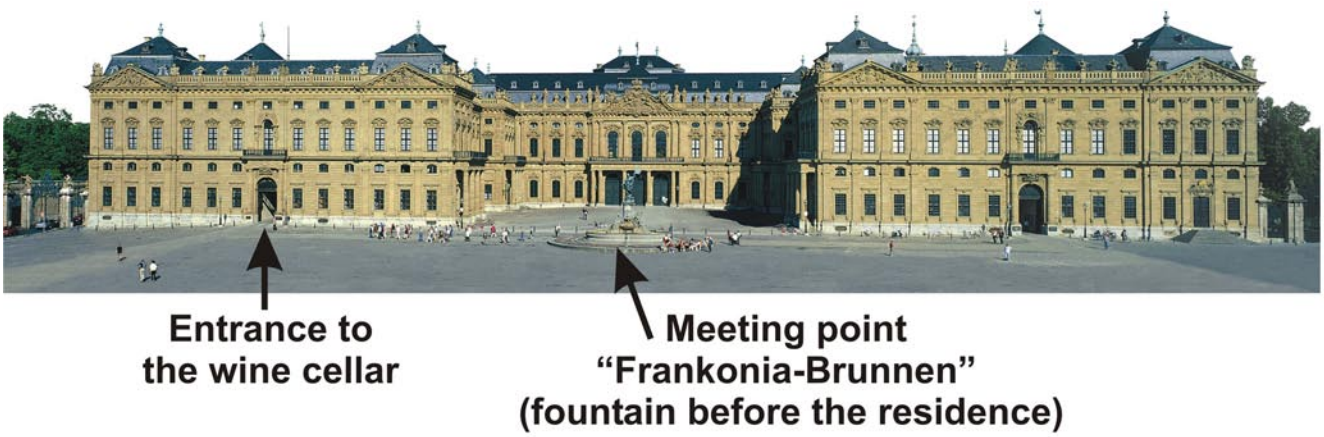
Contribution towards expenses: Participants, accompanying persons: **Euro 10,00**

Saturday, September 12, 2009, 7 pm

Farewell-Dinner Riverboat trip at the river Main

Meeting point: Alter Kranen – the Old Crane at the river Main. Admission by ticket only, you should pre-book at moment of your registration

Contribution towards expenses: Participants, accompanying persons: **Euro 50,00**



▲ **The former residence of the Würzburg prince-bishops is one of the most important baroque palaces in Europe and today it is on UNESCO's World Cultural Heritage list. Originally designed for Prince-Bishop Johann Philipp Franz von Schönborn by the then young and unknown architect Balthasar Neumann, it took sixty years to complete; the shell of the palace was built from 1720 to 1744 and the interior finished in 1780.**
Neumann's world-famous staircase, roofed by an unsupported vault, was decorated in 1752/53 by the Venetian Giovanni Battista Tiepolo with a ceiling fresco representing the four continents. The painting, measuring 18 x 30 meters, is one of the largest frescos ever created. The magnificent sequence of rooms begins with the Vestibule and Garden Hall and continues via the staircase and White Hall to the Emperor's Hall, also with frescos by Tiepolo. The vaulting of these rooms even withstood the devastating fire of 1945, while the ceilings and floors of the Imperial Apartments flanking the Emperor's Hall were destroyed. The furnishings and wall paneling had been removed beforehand enabling the rooms to be reconstructed.
Restoration was completed in 1987 with the reopening of the Mirror Cabinet. There are a total of over 40 palace rooms to visit, with a rich array of furniture, tapestries, paintings and other 18th century treasures.

IDMC-7 Scientific Program

Wednesday, September 9, 2009		
Lecture Hall 1 (HS216, Audimax)		
S1	**Opening Ceremony** **Welcoming addresses by:** **Krahe, Ralf** (Houston, USA, on behalf of the Congress Chairmen) **President of the University of Würzburg** **Vice president Prof. Dr. Heidrun Moll** **Dean of the Medical Faculty** **of the University Würzburg** **Vice-dean Prof. Dr. med. Manfred Gessler** **Special Lectures** Chairs: Grimm, Tiemo (Würzburg, Germany) Schoser, Benedikt (Munich, Germany)	17.00 – 19.00
S1-01	**A Hundred Years of Myotonic Dystrophy** Harper, Peter (Cardiff, UK)	17:30 – 18:15
S1-02	**Kenneth Ricker and PROMM/DM2** Moxley III, Richard (Rochester, USA)	18:15 – 19:00
	Welcome Reception Foyer of the Lecture Hall	19:00 – 21:00
Thursday, September 10, 2009		
Lecture Hall 1 (HS216, Audimax)		
S2	**Pathomechanisms in DM – Part 1** Chairs: Timchenko, Lubov (Houston, USA) Ranum, Laura (Minneapolis, USA)	08.00 – 12.30
S2-01	**Current understanding of pathomechanisms in DM** Thornton, Charles (Rochester, USA)	08:00 – 08:30
S2-02	**Multi-step deregulation of MBNL1 complex stoichiometry results in progressive RNA splice defects in myotonic dystrophy** Reddy, Sita (Los Angeles, USA)	08:30 – 08:45
S2-03	**Tau mis-splicing recovery in myotonic dystrophy type 1** Tran, Hélène (Lille, France)	08:45 – 09:00
S2-04	**A (CCUG)n-binding protein that may explain the lesser severity of DM2** Hammer, Caroline (Illkirch, France)	09:00 – 09:15
S2-05	**Identification of CUGBP1 mRNA Targets by CUGBP1/RNA Immuno-capture** Bachinski, Linda (Houston, USA)	09:15 – 09:30
S2-06	**Protein and micro-RNA binding behavior of DMPK messenger RNA** Wieringa , Be (Nijmegen, The Netherlands)	09:30 – 09:45
S2-07	**Identification of novel candidates that intervene in Myotonic Dystrophy Type 1 (DM1)** Francois-Xavier Laurent (Gif sur Yvette, France)	09:45 – 10:00
	Coffee break / Poster Viewing / Exhibition	10:00 – 10:30
S2-08	**The DMSXL transgenic mice carrying very large expansions exhibit molecular and physiological defects: an animal model for gene therapy experiments?** Gourdon, Geneviève (Paris, France)	10:30 – 10:45
S2-09	**A second example of RNA-gain-of-function disease: Sam68 and MBNL1 sequestration by CGG repeats in Fragile X Tremor Ataxia Syndrome** Sellier, Chantal (Illkirch, France)	10:45 – 11:00
S2-10	**Development of a muscleblind-like 1 (Mbnl1) overexpression model to assess the use of MBNL1 as a potential therapeutic for myotonic dystrophy** Chamberlain, Christopher (Minneapolis, USA)	11:00 – 11:15
S2-11	**Misregulation of diacylglycerol kinase eta (DGKη) splicing as a potential cause of neuropsychiatric symptoms in myotonic dystrophy type 1** Matsuura, Tohru (Okayama, Japan)	11:15 – 11:30
S2-12	**Study on the mechanisms of regulation and deregulation of hcTNT exon 5 inclusion** Behm-Ansmant, Isabelle (Vandoeuvre-les-Nancy, France)	11:30 – 11:45
S2-13	**MBNL1 associates with YB-1 in cytoplasmic stress granules** Ishiura, Shoichi (Tokyo, Japan)	11:45 – 12:00
S2-14	**Abnormal secretion of prostaglandin E2 inhibits differentiation of congenital myotonic dystrophy muscle cells** Furling, Denis (Paris, France)	12:00 – 12:15
S2-15	**Quantitative semi-automated expanded CUG repeats assessment: application to the DM-SXL mouse** Bassez, Guillaume (Paris, France)	12:15 – 12:30
	Lunch / Exhibition	12:30 – 14:00
	Poster Viewing with Presenters (odd numbers)	13:00 – 14:00
P1	**Poster Session 1: Basic Research – Part 1** Chairs: Müller-Reible, Clemens R. (Würzburg, Germany) Pearson, Christopher (Toronto, Canada)	13:00 – 14:00
P2	**Poster Session 2: Basic Research – Part 2** Chairs: Timchenko, Lubov (Houston, USA) Thornton, Charles (Rochester, USA)	13:00 – 14:00
P3	**Poster Session 3: Clinical Issues – Part 1** Chairs: Van Engelen, Baziel (Nijmegen, The Netherlands) Eymard, Bruno (Paris, France)	13:00 – 14:00
P4	**Poster Session 3: Clinical Issues – Part 2** Chairs: Udd, Bjarne (Helsinki; Finland) Reiners, Karlheinz (Würzburg, Germany)	13:00 – 14:00
P5	**Poster Session 4: Translation and Therapy** Chairs: Ishiura, Shoichi (Tokyo, Japan) Schneider-Gold, Christiane (Bochum, Germany)	13:00 – 14:00

S3	**Pathomechanisms in DM – Part 2** Chairs: Gourdon, Geneviève (Paris, France) Wieringa, Be (Nijmegen, The Netherlands)	14:00 – 16:15
S3-01	**Investigating cellular and molecular abnormalities in the central nervous system of mice carrying large CTG repeat expansions** Gomes-Pereira, Mario (Paris, France)	14:00 – 14:15
S3-02	**An unusual family co-segregating myotonic dystrophy type 1 and Charcot-Marie-Tooth disease present with an imperfect CTG repeat allele at the DM1 locus** Braida, Claudia (Glasgow, UK)	14:15 – 14:30
S3-03	**De novo base substitution mutations at the myotonic dystrophy type 1 locus** Couto, Jillian (Glasgow, UK)	14:30 – 14:45
S3-04	**Molecular pathophysiology and CNS effects in mouse models of myotonic dystrophy types 1 & 2** Kang, Yuan-Lin (Minneapolis, USA)	14:45 – 15:00
S3-05	**A novel role for the protein RFDM in restoring fusion of fetal DM1 muscle satellite cells** Pelletier, Richard (Quebec, Canada)	15:00 – 15:15
S3-06	**The role of microRNAs in the regulation of gene expression in Myotonic Dystrophy** Timchenko, Lubov (Houston, USA)	15:15 – 15:30
S3-07	**Study on human MBNL1 RNA binding properties** Vautrin, Audrey (Vandoeuvre-Les-Nancy, France)	15:30 – 15:45
S3-08	**Testing the role of CUGBP1 in skeletal muscle wasting in DM1** Ward, Amanda (Houston, USA)	15:45 – 16:00
S3-09	**Accumulation of Mutant DM2 (CCUG)n Transcripts Triggers Activation of PKR and ER Stress Response** Wojciechowska, Marzena (Houston, USA)	16:00 – 16:15
	Coffee break / Poster Viewing / Exhibition	16:15 – 16:45
S4	**Models for DM** Chairs: Maurice Swanson, Maurice (Gainesville, USA) Mahadevan, Mani (Charlottesville, USA)	16:45 – 19:15
S4-01	**Animal models for DM** Cooper, Tom (Houston, USA)	16:45 – 17:15
S4-02	**Muscleblind Loss-of-Function Models for Myotonic Dystrophy** Swanson, Maurice (Gainesville, USA)	17:15 – 17:30
S4-03	**Insights from mouse models of RNA toxicity** Mahadevan, Mani (Charlottesville, USA)	17:30 – 17:45
S4-04	**Mouse Models for Myotonic Dystrophy Type 2 (DM2) Lacking Aberrant Splicing Implicate Novel Cytoplasmic Pathomechanisms** Krahe, Ralf (Houston, USA)	17:45 – 18:00
S4-05	**CCUG RNA gain-of-function effects in a conditional mouse model of myotonic dystrophy type 2 (DM2)** Margolis, Jamie M. (Minneapolis, USA)	18:00 – 18:15
S4-06	**Mouse model of congenital myotonic dystrophy type 1** Srinivasan, Varadamurthy (Charlottesville, USA)	18:15 – 18:30
S4-07	**A Drosophila model for Myotonic Dystrophy Type 2 (DM2)** Bergmann, Andreas (Houston, USA)	18:30 – 18:45
S4-08	**Towards a Zebrafish Model of Myotonic Dystrophy** Todd, Peter (Michigan, USA)	18:45 – 19:00
S4-09	**Toxicity of noncoding CUG and CCUG repeats** Timchenko, Lubov (Houston, USA)	19:00 – 19:15

Friday, September 11, 2009

Lecture Hall 1 (HS216, Audimax)

S5	**Clinical Issues in DM** Chairs: Eymard, Bruno (Paris, France) Meola, Giovanni (Milan, Italy)	08:00 – 12:00
S5-01	**Unusual presentations and a large proportion of mild phenotypes expand the spectrum and epidemiology of DM2** Udd, Bjarne, (Tampere, Finland)	08:00 – 08:20
S5-02	**Brain imaging in DM** Day, John (Minneapolis, USA)	08:20 – 08:40
S5-03	**Congenital myotonic dystrophy: Canadian surveillance and cohort study** Campbell, Craig (London, Canada)	08:40 – 08:55
S5-04	**Spectrum of disease manifestations of juvenile myotonic dystrophy type 1 (JDM) patients** Luebbe, Elizabeth (Rochester, USA)	08:55 – 09:10
S5-05	**Visual function in Congenital and Childhood Myotonic Dystrophy Type 1** Ekström, Anne-Berit (Gothenburg, Sweden)	09:10 – 09:25
S5-06	**Sleep-Disordered Breathing in a cohort of 40 Italian Steinert's Dystrophy patients. A clinical and polisomnographic study** Morandi, Lucia (Milan, Italy)	09:25 – 09:40
S5-07	**Cerebral white matter affection in myotonic dystrophy type 1 and 2 - A Diffusion-Tensor-Imaging Study at 3T** Minnerop, Martina (Juelich, Germany)	09:40 – 09:55
	Coffee break / Poster Viewing / Exhibition	09:55 – 10:30
S5-08	**White Matter Microstructural Abnormalities in DM Observed with Diffusion Tensor Imaging** Franc, Daniel (Minneapolis, USA)	10:30 – 10:45
S5-09	**Depression in Myotonic dystrophy type 1: clinical and neuronal correlates** Lindberg, Christopher (Göteborg, Sweden)	10:45 – 11:00
S5-10	**French registry for myotonic dystrophies (DM1-DM2): toward the characterization of a large DM population** Guiraud-Dogan, Céline (Créteil, France)	11:00 – 11:15
S5-11	**Efficacy And Limitation Of Group Exercise For Swallowing Training In Myotonic Dystrophy** Saito, Akiko (Aomori, Japan)	11:15 – 11:30
S5-12	**General anesthesia in myotonic dystrophy type 2** Kirzinger, Lukas (Munich, Germany)	11:30 – 11:45
S5-13	**Double genetic trouble (DM2/FSHD) in a Sardinian DM2/PROMM family** Meola, Giovanni (Milan, Italy)	11:45 – 12:00

	Lunch / Exhibition	12:00 – 14:00
	Poster Viewing with Presenters (even numbers)	13:00 – 14:00
P1	**Poster Session 1: Basic Research – Part 1** Chairs: Müller-Reible, Clemens R. (Würzburg, Germany) Pearson, Christopher (Toronto, Canada)	13:00 – 14:00
P2	**Poster Session 2: Basic Research – Part 2** Chairs: Timchenko, Lubov (Houston, USA) Thornton, Charles (Rochester, USA)	13:00 – 14:00
P3	**Poster Session 3: Clinical Issues – Part 1** Chairs: Van Engelen, Baziel (Nijmegen) Eymard, Bruno (Paris, France)	13:00 – 14:00
P4	**Poster Session 3: Clinical issues – Part 2** Chairs: Udd, Bjarne (Helsinki; Finland) Reiners, Karlheinz (Würzburg, Germany)	13:00 – 14:00
P5	**Poster Session 4: Translation and Therapy** Chairs: Ishiura, Shoichi (Tokyo, Japan) Schneider-Gold, Christiane (Bochum, Germany)	13:00 – 14:00
S6	**TREAT-NMD / Marigold Foundation Working group Summaries**	14:00 – 15:00
S7	**Keynote Lecture** Chairs: Ashizawa, Tetsuo (Gainesville, USA) Krahe, Ralf (Houston, USA)	
S7-1	**Developing treatment for hereditary neuromuscular disease** Fischbeck, Kenneth H. (Bethesda, USA)	15:00 – 16:00
	Coffee break / Poster Viewing / Exhibition	16:00 – 16:30
	Walk to the Residence	16:30 – 16:50
	Visiting the Residence Lift available; admission by ticket only. Pre-booking at registration requested	17:00 – 18:45
	Winetasting Reception at the Cellar of the Residence Meeting point at the fountain before the Residence. Lift available; admission by ticket only. Pre-booking at registration requested	18:45 – 23:00

Saturday, September 12, 2009

Lecture Hall 1 (HS216, Audimax) (deutsche Simultanübersetzung)

S8	**Molecular and symptomatic therapy** Chairs: Puymirat, Jack (Quebec, Canada) Toyka, Klaus (Würzburg, Germany)	08:30 – 10:30
S8-01	**Molecular therapy for myotonic dystrophy: current status and future strategies** Wansink, Derrick G. (Nijmegen, The Netherlands)	08:30 – 09:00
S8-02	**IPLEX (rhIGF-I/rhIGFBP-3) Treatment of Myotonic Dystrophy Type-1 (DM1): A Safety and Tolerability Trial** Heatwole, Chad (Rochester, USA)	09:00 – 09:15
S8-03	**Reversal of myotonia and splicing defects by antisense oligomers in a transgenic mouse model of myotonic dystrophy type 1 (DM1)** Wheeler, Thurman (Rochester, USA)	09:15 – 09:30
S8-04	**Development of therapies against RNA toxicity in DM1** Yadava, Rahmesh (Charlottesville, USA)	09:30 – 09:45
S8-05	**Pentamidine reverses splicing defects associated with Myotonic Dystrophy Type 1 (DM1)** Warf, M. Bryan (Eugene, USA)	09:45 – 10:00
S8-06	**Chemically modified (CAG)n antisense oligonucleotides as molecular tools to silence toxic, expanded DMPK transcripts** Mulders, Susan (Nijmegen, The Netherlands)	10:00 – 10:15
S8-07	**Assays to screen for drugs to treat myotonic dystrophy** Brook, J David (Nottingham, UK)	10:15 – 10:30
	Coffee break / Poster Viewing / Exhibition	10:30 – 11:00
S9	**Heart and DM** Chairs: Ertl, Georg (Würzburg, Germany) Duboc, Denis (Paris, France)	11:00 – 12:30
S9-01	**Clinical Aspects of Cardiac Involvement in Myotonic Dystrophy: Current Knowledge and Future Directions** Groh, William J. (Indianapolis, USA)	11:00 – 11:15
S9-02	**Is Cardiac Involvement in Adult Survivors of Congenital- or Childhood-Onset DM1 Different than in Classical Adult-Onset DM1?** Groh, William J. (Indianapolis, USA)	11:15 – 11:30
S9-03	**Cardiac abnormalities in Congenital Childhood Myotonic muscular dystrophy (DM1)** Mishra , Shri (Los Angeles, USA)	11:30 – 11:45
S9-04	**High Prevalence of Brugada Syndrome in Patients with Steinert's Disease. A New Insight in the Pathophysiology of Arrhythmias in Steinert's Disease** Wahbi, Karim (Paris, France)	11:45 – 12:00
S9-05	**Protein Kinase C inhibition ameliorates the cardiac phenotype of a mouse model for myotonic dystrophy type 1** Kuyumcu-Martinez, Muge N. (Houston, USA)	12:00 – 12:15
S9-06	**The progression of Muscular Impairment Rating Scale (MIRS) and the development of cardiac conduction abnormalities in DM1** Antonini, Giovanni (Rome, Italy)	12:15 – 12:30

Lecture Hall 2 (HS224)

M1	**Patient Session in German / Vorträge für Patienten und Angehörige auf Deutsch**	08:30– 12:30
M1-1	**Einführung** Schoser, Benedikt (München)	08:30 – 09:00
M1-2	**Klinische Symptome der DM1 und DM2** (Klinischer Verlauf, CDM, Herz, Auge, Hirn, Muskel, endokrine Organe, Immunsystem) Schneider-Gold, Christiane (Bochum)	09:00 – 09:45
M1-3	**Grundlagen und Diagnose der DM1 und DM2 (Ursache, Genetik, Beratung, Pränataldiagnostik)** Kress, Wolfram (Würzburg); Grimm, Tiemo (Würzburg)	09:45 – 10:30
	Kaffeepause	10:30 – 11:00
M1-4	**Komplikationen und Behandlungsmöglichkeiten der DM1 und DM2** (Herz, Müdigkeit, Schmerzen, Operationen, Darm) Schoser, Benedikt (München)	11:00 – 11:45
M1-5	**Berichte von Patienten und Beantwortung von Fragen.** **Wie geht es weiter?**	11.45 – 12.30

Lecture Hall 3 (HS210)		
M2	**Patient Session in English** Organized by Moxley III, Richard (Rochester, USA)	08:30 – 12:30
	Lunch / Poster Viewing / Exhibition	12:30 – 13:30
Lecture Hall 1 (HS216, Audimax) (deutsche Simultanübersetzung)		
S10	**Genetic counseling** Chairs: Grimm, Tiemo (Würzburg, Germany) Schoser, Benedikt (Munich, Germany)	13:30 – 14:50
S10-1	**Genetic counseling in DM1** Rogers, Mark (Cardiff, UK)	13:30 – 14:10
S10-2	**Genetic counseling in DM2** Kress, Wolfram (Würzburg, Germany)	14:10 – 14:45
	Coffee break / Poster Viewing / Exhibition	14:45 – 15:30
S11	**Messages from the patient organizations** Chairs: Moxley III, Richard (Rochester, USA) Monckton, Darren G (Glasgow, UK)	15:30 – 16:45
S11-1	**Awards (award committee)**	15:30 – 16:00
S11-2	**Presentation of the patients organizations**	16:00 – 16:45
S12	**Conference Highlights** Chairs: Moxley III, Richard (Rochester, USA) Monckton, Darren G (Glasgow, UK)	16:45 – 18:00
S12-1	**Late-breaking Session**	16:45 – 17:15
S12-2	**Conference Highlights and Concluding Remarks** Wierenga, Be (Nijmegen, The Netherlands) Ashizawa, Tetsuo (Gainesville, USA) Brook, David (Nottingham, UK) Thornton, Charles (Rochester, USA) Krahe, Ralf (Houston, USA) Grimm, Tiemo (Würzburg, Germany) Schoser, Benedikt (München)	17:15 – 18:00
	Farewell-Dinner Riverboat Cruise on the Main River Meeting point: Alter Kranen – The Old Crane at the river Main. Admission by ticket only. Pre-booking requested	19:00 – 23:00

Thursday, September 10, 2009 (odd numbers) and Friday, September 11, 2009 (even numbers) 13.00–14.00 h	
Foyer of the lecture hall	
P1	Poster Session 1: Basic Research – Part 1 Chairs: Müller-Reible, Clemens R. (Würzburg, Germany) Pearson, Christopher (Toronto, Canada)
P1-01	Searching for trans-acting genetic modifiers of somatic mosaicism and disease severity in myotonic dystrophy type 1 Fernando Morales (San José)
P1-02	CTCF Induces Replication Fork Pausing Around DM1 Repeats Katharine A. Hagerman (Ontario)
P1-03	Study of DM1 associated RNAs using atomic force microscopy (AFM) Francois Meullenet (Nottingham)
P1-04	Disease-associated trinucleotide repeats form transcription-induced RNA:DNA hybrids K. Reddy (Ontario)
P1-05	CpG methylation proximal to the CTG/CAG tract of the DM1 locus in patients and transgenic mice A. López Castel (Ontario)
P1-06	Tissue-specificity of trinucleotide repeat instability and DNA replication in myotonic dystrophy type 1 JD Cleary (Ontario)
P1-07	Studies of the distribution of Muscleblind-like proteins in myotonic dystrophy J David Brook (Nottingham)
P1-08	A bi-chromatic fluorescent assay to measure splicing efficiency in Myotonic Dystrophy. Javier T Granados-Riveron (Nottingham)
P1-09	Validation of sensitivity and specificity of Tetraplet-Primed PCR (TP-PCR) in the molecular diagnosis of for Myotonic Dystrophy type 2 Claudio Catalli (Rome)
P1-10	Effect of RNAi directed against CUG repeats in a mouse model of DM1 Krzysztof Sobczak (Poznan)
P1-11	DNA methylation at the DM1 Locus John Day (Minneapolis)
P1-12	DMSXL mice carrying over 1000 CTG: characterization of the muscle function Fadia Medja (Paris)
P1-13	The splicing of MBNL1 is altered in DM1 muscles Camille Lemercier (Paris)
P1-14	Premature activation of the p16 stress pathway in congenital DM1 myoblasts Erwan Gasnier (Paris)
P1-15	The effort to obtain longer CTG triplet repeat DNA by using Saccharomyces cerevisiae Noboru Sasagawa (Tokyo)
P1-16	Altered splicing of CAMKIID in brain from patients with myotonic dystrophy type 1 Koichi Suenaga (Nishinomiya)
P1-17	Normal myogenesis and increased apoptosis in myotonic dystrophy type 1 muscle cells Emanuele Loro (Padova)
P1-18	Altered mRNA splicing of the MYH14 gene in the skeletal muscle of myotonic dystrophy type 1 patients Fabrizio Rinaldi (Rome)
P1-19	The role of Twist in DM1 Andrie Koutsoulidou (Nicosia)

P1-20	Cytoplasmic export of DM1 transcripts benefits muscle cell differentiation Nikolaos Mastroyiannopoulos (Nicosia)
P1-21	Cell model for repeat instability and senescence in DM1 Masayuki Nakamori (Rochester)
P1-22	Mis-splicing of microtubles-associated tau exon 10 is associated to a CELF proteins gain of function but not to a MBNL1 loss of function Claire-Marie Dhaenens (Lille)
P1-23	Specific micro-RNA processing alteration in DM Frédérique Rau (Illkirch)
P2	**Poster Session 2: Basic Research – Part 2** Chairs: Timchenko, Lubov (Houston, USA) Thornton, Charles (Rochester, USA)
P2-01	The role of microRNAs in the regulation of gene expression in Myotonic Dystrophy Lubov Timchenko (Houston)
P2-02	Abnormal expression of ZNF9 in myotonic dystrophy type 2 (DM2) Olayinka Raheem (Tampere)
P2-03	Proteomic analysis of DM2 human myotubes reveals alterations in mitochondrial components, in the unfolded protein response and in the ubiquitin proteasome system Francesco S. Rusconi (Milan)
P2-04	Progressions of (CTG) n expansions, muscular disability rating scale (MDRS), and abnormal glucose metabolism are age dependent in myotonic dystrophy type 1 (DM1) Masanobu Kinoshita (Tokyo)
P2-05	High-throughput screening to identify modulators of aberrant splicing in DM1 Debra O'Leary (San Diego)
P2-06	Subcellular localization of Drosophila Muscleblind changes from preferentially nuclear to cytoplasmic during muscle development M. Beatriz Jiamusi Trios (Burgesses-Valencia)
P2-07	DMPK-interacting proteins Sergio Salvatore (Padova)
P2-08	Transcriptional defects in DM1 result from nuclear exclusion of SHARP Sita Reddy (Los Angeles)
P2-09	Autoregulation of MBNL1: coupling of splicing regulation and intracellular localization Yoshihiro Kino (Saitama)
P2-10	Muscleblind-like proteins in normal and myotonic dystrophy muscle and their role in rapid diagnostic testing Ian Holt (Shropshire)
P2-11	The mechanisms of MBNL1 regulated splicing Andy Berglund (Eugene)
P2-12	What are RNA foci? Interactions of the mutant DMPK mRNA Shagufta Rehman (Charlottesville)
P2-13	Abnormal splicing of myomesin in DM muscle Michinori Koebis (Tokyo)
P2-14	Mathematical models of dynamic DNA in myotonic dystrophy Catherine Higham (Glasgow)
P2-15	Aberrant expression of microRNA in myotonic dystrophies Riccardo Perbellini (Milan)
P2-16	Abnormally expressed genes in statin induced myopathy appear dysregulated in myotonic dystrophy type 2 Mark Screen (Helsinki)
P2-17	Use of human embryonic stem cells as new model to decipher early pathological events involved in Myotonic Dystrophy type 1 Jérôme Denis (Evry)
P2-18	Altered isoform usage for MADS-domain transcription enhancer factor 2 (MEF2) in myotonic dystrophy and other neuromuscular disorders Linda Bachinski (Houston)
P2-19	Effects of mexiletine on cardiac parameters, muscles strength and myotonia in myotonic dystrophy type 1 Marta Panzeri (Milan)
P2-20	Non-radioactive detection of repeat expansions in DMPK and ZNF9 genes Martina Witsch-Baumgartner (Innsbruck)
P2-21	Global expression profiling of Myotonic Dystrophy type 1 (DM1) and type 2 (DM2) identifies novel effector genes and cellular pathways Mario Sirito (Houston)
P2-22	New Technique for Rapid and Reliable Analysis of Trinucleotide Repeats in Myotonic Dystrophy Type 1 Skrzypczak-Zielinska M., Sulek-Piatkowska A., Froster U. G. (Leipzig, Germany)
P3	Poster Session 3: Clinical Issues – Part 1 Chairs: Van Engelen, Baziel (Nijmegen) Eymard, Bruno (Paris, France)
P3-01	Myotonic dystrophy type 2 (DM2) in Italy: spectrum of clinical and laboratory findings Marta Panzeri (Milan)
P3-02	Risk of arrhythmia in type I Myotonic Dystrophy: the role of clinical and genetic variables Paola Cudia (Venice)
P3-03	CLCN1 mutations screening in Italian patients affected by myotonic dystrophy type 2 (DM2) Saverio Massimo Lepore (Rome)
P3-04	The correlation between oral dysfunction and videofuluoroscopic swallowing findings in myotonic dystrophy type 1(DM1) Hirokazu Furuya (Fukuoka)
P3-05	Frequency of DM1 and DM2 in Germany Tiemo Grimm (Würzburg)
P3-06	Hearing evaluation in DM2: a prospective study of 10 Patients Bruno Eymard (Paris)
P3-07	White matter pathology and neurocognitive correlates in adolescents with myotonic dystrophy type 1: A Diffusion Tensor Imaging study Jeffrey Wozniak (Minneapolis)
P3-08	Repetitive components of compound motor action potential in DM1 patients Anna Modoni (Rome)
P3-09	Muscle pathological changes and brain MRI findings in DM1 Chiara Ferrati (Padova)
P3-10	Subtle cognitive decline in Myotonic dystrophy type 1: a five-year follow up study Stefan Winblad (Göteborg)
P3-11	Test/retest and machine/machine reliability of Dual Energy X-ray Absorptiometry (DEXA) measurements in patients with DM-1 and DM-2. Shree Pandya (Rochester)
P3-12	Participation in physical activity by people with myotonic dystrophy Margaret Phillips (Derby)

P3-13	Clinical and biomolecular findings in a juvenile onset case of myotonic dystrophy type 2 Marzia Giagnacovo (Milan)
P3-14	Health-related quality of life in patients with myotonic dystrophy type 1 and myasthenia gravis: a comparative analysis Vidosava Rakocevic Stojanovic (Belgrade)
P3-15	Development of Scottish Myotonic Dystrophy management guidelines Cheryl Longman (Glasgow)
P3-16	Frequency of DM2 and DM1 mutations in the Finnish population Tiina Suominen (Tampere)
P3-17	Lipid metabolism alteration in myotonic dystrophies Anja Schmidt (Munich)
P4	Poster Session 4: Clinical Issues – Part 2 Chairs: Udd, Bjarne (Helsinki; Finland) Reiners, Karlheinz (Würzburg, Germany)
P4-01	Clinical, muscle pathology and FISH biomolecular findings correlation in 42 Italian patients with myotonic dystrophy type 2 Rosanna Cardani (Milan)
P4-02	Motor outcome measures in childhood and congenital DM1 Craig Campbell (London)
P4-03	Structural and functional brain abnormalities in myotonic dystrophy type 1 and 2 Robert Roebling (Ulm)
P4-04	The lived experience of patients with Myotonic Dystrophy Type 1 Maud-Christine Chouinard (Jonquiere)
P4-05	Quality of life and family impact of congenital and childhood DM1 Craig Campbell (London)
P4-06	Health supervision in Myotonic Dystrophy Type 1 Cynthia Gagnon (Jonquière)
P4-07	Chronic muscle stimulation reverts the abnormal sEMG pattern in Myotonic Dystrophy type 1 Carmelo Chisari (Pisa)
P4-08	Diagnostic odyssey of myotonic dystrophy type 2 (DM2) patients James Hilbert (Rochester)
P4-09	Ocular motor function in congenital and childhood Myotonic Dystrophy Type 1 Eva Aring (Göteborg)
P4-10	High impact symptoms in Myotonic Dystrophy Type-1 (DM1): A qualitative study Chad Heatwole (Rochester)
P4-11	Scaled down genetic analysis of myotonic dystrophy type 1 and type 2 Masayuki Nakamori (Rochester)
P4-12	Decision-making dysfunction in DM1 Nathalie Angeard (Paris)
P4-13	Hard to swallow: understanding the lived experience of Caregivers for individuals with Myotonic Dystrophy (DM1) and dysphagia Kori LaDonna (London)
P4-14	Quantitative isometric muscle strength at the ankle in myotonic dystrophy type 1 Jack Puymirat (Quebec)
P4-15	Gait and balance difficulties in individuals with Myotonic Dystrophy type 1 Elisabet Hammarén (Göteborg)
P4-16	No evidence of specific postprandial hyperlipidemia in Myotonic Dystrophy Hiroto Takada (Aomori)
P4-17	Characteristic features of oral and dental health in myotonic dystrophy T. Ishida Aomori, Japan)
P5	Poster Session 5: Translation and therapy Chairs: Ishiura, Shoichi (Tokyo, Japan) Schneider-Gold, Christiane (Bochum, Germany)
P5-01	Perceptions of professional, lay, and peer facilitators goal-setting and strategies used to promote social support and self-management behavior in face-to-face and online support groups for adults with either Multiple Sclerosis or Myotonic Muscular Dystrophy Leslie Krongold (Alameda)
P5-02	The lived experience of DM1 patients caregivers Maud-Christine Chouinard (Jonquière)
P5-03	Living with Myotonic Dystrophy Type 1 (DM1) Sufferers: How caregivers' experience differs according to gender Claudia Bouchard (Jonquière)
P5-04	Adapting and validating the Stanford Self-Management Program for people with DM1: preliminary results and lessons learned Cynthia Gagnon (Jonquière)
P5-05	Quality of life in myotonic dystrophy type 1 Eric Gagnon (Jonquiere)
P5-06	Functioning, disability and health-related quality of life in adults with Myotonic dystrophy type 1 (DM1) Marie Kierkegaard (Stockholm)
P5-07	Myotonic Dystrophy - A Scottish Perspective Anne Marie Taylor (Dundee)
P5-08	Role of oro-pharyngo-oesophageal scintigraphy in the evaluation of swallowing disorders in patients with Myotonic dystrophy type 1 (DM1) Venanzio Valenza (Rome)
P5-09	Myotonic dystrophy: a service improvement survey of quality of life, social integration and community support systems for patients and caregivers Margaret Phillips (Derby)
P5-10	In vivo drug screening of 170 natural compounds in a DM1 fly model Irma Garcia-Alcover (Paterna)
P5-11	Systemic delivery of antisense morpholino corrects ClC-1 splicing and reduces myotonia in a transgenic mouse model of DM1 Thurman Wheeler (Rochester)
P5-12	Pluripotent stem cells to explore mechanisms and treatments of monogenic diseases Cécile Martinat (Evry)
P5-13	RNA-based gene therapy to remove toxic expanded CUG-transcripts Denis Furling (Paris)
P5-14	High Throughput Screen Assays for Identifying Inhibitors of Protein-RNA Binding As a Potential Treatment for Myotonic Dystrophy Type-1 Catherine Chen (Bethesda)
P5-15	Test/retest reliability of regional lean body mass (LBM) measurements using Dual Energy X-ray Absorptiometry (DEXA) in patients with DM1 Shree Pandya (Rochester)

Abstracts

S1 Opening Ceremony

S1-01 A Hundred Years of Myotonic Dystrophy

Peter S Harper
Institute of Medical Genetics, Cardiff University, Heath Park, Cardiff, CF14 4XN, Wales, UK

The century that has passed since the first definitive descriptions of myotonic dystrophy in 1909 by Hans Steinert and Frederick Batten have taken us from initial recognition to a detailed, though still incomplete understanding of its molecular basis and pathogenesis. The principal landmarks along this path will be outlined, along with the broader lessons we can learn from research on myotonic dystrophy and from the condition itself. We now stand on the threshold of being able to transform our new understanding into meaningful therapy, opening a further chapter of history for myotonic dystrophy and those affected by it

S1-02 Kenneth Ricker and PROMM/DM2

Richard T. Moxley, III
University of Rochester Medical Center, Director, Neuromuscular Disease Center Department of Neurology, 601 Elmwood Avenue, Box 673 Rochester, NY 14642 USA

Following the discovery in 1992 that Steinert's Disease, myotonic dystrophy type 1 (DM1), results from an unstable CTG repeat expansion in the 3'untranslated region of the DMPK gene on chromosome 19, an unexpected opportunity occurred to discover another form of myotonic dystrophy. Dr. Kenneth Ricker and investigators at the University of Rochester identified another group of myotonic dystrophy patients without CTG repeat enlargement in the DMPK gene who had symptoms and findings similar to but distinct from DM1, and these patients were classified as having proximal myotonic myopathy (PROMM). A few years later nurtured by Dr. Ricker's determined efforts and the creative work of researchers at the University of Minnesota, the genetic locus of the PROMM was discovered and the disease was classified as myotonic dystrophy type 2 (DM2). It is caused by an unstable CCTG repeat expansion in intron 1 of the zinc finger protein 9 gene on chromosome 3. A historic overview of the discovery of DM2 and future opportunities to advance our understanding of its clinical manifestations and therapy will be reviewed.

S2 Pathomechanisms in DM – Part 1

S2-01 Current understanding of pathomechanisms in DM

Charles A. Thornton
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S2-02 Multi-step deregulation of MBNL1 complex stoichiometry results in progressive RNA splice defects in myotonic dystrophy

Sharan Paul 1, Warunee Dansithong 1, Ian Holt 2, Saloni Mittal 3, David Brook 3, Glenn Morris 2, Lucio Comai 4* and Sita Reddy 1*
1 Department of Biochemistry and Molecular Biology Institute for Genetic Medicine, Room 240, University of Southern California, Los Angeles, CA 90033, USA; 2 Wolfson Centre for Inherited Neuromuscular Disease, Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, SY10 7AG, UK; 3 Institute of Genetics, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK; 4 Department Molecular Microbiology and Immunology Institute for Genetic Medicine, Room 240, University of Southern California

To determine the origins of myotonic dystrophy (DM1) we immunopurified a 11-member RNA-independent MBNL1 complex from human myoblasts. MBNL1 complex stoichiometry is altered in DM1 myoblasts, which exhibit a graded decrease in steady-state MBNL1 levels and increased steady-state levels of 9 of its protein partners. In vivo modeling reveals a multi-step model for DM1: with MBNL1 decrease occurring first followed by a secondary increase in the levels of its protein partners. Experiments in human myoblasts reveal a third level of control occurring prior to MBNL1 complex deregulation. These events are unlinked to dicer drosha function, NKX2.5 induction or PKC alpha activation. Thus three, sequential processes independently dysregulate RNA splice site choice in DM1, acting both to mutually reinforce and expand the spectrum of RNA processing defects as a function of time. Our data support a multi-stage model for the origin and progression of DM1. Such a model provides a molecular explanation for the progressive and highly variable pathology observed in DM1.

S2-03 Tau missplicing recovery in myotonic dystrophy type 1

Hélène Tran 1,2, Claire-Marie Dhaenens 1,2, Capucine Trollet 3, Hélène Obriot 1, Dana Ghanem 1,2, Edwige Van Brussels 1,2, Nicolas Charlet 4, Mario Gomez-Perreira 5, Geneviève Gourdon 5, Denis Furling 3, Luc Buée 1,2, Marie-Laure Caillet-Boudin 1,2, Susanna Schraen-Maschke 1,2 and Nicolas Sergeant 1,2
1 Inserm, U837, Lille, France; 2 UniversitéLille2, IMPRT, JPARC, Lille, France; 3 Univ.-Paris6/ Inserm/ CNRS - Umrs974/ Institut de Myologie, Paris, France; 4 Inserm Avenir Group, IGBMC, Illkirch, France; 5 Inserm U781, Paris, France

Neurofibrillary degeneration (NFD) is a neuronal lesion often associated with cognitive impairment and common to several dementing diseases. Intraneuronal aggregates made of abnormally modified isoforms of microtubule-associated protein Tau characterize NFD. Etiological factors promoting Tau aggregation remain ill defined. In myotonic dystrophy type 1 (DM1), we showed an indirect mis-splicing of Tau associated to NFD. Here, we aim to uncover the molecular mechanisms leading to Tau mis-splicing. We show in vitro that the CTG expansions and MBNL1 invalidation reproduce Tau mis-splicing. In addition, ectopic expression of the different isoforms of MBNL1, along with the CTG expansions, partially restores Tau mis-splicing, showing a strong implication of MBNL1. Interestingly, we observe that the longest isoforms of MBNL1 were likely more efficient to restore Tau splicing. We therefore perform structure/function studies by generating truncated forms of MBNL1 and analyzing their function on Tau

splicing. The carboxy-terminal region of MBNL1 does not colocalize to intranuclear inclusions of CUG expansions nor modifies the alternative splicing of target transcripts. In sharp contrast, the amino-terminal region of MBNL1 (MBNL1-Nter) colocalizes with intranuclear CUG expansions, although it does not modify alternative splicing of target transcripts. More importantly, in the presence of the CUG expansions, MBNL1-Nter is sufficient to partially restore Tau mis-splicing. In conclusion, our results showed that MBNL1-Nter is sufficient to restore Tau mis-splicing. Moreover, preliminary experiments in a mouse model of DM1 showed a low toxicity and the colocalisation of MBNL1-Nter with the nuclear foci in the muscle fibres, suggesting that MBNL1-Nter might be considered as a potential therapeutic tool.

S2-04 A (CCUG)n-binding protein that may explain the lesser severity of DM2

Caroline Hammer 1, Ludovic Guigou 1, Laura Ranum 2, Jack Puymirat 3, Guillaume Bassez 4, Denis Furling 5 and Nicolas Charlet 1
1 IGBMC, U964, Illkirch, France ; 2 University of Minnesota, Minneapolis, USA; 3 Centre Hospitalier Universitaire de Québec, Québec, Canada 4 Faculté de Médecine de Créteil, Créteil, France ; 5 Institut de Myologie, UMR787, Paris, France

Although DM2 patients have a longer microsatellite expansion (75-11000 CCTG) than cDM and DM1 patients (50-4000 CTG), the disease course of DM2 patients is milder and there is no congenital form. Thus, the MBNL1 sequestration model alone is insufficient to explain the differences in pathogenesis between cDM/DM1 and DM2. We identified a protein, CCUG-BP1, that co-localizes with CCUG but not CUG repeats in RNA aggregates of muscle tissues from DM2 and DM1 patients. In transfected COS cells, eGFP-CCUG-BP1 did not co-localize with other types of RNA expanded repeats like (CGG)n or (AUUCU)n, causing FXTAS and SCA10 diseases respectively.
UV-crosslinking assays showed a direct interaction between purified recombinant CCUG-BP1 and CCUG repeats but not CUG repeats. Moreover, we found by gelshift assays that MBNL1 and CCUG-BP1 display roughly the same affinity for CCUG repeats. By western-blotting known amounts of purified recombinant protein along with protein extracts from healthy muscles, we found similar concentrations of MBNL1 and CCUG-BP1 in those tissues.
Taken together, these data suggest that CCUG-BP1 and MBNL1 compete for binding expanded CCUG repeats in DM2 muscle cells. We confirmed our hypothesis in vitro, as purified CCUG-BP1 is able to significantly decrease MBNL1 binding to CCUG repeats, even when the two proteins are in a 1 to 1 ratio. Also, transfection of muscle cells from a DM2 patient showed that overexpressed CCUG-BP1 prevents MBNL1 from being trapped into CCUG foci. To address whether CCUG-BP1 co-localization with CCUG foci is deleterious to the cell, we are currently determining the immobile fraction of CCUG-BP1 and MBNL1 in CCUG aggregates of transfected HeLa cells by FRAP experiments. Because CCUG-BP1 is involved in splicing regulation, we are investigating the splicing outcome of targets regulated by CCUG-BP1 but not MBNL1 in muscle tissues of control, DM1 and DM2 patients.
In conclusion, we propose a model in which the competition between CCUG-BP1 and MBNL1 for binding CCUG repeats would result in a higher soluble fraction of MBNL1 than in the case involving CUG repeats, leading to the less severe symptoms displayed by DM2 patients compared to the cDM/DM1 ones.

S2-05 Identification of CUGBP1 mRNA Targets by CUGBP1/RNA Immuno-capture

Linda L. Bachinski 1, Claudia Huichalaf 2, Keith A. Baggerly 1, Benedikt Schoser 3, Bjarne Udd 4, Lubov T. Timchenko 2 and Ralf Krahe 1
1 University of Texas M. D. Anderson Cancer Center, Houston, TX, USA; 2 Baylor College of Medicine, Houston, TX, USA; 3 Friedrich-Baur Institute, Ludwig-Maximillians-University, Munich, Germany; 4 Tampere University Hospital and Medical School, Tampere, Finland

Rationale: In myotonic dystrophy (DM) CUGBP1 is hyper-phosphorylated and steady-state protein levels are upregulated. In both its phosphorylated and unphosphorylated isoforms, CUGBP1 is a key regulator of alternative splicing and protein translation.
Methods: To identify CUGBP1 mRNA targets and to determine whether these targets are dysregulated in DM, we extracted RNA from CUGBP1 immunoprecipitates of ribonuclear protein fractions from normal and DM cells and hybridized these to Affymetrix exon arrays. To determine the normal targets of CUGBP1, we compared immunoprecipitated results to those from whole transcriptome.
Results: Captured probes were differentially enriched for targets from 5'ends, 3'ends, and alternative exons (overall p-value = 0.024 for top 50 probes). Of the 26 known CUGBP1 targets, we identified 14, most of which had enriched probes in the expected regions of the target genes. Known targets identified included CLCN1, MAPT, MEF2A, MELK, MTMR1, SRF, WEE1, AURKA, AURKB, CDC2, CEBPB, CSKN2B, MOS, and RBM9. Focusing on genes known to be important in muscle differentiation and maintenance, we identified novel candidate CUGBP1 mRNA targets from all three predicted target sites, namely 3', 5', and internal exons. Analysis identified many probes with significant differences between normal cells and DM. Interesting candidates included CEBPB and MEF2A (known CUGBP1 targets), CRYBB3 (lens crystalline), PPEF1 (Ca+2 binding), KCNN2 (Ca+2-activated K+ channel) and RBM6 (RNA binding motif protein).
Conclusion: Identification of CUGBP1 target mRNAs is an important step in the elucidation of pathways that are affected in DM by elevation of CUGBP1.

S2-06 Protein and Micro-RNA binding behavior of DMPK messenger RNA

Van den Broek W.J.A.A. 1, Mulders, S.A.M. 1, Gourdon, G. 2, Wansink, D.G. 1 and Wieringa, B. 1
1 Department of Cell Biology, Nijmegen Centre for Molecular Life Sciences, Radboud University Nijmegen Medical Centre, The Netherlands, 2 Inserm U781, Clinique Maurice Lamy, Hopital Necker-EM, 75015 Paris, France

The progressive nature of disease in DM1 can best be explained by an accumulation of problems caused by (i) somatic expansion of the unstable (CTG•CAG)n repeat and (ii) capture of cellular factors by DMPK transcripts with long (CUG)n tracts, resulting in cell stress accompanied by formation of abnormal RNA protein complexes. We present here different intrinsic and extrinsic conditions - of both natural and experimental nature - that modulate DM1 pathogenic effects at the RNA level.
RNA folding software predicts that the DMPK 3'UTR including the unstable (CUG)n tract engages in a complex 3D conformation, whose structure appears to depend on the context of the entire transcript sequence. Cell and animal models, which carry the (CTG.CAG)n repeat tract in a chimaeric context may therefore be not perfect models for mimicking the situation in patients. Theoretical and experimental tools helped us to explain how different segments in the mutant DMPK mRNA sequence could be involved in the formation of

higher order RNA-RNA or RNA-protein complexes. Candidate seed sequences or miRNA-responsive elements (MREs) were recognized in the 3'UTR of the DMPK mRNA, with the potential to form base-paired duplexes with miRNAs (miR-1/-206, -15, -103/-107), i.e. small, conserved RNAs of ~20 nucleotides that are cell type-dependent regulators of gene expression with a fundamental role in development, differentiation and protection to functional overload. We show that (i) overexpression of some of these miRNAs compromized translation of reporter constructs with the DMPK 3'UTR and (ii) expression-saturation with transgenic mRNAs that carry such seed elements may perturb normal miRNA control over known targets in the myogenic differentiation program (i.e., "miRNA sponge activity").
In parallel, we used myoblast-myotube cell cultures derived from DM mouse models to demonstrate that dose of the expanded DMPK mRNA determines protein factor binding, resulting in RNP aggregation and ultimately nuclear foci formation. Surprisingly, we find that certain heat shock proteins, the ubiquitin proteasome system and the general metabolic state of cells are important determinants of the number of foci formed.

S2-07 Identification of novel candidates that intervene in Myotonic Dystrophy Type 1 (DM1)

François-Xavier Laurent, Alain Sureau and Joëlle Marie
Gif sur Yvette

In Myotonic dystrophy type 1 (DM1), the sequestration of Muscleblind-like family proteins by the mutant DMPK mRNA and the hyperphoshorylation of CUG-BP1 result in a misregulation of the alternative splicing of various pre-mRNAs. However, several observations suggest that other factors could be involved in the DM1 pathogenesis.
Our project is to characterize protein complexes that interact with CUG repeats by affinity chromatography purification using in-vitro transcribed dsRNA containing CUG repeats. RNAs were biotinylated, bound to streptavidin agarose and incubated with nuclear extracts. Eluted proteins were separated by SDS-PAGE. The results show that several proteins are associated with the repeats according to the myogenic differentiation. Mass spectrometry analysis revealed numerous candidates, including dsRNA binding proteins, splicing regulators and mRNA export factors.
These candidates are tested for several features related to DM1:
1- Colocalisation with RNA foci using RNA-FISH in HeLa and C2C12 cells overexpressing the 3'UTR of DMPK containing 960 CTG repeats. The positive candidates will be tested in DM1 primary myoblasts.
2- Putative interaction with MBNL1 using co-immunoprecipitation in mammalian cells.
3- Investigation whether the candidates regulate the alternative splicing of pre-mRNAs misregulated in DM1 using minigenes. The characterisation of some candidates will be presented and discussed.

S2-08 The DMSXL transgenic mice carrying very large expansions exhibit molecular and physiological defects: an animal model for gene therapy experiments?

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1 Inserm U781, Hôpital Necker Enfants-Malades, UniversitéParis Descartes, Paris, France, 2 Institut de Myologie-Umrs974, UniversitéPierre et Marie Curie-Paris6/ Inserm/ CNRS, Paris, France, 3 Inserm U955, Département de Neurosciences, Facultéde Médecine, UniversitéParis XII, Créteil, France, 4 Facultéde Médecine, Atelier de Régénération Neuromusculaire, UniversitéPierre-Marie Curie Paris, France, 5 UMRS-956, Facultéde Médecine Pierre-Marie Curie, Paris, France, 6 Institut de Myologie, Hôpital PitiéSalpêtrière, Paris, France, 7 Département de Génétique Humaine, CHUQ (CHUL), Facultéde Médecine, Universitéde Laval, Québec, Canada

Several years ago, we developed transgenic mice carrying the DM1 locus covering about 45 kb of human DNA sequences with different CTG repeat lengths (20, 55 or 300 CTG). Mice carrying >300 CTG repeats showed very high levels of somatic and intergenerational instability biased towards expansion, which have been previously reported. Thanks to the CTG repeat instability over successive mouse generations and to large expansion events, we obtained, after 10 years of breeding, transgenic mice carrying >1000 CTG and up to 1800 CTG (mice with >1000 CTG are named DMSXL mice). The human DMPK transgene is expressed under the control of its own promoter and shows a pattern of expression similar to that observed in humans and the Dmpk endogenous gene. However, expression levels remain low for the DMPK transgene in mice and only homozygous mice show a phenotype. Mice homozygous for the transgene with very large repeats show a stronger phenotype than mice homozygous for ~500 CTG. Furthermore, they display abundant DMPK nuclear foci, Mbnl1 sequestration and splicing abnormalities. These mice also express the DMPK antisense, recently described, in different tissues including skeletal muscle, heart and brain. DMSXL homozygous mice are currently used in several collaborative gene therapy experiments, not only in muscle but also in systemic approaches. The molecular and physiological characterisation of the DMSXL mice in several tissues will be presented and discussed.
This work received financial support from ANR, AFM and Inserm.

S2-09 A second example of RNA-gain-of-function disease: Sam68 and MBNL1 sequestration by CGG repeats in Fragile X Tremor Ataxia Syndrome

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FXTAS is a neurodegenerative disorder of older adult carriers of premutation allele (50 to 200 CGG repeats) in the 5'UTR of the Fragile X Mental Retardation 1 gene (FMR1). The pathologic hallmark of FXTAS is the presence of ubiquitin positive intra-nuclear inclusions in neurons and astrocytes with a broad distrubution thoughout the brain.
We set up a cellular model in which transfection of expanded CGG repeats leads to accumulation of intra-nuclear CGG RNA aggregates visualized by RNA FISH experiments. In contrast to CUG or AUUCU foci, CGG aggregates are dynamic structures that enlarge over time and accumulate various proteins sequentially.
We found that a CGG-BP1 protein is sequestered by CGG repeats in cultured cells and FXTAS patient. Next, we identified the splicing factor Sam68 to specifically colocalize with CGG aggregates. FRAP ex-

periments demonstrated immobilization of Sam68 within CGG aggregates. Minigene responding to Sam68 also responds, but in an opposite direction, to CGG repeats, thus suggesting a functional sequestration and loss of function of Sam68. Finally, we shown late accumulation of hnRNP A, hnRNP G and MBNL1 within the larger CGG aggregates. Using various shRNA and mutants, we found that Sam68 recruitment is an early event required to the latter recruitment of hnRNP A, hnRNP G and MBNL1 through protein-protein interactions.
In conclusion, our results suggest that like CUG repeats, CGG expanded repeats form intra-nuclear RNA aggregates that sequester specific proteins resulting in loss of their functions. However, in contrast to CUG repeats, expanded CGG repeats sequester various proteins sequentially, among which depletion of Sam68 splicing activity leads to specific splicing alteration in FXTAS patients.

S2-10 Development of a muscleblind-like 1 (Mbnl1) overexpression model to assess the use of MBNL1 as a potential therapeutic for myotonic dystrophy

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Several lines of evidence support an RNA gain-of-function mechanism for myotonic dystrophy (DM) where CUG- or CCUG-repeat transcripts accumulate and sequester RNA binding proteins such as muscleblind-like 1 (MBNL1). These data strongly suggest a model where MBNL1 is sequestered by toxic RNA resulting in misregulation of downstream MBNL1 targets. This hypothesis has been supported by previous work demonstrating that delivery of Mbnl1 to HSALR murine TA muscle decreases myotonia, reverses splicing abnormalities, and re-distributes Mbnl1 throughout the nucleus. To validate MBNL1 as a potential multisystemic therapy for DM, the toxicity of MBNL1 overexpression must be determined. To address this question we are developing two Mbnl1 overexpression mouse models. In the first, an Mbnl1 transgene is regulated by the ubiquitous chicken β-actin promoter. The second model is a cre-inducible model in which Mbnl1 overexpression can be induced in specific tissues after removal of a floxed stop cassette. The toxicity of Mbnl1 overexpression will be determined by assessing viability, gross histological changes, and behavioral abnormalities. Inducible and/or constitutive Mbnl1 overexpressor mice will be crossed to DM mice to assess the potential of Mbnl1 overexpression to reverse CUG and CCUG induced phenotypes. Data from these experiments will be presented at the meeting.

S2-11 Misregulation of diacylglycerol kinase eta (DGKη) splicing as a potential cause of neuropsychiatric symptoms in myotonic dystrophy type 1

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Myotonic dystrophy type 1 (DM1) is a neuromuscular disease also affecting the central nervous system. Daytime sleepiness and personality change are among the most frequent complaints of DM1 patients and their families. In order to shed light on the molecular basis of a "brain disorder in DM1", we have completed a survey (Human Exon 1.0 ST array, Affymetrix) of mis-regulated splicing events on mRNAs from autopsied temporal cortex of DM1 and non-DM1 patients. We picked up a total of 10 candidate exons with a stringent condition, and found by RT-PCR that two were indeed aberrantly spliced, specific to DM1. One is aberrantly spliced diacylglycerol kinase eta (DGKη). Diacylglycerol kinase (DGK) regulates signal transduction processes controlling the balance between two bioactive signaling lipids, diacylglycerol (DG) and phosphatidic acid (PA), both of which regulate important enzymes such as PKC. There are ten DGK isozymes encoded by independent genes. DGKη belongs to Type II DGK containing PH domain at the N-terminal. We investigated the role of DGKηmissplicing in DM1 brain dysfunction. As a result,
1. We developed a minigene carrying the misspliced exon and confirmed the splicing regulatory elements.
2. In situ hybridization studies showed DGKηis expressed exclusively in dentate gyrus, which is part of the hippocampal formation.
3. Each DGKηsplice variant showed subcellular localization different from each other, suggesting the functional difference.
4. We demonstrated, in vitro, missplicing of DGKηin DM1 lead to the activation of ERK pathway, independently of its catalytic activity.
Recent studies showed strong genomic association between DGKηand bipolar disorder (Banum AE et al., Mol Psychiatry 2008). In addition, genetic mutations in the RAS/MAPK pathway have been known to cause widespread disturbance of central nervous system (Aoki Y et al, Hum Mutat 2008). Overall, it is speculated that DGKηmissplicing contributes to neuropsychiatric problems in DM1 through activating the ERK signal.

S2-12 Study on the mechanisms of regulation and deregulation of hcTNT exon 5 inclusion

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Aberrant alternative splicing of several pre-mRNAs has been reported to contribute to some of the DM symptoms. The MBNL and CELF protein families are frequently involved in regulation of the alternative splicing events which are altered in DM cells. The human cardiac troponin T (hcTNT) exon 5 is included at an abnormal rate in DM1 patients. Two MBNL1 sites and three CUG-BP sites were previously described upstream and downstream from exon 5, respectively. By sequence inspection, followed by footprinting experiments, site-directed mutagenesis and EMSA, we identified six additional intronic MBNL1 binding sites in the vicinity of hcTNT exon 5. We developed an in cellulo approach in order to test the functional importance of these new MBNL1 binding sites. By use of a mini-gene, we defined the hcTNT pre-mRNA regions which are required for normal exon 5 inclusion and to get the trans-dominant CUG repeat effect, respectively. We established the secondary structure of the overall hcTNT pre-mRNA region required for regulation and deregulation and defined the protein binding sites by footprinting assays. The newly identified MBNL1 binding sites are needed for both regulation and deregulation of exon 5 inclusion. We observed the involvement of other regulatory elements which do not bind MBNL1. We are currently identifying their protein partners. This will allow us to understand the functional interplay between MBNL1 and these other regulatory factors in the regulation of exon 5 inclusion and the consequences of MBNL1 sequestration by CUG/CCUG repeats.

S2-13 MBNL1 associates with YB-1 in cytoplasmic stress granules

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The muscleblind (MBNL) protein family is thought to be involved in the molecular mechanism of myotonic dystrophy (DM). Although it has been shown to have splicing activity, a broader function in cellular RNA metabolism has been implicated. In this study, we attempted to find the binding proteins of MBNL1 in order to elucidate its physiological function. First, we performed a GST pull-down assay using GST-MBNL140 as bait and a multifunctional DNA/RNA binding protein YB-1 was identified. MBNL1 formed an RNP complex with YB-1 in binding assays. YB-1 also showed a weak but significant effect on a-actinin splice site selection.
Interestingly, in response to stress, MBNL1 moved to cytoplasmic granules where it co-localized with YB-1, which was previously reported to be a component of stress granules. Endogenous MBNL1 exhibited two distinct localization sites in response to heat shock: one in the cytoplasm and the other in the nucleus. These results provide new insight into the dynamics of MBNL1 in response to stress, and they suggest a role for MBNL1 in mRNA metabolism in the cytoplasm.

S2-14 Abnormal secretion of prostaglandin E2 inhibits differentiation of congenital myotonic dystrophy muscle cells

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Congenital form of myotonic dystrophy type1 (cDM1) is the most severe form of the disease associated with CTG expansion over 1500 repeats and delayed muscle maturation. We have previously showed that the differentiation of myoblasts isolated from cDM1 patients is impaired. In the present study, we were interested to the potential role of soluble secreted factors in cDM1 differentiation defect. We found that the level of prostaglandin-E2 (PGE2) was increased in the medium of cDM1 muscle cells. This increase was correlated with a higher level of cyclooxygenase-2 (Cox-2) protein, a rate-limiting enzyme involved in the prostaglandin synthesis. Increased levels of PGE2 inhibit myogenic differentiation by decreasing the intracellular calcium concentration. Interestingly, exogenous addition of acetylsalicylic acid, an inhibitor of Cox enzymes, abolishes PGE2 abnormal secretion and restores the differentiation of cDM1 muscle cells. The up-regulation of the Cox-2 protein was found both in muscle cells in vitro and in the muscles biopsies from cDM1 patients. CUGBP1 is able to modulate the level of Cox-2 however increased level of CUGBP1 in cDM1 muscle cells seems to be necessary but not sufficient to increase Cox-2 expression suggesting that other factors may be involved in the up regulation of Cox-2 in cDM1. Altogether, our results suggest that altered autocrine mechanism leading to abnormal secretion of PGE2 may be involved in the delay of muscle maturation observed in cDM1.

S2-15 Quantitative semi-automated expanded CUG repeats assessment: application to the DM-SXL mouse.

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Myotonic dystrophy evaluation is challenging due to complex pathophysiological mechanisms that occur in various tissue and cell types. Among molecular abnormalities, one key biomarker of DM1 is the nuclear accumulation of toxic expanded CUG repeats, as "foci", that can be evidenced by fluorescent in situ hybridization (FISH). In view of the multi-organ involvement of DM1 and systemic delivery of new therapeutic approaches, we aimed at developing a standardized method to precisely quantify and monitor the nuclear foci load in specific tissues. In DM-SXL, a DM1 mouse model carrying a 1200-1750 CTG repeats expansion, we assessed the number, the size, and the nucleus surface occupied by foci accumulation. We found a high content of foci, as compared to the DM500 model, that significantly varies in nine different skeletal muscles, in ten brain regions and in a variety of tissues. Similarly, a heterogeneous anatomical distribution was observed within the TA muscle. We monitored the foci content in 1-month and 4 month-old mice and further investigated the cell-type (myonuclei, endothelial cells, satellite cells) of the foci accumulation in skeletal muscle. Interestingly, we evidenced a continuum in size for CUG repeats accumulation from small unit to large aggregates and showed their colocalization with Mbnl1 in skeletal muscle and brain of DM-SXL mice. This accurate and reproducible quantification method allows the characterization of multiple tissues of the DM-SXL model, thus providing a valuable tool to measure the efficiency of intramuscular or systemic therapy.

S3 Pathomechanisms in DM – Part 2

S3-01 Investigating cellular and molecular abnormalities in the central nervous system of mice carrying large repeat expansions

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Although traditionally regarded as a muscle disease, DM has emerged as a brain condition. Excessive daytime sleepiness, cognitive decline, personality disorders, reduced initiative, apathy and anhedonia have all been reported to a variable extent. Experimental evidence supports a trans-dominant effect of CUG-containing transcripts in DM1 pathogenesis. However, the molecular and cellular pathways, connecting the CTG repeat expansion to the debilitating neurological symptoms are still unclear. We have previously generated transgenic mice carrying

a highly unstable 300-CTG repeat expansion within the human DM1 locus. Region-specific RNA foci accumulation, abnormal steady-state levels of splicing regulators and misplicing of candidate genes have been detected in the CNS of mice carrying >1000 CTG repeats. Taking advantage of these animals, we have sought to investigate the molecular and cellular neuropathogenesis of DM1. (1) At the molecular level, epitope-specific Tau hyperphosphorylation has been detected in hippocampus, thalamus and striatum of transgenic mouse brains, while absent in other brain regions. We are currently investigating the molecular mechanisms underlying regional Tau hyperphophorylation. Novel disease intermediates and pathways, potentially affected by the CTG expansion in the CNS, have been identified by a global proteomic approach and are currently being validated. (2) At the cellular level, histopathological analyses have explored abnormalities in transgenic mouse brains. Ongoing functional assays will help to dissect the physiological consequences of the DM1 mutation in the CNS.
In conclusion, using these animals, we hope to provide new insight into the molecular and cellular pathways affected by the DM1 repeat expansion in the CNS.

S3-02 An unusual family co-segregating myotonic dystrophy type 1 and Charcot-Marie-Tooth disease present with an imperfect CTG repeat allele at the DM1 locus

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Background: Charcot-Marie-Tooth disease (CMT) is a genetically heterogeneous hereditary motor and sensory neuropathy of the peripheral nervous system. The DM1+CMT++ family is an unusual three-generation family in which all 14 patients co-segregate both myotonic dystrophy type 1 (DM1) and Charcot-Marie-Tooth disease (CMT) (LOD score = 8.03).
Objectives: Investigate the nature of mutation in the DM1+CMT++ family and in a number of other sporadic DM1 cases with an unusual molecular diagnosis.
Results: We identified a complex repeat array at the DM1 locus with the following structure: (CTG)x (GGC)3 G (CCG)20 (CCGCTG)14 (CTG)35. The pure CTG repeat tract at the 5'-end was unstable in the soma and in the germline, but the interrupted 3'-end appeared to be stable. Similar imperfect CTG repeat alleles containing CCGCTG and/or CCG repeats were also identified in other DM1 patients with an unusual molecular diagnosis.
Conclusions: The expanded (>86 repeats) CTG tract at the 5'- end of the array explains the classic DM1 symptoms. A novel effect on the downstream genes; and/or a novel RNA gain-of-function mediated by the presence of CCG and CGG repeats, analogous to the RNA gain-of-function observed in fragile X associated tremor ataxia syndrome, might explain the CMT symptoms in this family. Imperfect CTG repeat alleles are present in more DM1 patients than previously realized and probably accounts for some of the otherwise enigmatic symptomatic variation observed within and between DM1 families. A more detailed molecular characterization of the mutation in individual DM1 families, should lead to provision of more accurate prognoses and the development of effective therapies.

S3-03 De novo base substitution mutations at the myotonic dystrophy type 1 locus

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Background: Recently we showed that a large family with a very unusual DM1 diagnosis presented with an imperfect expanded CTG allele. In the current study, we investigated the prevalence of imperfect expanded CTG alleles by screening 142 typical DM1 patients.
Methods: Patients were investigated using a combination of PCR, restriction digests, cloning, and sequencing.
Results: We found eight patients with imperfect alleles, seven of which had a known family structure. In these cases we confirmed that the mutations that resulted in these imperfect alleles were de novo, as they were not present in the preceding generation. As all these mutations appear to have arisen as base substitutions, we estimated the base substitution rate to be 0.02% per base per generation.
Conclusions: This study shows that in addition to being a hot spot for expansion mutations, this locus is also a hotspot for base substitution mutations, with a rate that is 10-fold higher than the most mutable locus in the human genome. Thus far all mutations have been observed at the 3'end of the CTG allele. This polarity implicates a role for cis factors in promoting mutations at this locus. Correlation of genotype and phenotype information showed that in one family, individuals with these mutations presented with symptoms that were much less severe. Taken together, this work suggests that variant repeats are more common at the DM1 locus than previously expected, and might impact disease variation. Therefore, they should be accounted for in future studies of DM1.

S3-04 Molecular pathophysiology and CNS effects in mouse models of myotonic dystrophy types 1 & 2

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To assess if DM1 and DM2 differences are independent of the repeat motif and genetic context, but instead result from temporal and spatial expression patterns specific to the affected genes, we developed multisystemic transgenic mouse models of DM1 and DM2. TRE-(CCTG)300 expansion mice were crossed to BAC mice expressing the tet-inducible transactivator (tTA) under the control of either the endogenous human DMPK or ZNF9 promoter. Doubly transgenic DM1-CCUG [TRE-CCTG(300):DMPK-tTA] and DM2-CCUG [TRE-CCTG(300):ZNF9-tTA] mice show broad expression and multisystemic phenotypes in skeletal muscle and brain including RNA foci and splicing abnormalities.

DM is a multisystemic disease that significantly affects the CNS causing abnormal cognition, behavioral abnormalities and progressive memory problems. To investigate CNS RNA gain-of-function effects in DM1 and DM2, functional cerebellar optical imaging studies were performed on newly developed DM1-CCUG and DM2-CCUG mice and on the Mbnl1ΔE3/ΔE3 loss-of-function model of DM. Flavoprotein optical imaging studies of DM2-CCUG and Mbnl1ΔE3/ΔE3 mice in vivo show reductions in mGluR1 dependent parallel fiber-Purkinje cell long-term potentiation (PF-PC LTP). Additionally, initial studies show that the DM2-CCUG and Mbnl1ΔE3/ΔE3 mice exhibit a lack of mGluR1 dependent high frequency PF stimulation evoked patches of activation. DM1-CCUG mice lack LTP of the patches. These observations suggest that RNA gain-of-function effects in DM cause fundamental deficits in CNS plasticity which may contribute to the neurologic features of DM disease

S3-05 A novel role for the protein RFDM in restoring fusion of fetal DM1 muscle satellite cells

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S3-06 The role of microRNAs in the regulation of gene expression in Myotonic Dystrophy

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Myotonic Dystrophy 1 is a neuro-muscular disease caused by non-coding CTG repeat expansion in the 3'UTR of DMPK gene on chromosome 19q. We showed that CTG expansion causes DM1 pathology at the RNA level. The mutant CUGn RNA affects RNA-binding proteins, CUGBP1 and MBNL1, altering RNA processing in patients'tissues. CUGBP1 is a multifunctional protein which controls translation, splicing and RNA decay. CUGBP1 regulates RNA decay through the binding to ARE elements in the 3'UTRs of mRNAs. We recently found that CUGBP1 may also control RNA stability by delivery of microRNAs (miRs) to mRNAs and following inhibition of translation of these mRNAs. To elucidate the role of CUGBP1-miR pathway in DM pathology, we have compared global expression of miRs in myoblasts and fibroblasts from control, DM1 and DM2 patients. This analysis showed that the levels of three miRs are specifically altered in DM1 and DM2 myoblasts. MiRs let7A and let7I are reduced in both DM1 and DM2 myoblasts. MiR-365 is significantly increased in DM1 myoblasts, but not in DM2 myoblasts. We have found that CUGBP1 specifically binds to miRs 365 and let7A. These miRs have predicted RNA targets among several functional groups of genes which are significantly changed in DM1 and DM2. These genes regulate different functional pathways including cell adhesion, cell cycle, cytokines, DNA replication and tight junction. The elucidation of the role of the identified miRs in DM1 and in DM2 will help better understand pathological alterations and develop therapeutic approaches to cure DM1 and DM2.

S3-07 Study on human MBNL1 RNA binding properties

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In Myotonic Dystrophies (DM), MBNL1 binds to CUG/CCUG repeats and is sequestered in nuclear foci. This sequestration causes misplicing of several pre-mRNAs, which subsequently leads to some of the DM symptoms. For a better understanding of the RNA binding properties of MBNL1 and its various isoforms, we performed both SELEX and 3-hybrid experiments. For SELEX experiments we used a library of RNA stem-loop structures with a central 18-nt long randomized sequence and recombinant C-ter truncated MBNL1 protein (MBNL1ΔCt-GST). After 4 rounds of selection 53 different RNAs having a strong affinity for MBNL1ΔCt were identified. They all contain the (U/G)(U/C)GCUU sequence. 17 of them share an 11-nt long conserved sequence and a common folding that is preserved by base-pair compensations. These 17 RNAs contain only one MBNL1 binding sequence, which is located in the terminal loop. The 36 other RNAs are more heterogeneous and most of them contain multiple MBNL1 binding sites. Experimental analysis of their 2D structures revealed the presence of several non-canonical base-pairings. Therefore, MBNL1 can recognize different kinds of RNA motifs and the presence of one (U/G)(U/C) GCUU sequence can be sufficient for efficient MBNL1 binding depending on the context. We next compared the relative RNA binding affinities of each of the 9 MBNL1 isoforms. 3-hybrid tests were made with the SELEX RNAs, CUG repeats and pre-mRNAs MBNL1 binding sites. The SELEX RNAs were found to have the highest affinities. In addition, exon 4 was found to modulate the MBNL1 RNA binding properties.

S3-08 Testing the role of CUGBP1 in skeletal muscle wasting in DM1

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CUGBP1 protein levels are elevated in cardiac and skeletal muscle tissues from DM1 individuals. Elevated CUGBP1 may have pathological consequences for both its nuclear and cytoplasmic RNA processing activities. The role of elevated CUGBP1 in muscle wasting is supported by several correlations. First, constitutive overexpression of CUGBP1 in embryonic mice resulted in impaired myogenesis and muscle histology similar to DM1 patients. Second, there is a temporal correlation between CUGBP1 up-regulation and overt muscle wasting in a DM1 mouse model (EpA960/HSA-Cre) expressing RNA containing 960 CUG-repeats. Third, two MBNL1 depletion mouse models for DM1 (HSALR and Mbnl1ΔE3/ΔE3) show many pathological features of DM1, but neither demonstrate increased CUGBP1 levels nor muscle wasting. Fourth, DM2 patients do not have increased CUGBP1 levels and typically have much milder wasting. To test the hypothesis that CUGBP1 either contributes to or is primarily responsible for the muscle wasting in DM1, we have generated adult mice with inducible and skeletal muscle specific expression of CUGBP1. Transgenic mice with a modified reverse tetracycline transactivator gene driven from the myosin light chain 1/3 promoter/enhancer (MDAFrtTA) were crossed to a tet-responsive CUGBP1 responder line (TRECUGBP1). Bitransgen-

ic MDAFrtTA/TRECUGBP1 mice fed a doxycycline-containing diet showed high induction of CUGBP1 in all skeletal muscles tested including the gastrocnemius, quadriceps, triceps and tibialis anterior. In addition, bitransgenic MDAFrtTA/TRECUGBP1 mice have reduced body weight and aberrant splicing of CUGBP1-regulated events. Results from muscle histology and functional studies as well as implications for the role of CUGPB1 in muscle wasting will be presented.

S3-09 Accumulation of Mutant DM2 (CCUG)n Transcripts Triggers Activation of PKR and ER Stress Response

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Here we report that expression of mutant DM2 (CCUG)n transcripts, which are prone to form double-stranded (ds) hairpin structures, activates the interferon-inducible dsRNA-dependent protein kinase (PKR). PKR is a sensor of increased genotoxic and cellular stress, and its activation leads to the down-regulation of translation by phosphorylation of the eukaryotic translation initiation factor 2α(eIF-2α). By using primary human DM2 myoblasts and skeletal muscle from transgenic DM2-HSA-(CCTG)121 mice, we show that phosphorylation of PKR occurs at Thr446 and Thr451 in DM2 but not normal cells. PKR activation induces its nuclear translocation in DM2 cells, which in turn leads to phosphorylation of eIF-2αat Ser51 and increases its affinity to another translation initiation factor eIF-2B. Induction of ER stress in DM2 cells is further indicated by the upregulation of ER chaperons (GRP78 and GRP94), ER transmembrane proteins (PERK and IRE1), and heat-shock proteins (HSP70 and HSP90). We also show that activation of PERK, another ER stress sensor and eIF-2αkinase, occurs by phosphorylation at Thr980. PERK activation results in the release of BiP from the PERK/BiP complex present in normal cells. Finally, we show that attenuation of translation through eIF-2αactivates apoptosis as evidenced by cleaved caspase-3 and its target PARP-1. In conclusion, the activation of the cellular stress sensors PKR and PERK in response to the expression of the toxic DM2 RNA induces the ER stress response through two separate, but converging pathways centered on eIF-2α. Inactivation of eIF2-αis predicted to globally attenuate translation in DM2 cells.

S4 Models for DM

S4-01 Animal models for DM

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S4-02 Muscleblind Loss-of-Function Models for Myotonic Dystrophy

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Current disease models for both types of myotonic dystrophy (DM1, DM2) propose that expression of toxic (C)CUG RNAs adversely affects alternative splicing during postnatal development by impairing the activities of the muscleblind-like (MBNL) proteins while triggering enhanced phosphorylation and stability of CUGBP1. Although Mbnl1 isoform knockout mice develop several of the characteristic features of DM disease (myotonia, abnormal myofiber structure, particulate cataracts, RNA mis-splicing), loss of Mbnl1 does not lead to muscle wasting or to congenital DM (CDM). Since the MBNL loss-of-function model for DM predicts that toxic (C)CUG RNAs impair the activities of all MBNL proteins, we are developing Mbnl2 and Mbnl3 conditional knockout lines and results obtained from these models will be discussed. In addition, we have previously shown that Mbnl1 overexpression, following direct intra-muscular injection of recombinant adeno-associated virus (rAAV)2/1-Mbnl1, reverses myotonia and RNA mis-splicing in the HSALR model for DM. This therapeutic approach has now been extended to compare the systemic effects of rAAV-mediated overexpression of Mbnl1, Mbnl2 and Mbnl3.

S4-03 Insights from mouse models of RNA toxicity

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Rationale: Myotonic dystrophy type 1 (DM1) is the prototype for RNA mediated disease pathogenesis. We have developed inducible/reversible mouse models of RNA toxicity as models for DM1. Surprisingly mice overexpressing a normal DMPK 3'UTR develop DM1 phenotypes. We have now been developing an inducible mouse model expressing a mutant DMPK 3'UTR with (CTG)>200. We are using these models to study the mechanisms of RNA toxicity and to identify potential avenues for therapy.
Hypothesis: Studying the similarities and differences in mice with RNA toxicity due to a mutant DMPK 3'UTR and overexpression of the normal DMPK 3'UTR will provide better understanding about RNA toxicity.
Methods: We have used a variety of phenotypic and molecular assays including EMGs, ECGs, exercise testing, RT-PCR for RNA splicing, microscopy, western blotting, real-time RT-PCR for quantitative gene expression and mouse genetics to study these mice.
Results: The new mutant DMPK 3'UTR transgenic mouse model showed onset of myotonia and cardiac conduction abnormalities within 1-2 weeks of toxic RNA induction. In addition, the mice had many of the splicing defects found in DM1 patients (including Clcn-1, Tnnt3, Tnnt2, Fxr-1), RNA foci and MBNL1 sequestration. Upon stopping doxycycline, many of the mice reverted back to normal with loss of myotonia and correction of conduction abnormalities. The molecular changes including RNA splicing defects, MBNL1 sequestration and RNA foci also reverted. Data from ongoing studies looking at differences and similarities between DMPK3'UTR (CTG)>200 and DMPK3'UTR (CTG) 5 overexpressors with respect to phenotypic and molecular changes will be presented.

S4-04 Mouse Models for Myotonic Dystrophy Type 2 (DM2) Lacking Aberrant Splicing Implicate Novel Cytoplasmic Pathomechanisms

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We have generated transgenic mice expressing a mutant DM2 expansion of (CCTG)121 in intron 1 of the human skeletal actin (HSA) gene and knock-in mice expressing a mutant DM2 expansion of (CCTG)189 in intron 1 of Znf9. Both mouse models show muscle weakness and an overall muscle pathology associated with human DM, including aberrant type 2 muscle fibers, sarcoplasmic masses and central nuclei. The transgenic mice also have myotonia by EMG. Other phenotypic features include cardiomyopathy and sudden cardiac death, suggestive of arrhythmia, abnormalities of lens development and retinal atrophy, and azoospermia in males. Recapitulating pathological features that have been shown for DM patients, both of our transgenic and knock-in DM2 mice show nuclear foci that sequester Mbnl1, elevated steady-state levels of CUGBP1, mediated by phosphorylation through phospho-PKC, and dysregulation of CUGBP1 target genes. However, both DM2 mouse models lack aberrant splicing for Clcn1, Atp2a2a1, Ryr1, Tnnt3, Tnnt2, Ank2, Capzb and Fxr1. In addition, our DM2 mice show cytoplasmic foci and other cytoplasmic abnormalities, including activation of the ER stress response which is predicted to globally attenuate translation. Our mouse models indicate that DM-like progressive myopathy can result in the absence of aberrant splicing. They complement existing mouse models for DM and are uniquely suitable for the dissection of cytoplasmic pathomechanisms in DM.

S4-05 CCUG RNA gain-of-function effects in a conditional mouse model of myotonic dystrophy type 2 (DM2)

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We developed a tetracycline inducible murine model of DM2 to test the hypotheses that CCUG300 expansion transcripts expressed in the absence of the endogenous gene context, are sufficient to replicate the skeletal muscle features of myotonic dystrophy and that many of these features are reversible. Skeletal muscle from transgenic animals expressing transcripts with 300, but not 5, CCUGs show a number of phenotypic changes characteristic of myotonic dystrophy including: 1) variation in fiber size and central nuclei; 2) electrical myotonia; 3) ribonuclear inclusions; and 4) aberrant splicing of Capzb, Insr, and Clc1. RT-PCR assays show that the administration of doxycycline to 10 month old animals for 10 weeks turns off transgene expression and results in the loss of ribonuclear inclusions, a significant reduction in the number of central nuclei and the reversal of some of the splicing alterations. These studies show that expression of (CCUG) 300 expansion transcripts as part of an exogenous non-coding transcript is sufficient to cause a number of phenotypic characteristics of myotonic dystrophy and that some of the pathogenic changes are reversed when expression of the transgene is turned off.

Additional studies show variable expression of Cugbp1 in (CCUG) 300 mice, but no statistically significant increase in the mutant vs. control groups (p= 0.11). Combined FISH/IF studies in skeletal muscle show an increase of Cugbp1 levels in some nuclei, but no correlation with the presence or absence of CCUG foci. Unexpectedly, a dramatic increase in Cugbp1 protein (p = 0.0085), but not RNA, was found in a chloride channel knock-out mouse (Clcn1adr-mto), but not in other mouse models of muscle disease (mdx-/- and mdx-/-:utr-/-). Additionally, Mbnl1 and Cugbp2 were also elevated in the Clcn1adr-mto mice. In summary, overexpression of CUGBP1 is not a consistent feature in our (CCUG)300 model and upregulation of CUGBP1 is not unique to DM.

S4-06 Mouse model of congenital myotonic dystrophy type 1

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Rationale: Congenital myotonic dystrophy (CDM) is a devastating form of DM1 with a high neonatal mortality. No adequate animal models exist. The aim of this study was to generate a mouse model of CDM.
Methods: The model was generated using mice that carried a GFP-DMPK3'UTR transgene driven by a human DMPK promoter induced by administration of 0.2 % doxycycline. Homozygous pregnant transgenic mice were induced on d10 of gestation (onset of endogenous DMPK expression). For comparison we used uninduced transgenic mice, wildtype induced and uninduced mice, wildtype mice implanted with embryos homozygous for the transgene, and homozygous transgenic mice implanted with wildtype embryos. Embryos were collected at d12, 14, 16, 18 and the first day after birth. Expression analysis for myosin heavy chain isoforms, RNA-binding proteins and DMPK were performed. Neonatal outcomes were scored.
Results: Homozygous pups born to induced homozygous mothers were severely growth retarded with low birth weight, showed abnormal MHC expression patterns with predominance of embryonic isoforms, and had increased neonatal mortality as compared to the other groups. Notably, homozygous pups born to uninduced homozygous mothers or to induced wildtype mothers did not show growth retardation or early mortality. Wildtype pups born to induced homozygous mothers were growth retarded and showed increased neonatal mortality, clearly demonstrating a maternal effect. However, MHC expression patterns in these mice were not as severely altered as in homozygous pups suggesting a separate toxic RNA effect on muscle development.
Conclusions: This model exhibits some key features of muscle developmental defects in CDM and may be useful to study the molecular mechanisms of CDM.

S4-07 A Drosophila model for Myotonic Dystrophy Type 2 (DM2)

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In the fruit fly Drosophila melanogaster, we have generated two transgenic myotonic dystrophy type 2 (DM2) lines with 16 and 106 CCTG repeats, referred to as (CCUG)16 and (CCUG)106, respectively. Expression of (CCUG)106, but not of (CCUG)16, in the fly eye causes formation of (CCUG)n RNA foci. The external surface of (CCUG)106-expressing eyes is disorganized, whereas (CCUG)16-expressing eyes

appear normal. Photoreceptor neurons and cone cells in (CCUG)106-expressing retinae are irregularly aligned and may be reduced in number. We find that apoptotic cell death is increased in (CCUG)106-expressing eyes. Excitingly, inhibition of cell death by expression of the caspase inhibitor P35 in the (CCUG)106 model rescues the mis-alignment phenotype of photoreceptor neurons and cone cells. The external surface of (CCUG)106-expressing eyes also appears rescued by P35. These observations support the notion that expression of expanded (CCTG)n/(CCUG)n repeats in the fly eye is toxic causing inappropriate cell death which leads to misalignment and reduction in the number of photoreceptor neurons and cone cells. These data and ongoing research efforts will be presented at the conference.

S4-08 Towards a Zebrafish Model of Myotonic Dystrophy

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Myotonic Dystrophy Type I (DM1) is a multi-system, autosomal dominant disorder due to expansion of a CTG repeat sequence in the 3'UTR of the DMPK gene on chromosome 19q35. Patients with DM1 have 50 to 5000 CTG repeats, and the repeat size correlates with the age of onset and disease severity. In cases with large (>1800 CTG) repeats, the symptoms are dominated by congenital hypotonia and mental retardation. Over the past 15 years significant advances in our understanding of DM1 pathophysiology have occurred, with mounting evidence suggesting that the expanded CTG repeat sequence in the DMPK gene leads to an RNA mediated toxic gain of function. However, to date little progress has been made on how this or other mechanisms lead to the qualitatively different phenotype seen in congenital DM1. To better understand early developmental processes in congenital DM1, we are utilizing the Zebrafish, Danio rerio, to study brain and muscle development in the presence of an expanded CUG repeat containing mRNA. Initial experiments have evaluated whether direct injection of expanded CUG repeat containing mRNA can induce toxicity during early development. In addition, we are creating multiple transgenic Zebrafish lines that express different sized CTG repeats in the 3'UTR of a fluorescent reporter gene. In summary, these Zebrafish models under development are likely to serve as valuable tools for understanding early developmental events in DM1. Moreover, they represent an ideal system for future high throughput drug and genetic screens targeted at therapeutic development.

S4-09 Toxicity of noncoding CUG and CCUG repeats

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Myotonic Dystrophies type 1 and 2 are caused by expansions of noncoding RNA CUG and CCUG repeats which are dramatically increased in the patients'cells. The obvious approach to correct DM1 is to remove RNA CUG expansion by degrading the mutant DMPK mRNA. However, the biological "side effects" of degradation of mutant CUG/CCUGn RNAs are unknown. Studies of tet-inducible "reversible" DM1 mouse model showed that high levels of normal size CUG repeats (CUG5) are sufficient to cause myotonia, muscular dystrophy and cardiac conduction defects. These data suggest that the products of decay of the mutant CUG and CCUG RNAs may possess the same toxicity as un-degraded full-length mutant CUG/CCUG RNAs. To evaluate toxicity of CUGn / CCUGn RNAs during decay, we have generated tet-inducible cell models expressing short CUG25 and CCUG36 repeats and long CUG914 and CCUG100 repeats. We have found that induction of large amounts of short CUG25 or CCUG36 repeats in normal cells is sufficient to disrupt molecular pathways similar to disruptions observed in DM1/2 myoblasts and in DM1/2 muscle. Complete degradation of short or long CUG repeats removes their toxicity. We are using the generated cellular systems for the identification of proteins which regulate stability and decay of the mutant DMPK/ZNF9 transcripts to accelerate their decay without accumulation of short products of degradation. Identification of these regulatory proteins will help to develop approaches to reduce toxicity of CUG/CCUG expansions in DM1 and DM2 patients.

S5 Clinical Issues in DM

S5-01 Unusual presentations and a large proportion of mild phenotypes expand the spectrum and epidemiology of DM2

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Objective: To identify the larger range of clinical presentations of DM2 with emphasis on unusual and oligosymptomatic phenotypes and how these change the epidemiology of the disease
Methods: We have increased the ascertainment power of DM2 disease by the inclusion of new immunohistochemical techniques in the assessment of muscle biopsy samples, by screening of certain patient cohorts with other diagnoses for the DM2 mutation, and by using a low threshold for molecular genetic screening tests in patients even without myotonia on EMG.
Results: During 5 years of diagnostic efforts we have identified DM2 patients with peculiar phenotypes as well as a large proportion of patients with milder symptoms and few signs. The advanced clinical ascertainment procedures have identified new patient categories which all together have increased the prevalence of DM2 disease. The prevalence of DM2 in our study region of Central Finland is now considerably higher than the prevalence of DM1 disease.
Conclusions: The phenotypic spectrum of DM2 is definitely larger than previously reported and the average disease severity is milder. This change of parametric severity is most likely due to the fact that in the early stages of linkage studies of DM2 disease families with the most pronounced and marked phenotypes were enrolled. For some of the very rare and peculiar phenotypes uncovered bigenic complexities may be one explanation. Considering the ancient European origin of the mutation the prevalence of DM2 could be very similar in different European populations, although bottle-neck type of population backgrounds in Finland may have caused regionally higher prevalence of DM2 which has been shown also for certain regions in DM1.

S5-02 Brain imaging in DM

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S5-03 Congenital myotonic dystrophy: Canadian surveillance and cohort study

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Background: Congenital Myotonic Dystrophy (CDM) is a clinical manifestation of genetic anticipation in DM1 families. Currently there is an ongoing active surveillance and cohort study being conducted to determine the incidence of CDM in Canada. Clinical information and quality of life data is recorded in the cohort phase. This study will help provide a clear definition of CDM, identify a relationship between phenotype and genotype and identify the burden of illness when caring for a child with CDM both medically and within the family.
Methods: The prospective monthly surveillance has been initiated in March 2005 and will be complete in 2010. An incident case is a new genetically diagnosed case of CDM under the age of three years, which required hospital admission for greater than 72 hours due to neonatal symptoms directly related to DM1. All incident cases are invited to participate in the cohort study. Phone information, standardized questionnaires and reports from the primary physician are used for follow-up data.
Results: There have been a total of 91 cases reported, with 28 confirmed as CDM. Neonatal data was available for 26 cases with a mean number of trinucleotide repeat of 1236. Thirteen children are currently enrolled in the cohort study. Twenty one (21/26) children received nasogastric feeding therapy for a mean of 31 days. Seventeen (17/26) children received invasive ventilation for a mean of 24 days. Five (5/26) children died during the neonatal period, four due to withdraw of life support at a mean of 28 days.
Conclusion: Surveillance and prospective examination of CDM at a population level is important, as the impact of this rare disease is systemic, chronic and associated with significant morbidity and mortality in the newborn period.

S5-04 Spectrum of disease manifestations of juvenile myotonic dystrophy type 1 (JDM) patients

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Understanding the spectrum of JDM is essential to guide clinical care and future treatment trials. Objective: To characterize congenital DM1 (CDM) and JDM in a large, national cohort. Methods: Compared patient-reported manifestations of CDM (onset of symptoms < 4 wks to 1 yrs) and JDM (onset of symptoms 2-12 yrs.) patients in the NIH sponsored National Registry using T-tests and chi-square ($p<0.05$ indicated significance). Results: The Registry has currently enrolled 31 CDM and 24 JDM patients (~5% of all DM1 members). Average age at enrollment was 19.6 yrs in JDM and 7.3 yrs in CDM patients ($p<0.0001$). The most reported first symptoms were post-natal complications in CDM (83.9%) and grip myotonia in JDM (25.0%). No JDM patients used assistive devices, whereas CDM patients used canes (6.5%), ankle braces (35.5%), and leg braces (16.1%). JDM patients used less physiotherapy (100% versus 41.7%; $p<0.0001$) and less occupational (74.2% versus 33.3%; $p=0.002$) and speech therapies (77.4% versus 50.0%; $p<0.034$). Psychological counseling was used more by JDM patients (58.3% versus 6.5%; $p<0.0001$). Average CTG repeat size was greater in CDM (1499.5 ±434.1; n=22) than in JDM (656.9 ±267.6; n=15; $p<0.0001$). No deaths occurred for both subtypes after 0-7 yrs of follow-up. Conclusion: The spectrum of early onset DM1 is diverse. JDM patients exhibited myotonia more frequently as an initial symptom and had greater use of psychological counseling. JDM patients also reported less use of assistive devices. Further research is warranted to study the progression and potential treatment implications of JDM and CDM.

S5-05 Visual function in Congenital and Childhood Myotonic Dystrophy Type 1

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Objectives: To investigate visual function in a group of individuals with congenital and childhood DM1, to correlate the results to the size of the CTG repeat expansion and the onset form, and to compare the results to those of a control group.
Design: Cross-sectional study with age and gender matched control groups.
Participants and/or Controls: 49 individuals with severe and mild congenital and childhood DM1 and controls matched for age and sex.
Methods: The ophthalmologic examination included best corrected visual acuity, refraction, slit-lamp biomicroscopy, indirect ophtalmoscopy and flash VEP.
Main Outcome Measures: Visual acuity, refractive error, lens-, fundus- and VEP- pathology. Results: The study shows a higher prevalence of low visual acuity, hyperopia and astigmatism in the study population compared with the controls. The size of the CTG repeat expansion had an impact on BCVA in all subgroups with lower values in individuals with larger expansion size. In childhood DM1, individuals with high hyperopia and astigmatism had greater CTG repeat expansion size than those without. No true cataract was found. Subtle non-specific fundus changes were present in addition to VEP pathology.
Conclusion: Children and adolescents with DM1 have a variety of visual function pathologies and DM1 has an impact on the developing visual system, necessitating early ophthalmologic assessment and follow-up.

S5-06 Sleep-Disordered Breathing in a cohort of 40 Italian Steinert's Dystrophy patients. A clinical and polisomnographic study

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Objective: To examine the prevalence of sleep disorder breathing (SDB), excessive daytime somnolence (EDS) and fatigue in type 1 myotonic dystrophy (DM1), evaluating their association with clinical–genetic variables and their role as predictors of the risk of beginning nocturnal non invasive mechanical ventilation (NIMV).
Methods: 40 patients with genetically proven DM1 underwent neurological, cardiological and pneumological evaluation, including arterial blood gas analisys (ABG), lung function tests (PFT) and ecocardiography. All patients were also submitted to a complete polysomnographic study (PSG).
Severity of muscular involvement was assessed according to the 5 point Muscular Disability Rating Score (MDRS). EDS was defined by an Ep-

worth Sleepiness Scale score (ESS) greater than 9, fatigue by a Fatigue Scale score (FSS) greater than 36.
Results: PSG revealed a SDB in 24/40 pts (Cheyne Stoke breathing was found in 5/24).
EDS was present in 12 pts, not associated with any clinical, PSG, ABG or lung function test variable. Fatigue was present in 12 pts associated with daytime hypercapnia (p=0.05).
After PSG, PFT and ABG, 9 patients were submitted to NIMV based on evidence of nocturnal hypoventilation, sleep-apnea, or daytime hypercapnia. The need of NIMV was also associated with vital capacity (p=0.05) and strength of expiratory muscles as evaluate by Maximal Expiratory pressure (p=0.03).
Conversely, clinical variables (including age, sex, disease duration, BMI, MDRS, EDS and FSS) were not associated with the occurrence of NIMV.
Conclusions: The prevalence of SDB was around 50%, with a Cheyne Stoke respiration in 12.5%. EDS was not associated to any clinical or PSG variable, suggesting an independent genesis. Conversely fatigue was associated daytime hypercapnia.
We did not find any clinical variable associated to the risk of NIMV, therefore we recommend that DM1 patients should be routinely evaluated by PFT, ABG and PSG.

S5-07 Cerebral white matter affection in myotonic dystrophy type 1 and 2
- A Diffusion-Tensor-Imaging Study at 3T -

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Myotonic dystrophy type 1 (DM1) and 2 (DM2) are autosomal dominantly inherited neuromuscular disorders with multisystemic involvement. Although cognitive impairment and white matter changes (WMC) are more common in DM1, brain volume loss and WMC have also been described in DM2. Using voxel-based morphometry we previously found grey and white matter loss along cerebral midline structures in DM2. To further investigate WMC in DM1 and DM2, we used diffusion-tensor-imaging (DTI) and correlated changes with clinical data.
We compared 22 DM1 (m/f: 9/13, age 43.1 years (y), disease duration (DD) 13.2 y) and 22 DM2 patients (m/f: 12/10, age 52.5 y, DD 11.9 y) with age- and sex-matched healthy controls (m/f: 11/11, age 50.0 y). All subjects underwent clinical-neurological examinations and neuropsychological testing. Diffusion weighted images were obtained using a 3T MRI scanner with 60 gradient directions, Tract Based Spatial Statistics/FSL was used for both preprocessing and statistical analysis of DTI data (t-Test, pFWE<0.05).
FA changes throughout the whole brain, affecting associative fibres (cingulum, superior and inferior longitudinal fascicles, inferior fronto-occipital fascicles), commissural fibres (corpus callosum) and projecting fibres in brainstem and internal capsule, were more pronounced in DM1 than DM2; brainstem was exclusively affected in DM1.
DTI analyses demonstrated distinct cerebral white matter involvement in DM1 and DM2. These findings could be the morphologic substrate of cognitive impairment in both patient groups. Brainstem tegmentum affection in DM1 may be associated with hypersomnia and disturbed vigilance. Neuropsychological data analyses and correlation studies of brain morphometric and clinical data are currently underway.

S5-08 White Matter Microstructural Abnormalities in DM Observed with Diffusion Tensor Imaging

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Rationale: White matter abnormalities in the form of cerebral white matter hyperintensities have been observed in DM1 and DM2. Diffusion Tensor Imaging is a magnetic resonance imaging modality that measures the self-diffusion of water in tissues and can provide quantitative information about white matter organization and microstructure. In this preliminary study, we used diffusion tensor imaging to examine the impact of myotonic dystrophy on white matter microstructure.

Hypothesis: Measures of white matter microstructure are abnormal in myotonic dystrophy.
Methods: DM1ao, DM1eo and DM2 subjects were recruited from a university clinic. Controls were recruited from the community. There were five subjects in each of the 4 groups. Whole brain DTI (12 directions, 2mm thick slices), T1 and PD scans was collected on a Siemens Trio scanner. T1 and PD scans were segmented to provide a white matter mask which was applied to the registered fractional anisotropy maps. White matter FA was evaluated in total cerebral white matter and four anatomically defined, AC-PC aligned regions: a SUP(anterior to genu, superior to AC-PC plane), INF(anterior to genu corpus callosum, inferior to AC-PC plane), ACC(superior to top of corpus callosum), OCC(posterior to splenium, superior to AC-PC plane, inferior of top of corpus callosum). Comparisons were made between the MD and control groups.
Results: Largest FA reductions were observed in the DM1ao and the DM1co, with smallest differences in DM2. Both frontal and occipital regions were affected.
Conclusion: In this preliminary study, reduced white matter integrity was observed in DM subjects in multiple brain regions. These findings need to be verified in a larger sample. Future studies need to examine the relationship of white matter integrity and cognition.

S5-09 Depression in Myotonic dystrophy type 1: clinical and neuronal correlates

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Objective: To explore the prevalence and cause of depression in DM1.
Background: It is known that DM1 is a disorder associated with cognitive and brain dysfunction but the prevalence of depression has been debated. As yet, no study has shown any association between depression and clinical parameters associated with the disease. Methods: Thirty-one patients with DM1 and 47 subjects in a clinical contrast group containing patients with other neuromuscular disorders completed Beck Depression Inventory (BDI). Results were compared with normative collectives. Analysis of neurocognitive, genetic and clinical data was undertaken. Results: Signs of a clinical depression (BDI > 9) were prevalent in 32% of the patients with DM1, which was comparable with ratings in the contrast group. The depressive condition was exclusively mild to moderate in both groups. In DM1, we found high BDI ratings on somatic items in comparison to low ratings on cognitive and emotional content. A longer duration of clinical symptoms of DM1 was associated with lower scores on the BDI and higher educational levels were correlated with higher scores on depression. We also found a negative association with white matter lesions. Conclusion: Our findings suggest that there are significantly more DM1 patients than normative collectives showing signs of a clinical depression. The depressive condition is however mild to moderate and comparable

with ratings in other neuromuscular disorders. Results indicate that the awareness of the risk of depression and the need for intervention is at hand particularly early during the disease process

S5-10 French registry for myotonic dystrophies (DM1-DM2): toward the characterization of a large DM population

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The high variability and multisystemic involvement in myotonic dystrophies create particular challenges for both management and the design of therapeutic trials. Therefore, a database specifically dedicated to myotonic dystrophies summarizing the relevant informations in a large population of patients may be a valuable tool for promoting clinical research. The French database contains clinical and paramedical data collected in a standardized manner during the medical consultations of DM patients in several French neuromuscular centres. Informations include expansion size of the mutation, clinical history, clinical evaluation of neuromuscular and systemic signs, and professional and social consequences of the disease completed by an annual follow-up section. The database has already allowed us 1) to search for genotype/phenotype correlations, 2) to evaluate the prevalence of different symptoms in the French DM patients and 3) to look for potential interrelations of specific symptoms. In conclusion, the French database may be of great interest to initiate different clinical studies and to select patients for future therapeutic trials.

S5-11 Efficacy and limitation of group exercise for swallowing training in myotonic dystrophy.

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Objects: Although dysphasia is one of severe complication in myotonic dystrophy type 1 (MD), many patients with MD are not conscious of swallowing disturbance and often not constructive to guidance or rehabilitation for swallowing. It is important to lead a valid procedure for dysphasia in MD. The aim of this research is to study effects of a group physical therapy as a swallowing exercise in MD.
Methods: Thirteen inpatients with MD of our hospital were participated. Ten minutes-exercise planned by our nursing team was performed all together to recorded tape-sounds in a dining hall before lunch and dinner everyday during six months. Swallowing function was assessed by our checklist composed of ten observational points before and after the trial. Four of thirteen patients were examined by X-ray fluoroscopy for swallowing movements.
Results: No patients fell off the training. The score of the checklist was improved in ten of thirteen patients. Two patients kept the same score. There was no episode of suffocation or pneumonia according to dysphasia during the trial. However, X-ray fluoroscopy showed severe prolongation of pharyngeal stage and stagnation of a bolus around the piriform recess.
Conclusion: A group therapy is effective in MD, as an interesting procedure attracts them. Episodes of suffocation or pneumonia could be decreased according to arousing their attention to ignorance of swallowing disturbance. On the other hand, it is necessary to confirm their swallowing function by means of fluoroscopy because there are physical limitations for effects of training against dysphasia in MD.

S5-12 General anesthesia in myotonic dystrophy type 2

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Background: There have been no reported risks for general anesthesia in DM2. We assessed the frequency, type, and severity of perioperative complications under general anesthesia in DM2.
Methods: A retrospective multicenter study was conducted. Out of 302 DM2 patients, 134 participated by completing questionnaires. Additionally, their clinical records were reviewed.
Results: 121 patients had 340 operations in general anesthesia at an average age of 40.5 years. 132 (38.8%) general anesthesia were performed prior to DM2 onset, 187 (55.9%) after disease onset. 212 (62.4%) of the operative interventions were done before DM2 diagnosis was proven. In 120 (35.3%) interventions, DM2 was already diagnosed. The locations of surgery were lower abdomen (47%), peripheral (46.8%), upper abdomen (3.8%), thorax (1.8%), and brain (0.6%). The overall frequency of severe complications was 2 in 340 (1.65%). One incident was a postoperative development of rhabdomyolysis, hyperthermia, muscle weakness and renal failure, the other prolonged muscular weakness and renal failure.
Conclusions: Compared to DM1, in DM2 there is a low risk of developing severe complications perioperatively. The overall lower risk seems to be predominantly related to the minor respiratory involvement in DM2.

S5-13 Double genetic trouble (DM2/FSHD) in a Sardinian DM2/PROMM family

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Background: Myotonic dystrophy type 2 (DM2) and facioscapulohumeral muscular dystrophy (FSHD) are the two more common forms of muscular dystrophy seen in adults. DM2 is caused by a CCTG repeat expansion in intron 1 of ZNF9 gene on chromosome 3q21 while FSHD is caused by a deletion of a repetitive element on chromosome 4q35. Objective. We present clinical, histopathological and genetic data on a DM2 Sardinian family in which, DM2 abnormality occurs in combination with FSHD in two patients. Methods and Results. One patient (50 years old) has a phenotype with weakness affecting muscles usually involved in both DM2 and FSHD. Myotonia is not clinically detectable but revealed by EMG. Histopathological analyses of muscle sections show fibre size variability and central nuclei. No nuclear clumps are present. Genetic analyses show a DM2 expansion comprised between 1-8 kb and a 30 kb residual DNA fragment in the 4q35 region. FISH with (CAGG)5-probe in combination with MBNL1-immunofluorescence demonstrate the presence of nuclear foci of CCUG-

containing RNA co-localizing with foci of MBNL1. Two sisters presenting CCTG-expansion (1-8kb and 800bp-2.5kb) show a less severe PROMM-phenotype with a characteristic DM2 muscle histopathological pattern. Nuclear mutant-mRNA and MBNL1 are also present. His son (21 years old) has poor facial mimic movement and more severe weakness (MRC 3) and wasting than his father with FSH distribution. No myotonia is detectable by EMG. Genetic tests are positive for both DM2 and FSHD mutation. Each patient examined has been proven negative for mutation in CLCN1 gene. Conclusion. This is the first report on a DM2/PROMM family having 2 separate mutations in muscular dystrophy-related genes.

S7 Keynote Lecture

S7-01 Developing treatment for hereditary neuromuscular disease

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Over the past two decades, nearly 3000 disease genes have been identified. Each of these offers opportunities for understanding the disease mechanism and for developing targeted treatment. Four examples are Duchenne muscular dystrophy (DMD), spinal muscular atrophy (SMA), Friedreich's ataxia (FRDA), and spinal and bulbar muscular atrophy (SBMA).
DMD is caused by loss of the structural muscle protein dystrophin, usually through partial gene deletions that shift the translational reading frame. Approaches to treatment include gene replacement, exon skipping with antisense oligonucleotides, and pharmacologic suppression of mutations that prematurely terminate protein translation. Drugs that slow muscle degeneration or enhance muscle regeneration may also be beneficial.
SMA is an autosomal recessive motor neuron disease caused by deletion of the "survival motor neuron" (SMN1) gene. Because another partially functional version of the gene (SMN2) is present, SMA patients have a relative, not complete, loss of the SMN protein. SMN has normal functions in the splicing and axonal transport of mRNA, on which motor neurons may be particularly dependent. Cell culture and animal models have been used to develop treatment based on restoring SMN levels. One approach, stimulation of the SMN promoter with histone deacetylase (HDAC) inhibitors, has been effective in SMA mice and is now being tested in patients. Pre-clinical results with gene replacement are also encouraging, and assays have been developed for high throughput screening to identify other small molecules as potential treatment.
FRDA is an autosomal recessive disease caused by a GAA repeat expansion within an intron, leading to decreased levels of frataxin, a nuclear-encoded mitochondrial protein. Approaches to treatment include HDAC inhibition to increase frataxin expression, and anti-oxidants such as idebenone.
SBMA is an X-linked motor neuron disease caused by repeat expansion in the androgen receptor (AR) gene. The expanded CAG repeat leads to an polyglutamine tract expansion in the AR protein that causes both a loss of function and a toxic gain of function in the protein that is ligand (androgen) dependent. Approaches to treatment include reducing androgen levels and reducing levels of the toxic protein with HSP90 inhibitors and IGF-1.

While each of these diseases presents unique challenges and opportunities, the same general approach for developing pharmacological and biological therapy can be applied to other hereditary diseases where the underlying defects are known, including myotonic dystrophy.
S8 Molecular and Symptomatic Therapy

S8 Molecular and symptomatic therapy

S8-01 Molecular therapy for myotonic dystrophy: current status and future strategies

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One century after the first description of myotonic dystrophy and nearly 20 years following the discovery of the genetic basis for DM type 1, a cure for DM does not yet exist. In the clinic, treatment is dedicated to reduction of symptoms, without any opportunity to stop disease progression. In this lecture, I will give an overview of molecular therapeutic strategies developed in the past few years and discuss new potential avenues to slow, halt or maybe even revert clinical features of DM. Fortunately, fundamental research has greatly improved our understanding of the complex, molecular pathogenesis underlying the disease. Important targets for intervention in different molecular pathways involved in DM - at the DNA, RNA and protein level - have been identified. While a cure for DM is probably still years away, some recent developments based on these molecular targets are promising and will be discussed.

S8-02 IPLEX (rhIGF-I/rhIGFBP-3) treatment of myotonic dystrophy type-1 (DM1): A safety and tolerability trial

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RATIONALE: Insulin-like growth factor-I (IGF-I) promotes muscle regeneration, insulin sensitivity, protein synthesis, and function of dystrophic muscle. Studies in diabetes and hip fracture suggest that recombinant human IGF-I (rhIGF-I) complexed with IGF binding protein 3 (rhIGF-I/rhIGFBP-3) enhance activity and reduce the side effects of IGF-I.

OBJECTIVE: Evaluate the safety, tolerability, and feasibility of rhIGF-I/rhIGFBP-3 (IPLEXTM, Insmed Inc.) as a treatment for DM1.
METHODS: Fifteen DM1 patients received daily subcutaneous rhIGF-I/rhIGFBP-3 for 24 weeks. The first six (Cohort 1) received 0.5 mg/kg/day for eight weeks followed by 1.0 mg/kg/day for 16 weeks. Following approval of the safety monitoring committee, the next nine (Cohort 2) received consecutive 8-week treatments of 0.5, 1.0, & 2.0 mg/kg/day for a total of 24 weeks. Serial assessments of safety, muscle mass, and muscle function were performed.
RESULTS: All patients tolerated rhIGF-I/rhIGFBP-3 with no serious adverse events. The most frequent adverse event was a mild reaction at the injection site (n=8 patients). Hypoglycemia (n=3), lightheadedness (n=2) and transient papilledema (n=1) also occurred. Lean body (mainly muscle) mass [DEXA] increased by 1.9 +/- 1.6 kg (p=0.0007)

after 24 weeks of treatment. After this same period, final dosages of 1 and 2 mg/kg/day produced similar, three-fold elevations above basal levels of total IGF-I.
CONCLUSION: rhIGF-I/rhIGFBP-3 is safe and well tolerated in DM1. Despite a muscle wasting disease process, these data suggest that patients with DM1 retain capacity to accrue muscle in response to IGF-I. Further randomized controlled trials of this treatment are warranted.

S8-03 Reversal of myotonia and splicing defects by antisense oligomers in a transgenic mouse model of myotonic dystrophy type 1 (DM1)

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Objective: To test whether CAG repeat antisense oligonucleotides can (1) block the interaction of expanded CUG (CUGexp) RNA with binding proteins; and (2) reverse symptoms of DM1 in a transgenic mouse model.
Background: Morpholino oligomers are nucleic acid analogs that demonstrate prolonged stability and high affinity for RNA targets. Binding of morpholinos to RNA does not induce target cleavage by RNase H.
Design/methods: CAG25 is a 25-mer morpholino comprised of CAG repeats. CAG25 or control morpholino was injected into hindlimb muscle of mice that express CUGexp RNA. Entry of morpholino into muscle fibers was enhanced by electroporation in vivo.
Results: CAG25 is able to invade CUGexp hairpins and form stable heteroduplex in vitro. CAG25 prevents MBNL1-CUGexp binding, and also disrupts pre-formed MBNL1-CUGexp complexes in vitro. Intramuscular injection of CAG25 released MBNL1 protein from its sequestered site (assessed by immunofluorescence) and reduced nuclear inclusions of CUGexp RNA (fluorescence in situ hybridization). This led to (1) correction of RNA mis-splicing for all transcripts tested; (2) normalization of chloride current (patch clamp); (3) marked reduction or absence of myotonia; and (4) downregulation of total mutant RNA levels (Northern blot). Effects persisted up to 14 weeks after a single injection. Injection of control morpholino had no effect.
Conclusions/relevance: CAG25 morpholino reduces the toxicity of CUGexp RNA in a mouse model of DM1. Initial examination did not show off-target effects on endogenous CUG-containing transcripts, but this must be further evaluated. Application to human DM1 will require methods to enhance muscle uptake after systemic delivery.
Study supported by: University of Rochester Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (NIH/NS48843) and NIH (AR46806).

S8-04 Development of therapies against RNA toxicity in DM1

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Rationale: In DM1 cells, the CTG expansion causes the production of a RNA molecule that is trapped in the nucleus as ribonuclear foci thereby sequestering RNA-binding proteins and it is considered toxic to various cellular functions including myogenic differentiation and RNA splicing. One potential therapeutic approach is to get rid of the toxic RNA from the cells.
Methods: To identify effective small molecules and gene therapies we have developed new cell based systems amenable to high-throughput screening. Using a myoblast cell model expressing a GFP-DMPK 3'UTR transcript, and a luciferase reporter gene under the control of E-box elements, we generated cells that readily report on the presence of the RNA (GFP fluorescence), have RNA foci, sequester MBNL1, and have splicing defects and myogenic differentiation defects. We confirmed the usefulness of this system by testing a library of gene therapy compounds targeted against the mutant DMPK mRNA that were transiently transfected at a high efficiency (>70 to 80%).
Results: We identified several therapeutic molecules that significantly decreased or abolished GFP expression. This also resulted in a loss of RNA foci, redistribution of MBNL1 to the nucleoplasm, correction of RNA splicing defects and importantly, rescue of myogenic differentiation as assessed by increased myosin heavy chain (MHC) expression and myotube formation. Some of these molecules showed selectivity for the mutant DMPK 3'UTR mRNAs without affecting normal DMPK 3'UTR transcripts.
Conclusions: In addition to identifying novel therapeutic molecules, these experiments have established the utility of the cell based system for high-throughput screening.

S8-05 Pentamidine reverses splicing defects associated with Myotonic Dystrophy Type 1 (DM1)

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Current data suggests that if MBNL1 is released from sequestration to either CUG or CCUG repeat expansions, disease symptoms of DM1 or DM2 may be alleviated. As a step towards the goal of identifying a therapeutic for DM, small molecules that are known to bind structured nucleic acid were screened using a previously characterized MBNL1-CUG gel shift assay to test if any would disrupt the protein-RNA complex. We identified pentamidine as a compound that disrupts MBNL1's binding to CUG repeats. We show in cell culture that pentamidine reverses the mis-splicing of two pre-mRNAs affected in DM1. To directly visualize pentamidine's affect on MBNL sequestration, we saw that the small molecule significantly reduced the co-localization of MBNL with the CUG repeats in tissue culture recapitulating DM1. Furthermore, pentamidine partially rescued splicing defects of two tested pre-mRNAs in mice that express expanded CUG repeats. To increase efficacy, we are performing a structure activity relationship (SAR) with pentamidine and have found modifications that increase the affinity for CUG repeats. We are in the process of testing these compounds in the cell culture and mouse models for myotonic dystrophy, as well as testing to see if pentamidine can reverse splicing defects associated with expression of CCUG repeats.

S8-06 Chemically modified (CAG)n antisense oligonucleotides as molecular tools to silence toxic, expanded DMPK transcripts

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Expanded DMPK RNA plays a central role in the complex cascade of events in the molecular pathogenesis of DM1 and is therefore an attractive therapeutic target. We hypothesize that direct silencing of tox-

ic (CUG)n transcripts offers the most straightforward solution for improvement of DM1 features in patients.
Previously, PS58, a (CAG)7 antisense oligonucleotide (AON) was identified that is capable of silencing expanded (CUG)n transcripts in vitro in mouse and patient cells and in vivo in transgenic DM1 mouse models. As a follow up, we started a series of experiments to determine the influence of oligo chemistry (phosphate and sugar backbone modifications) and length (number of triplets) on (CAG)n AON silencing efficacy and specificity in our myoblast-myotube cell models from DM500 mice or DM1 patients. Based on microscopical analyses, we conclude that the chemical composition strongly determines subcellular localization of the AON and hence its ability to silence (CUG)n transcripts. Furthermore, we find that (CAG)n AONs of different lengths are active towards (CUG)n RNA, but that their specificity varies regarding (CUG)n segment length in the target RNA. This aspect is important, since we wish to minimize silencing of normal-sized DMPK transcripts and other (CUG)n-bearing transcripts in the cell. We also tested two authentic siRNAs directed towards the (CUG)n segment, but found hardly any silencing activity. Time-course experiments showed that (CAG)n AONs act quickly and with high affinity. Finally, we have started to unravel the molecular mechanism of (CAG)n AON silencing and will present recent data on this subject.

S8-07 Assays to screen for drugs to treat myotonic dystrophy.

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Myotonic Dystrophy (DM) is a progressive neuromuscular disorder with no available treatment. There is now a good understanding of the molecular basis of DM, which allows the development of rational assays to identify therapeutic compounds not currently available for this disorder. In order to find a drug to treat DM, robust biological assays are required for screening projects. We have developed an assay based on patient cell lines that will allow screens for compounds to treat DM.
We have established a series of cell lines from DM patients which have been stably transfected with a telomerase-expressing plasmid to allow continued growth in culture. Two of the lines have also been infected with an inducible plasmid to express MyoD, which promotes differentiation into myoblasts and myotubes. To assess the suitability of these cells in screens for compounds to treat DM, we have set up and analysed a series of 96-well plates in an in situ hybridization-based assay for nuclear foci. Counts of nuclear foci have been conducted and validated on a high content imaging system. We found that cell line KAGOTelo contained on average 8 foci. If a drug reduced the number of spots by 10%, with the number of spots in treated cells at 7.2 and a standard deviation between cells of 1.5 spots, we would have to score 93 cells of each type (control and treated) to have a 95% chance of finding a significant difference ($p<0.05$) between the two groups. For a second cell line, KBTeloMyoD, we would need to look at 81 cells per group to have a 95% chance of finding a significant difference. We believe this approach is sufficiently robust to use in medium-throughput compound screens for DM.

S9 Heart and DM

S9-01 Clinical Aspects of Cardiac Involvement in Myotonic Dystrophy: Current Knowledge and Future Directions

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Objective: To review the current state of knowledge and future directions regarding cardiac abnormalities in patients (pts) with myotonic dystrophy (DM) types 1 (DM1) and 2 (DM2).
Methods: Literature review and analysis from The Arrhythmias in DM1 Study.
Results: The presence of cardiac abnormalities in DM was recognized soon after the description of the disease by Steinert in 1909. Cardiac involvement occurs in both DM1 and DM2 but typically appears later in life and is less severe in DM2. The pathology observed is myocardial fibrosis and degeneration preferentially affecting the conduction system. The degenerative changes provide the abnormal substrate responsible for the primary cardiac clinical manifestation of arrhythmias. Arrhythmias including sinus node dysfunction, progressive heart block, atrial tachycardia, flutter, or fibrillation, and ventricular tachycardia or fibrillation can occur. Sudden death is believed to result from severe bradycardia after atrioventricular block or a ventricular tachycardia or fibrillation. The use of pacemakers has been recommended for DM pts with significant conduction abnormalities whether or not they are symptomatic because of the unpredictable progression of atrioventricular block. The use of implantable cardioverter-defibrillators (ICDs) has been reported in DM1 pts. A clinical history of arrhythmias and conduction abnormalities on the ECG identifies a DM group at high risk for sudden death. Questions requiring future studies include (1) further risk stratification in high arrhythmia risk pts, and (2) whether pacemakers and ICDs improve long-term outcomes.
Conclusion: Appropriate recognition and treatment of cardiac involvement is an important dimension in the overall care of the DM pt.

S9-02 Is Cardiac Involvement in Adult Survivors of Congenital- or Childhood-Onset DM1 Different than in Classical Adult-Onset DM1?

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Objective: The majority of myotonic dystrophy type 1 [DM1] patients [pts] survive into adulthood including those presenting with symptoms at birth or in childhood. It is unclear whether cardiac involvement in adult survivors of congenital/childhood-onset [Congen] DM1 is different than that in classical/adult-onset [Adult] DM1 patients.
Methods: Analysis from The Arrhythmias in DM1 Study, a prospective, observational registry of 406 adult pts with clinically- and genetically-verified DM1 followed for 6.6.3 yrs. 36 pts (8.9%) with senile-onset DM1 were excluded for the current analysis.
Results: Congen pts (58 of 406, 14.3%) were younger (31vs.42 yrs, $p<0.001$), had greater CTG expansion (1015vs.613 repeats, $p<0.001$), and had more severe skeletal muscle involvement by impairment rating scale (3.5vs.3.3, $p=0.04$) than the Adult pts (312 of 406, 76.8%). There was no difference in gender (male, Congen 45%, Adult 50%, $p=0.48$) between the two groups. At study entry, severe cardiac conduction abnormalities and arrhythmias were as frequent in the Congen (17.2%vs.25.3%, $p=0.19$) as Adult pts. During follow-up, Congen pts were as likely to have atrial tachyarrhythmias (10.3%vs.12.8%, $p=0.60$),

ventricular tachyarrhythmias (1.7%vs.1.9%, p=0.92), left ventricular dysfunction / heart failure (8.6%vs.8.3%, p=0.94), and require a pacemaker or defibrillator (10.3%vs.14.4%, p=0.41) as Adult pts. Congen pts had less all-cause deaths (12.1%vs.26.6%, p=0.02) but not less sudden cardiac deaths (6.9%vs.7.4%, p=0.90) than Adult pts.
Conclusion: Despite a younger age, Congen pts are at as high of a risk of cardiac conduction abnormalities, arrhythmias, and sudden death as Adult pts.

S9-03 Cardiac abnormalities in congenital childhood myotonic dystrophy (DM1)

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Introduction: Myotonic muscular dystrophies (DM1) and (DM2) are autosomal dominantly inherited diseases. DM1 and DM2 patients have been shown to exhibit variable levels of cardiac abnormalities and particularly conduction defects with an incidence of more than 50% (Clarke et al 2001,). While many studies have occurred that have investigated cardiac conduction defect issues in adult DM1 patients (Groh et al 2008), the exact incidence and prevalence of cardiac issues in congenital and childhood DM1 and DM2 patients is not well known.
Materials and Methods: Utilizing the International Myotonic Dystrophy Organization's (IMDO) database of 1,330 DM patients and their families, a survey was conducted on the portion of the database who had confirmed with IMDO they had children who were diagnosed with congenital and childhood DM1. Of the 214 who received the survey via e-mail, only 31 completed the survey and 2 of those people actually did not have children. Of the remaining 29 who completed the survey and had children with congenital childhood DM1, there were 6 confirmed diagnoses of various cardiac problems including but not limited to supraventricular tachycardia, murmur, bundle branch block, and fascicular block.
Results: This limited survey of a small sample size of 29 parents of children with congenital DM1 resulted in 6 confirmed children with some form of cardiac abnormality. There were 2 children who had myocardial infarctions, with one of those children having multiple infarctions that were related to a right bundle branch block and is on defibrillator. Other child was also diagnosed with a left anterior fascicular block. Two children were diagnosed with a heart murmur, and one of those children was also diagnosed with a mitral valve prolapse. One child was diagnosed with first-degree AV block. Another child was diagnosed with supraventricular tachycardia. This survey indicates that about 20% of the sample's congenital DM1 patients had confirmed diagnosis's of cardiac abnormalities.

Patient Number	Diagnosis
Patient 1	supraventricular tachycardia
Patient 2	first-degree AV block
Patient 3	Heart murmer and mitral valve prolapse.
Patient 4	Second degree AV block
Patient 5	Complete Heart block on defibrillator
Patient 6	multiple infarctions related to a right bundle branch block and left anterior fascicular block on defibrillator

Discussion: The incidence and prevalence of cardiac abnormalities in adult onset DM1 varies from 30-40%. The incidence and prevalence of those same abnormalities in congenital and childhood Myotonic dystrophy is not well understood. This preliminary study demonstrated that the incidence in this small sample size is approximately 20%. Further study with a larger sample size may yield more comprehensive data and a study is underway in our institution to further explore this issue.

S9-04 High prevalence of Brugada syndrome in patients with Steinert's Disease
A new insight in the pathophysiology of arrhythmias in Steinert's Disease

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Background: Steinert's muscular dystrophy, or DM1, is an inherited multisystemic disease with a high risk of sudden death. The mechanisms underlying sudden death and particularly ventricular arrhythmias in those patients are not clear.
Objective: To determine whether Brugada syndrome could be implicated in ventricular arrhythmias associated to DM1.
Design, Setting and Patients: We analyzed the electrocardiograms of 500 patients with Steinert's disease. Patients in whom a type 1 Brugada ECG pattern was identified were screened for a mutation in the SCN5A gene and underwent complete cardiac examination.
Main outcome measures: The prevalence of Brugada pattern among DM1 patients and the risk for ventricular arrhythmias in patients with both abnormalities.
Results: Seven patients with a type 1-Brugada pattern were identified, representing an 80-fold higher prevalence than expected when compared to the general population. SCN5A sequencing was normal in all patients. One patient died suddenly of ventricular fibrillation and four patients had severe ventricular arrhythmias.
Conclusions: The prevalence of Brugada syndrome among DM1 patients is much higher than expected. Patients presenting with the association of both diseases have a high risk for severe ventricular arrhythmias.

S9-05 Protein Kinase C inhibition ameliorates the cardiac phenotype of a mouse model for myotonic dystrophy, type 1

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Cardiac complications are the second most common cause of death in individuals with myotonic dystrophy type 1 (DM1). Developmentally regulated alternative splicing is disrupted in DM1 due to misregulation of the splicing regulators: muscleblind like 1 (MBNL1) and CUG binding protein 1 (CUGBP1). CUGBP1 is upregulated in DM1 due to activation of the protein kinase C (PKC) pathway, which hyperphosphorylates and stabilizes CUGBP1 protein.
We have previously generated a heart specific tamoxifen inducible DM1 mouse model in which 960 CUG repeat RNA is expressed in the context of DMPK 3'UTR (DMPK-960CUG). Within three weeks following induction of DMPK-960CUG RNA, these mice exhibited high mortality, conduction abnormalities, systolic and diastolic dysfunction as well as molecular changes seen in DM1 patients such as colocalization of MBNL1 with RNA foci and misregulated alternative splicing. Importantly, activated PKCα/βII and increased CUGBP1 lev-

els were evident within six hours after induction of DMPK-960CUG RNA in this mouse model.
In this study, we wanted to determine whether PKC activation is pathogenic in DM1. Therefore, we blocked PKC activity in a heart specific DM1 mouse model using a PKC inhibitor. Animals given the PKC inhibitor exhibited higher survival rate that correlated with reduced phosphorylation and decreased steady state levels of CUGBP1. Importantly, functional studies demonstrated that PKC inhibition ameliorated the cardiac conduction defects and contraction abnormalities that are reproduced in this mouse model. The inhibitor also improved alternative splicing changes regulated by CUGBP1. Taken together, our results suggest that pharmacological blockade of PKC activation mitigates DM1 cardiac phenotype and provides strong evidence for a pathogenic role of the PKC pathway in DM1 pathogenesis.

S9-06 The progression of Muscular Impairment Rating Scale (MIRS) and the development of cardiac conduction abnormalities in DM1.

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Background. Other than tachyarrhythmia, severe cardiac conduction defects are predictors of sudden death in DM1. Many DM1 patients with normal ECG at their first examination, develop cardiac changes during the disease course. Aim of our study was to identify predictors of development of cardiac conduction defects in DM1.
Patients and Methods. 167 patients with DM1 (M/F 99/68, aged 6-75 yrs, mean age 37.7+15.8, CTG range 60-1700) without cardiac abnormalities at baseline and submitted at least once a year to cardiac and neurological evaluation, were included in the study. Their follow-up (F-U) ranged from 1 to 28 yrs. (median 6 yrs.).
Results. During the F-U 24% of patients developed conduction delays, including AVB-I: 14%, AVB-II: 5.5%, Bundle Branch Block: 7.7%. Sixteen patients (9.5%) underwent PMK implantation for either AVB>I at Holter monitoring or trifascicular block. Seventeen patients (10%) died during the F-U (myocardial infarction 2 pts., sudden death 2 pts., heart failure 1 pts., respiratory failure 4 pts., other causes 8 pts.). Muscular impairment scale at baseline was >2 in 39 patients (23.2%). During the F-U, 55 patients (33%) showed a change of at least one grade in MIRS. Cox regression analysis showed that both baseline MIRS and MIRS progression during F-U, were independent predictors of cardiac conduction defects development (OR 1.53; 95% CI 1.05-2.02, p=0.05 and OR 3.2; 95% CI 1.2-8.3, p=0.013, respectively).
Conclusion. MIRS at baseline and its progression overtime are predictors of the occurrence of cardiac conduction defects in DM1.

S10 Genetic counseling

S10-01 Genetic counseling in DM1

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S10-02 Genetic counseling in DM2

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Compared to DM1 patients, there are major differences in how DM2 patients respond to genetic counseling. Many DM2 patients show relief after receiving a definitive molecular diagnosis, often after a long time of uncertainty and fruitless diagnostic efforts. Genetic counselors notice a tendency by primary care givers to push the complaints of DM2 patients into the psychological corner, at least in Germany. Some instructive examples will be described. As with any disorder of genetic origin, patients need some time to understand the implications of the diagnosis, but information about the mode of inheritance and the consequences for the family are normally well accepted. So far, there were no inquiries about prenatal diagnosis which underlines the general understanding of DM2 as a late onset disorder for which in contrast to DM1 no congenital or juvenile forms are known. Being aware of the natural history of DM1, affected parents (and attending pediatricians) nevertheless may pay more than usual attention to the developmental milestones of their offspring. As will be illustrated, there are occasional case histories of concerned parents in which hypotonia after birth –a not uncommon symptom in newborns –was misinterpreted as an early consequence of DM2. In addition to providing information concerning the genetic basis, mode of inheritance, and natural history of DM2, genetic counseling should include information on social aid programs and family support groups.
Poster Sessions

P1 Poster Session 1: Basic Research – Part 1

P1-01 Searching for trans-acting genetic modifiers of somatic mosaicism and disease severity in myotonic dystrophy type 1

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Somatic mosaicism in myotonic dystrophy type 1 (DM1) is age-dependent, tissue-specific and expansion-biased, features that very likely contribute toward the tissue-specificity and progressive nature of the symptoms. Previously, we performed a detailed quantitative analysis of somatic mosaicism in the blood DNA of DM1 patients showing that variation in the rate of somatic expansion contributes toward variation in age of onset. This allowed us to conduct a preliminary search for genetic modifiers of DM1, with genes in the DNA mismatch repair (MMR) pathway top of the list of candidates. Using bioinformatic analyses, we searched for polymorphisms in the MSH3, MSH2 and PMS2 MMR genes and used PCR and restriction digestion to screen

these polymorphisms in a cohort of Costa Rican, US and Scottish DM1 samples. From the 13 putative polymorphisms screened, we confirmed that only 11 were indeed polymorphic. Most of the polymorphisms analysed were located in the DHFR/MSH3 locus (9), one was in MSH2 and the other in PMS2. From our previous analyses, we obtained the residuals for age of onset from the correlation between age of onset and inherited allele length, and the residuals for the degree of somatic variation after correcting for the interaction between the inherited allele length and age at sampling. Although some of the associations produced marginally significant correlations, none of these associations remained significant after correcting for multiple testing. This could be for two reasons: Firstly, the sample size was not large enough; or secondly, these polymorphisms do not act as trans-acting genetic modifiers in DM1. We are now increasing the sample size and the number of polymorphisms to be analysed in order to generate a statistical model for DM1 that accommodates multiple variables to determine the contribution and effect of somatic variation, age at sampling, inherited allele length and MMR polymorphisms on the mutational dynamics of the CTG repeat and its relationship with the age of onset and the progressive nature of the disease.

P1-02 CTCF induces replication fork pausing around DM1 repeats

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P1-03 Study of DM1 associated RNAs using atomic force microscopy (AFM)

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Atomic force microscopy (AFM) uses a micrometre-sized tip probe to scan over a surface covered with a sample of interest. Biomolecular complexes such as protein/nucleic acid interactions can be easily imaged by AFM as the resolution of the technique falls within the nanometre range. The stochiometry of the biomolecular complexes can also be estimated because of the 3-dimensional resolution of the technology.
In this study, AFM was used to image various DM1-associated mRNAs containing normal and over expanded numbers of CUG or CAG repeats, as well as their interactions with full length MBNL1 (FL-MBNL1). As predicted by RNAfold, the RNA transcripts containing a large number of CAG or CUG repeats showed mostly a single stable double-stranded RNA hairpin, whereas transcripts containing only 11 triplet repeats did not show a distinctive single hairpin. Imaging of FL-MBNL1 interactions with transcripts containing either 140 CUG repeats in the DMPK-3'UTR context, or, only 95 CUG repeats revealed a binding orientation of the MBNL1 proteins from the base of the hairpin toward the loop of the hairpin. The dimensions analysis of the MBNL1-RNA complexes suggested that two MBNL1 proteins interact primarily at the base of the hairpin and that further binding occurs toward the hairpin loop. The volume analysis of the various RNAs imaged and of the FL-MBNL1/dsCUG RNA complexes revealed a spread distribution suggesting that further aggregation FL-MBNL1 proteins onto the dsRNA hairpin or, that association of several FL-MBNL1/dsCUG complexes occurs upon the binding of MBNL1 onto the dsCUG hairpin. These results provide new insights to the biomolecular mechanism underlying MBNL1 protein interaction with dsCUG RNA hairpins in DM1.

P1-04 Disease-associated trinucleotide repeats: form transcription-induced RNA:DNA hybrids

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P1-05 CpG methylation proximal to the CTG/CAG tract of the DM1 locus in patients and transgenic mice

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P1-06 Tissue-specificity of trinucleotide repeat instability and DNA replication in myotonic dystrophy type 1

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P1-07 Studies of the distribution of Muscleblind-like proteins in myotonic dystrophy

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Muscleblind-like proteins are important regulators of alternative splicing and their sequestration by expanded RNA foci in DM cells is thought to result in several splicing abnormalities. Apart from their nuclear role in splicing, recently, MBNL2 has been shown to recruit integrin RNA to focal adhesions in the cytoplasm. Since all Muscleblind-like proteins are structurally very similar, it is quite possible that they also have similar functions. In order to investigate whether MBNL1 also has any cytoplasmic function, the sub-cellular localisation of MBNL1 was investigated more closely. These studies suggest that MBNL1 is not confined to the nucleus but shuttles between the nucleus and the cytoplasm. Like several other RNA-binding proteins, this nucleocytoplasmic shuttling of MBNL1 is dependent on transcription. Experiments have been conducted to elucidate the mechanism of import and export of MBNL1 as well as the signals responsible for nuclear localisation and nuclear export of MBNL1. The ability of MBNL1 to shuttle between the nucleus and the cytoplasm implies that in addition to regulating alternative splicing, it may also be involved in mRNA transport across the nuclear envelope, or have cytoplasmic functions, such as translational regulation, mRNA stability and/or mRNA localization.

P1-08 A bi-chromatic fluorescent assay to measure splicing effciency in Myotonic Dystrophy

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Intron retention is one of several types of alternative splicing alterations that have been described in DM. Intron 2 of the muscle-specific chloride channel (CLCN1) and intron 19 of the sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA2) have been detected in anomalous mature cytoplasmic transcripts in DM cells.
We have developed a double reporter assay to detect variations in splicing efficiency under different conditions. The relevant splicing units (ie two consecutive exons separated by an intron) of CLCN1 and SERCA2 have been cloned in a plasmid vector encoding green fluorescent protein (GFP) at the 5'end and DsRed at the 3'end of the multiple cloning site. The intron in both instances contains either several stop codons (CLCN1) or a number of bases non-divisible by 3 (SERCA2), so if splicing of the intron takes place, both fluorescent proteins are translated from a single open reading frame, resulting in a chimeric product with green and red fluorescent activities. If no splicing occurs, the product only displays green fluorescent activity. This is because a) translation would cease at the first stop codon within the intron (CLCN1) or b) the red fluorescent protein would be encoded out-of-frame and would not be translated (SERCA2).
The system has been designed to allow quantitative analysis of these two splicing events. Stable cell lines have been generated from control and DM immortalized fibroblasts with the constructs. The efficiency of the splicing event, measured by the ratio red to green fluorescent activities, has been conducted on cultures of these cell lines. Preliminary data indicate that these cell lines are suitable for use in screens of compounds to restore DM-associated splicing abnormalities.

P1-09 Validation of sensitivity and specificity of tetraplet-primed PCR (TP-PCR) in the molecular diagnosis of for Myotonic Dystrophy type 2

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Background: Myotonic Dystrophy type 2 (DM2, OMIM #602688) is a multisystemic degenerative disease caused by a tetranucleotide (CCTG)n expansion in the ZNF9 gene. Routine testing strategies require the use of Southern Blot or Long Range PCR but technical difficulties, due to the presence of very large expansions and wide somatic mosaicism, greatly reduce the sensibility of present gold standard techniques.
Objective: Establish validity of Tetraplet-Primed PCR (TP-PCR) for a fast discrimination of positive and negative patients, according to other dynamic mutations testing strategies, as Friedreich's Ataxia, Huntington's Disease and Steinert's Disease, in order to simplify the diagnostic procedure for DM2.
Methods: We analyzed by TP-PCR 87 DM2 positive and 76 DM2 negative patients previously characterized by long-range PCR, including 37 DM1 positive patients in order to establish sensitivity and specificity of this technique. We then attempt a prospectic analysis of 25 patients with unknown genotype for which diagnostic or presymptomatic testing were asked.
Results: Our results show that TP-PCR is a fast, reliable and flexible technique, with a specificity and a sensitivity of almost 100%, with no positive or false negative results in retrospective nor prospective application.
Conclusions: We therefore consider that the use of this technique, in combination with the Short Range PCR, is sufficient to correctly establish the presence and the absence of ZNF9 exanded alleles in the molecular diagnosis of DM2.

P1-10 Effect of RNAi directed against CUG repeats in a mouse model of DM1

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Background: One strategy to treat DM1 is to accelerate the degradation of toxic RNA. In theory, two features could be exploited to achieve selective degradation of expanded CUG (CUGexp) transcripts: (1) multiplicity of binding sites within the expanded repeat; and (2) residence within the nucleus.
Objective: To further examine the feasibility, selectivity, and effectiveness of using siRNA to target the CUGexp transcript.
Methods: We used siRNA against the CUG repeat to test whether target susceptibility was influenced by expanded vs. non-expanded repeat size or nuclear vs. cytoplasmic RNA location. siRNA was injected into muscle of HSA-LR mice that express a transgene mRNA containing ~250 CUG repeats.
Results: A single injection of siRNA resulted in 70-80% downregulation of the CUGexp transcript. By comparison, there was slight reduction or no effect on six different endogenous mouse transcripts that contained non-expanded CUG repeats. There was marked reduction in the number and intensity of CUGexp foci in the nucleus, and decreased sequestration of Mbnl1 protein. Six different Mbnl1-dependent exons showed near-complete rescue of misregulated alternative splicing. Myotonia was significantly reduced.
Conclusion: These data suggest that the repeat tract itself may be an appropriate target for allele-selective RNAi. The basis for selectivity has not been determined. The results suggest that partial reduction of the CUGexp transcript could prove beneficial in DM1.

P1-11 DNA Methylation at the DM1 Locus

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Rationale: Previous studies have shown hypermethylation of DNA adjacent to the CTG repeat in subjects with congenital DM1, and have raised the possibility that methylation augments DMPK expression by interfering with CTCF binding. We investigated DM1 locus methylation in affected individuals to determine if methylation correlates with disease severity.
Methods: CpG methylation surrounding the DM1 CTG repeat was analyzed by bisulfite sequencing.
Results: Sequence ~1.5kb upstream of the CTG repeat is hypermethylated over a region of ~700bp in DNA from fibroblasts of subjects with a full range of disease severity: wildtype, adult-onset DM1, and congenital DM1. In the wildtype, the hypermethylated region transitioned to an unmethylated 600 bp region adjacent to the repeat domain. Incontrast, the mutant allele in congenital DM1 is hypermethylated throughout the 600 bp region. In both adult-onset DM1 subjects without craniofacial abnormalities, and subjects diagnosed in adulthood but manifesting stigmata of congenital onset (craniofacial abnormalities, high-arched palate), an intermediate pattern of methylation appears to correlate positively with increasing repeat number. The wildtype alleles in DM1 cell lines remain unmethylated. A 700bp region just downstream

of the repetitive elements remains relatively free of methylation, independent of the repeat size or clinical phenotype.
Conclusions: Methylation at the DM1 locus in congenitally affected individuals is increased in the region that is immediately 5'to the CTG repeat expansion, including a previously identified CTCF site. The region even further upstream is hypermethylated on all wildtype and affected alleles, and the region 3'to the CTG expansion is unmethylated in all normal and affected individuals. Although the degree of methylation immediately upstream to the CTG repeat appears to correlate with the size of the repeat expansion, the cause and effect relationships among repeat length, degree of methylation and clinical severity remain to be determined.

P1-12 DMSXL mice carrying over 1000 CTG : characterization of the muscle function

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Transgenic mice carrying 45kb of the human DM1 locus with 300 CTG repeats were developed by G. Gourdon's group several years ago. Recently, mice carrying over 1000 CTG (DMSXL) were obtained due to CTG instability over successive generations. In the present study, we have characterized both physiologic performances using global and non-invasive approaches and functional properties of the skeletal muscles of these transgenic mice. Significant changes in physiologic exercise performances were observed in 4-month-old DMSXL mice when compared to age-matched control mice as assess by wheel test, grip test and treadmill exercise. A significant decrease of force production was also measured in the tibialis anterior (TA) and the soleus muscles of the DMSXL mice. Interestingly, maximal tetanic force normalized to muscle mass (ie specific force) is modified in the TA muscles of the DMSXL mice. This result indicates that the reduction in absolute maximal tetanic force is not only the consequence of the decreased muscle mass suggesting that additionnal mechanism related to the large CTG expansion (>1000 CTG) may altered the contractiles properties of the DM1 muscles. The muscle function of the DMSXL mice is therefore qualitatively impaired.

P1-13 The splicing of MBNL1 is altered in DM1 muscles

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The abnormal accumulation of the mutant DMPK transcripts containing CUG expansions in nuclear foci contributes to the DM1 physiopathology. MBNL1 is a splicing factor that has been found to co-localize with nuclear foci of CUG expanded repeats. Expression of mutant DMPK mRNA sequesters MBNL1 leading to an alteration of its pre-mRNA splicing activities. Deregulation of MBNL1 activity is involved in many RNA splicing abnormalities that are characteristic of DM1. Interestingly, MBNL1 itself is also subject to alternative splicing. In human, ten isoforms of MBNL1 were described. In this study we were interested to determine if the splicing of MBNL1 is altered in the muscle of DM1 patients. We have analysed the expression of the different splice isoforms of MBNL1 in DM1 patients and during normal human muscle development. We found that the longer MBNL1-42 and -43 transcripts containing exon 6 and exons 6/8 represent the major isoforms in DM1 muscles, in contrast to control muscles where the MBNL1-40 transcripts lacking exon 6/8 are predominant. We also determined the cellular localisation of each isoform using GFP fusion proteins and nucleo-cytoplasmic fractionation. Their co-localization with the foci was also analysed in DM1 cells and MBNL1-immunoprecipitation assay are currently under development to identify MBNL1 partners.

P1-14 Premature activation of the p16 stress pathway in congenital DM1 myoblasts

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The myogenic program of the myoblasts isolated from congenital DM1 muscles is altered: the differentiation of the cDM1 muscle cells is impaired and their proliferative capacity is significantly reduced when compared to non-affected cells. The cDM1 myoblasts have not exhausted their proliferative capacity, as shown bt telomere measurments, but present a premature replicative arrest. A mechanism of premature senescence triggers this early arrest. We have shown that an early activation of the p16 stress pathway is responsible for this arrest: inactivation of the cdk inhibitor p16 in cDM1 myoblasts inhibits premature senescence and restores their proliferative capacity. An increased susceptibility to oxidative stress was suggested in cells containing expanded CTG repeats and changes in the oxidative status were reported in DM1 patients. The implication of an oxidative stress mechanism in the early activation of the p16 stress sensor pathway was examined. We found that low-oxygen environment increases the proliferative capacity of cDM1 cells and delays activation of p16 activation. The pathways implicated in p16 regulation in cDM1 cells are currently under investigation.

P1-15 The effort to obtain longer CTG triplet repeat DNA by using Saccharomyces cerevisiae

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Objective: It is widely known that CTG triplet repeat is very unstable. Generally we only obtain pure 130-150 CTG repeat that is constructed into cloning vectors in E.coli, although thousands of repeat is seen in DM patients. Many efforts have been paid for obtaining long CTG triplet repeat, and we still need to get it for establishing model organisms. Although Cohen et al. has reported an instability of CTG triplet repeat on yeast attificial chromosome (YAC) transformed into the budding yeast Saccharomyces cerevisiae, there still remains advantages for using YAC in the yeast than cloning vectors in E.coli. The eukaryote Saccharomyces cerevisiae has nucleosome structures in its genome, and this might affect a stability/instability of CTG triplet repeat. In this report, we tried to clone long CTG triplet repeat on YAC in the yeast.
Methods: Pure CTG triplet repeat was amplified by a modified PCR method. These repeat DNAs were ligated into the artificial chromosome pYAC4, and transformed yeasts were selected with uracil. Each independent clones was screened by PCR and electrophoresis to check the length of CTG repeat.
Results and conclusions: We obtained several clones that were survived in the synthetic medium minus uracil. PCR screening showed that we obtained clones with CTG triplet repeat, although their repeats indicated to be several tens to one hundred repeat. We still continue to check much clones to obtain longer CTG repeat DNA.

P1-16 Altered splicing of CAMKIID in brain from patients with myotonic dystrophy type 1

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Objective: In addition to myotonia, muscle weakness, and early-onset cataracts, congenital form of DM1 manifests mental retardation, while juvenile and adult-onset forms of DM1 show personality changes and behavioral problems. Downstream, more than 10 mRNAs have been reported to be aberrantly spliced in muscles from DM1 patients. However, the reports on aberrantly spliced mRNAs in brain have been quite limited. To explore the pathogenesis in DM1 brain, we investigated the splicing of genes in brain from DM1 patients and controls.
Methods: We used 23 brain specimens; 9 from DM1, 5 from other neurological diseases including ALS, OPCA, and DRPLA, 8 from normal control, 1 from normal fetal control. We examined by RT-PCR 20 candidate exons as well as exon 2 and 10 of tau and exon 5 of N-methyl-D-aspartate receptor 1(NMDAR1), the abnormal splicing of which were previously shown in DM1 brain.
Results: We found a significant increase of an alternatively spliced isoform of Ca2+/calmodulin-dependent protein kinase (CaMK) IId gene, delta9, in brain from DM1 patients. The expression of CaMKIId splice variants in brain is regulated developmentally: delta1 is dominantly expressed in adult, and delta4 is expressed in neonate. The induction of CAMKII delta9 isoform was reported during the differentiation of murine P19 embryonal carcinoma cells. The splicing of CaMKIId is also regulated developmentally in heart but the splicing of CaMKIId in DM1 heart did not differ significantly.
Conclusions: It is possible that the aberrant isoform of CAMKIId may be responsible for the pathogenesis of DM1 brain, although functional studies for this isoform are necessary.

P1-17 Normal myogenesis and increased apoptosis in myotonic dystrophy type 1 muscle cells.

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DM1 muscle distress is characterised by myotonia, progressive muscle weakness and wasting leading to distal muscle atrophy. The muscle pathogenesis is still controversial. A diffused splicing misregulation of various muscle specific proteins (IR, ClC, TnT, RyR1, SERCA, MBNL1), a progressive senescence of muscle precursors and an impairment of myogenic program have been all proposed to lead to atrophy and altered regeneration in DM1 muscle.
Eight primary human cell lines from adult-onset (6) and congenital DM1 patients (2), (CTG)n range 180-1850, were differentiated into aneural or contracting innervated myotubes. Morphological, immunohistochemical, RT-PCR and Western blotting analyses of several markers of muscle differentiation indicated that the degree of differentiation of aneural and innervated DM1 myotubes in vitro was comparable to that of age-matched controls. FISH and long-PCR confirmed the presence of altered CTG expansions in all the pathological muscle cells lines. In addition the normally differentiated DM1 myotubes displayed the splicing alteration of Insulin Receptor and MBNL1, typically associated to the DM1 phenotype.
We identified a drastic reduction of 15 days differentiated DM1 myotubes, compatible with type 1 (apoptotic) and 2 (autophagic) cell death processes. Consistently, we detected an increased presence of apoptotic (caspase 3 activation, citochrome c release and chromatin fragmentation) and autophagic markers (P62/LC3 pathway).
Our results indicate that the early steps of the myogenic program were unaffected by the CTG expansion and, for the first time, that a premature death of DM1 myotubes may impair the muscle mass maintenance-regeneration resulting in muscle atrophy and wasting in DM1 patients.
Study supported by PRIN 2005064759, AFM. 13360, TLT GGP07250. Muscle samples were provided by Telethon BiobankN°GTF05003.

P1-18 Altered mRNA splicing of the MYH14 gene in the skeletal muscle of myotonic dystrophy type 1 patients

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Background: Among the extramuscular symptoms, DM1 patients show moderate to severe hearing loss, usually sensorineural, detected after routine audiometric screening. The MYH14 gene encodes for a member of non muscular class II myosins (NMHCs) which are involved in cytokinesis, cell motility and polarity. The MYH14 gene localizes within the DFNA4 locus and is mutated in patients affected by autosomal dominant hearing loss.
Objective: In this work, we have investigated the alternative splicing and the expression of the MYH14 gene in the muscle tissues from DM1 patients (n=12) with different (CTG)n expansion grade.
Methods and Results: QRT-PCR results show a down regulation of the MYH14 mRNA in DM1 compared to control samples (n=4), with values ranging from 20% to 60%. The MYH14 gene has two alternatively spliced isoforms, an inserted isoform (NMHC II-C1) with an inclusion 24 bp in exon 6 a non-inserted isoform (NMHC II-Co). We therefore analyzed, by RT-PCR, the splicing pattern of this gene in our muscle samples. Results demonstrated that the level of NMHC II-Co isoform increases in DM1 muscle with higher (CTG)n triplets number compared to controls and DM1 samples with small expansions. Immunofluorescence analysis of the MYH14 protein revealed a strong expression in skeletal muscle fibers, with a sarcomeric localization, and no significant differences in protein localization between DM1 and controls.
Conclusion: Inclusion of MYH14 in the wide set of genes differentially expressed in the DM1 tissues, may help to understand another aspect of DM1 phenotype, hearing loss, that is present in some patients.
Study supported by Telethon grant #GPP07250 and AFM grant #13360.

P1-19 The role of Twist in DM1

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Myotonic Dystrophy 1 (DM1) is characterised by the inability of myoblasts to withdraw from the cell cycle and differentiate to form multinucleated myotubes. During myogenesis, the proliferating myoblasts are induced to exit the cell cycle and differentiate with the help of a family of basic helix-loop-helix (b-HLH) transcription factors that are known as myogenic transcription factors. Twist is a b-HLH transcription factor that was first determined in Drosophila as a major regulator of mesoderm formation. Many studies have demonstrated that Twist is involved in several pathways that control cell survival, differentiation, apoptosis and angiogenesis. Specifically, Twist has been reported to play a role in muscle cell regulation by inhibiting other myogenic regulatory factors, such as MyoD. We are interested in determining the role of Twist in DM1 and whether it participates in the inhibition of muscle cell differentiation. RNA and protein analysis was performed on normal and DM1 muscle cells. Preliminary results show that Twist levels are fluctuated during muscle cell proliferation and differentiation in DM1 cells. Moreover, the pattern of Twist expression levels in DM1 muscle cells is different compared to that of the wild type cells. These results indicate that Twist might be involved in the mechanism of inhibition of muscle cell differentiation and might play an active role in the development of the DM1 phenotype.

P1-20 Cytoplasmic export of DM1 transcripts benefits muscle cell differentiation

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Mutant DMPK transcripts are known to be retained in the nucleus of myoblasts and inhibit myogenesis in DM1. Nevertheless, it is still unclear how exactly the retention of the mutant transcripts induces this defect. We have recently created a novel cellular model in which the mutant DMPK 3´UTR transcripts were released to the cytoplasm of myoblasts by using the WPRE genetic element. As a result, muscle cell differentiation was repaired. Furthermore, this cellular model was exploited to investigate the effect of the levels and location of the mutant transcripts on muscle cell differentiation. Stable clones expressing a variety of nuclear and cytoplasmic mutant DMPK 3'UTR RNA levels were selected. The initial cell fusion (ICF) and myotube maturation (MM) of each clone were then measured. Experiments revealed that the total amount of mutant DMPK RNA was proportional to the inhibition of both the initial cell fusion and myotube maturation. This is the first time that the measurable levels of mutant DMPK transcripts were related to the degree of inhibition of muscle cell differentiation. It was also concluded that less inhibition of ICF occurs when mutant transcripts are localized in the cytoplasm of myoblasts, compared to those which are localized in the nucleus. Cytoplasmic inhibition, similar to that seen in the nucleus is observed only at high mutant DMPK RNA concentrations. On the contrary, the inhibition in MM seems to be similar, regardless whether the mutant DMPK 3'UTR transcripts are located in the nucleus or the cytoplasm. These results indicate that mutant DMPK RNA levels are proportional to the inhibition of muscle cell differentiation and that the repair seen in the induced nuclear export could be due to a repair seen in the initial cell fusion during myogenesis.

P1-21 Cell model for repeat instability and senescence in DM1

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Background: Previous work has shown repeat instability and premature senescence in primary DM1 cells.
Objective: To develop mammalian cell models to examine the somatic instability of expanded CTG repeats.
Methods: Constructs that contain uninterrupted highly-expanded CTG repeats were prepared by cell-free cloning. The repeat was cloned within the DMPK 3'UTR. The constructs were designed for conditional transcription of the repeat. Normal human fibroblasts were stably transfected with these constructs. Clonally derived cells were analyzed for repeat instability by small-pool PCR. Proliferative capacity of cells was assessed.
Results: Stably transfected cells showed distinct nuclear foci after induction of CUG transcription. Repeat instability was observed in proliferating cells, and exaggerated by transcription of expanded CUG repeats. Transcription of the repeat also led to premature growth arrest, whereas cells harboring a non-transcribed repeat continued to replicate.
Conclusion: Preliminary results from this model provide further evidence that instability of hyper-expanded CTG repeats, and effects on replicative life span, are increased by transcription of the repeat tract. This model provides a simple mammalian system to investigate the mechanisms for repeat instability and premature senescence in DM1.

P1-22 Mis-splicing of microtubule-associated tau exon 10 is associated to a CELF proteins gain of function but not to a MBNL1 loss of function

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DM1 is a multisystemic neuromuscular inherited disease characterized by a dynamic unstable CTG expansion in the 3'UTR of DMPK gene. The central nervous system is also affected. Main clinical symptoms include cognitive dysfunctions such as executive disabilities and facial emotional recognition difficulties. Neurofibrillary degeneration (NFD) is often observed in the limbic system of elderly DM1 patients. NFD corresponds to the intraneuronal aggregation of abnormally modified microtubule-associated Tau proteins. We and others previously described a modified splicing of Tau exon 2, 3 and 10 which may be explained by

the trapping of Muscleblind-like (MBNL) splicing regulators in CUG repeats or by a gain of function of CELF family members. Here, we analyzed the spatiotemporal distribution of NFD, the instability of the CTG expansion and focused on the splicing of Tau exon 10 in the brain of a DM1 patient originated from North of Spain, and compared the results to those previously obtained in three other DM1 patients. This patient was a 60 years old man, carrying a moderate expansion (150 CTG repeats). NFD was observed in the hippocampus and the most severely affected brain region was the amygdala. This brain structure of the temporal cortex is essential for the facial emotion recognition process and presence of NFD in this region may explain the alteration of this cognitive function. At the molecular level, the splicing of Tau exon 2,3 and 10 was modified in all brain regions studied. However, a reduced inclusion of Tau exon 10 was observed only in this Spanish DM1 patient, not in the three others. Although the maximal length of CTG repeats in the brain (1000) was lower than those observed in the three others (more than 3000), it could not explain the difference of Tau exon 10 splicing. Interestingly, in the brain tissue of the Spanish DM1 patient, we observed an increased amount of some CELF proteins that was not observed in the other DM1 patients. In vitro experiments in HeLa cells showed 1) that a reduced inclusion of Tau exon 10 is induced by long CUG tracks but not by MBNL1 loss-of-expression. 2) overexpression of ETR3 and CELF4 proteins, but not overexpression of CUGBP1, have a weak effect on Tau exon 10 splicing. However, 2-dimensional gel electrophoresis suggested a modified phosphorylation of CUGBP1 in DM1 brain. Together, our results suggest that Tau exon 10 splicing might be sensitive to a gain of one or more CELF proteins function, but does not exclude the involvement of other splicing factors.

P1-23 Specific micro-RNA processing alteration in DM

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Myotonic dystrophy (DM) is the most common form of adult muscular dystrophy. DM patients exhibit several symptoms including cardiac conduction abnormalities resulting in sudden death. DM is due to CUG or CCUG repeats expansion which accumulates in nuclear RNA aggregates that sequester the Muscleblind like 1 (MBNL1) splicing factor, leading to alternative splicing alterations.
Recently, it has been demonstrated that small RNA called microRNA (miRNA) play an important role in heart function. miRNA are cell type dependent regulators of gene expression by inhibiting mRNA translation or promoting mRNA degradation.
Using DNA microarray in a muscle cellular model of DM, we found specific misregulation of miRNA implicated in the regulation of genes important for cardiac conduction. These misregulations were confirmed by quantitative PCR in this model but also in DM patient's heart samples. Misregulation of miRNA targets was shown at mRNA level and at protein level in DM model cells. Moreover, by UV-Crosslinking experiments, we found that MBNL1 binds to miRNA sequence. Furthermore, miRNA processing is impaired in cellulo with overexpression of long CUG repeats or shMBNL1. These preliminary data suggest a role of MBNL1 in miRNA processing.
In conclusion, our results suggest that MBNL1 may regulate maturation of specific miRNA essential for heart conduction. This work may shed light on the molecular mechanisms of heart conduction defect in DM patients.

P2 Poster Session 2: Basic Research – Part 2

P2-01 The role of microRNAs in the regulation of gene expression in Myotonic Dystrophy

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Myotonic Dystrophy 1 is a neuro-muscular disease caused by non-coding CTG repeat expansion in the 3'UTR of DMPK gene on chromosome 19q. We showed that CTG expansion causes DM1 pathology at the RNA level. The mutant CUGn RNA affects RNA-binding proteins, CUGBP1 and MBNL1, altering RNA processing in patients'tissues. CUGBP1 is a multifunctional protein which controls translation, splicing and RNA decay. CUGBP1 regulates RNA decay through the binding to ARE elements in the 3'UTRs of mRNAs. We recently found that CUGBP1 may also control RNA stability by delivery of microRNAs (miRs) to mRNAs and following inhibition of translation of these mRNAs. To elucidate the role of CUGBP1-miR pathway in DM pathology, we have compared global expression of miRs in myoblasts and fibroblasts from control, DM1 and DM2 patients. This analysis showed that the levels of three miRs are specifically altered in DM1 and DM2 myoblasts. MiRs let7A and let7I are reduced in both DM1 and DM2 myoblasts. MiR-365 is significantly increased in DM1 myoblasts, but not in DM2 myoblasts. We have found that CUGBP1 specifically binds to miRs 365 and let7A. These miRs have predicted RNA targets among several functional groups of genes which are significantly changed in DM1 and DM2. These genes regulate different functional pathways including cell adhesion, cell cycle, cytokines, DNA replication and tight junction. The elucidation of the role of the identified miRs in DM1 and in DM2 will help better understand pathological alterations and develop therapeutic approaches to cure DM1 and DM2.

P2-02 Abnormal expression of ZNF9 in myotonic dystrophy type 2 (DM2)

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Objective: The host gene for DM2 repeat expansion mutation ZNF9 and its protein product is thought to be of no importance for the disease pathogenesis. However, recently, heterozygous Znf9+/- knockout mice were shown to develop a multiorgan phenotype with similarities to DM. This prompted for a comprehensive study to address in detail the expression of the gene transcript and ZNF9 protein in DM2 patient muscle.
Methods: The expression of ZNF9 mRNA and protein was assayed by microarray profiling, real-time RT-PCR, splice variant analysis. Immunofluorescence and western blot analysis were performed using three different ZNF9 antibodies.
Results: Our results indicate that ZNF9 is not normally expressed in DM2. First, there is a significant overall reduction at both the mRNA and protein levels, and an abnormal splice isoform with retention of intron 3 was detected in a small fraction of the mRNA. Second, in DM2 patients the subcellular localization of ZNF9 protein is different with less cytoplasmic and more membrane-bound protein. Third, in

the subpopulation of highly atrophic type 2 fibers, characteristic for the muscle pathology in DM2, ZNF9 protein expression was markedly increased. As a general finding we also observed that in differentiating myoblasts ZNF9 protein is localized mainly nuclear, while in the mature muscle fibers it is not nuclear but cytoplasmic and organized in sarcomeric striations in the Z-disc.
Conclusions: The reduction in the expression of ZNF9 appears to be related to the aberrant splicing of ZNF9. Retention of intron 3 gives rise to a mutant transcript with a premature termination codon and this abnormal transcript is likely to be subject to nonsense-mediated decay, explaining its low abundance. The cytoplasmic localization of ZNF9 and abnormal expression in DM2 patients, suggest that ZNF9 functions may not be restricted to transcription regulation and that the disease pathomechanisms may also involve abnormal ZNF9 expression in DM2.

P2-03 Proteomic analysis of DM2 human myotubes reveals alteration in mitochondrial components, in the unfolded protein response and the ubiquitin proteasome system

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Myotonic Dystrophy type 2 (DM2) is caused by a microsatellite amplification at the level of the DNA. The toxic RNA gain of function leads to the abnormal splicing pattern, largely responsible for the pathological condition. However to better define the functional changes occurring in DM2 patient derived myotubes cultures we though it is important to analyze also the global protein profile of the pathological state. To this aim we performed a quantitative proteomic analysis, comparing the cytosolic/membrane and the nuclear protein expression pattern of differentiated myotubes from DM2 patients with those from sister parallel control cultures. We cluster the proteins changing their expression in DM2 cultures in three major functional classes i) Mitochondrial components, with statistically significant reduction of the translational factor EFTu, and of two mitochondrial chaperones, HSP60 and HSP70, ii) the UPR (unfolded protein response) with variations of the levels of chaperones ERp29, and PDI, iii) the UPS (ubiquitin proteasome system) with changes of the 26S proteasome regulatory subunit 13 and COP9 signalosome subunit 4, and a reduction of Rad23B homolog. Changes affecting the UPS are supported by a global reduction of the ubiquitinated proteins profile. In addition, we found a decremental change in the level of the chloride intracellular channel 1 (CLIC1), a redox-regulated ion channel. The data were also partially validated by western blotting with specific antibodies. In conclusion our results suggest that DM2 cultures are exposed to a stress condition and the alteration observed might be a cellular homeostatic adaptation to the stress.

P2-04 Progressions of (CTG) n expansions, muscular disability rating scale (MDRS), and abnormal glucose metabolism are age dependent in Myotonic dystrophy type 1 (DM1)

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Background: DM1 is an autosomal dominant disorder characterized by a wide variety of clinical features. The mutation responsible for DM1 is an unstable expansion of a (CTG) n in the 3'untranslated region of a gene encoding DMPK. The somatic and mitotic instability of (CTG) n expansions has been reported. We therefore investigated whether the progressions of (CTG) n expansion in leukocytes, MDRS, and abnormal glucose metabolism might be age-dependent. Object and Methods: In 13 DM1 patients the (CTG) n length, the values of MDRS, and abnormal glucose metabolism (AGM) were examined twice at intervals of 3-10 years. 1) To determine the (CTG) n length, standard Southern blot analysis was performed using the method described previously. Briefly, the genomic DNA extracted from the leukocytes was digested with the restriction enzyme of Eco R I, then hybridized with a 32P-labeled cDNA 25 probe. 2) The MDRS described by Mathieu et al. were used to estimate the extent of muscular involvement. 3) Standard 75g oral glucose tolerance test was done according to the National Diabetes Data Group recommendations. Results: The initial (CTG) n length varied from approximately 230 to 1670 repeats. In all patients, the (CTG) n lengths had expansions with an average size of 70 repeats / year and the values of MDRS were elevated with an average values of 1.2. In 6 patients the extent of abnormal glucose metabolism was progressed. Conclusion: Both muscular involvement and AGM in DM1 might be progressed with age-dependent (CTG) n expansion

P2-05 High-Throughput Screening to Identify Modulators of Aberrant Splicing in DM1

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Aberrant splicing events caused by the entrapment of Muscleblind 1 (MBNL1) in nuclear foci with DMPK mRNA and the resulting increase in activity of CUG binding protein 1 (CUGBP1) contribute to the disease pathology of Myotonic Dystrophy type 1 (DM1). The identification of mechanisms that restore target gene splicing may lead the way to efficacious new DM1 therapeutics. Here we describe the assay development and high-throughput screening (HTS) of 670,000 small molecule compounds using two reporter gene assays in 1536-well format. A high content imaging screen was conducted monitoring splicing of a bichromatic minigene reporter construct sensing the inclusion of cardiac troponin T exon 5 in HEK293 cells overexpressing CUGBP1, using the Evotec Opera confocal imaging system. In parallel, the same library of compounds was screened against a luciferase minigene reporter monitoring intron 2 retention in the skeletal muscle-specific chloride ion channel, in immortalized DM1 patient-derived (DMPK CTG1000) myoblasts. The HEK293-based assay was designed to bias small molecule hits towards modulators of the CUGBP1 pathway, whereas the patient-derived cell system utilized a less CUGBP1-dependent reporter construct embedded in a DM1 cellular context. The combined hit lists of these two screens yielded 1600 compounds that were triaged in dose-response format against both HTS assays, as well as against alternative reporter constructs in DM1 pa-

tient fibroblasts and myoblasts, and toxicity filter assays. Functional genomics screens using the same assay systems will aid in our understanding of the aberrant splicing of DM1, as well as the mechanism of action for small molecule leads.

P2-06 Subcellular localization of Drosophila Muscleblind changes from preferentially nuclear to citoplasmic during muscle development

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Drosophila muscleblind gene, as similarly described for its human MBNL1 ortholog, regulates alternative splicing of defined pre-mRNAs. In the embryo, the protein is detected in the nucleus of pharyngeal, visceral and somatic muscles, as well as in the ventral nerve cord, and the Bolwig's organ (the larval photorreceptor system). However, little is known about the expression of Muscleblind during larval and adult stages, and whether the preferential nuclear localization is developmentally regulated. In this study, we have used a polyclonal antibody against all Muscleblind isoforms to describe throughout development the expression pattern of Muscleblind in different tissues. We have observed that Muscleblind expression in skeletal muscle and nervous system is maintained from embryo to adult. However, in muscular tissue a progressive change from a preferentially nuclear localization to preferentially citoplasmic occurs. Furthermore, using double staining with the Actin marker phalloidin we have observed that Muscleblind colocalizes with Z bands and M lines in adult sarcomeres. This suggests that Muscleblind, besides its known involvement in alternative splicing regulation, has additional molecular roles. We are currently working on detailed descriptions of Muscleblind expression in other tissues, and in identifying these new molecular roles.

P2-07 DMPK-INTERACTING PROTEINS

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BACKGROUND: Myotonic dystrophy type 1 (DM1) is caused by an expansion of CTG repeats at the 3'-UTR of the serine/threonine protein kinase DMPK. Expanded CTG-repeats are toxic since they are transcribed into an RNA molecule which is then sequestered within the nucleus in form of foci. RNA cytotoxicity is linked to the aberrant splicing of several developmentally regulated genes, including insulin receptor, muscle-specific chloride channel, cardiac troponin T, sarcoplasmic/endoplasmic Ca2+- ATPases and ryanodine receptor. Strong evidence in favour of the toxic gain-of-function is given by DM type 2 (DM2) which share many genetic and clinical features with DM1. DMPK pre-mRNA undergoes alternative splicing giving rise to many isoforms. However, it seems not involved in the splicing dysregulation of DM1. In the Swiss-Prot database, more than twelve human isoforms are listed (SW-Q09013) together with ten variants in mouse (SW-P54265). Such a variability in phenotypic expression of DMPK together with its differential subcellular targeting suggest that different splicing isoforms may be involved in different signalling pathways, possibly through DMPK-interacting proteins. On the other hand, the decreased amount of DMPK in DM1 patients and muscle pathology in DMPK knockout mice including impaired glucose/insulin metabolism, suggest that haploinsufficiency of DMPK may play some role in DM1 pathophysiology. METHODS: To obtain a better understanding of the DMPK function, we used a proteomic approach to characterize proteins co-segregating with DMPK in soluble complexes isolated from high-speed supernatant of rat muscles. DMPK has a very low abundance in tissues, and a comprehensive analysis of DMPK interacting-proteins is still lacking. We carried out experiments with DMPK in its native form to preserve its physiological stoichiometry with the potential partners. DMPK-containing complexes were isolated and immuno-detected by non-denaturing electrophoresis, gel-filtration, ionic-exchange chromatography and immunoprecipitation. RESULTS: DMPK peptides detected by high resolution mass-spectrometry (MS) were identified by matching them to protein sequences in the IPI data-base. On the whole, the DMPK coverage was 18%. We found several putative DMPK-binding proteins, including few heat shock proteins such as HSP20/HSPB5, HSP60/CPN60, HSP70, HSP90. Furthermore, we obtained evidence of a direct interaction between DMPK and alphaB-crystallin/HSPB5. Surprisingly, we did not detect by MS neither MKBP/HSPB2, nor previously described substrates of DMPK-mediated phosphorylation. CONCLUSIONS: Our results suggest that subcellular localization of the isoforms might be relevant to the specificity of DMPK interactions. Furthermore, the altered concentration of DMPK and/or the unbalance between its isoforms may be relevant factors to the pathogenesis of DM1, leading to secondary alteration of regulatory pathways mediated by different interactors.

P2-08 Transcriptional defects in DM1 result from nuclear exclusion of SHARP

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This study describes a novel mechanism by which CTG tract expansion impacts DM1 pathology. We demonstrate that the steady-state RNA levels of a panel of physiologically important RNAs in DM1 patients is unlinked to MBNL1, MBNL2 loss or the over expression of CUG-BP1 and hnRNPH, four alternative splice factors that have been implicated in the development of DM1 specific splice defects. Our data demonstrates that such transcriptional defects in DM1 occur as a consequence of the nuclear exclusion of the transcription factor, SHARP. Nuclear exclusion of SHARP occurs subsequent to MBNL1 complex deregulation. As aberrant localization of SHARP occurs as a late event in DM1 biology it is predicted to play a important role in DM1 disease progression.

P2-09 Autoregulation of MBNL1: coupling of splicing regulation and intracellular localization

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Although loss-of-function of MBNL1 has been postulated in the pathogenesis of DM, how the function of this protein is regulated is still unclear. Identification of the mechanism regulating the activity of MBNL1 would be important for both understanding of DM pathogenesis and therapeutic interventions.

Method: By using multiple minigenes, the splicing regulatory activities of MBNL1 splice isoforms and mutants were analyzed. Intracellular localization of these proteins was also compared. Fluorescent minigenes of MBNL1 exon 7 was established to test the relationship among the pattern of exon 7 splicing, intracellular localization and splicing activity of MBNL1. These minigenes were also used for identification of cellular factors that regulate the splicing pattern of MBNL1.
Results: A nuclear localization signal of MBNL1 was mapped to a region across exons 7 and 8. Inclusion of exon 7 determined both intracellular localization and splicing regulatory activity of MBNL1. Alternative splicing of exon 7 was regulated by MBNL1 itself, an expanded CUG repeat and several other cellular factors.
Conclusions: We found that the splicing regulatory activity and intracellular localization of MBNL1 are coupled by the alternative splicing of MBNL1 exon 7, which is regulated by MBNL1 itself. This suggests a functional feedback to maintain nuclear MBNL1 activity to a certain level. This mechanism might facilitate nuclear sequestration of MBNL1 by expanded repeats. In addition, the fluorescent minigenes established in this study might be used to screen drugs that modulate the functionality of MBNL1.

P2-10 Muscleblind-like proteins in normal and myotonic dystrophy muscle and their role in rapid diagnostic testing.

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There is strong evidence for the role of muscleblind-like protein 1 (MBNL1) in DM pathology, but the roles of the related proteins, MBNL2 and MBNL3, are much less clear. Using monoclonal antibodies specific for each of the three gene products, we found that MBNL2 decreased during human fetal development and myoblast culture, while MBNL1 was unchanged. In adult muscle, MBNL2 was elevated in immature, regenerating muscle fibres compared with mature fibres, supporting some developmental role for MBNL2 in muscle. MBNL3 was not detected in human tissue. Both MBNL1 and MBNL2 were partially sequestered by nuclear foci of expanded repeats in DM1 muscle biopsy compared with an aged-matched control.
In DM1, nuclear foci of CUG repeats accumulated at the periphery of nuclear splicing speckles. This can be used to distinguish between DM1 and DM2, since nuclear foci of CCUG repeats were widely dispersed in the nucleoplasm and not associated with nuclear speckles.
We have also investigated the diagnostic possibilities of nuclear foci in epithelial buccal cells. Foci were detected in juvenile onset DM1 patients and the method could be useful as a rapid, non-invasive diagnostic test for congenital myotonic dystrophy.
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P2-11 The mechanisms of MBNL1 regulated splicing

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To understand the mechanisms through which MBNL1 regulates splicing, we have used exon 5 of the cardiac troponin T (cTNT) pre-mRNA as a model system. In order to identify the nucleotides in the cTNT site that contribute most to recognition by MBNL1, we performed a doped SELEX experiment. Unlike traditional SELEX, which starts with a pool of uniformly random RNAs, doped SELEX begins with a population of RNAs synthesized such that they are biased towards a specific starting sequence, but each position is still allowed to vary. This experiment revealed YGCU motifs embedded in pyrimidines as high affinity sites for MBNL1. The cTNT site is currently the only known human MBNL1 binding site. To identify additional MBNL1 sites we analyzed the intronic and exonic sequences encompassing 24 exons known to be mis-spliced in DM1. We identified the locations of all instances of YGCU within the last 200 nucleotides of the upstream intron and the first 200 nucleotides of the downstream intron (if either intron was less than 400 nucleotides in length then only half of the intron was included). All 24 mis-spliced exons contain one or more instances of the YGCU motif in adjacent intronic sequence, and several contain instances within the exon as well. Interestingly, we found that the location of the motif appears to correlate with the type of splicing regulation exerted by MBNL1: positive regulation by MBNL1 if the motif(s) are downstream of the exon and negative if found upstream of the exon.

P2-12 What are RNA foci? Interactions of the mutant DMPK mRNA

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Rationale/Hypothesis: Aberrant RNA-protein interactions with the mutant DMPK mRNA may explain DM1 pathogenesis. Evidence for in vivo interaction of the mutant RNA with candidate RNA-binding proteins will help in studying the role of RNA foci and the mutant DMPK mRNA in DM1 pathogenesis.
Methods: Using a fluorescence assay to detect RNA-binding proteins and the mutant DMPK mRNA, we studied the expression and distribution of candidate RNA-binding proteins including MBNL1, CUGBP1, hnRNP-H, hnRNP-C, etc. in human fibroblasts, myoblasts and tissues as well as tissues from mouse models of RNA toxicity. We applied advanced microscopic techniques to assess in-vivo interactions between the mutant DMPK mRNA and RNA-binding proteins. Cellular fractionation followed by western blotting and RT-PCR were used to assess the distribution of these proteins and the mutant DMPK mRNA and to compare results between normal and DM1 cells.
Results: We assessed MBNL1 first. We observed nuclear and cytoplasmic distribution of MBNL1 in unaffected as well as DM1 cells. We confirmed the co-localization of MBNL1 to the mutant DMPK mRNA. In contrast to skeletal muscle tissue where MBNL1 sequestration was marked, in DM1 cells it was incomplete. Additionally, our assays demonstrated for the first time in-vivo interactions between MBNL1 and the mutant DMPK transcript.
Conclusions: This represents the first in vivo demonstration of interaction between MBNL1 and DMPK mutant RNA and provides proof of principle that this approach can be used to study relevant RNA-protein interactions. Utility of this approach in studying other RNA-protein interactions and drug discovery will be presented

P2-13 Abnormal splicing of myomesin in DM muscle

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Many studies confirmed that alternative splicing of genes is abnormally regulated in DM cells. Increased inclusion of chloride channel 1 (CLCN-1/CLC-1) exon 7A is associated with myotonia in DM1, a genetic disease caused by the expansion of a CTG repeat. In mouse models, myotonia as well as aberrant splicing of the mouse counterpart of CLC-1, Clcn1, can be induced by either over-expression of CUG repeat RNAs or knockout of Mbnl1, an RNA-binding protein sequestered by CUG repeats in DM1 cells.
Using exon array system, we identified abnormally spliced genes in DM1 muscle. One of these genes, myomesin is possibly involved in muscle weakness in DM muscle. The results provide molecular evidence for a novel mechanism for DM pathogenesis.

P2-14 Mathematical models of dynamic DNA in myotonic dystrophy

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BACKGROUND AND OBJECTIVES: Currently, DM1 patients, concerned about their own prognosis and their reproductive choices, have limited information available to them about how their disease will progress. This is because average allele length only accounts for 25% of the variance in age of onset. Thus, there is great potential for more sophisticated modelling and inference techniques to improve the prognostic value of genetic information. We aim to develop mathematical models to capture the key features of the mechanism underlying allele length evolution. These models can then be used to predict age of onset and severity of the symptoms with greater precision. This has potential for improving prognostic information for patients as well as providing a deeper understanding of the underlying biological process.
METHODS: These mathematical models, created using a range of deterministic and stochastic techniques, have biological parameters, some of which can be measured experimentally and some of which must be inferred indirectly. Parameter estimation and model comparison are important steps towards obtaining an explanatory model that can be used for simulation and prediction. We are developing modern Bayesian techniques involving Markov chain Monte Carlo in order to calibrate our models against the biological data, collected from small pool PCR analysis of allele length in blood cells from 145 DM1 patients, which reveals the extent and nature of the variation of allele length within and between patients.
RESULTS AND CONCLUSION: We report initial findings that the underlying biological mechanism consists of both expansions and contractions, with only a slight bias in favour of repeat expansion, and that the observed tendency towards expansion is the net result of many more expansions and contractions than previously thought.

P2-15 Aberrant expression of microRNA in myotonic dystrophies

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Rational and objects. MicroRNAs (miRNAs) are a class of short endogenous non-coding RNA molecules that post-transcriptionally regulate gene expression. Recent studies have demonstrated that miRNAs are required for muscle development and function, with crucial roles for miRNAs in regulating muscle cell proliferation and differentiation. Dysregulated expression of miRNAs has been functionally linked to muscle-related diseases, including cardiac hypertrophy, cardiac arrhythmias, and ischemia. Distinctive patterns of miRNAs expression were also described in many neuromuscular disorders such as Duchenne, Becker and facioscapulohumeral muscular dystrophies, but miRNAs have not been studied in myotonic dystrophy. Methods and results. We measured the expression of 24 specific miRNAs by TaqMan Real Time PCR (qPCR) method. This analysis was performed in muscle biopsies of DM1 (n=15), DM2 (n=9), and control (n=10) subjects. Upregulation of miR-1 and miR-335 and downregulation of miR-29b, miR-29c, miR-33 and miR-223 were observed in DM1 biopsies. In DM2 biopsies, upregulation of miR-34c was identified. The expression of these miRNAs was measured in patient-derived myoblast and myotube cultures. No significant differences between DM1, DM2 and control were observed, indicating that the observed miRNA dysregulations were not cell autonomous. Moreover, an aberrant tissue distribution of miR-1 was observed by in situ hybridization in muscle biopsies of DM1 patients. Conclusion. These results indicate that miRNAs are differentially expressed and localized in DM, suggesting a potential role in DM pathogenesis.

P2-16 Abnormally expressed genes in statin induced myopathy appear dysregulated in myotonic dystrophy type 2.

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BACKGROUND: A CCTG repeat expansion in intron 1 of ZNF9 causes a plethora of pathological pathway changes which manifests into Mytonic dystrophy type 2 (DM2). The treatment of hyperlipidemia associated with DM2 insulin resistance, has shown that patients have an increased incidence of statin induced myopathy.
OBJECTIVES: To examine the global gene expression changes in DM2 and to distinguish pathways that are dysregulated, thereby highlighting the main mechanisms in DM2 associated statin induced myopathy.
METHODS: We studied the gene expression profile in skeletal muscle biopsies of DM2 patients in two different DM2 related projects: DM2 associated lipidomics expression changes and specific statin induced myopathy expression changes. The oligonucleotide microarrays gene

expression changes that correlate are assessed by RT-PCR with the aim of identifying alternative splicing or aberrant splicing changes.
RESULTS: We have identified a number of genes which show expression changes in the microarray profile and through RT-PCR. We will attempt to link each candidate gene to lipidomic changes and statin induced myopathy specific for the DM2 pathology. We investigated 22 genes that showed expression increase or decrease in the different studies, including well validated target genes, such as MBLN1. 14 transcripts, including MYBPH, FBLN1, CREBBP, BCL2,UBE2D1, KAL1, APOD and MITF showed their expression changed in RT-PCR analysis.
CONCLUSION: We have identified a range of target genes, many of which have been previously linked to myotonic dystrophy and various muscle pathologies. These gene expression changes in critical candidate genes may give new insight into reasons why DM2 patients have an increase in incidence of statin induce myopathy.

P2-17 Use of human embryonic stem cells as new model to decipher early pathological events involved in Myotonic Dystrophy type 1.

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Human embryonic stem cell lines (hES), derived from an embryo during pre-implantation diagnosis (PGD) which express a disease-related mutated gene, could represent a relevant cellular model allowing analytic and therapeutic research for the disease.
Here, we took the opportunity of three existing hES cell lines affected by Myotonic Dystrophy type 1 (DM1) (ranging 400 to up than 1800 expanded CTG repeat in the 3' UTR of the DMPK gene) to address early developmental events associated with the mutation.
The three mutated and two control hES cell lines were specified towards homogeneous population of neural stem cells (NSC) that we are able to differentiate towards greatly enriched population of postmitotic neurons.
We found that in the mutant cell populations (NSC and neurons), the mRNA for the DMPK gene accumulates in nuclear aggregates (foci) and co-localized with muscleblind 1 (MBNL1) protein, a splicing factor as previously described in DM1 patients'cells. Validating the pertinence of this cellular model, our results suggest that, whereas considered as a late-onset disease, DM1 could be associated with early developmental alterations.
In order to identify new modulated genes in association with DM1 mutation, we performed a differential whole genome transcriptional analysis between mutant and control cultures. This gene expression profiling reveals a unique set of modulated genes, not yet known to be implicated in DM1 that could provide new insights both pathological process and protective pathways.
In conclusion, this study underlines the importance of PGD-derived ES cell lines as a tool to decipher molecular mechanisms of monogenic diseases and provides some significance about the potential of mutated human embryonic stem cells to mimic events occurred in adult patient cells.

P2-18 Altered Isoform Usage for MADS-Domain Transcription Enhancer Factor 2 (MEF2) in Myotonic Dystrophy and Other Neuromuscular Disorders

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Rationale: Because of their central role in muscle development and maintenance, the members of the MEF2 gene family represent excellent candidate effectors of the muscle pathology in myotonic dystrophy (DM).
Methods: We investigated the expression of the four MEF2 family members in skeletal muscle biopsies from normal individuals and patients with neuromuscular diseases (NMD) that feature either myotonia or dystrophy, including myotonic dystrophy types 1 and 2 (DM1, DM2). Since DM is characterized by a global splicing defect, we also investigated the alternative splicing of these four genes.
Results: We observed that both MEF2A and MEF2C, but not MEF2B and MEF2D were over-expressed in all NMD. Coordinate up-regulation of twelve MEF2-interacting genes was also observed. Usage of cassette exons 4 and 5 in both MEF2A and MEF2C showed a significant difference between DM and normal muscle, with the diseased muscle similar to the embryonic isoform. Additionally, when all non-DM neuromuscular disease biopsies were compared to normal, we saw similar significant differences in splicing for all genes. For MEF2C, the missplicing was significantly higher in DM than in the other NMD.
Conclusion: Our data confirm dysregulation of MEF2A and MEF2C for both expression and splicing in several neuromuscular disorders, including myotonic dystrophy. It also demonstrates that aberrant splicing in neuromuscular disorders is not dependent upon the presence of expanded repeats, and suggests that some aberrant splicing, even in DM, may be compensatory rather than primary.

P2-19 Effects of mexiletine on cardiac parameters, muscle strength and myotonia in myotonic dystrophy type 1

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Background: The use of mexiletine (Mxt) to treat myotonia in myotonic dystrophy (DM1) is controversial: data on efficacy is limited and there are concerns about potential cardiac adverse effects. In order to design a randomized trial to test the effects of Mxt on myotonia and on muscle strength in DM1 patients, it is mandatory to evaluate its tolerability and safety in such patients.
Aims: To determine the effects of long term treatment with Mxt on cardiac parameters, muscle strength and myotonia in DM1 patients.
Patients and methods: 57 patients with DM1, treated with Mxt (200mg tid for a mean period of 7.46 yrs), and 104 control patients with DM1 matched for age, gender, disease duration, disease severity, CTG expansion and follow-up duration, underwent repeated cardiac evaluation, including 12-lead-ECG, ECG-Holter. Muscle strength (MRC from 15 muscles) and myotonia (4-point Likert scale) were also evaluated.
Results: Number of deaths and PM implantations was similar in both groups ($p = 0.85$), such as progression of AV and IV conduction distur-

bances (mean PR: baseline 185.92 vs 190.82, follow-up 191.44 vs 192.7.0; mean QRSd: baseline 108.62 vs 105.41, follow up 112.64 vs 114.56 respectively in treated and untreated groups. $p > 0.99$ for all parameters). No major arrhythmias were identified by ECG-Holter. Side-effects were minimal (1 hypotension, 3 gastric discomfort). Myotonia improved significantly in the treated DM1. MRC decreased significantly and similarly in both treated and untreated DM1 patients ($p < 0.01$).
Conclusions: Mxt is safe and well tolerated and seems to be effective in relieving myotonia in DM1 patients but a controlled randomized trial is warranted.

P2-20 Non-radioactive Detection of Repeat Expansions in DMPK and ZNF9 Genes

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DMPK and ZNF9 genes contain tandem repeat sequences that when expanded cause myotonic dystrophies type 1 (DM1, Steinert disease) and type 2 (proximal myotonic myopathy, PROMM or Ricker syndrome). In the case of DM1 a [CTG]n trinucleotide repeat sequence in the 3' region of the DMPK gene on chromosome 19q13 is expanded to 51 up to several thousand repeats. Determination of the specific repeat number is necessary to detect anticipation and to assess prognosis with regard to minimal, classical, juvenile or congenital expression of the disease. In PROMM a [CCTG]n repeat in complex repeat region with [TG]n[TCTG]n[CCTG]n in the first intron of the ZNF9 gene on chromosome 3q21 is expanded from 75 to over 11.000 units, with an average of 5000 repeats. Analysis of repeat expansion in the DMPK gene and ZNF9 genes is fast, inexpensive, and reliable. Both the clinical sensitivity and the clinical specificity are >99%. We now report a highly sensitive Southern blot method without using radioisotope labelling. As first step standard PCR is used to detect possible presence of two normal alleles of different sizes; Patients with one allele are further investigated. For DM1, repeat primed PCR for DMPK is carried out, followed by Southern blot of the DMPK gene after digestion of genomic DNA with EcoRI and BglI. For PROMM, Southern blot involves hybridization of a long-range PCR product of the ZNF9 gene with a [CCTG]5 probe. The labeling of probes was achieved by PCR using dTTP with the glycoside digoxigenein and specific primers for DMPK gene or [CCTG]5 oligonucleotides. Hybridized probes were detected by anti-DIG antibody following manufacturer's instructions. Using this approach we get optimal resolution of bands in the gel for DMPK and smears in expanded PROMM as in view of somatic heterogeneity of expanded alleles. In conclusion using Dig labeled probes for detection of expanded alleles in the DMPK and ZNF9 genes is as reliable as using radioactive probes.

P2-21 Global expression profiling of myotonic dystrophy type 1 (DM1) and type 2 (DM2) identifies novel effector genes and cellular pathways

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Rationale: Despite similar mutations, DM1 and DM2 are clinically distinct. The basis for the differences is unknown. The prevailing paradigm is that DM is a toxic RNA disease, mediated by expression of mutant (CUG)DM1 and (CCUG)DM2 transcripts. RNAs accumulate in foci and interfere with splicing, transcription and translation of "effector"genes.
Objective: To identify effector genes shared and distinct between DM1 and DM2, we performed global mRNA expression profiling in DM1 (n = 10) and DM2 (n = 20) patients and normals (n = 6).
Methods: Expression profiling of skeletal muscle biopsies was performed with Affymetrix U133Plus2 microarrays. Differentially regulated genes were analyzed with Ingenuity Pathway Analysis (IPA) to identify dysregulated cellular pathways. We also performed a focused analysis of 349 manually curated genes involved in RNA metabolism.
Results: IPA identified 19 significantly dysregulated pathways. Among the three most significant, "calcium signaling"and "actin cytoskeleton"are well studied in muscle physiology. The third, "hepatic fibrosis/hepatic stellate cell activation", suggests a link between the fibrosis seen in DM and the TGF-beta pathway. Analysis of RNA-processing genes identified three groups with >30% enrichment ("alternative splicing", "A-complex associated", and "LSm proteins"). In all, 113/1,013 probesets (11%) representing 78 RNA-processing genes were dysregulated in DM patients, with significantly more genes (61 vs. 17) upregulated.
Conclusion: Consistent with their similar pathologies, DM1 and DM2 showed highly correlated gene expression profiles and shared enrichment of pathological pathways. Dysregulation of genes involved in RNA metabolism may underlie the observed multi-systemic phenotype that distinguishes DM from other inherited neuromuscular disorders. Overall, our data indicate a global trans-dominant effect of the toxic (CUG)DM1 and (CCUG)DM2 on gene expression.

P2-22 New Technique for Rapid and Reliable Analysis of Trinucleotide Repeats in Myotonic Dystrophy Type 1

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Molecular genetic testing of myotonic dystrophy type 1 (DM1) is based on the identification and determination of a CTG repeats expansion in the DMPK gene. This is usually done by Southern blot analysis – a time consuming and very laborious technique requiring high molecular weight DNA. The aim of our study was to develop a highly sensitive, rapid and cost effective molecular analysis characterizing the CTG repeat region of DMPK gene based on a two step PCR protocol.
1. For the detection of alleles of up to 100 repeats a quantitative fluorescent (QF) amplification with primers flanking the repeat region of the MD1 locus and 2 reference genes for standardization was used.
By this method it was possible to identify both homozygous and heterozygous DM1 alleles.
2. Long PCR was only performed if a single wild type allele was detected which gave a QF-PCR-signal of only half intensity the compared to a homozygous sample.
The results of using combined QF and Long PCR indicated high accuracy in comparison to Southern blot analysis. We conclude that our new rapid analysis is reliable for genetic testing of DM1 patients.

P3 Poster Session 3: Clinical Issues in DM – Part 1

P3-01 Myotonic dystrophy type 2 (DM2) in Italy: spectrum of clinical and laboratory findings

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Background: The increasing number of patients with genetically determined DM2 having very mild phenotypes and no EMG myotonia and no cataracts underlines the need to revisit diagnostic criteria for DM2.
Aims: To assess clinical and laboratory findings in a large cohort of Italian patients with genetically confirmed DM2.
Methods: We retrospectively looked at charts from 102 patients (71 males, 31 females) attending our Neuromuscular Clinic. We analytically looked for symptoms at onset, degree of muscle impairment (strength, atrophy and clinical and EMG myotonia) and severity of multi-organ involvement.
Results: 4/102 patients fulfilled the diagnosis of PDM. None of these had EMG myotonia. 12 (1.8%) had very mild phenotypes (high CK, occasional muscle pain) and the remaining had typical PROMM features. Age at onset was in the 3rd and 4th decade (65%) (mean age 39,3 ±11,1); in 15% onset was over 60. Most frequent symptom was lower limb weakness (52%). Clinical myotonia could be detected in 25% of patients. Cataracts were present in 60% of patients. 3 patients required PM implantation (2.8%) while 4 required NIV (3.9%).
Conclusions: Clinical presentation of DM2 varies widely. Initial diagnostic criteria including the triad of proximal muscle weakness, myotonia and cataracts is present only in the classical PROMM presentation. The diagnosis of PDM needs to be considered even when EMG myotonia cannot be detected and when cataracts are not seen. In a subgroup of patients cardiac or respiratory involvement may be the presenting and major symptom.

P3-02 Risk of arrhythmia in type I myotonic dystrophy: the role of clinical and genetic variables

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Rationale: Cardiac arrhythmias are a major life-threatening complication in DM1 being the second cause of premature death in affected patients. The development and the rate of progression of arrhythmias are unpredictable. The lack of prognostic factors useful to assess the arrhythmic risk is a serious problem in the management of DM1 patients. Some studies have attempted to find out the reliability of clinical and genetic variables (sex, severity of muscle involvement, familiarity for heart disease, CTG expansion) as prognostic indexes for the arrhythmic risk. However their results appeared controversial.
Objectives: To examine the association between the presence of arrhythmia in DM1 and clinical-genetic variables, evaluating their role as predictors of the arrhythmic risk
Methods. 245 DM1 adult patients (133 males and 112 females, mean age 44.57) from five clinics dedicated to neuromuscular disorders underwent clinical and non-invasive cardiological evaluation (standard 12-lead ECG and 24-hour ECG Holter). Severity of muscular involvement was assessed according to the five point muscular disability rating score (MDRS). Molecular study was performed according to standard methods. Data were analysed by univariate and multivariate models. Univariate relationships between the development of ECG abnormalities and potential predictors (sex, familiarity for heart disease, class of expansion, and MDRS) were explored using Pearson chi-square. Relationship between the development of ECG abnormalities and age was explored using Student T-test for equal variances. Normality of the distribution was assessed by Kolmogorov-Smirnov statistic. Cochran-Armitage test was used to evaluate if frequencies of ECG abnormalities increased with ageing or class of CTG expansion. Multivariate analysis was performed by logistic regression model. Cox & Snell R2 was computed to evaluate the goodness of fit of the model containing all the predictors. The significance level was established at $P \leq 0.05$, two sided.
Results. We found cardiac arrhythmias in 63 subjects, 40 of which required a device implant. Statistical analyses revealed that men had a more than double risk to develop arrhythmias compared to women ($p = 0.018$). The addition of each year of age caused an increased risk of arrhythmia equal to 3% ($p=0.030$). Subjects with MDRS 5 had a risk of arrhythmia 12 times higher than patients with MDRS 1-2 ($p<0.001$). Although all the variables considered were significantly associated with the arrhythmic risk, the multivariate analyses showed a Cox & Snell R2 coefficient of 0.14 and the logistic regression model revealed a low sensitivity (22%).
Conclusion. Male sex, age and muscular disability are strongly associated with development of arrhythmia in DM1. However, all these variables are weak predictors of the arrhythmic risk. These results suggest that other factors may be involved in the development of cardiac conduction abnormalities in DM1.

P3-03 CLCN1 mutations screening in Italian patients affected by myotonic dystrophy type 2 (DM2)

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Background: Myotonia occurs in DM1 and DM2 and is manifested as delayed skeletal muscle relaxation following voluntary contraction. In DM2 there is a prevalent proximal muscle compromission with weakness, pain and EMG detectable myotonia less symptomatic than in DM1. A high frequency of cosegregating CLCN1 recessive mutations has been reported among DM2 patients. The CLCN1 gene maps on chromosome 7q35 and when mutated causes myotonia congenita (recessive Becker disease and dominant Thomsen disease).
Objective and Methods: In this work we have performed a mutations screening of the CLCN1 gene in 36 genetically confirmed DM2 patients from Italy. The CLCN1 gene has been analyzed by direct sequencing of all exons and intronic/exonic junctions.

Results: This screening revealed the presence of the R894X mutation in one DM2 patient. Moreover, two novel missense mutations have been identified in heterozygous state in two additional DM2 patients. The P744T mutation is C to A transition in CLCN1 exon 18 (c.2232C>A), the G280E consisting in a G to A transition in CLCN1 exon 7 (840G>A). These nucleotidic substitutions have not been detected by RFLP analysis in 100 chromosomes from control subjects. Overall, the frequency of the CLCN1 mutation carriers in our DM2 patients is 8,3% and is significantly higher than what observed in the general population (approximatively 2%).
Conclusion: The high frequency of co-segregating CLCN1 mutations among DM2 patients supports the idea that the CLCN1 gene may contribute to the myotonia and perhaps muscle pain symptoms of the patients, eventually contributing to earlier diagnosis of DM2.
Study supported by Telethon grant #GPP07250 and AFM grant #1336

P3-04 The correlation between oral dysfunction and videofuluoroscopic swallowing findings in myotonic dystrophy type 1(DM1)

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Formerly, Leonard RJ reported the prolonged bolus transit times, shortened hyoid displacement and reduced pharyngeal constriction in the DM1 patients. We have reported that the patients of DM1 compensate for weak bite force by increasing their masticatory muscle activity while chewing. To make the factor of dysphagia in DM1 clear, we examined oral and swallowing function of the DM1 patients and compared the various factors referring to the dysphagia.
Materials and methods: Twelve DM1 patients (male 7, female 5, mean age 52.3y.o.) were enrolled as the subjects of the study. We measured the maximum bite force, masseter muscle activity, and tongue strength. Swallowing function was evaluated by the modified barium swallow procedure with videofluoroscopic examination (VF). Bolus transit time in oral and pharyngeal cavity, distance of hyoid bone elevation and ratio of pharyngeal area at bolus hold to pharyngeal area maximally constricted were measured.
Result: The mean maximum bite force of those patients was 110.8N and the mean masseter muscle activity was 34.8%MVC. The statistical analysis showed close relation between these factors ($p<0.05$). The mean maximum tongue strength was decreased and the mean oral and pharyngeal transit time is prolonged (11.1sec). The mean hyoid displacement was shortened and the mean ratio of pharyngeal constriction was also reduced. However, we couldn't find the significant correlation between oral dysfunction and VF findings.
Conclusion: The muscular weakness of oral and pharynx has the most significant effect on the dysphagia in the patient of DM1.

P3-05 Frequency of DM1 and DM2 in Germany

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Background: Myotonic Dystrophy Curshman-Steinert (DM1) is considered the most frequent muscular dystrophy in the central European population. Estimates of geo-graphic distribution and frequency of DM1 were established prior to the discovery of DM2 (PROMM). At that time, the prevalence of myotonic dystrophy as a single entity was estimated at 1:10.000 or higher.
Methods: We performed a retrospective study of DM1/ DM2 patients diagnosed in our laboratory between 1993 and 2006. Patients referred in or prior to 2001, testing negative for DM1, were subsequently reanalysed for DM2.
Results: The numbers of patients referred increased over the years and currently approaches 450 independent families per year. Initially, between 30 and 40% of patients tested positive for DM1/ DM2, but this figure declined during recent years. During the entire observations period, there were equal proportions of carriers of DM1 and DM2 mutations. This may reflect the overlapping features of the clinical phenotype of both conditions. Based on data of outside centres we estimate that about half of the total DM1/DM2 diagnostic testing in Germany is performed by the Wuerzburg laboratory, thereby excluding a significant bias in the ascertainment of DM1 and DM2 cases.
Conclusions: The increasing number of referred patients to our molecular genetic laboratory is likely to reflect the awareness and increasing knowledge about multisystemic myotonic dystrophies within the medical community. The observed declining number of positive tests may likewise reflect the increasing awareness of the high degree of phenotypic variability of the condition. This obviously leads to a less stringent and more liberal decision for molecular testing. The observed 1:1 proportion among DM1 and DM2 mutation carriers is obvious in our patient cohort and should be valid for all of Germany.
Based on our data, we currently estimate the prevalence of each, DM1 and DM2, in the central European population in the order of 1:20.000.

P3-06 Hearing evaluation in DM2 : a prospective study of 10 Patients

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Introduction: PROMM/DM2 is a autosomal dominant multisystemic disorder including myotonic muscular dystrophy, cataract, cardiac and CNS symptoms. Patients may complain of hearing loss but the frequency ; the significance of this symptom remain debated.
Objective: To study prospectively the hearing capacities of 10 patients with DM2.
Methods: A clinical examination and tonal audiometry have been performed in 10 DM2 patients from 8 families ; moreover, in the same population, brainstem auditory evoked responses (BAERS) have been studied in 4 patients. . DM2 was certified by molecular genetic.
Results: 10 DM2 patients were enrolled, 5 males and 5 females aged from 47 to 77 y. Age at onset of the disease was from 10 to 60 y. Initial symptoms were as follow: cataract (2), proximal weakness, isolated (4), with myalgia (1), isolated myalgia (2), myotonia (1). At he moment of the hearing evaluation, the disease symptoms were : proximal weakness (8), myotonia (9), atrioventricular conduction defect (2). Age at hearing evaluation was from 47 to 77 y. Hearing loss was a complain for all patients. Tympanic membrane was normal in all patients but one (otitis after-effect). Tonal audiometry revealed a sensorineural hearing loss in all patients, affecting mainly high frequencies : mean loss for 4000 and 8000 Hz, respectively -38 and -71 db. BAERS showed normal neural pathway.

Conclusions: All patients from a series of 10 DM2 prospectively studied complain of hearing loss. The main abnormality evidenced in all patients concerned tonal audiometry indicating a sensory neural defect with a predominant loss of high frequencies. The studies of the BAERS suggest a disease of inner ear. The high frequency of hearing loss argues in favour of a significant relation with DM2 but its pathophysiology remains obscure. Association of sensory-neural hearing impairment and proximal weakness and/or cataract, if not specific (such association may be found in mitochondrial diseases), is a clue for DM2 diagnosis.

P3-07 White matter pathology and neurocognitive correlates in adolescents with myotonic dystrophy type 1: A Diffusion Tensor Imaging study

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Objective: The goal of the current study was to utilize Diffusion Tensor Imaging (DTI) to characterize brain white matter status at the microstructural level in child and adolescent patients with Myotonic Dystrophy Type 1 (DM1) and to investigate relationships between white matter pathology and cognitive deficits.
Background: Central nervous system involvement is an important clinical component of Myotonic Dystrophy as demonstrated by neurocognitive and neuroimaging studies. The nature of white matter pathology in DM1 and its role in the frequently-observed neurocognitive deficits has been only minimally described in young patients. DTI is a sensitive MRI technique that characterizes white matter (normal-appearing or otherwise) at the microstructural level and provides scalar measures that correspond to level of underlying pathology. Thus far, the clinical significance of neuroimaging findings in DM1 is not well-established.
Methods: 8 children and adolescents between the ages of 10 and 17, diagnosed with DM1 and 8 age-matched, sex-matched control subjects were scanned on a Siemens 3T scanner. Subjects also completed a neurocognitive battery including measures of intelligence, working memory, executive functioning, processing speed, and memory.
Results: Overall IQ was significantly lower for DM1 patients (mean=75, SD=17.6) compared to control subjects (mean=103, SD=10.8). White matter fractional anisotropy (FA) was measured in inferior-frontal, superior-frontal, supra-callosal, and occipital regions of interest. Patients with DM1 had significantly lower FA in all four large regions of interest with p-values < .001 and very large effect sizes (d=2.6 to 3.2) in all four regions. Within the group of DM1 patients, significant correlations were observed between executive functioning (as rated by parents on the Behavior Rating Inventory of Executive Functioning or BRIEF) and FA in both inferior-frontal and superior-frontal region. The degree of white matter abnormality in these two frontal white matter regions correlated with the patients'reported levels of impairment in planning, organizing, self-monitoring, and inhibiting behavior.
Conclusion: The results indicate that child and adolescent patients with DM1 have profound abnormalities in white matter microstructural integrity and that these abnormalities correspond with cognitive dysfunction in these individuals.

P3-08 Repetitive components of compound motor action potential in DM1 patients

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Rationale: Neuromuscular excitability properties has been recently investigated in DM1, in relation to altered membrane depolarization, which might be due either to the loss of chloride channels or to change in sodium conductance in the motor axon or in muscle fibers.
Accordingly, recent molecular studies showed that the toxic n(CUG)RNAs accumulate in the subsynaptic nuclei of muscle fibers and in the motor neurons of DM1 patients, sequestering MBNL1 proteins and probably leading to a neuromuscular junction (NMJ) dysfunction.
Hypothesis: To describe the occurrence of compound motor action potential repetitive components (R-CMAP) after motor nerve stimulation, possibly related to neuromuscular hyperexcitability, in patients affected by myotonic dystrophy type 1 (DM1), and to perform genotype-phenotype correlations.
Methods: This study included 8 DM1 patients (5M, 3F) aged form 37 to 77 years. The degree of muscle weakness was assessed by MDRS scale. Exclusion criteria were: diabetes, risk factors for peripheral neuropathy, antimyotonia drugs. On each patients we performed sensory and motor nerve conduction study including sural nerve and tibial, peroneal, ulnar nerves, respectively. F-waves were recorded from tibial and ulnar nerves.
Results: Polyphasic CMAPs, in particular the neural type of R-CMAPs, were documented in 4 patients (2M, 2F; mean age: 42.74), carrying the largest n(CTG) expansions in leukocytes (ranging from 1000 to 1500). In these patients, a single nerve stimulation generate a CMAP closely followed by high number of extra-discharges producing repeated oscillations originating in the distal portion of the peripheral nerve as observed in conditions characterized by generalized peripheral nerve hyperexcitability.
Conclusions: Our findings are in agreement with recent molecular studies, documenting a spliceopathy affecting peripheral nerve terminals at NMJ in DM1. Indeed, a significant correlation was found between the occurrence of motor nerve hyperexcitability and the n(CTG) expansion in leukocytes, suggesting that the progressive accumulation of the toxic RNAs might interfere with the expression of different genes coding ion channels.

P3-09 Muscle pathological changes and brain MRI findings in DM1

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Rational. Cerebral and muscle impairment in myotonic dystrophy type 1 (DM1) is well documented but there are few reports on their correlations.
Objects. To determine the degree of involvement in brain and skeletal muscle in a series of DM1 patients molecularly defined in order to highlight possible correlations.
Materials and methods. 24 DM1 patients were recruited for the study. Age at study, age at disease onset and disease duration were recorded. Molecular characterization of CTG(n) expansion was done in genomic DNA from blood. Neuromuscular assessment was done by MIRS. A morphometric study of muscle biopsies included the quantitative evaluation of fibre atrophy and hypertrophy factor of both fibre types. All patients underwent brain MRI; MRI imaging were classified by the ARWMC (age related white matter changes) score in or-

der to quantify the pattern of distribution of white matter hyperintense lesions (WMHLs).
Results. Morphometric analysis of muscle showed an increased atrophy factor for both fibre types, especially of type 1 fibres in 14/24 cases. 20/24 patients had abnormal MRI imaging, showing scattered supratentorial, bilateral, symmetrical focal or diffuse WMHLs, with a typical temporo-insular diffuse subcortical pattern in most patients. No significant correlations were found between atrophy factors and MRI total lesion load of WMHL. A trend towards a more severe muscle impairment in patients harbouring larger CTG expansion was observed without reaching significativity.
Conclusions. A greater expansion size is confirmed as risk factor for more extensive cerebral and muscle impairment. Our study confirms that muscle and brain are independently involved.

P3-10 Subtle cognitive decline in Myotonic dystrophy type 1: a five-year follow up study

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Objective: To characterize the progression of cognitive decline in classical DM1 at a five-year follow-up examination. Background: Previous studies have indicated decline in executive attention, in agreement with frontal brain involvement. Methods: Repeated assessment was performed in 34 (mean age = 42, 18 female, 16 male) out of 47 DM1 patients in whom a previous neuropsychological assessment was performed five years earlier. Neuropsychological test included measurements of memory, attention, visuospatial function, arithmetic, verbal ability, speed and executive function. Statistical analysis was performed using the Wilcoxon signed-rank test. Results: Neuropsychological tests results associated with executive attention, visuoconstructive and verbal memory abilities were significantly worsened over time. The difference between results on separate test occasions, as measured by Cohen's d, was mild to moderate. Notably, not a single patient showed severe decline (comparable to dementia) in any examined cognitive domain. Conclusion: Our data confirms a subtle decline in executive attention function. However, our data also shows that visuoconstructive and verbal memory abilities was worsened over time, indicating, not only frontal brain dysfunction but also the possible involvement of other cortical and subcortial brain areas. These findings indicate the need for further longitudinal studies on cognition in DM1 and the development of interventions, both including proper medication and rehabilitation modules as to mitigate the progress of cognitive dysfunction. Subsequent analysis of the present data will determine if factors such as CTG repeat expansion size and muscle function is important for the rate of cognitive decline over time.

P3-11 Test/retest and machine/machine reliability of dual energy X-ray absorptiometry (DEXA) measurements in patients with DM-1 and DM-2.

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Objective: To document the test-retest reliability and machine/machine reliability of Dual Energy X-ray Absorptiometry (DEXA) measurements in patients with DM-1 and DM-2.
Background: DEXA measurements are frequently used in longitudinal natural history studies and multi-center therapeutic trials as an outcome measure to document changes in lean body mass in patients with DM. There are no data available in the literature documenting the test-retest reliability OR the machine/machine reliability of these measurements.
Methods: We performed repeated DEXA measurements on two separate occasions within 1 -36 hours on 15 patients with DM participating in clinical trials at our site. The measurements were made with instrumentation and software from the Lunar corporation, Madison WI. We also made repeated measurements on another 8 patients within a 24hour period on 2 separate machines, one from the Lunar corporation and the other from the Hologic corporation.
Results: Test/retest reliability:
The Intra class correlations (ICC) for the test/retest measurements were as follows:
DEXA total 0.99, DEXA Bone Mineral Content (BMC) 0.99, DEXA Fat 0.99, and DEXA Lean Body Mass (LBM) 0.99 Machine/Machine correlation. The Pearson correlation coefficients for the Machine/Machine measurements were as follows: DEXA total 0.99, DEXA Bone Mineral Content (BMC) 0.96, DEXA Fat 0.99, and DEXA Lean Body Mass (LBM) 0.93.
Conclusions: All the ICC's were greater than .90 which denotes excellent test/retest reliability. The excellent machine/machine correlation documents the reliability of measurements taken across several sites in a clinical trial and also the reliability of longitudinal measurements that may have been made over an extended period of time during which hardware and software changes may have occurred.

P3-12 Participation in physical activity by people with myotonic dystrophy

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Rationale: Physical activity has benefits for the general population, some of which may benefit people with myotonic dystrophy (pwDM). Further information on which activities pwDM are participating in and which barriers to activity exist could assist in future research and management.
Objectives: To compare pwDM with facioscapulohumeral (FSHD), limb girdle (LGMD) muscular dystrophy and unaffected controls as regards:
1. The types and degree of physical activity participated in.
2. Barriers to physical activity experienced.
Methods Postal questionnaires were sent to controls and patients with the above diagnoses from seven UK centres. The Physical Activity Scale for Individuals with Physical Disabilities (PASIPD) was the primary outcome measure, other measures were age, the Guy's Neurological Disability Scale(GNDS), and the Barriers to Physical Activity and Disability Survey (BPADS).
Results: 97 pwDM, 68 FSHD, 82 LGMD and 84 controls replied. Mean age for pwDM was 44.9, CI 41.5 years. Median PASIPD score was 9.7(IQR18.1) and median total number of barriers was 9(IQR6.5) for pwDM, both significantly different with all groups compared, $p<0.0001$ (Kruskal-Wallis test), the major difference being with controls - median PASIPD score of 21.8(IQR 25.4) and median total barriers of 4(IQR 5). The GNDS gave further insight into disabilities experienced by pwDM, e.g. 11.7% experienced urinary incontinence, 44.2% required a mobility aid and 18.5% required help regarding cognitive problems. Disability and barriers were associated with the degree of physical activity.
Conclusions: pwDM are participating in physical activity but degree of this, type of barriers and disabilities differ from the general population.

P3-13 Clinical and biomolecular findings in a juvenile onset case of myotonic dystrophy type 2

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Objective. Myotonic dystrophy type 2 (DM2) is a common adult onset muscular dystrophy caused by a dominantly transmitted CCTG expansion in intron 1 of ZNF9 gene. In DM2 there is no obvious evidence for an intergenerational increase of expansion size and no congenital cases have been reported. No DM2 patient younger than 14 years has been described and confirmed at biomolecular level. We describe a 14-year-old DM2 female patient who shows grip myotonia. The 45-year-old mother shows a more severe DM2 phenotype compared to her daughter with symptom onset at age 20 years. Methods and Results. Histological and immunohistochemical analysis of the patient's muscle biopsy do not show DM2 characteristic histological features. On the contrary, the mother's muscle presents internalized myonuclei in type 2 fibres, pronounced type 2 fibres atrophy and numerous nuclear clumps myosin fast-positive. Long-PCR on both muscle and blood samples reveals only a small increase in the CCTG repeat number through maternal transmission. FISH in combination with MBNL1-immunofluorescence on muscle sections shows the presence of mutant-mRNA and MBNL1 nuclear foci whose fluorescence intensity and area appear to be similar in the patient and her mother. Splicing analysis of the IR, ClCN1 and MBNL1 genes in muscle tissue demonstrates that the level of aberrant splicing isoforms is lower in the daughter than in the mother. Mutation screening of the CLCN1 and SCN4 genes is in progress to exclude a second genetic mutation in these ion channel genes responsible for myotonia in the young DM2 patient. Conclusion. This study reports for the first time the clinical, genetic and molecular characterization of a juvenile form of DM2.

P3-14 Health-related quality of life in patients with myotonic dystrophy type 1 and myasthenia gravis: a comparative analysis

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Objective: To evaluate the health-related quality of life (HRQoL) in patients with myotonic dystrophy type 1 (DM1) in comparison with HRQoL in patients with myasthenia gravis (MG).
Patients and methods: Forty-six patients with DM1 (25 women and 21 men, median age 43 years) and the same number of patients with MG, matched with the patients with DM1 for age, sex and education, underwent the MOS 36-item short-form health survey (SF-36), emotional functioning tests (Hamilton rating scale for depression and for anxiety), and the multidimensional scale of perceived social support. The severity of muscular involvement in patients was assessed with DASH questionnaire.
Result: The SF-36 mean scores profile of our patients with DM1 was lower than those of MG patients in all eight domains. In both groups of our patients the lowest scores were for limitation in usual role activities because of physical and emotional problems. The highest scores were observed for bodily pain and social functioning in both groups. The emotional assessment showed significantly higher scores in depression and anxiety in DM1 patients than in those with MG ($p<0.01$). Our patients with DM1 had significantly lower support than MG patients ($p<0.01$), and the acceptance of the disease was better in patients with MG than in those with DM1.
Conclusion: HRQoL was more severe impaired in DM1 patients than in those with MG. The major influence on HRQoL in DM1 and MG patients had severity of muscular involvement measured by DASH.
Key words: myotonic dystrophy, myasthenia gravis, health-related quality of life

P3-15 Development of Scottish myotonic dystrophy management guidelines

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Background: Guidelines for the management of Myotonic Dystrophy were developed by the four Scottish Clinical Genetic Centres in 1998. These were implemented by the Genetic Centres but not by other clinicians in Scotland.
Methods: We performed a critical appraisal of the literature on management aspects in Myotonic Dystrophy, from 1992 to 2008 inclusive. Discussions were held at a series of multidisciplinary meetings.
Results: We have produced national, evidence-based, guidelines for the management of adults with Myotonic Dystrophy in Scotland. The guidelines have been implemented in the six dedicated Myotonic Dystrophy clinics in Scotland. Data is collected electronically using a specially designed management database. Guidelines, and additional practical information, have been combined into a one page leaflet that has been circulated to General Practitioners and other clinicians managing Myotonic Dystrophy patients. We will describe the guideline development process and present the guidelines in full.
Conclusions: These guidelines will allow the most appropriate management of Myotonic Dystrophy within our health care system. The data collected will allow audit and future refinement of the guidelines, as well as providing a valuable clinical research resource.

P3-16 Frequency of DM2 and DM1 mutations in the Finnish population

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Prevalence of DM1 disease is estimated to be 1 in 8000 in most European populations, but for DM2 the prevalence has not yet been established. Estimations of the prevalence are based on different regional ascertainments and large scale population based studies have not been carried out.
To study the frequency of DM2 and DM1 expansion mutations we analyzed 4532 Finnish population control DNA samples obtained from anonymous blood donors. ZNF9 and DMPK gene allele sizes were analyzed using PCR and fragment analysis. The samples showing only

one allele were further analyzed by repeat-primed PCR (RP-PCR) to identify the DM1 and DM2 associated expansion mutations.
Results for allele sizes were obtained from 4511 samples for DM2 and 4520 for DM1 giving a very low failure percentage (0.46 and 0.26 respectively). 12.6% (572) of the samples showed one single allele for the ZNF9 locus and 20.7% (938) for the DMPK locus. With RP-PCR applied to these samples two DM2 and two DM1 mutation positive samples were identified. These results suggest an overall frequency of approximately 1 in 2250 for both DM2 and DM1 mutations. In addition, five larger ZNF9 alleles which seem to be unstable by the RP-PCR method were identified and one DM1 pre-mutation showing approximately 45 CTG repeats.
Our results implicate that DM2 mutation is much more frequent than previously suspected and is at least as frequent as DM1 because all DM2 mutation carriers will develop manifesting symptoms.

P3-17 Lipid metabolism alteration in myotonic dystrophies

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Background: There have been no systematic reports on lipid metabolism alteration in myotonic dystrophies. We assessed the frequency, type, and severity of lipid alteration in both types of DM.
Methods: A retrospective multicenter study was conducted. 169 DM2 patients and 35 DM1 patients participated by completing questionnaires. Additionally, their clinical and serological records, including blood lipid parameters for dyslipoproteinemia (cholesterol, HDL, LDL and TG) were screened and reviewed.
Results: 94 DM2 (70.1%) and 19 DM1 (54.3%) patients show evidence to suffer from dyslipoproteinemia. In DM2 and DM1 patients the age of onset of dyslipoproteinemia was 47.6y and 46.2y, respectively. Comparing both DM groups, all measured parameters did not differ statistically significant. Nevertheless, comparing all lipid parameters of both types of DM with an average German population data set, we could find a no significant discrepancy for DM1 patients (Cholesterol: p=0.435; HDL: p=0.129), but for DM2 patients (Cholesterol p=0.021; HDL: p=0.001). 70.1% DM2 and 54.3% DM1 patients suffered from dyslipoproteinemia, and of these 34% DM2 and 21.1% DM1 patients were treated with statins.
Conclusions: According to Robert-Koch-Institute, Germany, one third of German adults show elevated cholesterol levels, which is significantly lower than the rate found here in DM patients. With this high rate of dyslipoproteinemia in DMs, it seems to be obvious, that DM patients show a predisposition for this metabolic disorder. Furthermore, compared with data from Robert-Koch-Institute, DM patients (DM2 23.9%, DM1 11.4%) use significantly more statins than the German average. In addition, the rate of discontinuation is high based on side effects like augmented symptoms (hyperCKemia in 27.8% DM2, muscle pain or weakness in 33.3% DM2) in relation to former studies revealing statin side effects in one of 10000 patients. More details will be presented.

P4 Poster Session 4: Clinical Issues in DM – Part 2

P4-01 Clinical, muscle pathology and FISH biomolecular findings correlation in 42 Italian patients with myotonic dystrophy type 2

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Background. Muscle histopathology from DM2 patients shows type 2 nuclear clumps and preferential type 2 fibre atrophy. How these findings correlate with muscle weakness and myotonia and to the spliceopathy underlying this disorder is still unclear.
Objectives. To present muscle histopathology in a cohort of genetically confirmed Italian DM2 patients and to correlate these findings to FISH results, to the severity of muscle impairment, disease duration and age of the patient.
Methods. The degree of fibre size variability, centronucleation, small angulated fibres, type 2 fibre atrophy, nuclear clumps, connective tissue replacement were analytically looked for in the brachii muscle biopsies from 42 Italian patients with genetically confirmed DM2. Overall muscle impairment and presenting phenotypes (PROMM vs PDM vs paucisymptomatic) were recorded.
Results. Histopathology was similar in all samples: increased fibre size variation (100%), internal nuclei (95%) and pyknotic nuclear clumps (82%). Immunostaining for fast and slow myosin heavy chain confirmed a preferential type 2 fiber atrophy (93%) and nuclear clumps expressing fast myosin (type 2). FISH with (CAGG)5-probe in combination with MBNL1-immunofluorescence demonstrated the presence of nuclear foci of CCUG-containing RNA co-localizing with foci of MBNL1 in all samples examined. No correlation was found between histopathology findings from grade 3 MRC vs grade 5 MRC muscles and in patients with PDM (n=5), with very mild (7) or with typical PROMM phenotypes (30).
Conclusions. Muscle biopsy may show specific histopathology findings consistent with DM2 even when symptoms and signs are minimal. These findings emphasize the diagnostic role of muscle biopsy in DM2 showing that histochemistry and immunohistochemistry may target the biomolecular diagnosis by FISH.

P4-02 Motor Outcome Measures in Childhood and Congenital DM1

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Background: Children with DM1 often have compromised motor function. However, few investigations have documented the reliability and validity of motor outcome measures. Understanding functional and direct strength outcome measures is important for monitoring clinical change and ultimately for clinical trial readiness. The objective of this study was to study reliability of functional measures and test validity through comparison to actual activity and fatigue ratings.
Methods: Children age 5-20 years old with a genetically confirmed diagnosis of DM1 were identified from a single, pediatric neuromuscular clinic. Children could have either congenital or childhood onset DM1. Subjects underwent two testing sessions separated by 7-14 days. At each session children completed the PedsQL Fatigue Scale, 6 Minute Walk Test (6MWT), timed writing and typing tasks, myometry,

and grip strength. Subjects wore the Stepwatch Activity Monitor for 7 days following the first visit.
Results: A total of 12 children participated. The mean age was 11.5 years (5-19 years) with an average CTG repeat size of 1125. All children completed the 6MWT with good effort except two: one due to inability to walk and one due to anxiety. The test-retest reliability was excellent ($r=0.98$, $p<0.0001$). The writing task was completed by 10 subjects and had high reliability ($r=0.97$, $p<0.0001$), however the typing task was only completed by 8 subjects. Typing or writing tasks did not correlate well with grip strength. The parent report of fatigue, the average steps per day or the percent of time at low activity did not correlate with distance on the 6MWT.
Conclusion: The 6MWT and writing tasks were performed well and were very reliable over a short time interval in a wide range of ages and severity of DM1 children. However, these functional tests did not correlate well with strength, report of fatigue or activity level.

P4-03 Structural and functional brain abnormalities in myotonic dystrophy type 1 and 2

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Myotonic dystrophy type 1 and 2 (DM1/DM2) are slowly progressive, multisystemic diseases with a more benign course in DM2. Beside the cardinal symptoms of myotonia, muscle weakness and atrophy, these patients also present with cognitive deficits. Here we characterized the cerebral abnormalities of 20 DM1 and 9 DM2 patients with a genetically proven diagnosis, and compared them to matched normal controls using neuropsychological examinations, structural cerebral magnetic resonance imaging (MRI) and 18F-deoxy-glucose positron emission tomography (FDG-PET) in combination with statistical parametric mapping (SPM2). Deficits in non-verbal episodic memory, visuo-constructional abilities and psychomotor speed were characteristic for both DM1 and DM2 patients, with DM1 patients being more severely affected. White-matter lesions (WML) were found in more than half of the patients with a frontal predominance and their extent was correlated to psychomotor speed. Analysis of the brain parenchymal fraction (BPF) showed a decrease of the global grey matter in both patient groups. Voxel-based morphometry (VBM) suggested a widespread cortical atrophy most pronounced in the frontal and parietal lobes. Interestingly, VBM revealed a bilateral hippocampal atrophy that was correlated to deficits in non-verbal episodic memory, and to a clinical score including the number of CTG repeats, disease duration, and age. Furthermore, VBM indicated a pronounced change of thalamic grey matter signals. FDG-PET of the brain was performed using partial volume correction with matched MRIs. These results indicated a pronounced fronto-temporal hypometabolism occurring independently from cortical atrophy. All imaging abnormalities were found to be similar in both patient groups but were generally more pronounced for DM1 than for DM2 patients, revealing a widespread brain pathology in both diseases.

P4-04 The Lived Experience of Patients with Myotonic Dystrophy Type 1

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RATIONAL. Until now, those working with patients suffering from Myotonic Dystrophy Type 1 (DM1) can only partially understand what it is like to live with the illness. In reality, very few writings have illustrated patients'perceptions of degenerative neuromuscular disorders. Of the writings that do exist, none are from patients suffering from DM1. It is essential that healthcare professionals have a better understanding of the difficulties DM1 patients experience in various aspects of their lives.
OBJECTS. The main objective of the research study was to examine how DM1 affects the lives of those diagnosed with the disorder.
METHODS. Within the context of an explorative qualitative phenomenological approach, 10 participants with adult phenotype DM1 were extensively interviewed in order to describe what living with DM1 is like.
RESULTS. Analysis of the interview transcripts indicate that many 1) believe to have poor health; 2) deplore the rapid deterioration of their health when compared to what had been anticipated; 3) have difficulty accepting their loss of autonomy; 4) expressed that DM1 impacts all aspects of their lives, forcing them to live day-by-day. Participants said that one of the most negative effects of the disorder is that, in addition to restricting their daily activities, it also restricts their leisure, social and professional lives. Participants'self-perception and conjugal relationships are also negatively affected.
CONCLUSION. The results outline the major impacts of DM1 in the lives of patients and confirm that there is a need to re-evaluate and improve the services offered to DM1 patients.

P4-05 Quality of life and family impact of congenital and childhood DM1

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Background: DM1, in childhood, is a multi-system disorder impacting on many aspects of a child's life. Quality of life in pediatric DM1 and the impact of the disease on the family has not been well explored. Disease severity, medical and psychological problems may be determinants of QOL and impact on family functioning. The primary objective of this study was to determine QOL in pediatric DM1.
Methods: Children age 5-19 years old with a genetically confirmed diagnosis of DM1 were identified from a single, pediatric neuromuscular clinic. Subjects underwent a clinical interview, chart review and were administered the PedsQL (parent and self report versions), the PedsQL Family Impact Module, and the Child Behavior Checklist.
Results: A total of 12 children participated. The mean age was 11.5 years (5-19 years) with an average CTG repeat size of 1125. Seven children had congenital DM1 based on neonatal symptoms. Mean child report QOL scores for physical, emotional, social, and school were 60, 64, 67 and 59, respectively. Parent report on all the dimensions was considerably lower than child report except for the emotional dimension. There was no difference in mean total QOL between congenital and childhood DM1 or with CTG repeat size. Family Impact mean scores (combined activities and relationships domains) were 69 and this was positively correlated with mean total QOL ($r=0.65$ $p=0.02$). Difficulties with internalizing disorders on the CBCL were reported in 25% of subjects ($n=4$).
Conclusion: In pediatric DM1 QOL and family activities and relationships are compromised compared to normal populations. The sample is small so only limited conclusions can be drawn regarding variables that may impact QOL. This study will better inform larger investigations focusing these psychosocial outcomes.

P4-06 Health Supervision in Myotonic Dystrophy Type 1

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The complexity and variability of disease manifestations in DM1 undoubtedly pose a challenge for the clinical management of patients. The follow-up has been described as fragmented, inadequate or even deficient for many patients. Numerous physical and social factors contribute to this situation: the multisystemic manifestations of DM1 resulting in several disabilities, the low educational level and the low income of patients as well as their poor social support network. These factors emphasize the need for a comprehensive management approach and a health supervision checklist has been developed and validated in order to improve management. The identification of the systemic and social concerns and the elaboration of recommendations for treatment have been done according to a systematic review of literature as well as to the opinions of experts. From a total of 4212 articles, 847 relevant articles for this project were selected for review. A first version of the checklist was elaborated and researchers and clinicians recognized for their expertise have been invited to participate in a consensus acceptance procedure using the Delphi method (10 participants in the first round and 15 in the second round). Results of the Delphi Consultation and the DM1 checklist will be briefly presented.

P4-07 Chronic muscle stimulation reverts the abnormal sEMG pattern in Myotonic Dystrophy type 1

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Objects: To verify the effects of a electrical muscular stimulation on the surface EMG pattern in patients affected by Myotonic Dystrophy type 1 (MyD).
Methods: Five MyD patients were evaluated. A motor point stimulation protocol was bilaterally carried out on the tibialis anterior muscle (TA). Stimulation consisted of 10-s, 35 Hz pulse train, 0.1 msec in duration. A supramaximal stimulation was applied and the surface myoelectric signal was recorded. The averaged rectified value of the amplitude (ARV) was evaluated. A transcutaneous electrical stimulation was performed for 1 month on one leg. The pattern of stimulation consisted of a on-off period of 10 s at 30 Hz for 1h twice daily. The intensity of stimulation was chosen as that able to evoke a functional contraction. The contralateral side served as control.
Results: The patients bilaterally showed the typical decreasing ARV pattern. The electrical treatment consistently provoked a clear and complete recovery of the normal increasing ARV curve on the stimulated side. In particular we observed a complete reverse of the pathological finding after 15 days of stimulation in those patients without strength deficit on the TA; whereas the stimulation provoked the complete reverse of the abnormal trend after 30 days in those subjects with a mild strength deficit. On the other hand the non-stimulated leg showed a persistence of the abnormal sEMG trend.
Conclusion: The results obtained clearly demonstrated that the chronic electrical stimulation is able to modify the electrical properties of the sarcolemma in MyD. These findings appear of interest for the rehabilitative approach to these patients. Of course we need to corroborate these data increasing the casistic. Moreover it is going on the long lasting follow-up to verify the persistance of the effect.

P4-08 Diagnostic odyssey of myotonic dystrophy type 2 (DM2) patients

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Limited data are available about the diagnostic "odyssey" of DM1 and DM2 patients. Delays in diagnosis may have important clinical and therapeutic consequences. Purpose: To analyze the diagnostic delay in a large sample of DM patients enrolled in the NIH sponsored National Registry. Methods: Analyzed diagnostic delay (age of diagnosis minus age of first symptom) and medical history as reported by members ≥18 yrs old. Used T-tests and chi-square to compare data between DM subtypes ($p<0.05$ indicated significance). Results: Age of onset averaged 32.6 yrs in DM2 (n=99) compared to 25.3 yrs in DM1 (n=526; $p<0.0001$). The most common first symptom of DM2 patients was leg weakness (29.3%) compared to grip myotonia in DM1 (37.5%). Pain was reported as the first symptom in 5.1% of DM2 and 0.4% of DM1 patients ($p<0.0001$). Diagnostic delay was significantly greater in DM2 (15.0 yrs ±12.9) compared to DM1 patients (7.3 yrs ±8.3; $p<0.0001$). Compared to DM1, more DM2 patients had genetic testing (65.7% versus 54.0%; $p=0.032$), received EMG (87.8% versus 70.7%; $p=0.0004$) and underwent muscle biopsy (46.5% versus 11.4%; $p<0.0001$). More DM2 patients (50.5%) were the first members of the family diagnosed with DM compared to DM1 (38.8%; $p=0.03$). Conclusion: DM2 patients took 15 yrs to be diagnosed (double the time compared to DM1) and have undergone significantly more diagnostic exams including genetic testing, EMG, and muscle biopsies. Studies to assess these discrepancies and to measure their impact on burdens of disease, family planning, and symptom management are necessary.

P4-09 Ocular Motor Function in Congenital and Childhood Myotonic Dystrophy Type 1

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Purpose: To assess ocular motor function in congenital and childhood onset myotonic dystrophy type 1 (DM1) and correlate the results with CTG-repeat size, severity of the disease, myotonia and skeletal muscle function.
Methods: A cross-sectional, multidisciplinary investigation of strabismus, motility, smooth pursuits, saccades and eye-lids were performed on 49 individuals with a confirmed diagnosis of DM1 < 18 years of age, with >40 CTG expansion repeats. The results were correlated to myotonia of the grip, tongue or thenar muscle as well as gross motor function score. In addition, the ocular results were compared to those of an age and sex matched control group.
Results: Ocular motor abnormalities were common in the present study and the most frequent findings in the total group were altered eye-movements and affected eyelids. In the severe congenital subgroup, the highest frequency of strabismus was found and it was 14 times more common compared to controls. The most prominent finding in the mild congenital group was that the CTG-repeat size affected the eyelids. In the childhood onset group the positive correlation between CTG-repeat size and motility defects were the main findings.

The lower the gross motor function scores, the more strabismus and motility abnormalities.
Conclusion: We found a variety of ocular motor abnormalities in all three subgroups. The abnormalities did indicate CNS pathology, myotonia and ocular muscular dysfunction of same origin as skeletal muscles dysfunction i.e. central or molecular.

P4-10 High Impact Symptoms in Myotonic Dystrophy Type-1 (DM1): A Qualitative Study

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RATIONALE: There is a clear and urgent need to systematically identify the issues and symptoms most relevant to DM1 patients. The development of validated patient-relevant outcome measures has the potential to positively impact scientific research and patient management.
OBJECTIVE: To utilize qualitative research techniques to identify the disease-specific symptoms and issues that are most important to DM1 patients.
METHODS: Twenty genetically confirmed, adult, DM1 patients representing varied levels of disability were interviewed over a 12 month period. Each interview focused on obtaining insight into the DM1 issues that have the greatest impact on patient quality-of-life (QOL). Interviews were taped, transcribed, coded, and analyzed using a qualitative framework technique and three investigator consensus approach.
RESULTS: 1175 direct quotes were coded, 223 like themes were identified, and 12 subdomains (representing 4 larger symptomatic domains) were used to create a QOL model representing the most relevant symptomatic and psychosocial issues in DM1. This model included domains pertaining to physical health, mental health, social health, and DM1-specific health issues. DM1-specific health categories included: 1) sleep disturbances; 2) specific activity impairment; 3) pain, fatigue, myotonia, and atrophy; 4) gastrointestinal dysfunction; 5) communication impairment; and, 6) multisystemic organ dysfunction. Difficulty with ambulation was the most frequently mentioned issue of DM1 patients.
CONCLUSION: There are multiple themes and symptoms, some previously underrecognized, that play a key role in DM1 patient QOL. These issues must be carefully studied and identified in order to develop disease-specific outcome measures, improve DM1 clinical care, and maximize the impact of clinical research.

P4-11 Scaled down genetic analysis of myotonic dystrophy type 1 and type 2

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Background: Expanded repeats in DM are unstable in somatic cells. Studies to monitor repeat length in DM-affected tissue are limited by low DNA recovery from small biopsies samples.
Objective: To scale-down methods for genetic analysis of DM.
Methods: Southern blots were performed by using digoxigenin-labeled (CAG)7 or (CCTG)5 probes composed of locked nucleic acids (LNA). Genomic DNA was digested with 4-bp restriction enzymes, so that target fragments were comprised almost entirely of expanded repeats. For moderately expanded repeats, the DM1 locus was amplified by rolling circle amplification prior to Southern blotting. DNA was analyzed from DM1 tissues (n=26) and fibroblasts (n=2), DM2 muscle (n=4) and blood (n=10), and blood from healthy controls (n=12).
Results: Repeat length could be determined from only 50-100 ng of DNA. Clear bands were detected in all DM1 samples, with no false-positive signal in control samples. The repeat size was concordant with that determined by conventional Southern blot, which required 100-fold more DNA. CCTG expansions in DM2 were also detected by this method. As in DM1, the repeat size was considerably larger in DM2 muscle than blood.
Conclusion: Repeat length in DM can be determined from sub-microgram quantities of genomic DNA. Enhanced sensitivity may reduce the problem of false negative Southern blots in DM2. By reducing the amount of flanking sequence, the resolution on gels of expanded alleles is maximized. These methods may facilitate genetic analysis of tissue samples from individuals with DM.

P4-12 Decision-making dysfunction in DM1

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Background: Recent findings underscore compromised decision-making in the classical form of DM1 (Gaul et al., 2006). But not much is known about the emotional and/or executive processes involved. We hypothesized decision-making impairments in DM1 patients using the Iowa Gambling Task (IGT) (Bechara et al., 2004), one of the most widely used measures of emotional decision-making which stimulates real-life decision-making.
Method: We aimed to (1) investigate the emotional decision-making in adult DM1 patients (25-45 years-old with no mental retardation) using the IGT and (2) dissociate emotional from executive processes using the Stroop Test as a measure of cognitive inhibition, the Trail Making Test as a measure of cognitive flexibility and the digit span as a measure of verbal working memory.
Results: Our results show significant IGT impairments for the DM1 group associated with emotional insensitivity to future consequences, such that behaviour is guided by immediate prospect. Our results revealed no significant impairments concerning cognitive inhibition, cognitive flexibility or verbal working memory.
Conclusion: Our data lead to the suggestion that DM1 patients'poor decision-making abilities may arise from a reduced sensitivity to future consequences independently of executive functions. This «myopia for the future» could be related to nonadherent behaviors (the extent to which a person's behavior - taking medication, following a diet, and/or executing lefestyle changes, corresponds with agreed recommandations from a health care provider) which are frequently observed in DM1 population.

P4-13 Hard to Swallow: Understanding the Lived Experience of Caregivers for Individuals with Myotonic Dystrophy (DM1) and Dysphagia

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Rationale: Given the complex physical and cognitive challenges faced by some individuals with Myotonic Dystrophy (DM1) caregivers may provide physical, social, and emotional support. Moreover, the morbidity and mortality associated with dysphagia may make caregivers

responsible for supervising food intake and for recognizing, preventing, and treating choking episodes. For this study, a caregiver is defined as a spouse, family member, or friend who is familiar with the daily activities of an individual with DM1. To date, the literature neither includes a needs assessment nor a study of the social and emotional impact of dysphagia for DM1 individuals and their families.
Objectives: To understand the lived experience of DM1 caregivers, and to assess their perceptions of individuals with DM1 and dysphagia.
Methodology/Method: An interpretive phenomenological approach was used as the methodological framework for this study. Individuals with DM1 (n= 5-10) were asked to invite a caregiver to be interviewed. Interviews will be transcribed verbatim and read as a whole to determine emerging themes. Data collection will continue until no new information is forthcoming. To ensure authenticity, participants will review a list of themes compiled by the researchers.
Results/Future Directions: To date, one caregiver has been interviewed, and four more have consented to participate. The participant interviewed suggests that symptoms may socially impact individuals with DM1 and their families, but that dysphagia may be a lesser concern than other aspects of the condition. Participant recruitment and data collection will continue to determine if these findings resonate with other DM1 caregivers.

P4-14 Quantitative isometric muscle strength at the ankle in myotonic dystrophy type 1.

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The main objective of this study was to characterize the maximal isometric strength of ankle evertors and dorsiflexors with handheld dynamometer (HHD) in DM1 patients and to establish the inra- interreliability. Twenty-two patients from Quebec (mean age=41,1 ±13,8) and 24 from Lyon (mean age=41,6 ±10,2) were compared to 16 matched controls. An excellent reproducibility of the torque measurements was obtained for both centers in eversion (R2=0,94/Quebec; 0,89/Lyon) and dorsiflexion (R2 = 0,96/Quebec; 0,90/Lyon). There was a strong relationship between eversion and dorsiflexion strength profiles (R2-0,87; Quebec/0,80, Lyon). The differences between 3 groups of DM1 (mild, moderate, severe) and between them and controls were all statistically significant (p< 0,001). No statistical differences between sites were observed (p > 0.05). The rates of muscle strength decline in dorsiflexion (eversion) were 60% (47%), 77% (71%), and 87% (83%) for DM1 with mild, moderate, and severe impairments, respectively. The handheld dynamometer protocol showed unique discriminative properties suitable for longitudinall study and for multicentre therapeutic trial.

P4-15 Gait and balance difficulties in individuals with Myotonic Dystrophy type 1.

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Objective: The aim of this study was to map balance and gait deficits in relation to muscle strength, stumbles and falls.
Methods: All adult walking individuals with classical or late-onset genetically proven DM1 between 20-60 years of age were invited to participate in an assessment by the physiotherapist at the Neuromuscular Centre. Self assessments of walking difficulties and balance confidence were performed together with physical examination of Time to walk 10m with maximum gait speed, Timed Up& Go (TUG), One Leg Stance, Tandem Stance and lower extremity isometric muscle strength measured with handheld myometry.
Results: Fifty-one out of 72 eligible patients, 71% (20 male, 31 female, mean age 41.3 ±9.7 yrs) participated. Muscular Impairment Rating Scale (MIRS) showed median 4, range 1-5. Thirty-seven percent of the whole group had accidentally fallen four times or more during the last year (range = 0-60). Among the patients with MIRS ≥4 (n=36, m/f=18/18), balance confidence and reported walking ability were decreased, 72 % reported outdoor walking difficulty compared to 40% in the group MIRS ≤3 (n=15, m/f=2/13). Fifty-six percent of MIRS ≥4 admitted avoidance of activities due to fear of falling. There were statistically significant differences between the two groups in the physical assessments. The mean time to walk 10m with maximum gait speed was 8.3sec (9.3 and 6.2sec resp, p<0.01); TUG mean was 10.4sec (11.3 and 8.4sec resp, p<0.01) 53% didn't manage to stay 10sec on one leg (67% and 20% resp, p<0.001); 35% couldn't stay 10sec in tandem stance (44% and 13% resp, p<0.01). Foot dorsiflexor strength was mean 131.6 N, (90.2 and 222.4 N resp, p<0.001); Knee flexor strength was mean 100 N (88.9 and 125.8 N resp, p<0.001); Knee extensor strength was mean 277.4 N (254.1 and 328.3 N resp, p<0.01). The differences in hip abductors & flexors were smaller but still statistically significant. There were, as expected, significant differences between genders in the stronger muscles. However, no significant differences in strength between men and women could be detected in the weakest muscle groups (foot dorsiflexors and knee flexors).
Conclusions: A high percentage of the DM1 individuals at the clinic suffer from gait and balance difficulties, partly due to weak distal muscles. Thirty-seven percent of the whole study group had fallen four times or more during the last year. Many DM1 individuals with a MIRS ≥4 have difficulties with participation in outdoor activities and sports, which they avoid due to the risk of falling. Is it possible to diminish the risks of falling and increase the quality of life? This study was the basis of a recently conducted intervention study, aiming to evaluate the effects of functional balance exercise.

P4-16 No Evidence Of Specific Postprandial Hyperlipemia In Myotonic Dystrophy.

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Objects: Hyperinsulinemia in myotonic dystrophy type 1 (MD) has been well reported. Hyperlipemia is closely related to hyperinsulinemia as one of important risk factors for atherosclerotic complications. Recent studies suggested not only fasting but also postprandial hypertriglycemia was good predictor of hyperlipemia. The aim of this study was to investigate postprandial lipemia in MD by means of oral fat-loading test.
Methods: Fourteen patients with MD (five females, the mean age of 52.6 years) were examined. Eight patients were diagnosed as non-diabetes (NDMD) and six patients were diabetes mellitus (DMMD). Nine non-diabetic volunteers were also participated as controls. Twenty-five grams of fat as butter was taken orally. Serum triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL), and free fatty acid (FFA) were measured before and at 120, 180, and 240 minutes after

the fat-loading. Apo lipoprotein B48 (ApoB) before and at 120 minutes after the loading were also surveyed.
Results: Age or body mass index showed no significant difference between MD and controls. There was no significant difference of parameters except for FFA between MD and controls. FFA at 180 or 240 minutes after the loading in MD was significantly lower than that in controls. In comparison between NDMD and DMMD, FFA at all point during the examination and ApoB before the loading in NDMD were significantly lower than those in DMMD.
Conclusion: Our results suggested that MD had no specific postprandial hyperlipemia. The lower value of FFA in MD might reflect effects of insulin due to hyperinsulinemia.

P4-17 Characteristic features of oral and dental health in myotonic dystrophy

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Background: There are many oral and dental problems at bedside of patients with myotonic dystrophy type 1 (MD). The aim of this study is to evaluate the present condition of oral health in MD in order to care and preserve good health of them.
Methods: Eleven in-patients with MD had dental examination, and their states of oral health were appraised by Kikuya's assessment score (Clean; 0-3, Semi-clean; 4-6, Semi-dirty; 7-9, Dirty; 10 or more). Eight in-patients with Duchene progressive muscular dystrophy (DMD) and seven in-patients with other neuromuscular disorders were also participated as controls.
Results: Patients with MD showed a tendency for dirty condition of oral health compared to patients with DMD or others (the mean score of MD; 6.1, DMD; 3.8, others; 4.2). Patients with MD who took care of oral health by their own ability indicated a tendency towards dirty compared to patients with help for oral health care (without help; 6.8, with; 4.0). Patients with help for oral health care could preserve their conditions due to twice treatments for five minutes per day. Patients without help could relatively keep their states by treatments over ten minutes.
Conclusion: Our results suggested that oral conditions in MD were dirtier than those in other neuromuscular disorders and oral states in patients without help were dirtier than those in patients with. Positive intervention for oral health care in MD should be considered because their situations were related to specific problems of functional disabilities, structural abnormalities, and mentality or character in MD.

P5 Poster Session 5: Translation and Therapy

P5-01 Perceptions of professional, lay, and peer facilitators goal-setting and strategies used to promote social support and self-management behavior in face-to-face and online support groups for adults with either Multiple Sclerosis or Myotonic Muscular Dystrophy

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Rational: People with chronic health conditions spend their life managing their illness. About 20% of Americans have some type of chronic health condition, and may attend support groups for increased knowledge of their condition, self-disclosure, camaraderie, and inspiration to move forward with their lives. Positive outcomes from support group participation include social support, which has been associated with better physical health and psychosocial well-being, and self-management skills. Integrating self-management behaviors into participant's daily lives has been successful in diabetes, arthritis, asthma, lung and heart disease support group interventions.
Objective: The purpose of this study is to explore how support group facilitators for adults with neurological health conditions perceive their role in promoting self-management techniques and social support and what strategies are used to achieve these goals. This study asks the following questions: What role do facilitators play in motivating support group participants to practice self-management techniques? What strategies do facilitators employ to promote social support and self-management techniques? Do these strategies differ for professional, lay, and peer facilitators, face-to-face and online support groups, and Multiple Sclerosis and Myotonic Muscular Dystrophy groups?
Methods: Facilitators of both face-to-face and online support groups for either Multiple Sclerosis or Myotonic Muscular Dystrophy will complete a researcher-designed survey instrument.
Results: To date I have reviewed the literature, defined the purpose, theoretical framework, and research questions, as well as developed the instrumentation. Data will be collected and analyzed in the fall of 2009.

P5-02 The lived experience of DM1 patients caregivers

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RATIONAL. Myotonic Dystrophy Type 1 (DM1) not only generates several abnormalities in various human systems but also impedes social participation (work, leisure, social networking etc.) of the patients. It is essential to know how people adapt to the disorder in their everyday life and natural environment. Those who care for the DM1 sufferers at home play an important role. In addition to accepting the permanent physical changes that will occur, DM1 sufferers and their caregivers must also adapt daily routines, family roles, and responsibilities and find essential resources. OBJECTS. Largely implicated in the follow-up of DM1 patients, nurses from our Neuromuscular Clinic (Jonquiere, Canada) have developed a study that explores the realities of people who care for DM1 sufferers. METHODS. Through a qualitative descriptive study, approximately 30 DM1 patients caregivers participated in semi-directed interviews. RESULTS. The results indicate that: 1) the caregiver role is essential in the disease management; 2) caregivers'social, conjugal and family life will greatly be affected; 3) some caregivers experience significant emotional difficulties; 4) the assistance provided by caregivers is centered on emotional and informative support, surveillance, and support of the activities of daily living; 5) caregivers have a serious need for assistance with regards to home services. CONCLUSION. The study permitted: understanding that people who are close to DM1 sufferers endure several problems, mostly psychologically and interpersonally related; establishing a portrait of the help caregivers provide; observing caregivers'role as key informants for healthcare team; and identifying that many support services are not provided.

P5-03 Living with Myotonic Dystrophy Type 1 (DM1) Sufferers: How Caregivers'Experience Differs According to Gender

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RATIONAL. Several countries have decided to de-institutionalize health care and support offered to those suffering from chronic diseases. As a result, close family members or friends take on the responsibility. Even if care and support are generally provided by women, certain men, especially the spouse, also assume responsibility. Several studies carried out in the field of assuming of responsibility for people growing older have shown that men and women take on different roles and responsibilities when giving care. They also react differently to stress caused by caring for their spouse who is either sick or has functional incapacities. OBJECTS. The study aims to describe and compare how a person's gender affects the experience of caring for a person with DM1. METHODS. A descriptive qualitative research study carried out with 11 men and 7 women relates the dynamics of accepting responsibility to care for DM1 sufferers with the caregiver's gender. Semi-directed interviews gathered information about: 1) the reasons why people assume responsibility for taking care of their spouse who is suffering from DM1; 2) how support and care are given; and 3) the repercussions experienced by the caregiver for taking on such a responsibility. RESULTS. Preliminary analysis of the results indicates that there is a difference in how each gender experiences giving care for their spouse with DM1. CONCLUSION. The results suggest that caregivers'needs should be screened through regular follow-ups. How each gender experiences the situation should also be taken into consideration.

P5-04 Adapting and Validating the Stanford Self-Management Program for People with DM1: Preliminary Results and Lessons Learned

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RATIONAL. Those with Myotonic Dystrophy Type 1 (DM1) deal with the consequences and challenges associated with the disorder on a daily basis. Self-management is an approach that allows for people with chronic diseases to take charge of their condition. OBJECTS. A self-management program already used for other chronic diseases at the Patient Education Research Center at Stanford University (California) has been adapted to support DM1 patients to take control of their condition. The content has been adapted to correspond to the reality of DM1 patients, to be leaded by two healthcare professionals and to include the participation of family members. METHODS. After an adaptation of the content, the program was done with a group of five DM1 patients and their caregivers. Interviews and questionnaires were used before, during and after. Questionnaires were about main indicators developed by the Stanford team (fatigue, self-efficiency, pain, illness intrusiveness, etc.). RESULTS. The participants were very satisfied and judge that the content was pertinent to their reality, providing sufficient baggage for them to handle the disorder. The trainers observed that participants achieved a feeling of confidence encouraging active participation. Caregivers also noted a better understanding of the disorder, allowing them to act as a source of information and support for DM1 patients. The preliminary results also suggest a potential improvement of indicators once the program was completed. CONCLUSION. The results obtained through the preliminary validation confirm that the adapted self-management program is plausible and has the potential to bring positive outcomes to those with DM1.

P5-05 Quality of life in myotonic dystrophy type 1

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Quality of life (QOL) as an outcome measure in clinical trials is becoming increasingly important. QOL measurement provides a way of incorporating the patient's perspective of how myotonic dystrophy impact on their lives. However, few studies have looked at health-related and subjective quality of life in DM1. METHOD: This is a secondary data analysis based on a cross-sectional design on 200 subjects with myotonic dystrophy type 1. The study included adult and mild phenotypes from a larger study that occurred between 2002 and 2004. The study was conducted with the MOS 36-Item Short-Form Health Survey (SF-36) about health related quality of life (HRQL) and the Ferrans and Powers Quality of Life Index (QLI) about subjective quality of life. RESULTS: The results will present HRQL and subjective quality of life global score, sub-scores and comparison between the adult and the mild phenotype as well as their association. The following hypotheses were made: 1) a difference will be observed between the adult and mild phenotypes for both HRQL and subjective quality of life and; 2) a small association will be observed between the two types of quality of life for the two phenotypes

P5-06 Functioning, disability and health-related quality of life in adults with Myotonic dystrophy type 1 (DM1)

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Objective: To describe and analyse functioning, disability and health-related quality of life with regard to disease severity in adults with DM1.
Design: The study was cross-sectional in design.
Methods: Forty-one women and 29 men with DM1 (mean age 45, range 19-70 years) underwent clinical examinations, functional assessments and answered established questionnaires in order to gather information on weight, height and waist circumference; cardiovascular and respiratory functions; grip strength and dexterity; mobility and walking; physical activity levels; daytime sleepiness; activities of daily living (ADL) and health-related quality of life. Disease severity classification was based on the muscular impairment rating scale.
Results: Overweight, waist circumferences indicating risk for metabolic complications, and low cardio-respiratory fitness and physical activity levels were found in 30 to 40% of participants. Cardiac and respiratory functions were classified as normal for 63% and 26%, respectively. Excessive daytime sleepiness was reported by 42%. Sixteen per cent were dependent in personal ADL and 39% in instrumental ADL. Disabilities in grip strength, dexterity, mobility and walking were found and persons with severe disease performed significantly worse than persons with mild disease. Ratings of health-related quality of life concerning physical health and vitality were lower compared to Swedish normative values and persons with severe disease had lower scores on physical functioning and general health compared to persons with mild disease.

Conclusion: Disabilities and differences between persons with mild and severe disease were identified and this clinically-relevant information can be used for developing health services for people with DM1.

P5-07 Myotonic Dystrophy - A Scottish Perspective

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The management of myotonic dystrophy is problematic and informed by few high quality clinical studies. Patient engagement with medical interventions is variable. In 1997 clinical guidelines for Scotland were developed and an integrated care pathway introduced in 4 regional genetic centres based on the evidence available at that time. These included a recommendation for annual ECG monitoring in patients with significant muscle disease. Audit carried out in 1998, one year after their introduction, showed a rise in the percentage of patients undergoing ECG from 33% to 72%, and a rise in the percentage of patients in whom anaesthetic risks were discussed from 55% to 89%.
A new initiative in Scotland in 2006 granted further funding to address unmet need. This led to a re-evaluation of the management of Myotonic Dystrophy.
Objectives: We wished to audit current standards of care and determine how many patients remain under follow up and how many had defaulted from clinic appointments. We report the results of an audit of current practice against pre-existing guidelines, identify problems with the follow up of this group of patients and make recommendations for long term management.
Methods: All patients had molecular confirmation of diagnosis and were identified from clinical genetics databases and the database from a neurologist-led muscle service.
A detailed audit in one centre assessed whether the 1997 management guidelines had been followed.
All patients attending 3 centres had their notes reviewed and non-attendance documented.
Results: Audit of the guidelines in one centre found a low frequency of adherence to the guidelines across all management areas. The frequency of annual ECGs was 29%. Only 41% of patients were documented as having been warned about anaesthetic risks. In both cases, this is lower than before the 1997 guidelines were developed.
Across the 3 centres, 33 - 56% of patients were lost to follow up after publication of the guidelines.
Discussion: We have developed a clinician-led disease-specific clinic with links to other professionals all working to an evidence based management protocol. There is an aim to be in regular contact with 100% of the Scots population and collect common outcome data. Future audit will review whether this improves long term attendance at clinic. Prospective data collection from the Scottish population will also allow measurement of the outcomes from the new evidence based clinical guidelines.

P5-08 Role of oro-pharyngo-oesophageal scintigrapgy in the evaluation of swallowing disorders in patients with myotonic dystrophy type 1 (DM1)

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Rationale: Evaluation of the severity of dysphagia in DM1 patients is important, due to the high risk of ab-ingestis pneumonia in such patients. So far, dysphagia has been reported in literature in DM1 patients with variable proportions, ranging from 25 to 80% of cases.
In this regard, this study assessed the role of oro-pharyngo-oesophageal scintigraphy (OPES) in the evaluation of dysphagia and the correlated risk of ab-ingestis pneumonia in a representative cohort of DM1 patients.
Materials and methods: 38 DM1 patients (16 female, 22 male; mean age 51.5 yrs) were submitted to OPES. Patients were classified according to n(CTG)in leukocytes, severity of muscle weakness, degree of dysphagia evaluated by administration of a questionnaire and disease duration. Other aetiologies of dysphagia had been ruled out.
OPES, a well tolerated, simple, reproducible and safe method, provides both qualitative and quantitative informations about the swallowing phases; it is based on the rapid sequential acquisition of 480 images after the administration of 10 ml of water containing 37 MBq of 99mTc-Colloid. The evaluation of sequential scintigraphic images and the activity/time curves allow a qualitative (bolus fragmentation with multiple swallowing, naso-pharyngeal or pharyngo-oral refluxes, premature ingestion of the bolus, laryngo-tracheal aspiration) and a quantitative [oral, pharyngeal and oesophageal transit times (OTT, PTT, ETT), retention indexes (ORI, PRI, ERI) and tracheal aspiration percentage (TAP)] analysis of swallowing disorders. Results obtained in DM1 patients were compared to those obtained in 17 healthy volunteers.
Results: Only in DM1 group OPES qualitative analysis showed: premature ingestion of the bolus in 2 pts. (5%), multiple swallowing in 11 (29%), glossal propulsion deficit in 25 (66%), pharyngeal peristalsis deficit in 22 (58%), tracheal aspiration in 5 (13%), pharyngeal phlogosis in 22 (58%) and oesophageal phlogosis in 21 (55%). OPES quantitative analysis showed the following alterations: OTT in 22 patients (58%), ORI in 29 (76%), PTT in 25 (66%), PRI in 27 (71%); ETT in 14 (36%), ERI in 23 (60%); mean TAP was 5.8% (range: 2-12%). No correlation was found between quantitative parameters and n(CTG), degree of muscular impairment and degree of dysphagia, while a significant correlation was found between OTT, PTT, PRI and duration of disease.
Conclusion: This study documented that the occurrence of swallowing disorders is a rather common feature, yet often underestimated, in DM1 patients. Our study shows that the prevalent impairment of the swallowing processes involves the oro-pharyngeal phase and its severity seems to be only influenced by disease duration. Moreover, compared to other diagnostic tests, only OPES allows to quantify the amount of tracheobronchial aspiration. For these reasons OPES appears useful for follow-up and treatment of swallowing disorders and to monitor the risk of ab-ingestis pneumonia in DM1 patients.

P5-09 Myotonic dystrophy: a service improvement survey of quality of life, social integration and community support systems for patients and carers

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Rationale: The many impairments in people with DM1(pwDM) makes management complex, requiring heath and social services multidisciplinary working. Understanding the needs of pwDM should assist in delivering services.
Objectives: To delineate health and social care needs of pwDM, and compare this with services currently available.
Methods: pwDM, attending one of the three UK muscle clinics included in the study, were sent a postal questionnaire. This included questions regarding symptoms and use of services; the Community Integration Questionnaire (CIQ) and the Euroqol for the pwDM and a questionnaire regarding support received for the main carer. A postal questionnaire concerning support services was sent to the nine relevant social services departments.
Results: 104 pwDM replied, aged 16 to 74 years. Patients experienced a variety of symptoms, carers also noted apathy and bowel/bladder symptoms as problems. The median summary index from the five Euroqol domains was 0.62 (IQR-0.44), 26% having at least moderate problems in all domains, and median 50% (IQR-35.5%) self rated health on the visual analogue scale. Median CIQ score was 14 (IQR-8.39). One social services department replied, stating they did not consider specific disease related groups.
Conclusions: There was a range of health and quality of life related needs. Scores on the Euroqol were comparable to other groups with neurological disability. Scores on the CIQ were comparable to traumatic brain injury patients who have received no rehabilitation. The lack of replies from social services may indicate a lack of focus in needs of individual patient groups.

P5-10 In vivo drug screening of 170 natural compounds in a DM1 fly model

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Myotonic dystrophy type 1 (DM1) is the most common muscular dystrophy in adults (1/8000). At the molecular level, the mechanism of pathogenesis lies in the expansion of CTG trinucleotide repeats in the 3'UTR of the DM protein kinase (DMPK) gene. Toxicity of the repeats occurs by a RNA gain-of-function mechanism by which CUG-carrying RNA molecules fold to form an imperfect double-stranded RNA hairpin that sequesters RNA binding proteins into ribonuclear foci. In this work we performed a in vivo drug screening using Drosophila melanogaster as a DM1 model. These flies have been previously described as a validated DM1 model. Model flies are unable to fly compared with controls so we based our screening on this feature, seeking for compounds that are able to rescue this phenotype. By means of this screening 170 compounds have been tested from a naturally derived collection.

P5-11 Systemic delivery of antisense morpholino corrects ClC-1 splicing and reduces myotonia in a transgenic mouse model of DM1

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Objective: To test the effect on myotonia and ClC-1 expression of an antisense morpholino oligomer that is modified for systemic delivery and designed to suppress inclusion of exon 7a.
Background: Intramuscular injection of antisense morpholino targeting the 3´splice site of ClC-1 exon 7a can eliminate myotonia in mouse models of DM1 (Wheeler, 2007). However, muscle uptake of unmodified morpholino after systemic delivery is low. Morpholinos coupled to an octa-guanidine dendrimer (Vivo Morpholinos) are designed for enhanced tissue uptake after systemic delivery.
Design/methods: 200 µg of Vivo Morpholino (Gene Tools) was injected into tail vein of HSALR mice twice weekly for two months. The sequence of the morpholino was the same as that previously shown to suppress inclusion of ClC-1 exon 7a in mice. Control mice were injected with vehicle alone (saline). Treatment assignments were randomized. Injections and electromyography were blinded.
Results: ClC-1 splicing was corrected and myotonia was eliminated in all 3 hindlimb muscles tested. ClC-1 protein was restored to the muscle surface membrane. Identical dosing for one month produced a partial effect. Intravenous injection of saline had no effect.
Conclusions: By using a dendrimer-modified morpholino, whole-body correction of myotonia can be obtained in a mouse model of DM1. These results support the feasibility and effectiveness of systemic delivery of antisense morpholino as treatment for DM1.
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P5-12 Pluripotent stem cells to explore mechanisms and treatments of monogenic diseases

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The lack of existing models of pathologic tissues has rendered many important questions in disease pathogenesis inaccessible. Human embryonic stem cells derived from affected embryos during a preimplantation diagnostic, as well as the technical development to obtain human induced pluripotent stem cells generated from patients, offer the unique opportunity to have access to a large spectrum of disease-specific cell models. These new disease-specific cell models are applicable for a wide systemic mechanistic analysis ranging from functional studies at the cellular level to a large-scale functional genomics screening.
As a proof of principle, we demonstrated that PGD-derived hES cells and derivatives which, express the causal mutation implicated in the Myotonic Dystrophy type 1 (DM1), may mimic molecular defects associated to the pathology, such as the nuclear aggregation of mutant RNA. Using a whole-genome transcriptional analysis, we provided a list of DM1 specific biomarkers that could be considered as a robust DM1 signature. These new biomarkers as well as the already known DM1 biomarkers have been used to develop a high throughput genomics screenings based on a gene knockdown and overexpression

approach that should provide more insights into the molecular pathways implicated in the development of DM1. In parallel, by using a high content screening approach, a pilot drug screening experiment has been successfully conducted in order to identify new molecules which, due to their ability to disrupt the nuclear mutant RNA aggregation, might represent new therapeutic strategies.
Complementary to these genome-wide genetic studies, we focused on the analysis of the functional defect between nerve and muscle, leading, as indicated by the name it-shelf, to the myotonia symptom. We thus identified abnormal neuritic network in mutated hES cell line-derived motoneuron cultures that could affect the normal communication of this cell type with its muscular target. This hypothesis is actually tested using a nerve-muscle in vitro system.

P5-13 RNA-based gene therapy to remove toxic expanded CUG-transcripts

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DM1 is a RNA-mediated disorder caused by the expression of the mutant DMPK transcripts containing expanded CUG repeats. The DMPK expanded transcripts are retained into the nuclei as foci and alters the functions of splicing factors leading to misregulation of RNA homeostasis. Several therapeutic strategies are currently under development to target and destroy the mutant DMPK transcripts. We have developed a new RNA based gene therapy to get rid of the expanded CUG-mRNA. The efficacy of this tool was first evaluated in vitro using DM1 muscle cells containing 800 CTG. A reliable and efficient 80-90% reduction of the mutant DMPK mRNA was measured in the DM1 cells transduced with a lentiviral vector. The normal DMPK transcripts were not impacted and no obvious cellular toxicity was detected. In parallel, we observed a strong reduction in the number of foci per nucleus and a significant increase in the number of DM1 cells without aggregates. These results were confirmed in several cells lines harboring different CTG lengths. In addition, MBNL1 proteins were no more trapped and the aberrant pre-mRNA splicing were largely abrogated in DM1 myotubes. Restoration of the myogenic differentiation of the DM1 myoblasts confirms that this tool reduces or abolishes the cellular defects induced by the expanded CUG-repeats. This new RNA-based gene therapy approach is now being tested in mice model for DM1 and the precise mechanism of action is under investigation.

P5-14 High throughput screen assays for identifying inhibitors of protein-RNA binding as a potential treatment for Myotonic Dystrophy Type-1

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Objective: To develop a homogenous assay for detection of protein-RNA interaction between muscleblind-like 1 (MBNL1) and expanded CUG repeat RNA that is amenable to high throughput screen (HTS).
Methods: We used time-resolved fluorescence energy transfer (TR-FRET) technology to assay the binding of C-terminally His-tagged MBNL1 and a biotinylated 36-mer RNA Biot-(CUG)12. The assay was miniaturized to 1536-well plate format and validated against a small library of 1280 known pharmacologically active compounds (LOPAC, Sigma-Aldrich).
Results: The assay is highly sensitive and can reliably detect 20fMoles of MBNL1-CUG complex in 4ul reaction volumes. Results from the LOPAC screen yielded a signal-to-basal ratio, Z'factor and hit rate of 5.5, 0.87 and 0.6%, respectively.
Conclusion: The signal-to-basal ratio, Z'factors and hit rate indicate that the assay is robust and ready for HTS.

P5-15 Test/retest reliability of regional lean body mass (LBM) measurements using dual energy X-ray absorptiometry (DEXA) in patients with DM-1.

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Background: Total Lean Body Mass measurements using DEXA have been used in longitudinal natural history studies and therapeutic trials in patients with DM-1. Recently, correlations between Regional LBM and quantitative strength measurements have been documented by McDonald et al. The regional measurements are made by technicians using software provided with the DEXA instrumentation. The test/retest reliability of these regional measurements is not known.
Methods: Regional LBM measurements were performed twice over a 1-24 hour period on DEXA scans of 10 patients with DM-1 using the Hologic post processing software. The whole body scans were divided into 5 body segments- Trunk, Right and Left Arm and Right and Left Leg using well defined anatomical landmarks and standard procedure as reported in previous studies.
Results: The Pearson correlations between the 2 regional measurements for each of the areas were as follows: Trunk 0.998, R Arm 0.995, L Arm 0.984, R Leg 0.993, L leg 0.996, the p value for each of the correlations was < .0001.
Conclusions: All the correlations were greater than .90 which denotes excellent test/retest reliability. The excellent correlation documents the reliability of regional measurements derived from scans that may be made over an extended period of time as part of natural history studies and/or therapeutic trials.

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