IDMC-6

6th International Myotonic Dystrophy Consortium Meeting

September, 12-15, 2007
University of Milan
Milan – Italy

Università degli Studi di Milano

Policlinico San Donato
Istituto di Ricovero e Cura a Carattere Scientifico

Milano
Comune di Milano
Assessorato alla Salute

6th International Myotonic Dystrophy Consortium Meeting

IDMC-6 Index

Welcome to the 6th International Myotonic Dystrophy Consortium Meeting

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It's my great pleasure to welcome you to the european, industrious, economic, cultural and friendly city of Milan, for the 6th International Myotonic Dystrophy Consortium Meeting.

The IDMC-6 will follow on previous highly successful meetings in Quebec (2005), Glasgow (2003), Kyoto (2001), Chapel Hill (1999) and Paris (1997).

In these ten years, remarkable progress has been achieved in the research of DM and its related issues, which is expected to contribute to the better understanding of the pathomolecular mechanisms underlying physiopathogenesis of DM and the development of new therapeutic approaches.

In IDMC-6 there were so many abstracts submitted, that we were not able to fit every body in for oral presentations and will be having a poster session as well.

In the meeting there will be the participation of a huge number of young investigators and researchers, coming from Australia, Canada, Costa Rica, Cyprus, East Europe, France, Germany, Israel, Italy, Japan, Scandinavian, Spain, The Netherlands, UK and USA which will guarantee an outstanding meeting.

There will be innovations in the course of this congress, including a satellite symposium on "Genome-wide strategy in DM" with the aim to collect investigators for DNA studies on large scale; a special lecture on phenotype, genotype and molecular basis of DM will conclude the first day of the meeting.

Another change is represented by the participation for the first time of patients from Italy, Belgium and Greece as well as from France, UK and USA.

These patients will exchange their experiences about quality of life and can interact with clinicians and researchers during all congress.

At the end of the meeting, an interactive session between investigators, physicians, patients and representative of advocacy groups will be to discuss the patient's experiences, the guidelines and standards of care for DM. Myotonic Dystrophy Foundation Excellence in Research Award will be given to promising young researches and/or clinicians to encourage to continue and pursue research in the area of Myotonic Dystrophy. A AIM (Associazione Italiana di Miologia) young investigator Poster Award will be also given.

The highlight session summarizing the main aspects of research and therapeutic approach in DM will close the IDMC-6.

The timetable of scientific part of the meeting allows only a few social events: one special lecture on "Leonardo and Last Supper: restoration works", the visit to Last Supper, a "Pizzata" and a "Gala dinner" in a relaxed atmosphere.

The venue of the meeting at main campus of University, in the centre of Milan, is at walking distance from magnificent monuments, like Duomo Cathedral, Galleria, Scala Theatre and fashion district, and allows you to visit all these places in free time.

Finally I would like to thank the International Committee for allow me to organize IDMC-6 in Milan and for continuous help and suggestions and all those who actively participated in the organization of this meeting: our generous sponsors, mainly foundations from patients, who made this congress possible (especially supporting the participation of young investigators), the organizing agency ECON and at last but not least to you all (young investigators, researchers, physicians, patients, representative of advocacy groups) for coming here in Milan and contributing to what I am sure will be another great IDMC meeting.

Thanks (Grazie)

Giovanni Meola, MD
Chairman of IDMC-6

IDMC-6 Committees

Local organizing committee

Luigi De Ambroggi
University of Milan
Department of Cardiology
IRCCS Policlinico San Donato
San Donato Mil. – Milan

Valeria Sansone
University of Milan
Department of Neurology
IRCCS Policlinico San Donato
San Donato Mil. – Milan

Giovanni Meola
University of Milan
Department of Neurology
IRCCS Policlinico San Donato
San Donato Mil. – Milan
Chair IDMC-6

International committee

Tetsuo Ashizawa,
Department of Neurology,
University of Texas,
Galveston-Texas, USA

Geneviève Gourdon
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Paris, France

Darren G. Monckton
University of Glasgow
Institute of Biomedical and Life Sciences
Glasgow, UK

Nakaaki Ohsawa
Aino Institute For Aging Research
Ibaraki (Osaka), Japan

Jack Puymirat
CHU Laval Research Center
Quebec, Canada

Charles Thornton
Department of Neurology
University of Rochester
Rochester-NY, USA

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Hunter Lord Funds
Atlanta-Georgia, USA

Margaret Bowler
Myotonic Dystrophy Support Group
Nottingham, UK

Claude Bourlier
French Myotonic Dystrophy
Support Group
Evry, France

Sponsorship
The Organizing Committee is pleased to acknowledge the support of the following organizations in funding the Meeting: Centro per lo Studio delle Malattie Neuromuscolari – CMN (Italy); Association Française contre les Myopathies – AFM (France); The Marigold Foundation (Canada); The Hunter Foundation (USA); The Myotonic Dystrophy Support Group – MDSG (UK); Myotonic Dystrophy Foundation (USA); AINO Hospital Foundation (Japan); IRCCS Policlinico San Donato (Italy); Bayer HealthCare; Biotest; Boston Scientific; MarvecsPharma; Novartis; Pfizer; Sanofi Aventis; UCB CNS Innovators; Officine Smeraldo.

Patronages
The Organizing Committee is also pleased to acknowledge the patronage by: Università degli Studi di Milano; Comune di Milano – Assessorato alla Salute; Società Italiana di Neurologia (SIN); Associazione Italiana di Miologia (AIM); Società Italiana di Istochimica (SII)

Registration
The Registration desk is located in the foyer of the Auditorium.

Oral presentation
Each speaker will have a 10 minutes period for slides presentation with an additional 5 minutes for discussion.
The programme is packed and the session chairmen will stick rigorously to the schedule.
It is essential for the smooth running of the meeting, that the pen Drivers or CDs must be handed in at the "Slides center" at least one hour before the beginning of the session.
Speakers will have the opportunity to check their presentations on PCs available in the Slide Center.

Posters
Posters should be attached on their boards on Thursday, September 13 before 10.00 hrs and removed on Saturday, 15 September between 16.15 and 19.30 hrs.
Posters still hanging at the end of the removal time, will be discarded.

Lunches
Lunches will be served from Thursday to Saturday in the University Refectory, on the basement floor.
Lunch times are listed on timetable overview.
Teas, coffee and juices throughout the meeting will be served during break time, in the '700 Court, in front of Auditorium.

Language
The official language of the IDMC-6 is English. A simultaneous translation (English-Italian) will be provided only during the Interactive Session 8.

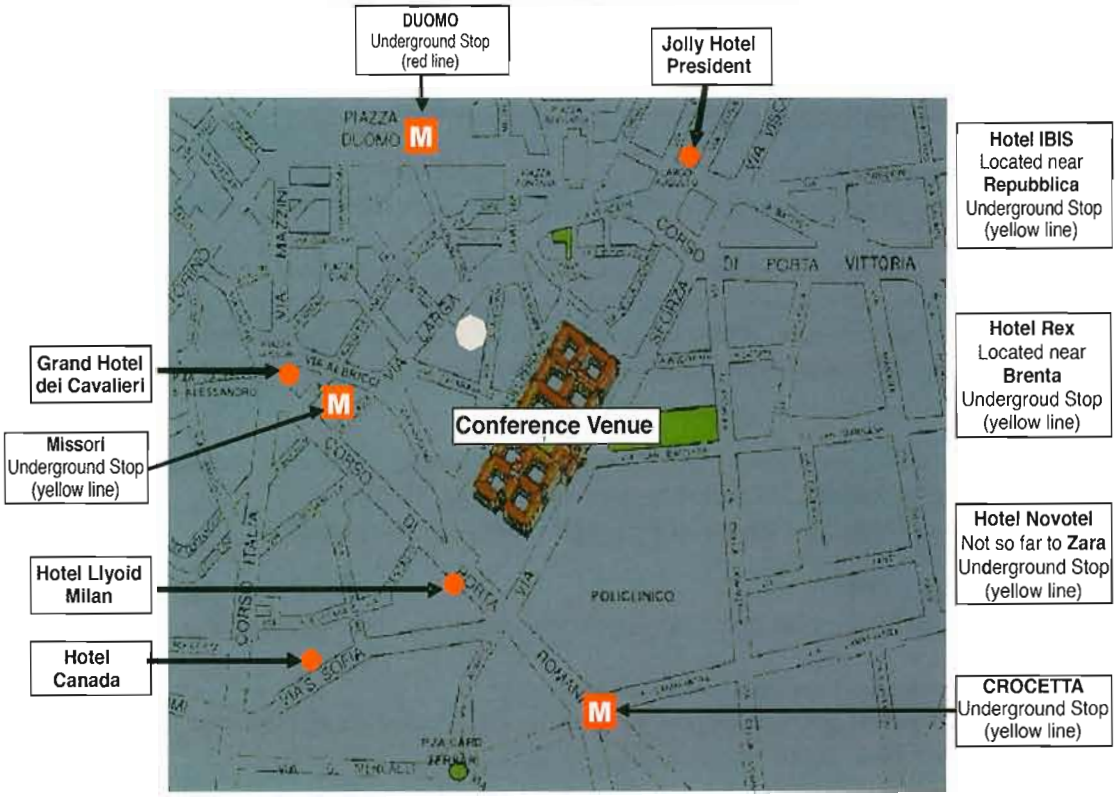
Continuing Medical Education
The Italian Ministry of Health has assigned to the Congress 13 CME credit points for Accreditation Medicine and Surgery.
Each Italian participant (Medical Doctor) will receive credits after submission of an evaluation form at the end of the Congress.

Persons living with myotonic dystrophy
The patients may attend to entire meeting if they wish. During the meeting, interactions between English, French and Italian patients with clinicians or researchers of the same language, are scheduled:

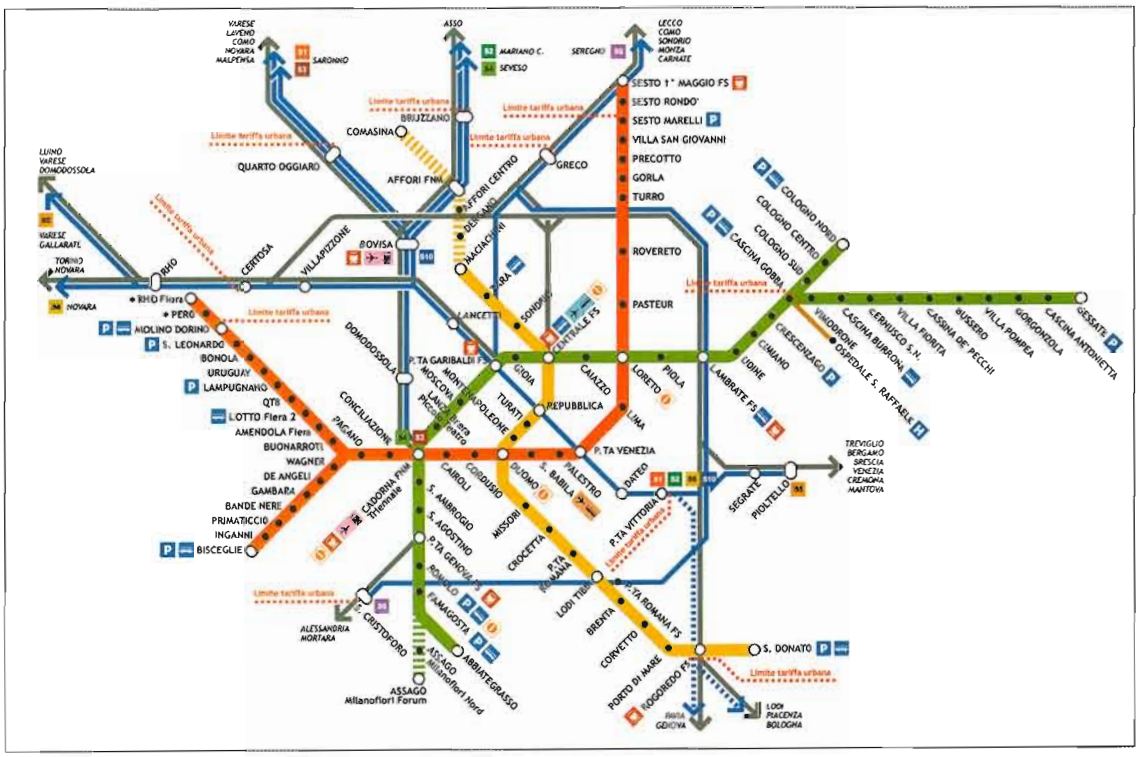
Thursday 13th	at 11.45-12.15 (continuing lunch time from 12.15-13.15)
Friday 14th	at 11.45-12.30 (continuing lunch time from 12.30-13.30)
Saturday 15th	at 12.00-12.45 (continuing lunch time from 12.45-13.45)

There will be opinion leaders, speaking the same language of the patients, who will summarize each session into a short informal discussion/questions + answers session to be held just before and during lunch. All questions from patients group will be discussed in a special meeting "**Interactive Session 8**" (Saturday 15th, at 16.30-18.30) where patients and relatives, advocacy groups could interact with all clinicians and researchers.

Map of University Main Campus, Hotels and Underground stops

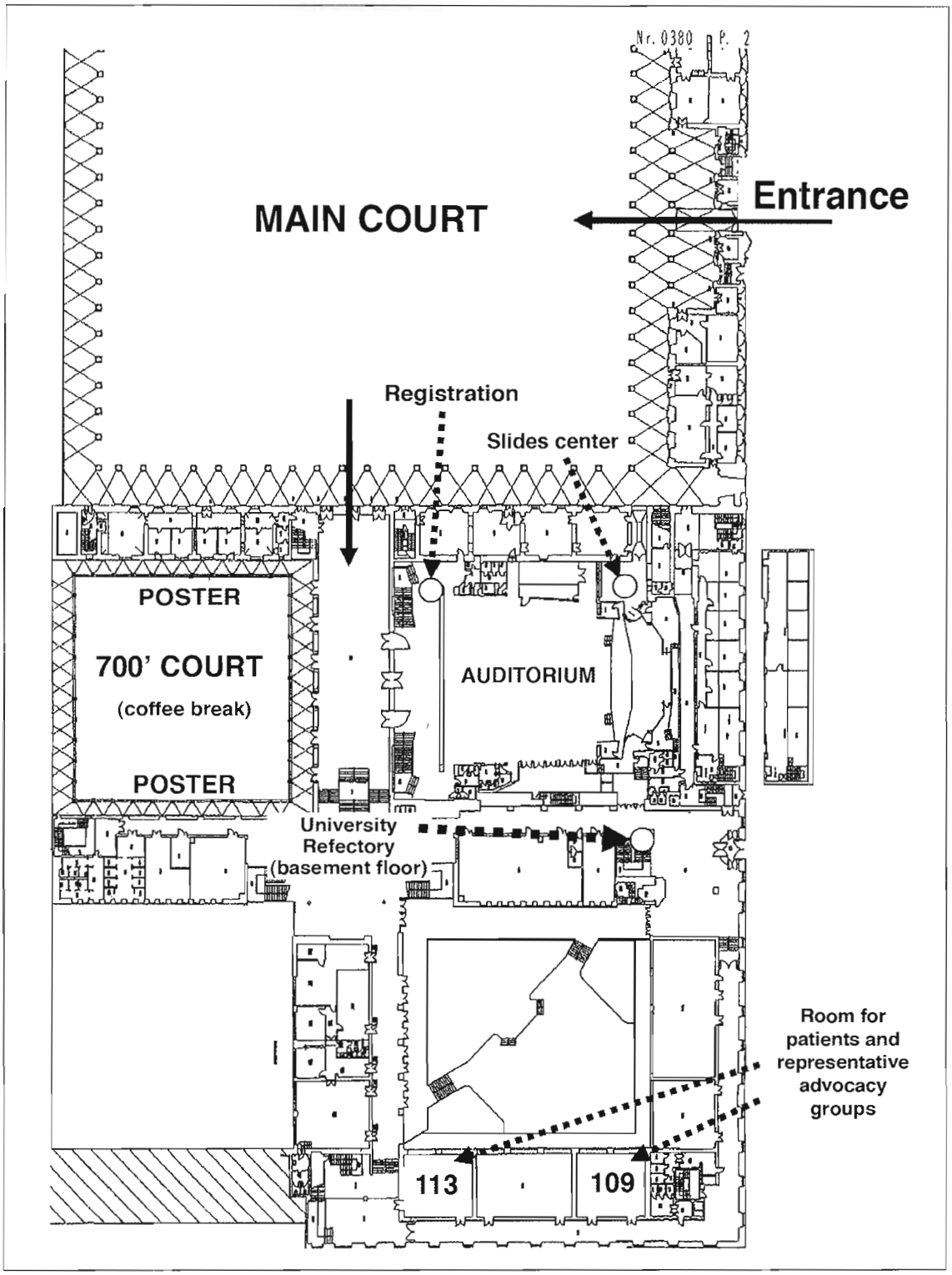


Underground - Metro



Meeting location

Università degli Studi di Milano
Via Festa del Perdono, 3/7



6th International
Myotonic Dystrophy Consortium Meeting

September, 12-15, 2007

Social activities

September 12, Wednesday – 20.00

Welcome Cocktail

September 13, Tuesday – 16.30-17.00

SPECIAL LECTURE
Leonardo and Last Supper: restoration works
dott. Ede Palmieri
Direttore Sopraintendenza per il Patrimonio Storico, Artistico e Etnoantropologico di Milano

or....Visit "Leonardo da Vinci's Last Supper"

Unfortunately only a limited number of tickets is available from Directory of "Cenacolo Vinciano". The reservation of the ticket is possible on Thursday , September 13, from 8.00 to 10.00 am at Reception Desk.

and at 20.00

PIZZATA (*Admission by ticket only. You should pre-book at moment of your registration*)

September 14, Friday - 20.00

Gala dinner in a relaxed atmosphere

(Admission by ticket only. You should pre-book at moment of your registration)

SCIENTIFIC PROGRAM

6TH INTERNATIONAL

MYOTONIC DYSTROPHY CONSORTIUM MEETING

(MILAN, 12-15 SEPTEMBER 2007)

September 12, Wednesday

Satellite Symposium — ***12.00 – 18.00***

A genome-wide strategy to define disease expressivity in DM
Allen D. Roses, MD
Senior Vice President, Pharmacogenetics
GlaxoSmithKline

Coffee break

Registration — ***17.00 – 18.30***

Welcome — ***18.30 – 19.00***

Prof. Giovanni Meola
Chairman of IDMC-6
Prof. Enrico Decleva
Chancellor of University of Milan
Prof. Virgilio Ferruccio Ferrario
Dean of Medical School - University of Milan
Prof. Giuseppe Rotelli
President of IRCCS Policlinico San Donato
Prof. Paolo Cabitza
Head of Department of Medical and Surgical Sciences
University of Milan – IRCCS Policlinico San Donato

Special lecture — ***19.00 – 20.00***

Phenotype, genotype, and molecular basis of Myotonic Dystrophy: past, present, and future
Prof. Giuseppe Novelli
Professor and Chair of Genetics
Tor Vergata University
Rome - Italy

Welcome Cocktail — ***20.00***

September 13, Thursday

Session 1 Oral session ***8.00 – 12.00***

DNA INSTABILITY AND MODELS OF MYOTONIC DYSTROPHIES
Session Chairs: Christopher Pearson and Be Wieringa

8.00 01 **Bidirectional transcripts at the DM1 locus and other triplet repeat diseases: implications for disease mechanisms**
SJ Tapscott, GN Filippova, S Mahoney, P Ladd, DH Cho

8.15 02 **Saturation effects of binding of miR-1 and miR-206 to the 3' UTR target site in DMPK mRNA: Yet another explanation for pathophysiological findings in cell and animal models for Myotonic Dystrophy type 1 (DM1)?**
Walther JA, A.van den Broek, Derick G Wansink and Bé Wieringa

8.30 03 **Modelling CUG expansion toxicity in Drosophila: what role for expansion context?**
G Le Mée, N Ezzeddine and O Aït-Ahmed

8.45 04 **Modelling Congenital Myotonic Dystrophy**
V Srinivasan, Q Yu and Mi Mahadevan

9.00 05 **CTG repeat "big jumps" in transgenic mice**
L Foiry, M Gomes-Pereira, A Nicole, S Tomé, C Junien, A Munnich and G Gourdon

9.15 06 **CUG length-dependent, brain region- and muscle type-specific splicing abnormalities in DM1 transgenic mice**
M Gomes-Pereira, A Huguet, A Nicole, J Acquaire, A Munnich, G Gourdon

9.30 07 **Insights from an Inducible/Reversible Mouse Model of RNA Toxicity in DM1**
MS Mahadevan, R S Yadava, V Srinivasan, Q Yu, C Frenzel-McCardell, J Puymirat, CA Thornton, AL Tucker, OW Prall, R.P Harvey

9.45 09 **Severe skeletal muscle wasting in a tissue-specific, inducible mouse model for Myotonic Dystrophy**
JP Orengo and TA Cooper

10.00 010 **Somatic mosaicism and genotype-phenotype correlations in DM1.**
F Morales, G Hogg, P Cuenca, G del Valle, R Brian, M Sittenfeld, T Ashizawa, A Wilcox, DE Wilcox and DG Monckton

Coffee break ***10.15 – 10.30***

10.30 011 **CTCF binding in cis regulates CAG/CTG repeat instability**
KA Hagerman, RT Libby, VV Pineda, R Lau, JD Cleary, BL Sopher, DH Cho, S Baccam, SJ Tapscott, GN Filippova, CE Pearson, AR. La Spada

11.45 012 **CNS RNA gain-of-function effects induced by CUG and CCUG transcripts**
R Daughters, Y Kang, D Tuttle, J Margolis, T Zu, M Moseley, J Day, M Swanson, L Ranum

11.00 013 **Pre-mutation allele pool in DM2**
LL Bachinski, T Czernuszewicz, LS Ramagli, T Suominen, O Raheem, MD Shriver, CA Thornton, B Udd, MJ Siciliano & R. Krahe

11.15 014 **A Transgenic Mouse Model for Myotonic Dystrophy Type 2 (DM2)**
R Krahe, M Sirito, M Wojciechowska, S Hajibashi, SE Olufemi, DR Mosier, O Raheem, R Randen, B Udd, & LL Bachinski

11.30 015 **Reversible multisystemic mouse models od DM1 and DM2**
Y Kang, J Margolis, J Day, L Ranum

Session 1 Poster session *11.45 – 12.15*

DNA INSTABILITY AND MODELS OF MYOTONIC DYSTROPHIES
Session Chair: Genevieve Gourdon

P1 **Sustained expression of CUG repeat RNA in Drosophila muscles is degenerative**
A Garcia-Lopez, L Monferrer and R Artero

P2 **Cis-elements, DNA replication and repeat instability at the human myotonic dystrophy type 1 locus**
JD Cleary, L Foiry, G Gourdon & CE Pearson

P2B **CTG repeat instability in transgenic mice: investigating the role of Msh2 and Ligase I**
S Tomé, L Foiry, A Huguet, DW Melton, A Munnich and G Gourdon

P3 **Progressive atrophy of the skeletal muscles in DM1 mice**
A Vignaud, A Ferry, G Gourdon, A Huguet , GS Butler-Browne and D Furling

P4 **Variables acting upon the ctg expansion over time in DM1 patients**
A Lopez de Munain, Cobo AM,Poza JJ

P5 **Identification of abnormal gene expression in myotonic dystrophy type 1 using a human PGD-derived embryonic stem cell line exhibiting intranuclear foci**
G Pietu, C Rochon, K Giraud-Triboult, L Kassar-Duchossoy, D Furling, J Denis, B Champon, C Martinat, K Sermon, M Peschanski

P6 **CUG repeats in DM1 are located within a retained intron of the DMPK 3'UTR**
ET Wang, D Housman, C Burge

P6B **Human DNA Ligase I in the replication, repair & instability of CTG/CAG repeats**
GB Panigrahi, A Lopez Castel, DW Melton & CE Pearson

Lunch *12.15 – 13.15*

Session 2 Oral session 13.15 – 16.15

CELLULAR AND MOLECULAR ASPECTS OF MYOTONIC DYSTROPHIES
Session Chairs: Tee Ashizawa and Jack Puymirat

13.15 016 **Form and function of short (CUG)n-RNA in cells transcribing extended CTG repeats characteristic of myotonic dystrophy type 1**
G Pall and A Hamilton

13.30 017 **Defective mRNA in myotonic dystrophy accumulates at the periphery of nuclear splicing speckles.**
I Holt, S Mittal, D Furling, GS Butler-Browne, JD Brook, GE Morris

13.45 018 **A bioinformatics approach to identify novel genes mis-spliced in myotonic dystrophy**
R Voelker & JA Berglund

14.00 019 **Cytoplasmic CUG RNA foci are insufficient to result in aberrant RNA splicing**
P Sarkar, C Wolf , W Dansithong, S Paul, A Chiang, D Branco, MC Sherwood, I Holt, GE Morris, L Comai, CI Berul and S Reddy

14.15 020 **Aberrantly spliced alpha-dystrobrevin alters alpha-syntrophin binding in myotonic dystrophy type 1**
M Nakamori, T Kimura, T Matsumura, H Fujimura, MP Takahashi and S Sakoda

14.30 021 **Amphiphysin 2 splicing alteration as a possible cause of muscle atrophy in myotonic dystrophic patients**
C Hammer, L Guigou, C Sellier, C Fugier, MC Hummel, C Thibault, N Sergeant, J Laporte, D Furling, B Udd and N Charlet-Berguerand

14.45 022 **The Response to Serum Starvation in DM1 Lens Cells Involves FGF Receptor Signalling and Ca2+ Channel Activation.**
JD Rhodes, S L Russell and A R Prescott

15.00 023 **Inhibition of prostaglandin E2 (PGE2) production restores the differentiation of congenital human myotonic dystrophy type 1 myoblasts**
J Puymirat, D Beaulieu, P Chapdelaine

15.15 024 **Significant alteration of gene expression in DM1 myoblasts is elicited by molecular events unlinked to RNA splice defects**
W Dansithong, S Paul, K Promnares, MP Takahashi,L Comai and S Reddy

15.30 025 **Mutant DMPK transcripts activates Notch signaling, impairing myogenesis in myotonic dystrophy type 1**
Xu W, Gao R, Takeuchi T, Furihata M, Puymirat J, Furling D, Ashizawa T and PS Sarkar

15.45 026 **Differential expression of elongation factor 1 alpha (EF1A) in DM muscle cells.**
R Pelletier, F Hamel and J Puymirat

16.00 027 **Spinocerebellar ataxia type 10 – parallel with and disparity from myotonic dystrophies in the RNA-mediated pathogenic mechanism**
MC White, R Gao, W Xu, SF Edwards, S Raskin, HAG Teive, G Schuster, HY Zoghbi, PS Sarkar, T Ashizawa

Coffee break ***16.15 – 16.30***

Special lecture: Leonardo and Last Supper: restoration works ***16.30 – 17.00***

dott. E De Palmieri
Direttore Sopraintendenza per il Patrimonio Storico, Artistico e Etnoantropologico di Milano

Visit to "Last Supper" ***17.30***

"Pizzata" ***20.00***

September 14, Friday

Session 2 Poster session ***8.00 – 8.45***

CELLULAR AND MOLECULAR ASPECTS OF MYOTONIC DYSTROPHIES
Session Chair: Stephen Tapscott

P7 **The p16 pathway mediates premature senescence of DM1 myoblasts**
Bigot A, Francois V, Butler-Browne GS, Mouly V and Furling D

P8 **Differential expression of splicing regulators and effects of CUG repeats in DM1 cerebral cell models**
D Ghanem, O Leroy, H Tran, CM Dhaenens, S Schraen-Maschke, B Sablonnière, L Buée, A Andreadis, N Sergeant, ML Caillet-Boudin

P9 **Visualization of the alternative splicing in living cells**
D Furutama, T Tanaka, R Sakai, T Maeda, T Hanfusa, N Ohsawa

P10 **In vitro study of DM1 primary myotubes**
E Loro, A Botta, C Catalli, V Romeo, F Rinaldi, C Angelini, L Vergani

P11 **Woodchuck post-transcriptional regulatory element induces nuclear export of myotonic dystrophy transcripts and repairs muscle cell differentiation**
NP Mastroyiannopoulos, E Chrysanthou, J Uney, MS Mahadevan & LA Phylactou

P12 **Analysis of MTMR1 pre-mRNA splicing in DM1 and DM2 muscle biopsies**
M Santoro, A Modoni, M Masciullo, E Ricci, PA Tonali, G Silvestri

P13 **Oxidative stress in DM1: role of NFKB and related proteins**
Valenti F, Aguennouz M, Musumeci O, Rodolico C, Lanzano N, Ciranni A, Crupi R, Toscano A, Vita G

P14 **Comparative studies of DM1 and DM2 in muscular biopsies**
S Salvatori, M Fanin, S Furlan, A Picard, E Pastorello, V Romeo, CP Trevisan and C Angelini

P15 **Abnormal expression of DMPK substrate phospholamban in DM2**
O Raheem, J Holmlund-Hapf, T Suominen, A Vihola, H Haapasalo, R Krahe and B Udd

P16 **Differences in aberrant expression and splicing of genes involved in Ca2+ metabolism between DM2 and DM1**
A Vihola, M Sirito, LL Bachinski, S-E Olufemi, O Raheem, T Suominen, B Udd and R Krahe

P17 **A variably spliced region of Ryanodine receptor 1 may be involved in excitation-contraction coupling.**
T Kimura, M Nakamori, MP Takahashi, H Yoshikawa, S Sakoda and AF Dulhunty

P18 **The CTG repeat expansion size correlates with the splicing defects observed in muscles from myotonic dystrophy type 1 patients**
Botta A, Rinaldi F, Catalli C, Bonifazi E, Loro E, Vergani L, Romeo V, Angelini C, Novelli G

P19 **Gene expression analysis in Myotonic Dystrophy: Indications for a common molecular pathogenic pathway in DM1 and DM2**
Rinaldi F, Botta A, Vallo L, Bonifazi E, Gambardella S, Mancinelli E, Angelini C, Meola G, Novelli G

Session 3 Oral session *8.45 – 11.45*

RNA-BINDINGS PROTEINS

Session Chairs: Charles Thornton and Tom Cooper

8.45 028 **Role of PKC pathway in DM1 pathogenesis by regulating CUGBP1 phosphorylation and steady state levels**
NM Kuyumcu-Martinez and TA Cooper

9.00 029 **Signal transduction pathways regulating CUGBP1 RNA-activity in DM1 patients**
E Salisbury, K Sakai, B Schoser, H Nguen, M Gu, CH Huichalaf, L Wang, NA Timchenko, L Timchenko

9.15 030 **MBNL binds similar RNA structures in the CUG repeats of myotonic dystrophy and its pre-mRNA substrate cardiac troponin T**
MB Warf and JA Berglund

9.30 031 **The RNA binding specificity of Drosophila muscleblind**
E Goers and JA Berglund

9.45 032 **The overexpression of MBNL1 fetal isoform observed in myotonic dystrophy brain does not modified tau splicing**
C Dhaenens, S Schraen-Maschke, V Vingtdeux, H Tran, D Ghanem, O Leroy, J Delplanque, E Vanbrussel, A Delacourte, P Vermersch, CA Maurage, H Gruffat, A Sergeant, M Mahadevan, S Ishiura, L Buée, TA Cooper, M-L Caillet-Boudin, N Charlet-Berguerand, B Sablonnière, N Sergeant

10.00 033 **Biochemical analyses of MBNL1 complexes in myotonic dystrophy**
S Paul, W Dansithong, I Holt, JD Brook, MP Takahashi,GE Morris, L Comai and S Reddy

10.15 034 **Multiprotein complexes as targets for RNA CCUG repeats expanded in patients with DM2**
E Salisbury, B Schoser, C Schneider-Gold, G-L Wang, NA Timchenko, L Timchenko

Coffee break ***10.30 – 10.45***

10.45 035 **MBNL3 Down- Regulates MEF2 dependent Genes by Alternative Splicing of MEF2 Transcription Factor**
KS Lee, Y Cao, SJ Tapscott and EH Wang

11.00 036 **Structural determinants for the molecular properties of MBNL proteins**
Y Kino, Y Oma, H Onishi, N Sasagawa, N Nukina, S Ishiura

11.15 037 **Proteins that interact with MBNL1**
H Onishi, Y Kino, N Sasagawa, S Ishiura

11.30 038 **Interactions of Muscleblind Proteins with Splicing Target and Pathogenic RNAs**
Y Yuan, S Compton, K Sobczak, M Stenberg, C Thornton, J Griffith, M Swanson

Session 3 Poster session ***11.45 – 12.30***

RNA-BINDINGS PROTEINS
Session Chair: Maurice Swanson

P20 **Zebrafish knock-down model for muscleblind-like 2**
LE Machuca-Tzil and JD Brook

P21 **Functional studies of Muscleblind-like protein 1 (MBNL1)**
S Mittal and JD Brook

P22 **Characterization of proteins that bind to the CUG repeats**
J Marie and F-X Laurent

P23 **Colocalization of ribonuclear inclusions and MBNL1 foci with no impairment of in vitro adult DM1 and DM2 myoblasts differentiation**
R Cardani, E Mancinelli, E Bonifazi, A Botta, G Novelli, G Meola

P24 **A putative role of ribonuclear inclusions and MBNL1 in the impairment of gallbladder smooth muscle contractility with colelithiasis in myotonic dystrophy type 1.**
R Cardani, E Mancinelli, G Saino, L Bonavina, G Meola

P25 **Study on differential binding properties of MBNL1 isoforms and of CELF proteins on RNAs containing CUG repeats or potential splicing target sequences.**
N Marmier-Gourrier, A Vautrin, N Charlet, I Behm-Ansmant and C Branlant

P26 **Studies on the RNA recognition properties of MBNL1 and CUG-BP1**
A Vautrin, N Marmier-Gourrier, I Behm-Ansmant and C Branlant

P27 **RNAi-mediated silencing of Drosophila muscleblind**
JM Fernandez-Costa and RD Artero

P28 **The function and expression of K02H8.1 (CeMBNL), the oltholog of mammalian MBNLs**
N Sasagawa, E Ohno, Y Kino and S Ishiura

P29 **Imaging mRNAs involved in Myotonic Dystrophy type 1 (DM1) using Atomic force Microscopy (AFM)**
F Meullenet, S Allen, S Tendler and D Brook

P30 **Loss of Tau exon 2/3 inclusion in DM1 implies the carboxy-terminal tail of MBNL1**
H Tran, CM Dhaenens, D Ghanem, N Charlet, TA Cooper, A Andreadis, E Van Brussels, B Sablonnière, L Buée, ML Caillet-Boudin, S Shraen-Maschke and Nicolas Sergeant

Lunch — *12.30 – 13.30*

Session 4 Oral session — *13.30 – 15.45*

CHARACTERIZATION AND MANAGEMENT OF SYSTEMIC ASPECTS OF MYOTONIC DYSTROPHIES
Session Chairs: John Day and Bruno Eymard

13.30 039 **Neuropsychological and Adaptive Skills in Myotonic Dystrophy type 1- A Study on 57 Individuals with Congenital and Childhood Forms**
L Hakenäs-Plate, A-B Ekström, M Tulinius, E Wentz

13.45 040 **Autism Spectrum Disorders in Myotonic Dystrophy type 1-A Study on 57 Individuals with Congenital and Childhood Forms**
A-B Ekström, L Hakenäs-Plate, M Tulinius, E Wentz

14.00 041 **Cognitive deficits bridging ages: a behavioural phenotype in Myotonic dystrophy type 1?**
S Winblad, A-B Ekström, L Hakenäs-Plate, M Tulinius, E Wentz, C Lindberg

14.15 042 **Cognitive impairment in myotonic dystrophy type 1 (DM1): a longitudinal follow-up study**
A Modoni, C Marra, MG Vita, M Masciullo, PA Tonali, E Ricci, G Silvestri

14.30 043 **Cross-Sectional Analysis of CNS Imaging and Function in DM1 and DM2.**
JW Day, J Dalton, JR Wozniak, D Franc, L Hemmy, KO Lim, LPW Ranum

14.45 044 **DM2 is predicted by combined type 2 fibre centronucleation and atrophy**
G Bassez, E Chapoy, S Bastuji-Garin, H Radvanyi-Hoffman, F-J Authier, J-F Pellissier, B Eymard, RK Gherardi

15.00 045 **Myotonic dystrophy type 2 (DM2); a diagnostic alternative to fibromyalgia**
S Auvinen, Suominen T, Hannonen P, Bachinski L, Krahe R, Udd B

15.15 046 **Disproportionately high prevalence of co-segregating CLCN1 mutations among myotonic dystrophy type 2 patients from Finland and Germany**
T Suominen, B Schoser, O Raheem, S Auvinen, M Walter, R Krahe, H Lochmüller, W Kress and B Udd

15.30 047 **Mapping of muscular involvement of lower legs in myotonic dystrophy : a magnetic resonance study and correlation to clinical data**
C Côté, H Bassem, JM Janier, L Herbert and J Puymirat

Coffee break ***15.45 – 16.00***

Session 5 Oral session ***16.00 – 17.45***

CHARACTERIZATION AND MANAGEMENT OF SYSTEMIC ASPECTS OF MYOTONIC DYSTROPHIES
Session Chairs: Nakaaki Ohsawa and Bjarne Udd

16.00 048 **Pacemakers do not prevent sudden death in myotonic dystrophy type 1**
WJ Groh, MR Groh, RM Pascuzzi

16.15 049 **Cardiac Arrhythmias in Type I Myotonic Dystrophy patients with Sleep Apnoea An Implantable Monitoring Device Prospective Study**
K Wahbi, A Lazarus, B Eymard, C Meune, J Varin, P Laforêt, D Duboc

16.30 050 **Progression of muscle weakness and cardiac involvement in myotonic dystrophy type 1 (DM1) vs type 2 (DM2): a longitudinal study.**
V Sansone, S Briganti, M Panzeri, R Mauro, A Degrate, V Montericcio, L De Ambroggi and G Meola

16.45 051 **Cardiac Involvement in DM2 : A 30 Patients Follow-up Study**
K Wahbi, G Bassez, C Meune, P Laforêt, A Lazarus, H Radvanyi, B Eymard, D Duboc

17.00 053 **Gastrointestinal (GI) symptoms in myotonic dystrophy type 1 (DM1) patients enrolled in the NIH Registry**
J Hilbert, W Martens, A Parkhill, A Smirnow, R Moxley III & the Registry Scientific Committee

17.15 054 **Erectile dysfunction in myotonic dystrophy type 1 (DM1)**
A Clemenzi, AF Radicioni, E Bucci, P Latino, S Morino, M Garibaldi, A Di Pasquale, A Anzuini, A Lenzi, G Novelli, G Antonini

Gala dinner ***20.00***

September 15, Saturday

Session 4 Poster session ***8.00 – 9.00***

CHARACTERIZATION AND MANAGEMENT OF SYSTEMIC ASPECTS OF MYOTONIC DYSTROPHIES

Session Chair: Bjarne Udd

P31 **Investigation of dementia in a patient with Myotonic Dystrophy type 1: is it a DM1 associated phenomenon or is it Alzheimer's disease?**
C Lindberg and S Winblad

P32 **Cerebrospinal fluid tau and amyloid β42 protein in patients with myotonic dystrophy type 1 (DM1)**
S Winblad, JE Månsson, K Blennow, C Jensen, L Samuelsson, C Lindberg

P33 **Cognitive and personality profile in Myotonic Dystrophy type1 (DM1)**
A Sistiaga, I Urreta, M Jodar, AM Cobo, J Emparanza, J Poza, A Imaz, JF Martí-Masso y A López de Munain

P34 **Intellectual functioning in a large sample of adult and late-onset DM1 patients**
S Jean, L Richer, J Mathieu

P35 **Comprehensive evaluation of sleep-wake cycle and daytime somnolence in myotonic dystrophy type 1 (DM1)**
MB Panico, V Pisani, F Placidi, A Romigi, F Izzi, F Corte, MG Marciani, R Massa

P36 **Cognitive impairment and psychiatric disorders in the juvenile form of myotonic dystrophy**
M Douniol, O Lanthier-Gazzano, A Jacquette, A Afenjar, N Angeard, D Héron, M Plaza, D Cohen

P37 **Psychopathological and cognitive characteristics in DM1 and DM2**
A Palmieri, V Romeo, F Squarzanti, E Albertini, C Borsato, E Pegoraro, C Angelini

P38 **Theory of mind and cognitive disorders in myotonic dystrophy type 1 (DM-1): a preliminary study to understand non-compliance with home ventilation treatment in Steinert's population**
V Havet, P Allain , I Pénisson-Besnier, I Richard, N Meslier

P39 **The block of Ca-dependent K+channels reduces myotonia in Steinert disease: an in vivo pharmacological study**
C Chisari, R Licitra, B Rossi

P40 **Unbalance in Myotonic Dystrophy-1 may follow cervical ataxia and respond to exercise**
L Tesio, C Chessa, M Atanni

P41 **Quantitative evaluation of muscle degeneration in DM1 patients using MRI**
B Hiba, LJ Hébert, C Vial, J Saulnier, M Nejjari, JF Remec, C Coté, F Bouhour, J Puymirat and M Janier

P42 **Different muscle MRI features in myotonic dystrophy type 1 and 2**
C Borsato, V Romeo, E Albertini, C D'Ascenzo, F Squarzanti, A Palmieri, V Beltrame, R Dal Borgo, R Stramare, M Fanin, E Pegoraro, C Angelini

P43 **Myotonic Dystrophies type 1 and type 2: MRI and SPECT comparative study**
V Romeo, P Zucchetta, C Ferrati, L Antunovic, R Manara, E Pegoraro, C Angelini

P44 **Myotonic dystrophy type 2: clinical, neurophysiological and muscular features of a family with short CCTG expansion**
S Lucchiari, S Corti, S Pagliarani, M Servida, E Fruguglietti, M Moggio, N Bresolin, GP Comi

P45 **Weakness and fatigue more than myotonia affect physical and mental perception of quality of life in patients with myotonic dystrophies**
M Panzeri, V Sansone, S Gandossini, INQoL Group, MR Rose and G Meola

P46 **Muscle pathology in myotonic dystrophies – an ultrastructural study**
A Nadaj-Pakleza, A Lusakowska, E Szmidt-Salkowska, A Sulek, W Krysa, M Rajkiewicz, A Kaminska

Session 5 Poster session *9.00 – 9.45*

CHARACTERIZATION AND MANAGEMENT OF SYSTEMIC ASPECTS OF MYOTONIC DYSTROPHIES

Session Chair: Denis Duboc

P47 **ECG-Holter monitoring is a valuable tool to screen Myotonic Dystrophy type 1 (DM1) patients for advanced conduction defects**
E Bucci, C Balla, F Marrara, D Santini, A Clemenzi, P Latino, S Morino, M Testa, G Antonini

P48 **Different phenotypic expression and CTG repeat expansion size in myotonic dystrophy type 1 patients**
S Contardi, F Pizza, P Avoni, R Liguori

P49 **RAMYD (Risk of Arrhythmias in MYotonic Dystrophy) study: the design**
M Pace, A Dello Russo, M Casella, F Mangiola, A Modoni, G Silvestri, E Ricci, G Nigro, L Politano, MG Bongiorni, P Melacini, P Della Bella, F Bellocci

P50 **RAMYD study: baseline cardiological characteristics.**
M Pace, A Dello Russo, M Casella, F Mangiola, M Vaccarella, A Modoni, G Silvestri, E Ricci, R Vannicelli, G Nigro, L Politano, MG Bongiorni, P Melacini, P Della Bella, F Bellocci

P51 **RAMYD study preliminary results: electrophysiological study and devices implant**
M Pace, A Dello Russo, M Casella, G Nigro, MG Bongiorni, P Melacini, P Della Bella, F Bellocci

P52 **Comparison between electroanatomic mapping vs cardiac magnetic resonance imaging in myocardial substrate study in Myotonic Dystrophy type 1**
C Bisceglia, M Pace, M Casella, A Dello Russo, R Biddau, M Vaccarella, G Pelargonio, F Mangiola, F Bellocci

P53 **Assessment of noninvasive ventilation in myotonic dystrophy type 1**
MA Hamon, N Meslier, F Dubas, JL Racineux, I Pénisson-Besnier

P54 **Ventilatory function in patients with myotonic dystrophy type 1.**
MG Di Gregorio, M Scutifero, F Spina, R Russo, A Palladino, G Fiorentino and L Politano

P54B **Artificial ventilatory management in myotonic dystrophy at a Japanese hospital for chronic neuromuscular disorders**
S Kon, Y Oyama, H Takada

P55 **Is heart rate variability a prognostic indicator in patients with dystrophia myotonica type 1**
A Palladino, M Scutifero, VM Ventriglia, P Sannino, MG Di Gregorio, R Russo, G Nigro, L Passamano, VR Petretta, V Cozza, E Bonifazi, G Novelli, G Nigro and L Politano

P56 **A cross-sectional study for glucose intolerance of myotonic dystrophy**
T Matsumura, H Iwahashi, MP Takahashi, T Saito, H Fujimura, S Shinno

P57 **Intracellular insulin mediated signalling in myotonic distrophy type 1 (DM1)**
P Latino, P Castri, E Bucci, A Clemenzi, S Morino, A Di Pasquale, A Fornasiero, M Garibaldi, L Iacovelli, F Orzi, G Antonini

P58 **Multidisciplinary study in patients with myotonic dystrophy type 1**
N Olivero, T Mongini, L Vercelli, G Gai, A Mattei, F Conrotto, A Cicolin, A Farri, L Palmucci

P59 **Abnormal ß-cell function in myotonic dystrophy type-1**
F Bouhour, A Brac de la Perrière, H Bassem, C Vial, M Janier, J Puymirat

P60 **Insulin resistance in patients with myotonic dystrophy type 1**
V Rakocevic-Stojanovic, S Popovic, S Peric, A Nikolic, I Basta, Z Stevic, Z Tasic, D Lavrnic

P61 **Advanced oxidation protein products in serum of patients with myotonic disease type 1: correlation with extra-muscular phenotype**
M Falorni, L Volpi, M Mancuso, A Rocchi, G Malvaldi, A Pompella, A Paolicchi , G Siciliano

P62 **Intraocular pressure and central corneal thickness study in patients with Steinert myotonic dystrophy**
N Rosa, M Lanza, C Irregolare, A Palladino, L Passamano, F Spina, MR Cecio, L Politano

P63 **A case of patient with coexistent Thomsen's disease and benign hyperbilirubinemia (e.g. Gilbert syndrome).**
HS Muradyan and SG Khachatryan

Session 6 Oral session ***9.45 – 12.00***

SPECIAL ASPECTS OF MANAGEMENT
Session Chairs: Laura Ranum and Giovanni Meola

9.45 055 **Protocol development for Preimplantation Genetic Diagnosis (PGD) of Myotonic Dystrophy Type 1 (DM1) in the UK: experience from 25 cycles**
Kakourou G, Dhanjal S, Mamas T, Doshi A, Gotts S, Serhal P, Ranieri DM, Delhanty JDA, Harper JC, SenGupta SB

10.00 056 **Congenital myotonic dystrophy: Canadian incidence and cohort study.**
C Campbell, SL Venance, P Jacob, V Siu

10.15 057 **Presymptomatic testing in Myotonic Dystrophy type I - 6-year experience for 131 candidates**
A Jacquette, C Colas, M Gargiulo, P Laforêt, B Eymard, J Feingold, H Radvanyi, D Héron

Coffee break ***10.30 – 10.45***

10.45 058 **It's genetic, but what does that mean?**
C Downing

11.00 059 **Review of children diagnosed with myotonic dystrophy over 30 years**
J Fenton-May, C Sampson, MT Rogers

11.15 060 **Neuropsychological Profile in the childhood form of DM1**
N Angeard, M Gargiulo, A Jacquette, H Radvanyi, B Eymard and D Héron

11.30 061 **The Saguenay Myotonic Dystrophy Integrated Care Pathway: Development and preliminary validation**
C Gagnon, M-C Chouinard, S Jean, J Mathieu

11.45 062 **Myotonic dystrophy type 2 in Japan: distinct ancestral origin from Caucasian families**
Y Amakusa, T Matsuura, T Saito, T Kimura, O Yahara, H Aizawa, Y Ikeda, JW Day, LPW Ranum, K Ohno

Session 6 Poster session ***12.00 – 12.45***

SPECIAL ASPECTS OF MANAGEMENT
Session Chairs: Giuseppe Novelli and Ralf Krahe

P64 **A care-card for myotonic dystrophies: improving management and follow-up**
V Sansone, R Mauro, M Panzeri and G Meola

P65 **From initial symptoms to genetic confirmation: what is the time-lag for myotonic dystrophies in Italy?**
V Sansone, R Mauro, M Panzeri and G Meola

P66 **Determinants of genetic knowledge in patients with myotonic dystrophy type 1 (DM1)**
L Laberge, M Perron, J Mathieu, J Auclair, M Gaudreault, S Veillette

P67 **Myotonic dystrophy unlinked to DM1 and DM2 mutations in three siblings**
V Pisani, A Botta, E Bonifazi, MB Panico, C Rocchi, GA Marfia, F Sangiuolo, G Bernardi, G Novelli, R Massa

P68 **Self-reported health problems and health habits in myotonic dystrophy type 1: a patient-oriented perspective**
MC Chouinard, C Gagnon, L Laberge, S Jean, J Mathieu

P69 **What do patients with Myotonic Dystrophy Type 1 know about their disorder: the first study in Bashkortostan (Russia)**
L Akhmadeeva, H Derevyanko, R Magzhanov

P70 **Development of Orofacial Dysfunction in Young Individuals with Myotonic Dystrophy type 1**
L Sjögreen, M Engvall, A-B Ekström, S Kiliaridis, M Tulinius, A Lohmander

P71 **Molecular analysis of a family co-segregating myotonic dystrophy type 1 and Charcot-Marie-Tooth disease**
C Braida, F Spaans, CG Faber, HJM Smeets, P Hofman, CEM de Die-Smulders and DG Monckton

P72 **An improved method for Southern DNA and Northern RNA blotting using a Mupid®-2 Mini-Gel electrophoresis unit for diagnosis of DM1 and 2**
H Furuya, T Yamada, K Ikezoe, T Arahata, Y Fukumaki, N Fujii

P73 **Myasthenic phenotype as possible manifestation of myotonic dystrophy**
V Milic Rasic, J Mladenovic, R Dimitirijevic, V Dobricic, S Romac

P74 **Clinical and Neuroimaging features of Myotonic Dystrophy in childhood**
K Gorni, S Orcesi, C Uggetti, E Fazzi, A Berardinelli

Lunch ***12.45 – 13.45***

Session 7 Oral session ***13.45 – 16.00***

THERAPEUTIC TRIALS AND FUTURE ADVANCES
Session Chairs: Dick Moxley and Giovanni Meola

13.45 063 **Correlation between Measures of Muscle Mass, Strength, Function and Quality of Life (QOL) in Patients with Myotonic Dystrophy Type 1 (DM-1): Implications for Clinical Trials.**
S Panda, N Dilek, B Martens, C Quinn, M McDermott, C Heatwole, C Thornton, R Moxley III

14.00 064 **Cardiac Safety and Efficacy of Mexiletine in Myotonic Dystrophy Type 1 (DM1)**
R Moxley III, E Logigian, M McDermott, W Martens, S Pandya, A Wiegner, C Thornton, R Tawil, R Moxley IV, C Barbieri, C Annis, N Dilek

14.15 065 **Effects of Mexiletine on myotonia, muscle strength, and cardiac parameters in myotonic dystrophies over time**
V Sansone, S Briganti, M Panzeri, R Mauro, A Degrate, L De Ambroggi and G Meola

14.30 066 **Safety and Tolerability of Recombinant Human Insulin-Like Growth Factor 1 Complexed with IGF Binding Protein 3 (rhIGF1/IGFBP3) in Myotonic Dystrophy Type 1 (DM1)**
C Heatwole, W Martens, C Quinn, J Hilbert, S Pandya, C Jackson, C Thornton, M McDermott, R Moxley

14.45 067 **Development of RNA-based therapeutic model system for myotonic dystrophy (DM)**
RS Yadava, EG Ames, V Srinivasan and MS Mahadevan

15.00 068 **Oligonucleotide-mediated silencing of expanded DMPK transcripts in a DM1 myoblast-myotube cell model**
SAM Mulders, WJAA van den Broek, G Gourdon, Bé Wieringa and DG Wansink

15.15 069 **Correction of ClC-1 splicing eliminates the myotonia in mouse models of myotonic dystrophy**
TM Wheeler, JD Lueck, MS Swanson, RT Dirksen and CA Thornton

15.30 070 **Biochemical screening methods to find inhibitors of RNA/protein interaction: CUGexp/MBNL1 example**
K Sobczak and CA Thornton

16.00 071 **Antisense RNA-based gene therapy reverses muscle atrophy in a mouse model of myotonic dystrophy type 1**
J Puymirat, G Doucet, A Vignaud, A Huguet , D Furling, G Gourdon, A Ferry

Session 7 Poster session *16.00 – 16.15*

THERAPEUTIC TRIALS AND FUTURE ADVANCES

Session Chair: Benedikt Schoser

P75 **Reduced oxidative stress markers after cysteine donor enriched dietary intake in patients with myotonic dystrophy type 1**
L Volpi, M Falorni, C Carlesi, G Ricci, M Mancuso, L Petrozzi, M Franzini, A Paolicchi, G Siciliano

P76 **Dehydroepiandrosterone in myotonic dystrophy type 1**
I Pénisson-Besnier, M Devillers, R Porcher, D Orlikowski, V Doppler, C Desnuelle, X Ferrer, MC Arne Bes, F Bouhour, C Tranchant, E Lagrange, A Vershueren, D Uzenot, C Vial, A Labarre Vila, J Pouget, B Eymard, D Annane

P77 **Abnormal glucose metabolic disorder in myotonic dystrophy type 1 (DM1): hyperinsulinemia in DM1 were inhibited using voglibose**
M Kinoshita, M Shigeta, K Hirose

P78 **Characterization of MBNL1 RNA ligands and search for molecules disrupting the (CUG)n-MBNL1 interaction**
L Guigou, P Villa and N Charlet

P79 **High-throughput screen of chemical compounds to identify candidates that relieve the nuclear retention of CUG-rich mRNA**
E Querido and P Chartrand

P80 **Non Invasive Assessment of Mouse Muscle Volume Using 3D µ-Echography**
M Nejjari, M Janier, G Gourdon, J Puymirat and B Hiba

Coffee break ***16.15 – 16.30***

Session 8 INTERACTIVE SESSION AND HIGHLIGHTS

Interactive session: Special meeting with advocacy groups, patients and families 16.30 – 18.30

- **Representative advocacy groups**
  - Shannon M. Lord
    Hunter Research Fund (USA)
    How the Myotonic Dystrophy Foundation started

  - Margaret Bowler
    Myotonic Dystrophy Support Group (UK)
    Encouragement to look after our own heath needs day by day (patients and carers)

  - Claude Bourlier
    French Myotonic Dystrophy Support Group (France)
    Living with a DM1, patient point of view

  - Toba Balaban
    DM2 Support/Advocacy Group (International)
    Myotonic Dystrophy Toronto Regional Support Group (Canada)
    The DM2 Experience: Web-based Patient Support

  - Caterina Campanelli
    Associazione Italiana contro le Miopatie Rare – AIM (Italy)
    Migliorare la conoscenza dei bisogni fondamentali delle famiglie colpite da miopatie per fornire loro risposte più efficaci

- **Questions from patients group to investigators and physicians**
- **Myotonic Dystrophy Foundation Excellence in Research Awards**
  - *John Brekka*
- **Young Investigator's Poster Award**
  - *Representative AIM (Associazione Italiana di Miologia)*
- **Guidelines and standards of care for DM**
  Dick Moxleyand Chairman

Highlight: Chris Pearson, Ralf Krahe, Bruno Eymard, Dick Moxley ***18.30 – 19.30***

Concluding Remarks ***19.30***


IDMC-6
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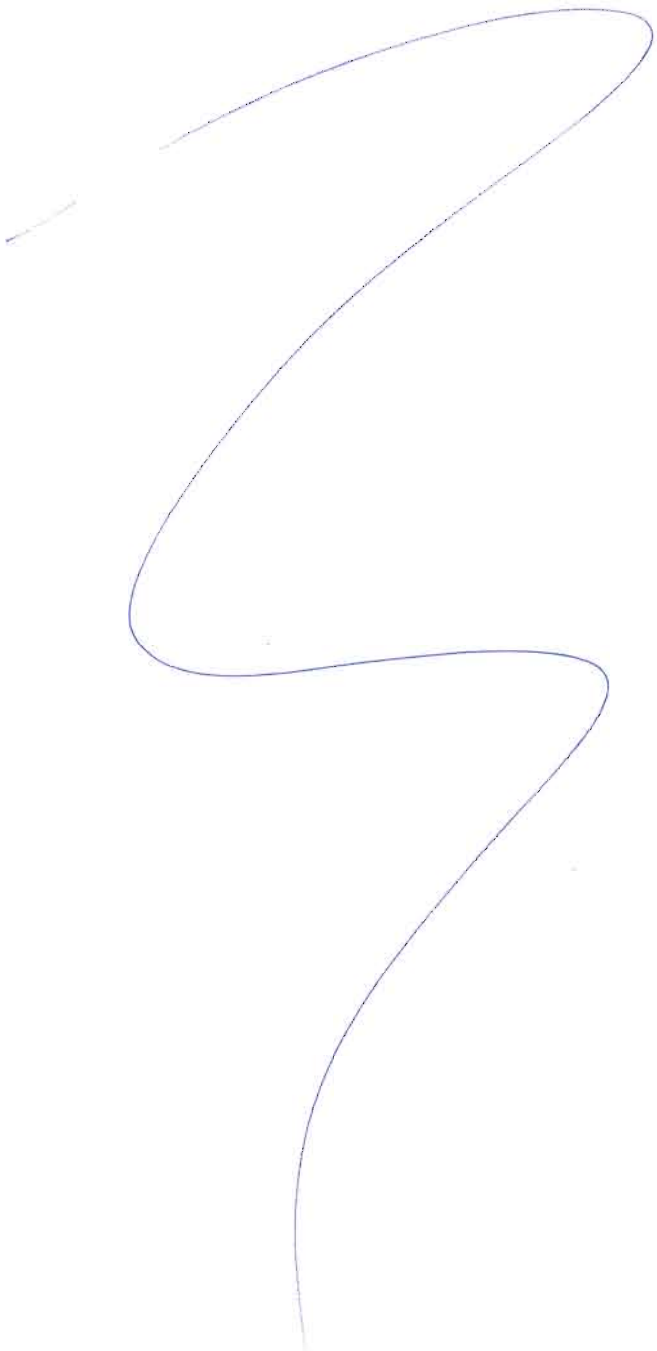
D elight to welcome in
M ilan as the perfect host for the
C onsortium meeting



ORAL COMMUNICATIONS

001
BIDIRECTIONAL TRANSCRIPTS AT THE DM1 LOCUS AND OTHER TRIPLET REPEAT DISEASES: IMPLICATIONS FOR DISEASE MECHANISMS

S.J. Tapscott, G.N. Filippova, S. Mahoney, P. Ladd, D.H. Cho

Division of Human Biology, Fred Hutchinson Cancer Research Center, Seattle, WA USA

Triplet repeats in the genome are associated with numerous human diseases. In an effort to characterize possible functional roles of triplet repeat sequences, we have identified an insulator element incorporating the CTG repeat at the DM1 locus. Bidirectional transcription through this region is associated with small RNA fragments, consistent with processing of double stranded RNA. In addition, the nucleosome associated with the repeat has heterochromatin associated modifications, H3K9 methylation and HP1 recruitment. Therefore, the nucleosome on the wild-type repeat has modifications normally associated with heterochromatin. Expansion of the repeat increases the region of heterochromatin and results in spreading of the heterochromatin beyond the CTG repeat. We are now extending our studies to other repeat loci and developing a general model of triplet repeats in the genome.

002
SATURATION EFFECTS OF BINDING OF MIR-1 AND MIR-206 TO THE 3' UTR TARGET SITE IN DMPK MRNA: YET ANOTHER EXPLANATION FOR PATHOPHYSIOLOGICAL FINDINGS IN CELL AND ANIMAL MODELS FOR MYOTONIC DYSTROPHY TYPE 1 (DM1)?

W.J.A.A. van den Broek, D.G. Wansink and B. Wieringa

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

Currently the best explanation for the disease symptoms in DM1 is an RNA gain-of-function effect, mainly involving misregulation of alternative splicing of certain RNAs. Still, it is not well resolved how in patients with DM1 the somatic expansion of the DMPK locus and the ensuing production of misspliced gene products, affect muscle and brain and are coupled to muscle wasting and neurodegeneration. Also there is sometimes an apparent discrepancy between expanded DMPK mRNA-repeat dosage and manifestation of DM1-like pathogenesis in animal models. This raises the question whether additional factors could be involved in modulating the pathogenic effects at the RNA level.

We examined the role of microRNAs (miRNAs), small conserved RNA molecules of ~20-22 nucleotides that are widespread regulators of gene expression and known to play a fundamental role in development and differentiation. Particularly interesting reciprocal relationships have been revealed for myogenic factors and miRNAs that belong to the miR-1 and miR-133 families in muscle and heart development and in response to functional overload. Even alternative splicing of splicing factors during muscle development is under the influence of miRNAs.

We report that miR-1 and miR-206 are predicted to form imperfectly base-paired duplexes with an evolutionary conserved "seed sequence" or microRNA-responsive element (MRE) in the 3' UTR segment of the Myotonic Dystrophy Protein Kinase mRNA. Using luciferase and GFP-based reporter cell models, we show that overexpression of both miRNAs compromised translation of reporter constructs with the 3' UTR. Reciprocally, preliminary data indicate that expression-oversaturation with the seed element in the 3' UTR of the DMPK mRNA in a transgenic animal model for DM1[1] may perturb normal miRNA control over known targets in the myogenic differentiation network. Our findings may help to explain pathophysiological findings in some of the currently existing cell and animal model for DM1, and improve our understanding of the complex role of RNA pathways in the pathophysiology of DM1.

Reference

1. O'Cochlain *et al.* (2004) *Hum. Mol. Genet.* 13, 2505-2518.

003
MODELLING CUG EXPANSION TOXICITY IN DROSOPHILA: WHAT ROLE FOR EXPANSION CONTEXT?

G.L. Mée, N. Ezzeddine and O. Aït-Ahmed

Institut de Génétique Humaine, UPR 1142 CNRS; France

Evidence for an RNA gain of function toxicity has now been provided for an increasing number of human pathologies including DM1. However the mechanisms that underlie the RNA pathogenicity seem to be more complex than expected and are still controversial.

We report here the modelling of the DM1 associated CUG expansion toxicity in *Drosophila*. Transgenic flies that express inducible repeats of various type (CUG or CAG) and length (16, 240, 480 repeats) were generated. Surprisingly, cytotoxicity as assessed by examining viability and eye neurodegeneration was observed in a single line $(CTG)_{240.4}$. Therefore repeat size is not the primary determinant in the toxicity of the expanded RNAs. Moreover quantitative RT-PCR analyses ruled out RNA level as the key factor for the toxicity.

These data prompted us to perform a systematic analysis of the insertion context in all the transgenic lines. We found that the $(CTG)_{240.4}$ transgene lies within an intron of a gene that encodes a zinc finger protein (ZNF). Surprisingly *ZNF* exonic sequences colocalized with the $(CUG)_{240}$ expansion in nuclear inclusions. We provide evidence that the $(CUG)_{240}$ induced phenotype could be accounted for neither by a partial loss of function nor by a simple overexpression of the *ZNF* endogenous gene. How the mutant RNA contributes to the phenotype of the

$(CTG)_{240.4}$ line is obviously the issue raised by this work. We believe that a confrontation of data provided by different contributors on different models were they controversial, is an absolute requirement in the objective of gaining insight into the molecular mechanisms of such a complex pathology. It is with this specific aim that we wish to present our paper and discuss our data.

004
MODELLING CONGENITAL MYOTONIC DYSTROPHY

V. Srinivasan, Q. Yu and M. Mahadevan

Department of Pathology, University of Virginia, Charlottesville, Virginia, USA

Myotonic dystrophy type I (DM1) is caused by an expanded CTG repeat in the 3'UTR of the DMPK gene which generates a toxic RNA implicated in the pathology of the disease. The congenital form is characterized by increased neonatal mortality (25%) and has been shown to be inherited exclusively from the mother. We have generated a tetracycline-inducible transgenic mice over expressing the toxic DMPK 3' UTR mRNA. These mice display major clinical features of adult DM1. We are trying to model congenital DM1 using these mice. We conducted combinations of matings between transgenic mice and wild type FVB strain mice. The matings were designed such that we could vary the dosage of the transgene inherited by the offspring, by changing the parental genotype, for studying the parent of origin effect on the offspring. We administered doxycycline to the pregnant mother on the 10th day to turn on the transgene in the embryo. The different genotypes of offspring as a result of the breedings were either homozygotes or heterozygotes born to affected or wild type mothers. Mortality, before three weeks postnatally was (> 80 %) among homozygous offspring with severe growth retardation in survivors. In heterozygous offspring born to affected mothers mortality was variable (5-75%) with some growth retardation. There was no mortality in heterozygous offspring born to wild type mothers. The above results indicate that it could be a parent of origin effect. An alternate conclusion is that it could be a gene dosage effect or a combination of both. In order to address this question we are conducting further experiments. Our experiments are also directed to find abnormal gene expression in the affected mice versus the wild type mice.

005
CTG REPEAT "BIG JUMPS" IN TRANSGENIC MICE

L. Foiry, M. Gomes-Pereira, A. Nicole, S. Tomé, C. Junien, A. Munnich and G. Gourdon.

INSERM U781, Hôpital Necker-Enfants Malades, Paris, France.

Since the first identification of a trinucleotide repeat expansion as the genetic basis of a human disease, many studies have focused on the mechanisms underlying trinucleotide repeat instability. The use of various genetically and biochemically defined systems has provided valuable insight into the factors affecting the instability of repetitive DNA sequences. Transgenic mice have been generated to provide mammalian model systems for the assessment of repeat biology *in vivo* and to study repeat instability in the germline or during somatic development. Investigators first attempted to introduce disease gene cDNAs containing expanded triplet repeats into the mouse genome. Despite repeat lengths being in the range known to display instability in humans, the expanded tracts were stable in mice. At that time, these results raised the question of the adequacy of mice to model trinucleotide repeat instability. In the second generation of mouse models, intergenerational and somatic instability was observed using longer CAG/CTG tracts, or moderately sized expansions within their native genomic DNA context. These encouraging results demonstrated that trinucleotide repeat dynamics could be recreated in mice, opening new avenues towards the understanding of the molecular mechanisms of repeat length mutations in mammalian cells. However, in all mouse models, the magnitude of intergenerational expansions remained much smaller than that detected in patients ("big jumps" of several hundred of CTG in DM1 for example) raising once more questions on the difference between the metabolism of trinucleotide repeats in mice and human. The observation of expansion big jumps in our transgenic mice has provided further information to address, at least in part, this question.

006
CUG LENGTH-DEPENDENT, BRAIN REGION - AND MUSCLE TYPE-SPECIFIC SPLICING ABNORMALITIES IN DM1 TRANSGENIC MICE

M. Gomes-Pereira,[1] A. Huguet,[1] A. Nicole,[1] J. Acquaire,[1] A. Munnich,[1] G. Gourdon[1]

[1]*Inserm U781, Hôpital Necker Enfants Malades, Paris, France*

We have previously generated DM300 transgenic mice to investigate the molecular mechanisms of trinucleotide repeat expansion and the molecular pathogenesis of DM1. The DM300 mice were created by random insertion of a large fragment of genomic DNA derived from the human *DM1* locus, containing a highly unstable 300-CTG expansion within the *DMPK* gene. The DM300 mice recreate important aspects of the disease pathogenesis, including nuclear RNA foci accumulation in multiple tissues, muscle abnormalities (such as myotonia and myopathy) and misdistribution of tau protein isoforms in brain. Large intergenerational repeat expansions (>200 CTG in one single transmission) have been recently reported in our mice, resulting in very large trinucleotide sequences associated with a strong phenotype. These mice show obvious splicing abnormalities in the skeletal muscle and the central nervous system. Interestingly, misplicing events appear to be muscle- and brain region-specific and seem more pronounced in mice carrying longer repeats. Successive intergenerational CTG "big jumps" have provided us with mice carrying trinucleotide repeat sizes ranging from 600 up to >1250 CTG, offering us the unique opportunity to assess the consequences of increasing CTG repeat sizes on *DMPK* expression and the sensitivity of multiple mispliced candidate genes to toxic CUG repeats. In addition, we are currently investi-

gating the relationship between key splicing factors and abnormal RNA metabolism detected in our model. Transgenic mice carrying very large CTG repeat expansions provide a useful tool to dissect the molecular pathogenesis of DM1 and to assess novel therapeutic schemes.

O07
INSIGHTS FROM AN INDUCIBLE/REVERSIBLE MOUSE MODEL OF RNA TOXICITY IN DM1

M.S. Mahadevan,[1] R. S. Yadava,[1] V. Srinivasan,[1] Q. Yu, [1]C. Frenzel-McCardell,[1] J. Puymirat,[2] C.A. Thornton,[3] A.L. Tucker,[4] O.W. Prall,[5] R.P. Harvey[5]

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A prevailing idea in the DM field is that DM is the first example of a disease caused by a toxic RNA molecule. To study this idea, we recently developed the first inducible transgenic mouse model of RNA toxicity for DM type 1 (DM1). In this model, we have demonstrated that expression of the DMPK 3'UTR mRNA results in key features of adult DM1such as myotonia, cardiac conduction defects, RNA splicing defects and histopathology and that silencing toxic RNA expression reverses many of these effects. Using this model we have begun to focus on other aspects of RNA toxicity beyond RNA splicing defects. Also, this is the first model of RNA toxicity resulting in cardiac phenotypes and as such we have focused much of our efforts on understanding the molecular basis of RNA toxicity in the heart. This has provided us with new insights into RNA toxicity. The results of ongoing experiments looking at molecular mechanisms of RNA toxicity will be presented with an emphasis on the effects of the toxic DMPK 3'UTR mRNA on the heart.

O09
SEVERE SKELETAL MUSCLE WASTING IN A TISSUE-SPECIFIC, INDUCIBLE MOUSE MODEL FOR MYOTONIC DYSTROPHY

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Although many symptoms in DM1, such as myotonia and insulin resistance, are explained by particular misregulated splicing events, the mechanism by which skeletal muscle degeneration arises remains unknown. In order to study the onset and progressive nature of skeletal muscle pathology our laboratory has created two transgenic mouse lines, expressing either 960 CUG repeats (EpA960) or no repeats (EpA0) within exon 15 of the *DMPK* gene in an inducible and skeletal muscle-specific manner. EpA lines express exon 15 of the *DMPK* gene with or without CUG repeats, when a floxed concatemer of three polyadenylation sites is removed by a Cre-mediated recombination event. Inducibility and tissue specificity is achieved by crossing EpA lines with mice expressing a skeletal muscle-specific modified Cre protein, which becomes activated in the presence of tamoxifen. EpA960/HSA-Cre bitransgenic mice express large amounts of DMPK-CUG960 RNA from the recombined allele post tamoxifen administration. Seventy percent of animals exhibit severe and progressive muscle wasting as assessed by muscle function tests, gross appearance and MRI. Histological abnormalities including increased central nuclei, variation in fiber size and atrophy of muscle fibers are seen in bitransgenic mice post tamoxifen. EMG studies indicate bitransgenic mice produce waxing and waning myotonic runs, characteristic of DM. Muscle tissue contains intranuclear RNA foci which colocalize with Mbnl. EpA960/HSA-Cre mice exhibit elevated levels of CUGBP1 and CUGBP2 proteins and have misregulated alternative splicing of pre-mRNAs as seen in individuals with DM1. With this model we are investigating pathogenic mechanisms involved in skeletal muscle wasting.

O10
SOMATIC MOSAICISM AND GENOTYPE-PHENOTYPE CORRELATIONS IN DM1

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Somatic mosaicism in DM1 is age-dependent, tissue-specific and expansion-biased, features that very likely contribute toward the tissue-specificity and progressive nature of the symptoms. We showed previously that logarithmic transformation of the estimated inherited allele length accounts for about 70% of the variation in age of onset, establishing that the progenitor allele is the major modifier of the age of onset in DM1. In order to better understand the dynamics of somatic variation and its contribution toward the age of onset, we used small-pool PCR to perform a detailed quantitative analysis of the degree of somatic variation in over 140 DM1 patients, generating a database of over 30,000 *de novo* variant alleles. These analyses revealed that patients carrying 50-100 repeats tend to have a relatively low degree of somatic variation in blood with a characteristically highly skewed distribution in which most alleles have only accumulated small changes. In contrast, in patients carrying more than 150 repeats, the allele length increases in nearly all cells in a more synchronised pattern. These data thus confirm that the mutation rate is highly dependent on the progenitor allele length. The second major modifier of the degree of somatic mosaicism observed is age at sampling, which acts synergistically with progenitor allele length to account for ~70% of the variation in somatic mosaicism. Most interestingly, these analyses revealed strong evidence for the contribution of variation in somatic mosaicism with variation in the age of onset *i.e.* earlier than expected age of onset in patients in whom the repeat expands more rapidly.

O11
CTCF BINDING IN CIS REGULATES CAG/CTG REPEAT INSTABILITY

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Since the discovery of disease-associated CAG instability, cis-acting sequence elements, genomic context, and epigenetic modifications have been thought to contribute to instability. Drastically different levels of repeat instability at different disease loci with identical repeats (spinobulbar muscular atrophy (SBMA) vs. spinocerebellar ataxia type 7 (SCA7) and myotonic dystrophy (DM1)) strongly supports the existence of cis-acting DNA elements that promote instability. Similarly, the distinct patterns of repeat instability between tissues of the same patient argue for tissue-specific epigenetic or *trans*-factor regulation. However the mechanistic basis of this is yet to be described. Binding sites for CTCF proteins are near unstable repeat tracts at many disease loci including SCA7 and DM1. Using a SCA7 mouse model with (CAG)92, we tested the role of the 3'-CTCF binding site by assessing germline and somatic repeat instability in mice with either a wild-type or mutant CTCF binding site, respectively able or unable to bind CTCF protein. Transmitted and somatic instability were significantly enhanced in mice with mutant sites. CpG methylation, ablating CTCF binding, also enhanced instability. Our results implicate this CTCF binding site as a cis-element regulating CAG/CTG instability, and indicate that CpG methylation is an epigenetic regulator of this element. Understanding how CTCF contributes to instability and pathogenesis is critical, as many repeat-disease loci including DM1 have CTCF sites proximal to the repeats.

O12
CNS RNA GAIN-OF-FUNCTION EFFECTS INDUCED BY CUG AND CCUG TRANSCRIPTS

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To understand the effects of CCUG and CUG expansion in the CNS we generated transgenic mouse models of DM2 (TRE-CCTG:ZNF9-tTA) and SCA8 (SCA8 CTG BAC-EXP) in which expression of expansion transcripts is driven by the endogenous human promoters. Consistent with RNA gain of function effects RNA foci are found in human and mouse DM2 and SCA8 brain. We previously reported a loss of cerebellar GABAergic inhibition in our SCA8 mice. Further analysis of *GABA-A transporter 4 (Gabt4)* was performed because it was identified as a CUGBP1 target by crosslinking/immunoprecipitation (CLIP) and because upregulation of *Gabt4* may reduce synaptic GABA and explain the loss of inhibition in our mice. *Gabt4* is significantly upregulated at the RNA ($p<0.001$) and protein ($p<0.001$) levels in both SCA8 BAC-Exp$^{+/-}$ and Mbnl1$^{\Delta E3/\Delta E3}$ animals with a similar trend in human SCA8 brain. Similarly, *Gabt4* RNA levels are increased in DM2 mouse brain ($p<0.001$) with preliminary data showing a trend toward increased protein in DM2 (human and mouse). Upregulation of endogenous *GAT4* in human SKNSH cells is triggered by overexpression of either CUG expansion transcripts ($p<0.001$) or CUGBP1. Additionally, increases in *GAT4* caused by the CUG expansion transcripts can be rescued by Mbnl1 overexpression, which changes alternative splicing and introduces a premature stop codon predicted to lead to nonsense-mediated decay.

Gabt4 is a novel gene dysregulated by CUG and CCUG transcripts. These data demonstrate that dysregulation of MBNL1/CELF pathways, which play a prominent role in DM skeletal muscle pathogenesis also contribute to the CNS changes found in DM2 and SCA8.

O13
PRE-MUTATION ALLELE POOL IN DM2

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DM1 and DM2 are caused by microsatellite expansions. For DM1 there is a reservoir of pre-mutation alleles. There have been no reports of pre-mutation alleles for DM2, and the minimum pathogenic size is unknown. The $(CCTG)_{DM2}$ is part of a complex motif: $(TG)_{12\text{-}26}(TCTG)_{7\text{-}12}(CCTG)_{3\text{-}9}(G/TCTG)_{0\text{-}4}(CCTG)_{4\text{-}15}$. DM2 expansions are as large as 40 kb with an uninterrupted $(CCTG)_n$; the smallest reported DM2 expansion is $(CCTG)_{75}$. To address questions of pre-mutation alleles in the population, the smallest pathogenic size, and the possible role of the DM2 repeat as a modifier in other neuromuscular diseases (NMD), we cloned and sequenced unusually large but still amplifiable alleles (n=12), along with more typical ones from both normal and disease populations (n=20). We identified 2 classes of large alleles: one, more common in African Americans, had multiple (CCTG) tracks, separated by up to 4 interruptions with a total of $(CCTG)_{32}$; the other had uninterrupted $(CCTG)_{22\text{-}33}$. We also identified 2 DM2 patients with amplifiable expansions in PBL of 380 bp and ~500 bp with $(CCTG)_{55}$ and $(CCTG)_{100}$, respectively. Instability of these uninterrupted alleles was confirmed by small-pool PCR; they were significantly more unstable than normal controls. We conclude that the $(CCTG)_{22\text{-}33}$ alleles represent a pre-mutation pool. The minimum pathogenic size can be $(CCTG)_{55}$, at least in PBL. Because 2 "pre-mutation" alleles were identified in a clinical population with non-DM NMD diagnosis, these alleles may have phenotypic consequences, either alone or as modifiers.

O14
A TRANSGENIC MOUSE MODEL FOR MYOTONIC DYSTROPHY TYPE 2 (DM2)

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DM is caused by unstable microsatellite expansions -- in DM1 a (CTG)n expansion in the 3' UTR of *DMPK* in chromosome 19q13.3, and in DM2 a (CCTG)n expansion in intron 1 of *ZNF9* in 3q21.3. The prevailing paradigm is that DM is a toxic RNA disease, mediated by the mutant expansion of normally polymorphic (CTG)n-like repeats. Transcription of these repeats is both necessary and sufficient to cause disease. Mutant RNAs accumulate in foci and interfere with RNA splicing, transcription and/or translation of "effector" genes. In spite of similar mutations, DM1 and DM2 are clinically distinct diseases. The underlying molecular basis for the differences is as yet unknown. To date, there are no mouse models for DM2. Analogous to the DM1-HSAtg mouse with $(CTG)_{250}$, which has proven the most informative model for DM1, we have generated transgenic mice expressing a mutant DM2 expansion of $(TG)_{20}(TCTG)_{12}(CCTG)_{121}$ in intron 1 of the human skeletal actin (*HSA*) gene. Our DM2-*HSA*tg mice have ribonuclear inclusions that sequester Mbnl1 and 2. They have myotonia by EMG, muscle weakness and wasting, a muscle pathology associated with human DM, including aberrant type 2 muscle fibers, sarcoplasmic masses and central nuclei. Other phenotypic features include cardiomyopathy and sudden cardiac death, suggestive of arrhythmia. Although none of our mice have overt cataracts at this time, abnormalities of lens development are present. Our mice complement the existing models for DM and are uniquely suitable for dissection of the mechanisms responsible for the DM2 phenotype.

O15
REVERSIBLE MULTISYSTEMIC MOUSE MODELS OF DM1 AND DM2

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To test the toxicity of CCUG expansion transcripts, TRE-(CCTG)300 and TRE-(CCTG)5 mice were generated and crossed to transgenic mice expressing the tet-inducible transactivator in skeletal muscle (m-tTA). Doubly transgenic mice (TRE-CCTG:m-tTA) expressing the expanded, but not control transcripts, show increased central nuclei, RNA foci, alternative splicing changes (*insulin receptor* & *muscle titin*), and electrical myotonia. Turning off transgene expression in 10 month old TRE-(CCTG)300:m-tTA mice for 8 wks results in significant reductions of central nuclei, myotonia and RNA foci. Additional cell culture studies show RNA foci are no longer detectable 24 hrs after turning off expression of the CCUG expansion transcripts. These data show that transcripts with 300 CCUGs are sufficient to generate the hallmark DM skeletal muscle features and that these effects are reversible.

Hypotheses to explain DM1 and DM2 differences include: altered expression of genes at the DM1 locus; effects of the DMPK-3'-UTR; differences in toxicity of the CUG and CCUG motifs. To test 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, the TRE-CCTG mice were crossed to BAC transgenic mice expressing the tTA under the control of either the endogenous human *DMPK* or *ZNF9* promoter. Doubly transgenic CCTG(300): DMPK-tTA and CCTG(300):ZNF9-tTA mice show broad expression and multisystemic phenotypes including myotonia, central nuclei and RNA foci in skeletal muscle, and in brain electrophysiological changes, *Tau* E10 exclusion, and RNA foci. Further characterization of these models will help define the molecular mechanisms underlying the multisystemic features of DM1 and DM2 and the similarities and distinctions between these disorders.

O16
FORM AND FUNCTION OF SHORT (CUG)N-RNA IN CELLS TRANSCRIBING EXTENDED CTG REPEATS CHARACTERISTIC OF MYOTONIC DYSTROPHY TYPE 1

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Using a cell-based model system we have discovered that cells expressing 'toxic' mRNA containing expanded (CUG) repeats (>50) characteristic of DM1 produce short (CUG)n-RNA fragments, where n is the number of repeats. These short fragments can be detected in a strikingly regular, albeit complex, pattern and may represent intermediate products of processing/degradation of the 'toxic' repeat-containing transcripts.

We have inferred from this observation that there is cellular machinary that can process/degrade 'toxic' (CUG)n repeat-containing transcripts.

The complex mixture of short (CUG)n RNA observed is not a uniformly spaced ladder ascending in increments of a single nucleotide or in multiples of three. Instead, it is composed of pairs of RNA fragments that differ in size by approximately 2 nucleotides. In turn, each pair is apparently separated from the adjacent pairs by an average of 4-5 nucleotides.

Observation of the short (CUG)n has been made possible by the development of new RNA detection methodology that allows upto 100-fold greater sensitivity in comparison to conventional UV cross-linking (Pall *et al.*, *Nucleic Acid Res.*, 2007 35(8)).

Currently, I am using RNA interference (RNAi) technology to explore candidate proteins that may contribute to the production of the short (CUG)n fragments. These candidates include proteins known to interact with CUG repeats e.g. CUG-BP and MBNL1 and 2; proteins involved in the production of short RNA i.e. components of the RNAi pathway and in the degradation of mRNA.

O17
DEFECTIVE MRNA IN MYOTONIC DYSTROPHY ACCUMULATES AT THE PERIPHERY OF NUCLEAR SPLICING SPECKLES

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Nuclear speckles are storage sites for small nuclear RNPs (snRNPs) and other splicing factors. Current ideas about the role of speckles suggest that some pre-mRNAs are processed at the speckle periphery before being exported as mRNA. In DM1 myotonic dystrophy, the export of mutant DMPK mRNA is prevented by the presence of expanded CUG repeats which accumulate in nuclear foci. We show that these foci accumulate at the periphery of nuclear speckles. In DM2 myotonic dystrophy, mRNA from the mutant ZNF9 gene is exported normally, because the expanded CCUG repeats are removed during splicing. We show that the nuclear foci formed by DM2 intronic repeats are widely dispersed in the nucleoplasm and not associated with either nuclear speckles or exosomes. We hypothesize that the expanded CUG repeats in DMPK mRNA are blocking a stage in its export pathway that would normally occur at the speckle periphery. Localization of the expanded repeats at the speckle periphery does not appear to be involved in their pathogenic effects, because DM1 and DM2 are quite similar clinically. This observation makes possible an antibody method for distinguishing DM1 and DM2 cells. This study was supported by the UK Muscular Dystrophy Campaign and Association Francaises contre les Myopathies.

O18
A BIOINFORMATICS APPROACH TO IDENTIFY NOVEL GENES MIS-SPLICED IN MYOTONIC DYSTROPHY

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The actual number of splicing events that are mis-regulated in myotonic dystrophy (DM) is currently unknown. To date approximately 24 specific splicing defects have been associated with DM. Together, these molecular defects can potentially account for many of the pathologies associated with DM. However, several major disease phenotypes cannot yet be correlated with specific mis-splicing events. Most of the DM related mis-splicing events have been identified through directed studies of transcripts known to be alternatively spliced in tissues affected by DM. Such targeted studies have provided key insights into understanding the molecular details of DM pathology; however, a broader approach should reveal additional transcripts affected in DM.

Towards the goal of identifying additional mis-regulated splicing events in DM, we have developed *ab initio* methods for predicting alternatively spliced exons that are regulated by the splicing factors, MBNL and CUG-BP. We recently published the results of an analysis of intronic sequences that are both highly conserved between mammals and that are enriched near alternatively spliced exons. This study successfully identified motifs matching binding sites for known splicing factors including FOX1 and QKI. Amongst the potentially novel motifs discovered were several containing a core UGC and/or CUG sequence: UGCUUG, UGCUG, and UUCUG. All of these resemble MBNL and/or CUG-BP binding sites. Overall, several hundred skipped human exons contain highly conserved instances of these motifs in their flanking introns. We are currently testing several of these alternatively spliced exons to determine if they are mis-spliced in tissue from DM patients.

O19
CYTOPLASMIC CUG RNA FOCI ARE INSUFFICIENT TO RESULT IN ABERRANT RNA SPLICING

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Expanded CUG tracts form both nuclear and cytoplasmic aggregates in DM1 cells. The relative significance of these aggregates in DM1 pathology is unknown. To test the toxicity of expanded CUG repeats in the heart we developed and analyzed mice that express a beta galactosidase cassette in which expanded CTG repeats are located 3' of the termination codon under the control of the alpha myosin heavy chain promoter. Transgenic mice demonstrate CUG foci exclusively in the cytoplasm of cardiac cells. As a key pathological consequence of expanded CUG tracts is aberrant RNA splicing, which is hypothesized to result from either the abnormal sequestration of MBNL1 in CUG foci or elevated levels of CUG-BP1, we tested the ability of cytoplasmic CUG foci to elicit these changes. We observe both MBNL1 sequestration in the cytoplasmic foci and increased steady-state CUG-BP1 levels in adult transgenic mouse hearts. Importantly, these defects are insufficient to result in abnormal RNA splicing or a severe cardiac phenotype. These data demonstrate that cytoplasmic focus formation is insufficient to result in DM1 pathology. Our data are consistent with the hypothesis that nuclear aggregation of expanded CUG repeats may specifically abolish MBNL1 activity in RNA splicing by either altering the biochemical properties of MBNL1 within the nucleus or result in aberrant activity or stoichiometry of functionally relevant MBNL1- nuclear protein partners.

O20
ABERRANTLY SPLICED ALPHA-DYSTROBREVIN ALTERS ALPHA-SYNTROPHIN BINDING IN MYOTONIC DYSTROPHY TYPE 1

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In myotonic dystrophy type 1 (DM1), aberrant mRNA splicing of several genes has been reported to possibly explain some of the symptoms, including myotonia and insulin resistance. The cause of the muscle wasting, which is a cardinal feature of the disease, is however still unknown. Alpha-dystrobrevin is a key component of the dystrophin-glycoprotein complex in striated muscle and plays an important role in maturation and signal transduction. In addition, alpha-dystrobrevin is known to undergo extensive developmental or tissue-specific splicing.
We investigated the alternative splicing of alpha-dystrobrevin in skeletal and cardiac muscle of DM1 patients. Alpha-dystrobrevin mRNA including exons 11A and 12 (variable region 3) was increased in both skeletal and cardiac muscle of DM1 patients. Furthermore, immunoblotting demonstrated that the aberrantly spliced alpha-dystrobrevin in DM1 muscle was significantly increased, and immunohistochemistry showed that it was localized to the sarcolemma. The aberrantly spliced alpha-dystrobrevin isoform, expressed in cultured cells, showed increased binding with alpha-syntrophin. Since alpha-syntrophin coordinates the assembly of signaling molecules and is suggested to control fiber type and size, it is speculated that the aberrantly spliced alpha-dystrobrevin including the variable region 3 contribute to the muscle pathogenesis in DM1.

O21
AMPHIPHYSIN 2 SPLICING ALTERATION AS A POSSIBLE CAUSE OF MUSCLE ATROPHY IN MYOTONIC DYSTROPHIC PATIENTS

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Myotonic Dystrophies (DM) are multi-systemic diseases characterized by specific splicing alterations, among which some have been correlated with the disease phenotype. Notably, aberrant inclusion of Insulin Receptor exon 11 and Chloride Channel exon 7 cause insulin resistance and myotonia respectively. However, origins of other DM phenotypes such as dystrophy steel remain unknown.
In order to shed light on the DM pathogenesis, we have completed a genome-wide screen (Exon Arrays, Affymetrix) of mis-regulated splicing events on RNAs from primary cultures derived from muscle of DM and non-DM patients. We found splicing misregulation of 20 candidates, among which Amphiphysin 2.
Amphiphysin 2 is necessary for the biogenesis of muscle T-tubules, responsible for the initation of muscle contraction. Amphiphysin 2 knock-out in mouse and Drosophila give rise to skeletal muscle atrophy. Importantly, loss-of-function mutations also lead to centronuclear myopathies.
1 – We confirmed by RT-PCR and qPCR, on a cohort of CDM, DM1 and DM2 patients, Amphiphysin 2 splicing alterations. Our results suggest a correlation between degree of Amphiphysin 2 splicing changes and severity of the dystrophy.
2 – We are assessing, *in cellulo* and *in vitro*, the function of CELF and MBNL1 splicing factors on Amphiphysin 2 splicing regulation.
3 – Importantly, studying the function of Amphiphysin 2 isoforms we demonstrated, *in cellulo* and *in vitro*, a loss-of-function of the DM isoforms.
4 – Finally, to test if re-establishing correct splicing of Amphiphysin 2 reverses DM muscle pathology, we are currently producing lentivirus expressing MBNL1 or correct Amphiphysin splicing isoforms to infect DM myotubes.
Overall, our results propose Amphiphysin 2 splicing alteration as a possible cause of muscle atrophy in Myotonic Dystrophic patients.

O22
THE RESPONSE TO SERUM STARVATION IN DM1 LENS CELLS INVOLVES FGF RECEPTOR SIGNALLING AND CA2+ CHANNEL ACTIVATION

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Cataract is a characteristic feature of myotonic dystrophy type 1 (DM) and recent evidence shows that DM1 lens cells have reduced growth and an impaired ability to withstand stress (Hum.Mol.Genet., 2006). We have therefore investigated the mechanisms of proliferation and cell death in DM lens cells. Six cell lines were derived by SV40 transformation of lens capsulorhexis specimens obtained during cataract surgery. Two age matched lines from non-DM cataracts and 4 from DM1 cataracts were used. Conditioned serum free media (CM) were harvested from both cell types and their effect on cell growth was determined using the untransformed, non-DM lens cell line FHL124. DM CM stimulated growth in FHL124 cells up to 3 fold more than CM from control cells. The stimulation was inhibited by SU5402 (FGFR1 inhibitor) and wortmannin (PI3K inhibitor). CM induced FGF receptor (FGFR) phosphorylation in FHL124 cells and increased the level of pERK and pAKT. Interestingly DM cells did not survive when FGF signalling was inhibited by SU5402 (10μM). Additionally DM CM induced an increase in intracellular Ca^{2+} greater than that produced by control cell CM. The Ca^{2+} increase persisted in the presence of thapsigargin but was abolished in the absence of external Ca^{2+} indicating channel activation. The cell lines that induced the greatest growth stimulation also induced the largest Ca^{2+} influx. Transcription analysis (Affymetrix

and PCR) strongly indicates that increased glutamate production and signalling via glutamate/GABA receptors occurs in DM lens cells. Under stress conditions (ie. serum deprivation) DM lens cells produce factors that activate FGF signalling (survival) and Ca^{2+} influx possibly via ionotropic glutamate receptors.

O23
INHIBITION OF PROSTAGLANDIN E2 (PGE2) PRODUCTION RESTORES THE DIFFERENTIATION OF CONGENITAL HUMAN MYOTONIC DYSTROPHY TYPE 1 MYOBLASTS

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One characteristic of myotonic dystrophy type 1 (DM1) is the presence of a congenital form (CDM1), which differs from the adult form by the presence of a delay in the development of the skeletal muscle. To date, little is known about the mechanisms by which the expansion causes the delay in CDM1 muscle development. We hypothesized that the delay in muscle development is triggered by a soluble factor produced by CDM1 myoblasts. We used human CDM1, DM1 and DM2 myoblast cultures to identify a soluble factor produced specifically by CDM1 myoblasts. We show that culture medium conditioned by CDM1 but not by DM1 or DM2 myoblasts blocked the differentiation of normal myoblasts. Fractionation analysis of the culture medium revealed that the effect coincides with the fraction containing proteins with a *Mr* <5 kDa. We identified PGE2 as a potential candidate. The level in PGE2 activity was specifically increased in the culture medium of CDM1 myoblasts, and this was associated with a concomitant increase in COX-2 protein, an enzyme involved in prostaglandin (PG) synthesis. Treatment of normal myoblasts with PGE-2 blocked their differentiation. Because COX-2 inhibition also alters cellular production of other PGs, we targeted microsomal PGES (mPGES-1), an enzyme that acts downstream of COX-2 and that affects PGE2 production only. Inhibition of mPGES-1 by specific shRNAs completely abolished the production of PGE2 in the culture medium and restores the ability of CDM1 myoblasts to fuse. Finally, we show that the level of Cox-2 is specifically increased in skeletal muscle derived from CDM1 but not in DM1 or DM2. *Conclusions*. The targeting of mPGES-1 seems to be an appropriate approach to develop a therapy to treat the delay in muscle development observed in CDM1.

O24
SIGNIFICANT ALTERATION OF GENE EXPRESSION IN DM1 MYOBLASTS IS ELICITED BY MOLECULAR EVENTS UNLINKED TO RNA SPLICE DEFECTS

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Expression analysis reveals that in addition to RNA splice defects DM1 cells display a key set of aberrantly expressed genes including those that play an important role in myogenesis, ion channel function and muscle structure. Down regulation of either MBNL1, MBNL2 or elevated steady-state levels of CUG-BP1 in normal myoblasts is not sufficient to elicit the alterations in gene expression observed in DM1 myoblasts. In striking contrast siRNA mediated inactivation of a homolog of a drosophila transcription factor with known RNA binding domains in normal myoblasts serves to recapitulate a large fraction of DM1 specific gene transcription defects. These data demonstrate that that defects in RNA splicing and gene expression in DM1 are a consequence of independent molecular defects.

O25
MUTANT *DMPK* TRANSCRIPTS ACTIVATES NOTCH SIGNALING, IMPAIRING MYOGENESIS IN MYOTONIC DYSTROPHY TYPE 1

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Myotonic Dystrophy type 1 (DM1) is an autosomal dominant neuromuscular disease. DM1 phenotypes manifest as the result of progressive expansion of CTG repeats at the 3' UTR of DMPK on chromosomal locus 19q13.3. The myogenic defects in congenital DM1 (CDM1) is characterized by dysfunctional skeletal muscle, consists of immature and short muscle fibers and abnormally high population of satellite cells; in contrast, the efficacy of postnatal skeletal muscle regeneration is diminished in adult DM1 patients. Importantly, the ability of CDM1 myoblasts to progress toward myogenic lineage was found to be drastically low when cultured *in vitro*. Although numerous cell-culture and animal models have provided important insights into the complex DM1 etiology, the mechanism by which massive CTG repeat expansions impair myogenesis has remained largely unknown. We have identified a novel E3 ubiquitin ligase and a Notch signaling regulator, Mind bomb 2 (MIB2) which is sequestered by the expanded CUG RNA sequences in DM1. We will present evidence to show that MIB2 sequestration results in stark upregulation of Notch signaling in DM1 and that chronically activated Notch signaling disrupts myogenesis in DM1. Our results provide important new insights into the mechanism by which massive expansion of CTG sequences impairs myogenesis, diminishes oxidative capacity of skeletal muscle and provides possible avenues for the development of pharmacological interventions for DM1.

O26
DIFFERENTIAL EXPRESSION OF ELONGATION FACTOR 1 ALPHA (EF1A) IN DM MUSCLE CELLS

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The RNA gain-of-function model predicts that the $(CUG)_n$ and $(CCUG)_n$ repeats carried by DM1 or DM2 mutant transcripts bind and sequester proteins, resulting in an alteration or inhibition of their function. Some of the phenotypes exhibited by the patients, however, are different in each disease such as the absence of a congenital form in DM2. This suggests that there are both common and distinct mechanisms involved in the pathogenesis of type 1 and type 2 myotonic dystrophies. We hypothesized that a different set of proteins may bind to the DM2 repeats relative to DM1, which would explain the dissimilarities between the two forms of the disease. We set out to isolate and characterize novel proteins with affinity for either (CUG)n or (CCUG)n repeats, using an *in vitro* bead capture approach along with nuclear extracts. One of the proteins we isolated is elongation factor 1 alpha (EF1A).
EF1A catalyzes the first step of the protein elongation cycle by carrying the aminoacyl-tRNA on the A site of the ribosome, which contains the growing polypeptide chain as peptidyl-tRNA. In the mouse, EF1A is the embryonic form, expressed in skeletal muscle until day 28 after birth. Its homolog, EF1A-2/S1 (elongation factor 1 alpha 2) is the only form expressed in adult heart, neurons and skeletal muscle. EF1A-2/S1 is deleted in the *wasted* mouse, which displays a muscle wasting phenotype reminiscent of DM1. We quantified by western blotting the level of expression of both isoforms in adult and fetal muscle biopsies. Both EF1A and EF1A-2/S1 are overexpressed in adult DM1 and DM2 patient biopsies, while they are below detectable levels in normal muscles. We also determined that EF1A levels are decreased in fetal DM1 biopsies relative to normal controls. Similarly, the levels of EF1A are lower in DM1 myoblasts *in vitro* compared to a normal control.
We are currently investigating whether EF1A and/or EF1A-2/S1 contribute to the DM pathology. In addition to its function in protein elongation, EF1A has numerous moonlighting functions. For example, it is essential for the regulation of the actin skeleton (through F-actin bundling) and cell morphology. It also participates in oxidative stress-induced apoptosis, microtubule severing, diabetes, and ubiquitin mediated protein degradation. These experiments should shed light on the potential role of EF1A in the pathophysiology of DM1 and DM2.

O27
SPINOCEREBELLAR ATAXIA TYPE 10 – PARALLEL WITH AND DISPARITY FROM MYOTONIC DYSTROPHIES IN THE RNA-MEDIATED PATHOGENIC MECHANISM

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The pathogenic mechanisms of Myotonic dystrophy types 1 and 2 (DM1 and DM2) have primarily been attributed to repeat-expansion RNA mediating trans-dominant gain of function. Similar gain-of-function mechanisms by repeat-expansion RNA have been implicated in fragile X tremor ataxia syndrome (FXTAS), spinocerebellar type 8 (SCA8) and Huntington's disease like 2 (HDL2), in which expanded repeats are located within non-coding region.
Spinocerebellar ataxia type 10 (SCA10) is an autosomal dominant neurodegenerative disorder with the core feature of progressive cerebellar ataxia, which is variably associated with epilepsy and other extra-cerebellar neurological abnormalities. The genetic mutation of SCA10 is an expansion of ATTCT repeats within intron 9 of the *ATXN10* gene on chromosome 22q13.31. The mechanism by which the ATTCT expansion causes the SCA10 phenotype is unknown. We have shown that neither a gain nor a loss of function of ataxin-10 protein is likely the pathogenic mechanism of SCA10. We present data suggesting that in SCA10, the mutant *ATXN10* transcripts encoding expanded AUUCU sequences are the principal pathogenic molecules that trigger neuronal apoptosis. The expanded AUUCU RNA forms a complex with an RNA-binding protein, hnRNP K, which leads to diminished cellular hnRNP K activity. This loss of hnRNP K function results in massive translocation of PKC ‰ to the mitochondria and caspase 3-mediated activation of apoptosis. Together, these results provide a key mechanism for neuronal apoptosis and neurological deficiencies in SCA10 and clues for development of therapeutic strategies.
The pathogenic mechanism of SCA10 resembles DM1 and DM2 in that the RNA containing the non-coding expanded repeat accumulates in cells and sequesters key protein(s). However, SCA10 differs in the key protein and downstream mechanisms that lead to neurological dysfunctions.

O28
ROLE OF PKC PATHWAY IN DM1 PATHOGENESIS BY REGULATING CUGBP1 PHOSPHORYLATION AND STEADY STATE LEVELS

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CUGBP1 is involved in pathogenesis of DM1 as a splicing and a translation regulator. CUGBP1 protein levels decrease to barely detectable levels during heart and skeletal muscle development. However, protein levels are abnormally increased in DM1 myoblasts, heart and skeletal muscle tissues. CUGBP1 overexpression in mice reproduces alternative splicing and translational abnormalities of certain genes observed in DM1. The mechanism for elevated CUGBP1 in DM1 is unclear. Here we show that the basis for increased CUGBP1 levels in DM1 is mediated by phosphorylation-induced protein stability. We found that CUGBP1 was hyper-phosphorylated in DM1 cells and COS M6 cells expressing expanded DMPK-CUG RNA, DM1 tissues, and heart tissues from an inducible DM1 mouse model. CUGBP1 hyper-phosphorylation required PKC activity and PKCα and PKCβII directly phosphorylated CUGBP1 *in vitro*. Furthermore, stimulation of PKC activity in cultured cells resulted in CUGBP1 hyper-phosphorylation as well as increased protein half-life. In support to this result, PKCα/βII activity was increased in heart tissues from individuals with DM1, the DM1 mouse model, and in DM1 skin fibroblasts and COS M6 cells expressing expanded CUG repeat RNA. A time course study in the DM1 inducible mouse model demonstrated that PKCα/βII activation, CUGBP1 hyper-phosphorylation, and elevation of CUGBP1 occurred within 6 hours following induction of expanded CUG repeat RNA. In addition, hyper-phosphorylation of CUGBP1 and increased PKC activity correlated with increased CUG-BP1 in embryonic and postnatal heart development. These results suggest that the PKC pathway normally regulates CUGBP1 stability during development and is aberrantly activated in DM1.

O29
SIGNAL TRANSDUCTION PATHWAYS REGULATING CUGBP1 RNA-ACTIVITY IN DM1 PATIENTS

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DM1 is a multisystemic disease associated with skeletal muscle weakness, progressive wasting and myotonia. DM1 is caused by an expansion of untranslated CTG repeats expression of which results in accumulation of RNA CUG repeats. Excessive amounts of CUG repeats misregulate RNA processing through increase of CUGBP1 and reduction of MBNL1. Unscheduled elevation of CUGBP1 *in vivo* causes muscular dystrophy and a delay of muscle development and differentiation, characteristics of the most severe form of disease, congenital DM1. CUGBP1 is a multifunctional protein regulating translation, mRNA stability and splicing. We have found that the binding of CUGBP1 to different mRNA targets is regulated by specific phosphorylation at different residues within CUGBP1 molecule. Studies in cultured cells showed that differential phosphorylation of CUGBP1 changes its RNA-binding affinity at different stages of normal and DM1 myogeneses directing CUGBP1 to different groups of RNAs – targets of CUGBP1. We have identified PI3K-Akt and cyclin D-cdk4 pathways as pathways operating upstream of CUGBP1 and as pathways which are altered in DM1 cells. Our data demonstrate that the normalization of cyclin D3-cdk4 in DM1 cells leads to a partial correction of the differentiation of DM1 myoblasts. The elucidation of signal transduction pathways regulating CUGBP1 activity will help to develop approaches for down-regulation of CUGBP1 in DM1 patients to normal levels and reversing DM1 symptoms associated with the CUGBP1 increase.

O30
MBNL BINDS SIMILAR RNA STRUCTURES IN THE CUG REPEATS OF MYOTONIC DYSTROPHY AND ITS PRE-MRNA SUBSTRATE CARDIAC TROPONIN T

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The current model for myotonic dystrophy (DM) is that the sequestration of MBNL to CUG/CCUG repeats and/or the over-expression of CUG-BP cause mis-splicing of various pre-mRNAs, which subsequently leads to disease symptoms in DM. However, the binding requirements and specificity of MBNL for both its endogenous targets and for RNA expansions remains unclear. We demonstrate that MBNL can bind short structured CUG and CCUG repeats with high affinity and specificity. Only six bases pairs are necessary for MBNL binding: two pyrimidine mismatches and four guanosine-cytosine base pairs in a stem. MBNL has a preference for pyrimidine mismatches, but many other mismatches are tolerated with decreased affinity. We also demonstrate that MBNL binds a helical region of a stem-loop in the endogenous pre-mRNA target, intron 4 of the cardiac troponin T (cTNT) pre-mRNA. The stem-loop contains two mismatches and resembles both CUG and CCUG repeats. We show that mutations previously known to disrupt MBNL's effect on splicing destabilize the stem and significantly reduce binding of MBNL. This suggests that MBNL may bind all of its RNA substrates, both normal and pathogenic, as structured stem-loops containing pyrimidine mismatches.
For exon 5 of the cTNT pre-mRNA, MBNL acts as a splicing repressor and may do so by blocking the access of other splicing factors at the 3' end of the intron. The binding site of MBNL appears to overlap with the binding site of the splicing factor U2AF65. To determine if U2AF65 and MBNL compete *in vitro* for binding to this 3' splice site, a 50 nucleotide RNA derived from intron 4

of the cTNT pre-mRNA was used in a crosslinking assay. We found that MBNL and U2AF65 compete for binding to this RNA, supporting a model in which MBNL blocks the use of this 3' splice site. We are currently studying other exons for which MNBL acts as a repressor to determine if the mechanism through which MBNL represses the inclusion of exon 5 of the cTNT pre-mRNA is applicable to other exons negatively regulated by MBNL.

O31
THE RNA BINDING SPECIFICITY OF DROSOPHILA MUSCLEBLIND

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In Myotonic Dystrophy type 1 (DM1), the RNA binding protein, muscleblind, binds expanded CUG repeats and is sequestered away from its normal cellular RNA targets. This sequestration event is at least partially responsible for the symptoms of DM1. To better understand the RNA binding of muscleblind, we have characterized the RNA binding affinity and specificity of the *Drosophila* muscleblind (Mbl) protein. Using a variety of CUG mutant RNAs and SELEX (systematic evolution of ligands by exponential enrichment)-identified RNAs, we have elucidated key aspects of the RNA structure and sequence that are important for specific RNA binding by Mbl. The mutagenesis of double-stranded CUG repeats demonstrated that Mbl requires pyrimidine-pyrimidine mismatches for binding while the human protein (MBNL) tolerates other types of mismatches, but shows the same general binding trends. The identity and location of the C-G and G-C base pairs within the CUG repeats is also essential for Mbl binding. Using SELEX we identified RNAs that bind to MBL with high affinity compared to the CUG repeats (K_d s of 2.0-0.4 nM for the SELEX RNAs versus 400-500 nM for the CUG repeats). The high affinity SELEX RNAs are highly structured and contain a five nucleotide consensus sequence of AGUCU that is always predicted to be base paired in the RNA structures. Mbl footprinting with one of the SELEX RNAs showed that Mbl recognizes both double-stranded and single-stranded regions of the RNA. Interestingly, one paired (within the consensus) and two unpaired guanosines show the strongest footprint with Mbl and mutation of any of the three guanosines eliminates Mbl binding. Our SELEX results show that Mbl can recognize complex secondary structures and that guanosines play an important role in Mbl binding.

O32
THE OVEREXPRESSION OF MBNL1 FETAL ISOFORM OBSERVED IN MYOTONIC DYSTROPHY BRAIN DOES NOT MODIFIED TAU SPLICING

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In Myotonic Dystrophy of type I (DM1), the mutant DMPK mRNA bearing expansion of CUG repeats sequesters the splicing factor MBNL1, supporting a loss of MBNL1 function. MBNL1 is highly expressed in muscle and heart but less is known about its expression in the human brain. Moreover, in the brain of affected individuals, we previously described a reduced inclusion of Tau exons 2 and 3. Herein, we described MBNL1 splicing in the human brain, and its alteration in DM1 brain, characterized by an enhanced expression of a major MBNL1 fetal-isoform. A reduced inclusion of Tau exons 2/3 is induced by long CUG repeats and the loss of MBNL1 expression, but not modulated by MBNL1 ectopic expression. Our results demonstrate that the modified splicing of MBNL1 and Tau in DM1 brain both result from a trans-dominant effect of CUG repeats but is not a consequence of the modified splicing of MBNL1 itself.

O33
BIOCHEMICAL ANALYSES OF MBNL1 COMPLEXES IN MYOTONIC DYSTROPHY

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MBNL1 is an alternative splice regulator that has been demonstrated to aggregate with the expanded CUG RNA in DM1 patient cells. To examine the mechanism by which MBNL1 aggregation results in aberrant RNA splice site choice in DM1 we have utilized MBNL1 specific monoclonal antibodies to identify proteins that interact with endogenous MBNL1 in normal human myoblasts using affinity purification and mass spectrometry. This analysis identified three groups of proteins that are relevant to spliceosome function, including alternative splice regulators, RNA helicases and spliceosome core components. The functional significance of these interactions was tested using a panel of biochemical and cell based assays. This set of experiments reveals that MBNL1 displays differential solubility and aberrant complex formation in DM1 myoblasts. In conjunction with changes in MBNL1 complex formation we observe altered stoichiometry of several MBNL1 interacting proteins that are sufficient to cause aberrant RNA splice site choice in DM1 cells.

O34
MULTIPROTEIN COMPLEXES AS TARGETS FOR RNA CCUG REPEATS EXPANDED IN PATIENTS WITH DM2

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Myotonic Dystrophy 2 (DM2) is a multisystemic skeletal muscle disease caused by an expansion of CCTG repeats, transcription of which results in accumulation of un-translated CCUG RNA. DM2 is similar but not identical to Myotonic Dystrophy 1, caused by an expansion of CUG repeats. In DM1 patients RNA CUG repeats target specific RNA CUG-binding proteins, CUGBP1 and MBNL1. To address the mechanisms responsible for specific features of DM2, we have searched for specific CCUG-binding proteins. These studies have identified multiprotein complexes as new targets which are affected by expanded RNA CCUG repeats. Purification of these complexes and mass spectroscopy of their components showed that CCUG repeats interact with the 20S proteasome and the complex containing CUGBP1 and eukaryotic translation factors 2, eIF2. Consistent with biological functions of the 20S proteasome and the CUGBP1-eIF2 complexes, the proteasome-dependent degradation of short-lived proteins and translation of known CUGBP1 targets are altered in DM2 cells. We have found that expression of CCUG repeats in normal myoblasts reproduces DM2-like changes of the protein misregulation. Thus, these new data demonstrate that the expanded RNA CCUG repeats target multiprotein complexes, including the 20S proteasome and the CUGBP1-eIF2 complexes. The interaction of the CCUG repeats with these complexes changes protein turnover and contributes to the development of DM2 pathology.

O35
MBNL3 DOWN-REGULATES MEF2 DEPENDENT GENES BY ALTERNATIVE SPLICING OF MEF2 TRANSCRIPTION FACTOR

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Proteins of the mammalian muscleblind-like (MBNL) family are believed to be regulators of myogenesis and are implicated in the neuromuscular degenerative disorder myotonic dystrophy. While the *Drosophila melanogaster* muscleblind protein is required for terminal muscle differentiation, the MBNL3 protein functions as an inhibitor of myogenesis. The phenotype of myotonic dystrophy has been linked to disrupted regulation of alternative splicing but the mechanism is still unknown. Our study is aimed to determine whether the inhibitory property of MBNL3 in muscle differentiation involves changes in the alternative splicing of muscle genes. Microarray analysis of MBNL3 over-expressing C2C12 cells revealed that MEF2 target genes, such as Mylpf, Atp2a2, Tnnt1 and Hrc were down-regulated. We have found that the splicing pattern of MEF2 upon muscle differentiation in MBNL3 expressing C2C12 cells differs from control cells but is similar to C2C12-CUG200 cells, a tissue culture model of myotonic dystrophy. These results suggest that alterations in MEF2 splicing by MBNL3 lead to a down regulation of MEF2 target genes and may contribute to the muscle wasting and weakness of myotonic dystrophy.

O36
STRUCTURAL DETERMINANTS FOR THE MOLECULAR PROPERTIES OF MBNL PROTEINS

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MBNL proteins have emerged as targets of expanded CUG/CCUG repeats. An important question is to what extent the loss of MBNL functions can explain the pathogenesis of DM1 and DM2. To address this question, it is essential to clarify the normal function as well as molecular properties of MBNL proteins.

Here we report a structure-function analysis of MBNL proteins using multiple MBNL isoforms as well as MBNL mutants. Such analysis would be important particularly because multiplicity of MBNL paralogs and splice isoforms have complicated the understanding of these proteins. MBNL proteins have an N-terminal RNA-binding region with four C3H-type zinc fingers and a C-terminal region with high variability. The N-terminus region of MBNL1 was sufficient for its splicing regulation of the *Clcn1* minigene, and mutations in the conserved zinc finger motifs disrupted its regulatory activity. In the *Clcn1* splicing, both MBNL1 and MBNL2 similarly repressed the inclusion of exon 7A, while these proteins acted differently in the case of *Actn1* splicing. Thus, splicing regulation can be different among MBNL paralogs. In the C-terminus region of MBNL proteins, we found a novel peptide motif that is conserved through evolution from nematode to human. This motif was essential for the nuclear localization of an MBNL1 isoform, MBNL1-42, even though this motif is located outside of the alternative exon uniquely included in MBNL1-42. Mutation analysis revealed critical residues for the nuclear localization. These results illustrate the structural determinants for the molecular properties and functions of MBNL proteins.

O37
PROTEINS THAT INTERACT WITH MBNL1

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In DM1 and DM2, the repeat expansions occur in untranslated regions of the genome, thus abnormal RNA foci appear in nucleus of patient's cells. MBNL1, a human homologue of Drosophila muscleblind, was discovered to be sequestrated in the foci. A series of studies support the hypothesis that a dysfunction of MBNL1 is related to DM onset. Although MBNL1 is known mainly as a splicing factor, the physiological function of MBNL1 is mostly remained unknown. To investigate it, we started screening of interacting proteins of MBNL1. By GST-pulldown assay, we identified several proteins with MALDI-TOF-MASS and LC-MS/MS systems. The candidates of binding proteins were such as RNA binding proteins, tRNA synthetases, and a glucosidase and many ribosomal proteins. In this profile, we focused our attention on a multifunctional nucleic acid binding protein, and a DEAD-box RNA helicase. In a minigene splicing reporter assay, the former functions with MBNL1 cooperatively. Interestingly, when subjected to oxidative stress or heat stress, MBNL1 colocalized at stress granules in the cytoplasm. These results suggest a novel role of MBNL1 in translational regulation or in other mRNA metabolic event.

O38
INTERACTIONS OF MUSCLEBLIND PROTEINS WITH SPLICING TARGET AND PATHOGENIC RNAS

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The MBNL proteins are RNA processing factors that regulate the alternative splicing of specific exons during postnatal development. The protein sequestration model for DM predicts that MBNL proteins have a higher affinity, and/or interact differently, with the CUG and CCUG repeat expansions in mutant DMPK and ZNF9 transcripts compared to their normal splicing targets. Here, we demonstrate using a combination of filter binding and gel shift analysis that MBNL1 possesses similarly high affinities (5-11 nM) for dsCUG, dsCAG and a skeletal muscle splicing target, the Tnnt3 F exon region. The MBNL1 binding site in Tnnt3 intron 8, upstream of the F exon, was mapped to a GC-rich hairpin containing a pyrimidine mismatch while chemical/enzymatic structure probing and electron microscopy revealed that MBNL1 forms a stacked ring structure when bound to dsCUG. In agreement with previous studies, the MBNL1 N-terminal region is essential for RNA binding. However, the C-terminal region mediates homotypic interactions which may stabilize inter-ring interactions. These observations suggest that MBNL sequestration by dsCUG and dsCCUG RNAs results from the formation of a novel RNA-protein complex and highlight the similarity in the binding sites for MBNL proteins on splicing precursor and pathogenic RNAs.

O39
NEUROPSYCHOLOGICAL AND ADAPTIVE SKILLS IN MYOTONIC DYSTROPHY TYPE 1- A STUDY ON 57 INDIVIDUALS WITH CONGENITAL AND CHILDHOOD FORMS

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Myotonic dystrophy type 1 (DM1) is one of the most common inherited neuromuscular diseases. In the present study, the aim was to investigate the cognitive and adaptive level in children and adolescents with congenital (severe and mild form), childhood and classical DM1. Fifty-seven children and adolescents with DM1 participated in the study. Cognitive level was assessed in fifty-two individuals by using the Griffiths Mental Development Scale or the Wechsler Scales. The parents of all 57 individuals agreed to be interviewed regarding their child's adaptive level by using the Vineland Adaptive Behaviour Scales. Ninety-five percent of the subjects with severe congenital DM1 had mental retardation (MR). In mild congenital DM 1 and childhood DM1 83% and 89% had MR respectively. There was a relationship between DM1 form and IQ, the more severe form of DM1 the lower IQ. Significantly higher Verbal IQ compared with Performance IQ was found in severe congenital and childhood DM1. The Vineland Adaptive Behavior Scales showed poor results and there was a positive relationship between cognitive and adaptive levels regarding the severe congenital DM1 and childhood DM1. The conclusion is that children and adolescents with DM1 exhibit, in most cases, regardless of DM1 form, significant cognitive and adaptive problems.

O40
AUTISM SPECTRUM DISORDERS IN MYOTONIC DYSTROPHY TYPE 1- A STUDY ON 57 INDIVIDUALS WITH CONGENITAL AND CHILDHOOD FORMS

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Myotonic dystrophy type 1 (DM1) is a neuromuscular disorder also affecting the central nervous system. The aims of the present study were to describe the neuropsychiatric problems in fifty-seven children and adolescents (26 females; 31 males), to estimate the size of the CTG-expansion and to correlate the molecular findings with

the neuropsychiatric problems. The following instruments were used: Autism Diagnostic Interview-Revised (ADI-R), Five to Fifteen, Griffiths Mental Development Scales, and the Wechsler Scales. Based on age at onset and presenting symptoms, the children were divided into four DM1 groups; severe congenital (n=19), mild congenital (n=18), childhood (n=18), and classical DM1 (n=2). Forty-nine percent had an autism spectrum disorder (ASD) and autistic disorder was the most common diagnosis present in 35%. ASD was significantly correlated with DM1 form; the more severe form of DM1, the higher the frequency of ASD. The rate of ASD increased with increasing CTG repeat expansions. ASD and/or other neuropsychiatric disorders such as attention deficit hyperactivity disorder, and Tourette´s disorder were found in 54 % of the total DM1 group. In conclusion, awareness of ASD comorbidity in DM1 is essential. Further studies are warranted to elucidate the molecular and structural aetiology causing neurodevelopmental symptoms such as ASD in DM1.

O41
COGNITIVE DEFICITS BRIDGING AGES: A BEHAVIOURAL PHENOTYPE IN MYOTONIC DYSTROPHY TYPE 1?

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It is known that Myotonic dystrophy type 1 (DM1) is a disorder associated with cognitive and behavioural deficits. We compared results on neuropsychological tests and ratings on adaptive behaviour of patients with the congenital, childhood and adult variants of DM1. Standardised tests and rating procedures were used: the Wechsler Intelligence Scales, Vineland Adaptive Behaviour Scales, Temperament and Character Inventory, and an extensive neuropsychiatric evaluation. We also explored correlations between measures and CTG repeat expansion size.
We found striking similarities between different subgroups of DM1, regarding low Performance IQ, but also significantly lower results on tests measuring attention, speed, arithmetic and visuospatial functions. When comparing behavioural profiles, the subgroups showed a pattern of avoidance and deficits in social interaction. Correlation between size of CTG-repeat expansion and cognitive/behavioural measures were found in subgroups. In summary this comparison exhibits, striking similarities between the different subgroups of DM1 according to several neuropsychological and behavioural measures but also an association with CTG repeat expansion size. These data underlines the importance of conducting further research on a possible behavioural phenotype in DM1.

O42
COGNITIVE IMPAIRMENT IN MYOTONIC DYSTROPHY TYPE 1 (DM1): A LONGITUDINAL FOLLOW-UP STUDY

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Central Nervous System involvement occurs in most myotonic dystrophy type 1 (DM1) patients: about two years ago we performed a neuropsychological study documenting an ageing-related decline of frontal and temporal cognitive functions in adult forms of DM1. No correlations were found between cognitive impairment and either n(CTG) in leukocytes or severity of muscle involvement. Here we present a 2 years follow-up neuropsychological study in order to verify the progression of this focal cognitive impairment. We re-evaluated 34 out of 70 DM1 patients studied. Patients were divided into 4 groups according to genotype. All groups showed a comparable mean educational level (9±4 yrs).
The neuropsychological test battery included MMSE, memory, linguistic, level, praxis, attentional and frontal-executive tasks. Statistical analysis was performed by One way MANOVA with repeated measures analysis.
Statistical analysis allowed us to separate the worsening performances from those resulted unvarying from the first evaluation: the whole patients showed a significant deterioration (p=0.01) in their linguistic and executive function abilities, in agreement with the predominant fronto-temporal involvement in DM1 patients.
Moreover, among the larger groups of patients (E2 and E3), we identified two different cognitive behaviours: patients belonging to E2, who have the highest mean age, got scores lower than E3 patients, with particular regard either to linguistic and executive tasks.
Therefore, our data confirm our previous hypothesis that in adult DM1 patients the cognitive damage is confined to fronto-temporal functions, with a tendency towards decline with aging.

O43
CROSS-SECTIONAL ANALYSIS OF CNS IMAGING AND FUNCTION IN DM1 AND DM2

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Genetic and clinical similarities of DM1 and DM2 have supported the toxic gain-of-function RNA mechanism as the cause of myotonia, muscular dystrophy, iridescent cataracts, insulin insensitivity, cardiac conduction defects, hypogammaglobulinemia, testicular failure, and other myotonic dystrophy features. Although CNS MRI abnormalities have been reported in older subjects with DM1 and DM2, the functional significance of these changes and their connection to the pathogenic CUG and CCUG expansions has remained unclear.

To further define the CNS changes in DM1 and DM2, we performed cross-sectional imaging and neuropsychological studies in 25-45 year old subjects with adult-onset DM1, congenital or childhood onset DM1, DM2, and controls. To expand the age range we also studied 8-18 year-old children with early-onset DM1. Macrostructural and microstructural changes were assessed with volumetric MRI and DTI methods, and compared to a comprehensive neuropsychological battery in all subjects.
We found reduced frontal lobe grey matter volume, and reduced frontal white matter fractional anisotropy (FA) in all DM groups compared to controls. In early onset DM1 the volumetric and FA abnormalities were also present posteriorly, and there was reduced full-range IQ that was not present in adult-onset DM1 or DM2. Executive function was impaired in all DM groups, and multivariate analysis showed a significant correlation between executive function disturbance and frontal FA abnormality.
Comparing the clinical DM1 and DM2 CNS abnormalities to those of multisystemic transgenic mice will help define the etiology of the CNS features of these diseases.

O44
DM2 IS PREDICTED BY COMBINED TYPE 2 FIBRE CENTRONUCLEATION AND ATROPHY

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DM2 CCUG expansions form pathogenic ribonuclear accumulations that are detectable by *in situ* hybridization (ISH). Clinical DM2 diagnosis is often overlooked due to poorly specific presentation and muscle biopsy shows a "denervation-like" pattern of unknown specificity, combining: (1) increased fibre size variation, (2) centronucleation, (3) small angulated fibres, (4) type 2 fibre atrophy, and (5) nuclear clumps. Here, we checked the presence of these alterations in a series of 2100 consecutive muscle biopsies in patients selected for unidentified myopathy, no inflammation nor neuropathy. Then, we used automated [CCUG]8 ISH as a gold standard to evaluate the value of each histological feature for DM2 detection. Among 104 included patients, ISH disclosed 8 DM2+ and 96 DM2- cases. Multivariate analyses identified the combination of "type 2 fibre atrophy" and "centronucleation" as the most relevant (Se=1.0, Sp=0.92), whereas these changes were mutually exclusive in non-DM2 patients ($p<0.0001$). Relevance of the combination was confirmed in an additional independent series (15 DM2+ vs 17 DM2-). Further investigation uncovered that centronucleation selectively affects type 2 fibres in DM2, and, conversely, type 1 fibres in DM1 ($p<0.0001$). These results designate DM2 as a type 2 fibre disease. They may strongly facilitate routine detection of DM2 and further substantiates a distinct muscle pathophysiology.

O45
MYOTONIC DYSTROPHY TYPE 2 (DM2); A DIAGNOSTIC ALTERNATIVE TO FIBROMYALGIA

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Background. Myotonic dystrophy type 2 (DM2) is a new, dominantly inherited, multisystem disorder, which has recently shown to be caused by a very large CCTG-expansion in chromosome 3q21. Besides variable muscle weakness, myotonia and cataracts prominent features in DM2 are variable myalgic pains and stiffness of muscles In spite of difficult muscular symptoms the clinical picture of DM2-patient might be mild or quite normal. The myalgic pains of DM2 and fibromyalgia(FM) resembles each others. *Methods.* 90 fibromyalgia patients, diagnosed at a specialized center, were randomly invited to participate in genetic testing for DM2 mutation. As the control cohorts we tested 70 long-QT syndrome patients for DM2 mutation as a modifier gene for phenotype variation and 200 normal controls. *Results.* 63 fibromyalgia patients participated. Two patients (3.2%) tested to be positive for the DM2 mutation and have DM2 disease as the cause of their muscle symptoms, whereas in the control cohorts of 70 long-QT syndrome patients and 200 normal individuals there were no DM2 mutations. *Conclusions.* The results of this study confirm our hypothesis of missed DM2 diagnosis among fibromyalgia due to similar clinical symptoms. DM2 is an important differential diagnosis to consider in patients with fibromyalgia symptoms, not at least because fibromyalgia is a major general health issue due to its frequency. DM2 is the first genetic disorder to be distinguished from fibromyalgia.

O46
DISPROPORTIONATELY HIGH PREVALENCE OF CO-SEGREGATING CLCN1 MUTATIONS AMONG MYOTONIC DYSTROPHY TYPE 2 PATIENTS FROM FINLAND AND GERMANY

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Clinical core features of DM2 are myotonia, proximal muscle weakness, myalgia and cataracts with a very variable phenotype. As part of the disease pathomechanism DM2 mutation causes aberrant splicing of different genes including *CLCN1*. Primary mutations in *CLCN1* cause congenital myotonia, characterized by myotonia and muscle hypertrophy. Several families with co-segregation

of DM2 and heterozygous recessive *CLCN1* mutations have been reported. To clarify whether co-segregation of frequent recessive *CLCN1* mutations have a modifier effect on the DM2 phenotype we analysed 200 Finnish and German DM2 patients, 350 controls and 100 DM1 patients for three *CLCN1* mutations (R894X, F413C and A531V). We found *CLCN1* mutations in 5% of DM2 patients compared to 2% in the controls; a statistically significant difference (p-value of 0.047, Fisher´s Exact Test). DM2 patients with co-segregating *CLCN1* mutation had more prominent myotonia and myalgia. The frequency of *CLCN1* mutations in DM1 patients (1%) did not differ from the controls. The modifier effect of *CLCN1* mutations is specific for DM2 and does not apply to DM1.

O47
MAPPING OF MUSCULAR INVOLVEMENT OF LOWER LEGS IN MYOTONIC DYSTROPHY : A MAGNETIC RESONANCE STUDY AND CORRELATION TO CLINICAL DATA

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We performed magnetic resonance imaging (MRI) of lower legs of patients with myotonic dystrophy type 1. The aim of the study was to evaluate if a typical pattern of muscular involvement could be identified, and if correlation exists between severity of MRI anomalies and clinical findings. MRI findings were edema and fatty degeneration, ranging from mild to complete fatty replacement of the muscle. In most patients, the medial gastrocnemius and soleus muscles were involved earliest and most severely, whereas posterior tibial and popliteus muscles were generally spared. If severity of MRI findings tend to correlate with clinical examination for muscle weakness, some patients have objectivated weakness without MRI alteration of corresponding muscles.

O48
PACEMAKERS DO NOT PREVENT SUDDEN DEATH IN MYOTONIC DYSTROPHY TYPE 1

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Sudden death (SD) can occur as a consequence of the cardiac involvement associated with myotonic dystrophy type 1 (DM1). Pacemakers (PM) have been recommended for patients (pts) with significant cardiac abnormalities whether or not they are symptomatic because of an unpredictable progression of atrioventricular block. Although many pts with DM1 receive PM it is unclear if the practice confers protection from SD. We examined the relationship between PM and SD in a DM1 population. *Methods*. The Arrhythmias in DM1 Study is a prospective observational registry of pts with clinically and genetically verified DM1 followed at 23 U.S. neuromuscular specialty clinics. *Results*. 406 pts with DM1 (205 men, age 42±12 years, CTG repeat 629±386) were followed for 5.6±2.3 years. Nine pts had a PM prior to study entry with 32 receiving a PM 2.4±2.2 years after entry. Twenty-seven of the 41 PM were implanted in pts with either no or minimal symptoms because of concern with conduction abnormalities. Pts with PM were older (49±13 vs. 41±12 years) and more likely male (13.7% vs. 6.5%). During follow-up, there were 81 (20.0%) all-cause deaths with 27 (6.7%) of these sudden and unexpected. All-cause death (41.5% vs. 17.5%, $p<0.001$) and SD (14.6% vs. 5.8%, $p=0.04$) was greater in pts with PM compared to pts without PM. Electrocardiographic monitoring during attempted resuscitation was available in 3 of the 6 pts with PM who had SD and showed ventricular fibrillation in 1 and pacing without capture (asystole) in 2. *Conclusions*. PM do not decrease all-cause or SD but, in fact, are associated with worse mortality. These findings support consideration for an implantable cardioverter-defibrillator instead of a PM if an antiarrhythmia device is indicated.

O49
CARDIAC ARRHYTHMIAS IN TYPE 1 MYOTONIC DYSTROPHY PATIENTS WITH SLEEP APNOEA. AN IMPLANTABLE MONITORING DEVICE PROSPECTIVE STUDY

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The long-term relationship between cardiac arrhythmias and sleep apnoea in myotonic dystrophy (DM1) is unknown. Pacemakers enabling the long-term monitoring of electrocardiographic and ventilation parameters were implanted in 20 patients with DM1 (mean age = 42±11.6 years) and His-ventricle (HV) interval ?70 ms, measured during invasive electrophysiologic testing. After a 40±12.3 months of follow up, episodes of sleep apnoea were observed in all patients and 17 patients (85%) presented with sleep apnoea syndrome. Arrhythmias were recorded by the pacemaker in 17 patients (85%). Fourteen developed arrhythmic episodes either in absence or in presence of concomitant sleep apnoea (conversely, the majority of sleep apnoea episodes were not associated with concomitant arrhythmias). For the other 3, simultaneous sleep apnoea was never observed. In the 5 patients regularly treated by non invasive positive pressure ventilation, a decrease in the numbers of SA and arrhythmic episodes was observed. In conclusion, a high incidence of arrhythmias and sleep apnoea was observed. While arrhythmias are generally attributable to an organic substrate in DM1, they could be sometimes precipitated by functional triggers, as in the case of sleep apnoea.

O50
PROGRESSION OF MUSCLE WEAKNESS AND CARDIAC INVOLVEMENT IN MYOTONIC DYSTROPHY TYPE 1 (DM1) VS TYPE 2 (DM2): A LONGITUDINAL STUDY

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Objective. To determine rate of progression of muscle weakness and severity of cardiac involvement in DM2 compared to DM1 over time.
Background. There are suggestions that DM2 may have a more favourable prognosis compared to DM1.
Methods: 70 patients with moderately-severe adult DM1 (mean age 45.1 years±14.4; disease duration 15.3±12.4; CTG range 600-800) and 26 patients with genetically-determined DM2 (mean age 56.6 years±12.8; 19 years±9.9 disease duration) were subjected to:(i)Manual muscle strength testing (MRC);(ii)EKG;(iii)24-hour Holter-EKG;(iv)2D echocardiograms at initial examination and every 6 months. DM1 and DM2 mean follow-up: 3.8 years±3 and 6 years±4 respectively.
Results. (i)Muscle weakness significantly worsens in both DM1 (from 129 to 125.3, $p< 0.0001$) and DM2 (from 139.7 to 137.2, $p= 0,024$) but with a faster rate in DM1 vs DM2;(ii) PR interval and QRS duration (QRSD) do not change significantly over time in both groups but in DM1 mean PR and QRSD are significantly longer ($p=0,001$). QRSD increases significantly in DM1 over time ($p<0,05$);(iv)Ejection fraction is unchanged over time in both groups;(v)2/26 patients (7.7%) with DM2 (ages at implantation 71 and 69) and 10/70 patients (7%) with DM1 (mean age of implantation 45.7 years±4.2) required permanent PM implantation;(vi)Final follow-up assessments significantly correlate to severity of involvement at initial assessments for both groups of patients.
Conclusions. The older age of PM implantation in DM2 patients compared to DM1,the low rate of major cardiac conduction defects and slower rate of progression of muscle weakness in these patients suggest that DM2 is a syndrome with a more favourable prognosis.

O51
CARDIAC INVOLVEMENT IN DM2: A 30 PATIENTS FOLLOW-UP STUDY

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Background. DM2 may be associated with cardiac involvement including severe conductive or rhythm disturbances. The aim of this study was to perform a systematical screening to evaluate their real prevalence. *Methods*. consecutive patients with genetically proven DM2 were included. Cardiac management was the same than for DM1 patients, including repeated left ventricular function assessment by echography and annually ECG, 24-hours holter ECG monitoring. Invasive electrophysiologic testing was performed in case of presyncope, syncope or significant ECG conductive defects. *Results*. 30 patients were enrolled (men 16, women 14, mean age 60.0±12.0 years). All had appropriate cardiac investigations during the last two years and five died during follow up of extra cardiac disease. Fourteen patients (46%) had conductive disturbances: 2 had complete AV block requiring pacemaker implantation, 6 had infrahissian AV block proved by invasive electrophysiological testing with HV interval <70 ms, 3 had first degree AV block on ECG, and 3 had bundle branch block. One patient had sinusal bradycardia. Ten patients (33%) had supraventricular arrhythmia : atrial fibrillation/flutter were induced by invasive atrial stimulation in 6 patients and 4 others had supraventricular extrasystolia in 24h-holter ECG monitoring. Eight patients (26%) had left ventricular hypokinesis : 4 had severe dilated cardiomyopathy, two of them associated with severe aortic stenosis and acute myocarditis, 4 had mild left ventricular dysfunction. *Conclusions*. the prevalence of cardiac involvement is high in DM2 patients, including severe complications. Its suggests that all patients should be referred for cardiac evaluation each year.

O53
GASTROINTESTINAL (GI) SYMPTOMS IN MYOTONIC DYSTROPHY TYPE 1 (DM1) PATIENTS ENROLLED IN THE NIH REGISTRY

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GI symptoms are a serious complaint in DM1 patients. The exact pathophysiology of GI disturbances remains unclear and limited information is available about the overall prevalence of symptoms and treatments. *Purpose*. To study the GI symptoms and medications used by patients in the NIH Registry. *Methods*. Analyzed the clinical and molecular data of DM1 patients with GI symptoms and without GI symptoms (controls). All patients were enrolled in the NIH Registry of DM and FSHD Patients and Family Members. *Results*. The Registry currently contains 479 DM1 patients (51% female). Of these patients, 75% (n=361) reported GI symptoms. The most common complaints were dysphagia (68%), gastro-esophageal reflux disease (GERD; 48%), and constipation (43%). Patients with GI symptoms were more overweight and had a longer duration of DM symptoms compared to controls ($p<0.05$). There were no significant differences in regard to age of onset or CTG size. Data indicated that symptomatic patients were taking a total of 131 GI medications. Of these medications, 63% were acid blockers (histamine antagonists or proton pump inhibitors), 9% were for constipation, and 7% were prokinetics. *Conclusions*. The Registry database suggests that GI symptoms relate to excess weight and duration of the

disease. The high prevalence of GI complaints indicate a need for improvements in symptomatic management, including studies of the effectiveness of different pharmacological, nutritional, otolaryngological, and GI therapies. Future investigations of the underlying cause(s) for GI dysfunction in DM1 are needed.
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O54
ERECTILE DYSFUNCTION IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Though impotence is a frequent complaint in DM1 patients, there are few data on its prevalence or relationship with clinical, endocrinological and genetic characteristics. In a case-control study 31 continuous patients with non-congenital DM1 and without physical and psychiatric diseases which could interfere with sexual activity, completed an internationally validated self-administered questionnaire (IIEF) for erectile dysfunction (ED) and sexual hormone levels (Testosterone, FSH, LH and PRL). IIEF showed that DM1 patients scored significantly lower than controls in erectile function, orgasmic function, intercourse satisfaction and overall satisfaction. By contrast, sexual desire was similar in patients and controls. Impotence occurred in 22 patients (71%) and three controls (9.7%). It was severe in eight patients, moderate in four and slight in ten. Disease severity and duration showed a direct correlation with frequency of impotence, even after having adjusted the relationship on potential confounding factors (i.e. age).
CTG expansion did not show correlations with ED.
DM1 patients showed a significant increase in both FSH and LH in DM1 compared with controls ($p<0.01$). PRL and testosterone were similar in patients and controls.
FSH and LH levels in DM1 patients directly correlated with disease duration ($p<0.04$), CTG expansion ($p<0.01$) and severity of disease ($p<0.01$). Age of patients showed no correlation with sexual hormone levels.
Patients with impotence displayed higher values of both FSH and LH ($p<0.01$) than patients with normal IIEF scores.

O55
PROTOCOL DEVELOPMENT FOR PREIMPLANTATION GENETIC DIAGNOSIS (PGD) OF MYOTONIC DYSTROPHY TYPE 1 (DM1) IN THE UK: EXPERIENCE FROM 25 CYCLES

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PGD allows couples with a known disorder to screen their embryos with a view to achieve an unaffected pregnancy.
The UCL Centre for PGD is currently the only centre routinely offering PGD for DM1 in the UK. We present our experience since October 2004. Reasons for referral included the presence of affected family members, patients' objections to abortion, experience of pregnancy termination, loss of a congenitally affected child or infertility.
Four protocols have been developed for diagnosis in 25 cycles. Five cases were cancelled before oocyte retrieval due to either poor response to the IVF treatment or hyperstimulation. In two cases only two embryos were available for biopsy and therefore PGD was cancelled.
From the remaining 18 cycles, 223 oocytes were collected, 203 inseminated, 128 fertilized and 126 embryos were of sufficient quality for biopsy on day 3. Thirteen embryos were rebiopsied on day 4. A diagnosis was achieved for 88 embryos (32 unaffected, 56 affected). Twenty-four embryos were transferred in 13 cycles and 6 pregnancies were established. The pregnancy rate per cycle leading to oocyte retrieval was 33.3% and per cycle with embryo transfer 46.15%. Six healthy infants have been born and one pregnancy is ongoing.
Our protocols have maximised the efficiency and accuracy of diagnosis and also reduced the genetic work-up time from six months to 48hrs from receiving blood from the couple. PGD for DM1 is an effective and practical option and should be presented as another available alternative to prenatal diagnosis during counselling of couples at risk.

O56
CONGENITAL MYOTONIC DYSTROPHY: CANADIAN INCIDENCE AND COHORT STUDY

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Background. Congenital Myotonic Dystrophy (CDM) is the symptomatic manifestation of DM1 in a neonate. Much of the knowledge of the epidemiology, clinical features and outcome of CDM continue to be based on single centre case series. A national Canadian study has been initiated to address these issues.
Method. The study has two phases. The first, started in March 2005, is a prospective, monthly surveillance to identify incident cases of CDM. A case is defined as a genetic confirmation of DM1 in any child who has neonatal respiratory or feeding dysfunction requiring hospitalization. The second phase is a 5 year natural history cohort of incident cases examining mortality, morbidity, development and quality of life.
Results. In the first 2.5 years 59 cases have been reported with only 16 meeting criteria and being incident cases. Five children have died, 3 from withdrawal of ventilatory support at 1 month (2 cases) and 6 months. The CTG repeat size ranged from 600-2300. In 7 of the cases the child was the index case for the family and in the other 9

cases where the mother knew her DM1 status only 3 parents chose prenatal testing. Six children did not need ventilation but had feeding dysfunction requiring hospitalization. Excluding those who died the mean length of ventilation was 26 days (3-79 days, n=5). The majority of eligible children have joined the cohort study.
Conclusions. The epidemiology, clinical features and outcome measures of CDM based on this population-based prospective cohort study will help to provide valuable information for families, health care providers and research efforts.

O57
PRESYMPTOMATIC TESTING IN MYOTONIC DYSTROPHY TYPE I 6-YEAR EXPERIENCE FOR 131 CANDIDATES

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Objectives. (1) To report a 6-year experience of presymptomatic testing in 131 candidates for Myotonic Dystrophy type I (DM1), (2) to compare their characteristics and outcomes with previous reported data about Huntington's disease (HD), autosomal dominant cerebellar ataxias (ADCA) and Facio-Scapulo-Humeral muscular dystrophy (FSH), (3) to discuss our experience for taking care of children asking for the test.
Methods. We collected data on presymptomatic testing for DM1 (n=131) in our clinical center from 1997 to 2003, with a protocol characterized by multistep procedure in a multidisciplinary team. 6 of the patients were younger than 18 years.
Results and discussion. The applicants' characteristics were similar in DM1 and HD, ADCA or FSH, showing a predominance of women and family planning as the most frequent reason for seeking presymptomatic testing. We observed a higher rate of completing the presymptomatic testing program in DM1 than in other previously studied diseases, probably due to the potential benefit such as prenatal diagnosis and cardiac follow-up. Surprisingly, 30 patients out of 131 (23%) presented symptoms of DM1 without complaint. If we include these patients, the rate of non-carriers was 64%. Whatever the condition, the psychological consequences of the result must be considered before the test is performed, in adults and moreover in children (over 10 years). Our protocol by multistep procedure and multidisciplinary team allows to clarify stakes and motivations and to anticipate consequences of the molecular result.

O58
IT'S GENETIC, BUT WHAT DOES THAT MEAN?

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This paper draws on a study of parents in DM families that has revealed their uncertainty about genetic aspects of DM, such as whether being tested negative means that the condition can still manifest in subsequent generations. One reason for this is the variable nature of the condition. Another reason is that in contrast with families affected by other genetic disorders, contact with clinical genetics services is fragmentary. It will be suggested that this should form part of the care plan for all families in order for them to make informed decisions.

O59
REVIEW OF CHILDREN DIAGNOSED WITH MYOTONIC DYSTROPHY OVER 30 YEARS

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Children diagnosed with myotonic dystrophy in Wales over the past 30 years have been reviewed to clarify the natural history of this condition for both the congenital and childhood forms. The data collected on the children during this period will be presented. The study will outline the natural history, variation and epidemiology in the condition. The clinical data will be correlated with the DNA and family information to identify links which will help provide prognostic data for future families.

O60
NEUROPSYCHOLOGICAL PROFILE IN THE CHILDHOOD FORM OF DM1

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Background. previous studies have shown variable degree of cognitive impairment in the childhood form of DM1 (Steayert *et al*., 1997; Gossens *et al*., 2000). It ranges from mental retardation to subnormal intelligence but dysfunction in verbal working memory and visuospatial abilities. Negative correlation with the CTG expansion size has also been reported (Angeard *et al*., 2007).
Method: we present here the results of a neuropsychological study on 20 subjects aged from 6;11 to 17 years with the childhood phenotype and no mental retardation (inclusion criteria).
Results. concerning global cognitive abilities, the mean FSIQ was in the subnormal range with a significant dissociation between verbal (VIQ = 91) and non-verbal scores (PIQ = 78). In neuropsychological measures focusing on mental flexibility, cognitive inhibition and planning, the sample study scored significantly lower than the normative reference population. Concerning memory, we observed a dissociation depending on the nature of the information to be treated: in the memorization of a list of words or a story, subject obtain normal scores in immediate and delayed recall but impaired performances for the geometrical figure.
Conclusions. the deficit in executive function as planning, resistence to interference or mental flexibility observed in the children could be related to those observed in adults and might correlate with a central nervous

system involvement like dorsolateral frontal cortex and right parietal lobe dysfunction (Meola, 2003). Short-term and long-term verbal memory's efficiency could be used to elaborate well-adapted rehabilitation.

O61
THE SAGUENAY MYOTONIC DYSTROPHY INTEGRATED CARE PATHWAY: DEVELOPMENT AND PRELIMINARY VALIDATION

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Myotonic dystrophy type 1 (DM1) is a complex disorder requiring a large spectrum of primary, secondary and often tertiary healthcare services. Many factors, such as health (multisystemic disease), economic (low income), social (poor social support network), and educational (low educational attainment) circumstances, as well as a lack of knowledge of the disease by health-care professionals, warrant the need of a multidisciplinary systematic approach in the management of DM1. Using interdisciplinary teamwork and evidence-based literature review, we developed a disease management program, a well known method of care delivery for several chronic diseases, adapted specifically to DM1. Within the framework of this disease management program, we want to implement an Integrated Care Pathway (ICP), an interdisciplinary care plan outlining the optimal sequencing and timing of interventions. The Saguenay DM1 ICP (SagDM1-ICP) will include multidisciplinary activities, tests, clinical assessments, and decision trees that will help to prompt decisions based on clinical practice guidelines. A presentation of the preliminary version of the SagDM1-ICP and its development procedures will be done along with procedures for the validation process. The challenge is to incorporate all the aspects of the disease including impairments, disabilities as well as social participation and environmental aspects to ultimately improve the quality of life of DM1 patients and their families.

O62
MYOTONIC DYSTROPHY TYPE 2 IN JAPAN: DISTINCT ANCESTRAL ORIGIN FROM CAUCASIAN FAMILIES

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Myotonic dystrophy type 2 (DM2) is an autosomal dominant, adult-onset muscular dystrophy characterized by myotonia and multisystemic features, caused by expansion of the tetranuceotide CCTG repeat in intron 1 of the zinc finger protein 9 (ZNF9) gene on chromosome 3q21. The size of expansion is extremely large and variable, ranging from 75 to 11,000 repeats, with a mean of 5,000. This unprecedented size and somatic heterogeneity of the expansion make the molecular diagnosis of DM2 more complicated. DM2 is also clinically variable, initially described as "proximal myotonic myopathy", or "proximal myotonic dystrophy", or "myotonic dystrophy with no CTG expansion". Further studies suggested that most genetically confirmed DM2 patients were Caucasians, and were considered to arise from a single ancestral origin. No DM2 mutation, to date, has been identified in sub-Saharan or east-Asian populations.

Herein, we report the Japanese family with the DM2 mutation. No consanguinity or genetic admixture with other ethnicities is documented. Three individuals are considered affected in the second generation without a congenital form. The cardinal clinical feature of the proband was a combination of adult-onset proximal muscle weakness and myotonia, consistent with the typical DM2 phenotype.

Cataracts, diabetes, hypogammaglobulinemia, and cardiac conduction defects were also seen. PCR-amplification of the DM2-repeat detected a single normal allele at 228 bp and subsequent Southern blot analysis showed an expanded DM2 allele of 18.1kb (corresponding to 3400 repeat) in the proband. To our knowledge, this is the first DM2 family identified in east-Asian population. Although DM2 mutations were reported in non-European populations including Morocco, Algeria, Lebanon, Afghanistan and Sri Lanka, all reported DM2 patients were considered to arise from a single common founder because they shared an identical haplotype. To investigate the ancestral origin of Japanese DM2, we performed a haplotype analysis of the Japanese DM2 family using SNP and short tandem repeat markers flanking the DM2 CCTG repeat. Our data indicate this family has an expansion-associated haplotype distinct from that commonly found in DM2.

O63
CORRELATION BETWEEN MEASURES OF MUSCLE MASS, STRENGTH, FUNCTION AND QUALITY OF LIFE (QOL) IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1 (DM-1): IMPLICATIONS FOR CLINICAL TRIALS

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Objective. To document the correlations between measures of muscle mass, muscle strength, function and QOL in DM-1. *Background*. Research funding agencies and Regulatory agencies are increasingly requiring clinical outcome measures that are reliable, responsive and reflective of all domains of health and disease in therapeutic trials. For an optimum choice of outcome measures, it is essential that we understand the relationships- both cross sectional and longitudinal- between these measures from various domains. *Methods*. Patients participating in various trials at our center routinely undergo evaluations of muscle mass (DEXA) muscle strength (MMT and MVICT), function

(Timed Function Tests) and QOL (SIP) at baseline and follow up evaluations. *Results.* Data from 50 genetically confirmed DM-1 patients who had undergone all of the above evaluations at baseline were analyzed using Pearson correlation coefficients. Statistically significant (p<.0001) relationships were documented for measures of leg muscle strength (MMT) and time to go 30' (r=.74), ascend and descend 4 steps (r=.60) and SIP – physical domain (= 64) from the cross sectional data. Longitudinal relationships are still being analyzed. *Conclusions.* We have documented the relationships between various outcome measures in DM-1. This allows us to make appropriate informed decisions about the choice of outcome measures for various clinical trial phases.

064
CARDIAC SAFETY AND EFFICACY OF MEXILETINE IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Background. Mexiletine is an anti-arrythmia medication which has shown promise as an anti-myotonia agent in small pilot studies in DM1. Two dosages of mexiletine were given to moderately affected, ambulatory DM1 subjects in a randomized, double-blind, placebo controlled clinical trial. *Methods.* Randomized patients received mexiletine and placebo for alternate 7 week periods. Dosages were 150 mg three times/day (TID) and 200 mg TID (20 pts each). EKGs were obtained before the first drug dose, on the first two days after starting each drug phase, at 3 weeks, and at 7 weeks. *Results.* Patients tolerated the full 7 week course of mexiletine at both doses without any serious adverse events, and there were no significant alterations in EKG measures. Both doses produced significant decreases in grip myotonia (90%-5% relaxation time dropped 38% at 150mg TID, p<.05; 59% at 200mg TID, p<.01). Mean changes are summarized in the following table which compares specific time intervals in resting EKGs at baseline to those obtained after 7 weeks of treatment for each patient (positive and negative values indicate increased and decreased measures, respectively). *Conclusions.* Mexiletine at dosages of 150 mg and 200 mg TID for 7 weeks was safe in terms of cardiac EKG parameters and was associated with reduced grip myotonia in a cohort of 40 patients with DM1.

065
EFFECTS OF MEXILETINE ON MYOTONIA, MUSCLE STRENGTH, AND CARDIAC PARAMETERS IN MYOTONIC DYSTROPHIES OVER TIME

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Objective. To assess the effects of mexiletine on myotonia, muscle strength, and cardiac parameters in patients with myotonic dystrophy types 1 (DM1) and 2 (DM2) over time. *Background.* Although mexiletine's antimyotonic effects and safety are described in patients with DM1, there is no standard treatment for myotonia in myotonic dystrophies. *Methods.* 36 patients with moderately-severe adult DM1 were treated with mexiletine 200mg tid and compared to age-, disease-duration and MRC- matched 34 untreated patients with moderately-severe DM1.10 patients with genetically-determined DM2 were treated with mexiletine 200mg tid and compared to 16 untreated patients with age-,disease-duration and MRC-matched genetically-determined DM2. Manual muscle strength (MRC), myotonia self-assessment scales (0 -5 scale) and cardiac parameters (PR interval; QRS duration,QRSD; heart rate, HR;ejection fraction, EF) were determined before and after long-term mexiletine treatment (DM1: mean treatment duration 7.5 years±3.7; DM2: 5.2±3.5 years). *Results.* Myotonia improved significantly in both treated DM1 and DM2 patients (p>0.0001).MRC decreased significantly in both treated and untreated DM1 and DM2 patients (p<0.001). Initial and final PR, QRSD, HR, EF were similar in the treated and untreated DM1 and DM2 groups. Side-effects were minimal. *Conclusions.* Mexiletine appears to be safe and well-tolerated. The behaviour of cardiac parameters over time seems to be independent of treatment. Myotonia on self-assessment scales proves to improve significantly in both DM1 and DM2. However, our data indicate that mexiletine cannot maintain muscle strength over time. Further studies quantifying myotonia and patient satisfaction reports are needed.

Table. (abstract 064)

Measure (mean change)	*150 mg TID (n=17)*	*Placebo (n=19)*	*200 mg TID (n=19)*	*Placebo (n=18)*
PR (ms)	- 0.9 (p>.75)	- 4.9 (p>.16)	+6.5 (p>.08)	+3.6 (p>.13)
QTc (ms)	+0.9 (p>.87)	+11.2 (p>.17)	+1.5 (p>.73)	- 6.7 (p>.17)
QRSD (ms)	- 0.5 (p>.69)	- 0.4 (p>.83)	+3.3 (p>.05)	+1.2 (p>.21)
Rate (bpm)	+0.82 (p>.75)	-0.95 (p>.66)	0.0 (p=1.0)	- 2.67 (p>.28)

O66
SAFETY AND TOLERABILITY OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR 1 COMPLEXED WITH IGF BINDING PROTEIN 3 (RHIGF1/IGFBP3) IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Objective. To evaluate the safety and tolerability of rhIGF1/IGFBP3 (IPLEXTM, Insmed Inc.) in DM1. *Background.* IGF1 is a potent autocrine/paracrine factor that promotes muscle growth and differentiation. This factor can overcome insulin resistance and deficient protein synthesis in DM1 muscle (Furling *et al.*, 1999). In a previous study, rhIGF1 improved insulin action and muscle atrophy in DM1 patients, although side effects occurred (Vlachopapadopoulou *et al.*, 1995). Therapeutic use of rhIGF1 may be limited by high peak levels and short duration of action. Compared to rhIGF1, rhIGF1/IGFBP3 is a longer acting preparation that may increase rhIGF1 activity with fewer side effects.
Methods. Six DM1 patients were treated with rhIGF1/IGFBP3 for 24 weeks. Dosages were 0.5 mg/kg/day for eight weeks followed by 1.0 mg/kg/day for 16 weeks. During this period, each patient received safety monitoring including serial DEXA scans, physical examinations, EKGs, echocardiograms, urine analyses, chest/neck x-rays, abdominal ultrasounds, and laboratory profiles. *Results.* Patients tolerated the full course of rhIGF1/IGFBP3 without serious adverse events. One patient experienced transient injection erythema. One patient developed gynecomastia. Otherwise, no significant safety concerns were identified. *Conclusions.* rhIGF1/IGFBP3 for 24 weeks at dosages of 0.5 mg/kg/day followed by 1.0 mg/kg/day was safe and well tolerated in a small cohort of patients with DM1. Longer-term randomized controlled trials are necessary to determine the efficacy of rhIGF1/IGFBP3 in DM1.

O67
DEVELOPMENT OF RNA-BASED THERAPEUTIC MODEL SYSTEM FOR MYOTONIC DYSTROPHY (DM)

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Considerable evidence suggests that the DM1 mutation, an expansion of the CTGn repeats in the DM protein kinase gene (DMPK) results in the synthesis of a toxic RNA molecule that causes problems presumably through aberrant RNA-protein interactions. One potential therapeutic approach is to get rid of the toxic RNA from the cells. The RNA molecules (antisense/ribozyme/siRNA) can be designed to specifically knock-down the expression of their target RNAs and have potential applications for RNA based therapeutics. Although improved rational designs have enhanced the ability to generate effective siRNAs, they still do not ensure that a single predicted siRNA will silence the target gene with highest accuracy. To overcome this discrepancy, we are screening numerous siRNAs targeted against the mutant DMPK mRNA in a cell culture model system. In transfection experiments, effective siRNA molecules have been identified on the basis of their ability to reduce the expression of 3'UTR RNA that is fused to a reporter gene (3'UTR RNA of DMPK fused to either lacZ or GFP). These cells and the corresponding phenotypic assays are a quick and powerful tool for efficiently screening potential siRNAs for their specificity against the mutant 3'UTR. This approach allows us to identify not only the most effective RNAs, but also those that produce the partial suppression of the target gene and generate novel, potentially therapeutic reagents. Results from these primary screens will be presented.

O68
OLIGONUCLEOTIDE-MEDIATED SILENCING OF EXPANDED DMPK TRANSCRIPTS IN A DM1 MYOBLAST-MYOTUBE CELL MODEL

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It is generally accepted that DM1 is caused by an RNA-mediated pathogenic cascade, with (CUG)n-expanded DMPK RNA as the "toxin" responsible for the multisystemic features. Hence, expanded DMPK transcripts form a target for therapeutic purposes. We hypothesize that silencing of transcripts from the mutant allele will ameliorate DM1 features in patients.
We have established a conditionally-immortalized SV40-Tagts myoblast-myotube cell model derived from DM500 mice that carry a transgene of the human DM1 locus with 500 CTG triplets. DM500 myoblasts and myotubes express high levels of (CUG)500 human DMPK (hDMPK) transcripts - next to endogenous mouse DMPK (mDMPK) mRNA - and contain typical DM1 nuclear (CUG)n RNA inclusions. We used this model to test artificial antisense oligonucleotides (AONs) with different chemical modifications and sequences for their potential to silence toxic hDMPK RNA expression. By use of Northern blotting and RT-PCR monitoring, we identified oligos that have high specificity towards toxic hDMPK RNA, but leave mDMPK transcripts unharmed. Treatment of DM500 cells with these oligos resulted in reduction of the number of ribonuclear foci and near normalization of aberrant splicing of certain transcripts. Next, we used MyoD-transfected fibroblasts of cDM1 patients to show that one oligo specifically silenced products from the expanded (CTG)1500 DMPK allele, but not the normal (CTG)11 DMPK allele. Currently, we are investigating the cellular mechanism underlying oligo action. Depending on progress, we will also report on AON treatment in DM500 mice.

O69
CORRECTION OF CLC-1 SPLICING ELIMINATES THE MYOTONIA IN MOUSE MODELS OF MYOTONIC DYSTROPHY

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The physiological basis of myotonia in myotonic dystrophy type 1 (DM1) has not been determined. Expression of chloride channel 1 (ClC-1) is reduced in DM1 muscle, but it is unclear whether this can account for the myotonia. The molecular basis of the chloride channelopathy is also unclear. This defect may result from misregulated alternative splicing of ClC-1, owing to inappropriate inclusion of exon 7a (Mankodi, 2002, Charlet-B, 2002), or reduced transcription of the ClC-1 gene (Ebralidze, 2004). To evaluate the role of chloride channelopathy in DM-associated myotonia, and the role of ClC-1 splicing in chloride channel deficiency, we attempted specific repression of exon 7a splicing in mouse models of DM1. A morpholino antisense oligonucleotide (AO) targeting the 3' splice site of exon 7a was injected into tibialis anterior of HSA^{LR} transgenic mice that express CUG^{exp} RNA or $Mbnl1^{\Delta E3/\Delta E3}$ mice that are homozygous for a targeted disruption of *Mbnl1*. Entry of morpholino into muscle fibers was enhanced by electroporation *in vivo*. RT-PCR analysis showed that AO had the intended effect of repressing the inclusion of exon 7a, whereas control oligonucleotide (CO) having similar base composition had no effect. The AO re-established the full-length reading frame in ClC-1 mRNA, upregulated the level of CLC-1 mRNA, restored the expression of ClC-1 protein at the sarcolemma, and eliminated the myotonia, as determined by a blinded EMG examiner. Effects persisted for up to 8 weeks after a single injection. The CO had no effect. These observations indicate that myotonia in two mouse models of DM does indeed represent a chloride channelopathy that results from abnormal alternative splicing of ClC-1. Antisense-induced exon skipping is a viable therapeutic strategy for correcting the alternative splicing defects in DM. This study was supported by University of Rochester Senator Paul D. Wellstone Muscular Dystrophy Cooperative Research Center (NIH/NS48843) and NIH (AR46806).

O70
BIOCHEMICAL SCREENING METHODS TO FIND INHIBITORS OF RNA/PROTEIN INTERACTION: CUGEXP/MBNL1 EXAMPLE

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Interactions between mutant DMPK mRNA and RNA binding proteins are key events in DM1 pathogenesis. For example, MBNL proteins have a high-affinity interaction with the CUG expansion (CUG^{exp}) tract in DMPK mRNA. Sequestration of these proteins in nuclear foci leads to functional deficiency of MBNL1. CUG-BP1 also interacts with poly(CUG) RNA. Although this protein is not sequestered in DM1 foci, upregulation of CUG-BP1 observed in DM1 may result from transient or indirect interactions with CUG^{exp} RNA. Compounds that inhibit CUG^{exp} RNA – protein interactions may therefore provide therapeutic benefit in DM1, if they can selectively release sequestered proteins without blocking other essential RNA-protein interactions. To identify compounds having these properties, we developed and optimized conditions for three biochemical screens, of which two are suitable for high throughput screening of large compound libraries. These screens employ interaction of CUG^{exp} RNA with recombinant MBNL1 protein. The readouts are based on changes of fluorescence polarization, enzymatic complementation of an MBNL1-fusion protein, or filter binding in a plate format. The sensitivity of each assay was tested using inhibitors of CUG^{exp}/MBNL1 complex formation. To the extent that these screens identify CUG^{exp}-binding compounds, they also may identify compounds that inhibit proteins other than MBNL1 from interacting with the mutant DMPK mRNA.

O71
ANTISENSE RNA-BASED GENE THERAPY REVERSES MUSCLE ATROPHY IN A MOUSE MODEL OF MYOTONIC DYSTROPHY TYPE 1

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In previous work, we have developed an antisense RNA capable of lowering preferentially the levels of mutant DM1 transcripts in human primary DM1 myoblast cultures. This effect was associated with a complete restoration of DM1 myoblast functions. To determine whether antisense RNA can target mutant DM1 transcripts *in vivo*, we monitored the effect of a single intramuscular injection of AAV6/ antisense RNA into the tibialis anterior (TA) of DM1 mice bearing 300 CTG repeats. The major TA muscle abnormalities observed in these untreated mice consist in a 30% decrease in the TA volume. In contrast, no splicing abnormalities were observed for the insulin receptor and chloride channel pre-RNAs, as observed in patients with DM1. A 55 to 60% decrease in the levels of the hDMPK mRNAs was observed in the TA, one month a single intramuscular injection of AAV6/antisense RNA. This effect was associated with a 8 to 15% increase in TA muscle volume. Finally, no inflammatory or immune response was observed in the injected muscle. No effect on hDMPK mRNA was noticed in the opposite TA injected with an control antisense. These data showed that antisense RNA can restore up to 50% of muscle volume and provides the rationale for a gene therapy for DM1 based on AAV6/antisense RNA. This work was supported by an AFM grant to Jack Puymirat. Jack Puymirat is the coordinator of a French-Canadian program on DM1

P1
SUSTAINED EXPRESSION OF CUG REPEAT RNA IN DROSOPHILA MUSCLES IS DEGENERATIVE

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Non-coding CUG repeat expansions interfere with the activity of human Muscleblind-like (MBNL) proteins contributing to myotonic dystrophy type 1 (DM1). To understand this toxic RNA gain-of-function mechanism we developed a *Drosophila* model expressing 60 and 480 CUG repeats in the context of a non-translatable RNA. These flies reproduced aspects of the DM1 pathology, most notably nuclear accumulation of CUG transcripts, spliceopathy, and diminished Muscleblind function *in vivo* through aberrant binding to CUG repeats. Here we further explore the histopathology developed by DM model flies. Myosin heavy chain driven expression of 480 CUG repeat RNA to the adult indirect flight muscles (IFM) showed an age-dependent tendency to position wings upheld. These flies were flightless (n=274) and showed alterations in IFM, whereas those expressing (CUG)60 RNA did not (0% flightless, n=204). 2-3 day old flies expressing (CUG)480 RNA developed muscle alterations including vacuolization and reduction in fiber size. We measured cross-sectional area of dorso longitudinal muscle 45e. Average size of muscle 45e decreased to approximately 45% of normal when expressing 480 CUG repeat transcripts. The phenotype was degenerative as 38-day old flies had smaller IFM packages, muscles were occasionally missing, and vacuoles increased in average volume. In contrast, (CUG)60 RNA did not appreciably affect muscle organization. Continued targeted expression of CUG repeat RNA to the IFM, therefore, was degenerative, which constitutes an additional hallmark of DM1 pathology.

P2
CIS-ELEMENTS, DNA REPLICATION AND REPEAT INSTABILITY AT THE HUMAN MYOTONIC DYSTROPHY TYPE 1 LOCUS

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Trinucleotide repeat instability is the cause of a growing number of human disorders including myotonic dystrophy type 1 (DM1). While a variety of DNA metabolic processes likely contribute to instability, the pronounced instability that occurs within proliferative tissues and during rapid proliferation supports a role for DNA replication. The distinct differences in instability between tissues suggest a role for cis-elements. Proliferation was shown to be required for repeat instability in cultured human DM1 patient-derived fibroblasts; this instability was enhanced by agents known to alter DNA synthesis and replication fork progression. We have determined the replication profile at the human DM1 chromosomal locus by quantitative competitive PCR analysis of nascent DNA derived from patient-derived fibroblasts and from transgenic mice with 45 kb of human DM1 locus with 300 CTG repeats. In humans, this analysis suggests that the DM1 locus is located within a region of abundant replication activity, with replication origins located proximal to the repeat tract. Interestingly, reduced replication activity is associated with the expanded DM1 repeat tract and is demarcated by flanking CTCF binding sites. Analysis of transgenic mice indicates differences in the replication profile between tissues. These data support a proximal origin of replication at the expanded unstable DM1 repeat tract such that the CAG repeat is the lagging strand template. Changes in the replication origin location, utilization or replication fork progression may be a significant factor in repeat instability.

P2B
CTG REPEAT INSTABILITY IN TRANSGENIC MICE: INVESTIGATING THE ROLE OF MSH2 AND LIGASE I

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DM1 is associated with a highly unstable CTG repeat in the 3' UTR of the *DMPK* gene. Intergenerational and somatic instability is observed in our transgenic mice carrying a large human genomic DNA fragment, with more than 300 unstable CTG repeats. We have previously shown that DNA mismatch repair (MMR) proteins Msh2 and Msh3 are the major players in the generation of trinucleotide repeat expansions in our model but the mechanism involved remains elusive. In order to define the role of the MMR proteins in CTG repeat instability, we crossed the DM300-328 mice with mutant mice, deficient for the Msh2 ATPase activity (Gua-674-to-Ala). This missense mutation in Msh2 ATPase domain does not affect Msh2 binding to mismatches, but does not allow normal MMR to proceed. Preliminary results showed a decrease in the frequency of expansions in *Msh2 ATPase* mutant mice, for both male and female transmissions. These data suggest that Msh2 binding and the stabilisation of alternative DNA structures are not sufficient to induce the formation of expansions and that MMR activity might be necessary for triplet repeat expansions. Somatic instability is under investigation.To shed light on the CTG repeat expansion mechanisms, we have investigated the role of Ligase I by crossing DM300-328 mice with mutant mice for the *Ligase* I gene (46BR, Arg-771-to-Trp). We obser-

ved that the decrease of Ligase I activity reduced the frequency of expansions upon female transmissions. Neither male transmissions nor somatic instability were affected by the mutation. These results suggest a role for Ligase I in triplet repeat expansion during oogenesis, but not in spermatogenesis or in the soma.

P3
PROGRESSIVE ATROPHY OF THE SKELETAL MUSCLES IN DM1 MICE

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Myotonic dystrophy type 1 (DM1) is characterized by a wide spectrum of clinical manifestations affecting skeletal muscle (progressive weakness, muscle wasting, myotonia). Transgenic mice, carrying the CTG expansion and producing an abnormal human DMPK with 350 repeats has been developed by G. Gourdon's group to study DM1. These mice reproduce some features of the human disease, such as myotonia. In the present study, we have characterized the functional properties of the skeletal muscles of these transgenic mice. A progressive decrease of force production (weakness) with age was measured in the *tibialis anterior* (TA) of the DM1 mice. This weakness is progressive and a significant 30% decrease in the force was measured in 10-month old DM1 mice. However, the ratio force/mass did not seem to be different between non-transgenic and transgenic mice, indicating that the weakness is caused by a loss in muscle mass. To determine if the muscle wasting in DM1 mice is associated with an active process, we have examined by northern blot the expression of the atrogin-1, a muscle-specific ubiquitin-ligase required for muscle atrophy. Our results showed that the expression of atrogin-1 is significantly increased in both 3- and 10-month old transgenic mice when compared to age-matched control. Its expression also increases with age confirming the progressive muscle atrophy of the DM1 mice. In conclusion this mouse model reproduces the progressive muscle atrophy that is observed in human patients and may be used to evaluate therapeutic strategies.

P4
VARIABLES ACTING UPON THE CTG EXPANSION OVER TIME IN DM1 PATIENTS

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Aims of the study. analyse the factors influencing the expansion over time of the CTG repeat in patients with myotonic dystrophy type I.
Patients and methods. 91 DM1 patients with at least two CTG repeat blood measures by Southern blot at different ages. The different DNA samples taken from one given patient were run simultaneously in a gel.
Results. Thirty two patients showed an increase in the CTG repeat in the second and/or posterior DNA samples whereas it remained stable in 59. The initial number of CTG repeats was significantly higher in the patients with an expansion over time (mean= 986 CTG) than in patients with a stable CTG (mean= 586 CTG). Moreover, in the patients who expanded over time, this increase was higher among those carrying the highest CTG expansions. We also observed a higher CTG increase over time in the patients with a higher time interval between blood samples (>10 years). However, nor the patient's age at the first DNA sampling neither the patient's sex or the sex of his parent were related with the CTG increase over time.
Conclusions. an increase over time in the CTG number was observed in 35% of our DM1 patients. Such an expansion is higher among patients showing the highest CTG repeats and with a higher time interval between DNA samplings. The increase is not continuous during lifetime but rather by jumps.

P5
IDENTIFICATION OF ABNORMAL GENE EXPRESSION IN MYOTONIC DYSTROPHY TYPE 1 USING A HUMAN PGD-DERIVED EMBRYONIC STEM CELL LINE EXHIBITING INTRANUCLEAR FOCI

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The existence of hES cells lines derived from embryos discarded at the time of a Pre-implantation genetic diagnosis (PGD) procedure because of the presence of a pathological mutation, offers the opportunity of an *in vitro* modeling of molecular defects associated with a mutated gene.
Here, we took the opportunity of an existing hES cell line affected by Myotonic dystrophy type 1 (DM1) [1] to address early developmental events associated with the mutation.
DM1 is a dominantly inherited multisystemic disorder including myotonia, muscle dystrophy and extramuscular symptoms. The genetic basis is an expanded trinucleotide repeat (CTG)n situated in the 3'-untranslated region (3'-UTR) of the *Dystrophy Myotonic Protein Kinase* (DMPK) gene. Although the exact physiopathological mechanisms leading to DM1 are still not well established, the most common hypothesis states that the enlarged CUG-containing transcripts that accumulate as foci in the nuclei of both cultured cells and biopsy tissue could exert a trans-dominant effect that disrupts splicing and possibly other cellular functions.
In this study, the mutant (VUB03_DM1) and two control (SA01 and VUB01) hES cells were specified towards neural (NPC) and mesodermal precursors (MSC) which would give rise after lineage-specific differentiation to two cell types affected by the pathology, namely the central nervous system and skeletal muscle.
In mutant cells, the accumulation of the abnormal RNA in nuclear foci was detected both in NPC and MSC populations, but not at the undifferentiated stage.
By comparing the transcription profiles of the mutant and two native hES cell lines by a differential screening on

whole-genome Affymetrix DNA chips and by Q-PCR, we identified abnormal expression of 14 genes related to the alteration of the expression of the DM1 mutant gene.
Among the modulated genes, two of them, *INSR* (Insulin receptor) and *SERCA2* (sarcoplasmic/endoplasmic reticulum CA2+ ATPase 2) are already known to be related to DM1. Two others have been found to be implicated in axonal guidance and spatial organization of the neuromuscular junction, suggesting a defect already in the development process, even though the clinical onset of DM1 (related to the mutant present in the cell line) occurs close to adulthood. One gene is a member of the slit family and the other an ephrin receptor.
In conclusion, the analysis of the mutant cells revealed that the impact of alteration the gene expression was detected at early stages of the developmental disease process and allowed the identification of new potential molecular mechanisms of the disease. These abnormally expressed genes may also represent a valuable tool to search for treatments by high throughput screening of potentially therapeutic compounds.

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Reference

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P6
CUG REPEATS IN DM1 ARE LOCATED WITHIN A RETAINED INTRON OF THE DMPK 3'UTR

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A trinucleotide repeat expansion in the 3' untranslated region of DMPK is responsible for type 1 myotonic dystrophy. Here, we report that the CUG repeats are entirely contained within a retained intron in the DMPK 3'UTR. The 5' (donor) splice site of this intron is relatively weak (Maximum Entropy Splice Site Score = 1.6 bits) and occurs in human, chimpanzee, and dog. The 3' (acceptor) splice site, which has been described previously in an exon-skipping event, is of medium strength (6.7 bits) and occurs in human, rhesus, and chimpanzee. We have observed that the non-retained isoform accounts for approximately 5-10% of total DMPK mRNA in human cells, and are currently assessing intron retention levels in DM1 cells. We are also evaluating whether decreasing the retention of this CUG repeat-containing intron can lessen the myotonic dystrophy phenotype, as assessed by morphological changes, gene expression, and pre-mRNA splicing in a C2C12 cell culture model of myotonic dystrophy. These findings suggest a potential route towards DM1 therapy in which intron retention levels in the 3'UTR of DMPK are modulated.

P6B
HUMAN DNA LIGASE I IN THE REPLICATION, REPAIR & INSTABILITY OF CTG/CAG REPEATS

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Instability of (CTG)•(CAG) repeats, the genetic basis for myotonic dystrophy, can arise during DNA replication, repair or recombination. Sealing of nicks by DNA ligase is a final step in these processes. Of the five mammalian ligases, ligase I (LigI) has been demonstrated to act at replication forks. LigI-deficiencies arrest fork progression. We investigated the role of human LigI in the replication of CTG/CAG repeats, the processing of slipped-CTG/CAG intermediates of instability and repair of G-T mismatches. We used extracts of cells derived from a patient (46BR) containing a mutant LigI (Arg771-to-Trp) retaining only 3-5% of normal activity. Repeat tracts were particularly sensitive to replication by the defective LigI, yielding increases of aberrantly processed Okazaki fragments and reduced replication efficiencies. This effect was highly sensitive to replication direction with CTG as the worst lagging strand template, and CAG being poor but worse than a non-repetitive DNA control. Replication-mediated instability on contraction-biased templates (CTG as lagging strand template) and expansion-biased templates (CAG as lagging strand template) was unaffected by LigI status. However, any instability events that did not complete replication would not have been scored. Repair of slipped-CTG/CAG intermediates and G-T mismatches was enhanced by cell extracts deficient in LigI compared to extracts with a wildtype LigI. The reduced repair efficiency in the presence of the wildtype LigI is consistent with a nick requirement for repair. Together our results suggest that if LigI is involved in the expansions or contractions of CTG/CAG repeats it's role may be exerted in a replication-dependant manner, in that sites of replication initiation during developmental windows or in specific tissues displaying instability may be more susceptible to LigI-mediated mutations or completion of replication.

P7
THE P16 PATHWAY MEDIATES PREMATURE SENESCENCE OF DM1 MYOBLASTS

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Large CTG repeats affect the differentiation program of the myogenic precursor cells. Molecular alterations induced by the CTG mutation were retained in satellite cells isolated from congenital cDM1 muscles as shown by the presence of nuclear foci, splicing defects and somatic instability of the CTG expansion. We showed that the proliferative capacity of the cDM1 myoblasts was significantly reduced when compared to non-affected cells. However the proliferative arrest of cDM1 myoblasts is not correlated with an excessive reduction of the telome-

re length since the cDM1 myoblasts stop dividing with larger telomere than control. This result indicates that the cDM1 myoblasts have not exhausted their proliferative capacity but have a premature replicative arrest. Analysis of several markers suggests that a mechanism of premature senescence triggers this early arrest. We found that an early accumulation of the cdk inhibitor p16 is associated with this phenotype in cDM1 cells. We show that an inactivation of p16 activity in cDM1 myoblasts inhibits premature senescence and restores their proliferative capacity. cDM1 cells with inactive p16 achieve the same number of division than that of control cells indicating that early activation of p16 pathway is responsible for premature senescence of cDM1 cells. In addition, we have measured an accelerated telomere shortening at each division in cDM1 myoblasts. The re-expression of the telomerase, the enzyme that extents the telomere, in cDM1 myoblasts seems to lead to an increase of their proliferative lifespan. Altogether, our results indicate that telomeric dysfunction could be responsible for the early activation of p16 in cDM1 cells leading to the premature senescence of DM1 myoblasts.

P8
DIFFERENTIAL EXPRESSION OF SPLICING REGULATORS AND EFFECTS OF CUG REPEATS IN DM1 CEREBRAL CELL MODELS

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To gain a better understanding of the pathological mechanisms that lead to a modified alternative splicing of Tau in DM1 brains, we looked for cellular models of cerebral-type able to illustrate these mechanisms. 6 isoforms of Tau are expressed in a control adult human brain whereas only the isoform lacking the 3 alternative exons 2, 3 and 10 is expressed in foetal brain. Interestingly, in adult DM1 brain, only the foetal Tau isoform is expressed.
Here, we show that human neuronal cells (undifferentiated or Retinoic acid-differentiated SY 5Y, NT2 and Kelly cells) mainly synthesized the foetal Tau RNA form, whereas human glial cells (T98, CCF-STTG1 and U118) as well as control (HeLa) cells expressed the adult Tau RNAs. This difference in Tau alternative splicing pattern suggested the existence of a different pool of splicing regulators of Tau. Nevertheless, no striking differences in the expression of regulators reported to influence Tau splicing could be evidenced among the factors studied. Only factors (ETR-3, CELF-4 and MBNL1) belonging to the two families of splicing regulators involved in DM1 were differently expressed in the various cell-lines. ETR-3 and CELF4 transcripts were detectable only in neuronal cells, and agreed with the role of ETR-3 in Tau exon 2 exclusion. Moreover, as reported for control/DM1 brains, MBNL1 splicing pattern differed between cells: the expression of foetal MBNL1 transcripts was increased in neuronal cells compared to other cells. Interestingly, a reduced inclusion of Tau exons 2/3 using Tau minigenes was induced by CUG960 repeats in all cell-types whatever their endogenous Tau splicing profile was. Effect of CUG960 repeats on the splicing regulators will also be described. In conclusion, our preliminary results suggested that the comparison between glial and neuronal cells constitutes an interesting approach for studying alternative splicing alterations implicated in DM1 brain.

P9
VISUALIZATION OF THE ALTERNATIVE SPLICING IN LIVING CELLS

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To discover therapeutic tools for myotonic dystrophy, it is important to develop a rapid and convenient screening system to detect aberrant alternative splicing events, especially in living cells. Here we developed the "alternative splicing detector" which is designed for the alternative expressions of fluorescent proteins. The "alternative splicing detector" plasmids were constructed with a minigene including known alternative splicing exons, for example Exons 10-12 of insulin receptor gene, and cDNA of fluorescent proteins inserted to the exons. After transfection of these plasmids, fluorescent signals will be detected. By an alternative splicing, distinct fluorescent proteins will be expressed. This system will allow the rapid screening of drugs, which could normalize aberrant splicing events, and give insight to the mechanisms of the alternative splicing.

P10
IN VITRO STUDY OF DM1 PRIMARY MYOTUBES

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Myotonic dystrophy 1 (DM1) is a complex multisystemic disorder linked to a (CTG)n expansion in 3'-UTR region of the DMPK gene. In muscle tissue myotonia and insulin resistance have been associated to altered splicing of chloride channel-1 (ClC-1) and insulin receptor (IR) mRNAs, due to pleiotropic effect of mutant DMPK.
Many publications claimed an inhibition of differentiation in DM1 myoblasts, maybe due to anomalous RNA-protein interactions compromising many key myogenic factors. In our laboratory primary human cell lines from DM1 patients with different $(CTG)_n$ (from 180 to 1500) and one from a congenital DM1 patient were established and differentiated into polynucleated myotubes. *In vitro* muscle maturation of DM1 primary cells was monitored by morphological and molecular analysis in undifferentiated and 10 days differentiated (aneural) myotubes. The increased myogenic markers expression was correlated with the splicing pattern of IRA/B and MBNL1 genes in myoblasts, aneural myotubes and DM1 mature muscle.
Further maturation was reached by innervation with rat embryo spinal-cord (mature myotubes). Innervated myo-

tubes were tested by FISH and immunohistochemistry. We observed that differentiation and innervation of DM1 primary myoblasts were unaffected by the CTG expansion and that both aneural and mature myotubes express pathological hallmarks of DM1 muscle. Therefore we conclude that *in vitro* culture of DM1 muscular cells are a useful tool for the study of pathophysiological mechanisms in DM1 affected tissue.

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P11
WOODCHUCK POST-TRANSCRIPTIONAL REGULATORY ELEMENT INDUCES NUCLEAR EXPORT OF MYOTONIC DYSTROPHY TRANSCRIPTS AND REPAIRS MUSCLE CELL DIFFERENTIATION

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CTG trinucleotide repeat expansions in the 3' untranslated region (3' UTR) of the myotonic dystrophy protein kinase (DMPK) gene are responsible for the myotonic dystrophy (DM). Mutant DMPK transcripts aggregate in the nucleus and are thought to trigger dominant effects by interacting with RNA binding proteins, which among other things result to the inhibition of muscle cell differentiation. The woodchuck post-transcriptional regulatory element (WPRE) is a cis-acting module that can enhance transgene expression at the post-transcriptional level. It is believed that when inserted downstream of a cDNA, it may improve gene expression by increasing RNA export. Since the major mechanism of DM pathogenesis is the nuclear retention of mutant DMPK transcripts, WPRE was exploited to transfer the mutant transcripts to the cytoplasm. Therefore, a series of constructs were produced expressing the wild type or the mutant DMPK 3' UTR with or without the WPRE sequence. Following transfection of these constructs in mouse C2C12 myoblasts, RNA and protein analysis was carried to detect transcript transport to the cytoplasm and alterations in muscle differentiation, respectively. WPRE stimulated the export of DM transcripts to the cytoplasm of myoblasts and repaired defective muscle cell differentiation. Current work aims at determining the molecular changes which occur as a result of the nucleocytoplasmic transport of the mutant DMPK RNA molecules. Furthermore, by collecting a large number clones expressing the mutant DMPK 3' UTR and WPRE sequence, the effect of nuclear or cytoplasmic mutant DMPK intensity on the inhibition of differentiation is currently being determined. This is the first demonstration of a mechanism by which the nuclear retained transcripts were liberated to the cytoplasm. The exploitation of this powerful method would be beneficial for the investigation of DM.

P12
ANALYSIS OF MTMR1 PRE-MRNA SPLICING IN DM1 AND DM2 MUSCLE BIOPSIES

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We analyzed *MTMR1* pre-mRNA splicing pattern by RT-PCR studies in muscle biopsies from adult-onset myotonic dystrophy type 1 (DM1), type 2 (DM2) patients and controls.
Moreover, we analyzed MBNL1 and CUG-BP1 protein levels by Western blot analysis.
Our results showed the occurrence of aberrant *MTMR1* splicing in both forms of myotonic dystrophy compared to controls, characterized by an increase of the fetal A and B versus the adult *MTMR1* C mRNA isoform. Furthermore the appearance of an aberrant G isoform was detected in DM1 patients carrying expansions greater than 1200 CTG in leukocytes.
CUG-BP1 steady-state levels were significantly increased only in DM1 muscle biopsies in comparison to controls, while the levels of MBNL1 1 resulted comparable in the three groups studied.
This study indicates the occurrence of a mis-regulation of *MTMR1* pre-mRNA splicing not only in DM1 but also in DM2 muscle tissues, confirming the existence of a common pathogenic mechanism for both forms of myotonic dystrophy related to the abnormal splicing of specific pre-mRNAs caused by the toxic gain of function of RNAs transcribed from the expanded alleles.
CUG-BP1 does not appear to modulate the aberrant *MTMR1* expression in DM adult muscle tissues, since the mis-regulation of *MTMR1* pre-mRNA was evident in both forms of myotonic dystrophy, despite the presence of significant differences in CUG-BP1 levels between DM1 and DM2 muscle biopsies. Nevertheless, the higher steady-state levels of CUG-BP1 detected only in DM1 muscle tissues supports that its increase contributes to the pathogenesis in this form of myotonic dystrophy.

P13
OXIDATIVE STRESS IN DM1: ROLE OF NFKB AND RELATED PROTEINS

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Myotonic dystrophy type 1 (DM1) is a complex multisystemic disorder characterized by myotonia, facial and distal limb muscle weakness, cataracts, frontal alopecia, testicular atrophy, cardiac conduction abnormalities, and diabetes mellitus. The pathogenic mechanisms of multisystemic involvement of DM1 are still unclear but recently it has been postulated that the pathogenesis is due to aberrant regulation of RNA alternative splicing. Expanded CTG repeats in DMPK gene have been demonstrated to increase the susceptibility of cells to oxidative stress. We investigated in the muscle samples from 16 patients with DM1, the activation of nuclear transcription

factor NF-κB, the activity of glutatione peroxidase (GPX) and hydrogen peroxide (HP). We studied also the expression of thioredoxine peroxidase (TPX), a CUG rich protein, which is induced by NF-κB and involved in the response to oxidative stress. Cytoplasmic immunoreactivity for the activated form of NF-κB was found in all regenerating fibers and in 30 to 35% of necrotic fibers. NF-κB-DNA binding activity studied by electrophoretic mobility shift assay (EMSA) was found 13-fold increased in DM1 patients. GPX activity and HP were increased and correlated with the CTG expansion. The expression of TPX resulted reduced in DM1 muscles suggesting a disturbed regulation at the trascription level. Our data suggest that the increased oxidative stress in DM1 muscles could be due to altered expression of antioxidant enzymes, such as TPX, which are induced by NF-κB.

P14
COMPARATIVE STUDIES OF DM1 AND DM2 IN MUSCULAR BIOPSIES

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Myotonic dystrophy (DM) is a heterogeneous condition which includes two forms, DM type 1 (DM1) and DM type 2 (DM2). In addition to muscle signs, such as myotonia, muscle wasting and weakness, they share other common clinical features, as hyperinsulinemia and intolerance to glucose, heart conduction defects, cataract, and hypogonadism.

In DM1 and DM2 the two causative mutations reside in different genetic loci and the two conditions are similar, but clinically distinguishable. It has been suggested that they derive from a common pathogenic mechanism, since several mutant RNA transcripts undergo altered alternative splicing. This study is directed to investigate possible differences in DM1 and DM2.

We performed an immunoblot analysis of DMPK and TnT proteins and an expression study of insulin receptor RNA in muscle biopsies from DM1 and DM2 patients.

We found that: a) the expression level of DMPK was decreased in DM1 but unchanged in DM2 muscles; b) the proportion between troponin T isoforms was significantly unbalanced toward higher molecular weight species in DM1 rather than in DM2; c) the RNA coding for the insulin-independent isoform of insulin receptor was significantly increased in both conditions and depended on fibre type composition.

The findings of our comparative study on the complex biochemical aspects of the two diseases underline some molecular and biochemical differences between DM1 and DM2.

P15
ABNORMAL EXPRESSION OF DMPK SUBSTRATE PHOSPHOLAMBAN IN DM2

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Introduction. Myotonic dystrophy type 1 (DM1) is caused by an expanded (CTG)n repeat sequence in the 3´ untranslated region of a protein kinase (*DMPK*) gene in chromosome 19q13.3. The role of DMPK for pathogenesis in DM1 is unsettled. Myotonic dystropy type 2 /PROMM (DM2) is caused by an expanded (CCTG)n repeat sequence CCTG in intron 1 of the gene ZNF9 in chromosome 3q21.3. DMPK is involved in Ca^{2+} metabolism of muscle cells by phosphorylating phospholamban. Phospholamban is a transmembrane protein that interacts with SERCA1 to inhibit its activity at low Ca^{2+} concentrations. SERCA1 is expressed in fast muscle fibers, of which a subset is highly atrophic in DM2. *Objectives*. The objective of this study was to study the pathomechanisms in DM2 disease by comparing the pattern and level of phospholamban expression in DM1, DM2 and normal muscle samples. *Methods*. Expression pattern and levels of phospholamban in DM muscle tissue was studied with gene expression profiling (Affymetrix platform), immunohistochemical and Western blot methods. *Results*. Phospholamban mRNA levels are highly upregulated in DMs. Nevertheless, on protein level the membrane expression was weaker in DM2 samples compared to the controls, and the perinuclear localization of phospholamban present in normal muscle samples was absent in DM2 samples. *Conclusions*. The molecular pathogenesis of myotonic dystrophies has been associated with aberrant splicing of other genes caused by accumulated mutant RNA-expansions. Previously abnormal splicing of genes involved in Ca^{2+} metabolism such as RYR1 and SERCA1 has been shown. Our results indicate an abnormality in the expression of phospholamban in DM2 that may be part of a larger Ca^{2+}-handling problem in DM2.

P16
DIFFERENCES IN ABERRANT EXPRESSION AND SPLICING OF GENES INVOLVED IN CA^{2+} METABOLISM BETWEEN DM2 AND DM1

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Aberrant splicing of multiple *effector* genes has been sug-

gested to cause the complex phenotype in DM1 and DM2. However, the reasons for muscle weakness and wasting, and the differential muscle and muscle fiber type involvement in these two diseases are not understood.
Since Ca2+ plays a central role in muscle function, we focused on the genes and proteins involved in Ca2+ metabolism, including Ca2+ channels, Ca2+ binding proteins, and transcription factors regulating their expression. The purpose of the study was to identify abnormal expression and splicing, with special emphasis on differences between DM2 and DM1 to explain their divergent muscle pathologies. mRNA expression and splicing was analyzed by microarray expression profiling and RT-PCR, while protein expression was studied with immunohistochemistry and western blotting, using muscle biopsy material or primary myoblasts. *SERCA1* and *RYR1* were aberrantly spliced in both DM2 and DM1. However, the relative proportion of the abnormal isoforms for both was greater in DM2. In addition, a number of other genes involved in Ca2+ regulation, such as *CASQ1*, showed abnormal expression in these diseases. While DM1 and DM2 share many aspects of muscle pathology, there are specific differences in histology, as well as muscle and fiber type involvement. Here, we present molecular differences in both expression and splicing of genes involved in muscle Ca^{2+} metabolism, including channels and regulatory proteins, that correlate with these phenotypic differences.

P17
A VARIABLY SPLICED REGION OF RYANODINE RECEPTOR 1 MAY BE INVOLVED IN EXCITATION-CONTRACTION COUPLING

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We have recently reported that the juvenile splice variant (ASI(-)) of ryanodine receptor 1 (RyR1) is over expressed in adults suffering from Myotonic dystrophy type 1 (DM1) and that the recombinant ASI(-) RyR1 is less active than the ASI(+) RyR1. We have further shown, using peptide competition studies, that the ASI region of the RyR1 is likely to contribute to an inhibitory inter-domain interaction. Peptides ASI+/- include highly positively charged residues similar to */A/* region of dihydropyridine receptor (DHPR) II-III loop which has been reported to activate RyR1. In this study we tested if ASI peptides are structurally and functionally equivalent to the */A/* peptide of the DHPR II-III loop. NMR studies showed that both ASI and */A/* peptides contain an a-helical structure with a similar alignment of basic residues. The ASI peptides activated RyR1 in a similar manner to the */A /*peptide. Substitution of neutral residues for some of the basic residues prevented its activation of RyR1. Competition studies showed that peptide */A/* prevented activation by the ASI peptides. The results suggest that the */A /*region of the II-III loop activates the RyR1 by interrupting an inter-domain interaction between the ASI region and other binding partners. The DHPR II-III loop is critical for excitation-contraction (EC) coupling where depolarization of the transverse tubule membrane is transmitted by DHPR to the RyR1. We therefore speculate that the inter-domain interaction is involved in EC coupling.

P18
THE CTG REPEAT EXPANSION SIZE CORRELATES WITH THE SPLICING DEFECTS OBSERVED IN MUSCLES FROM MYOTONIC DYSTROPHY TYPE 1 PATIENTS

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A typical features of myotonic dystrophy type 1 (DM1) tissues is the disruption of the normal developmental regulated splicing, which results in the preferential expression of fetal isoforms deleterious for adult tissues. In this study, we investigated the effects of CTG expansion length on the degree of splicing misregulation and foci formation in muscles from DM1 patients. To this purpose, we selected 6 muscle biopsies from DM1 patients with an expansion below 500 repetitions (group A) and 6 muscle samples from DM1 patients carrying a mutation up to 1000 CTGs (group B). RNA-FISH experiments on muscle sections, showed a clear correlation between the number of ribonuclear foci and the severity of DM1 mutations. Splicing analysis of the IR, Clcn1, MBNL1 and SercA1 genes demonstrated that the level of aberrant splicing isoforms is strikingly increased in group B patients, which also present a more severe muscle impairment. In addition, a significant correlation was also observed in the extent of abnormal splicing of all the gene analyzed, suggesting that a common mechanism might contribute to their splicing misregulation. These data indicate that the CTG repeat length as a direct effect on the spliceopathy typical of DM tissues and provide a useful link between the genotype and the clinical phenotype of DM1 patients.

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P19
GENE EXPRESSION ANALYSIS IN MYOTONIC DYSTROPHY: INDICATIONS FOR A COMMON MOLECULAR PATHOGENIC PATHWAY IN DM1 AND DM2

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The most accepted explanation for DM1 and DM2 pathogenesis is a change of function of DMPK and ZNF9 due to expanded RNAs retained in the nucleus and accumulating in ribonuclear foci. To analyze other possible molecular differences/similarities in the pathogenetic mechanisms leading to both disorders, we compared the expression profile of muscle biopsies from DM1 (n=4) and DM2 (n=4) patients to other control subjects (n=4). DM muscle tissues showed an abnormal splicing pattern of the *Clcn1* and *IR-A* genes and a marked reduction in *Clcn1* and *Sp1* transcript levels. No essential differences were observed in the *MBNL1*, *CUGBP1* levels as well as in the splicing pattern of the *MTMR1* gene. Then, we performed macroarray analyses to profile expression of 96 genes encoding muscular/CNS-related ion channels and transporters. The results of macroarray analyses reveal that DM1 and DM2 have a similar expression profile, which reflects a common muscle pathology origin. The differential expressed genes are important in calcium and potassium metabolism, anions transport and in the mitochondrial functions. Our results indicate that the DM1 and DM2 overlapping clinical phenotypes may derive from a common trans acting mechanism that traps and misregulates shared genes and proteins. However, additional studies are necessary to clarify how their alteration acts synergistically with the CLCN1 reduction and leads to EMG myotonia, which is more pronounced in DM1 compared to DM2 patients.

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P20
ZEBRAFISH KNOCK-DOWN MODEL FOR MUSCLEBLIND-LIKE 2

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Muscleblind-like (MBNL) is a family of three zinc finger proteins which have been implicated in the pathogenesis of Myotonic Dystrophy (DM). Transcribed repeats are retained within the nuclei of DM cells, appearing as foci associated with MBNL1, 2 and 3. It has been suggested that the sequestration of MBNL proteins by repeat expansion transcripts plays a key role in determining DM pathophysiology, including splicing abnormalities.
We have examined the *in vivo* role of *mbnl2* using a loss-of-function approach. We have showed that introducing *mbnl2* translation-blocking antisense molecules (morpholinos) in zebrafish embryos results in a dose-dependent specific phenotype, which consists of eye, brain and somite defects, as well as abnormalities of cardiac structure and function. The movements of *mbnl2*-morphants are restricted and uncoordinated, with exhaustion of avoidance response. Knockdown of zebrafish *mbnl2* produces splicing abnormalities and muscle defects, similar to those observed in DM. We were able to rescue this phenotype by the introduction of either the zebrafish or the human version of MBNL2.
The above results indicate that the sequestration of MBNL2 is important and must be considered when developing therapeutic approaches to DM. Additionally, a broader understanding scope of *mbnl2* function and relevance during development was derived from our studies.

P21
FUNCTIONAL STUDIES OF MUSCLEBLIND-LIKE PROTEIN 1 (MBNL1)

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Increasing evidence suggests that the molecular mechanism that underlies Myotonic Dystrophy pertains to the toxic expanded RNA repeats in cells of DM patients. Expanded $(CUG)_n$ and $(CCUG)_n$ RNAs are retained in DM1 and DM2 cells, respectively. Muscleblind-like proteins (MBNL1-3) are sequestered in DM cells by these expanded RNA transcripts and this functional inactivation of MBNL is believed to result in several developmentally-regulated alternative splicing defects, since MBNL proteins are important regulators of alternative splicing. However, the exact mechanism by which MBNL proteins regulate alternative splicing remains unknown so far. In order to get a clearer picture of MBNL1 function, we studied its sub-cellular localisation in a series of experiments that suggest a unique role of MBNL1 in shuttling. Furthermore, we have also tried to identify proteins that interact with MBNL1 using high throughput yeast two-hybrid analysis. Several MBNL1 interacting proteins have been identified and are currently being validated. We will report the outcomes of both studies.

P22
CHARACTERIZATION OF PROTEINS THAT BIND TO THE CUG REPEATS

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Muscular dystrophy type 1 (DM1) is a neuromuscular disease caused by a CTG expansion in the 3' untranslated region of the DMPK gene. One major feature in DM1 patients is the specific misregulation in alternative splicing of several pre-mRNA expressed in muscle and brain (cardiac troponin T, insulin receptor, muscle-specific chloride channel, tau ...). It has been proposed that the expansion of CTG sequesters and induces modifications of RNA binding proteins that are required for splicing. Two classes of proteins have been identified: the muscleblind-like proteins (MBNL) and the CUG-binding proteins (CUG-BP1). Both types of proteins have been shown to regulate splicing of several transcripts that are misregulated in DM1 patients. However, several observations argue for a more complex model than the one involving the sole MBNL and CUG-BP proteins. Thus, we have decided to investigate the repertoire of proteins in myogenic nuclear extracts that assemble onto CUG repeats using affinity chromatography. Several plasmids containing CUG or CAG repeats of different length (from 14

to 95 repeats) have been constructed. After transcription, the RNAs have been biotinylated and bound to streptavidine agarose. Beads were incubated with nuclear extracts and after washing, the proteins were analysed onto SDS-PAGE or 2D-gel electrophoresis. Preliminary results show that several proteins are specifically associated with the repeats according to the myogenic differentiation. Work is currently in progress to identify these proteins.

P23
COLOCALIZATION OF RIBONUCLEAR INCLUSIONS AND MBNL1 FOCI WITH NO IMPAIRMENT OF IN VITRO ADULT DM1 AND DM2 MYOBLASTS DIFFERENTIATION

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Myotonic dystrophies (DMs) are repeat expansion diseases in which expanded CTG (DM1) and CCTG (DM2) repeats cause the pathology. Mutant transcripts aggregate in multiple ribonuclear inclusions (RIs) which can bind specific RNA-binding proteins, such as muscleblind-like proteins (MBNLs), leading to a reduction in their activity. MBNLs are homologous to *Drosophila* muscleblind proteins that are essential for the terminal differentiation of muscle and photoreceptor. In our study we have examined the nuclear accumulation of mutant mRNA and the distribution of MBNL1 expression during DM1 and DM2 myoblasts differentiation, and whether these inclusions impair early and late muscle differentiation. Human satellite cells were isolated from muscle biopsies of adult DM patients (3 DM1; 4 DM2) and from four healthy subjects used as controls. By using FISH in combination with MBNL1-immunofluorescence (IF) it appears that both in DM1 and DM2 cells MBNL1 nuclear foci, which represent high concentration of protein, co-localize precisely with RNI both at myoblast and myotube stage. No RIs or MBNL1 nuclear foci are present in control cells. Moreover, IF and Western Blot analysis, showed no abnormalities in the expression of several markers of muscle differentiation in sister pathological cultures as compared to controls. MBNL1 nuclear sequestration by RIs at myoblast and myotube stage seems to not impair the *in vitro* DM muscle differentiation.

P24
A PUTATIVE ROLE OF RIBONUCLEAR INCLUSIONS AND MBNL1 IN THE IMPAIRMENT OF GALLBLADDER SMOOTH MUSCLE CONTRACTILITY WITH COLELITHIASIS IN MYOTONIC DYSTROPHY TYPE 1

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant multisystemic disorder characterized by myotonia, progressive muscular weakness and wasting. However, other organs and systems may be involved. DM1 is caused by expansion of unstable trinucleotide (CTG) repeats at 3' untranslated region of the DMPK gene on chromosome 19q13.3. Mutant transcripts are retained in muscle nuclei producing ribonuclear inclusions (RIs) which interact with RNA-binding proteins, such as muscleblind-like protein 1 (MBNL1), leading to a reduction in their activity. The reduced MBNL1 activity has been associated to skeletal and cardiac muscle dysfunction. It has been reported that 25 to 50% of DM1 patients have abdominal symptoms due to cholelithiasis or gallstones. Since impaired gallbladder motility plays an important role in gallstones formation, we have analyzed by FISH combined with MBNL1-immunofluorescence, the gallbladders obtained from a woman affected by DM1 who required a cholecystectomy at the age of 30. Gallbladders obtained from two no-DM1 subjects have been used as control. IRs and MBNL1 foci accumulate and colocalize in nuclei of DM1 gallbladder smooth muscle cells. Slides from control subjects were completely negative. These results suggest that nuclear accumulation of MBNL1 and IRs may have a direct adverse effect on gallbladder smooth muscle contractility and thus contribute to gallstones formation in DM1 patients.

P25
STUDY ON DIFFERENTIAL BINDING PROPERTIES OF MBNL1 ISOFORMS AND OF CELF PROTEINS ON RNAS CONTAINING CUG REPEATS OR POTENTIAL SPLICING TARGET SEQUENCES

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Splicing factor MBNL1 exists as a series of alternative splicing isoforms in human cells. In order to compare their RNA binding properties, we performed an *in vivo* three-hybrid study, using a wide range of RNAs, corresponding to double-stranded repeated sequences or single-stranded potential splicing target sequences. Our results show that all MBNL1 isoforms interact with CUG repeats. We also tested the relative binding affinities of MBNL1 isoforms on SELEX selected RNAs (see poster Vautrin *et al*). As this SELEX study was performed with a C-ter truncated recombinant MBNL1 protein, we tested the interaction of the full length MBNL1 protein and of the different MBNL1 isoforms with the same RNAs. Data of three-hybrid are in good agreement with the results of gel-shift assays performed with the recombinant C-ter truncated MBNL1. Moreover, the C-ter end of MBNL1 does not seem to be required for RNA/protein interactions, since identical three-hybrid results were obtained with both truncated and full length MBNL1 proteins.

Interestingly, we observed a significative difference of binding properties of isoforms containing or not exon 4, suggesting that the distance between the zinc finger motifs 2 and 3 can be important for RNA binding. Concerning CELF protein family, none of the RNAs tested was recognized using the three-hybrid test, except UG repeats. We also showed that the RRM2 motif and 70 a of the divergent domain of CUG-BP1 are sufficient to ensure RNA binding *in vitro*.

P26
STUDIES ON THE RNA RECOGNITION PROPERTIES OF MBNL1 AND CUG-BP1

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Aberrant alternative splicing of several pre-mRNAs has been reported to contribute to some of the DM1 symptoms. In many cases, the MBNL and CELF protein families have been involved in the alternative splicing regulation of the affected pre-mRNAs. The goal of our study is to bring information on alternative splicing regulation of pre-mRNAs by studying the RNA recognition properties of MBNL1 and CUG-BP1. We first focused our study on natural RNA targets of MBNL1 and CUG-BP. By *in vitro* transcription, we produced RNAs containing from 16 up to 51 CUG repeats, as well as fragments of the human cardiac troponin T pre-mRNA, which contain an exon regulated by the CUG-BP/MBNL protein pair. The CUG-BP and MBNL binding sites in the different tested RNAs were mapped by footprinting assays, site-directed mutagenesis and gel-shift experiments. Our second strategy for a better definition the RNA binding properties of MBNL1, was to perform a SELEX experiment using a library of RNAs with a 18 nucleotide randomized sequence and a C-ter truncated recombinant MBNL1 protein. The truncated version of MBNL1 contains the 4 Zn fingers required for RNA binding. After 6 rounds of selection we identified several RNA sequences with strong affinity for the C-ter truncated MBNL1. The affinity of MBNL1 for these RNAs was confirmed by gel-shift experiments and the RNA determinants for MBNL1 binding are currently investigated by site-directed mutagenesis. Analysis of the secondary structure and MBNL1 footprinting is underway for the different selected RNAs. The sequence identified will be used for a computer-assisted human genome screen to identify other pre-mRNAs regulated by MBNL1.

P27
RNAI-MEDIATED SILENCING OF DROSOPHILA MUSCLEBLIND

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Expanded CTG repeats in the 3' untranslated region of *DMPK* cause Myotonic Dystrophy (DM). A biochemical hallmark of the disease is misregulated alternative splicing of defined pre-mRNAs. Human Muscleblind-like (MBNL1-3) proteins are alternative splicing regulators that have been shown critical to the pathogenic pathway. MBNL proteins aberrantly bind mutant *DMPK* transcripts being depleted from their physiological targets. *Drosophila muscleblind* primary transcripts undergo alternative splicing giving rise to four transcript isoforms encoding proteins MblA, MblB, MblC and MblD. Mature transcripts share the first two exons, including the beginning of the open reading frame, but differ in the use of downstream exons. We previously showed that Muscleblind isoforms are not functionally redundant in *Drosophila* thus suggesting that may perform different molecular roles. In order to uncover these potentially distinct molecular functions we are developing transgenic flies that express isoform-specific interfering RNAs as well as control flies expressing double-stranded RNA complementary to a common region. These constructs are under the control of the binary Gal4/UAS system that allows targeting RNA interference to specific tissue types. We have already found that RNAi-mediated silencing of *Drosophila muscleblind* enhances toxicity to CUG repeat RNA in *Drosophila* flies, which supports that the silencing of *muscleblind* is effective. An update on isoform-specific phenotypes will be provided at the meeting.

P28
THE FUNCTION AND EXPRESSION OF K02H8.1 (CEMBNL), THE OLTHOLOG OF MAMMALIAN MBNLS

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The nematode Caenorhabditis elegans has an ortholog to mammalian MBNLs. The ortholog, K02H8.1, has a homology of around 50% to human MBNLs. K02H8.1 has two zinc finger domains like fruitfly muscleblind, although human MBNLs have four zinc finger domains.
To characterize the function of K02H8.1, we performed the yeast three-hybrid experiment to characterize the binding of K02H8.1 to bait RNAs. We found that binding affinities of K02H8.1 to CUG repeat and CCUG repeat were high, that were comparable to human MBNLs, although we also noticed several minor differences in binding preferences between K02H8.1 and human MBNL.
In addition, in the course of cDNA cloning, we obtained six splicing isoforms of K02H8.1. Three of them had more extra upstream exons, which is not registered in the database. We used SL1 leader RNA sequence as a forward primer, demonstrating that our all six isoforms have full 5'- sequence.
The extra upstream exon, exon 0, is separated with exon 1, by an intermediate 12Kb intron. The length of 12Kb is exremely long, compared with other introns.
We performed the promoter analysis by using promoter-GFP fusion construtcs. We could not see any K02H8.1 expression in muscle. Instead, we detected significant GFP expressions in neurons around phalynx.

P29
IMAGING MRNAS INVOLVED IN MYOTONIC DYSTROPHY TYPE 1 (DM1) USING ATOMIC FORCE MICROSCOPY (AFM)

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Atomic force microscopy (AFM) has recently become an established imaging tool within the nanotechnology world. Unlike conventional optical microscopy, AFM uses a micrometre-sized tip probe that scans over a flat surface where the sample of interest is dispersed. As the probe scans the surface, the change of height caused by the encounter of the sample is detected through a laser detection system and the data collected are further processed to re-create an image with the sample. In the past decade, AFM has been successfully used for imaging biological samples such as nucleic acid, proteins and their subsequent complexes.

In this study, AFM has been used to image mutant DM mRNA molecules that contain an over-expanded number of CUG repeats (140 repeats) and also their interaction with MBNL1 proteins. Results show that DM mRNAs containing 140 CUG repeats form a very stable double-stranded RNA hairpin that can be easily imaged in air using AFM. We have also imaged the interaction of these mRNA with MBNL1 proteins and a distinctive motif of the binding to the CUG hairpin is recognisable. Theses results are the very images showing MBNL1 proteins binding to mutant DM mRNAs. The results show that AFM could later be used as system to understand better the molecular mechanisms underlying Myotonic Dystrophy as well as understanding the mechanism of action of potential therapies.

P30
LOSS OF TAU EXON 2/3 INCLUSION IN DM1 IMPLIES THE CARBOXY-TERMINAL TAIL OF MBNL1

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Neurofibrillary degeneration is characterized by an intraneuronal aggregation of abnormally modified microtubule-associated Tau. The pathological lesion is often observed in the brain of patients suffering of DM1 and DM2 and we previously showed a modified splicing of Tau consisting of a reduced inclusion of Tau exon 2 and exon 3 in both diseases. Herein, we show in HeLa cells that long CUG repeats induced a reduced inclusion of Tau exons 2/3 of the endogenous pre-mRNA as well as those of a Tau minigene. Trans-dominant effect of CUG repeats is mediated by the loss of function of MBNL1, a protein with the double property of double-stranded RNA binding and splicing factor activities. We show that the loss of MBNL1 expression by siRNA repressed the inclusions of Tau exons 2/3 inclusion. In contrast, Tau exons 2/3 splicing was unmodified by ectopic expression of MBNL1. Preliminary analysis of MBNL1 structure/function relationship indicated that the carboxy-terminal tail of MBNL1 is essential for MBNL1 splicing factor activity since MBNL1 protein deleted of this domain (MBNL1¢CT) binds CUG repeats but do not modify the splicing of hcTNT exon 5, a known target of MBNL1. Tau splicing is not modified by MBNL1¢CT but when MBNL1¢CT is co-expressed with long CUG repeat it restores the inclusion of Tau exon 2/3 splicing as observed in the adult human brain. Altogether, our results show that the modified splicing of Tau in DM1 is directly linked to the aetiology of the disease and is possibly mediated by the loss of function of the carboxy terminal tail of MBNL1.

P31
INVESTIGATION OF DEMENTIA IN A PATIENT WITH MYOTONIC DYSTROPHY TYPE 1: IS IT A DM1 ASSOCIATED PHENOMENON OR IS IT ALZHEIMER'S DISEASE?

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Our male patient presently aged 79 was first investigated at age 69 years. He had then 4 years previously noted difficulties in running and the following years slight swallowing difficulties. At examination he had slight proximal and distal muscle weakness and atrophy in upper and lower extremities, atrophy in masseter and temporalis muscles, slight nasal speech and reduced movement of the soft palate. Deep tendon reflexes were normal and babinskis sign absent. A cataract operation was performed bilaterally at ages 68 and 70 years.

At age 72 he had deteriorated, use a cane, and could no longer climb stairs. Speech and swallowing was moderately affected. Progressive hand muscle weakness and now grip- and percussion myotonia was evident.

Presently at age 79 years he has moderate to severe dysarthria, profound muscle weakness in upper and lower extremities, uses a wheelchair but can manage standing transferring from chair and walk a few steps indoor. ECG has showed deterioration with increased PQ time exceeding 300ms and episodes of AV block II type 2 necessitating a pacemaker implantation at age 77.

DM1 was DNA verified at age 75, and impairment of memory has been noted since that age, but has become troublesome the last year.

A neurophychological investigation was performed at age 75 years. This revealed impaired immediate verbal memory and arithmetic as well as reduced psychomotor speed but showed no general cognitive reduction.

A new neuropsycholgical investigation as well as a CT scan of the brain will be performed shortly. A recent lumbar pucture showed a marked increase in CSF Tau 980 ng/L (<400) and P-Tau 121 ng/L (<80) while Amyloid Beta 500 ng/L (>450) was normal.

We discuss the possible causes of dementia: either he suffers a dementia caused by DM1 or alternatively he has Alzheimer's disease. This may be of importance since in the latter instance there is potential pharmacologic treatment available.

P32
CEREBROSPINAL FLUID TAU AND AMYLOID β42 PROTEIN IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Myotonic dystrophy type 1 (DM1) is associated with brain morphology changes including neurofibrillary degeneration. The authors examined cerebrospinal fluid (CSF) markers indicative of neuronal degeneration and amyloidogenesis; total tau (T-tau), phosphorylated tau (P-tau) and , amyloid1-42 (A,42), in 31 patients with DM1. Associations between CSF markers and CTG repeat expansion size, brain MRI findings, and neuropsychological test results were analyzed. As compared to matched controls A,42 was significantly decreased (p=0.001), while levels of T-tau were increased (p<0.001). No difference was found between measures considering P-tau levels. At present the clinical implications of these findings is unclear, due to a considerable overlap between CSF values of DM1 patients and healthy controls, but also considering modest associations between CSF markers and other measures. However notably, the Tau pathology, as seen in DM1, differs from Alzheimer's disease, considering the lack of increased levels of P-tau.

P33
COGNITIVE AND PERSONALITY PROFILE IN MYOTONIC DYSTROPHY TYPE1 (DM1)

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Objective. DM1 is a slowly progressive myopathy characterized by varying multisystemic affectation. Central nervous system (CNS) involvement in DM1 was described long ago but methodological problems have made it difficult to extract definitive conclusions. Consensus regarding personality defects is even weaker. The aim of this study was to define cognitive and personality pattern associated with DM1 patients, and to analyze both the relationship between such patterns and the association of these clinical patterns with the underlying molecular defect. *Participants and method.* Through comprehensive neuropsychological testing and personality assessment with MCMI-II (Millon), we have examined 121 molecularly confirmed patients and 64 control subjects. We employed a multiple linear regression model to assess the effect of each variable on cognition and personality. *Results.* Patients performed significantly worse than controls in tests measuring executive function and visuoconstructive abilities. In the personality profile, some paranoid traits associated with the cognitive profile stood out. There was a significant negative correlation between the CTG expansion size and most of the relevant neuropsychological and personality measures. *Conclusions.* Besides muscular symptomathology, there is a significant involvement of the CNS in DM1 related to the molecular defect. CNS impairment may predominantly affect fronto-parietal lobe, area on which future neuropathological or functional imaging studies should be centred.

P34
INTELLECTUAL FUNCTIONING IN A LARGE SAMPLE OF ADULT AND LATE-ONSET DM1 PATIENTS

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Background. There are evidences for a generally lower IQ level in DM1 population with no clear sign of an intellectual decline over time. Mental deficiency is also reported from studies which included the whole clinical spectrum of DM1, even if it's commonly accepted that individuals with congenital onset have the lowest IQs. *Objectives.* To describe the intellectual functioning in a large sample of adult and late-onset DM1 patients and reappraise its relationship with the (CTG)n, age at onset of symptoms, affected parent's sex and disease duration. Methods. A 7-subtest WAIS-R was used to estimate Full Scale IQ (FSIQ) in 151 adult and 37 late-onset DM1 patients. *Results.* FSIQ ranged from 61 to 119 (M=82.6, SD=8.4) where the late-onset subgroup had a higher mean FSIQ (88.7 vs 81.1, p<0.001). Classification of FSIQ showed that 82% of the total sample was below the Average range of intellectual functioning. Furthermore, 64.4% had a FSIQ significantly lower (>1 SD) than the normative sample mean. FSIQ significantly declined with both the increase of the (CTG)n (p<0.001) and the disease duration (p=0.005). FSIQ was not linked to the age at onset of symptoms nor the affected parent's sex. Even though people with late-onset DM1 are more likely to have a normal IQ profile, these results suggest that poor intellectual ability is an extremely common condition among adult-onset DM1 patients, even in the least severe forms. Moreover, the correlation between FSIQ and disease duration may be an argument supporting the hypothesis of a global decline in cognitive abilities over time, but this needs to be confirmed by a longitudinal design.

P35
COMPREHENSIVE EVALUATION OF SLEEP-WAKE CYCLE AND DAYTIME SOMNOLENCE IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

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Sleep complaints and excessive daytime somnolence (EDS) have been described in DM1 patients. Most previous studies have been addressed to selected DM patients complaining EDS, evaluating sleep quality by means of subjective questionnaires. The aim of our study was to investigate the sleep-wake cycle in consecutive, unselected, DM1 patients, by means of objective and subjective data, in order to obtain an overall and comprehensive framework of the relationship between sleep disorders and DM1. Fifteen consecutive DM1 patients (mean age 40 y.o., range 26-54) were enrolled. Subjective quality of sleep was assessed by means of the Pittsburgh Sleep Quality Index (PSQI). The Epworth Sleepiness Scale (ESS) and the Daytime Sleepiness Scale (DSS) were performed in order to evaluate the subjective daytime somnolence. All patients underwent 24-hour polysomnographic monitoring (a-PSG) followed by the Multiple Sleep Latency Test. The montage of a-PSG consisted of 8 EEG, 2 EOG and 3 EMG channels, oximetry, oronasal airflow and plethysmography. Only two patients showed a PSQI indicative of a poor quality of sleep. One patient presented a pathological ESS score and 3 subjects showed a DSS >7 (cut-off). The MSLT was <8 in 3 patients and Sleep Onset Rem Sleep was not observed. Regarding a-PSG data 3 patients showed a AHI >10, whereas a PLMI >5 was found in 10 subjects. The sleep architecture did not show gross alterations except for a mild reduction of the sleep efficiency. Finally, the MSLT score did not correlate with subjective scales or with nocturnal PSG data. The incidence of EDS in our patients seems lower than in previous studies, with a discrepancy between subjective and objective data. Finally, the prevalence of PLMs appears more elevated than in age-matched controls.

P36
COGNITIVE IMPAIRMENT AND PSYCHIATRIC DISORDERS IN THE JUVENILE FORM OF MYOTONIC DYSTROPHY

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Recently, have been described some particularities in the cognitive and psychiatric profile of child and adolescent with the juvenile form of the myotonic dystrophy. Most children (63%) met criteria for at least one DSM IV diagnosis. The most prevalent diagnosis is Attention Deficit with Hyperactivity Disorder, the attention deficit is the most impaired subtype. A bimodal distribution of IQ was found: one group with mental retardation (IQ=60) and one group without mental retardation. This group is characterized by a discrepancy between verbal IQ and performance IQ (vIQ > pIQ). The majority of Steinert patients have learning disabilities, due to reading and spelling impairment (specific difficulty in extracting information, despite of normal phonology and normal word identification). The learning disabilities could partially be explained by attention deficit, mental retardation, visuo-spatial deficit and partially by reading impairment even in subjects without mental retardation. Severity of learning disabilities and full scale IQ were correlated with longer mutation size and maternal transmission

P37
PSYCHOPATHOLOGIC AND COGNITIVE FEATURES IN DM1 AND DM2

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Previous investigations of cognitive function in Myotonic Dystrophy type 1(DM1) evidenced an impairment in executive function; in DM1 psychopathological assessment documented avoidant and obsessive-compulsive personality traits. To date only limited data are available on DM2/PROMM, suggesting similar neuropsychological and neuropsychyatric profile respect to DM1.
We characterized psychopathology and personality pattern in a cohort of 12 DM1 (9 males, 3 females, mean age 40,5±11,8 years) and 8 DM2 (3 males, 8 females, mean age 51,6±14,3 years) diagnosed on clinical molecular basis.
All our patients were evaluated with Structured Clinical Interview for DSM-IV Personality Disorders (SCID-II) and Rorschach Inkblot test using Exner's Comprehensive System. Furthermore, an extensive neuropsychological battery was performed in both groups.
Three DM1 patients and three DM2 patients showed personality disorders, in particular both DM1 and DM 2 exhibited an obsessive-compulsive personality traits. Rorschach test evidenced that both DM 1 and 2 showed emotional avoidance and low stress-tolerance. Avoidant beahviours, distortion of reality, impaired capacity to connote affective relations, lack of introspective abilities, depressive features characterized more DM1 than DM2. Only one DM1 patient showed high perceptual-thinking defect.
These findings are helpful in better defining and understanding the peculiar cognitive and personality patterns of both DM1 and DM2. Major neuropsychiatric involvement might be linked to the genetic mechanism.

P38
THEORY OF MIND AND COGNITIVE DISORDERS IN MYOTONIC DYSTROPHY TYPE 1 (DM-1): A PRELIMINARY STUDY TO UNDERSTAND NON-COMPLIANCE WITH HOME VENTILATION TREATMENT IN STEINERT'S POPULATION

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Myotonic dystrophy type 1 (MD-1) is an autosomal

dominant multisystem disorder associated with neuropsychological abnormalities. Mental retardation, executive dysfunction and personality disturbances have been reported in this population.
The objective was to investigate further social cognition in MD-1 patients using two Theory of Mind (TOM) tasks in order to understand non-compliance with home ventilation treatment in Steinert's population.
The performances of 25 patients with MD-1 and 10 matched controls were compared on two tasks designed to investigate understanding of other people's mental states: The 'Reading the Mind in the Eyes' test (Baron-Cohen *et al.*, 2001) and the 'Character Intention Task (Brunet *et al.*, 2003). MD-1 patients and controls were also given several neuropsychological tasks.
Compared to healthy controls, patients were impaired in both TOM tasks. In post-hoc analyses, two subgroup have been compared on TOM and intellectual tasks:10 MD-1 with good compliance in home ventilation (MD-1 GCHV) and 9 MD-1 with bad compliance (MD-1 BCHV). Significant differences have appeared between these two groups. These data suggest that patients MD-1 may have specific social intelligence disturbances and particularly MD-1 BCHV subgroup.
Interest for social cognition should be more frequently considered in MD-1 therapy. Nevertheless, future work should recruit more patients in order to complete analysis.

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P39
THE BLOCK OF CA-DEPENDENT K⁺CHANNELS REDUCES MYOTONIA IN STEINERT DISEASE: AN *IN VIVO* PHARMACOLOGICAL STUDY

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Many studies have been carried out to clarify the mechanism underlying the abnormalities of sarcolemma in Myotonic Dystrophy type1 (MD) but univocal results have not been reported.
The first clinical evidence of specific channels involvement was suggested after the observation that the local treatment of muscle with apamin, a specific blocker of Ca-activated K^+-channels (SK), reduced the "myotonic runs" in MD (Beherens *et al.*,1994). Recently we showed a characteristic surface EMG pattern, strictly leaked to myotonia, in MD patients (Chisari *et al.*,2001).
In this study we evaluated the effect of the local administration of apamin on sarcolemma excitability alteration recorded through surface and needle EMG.
We applied a stimulation protocol to 8 MD patients and recorded an amplitude parameter (ARV) of surface EMG before and after the local injection of 50 Ìl of 10 ÌM apamin. Moreover, to verify the reliability of our approach, in two patients we recorded the needle EMG "myotonic runs" before and after the local injection of apamin.
According to Beherens *et al.*, we observed a clear reduction of myotonic discharge recorded by means of needle EMG. On the other hand, in 2 out of 8 patients we observed a complete but transient normalization of the characteristic surface EMG pattern.
This work confirmed the role of SK in sarcolemma "instability" represented by the needle EMG "myotonic runs" and did not rule out the hypothesis that SK could play a specific role in the genesis of phenotypic expression of myotonia in MD. Of course further studies need to validate this hypothesis but we consider this approach a good starting-point to study *in vivo* muscle functional alteration in Myotonic Dystrophy type1.

P40
UNBALANCE IN MYOTONIC DYSTROPHY-1 MAY FOLLOW CERVICAL ATAXIA AND RESPOND TO EXERCISE

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Patients with myotonic dystrophy-type 1 (DM-1) often fall, due to "intrinsic" mechanisms (eg legs "giving out") for unknown reasons (Wiles CM.JNNP 2006;77:393-396). A case is presented, in which neck position sense was impaired. DA was a woman, 57 yrs old, employed. DM-1 was diagnosed at age 35. She had fallen 12 times in 2 months. She felt unsteady (DHI_{sf} inventory 7/13, normal=13/13). Strength was 4/5 at the lower limbs and on the neck extensors, 2 to 4/5 at the upper limbs, 0 to 2/5 on the neck flexors. Neck position sense was tested through a goniometric helmet. The head was placed at target angles of 20% or 70% of the full range of motion, in opposite directions of yaw, pitch or roll, and then replaced to neutral. Repositioning was asked for 6 times for each target, in a balanced design. Overall error (% of target) was +29+45 (normal <5+5). On force platforms, stability in standing was low (EquiTest® SOT score 33%, normal >70); the capacity to lean around towards the limits of stability,LOS, was also low (Balance Master® LOS 57%, normal >85). Eleven 1-hr exercise sessions were held in 6 days, based on hands-on strenghtening of the neck and the shoulder girdle. The DHI_{sf} score rose to 11/13. Head repositioning errors declined to +17±24. The SOT rose to 56 and the LOS rose to 85. During the following 3 months the patient reported no falls. Neck position sense is crucial to balance (Brandt T. JNNP 2001;71:8-22) and it relies upon muscle spindles. In DM-1 severe alterations of neck spindles have been demonstrated (Swash M. Clin Neuropathol 1983;2,2:75-783). The case suggests that this may lead to unbalance in DM-1, and that a short program of neck strenghtening may be beneficial.

P41
QUANTITATIVE EVALUATION OF MUSCLE DEGENERATION IN DM1 PATIENTS USING MRI

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* *Jack Puymirat is the coordinator of the Canadian-French project on DM1 funded by the AFM.*

MRI is a very promising technique for muscle degeneration exploration in DM1 patients, even though, only some descriptive or qualitative results were reported in the literature. We propose a quantitative method for the assessment of the Tibias Anterior (TA) muscle degeneration in DM1 patients using MRI.

14 DM1 patients were included in the study. Lower legs MRI examination was performed subsequent to genetic testing. TA muscular relative isometric strength (i.e. torque muscle) was also assessed for left and right legs using a hand-held dynamometer.

MRI examination was performed on a 1.5T whole body MR-system. 3D high spatial anatomical images were acquired in the transversal orientation, covering the legs from knee to ankle. The same geometric parameters were used to acquire MR images using the following pulse sequences: T1 weighted Turbo Spin Echo (TSE); DP/T2 weighted TSE; 3-point Dixon.

The TA muscle borders were manually delineated at each of T1 weighted slices in order to delineate its volume. Delineation between normal and diseased tissue was obtained by using an adapted threshold. Similarly, the oedematous area volume was assessed from T2 weighted images. Finally, Grey-level average values measured from fat and water images, obtained using a 3-point Dixon sequence, were used to define the fat to water ratio in the muscle.

Normal and degenerated tissue volumes, oedema volume and fat to water ration were obtained for TA muscles in this study. An excellent correlation was found between normal tissue volume and muscle strength.

This study validates the use of MRI for quantitative assessment of muscle degeneration in DM1 patients. This method could be very useful for muscle disease evolution assessment and for therapy effect quantification.

P42
DIFFERENT MUSCLE MRI FEATURES IN MYOTONIC DYSTROPHY TYPE 1 AND 2

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Objective. To determine different muscle MRI patterns in Myotonic Dystrophy type 1 (DM1) and 2 (DM2). *Materials*. We studied 7 patients affected with DM1 and 5 affected with DM2. *Methods*. Diagnosis of DM1 and DM2 was confirmed by molecular-genetic analysis. A muscle MRI (T1 and STIR sequences) study was performed in upper and lower limbs. *Results*. All DM1 patients showed abnormal muscle MRI findings (T1 sequences) in lower limbs: the anterior compartment of thighs and the posterior compartment of the legs presented fibro-fatty changes: vastus medialis, intermedius and lateralis, medial head of the gastrocnemius and soleus were particularly affected. Myoedema (STIR sequences) was present in lateral head of the gastrocnemius. DM2 patients showed different radiological involvement: fibro-fatty changes were less severe and localized to the trunk and pelvic muscles (erector spinae muscles and gluteus maximus). No myoedema was seen. *Discussion and conclusions*. DM1 and DM2 patients presented different radiological features. DM1 patients presented an involvement of the anterior compartment of the thighs (semilunar anterolateral perifemoral area of fibro-fatty changes) and of the posterior compartment of the calves (medial head of gastrocnemius and soleus) with myoedema localized to the lateral head of gastrocnemius. DM2 patients showed a less severe muscle involvement with slight fibro-fatty changes localized to erector spinae muscles and pelvic girdle. These different radiological aspects could reflect different clinical features in DM1 and DM2 patients who respectively present distal and proximal muscle weakness.

P43
MYOTONIC DYSTROPHIES TYPE 1 AND TYPE 2: MRI AND SPECT COMPARATIVE STUDY

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Objective. to study brain involvement in two cohorts of DM1 and DM2 patients by morphological (MRI) and perfusional (SPECT) investigation; to compare the results. *Methods*. we recruited 33 DM1 and 9 DM2 patients. We studied 27/33 DM1 through MRI and 22/33 DM1 through SPECT; 7/9 DM2 underwent MRI, SPECT was performed in all DM2. White matter abnormalities encountered in MRI scans were graded on T2 images according to the ARWMC score; the scores were computed on both hemispheres. SPECT scans were acquired on iterative mode; complete tridimensional reconstruction was obtained from single transverse, coronal and sagittal images; voxel per voxel tracer uptake computed analysis was bilaterally done to test frontal, temporal, parietal lobes and 6 specific Brodmann areas, normalizing signal by maximal cerebellum uptake and volumes by Talairach technique. Comparison of the results was possible in 17/33 DM1 and in 7/9 DM2 patients. *Results*. in DM1 MRI showed major, supratentorial, bilateral, focal or diffuse involvement, with frequent specific pattern of white matter abnormality; SPECT showed global or focal hypoperfusion; usually of mild degree. In DM2 MRI documented similar

white matter abnormalities, but at a lesser degree and with no temporal involvement; SPECT showed minor changes. Comparison did not evidence tight pairing of the two methods. *Conclusions*. MRI and SPECT showed major involvement in DM1 than in DM2 cohort. The different methods possibly reflect different structural and perfusional changes in Myotonic Dystrophies.

P44
MYOTONIC DYSTROPHY TYPE 2: CLINICAL, NEUROPHYSIOLOGICAL AND MUSCULAR FEATURES OF A FAMILY WITH SHORT CCTG EXPANSION

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Myotonic dystrophy type 2 (DM2/proximal myotonic myopathy, PROMM) is an autosomal dominant multisystemic disorder. Clinically, DM2 presents with myotonia, muscular dystrophy, cataracts, diabetes, testicular failure (hypogonadism), and cardiac conduction defects. No congenital case has ever been observed. The underlying gene is ZNF9, coding for a zinc-finger protein involved in CAP-independent translation of cellular transcripts. The pathologic allele consists in an expansion in intron 1 of the complex repeat tract (TG)n(TCTG)n(CCTG)n. In unaffected individuals the repeat is up to 27 CCTG-long, whereas in patients 75-11000 CCTG repeats were observed, and often more than one expanded allele can be detected in a single patient. Still debated is the smallest pathogenic size of the expansion.
The proband, a 31 year-old-male patient, complained of "stiffness" at the lower limbs during running, since his childhood. Similar symptoms were described by his sister, while the mother showed a mild increase of CPK. He had clinical myotonia at the hands and orbicularis oculi, proximal upper and lower limb muscle weakness. EMG showed myotonic discharges, the main feature of a biceps brachii muscle biopsy was the finding of many central nuclei. DM1 analysis was negative while the patient and his mother showed a short expanded fragment, formerly 82 CCTG repeats. This molecular diagnosis is based on a long-PCR assay using the same primer pairs by which is amplified the probe 32P-marked for the following Southern blot hybridisation.
This family contributes to define clinical and instrumental features associated with very short CCTG expansion within ZNF9 gene.

P45
WEAKNESS AND FATIGUE MORE THAN MYOTONIA AFFECT PHYSICAL AND MENTAL PERCEPTION OF QUALITY OF LIFE IN PATIENTS WITH MYOTONIC DYSTROPHIES

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Objective. (i) To assess health-related Quality of Life (QoL) in patients with myotonic dystrophy type 1 (DM1) and type 2 (DM2) using INQoL; (ii) to test reliability of INQoL Italia in DM; (iii) to correlate QoL perception with muscle weakness and cognitive/behavioural abnormalities. *Background*. Previous studies using SF-36 indicate that Health-related QoL is severely impaired in myotonic dystrophy type 1 and it is negatively influenced by severity and duration of disease as well as by specific cognitive deficits and changes in emotional functioning. Other studies suggest that internal consistency and reliability of self-assessment scales may be questionable in patients with DM1. *Methods*. We administered INQoL Italia to 106 patient with moderately severe DM1 (CTG range 100-650, n = 73) and genetically-confirmed DM2 (n = 33) at baseline and at 3-weeks interval and determined: (i) muscle strength (MRC); (ii) MMSE; (iii) WAIS-R profile, IQ; (iv) apathy (apathy scale). Qualitative analysis of individual free comments on the disease was also performed. *Results*. (i) No statistical difference was observed in test-retest results in each INQoL item and in total INQoL index for both DM1 and DM2, $p>0.98$; (ii) A small percentage of patients (20%) made comments on the disease and 25% of these dealt with impact of disease on their daily activities; (iii) muscle symptoms (weakness and fatigue: R = 0.8, $p<0.001$) significantly correlate to INQoL total scores; (iv) INQoL index does not correlate with years of education, MMSE, apathy and IQ. *Conclusions*. Internal consistency and reliability apply to INQoL Italia. Weakness and fatigue are the most important symptoms affecting QoL perception in our DM population. This information is crucial to target potential treatment strategies in DMs using INQoL as a tool to assess potential change in perception of QoL.

P46
MUSCLE PATHOLOGY IN MYOTONIC DYSTROPHIES – AN ULTRASTRUCTURAL STUDY

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Genetic testing is considered as the only reliable diagnostic definition in myotonic dystrophies (DMs). DMs encompass at least 2 forms: myotonic dystrophy type 1 (DM1) and myotonic dystrophy type 2 (DM2). However, a considerable number of patients with the genetically

proven type of the disease have unusual phenotypic presentations. Relatively little is known about the muscle histopathological and ultrastructural changes in both DM1 and DM2 patients.
We evaluated muscle biopsies from 19 patients with the genetically confirmed form of the disease (16 DM2 and 3 DM1 cases). All muscle biopsies had been taken for diagnostic purposes before genetic testing was available at our center.
Light microscopy confirmed the pattern of muscle pathology described previously in DM1 and DM2.
In electron microscopy multiple small (>20 Ìm) angulated fibers, with minimal remaining contractile elements and nuclear clumps were observed in all DM2 biopsies. No evidence of muscle denervation was found. Other myodegenerative changes consisted of IBM-type inclusions, vacuoles with myelin figures, honey comb-like structures, cytoplasmic bodies and lipofuscin accumulation. In the DM1 group sarcoplasmic masses were present in 1 biopsy. Only few severely atrophied nuclear clump fibers were found in one DM1 biopsy. Additional changes included vacuoles and abnormal sarcomeric organization (Z-line streaming, core-like structures).
Our study indicates, that a variety of ultrastructural abnormalities can be identified in skeletal muscle in myotonic dystrophy.
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P47
ECG-HOLTER MONITORING IS A VALUABLE TOOL TO SCREEN MYOTONIC DYSTROPHY TYPE 1 (DM1) PATIENTS FOR ADVANCED CONDUCTION DEFECTS

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Yearly 12-lead-electrocardiogram (ECG) is recommended to identify cardiac conduction defects which are among the most important complications in DM1. Twenty-four hour Holter monitoring (ECG-H) is usually considered clinically valuable when symptoms suggest intermittent arrhythmia. In our experience, transient 2nd degree AV block (AVB), which is considered an indication to pace-maker (PM) implantation, was revealed by ECG-H in some patients with normal ECG. We evaluated the contribution of ECG and ECG-H in detecting cardiac conduction abnormalities for which PM implantation is recommended. Sixty-two DM1 patients were followed for a median period of 33 months, during which they were yearly submitted to cardiac evaluation, ECG and ECG-H. ECG at baseline showed no abnormality in 44 patients, 1st degree AVB in 13, 1st degree AVB + Bundle-Branch-Block in three, and atrial fibrillation (AF) in two. During the follow-up ECG detected *de novo* 1st degree AVB in 8/44 patients. PM was implanted in the three patients with 1st degree AVB + Bundle-Branch-Block. Out of the remaining 59 patients, ECG-H showed, during the follow-up, transient advanced 2nd degree AVB in four patients (2 pts. with normal P-R, 2 with 1st degree AVB at ECG), and low ventricular rate AF in one. PM was then implanted in these five patients, according to current guidelines. ECG-H revealed that approximately 8% of DM1 population developed conduction defects, which could not have been identified with ECG only, and for which PM implantation was indicated. ECG-H should be considered a valuable tool to identify conduction defects in DM1 patients, even when ECG is normal.

P48
DIFFERENT PHENOTYPIC EXPRESSION AND CTG REPEAT EXPANSION SIZE IN MYOTONIC DYSTROPHY TYPE 1 PATIENTS

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Type 1 myotonic dystrophy (DM1) is a genetic autosomal dominant disease and the commonest muscular dystrophy in adults. The mutation is a CTG trinucleotide expansion on 19q13.3. The reliable hypothesis assumes that there is a correlation between the length of the tract and the severity of the disease. We report the preliminary results of a genotype-phenotype correlation in 16 patients with DM1. We observed some phenotypic differences in subjects with the same number of CTG repetions. Patients underwent neurological examination, electromyography, muscle biopsy, genetic analysis with DNA extracted from blood, muscle tomography, cardiologist, pneumology, oculist, endocrinologist, psychological tests. Fourteen patients were treated with mexiletine. A descriptive statistical analysis was performed. The analysis of 11 patients showed direct correlation between triplet size, muscular disability, systemic dysfunctions and inverse correlation between number of repeats, age of onset of the disease and intelligence quotient. In 5 patients these symptoms didn't correlate with repeat size; 2 patients had small CTG expansion but a severe neuromuscular impairment with early onset associated to cataract and cardiorespiratory disfunction; 3 patients had a long expanded tract but mild symptoms. The variability of phenotype could be explained by different CTG expansion in various tissues. All the patients treated with mexiletine reported clinical improvement of myotonia, so we also underline the efficacy of this drug in DM1.

P49
RAMYD (RISK OF ARRHYTHMIAS IN MYOTONIC DYSTROPHY) STUDY: THE DESIGN

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Background. Myotonic dystrophy type 1 (DM1) is multisystem disease involving also the heart with an increased incidence of sudden death (SD) mainly related to development of conduction blocks and to major ventricular tachyarrhythmias. *Aim of the study.* To assess the 2-year

cumulative incidence and the value of non-invasive and invasive findings as predictive factors for SD, resuscitated cardiac arrest, ventricular fibrillation, sustained ventricular tachycardia, and severe sinus dysfunction or high-degree atrio-ventricular block. *Study design.* More than 500 DM1 patients with a genetical and neurological evaluation will be evaluated at baseline with a clinical interview, 12-lead standard ECG, 24-hour ECG and echocardiogram. Conventional and non-conventional indications to electrophysiological study (EPS), PM, implantable cardioverter defibrillator (ICD) or loop-recorder (LR) implantation have been developed. In the case of an indication to either EPS, PM, ICD or LR implant at baseline or at follow-up, the pt will be referred for the procedure. At the end of 2-years follow-up, all the candidate prognostic factors will be tested for their association with the end-points. *Conclusion.* We believe that the results of this large and prospective trial will substantially contribute to improve the diagnostic and therapeutic management of cardiac involvement in DM1.

P50
RAMYD STUDY: BASELINE CARDIOLOGICAL CHARACTERISTICS

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Purpose. We want to analize baseline cardiological data among RAMYD study population. *Materials and methods.* 545 patients with a neurological and genetical evaluation, were enrolled and performed a complete cardiological evaluation (standard 12 leads ECG, 24 hours ECG, echocardiogram). *Results.* Standard 12?leads ECG documented sinus bradycardia < 50bpm in 4.1% of patients, AV conduction defects in about 22.5%, IV conduction defects in 29,6% and atrial tachyarrhythmias in 2,8% of patients. In one patient a sustained ventricular tachycardia (SVT) was documented.
Baseline 24 hour ECG data showed a first degree AV Block in 19.9%, an high degree AV block in 3.3%, a Left Bundle Brunch block in 7.1%, a Left Anterior Hemiblock in12.4% and a bifascicular block in 3.1% of patients. Isolated premature ventricular beats > 700/24 hours were recorded in 8.5% of patients while ventricular couplets and non SVT in 8.7% of cases. 4 patients had experienced long pauses (> 3sec), 7 patients had atrial arrhythmias. Echocardiography showed a structurally normal heart in most cases. An ejection fraction < 50% was documented in 5% of patients. *Conclusions.* conduction system abnormalities had a predominant role and an early identification also with simple instrumental cardiological exams and a scrupulous follow-up, may prevent sudden cardiac death.

P51
RAMYD STUDY PRELIMINARY RESULTS: ELECTROPHYSIOLOGICAL STUDY AND DEVICES IMPLANT

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Purpose. To evaluate the importance of the electrophysiological study (EPS) and of devices implant in the RAMYD study population. *Materials and Methods.* To undergo EPS we select patients with one of the following: symptoms as palpitations or syncope; family history of sudden death, pacemaker (PM) or implantable cardioverter-defibrillator (ICD) implant; PQ interval >240ms; 2nd/3rd AV block; left bundle branch block or fascicular block associated right bundle branch block; RR pauses >3sec; frequent ventricular arrhythmias. If HV was >70 msec a PM, if 50<HV<70 msec a cardiac event recorder and if syncopal sustained ventricular tachycardia (SVT) or ventricular fibrillation (VF) was induced an ICD was implanted.
Results. Among 545 patients, 118 (21.5%) underwent EPS. HV >70 msec was recorded in 32.2% and AH>140 msec in 30.3% of patients. An abnormal 1:1 AV conduction was detected in 4.6% and a sinus node dysfunction in 8.5% of patients. During programmed stimulation sustained atrial arrhythmias were induced in 5.6% and syncopal SVT or VF in 19.9% of patients. The results of EPS indicated a PM implant in 9.5% an ICD in 4.7% and a cardiac event recorder in 3.3% of patients.
Data collected by devices monitoring showed a high degree AV block in 26% and malignant VT in 12% of implanted patients. 87% of major cardiac events were recorded on patients with at least 24 month follow-up.
Conclusions: the progression of bradyarrhythmias and the occurrence of SVT in DM1 patients may require an early EPS and device implant.

P52
COMPARISON BETWEEN ELECTROANATOMIC MAPPING VS CARDIAC MAGNETIC RESONANCE IMAGING IN MYOCARDIAL SUBSTRATE STUDY IN MYOTONIC DYSTROPHY TYPE 1

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Background. In Myotonic Dystrophy type 1 (MD1) patients there is an alterated cardiac substrate that clinically presents with conduction abnormalities and arrhythmias. Aim of this study is detect early myocardial alterations using electroanatomic mapping and cardiac magnetic resonance (cMRI) in MD1 population without evidence of bradi or tachy-arrhythmias. *Methods.* Fourteen MD1

patients underwent electrophysiological study (EPS) and right ventricular (RV) electroanatomic mapping with CARTO system. RV unipolar voltage (UNI-v), bipolar voltage (BI-v) (normal value > 1,5 mV) and bipolar potential duration (Bi-dur) were misured; RV was divided in 4 regions: apex, septum, free wall and outflow tract. All patients underwent cMRI with RV structural alterations analysis (edema, fibroadipose infiltration and delayed enhancement). *Results*. CARTO mapping analysis evidenced at least one alterated potentials region (BI-v <1,5 mV) in a statistically significant greater number of patients (8/14) than cMRI did (2/14) (p=0.046). Comparing alterated myocardial substrate presence at CARTO mapping vs cMRI in the 4 RV regions a statistically significant difference was found for outflow tract (p=0.033). No statistically significant differences were found for apex, septum and free wall. *Conclusion*. CARTO electroanatomic mapping seems to be more accurate in detecting presence of alterated electrical substrate in RV outflow tract than cMRI in MD1 patients without evidence of arrhythmias. Such electrical alterations presence could help in identifying a preclinical stage in MD1 related myocardiopathy.

P53
ASSESSMENT OF NONINVASIVE VENTILATION IN MYOTONIC DYSTROPHY TYPE 1

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Objectives. To assess tolerance and efficacy of noninvasive ventilation (NIV) in a population of DM1 patients with sleep disordered breathing (SDB). *Methods*. Descriptive analysis of retrospective and prospective data in 64 consecutive patients (30 M/34 F; 41 ± 13 years) referred for DM1: muscular impairment (MIRS), number of CTG repeats, daytime sleepiness (Epworth sleepiness scale ESS), spirometry, maximal inspiratory and expiratory mouth pressures, and arterial blood gases. Respiratory polygraphy or polysomnography was performed in patients with either capnia > 44mmHg, VC < 50% pred., excessive sleepiness complaint or ESS > 10. NIV was initiated in patients with significant SDB associated with daytime hypercapnia and/or excessive sleepiness. *Results*. ESS was > 10 in 20 patients (31%). Daytime hypercapnia was found in 34 patients and VC was < 50% in 6 patients. SDB were disclosed in 28 of the 32 patients (87.5%) investigated for. VNI was initiated in 24 patients: it failed rapidly in 4 (intolerance 3, worsening of nocturnal hypoxemia 1), it was stopped later on (mean delay 17 months) in 7 patients because of poor use (<3 h/night) [NIV- group]; it was maintained in 13 patients [NIV+ group], follow-up 21±13.3 months. In NIV+ group, mean use was 7 h/night, mean satisfaction score was 7.2/10, all nocturnal parameters improved whilst daytime capnia and ESS did not change. Capnia increased (p 0.043) in NIV- group. No predictive factor for compliance with NIV was found. One patient died (intracranial hemorrhage) in NIV+ group, 1 tracheostomy was performed in NIV- group. *Conclusions*. Prevalence of SDB is very high in targeted patients. NIV was considered in more than 1/3 patients. No predictive factor for compliance with NIV (54% in our series) could be found.

P54
VENTILATORY FUNCTION IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Restrictive respiratory syndrome - often associated with sleepiness and attention deficit - is a frequent feature in myotonic dystrophy type 1 (DM1). It is usually progressive and often requires assisted mechanical ventilation (AMV). Aim of the study was to evaluate the effects of AMV on diurnal sleepiness and obstructive apnoea sleep syndrome in DM1 patients.
To this aim 136 patients – 89 males and 47 females (mean age 47,3 years) - have been consecutively enrolled from January 2003 to May 2007 and periodically followed by cardiological and respiratory assessments.
Respiratory involvement was investigated by clinical evaluation, BMI, EGA, spirometric tests, MIP and MEP evaluations. Diurnal sleepiness was evaluated by ESS (Epworth Sleepiness Scale).
Results. Thirty-six patients (26.4%) showed a moderate-severe restrictive syndrome, with forced vital capacity values (FVC) less than 60%, mild hypoxia, MIP < 60cmH2O and MEP<50 cmH2O. Thirteen out of them (36.1%) showing BMI>25 and ESS > 12, were investigated by nocturnal polysomnography. The analysis of the polysomnographic records revealed central hypo-apnoea and apnoea during the phases 1 and REM of the sleep, and alveolar hypoventilation. Seven of them (53.8%) showing AHI >10, were addressed to nasal nocturnal AMV by BiPAP. A re-evaluation after 3 months of treatment showed a reduced diurnal sleepiness (ESS<12) and a concomitant improvement of the sleep quality in all patients. These data suggest that AMV by BiPAP can be considered useful in improving both sleep quality and diurnal sleepiness in DM1 patients.

P54B
ARTIFICIAL VENTILATORY MANAGEMENT IN MYOTONIC DYSTROPHY AT A JAPANESE HOSPITAL FOR CHRONIC NEUROMUSCULAR DISORDERS

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Background. Respiratory problems are recognized to be a major complication in patients with myotonic dystrophy (DM). Some patients are under ventilatory support.
Objects: The aim of this study was to clear the clinical characteristics of DM patients under ventilatory management with tracheotomy (TIPPV) who had been for a long period in a Japanese hospital for chronic neuromuscular disorders. *Methods*. Patients with adult type of DM who had been over six months in our hospital and had TIPPV during a period from 1998 to 2007 were retrospectively

investigated. *Results*. Forty-four cases were picked up as long period inpatients. Sixteen of 44 patients had TIPPV. The mean age of having tracheotomy was 57.1 years.
The common cause of tracheotomy was aggravation of respiratory infection, followed by aggravation of chronic respiratory failure or difficulty of airway clearance. Eight of TIPPV patients were still alive, and one patient left our hospital to live at home. The mean duration of TIPPV management was 44.1 months. One patient was supported by ventilator only during night, and one extricated himself from ventilatory support. Eight of TIPPV patients were dead. The mean age at death was 62.1 years, and the mean duration from tracheotomy to death was 45.6 months. The most common cause of death was congestive heart failure, however, varied causes such as multiple organ failure, aggravation of respiratory infection, renal failure or sudden death followed the prior.
Conclusion: Mean age at tracheotomy was similar to that of death in natural course. Prolongation of life over 45 months was possible by means of TIPPV management. Varied cause of death suggested that multiple organs could be involved in DM.

P55
IS HEART RATE VARIABILITY A PROGNOSTIC INDICATOR IN PATIENTS WITH DYSTROPHIA MYOTONICA TYPE 1

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Myotonic dystrophy type 1 (DM1) - the most frequent adult muscular dystrophy - is a progressive, systemic, autosomal dominant disorder affecting 1:8000 individuals. The heart is commonly involved in DM1 with myocardial fibrosis and degeneration, preferentially affecting conduction system. Atrio-ventricular blocks of different degrees and/or atrial or ventricular tachy-arrhythmias are the most common clinical manifestations, with a potential risk of cardiac sudden death. Heart rate variability (HRV) is one of the most popular parameters used to assess the autonomic tone. HRV has been reported as a strong predictor of cardiovascular mortality. It has been proposed that a decrease in HRV is a powerful predictor of morbidity and mortality consequent to arrhythmic complications.
Aim of this study was to assess the use of HRV analysis as a prognostic indicator in a population of patients with Dystrophia Myotonica type 1. We show that HRV analysis is negatively related with age, as it declines as age of patients increases. SDNN seem to be the most sensitive parameter among the time domain indexes (SDANN, SDNN, and RSMMD), although no statistical correlation was found with the occurrence of arrhythmias.

P56
A CROSS-SECTIONAL STUDY FOR GLUCOSE INTOLERANCE OF MYOTONIC DYSTROPHY

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We made a cross-sectional study to reveal pathophysiology for glucose intolerance of myotonic dystrophy (DM). Participants were 85 DM patients and 734 general controls (Ctr), who had not been diagnosed as diabetes mellitus nor received diabetic medicine. Oral 75g glucose tolerance test (OGTT) was done for all participants. Insulin tolerance test (ITT) was also done in 35 DM patients. Adiponectin was examined in 50 DM patients and 557 Ctr. Statistical analyses after correcting with sex, age and body mass index, showed no difference in fasting blood glucose (FBS), visceral fat area, and blood pressure between DM and Ctr. However hemoglobin A1c, triglyceride, adiponectin, fasting immunoreactive insulin, homeostasis model assessment-insulin resistance (HOMA-IR) and insulinogenic index were higher in DM. The prevalence of diabetes mellitus in those having 90-110mg/dl and over 110mg/dl of FBS were 18.2% and 87.5% in DM, respectively. Furthermore, even those with low FBS, many DM patients presented hyperinsulinemia. Rapid deterioration of insulin productivity and HOMA-IR was observed in parallel with elevation of FBS for DM patients. In addition, K value of ITT was lower compared to previous study and was not correlated with FBS. Our results suggested that DM exhibits insulin resistance, which is compensated by hyperinsulinemia at an early disease stage. Glucose intolerance should be taken care from initial stage in DM and strict managements should be considered when FBS exceed 90mg/dl.

P57
INTRACELLULAR INSULIN MEDIATED SIGNALLING IN MYOTONIC DISTROPHY TYPE 1 (DM1)

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Insulin receptor (IR) levels and binding are reduced in DM1. The stimulated IR promotes the intracellular insulin signalling, via the cascade of three messengers: Phosphatidylinositol 3 kinase (IP3-K), Protein kinase B (PKB/AKT) and Glycogen-synthase Kinase 3 (GSK3-,). The same cascade may be stimulated by intracellular Ca++ via direct activation of IP3-K.
Aim of the study is to investigate the intracellular insulin-mediated signalling in 10 DM1 patients without diabetes or insulin-resistance evidence and age-matched controls. We measured the AKT activity in Lymphocytes stimula-

ted *in vitro* with human recombinant insulin (H-I). Moreover we evaluated AKT after stimulation with Ionomycin plus 12-O-tetradecanoylphorbol-13-acetate (I-TPA), which, promoting intracellular Ca++ influx, are able to activate the same insulin pathway by-passing the IR. Lymphocytes, incubated at 37°C, were stimulated with H-I for 5, 15 and 30 minutes or with I-TPA for 5 minutes; a percentage of cells were incubated and not stimulated (0 minutes) and used as baseline. Proteins extracted from cell lysates were electrophoresed and transferred to a supported nitrocellulose membrane. Filters were incubated with primary (anti-phospho-AKT) and secondary antibodies, and the immune complexes were detected by chemiluminescence blotting substrate kit. AKT phosphorilation was reduced in DM1 insulin-stimulated cells, while it showed no change in I-TPA stimulated cells.

Our results show that intracellular insulin signalling, though functionally preserved, is negatively influenced in DM1 subjects without clinical evidence of diabetes or insulin resistance.

P58
MULTIDISCIPLINARY STUDY IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Myotonic Dystrophy type 1 (DM1) is a complex multisystemic disorder caused by an expansion of a CTG repeat located in a 3' untranslated region of DMPK (myotonic dystrophy protein kinase) on chromosome 19q13.3. While 5-34 CTG repeats are observed in normal alleles, their number may reach 50-4000 in DM1. This expansion causes myotonia, muscular dystrophy and multisystemic manifestations.

Aim of the study was to evaluate cardiac, pulmonary and endocrine involvement in a group of patients with DM1 and correlate genetic defect and severity of the disease, if possible.

Twenty-eight adult patients with DM1 (age 24-69), genetically confirmed, were divided into 1) mild form with 50-150 CTG repeats (5 patients), 2) classic form with 150-1000 CTG repeats (21 patients) and 3) severe form with more than 1000 CTG repeats (2 patients). They underwent clinical examination with evaluation of muscle strength, determination of thyroid function, glucose metabolism and sex hormones, cardiological evaluation (Holter monitoring, echocardiography), pulmonary function tests (spirometry, respiratory muscles efficiency), pharyngeal evaluation with optic fibres and videofluorography

Polysomnography was performed in 16 patients, 5 of whom showed obstructive sleep apnea syndrome.

In 9 patients arrhythmias/conduction defects were found, in 3 left ventricular hypertrophy. In 7 patients pulmonary restrictive defect was diagnosed. Pharyngeal dysfunction was present in 16 patients.

We observed a correlation between the size of the triplet expansion and the clinical expression: in particular the 5 patients with mild form showed no heart or lung involvement, whereas both patients with the severe form presented arrhythmias and pulmonary restriction.

P59
ABNORMAL β-CELL FUNCTION IN MYOTONIC DYSTROPHY TYPE-1

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Myotonic dystrophy type 1 (DM1) is often complicated by abnormal glucose homeostasis. To assess insulin sensitivity and secretion, we studied 20 DM1 patients, and 6 healthy subjects with no history of diabetes (control subjects) with oral glucose tolerance test (OGTT). The homeostasis model assessment of insulin resistance (HOMA-IR) was used to estimate insulin sensitivity. ß-cell function was quantified as the ratio of the incremental insulin to glucose response over the first 30 min during the OGTT. We have also correlated glucose homeostasis with distal limb muscle weakness. No patient had impaired fasting glucose (4,3±0,6 mmol/l) and/or impaired glucose tolerance (2-hour glucose; 6,9±2 and 5,2±1,4 mmol/l for normal and DM1 patients). Insulin sensitivity was not different to that of control subjects (13,5±4,6 and 13,9±6,6 pmol/mmol, for control and DM1 patients). Increasing ß-cell function was identified during the first 30 min of the OGTT (249±210 and 839± 850pmol/mmol, for control and DM1 subjects). Moreover there is a significant correlation between the CTG repeat length and the increase in ß-cell function (1276±992 pmol/mmol for DM1 patients with a CTG repeat length above 1000 compared with 358±244 for DM1 patients with a CTG repeat length less than 1000, $p<0.03$). Finally, there was no correlation between increased ß-cell function and distal limb muscle weakness. Conclusion. 1) Insulin resistance is not a major metabolic alteration in this group of DM1 patients; 2) ß-cell function dysfunction appears to be the most important abnormality observed in our DM1 patients; 3) Abnormal ß-cell function does not seem to correlate with muscle weakness.

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P60
INSULIN RESISTANCE IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Myotonic dystrophy type 1 (MD1) is caused by an expanded CTG trinucleotide repeat in the 3' UTR of the dystrophia myotonica-protein kinase (*DMPK*) gene. In MD1, RNA containing the CUG expansion accumulates in ribonuclear foci and has been associated with dysregulation of RNA-binding proteins that have been proposed to result in a novel pathogenic mechanism via a *trans*-domi-

nant effect on pre-mRNA alternative splicing. This mechanism underlies misregulated splicing of the insulin receptor and the muscle-specific chloride-channel pre-mRNAs in MD1, leading to insulin insensitivity and myotonia. The aim of this study was to evaluate the presence and frequency of insulin resistance (IR) in MD1 patients. Twenty-five patients with MD1 were in an experimental group. Ten control subjects were recruited from the patients with neurological diseases that have no connection with the presence of IR or diabetes mellitus. An oral glucose tolerance test (OGTT) was performed in all patients. Glucose and insulin concentrations were measured early in the morning in both groups and then we calculated HOMA IR index. Glucose concentrations in 120th minute of OGTT and body mass index were normal in all patients. Statistically significant increase in fasting glucose levels and insulin concentrations were measured in MD1 group than in control subjects. Although, glucose concentrations were in the normal range of values in MD1 patients, insulin concentrations were higher than normally expected. Also, we found an increase in HOMA IR index values in patients with MD1. Our results show the presence of IR in our population of MD1 patients, although fasting glucose levels and OGTT were normal in all patients. We didn`t find a significant relationship between the presence of IR and the severity of the disease or age in our patients with MD1.

P61
ADVANCED OXIDATION PROTEIN PRODUCTS IN SERUM OF PATIENTS WITH MYOTONIC DISEASE TYPE 1: CORRELATION WITH EXTRA-MUSCULAR PHENOTYPE

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disorder linked to a monoallelic expansion of the CTGn repeat in the 3' untraslated region of the DM protein kinase gene. Although the pathogenetic mechanism underlying the multisystem involvement in myotonic dystrophy is still unclear, a role of oxidative stress in this disease has been suggested. We investigated 39 patients affected by MD to evaluate serum gamma-glutamyl transpeptidase (GGT), GGT fraction bound to low density lipoproteins (LDL-GGT) and advanced oxidation protein products (AOPP) levels, as indicators of oxidative stress. Total GGT (127±18.98 mU/mL vs.11±3.05 mU/mL; p=0.0005) and absolute LDL-GGT (19.08±6.27 mU/mL vs. 1.86±0.83 mU/mL; p=0.0021) values were increased in MD, with higher levels of GTT in older patients (p=0.0117). Plasma AOPP levels were significantly higher in patients than in controls (52.08±5.86 mol/L vs. 29.9±4.67 mol/L; p=0.021), with higher values in those patients with extra-muscular signs of the disease; AOPP showed a significant correlation with serum GGT levels (r=0.5831; p=0.0022), but not with age. The concomitant increment of gamma-glutamyl transpeptidase and advanced oxidation protein products indicates a possible role of oxidative stress in the pathogenesis of myotonic dystrophy type 1, while the association of increased advanced oxidation protein products levels with extra-muscular signs of the disease suggests that individual susceptibility to oxidative stress can modulate the extra-muscular phenotype of the disease.

P62
NTRAOCULAR PRESSURE AND CENTRAL CORNEAL THICKNESS STUDY IN PATIENTS WITH STEINERT MYOTONIC DYSTROPHY

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Ocular involvement other than lens opacities has not extensively investigated in patients affected by Steinert myotonic dystrophy. Purpose of the study was to evaluate the intraocular pressure (IOP) in patients affected by Steinert Disease (DM1). To this aim, IOP and central corneal thickness (CCT) were studied in 62 (DM1) patients and in 108 age and sex matched normal subjects. IOP was measured by the Goldman applanation tonometry (GAT), histeresis (HS) by the Ocular Response Analyzer and CCT by the Pentacam. *Results*. Compared to the healthy subjects, DM1 showed lower IOP values (12.74±6,2 mmHg vs 14.85±3.01 mmHg) (p<0.001), and a thicker cornea (576.96±36,59 μm vs 555.4±33,06 μm). The differences were statistically significant (p<0.001). Interestingly these findings were not associated with significant changes in HS (p= 0.03). *Conclusions*. Lower IOP values found in patients with DM1 are not related – as expected - to the different corneal thickness or HS, but may be consequent to an increased outflow, caused by ciliary muscle myotonia.

P63
A CASE OF PATIENT WITH COEXISTENT THOMSEN'S DISEASE AND BENIGN HYPERBILIRUBINEMIA (E.G. GILBERT SYNDROME (GS))

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Results. A 20 year-old male patient had onset of prolonged muscle contraction from age 15. Myotonia predominates in the upper extremities, causing difficulty in ambulating. Movements begins slowly and with difficulty, especially after prolonged rest. Although motor function improves to a normal level with continued exercise. He has muscular hypertrophy as a result of continuous involuntary exercise. ENMG- repetitive nerve stimulation cause progressive decline in successive evoked muscle action potentials as a result of increased muscle fiber refractoriness (with proper sound). From age of 9 mild lymphocytosis (41-46) and mild hyperbilirubinemia without transaminase (ALT/AST) elevation was found and persisted without any significant change until now (total 21.3-34.7 mkmol/L, indirect 19-34.7 mkmol/L). No relation between severity of Thomsen's disease and levels of bilirubin was found. Viral hepatites were ruled out. Repeated abdominal ultrasonography showed no abnormality.

P64
A *CARE-CARD* FOR MYOTONIC DYSTROPHIES: IMPROVING MANAGEMENT AND FOLLOW-UP

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Objective. To provide (i) information on the disease for the patients and relatives and for their doctors; (ii) information on management of symptoms for each organ involved; (iii) a diary in which each follow-up visit is updated. *Background.* Although the most frequent muscular dystrophy of adulthood, myotonic dystrophies are still underdiagnosed by general practioners. The multisystem feature of DMs may mask symptoms related to the disease; knowledge of the degree and frequency of different organs involved is helpful for a better management. Patients with DMs are often reluctant to come to outpatient visits and follow-up is difficult to be maintained regularly. *Methods.* The informational booklet will consist of a lay description of the disease, transmission and heredity, emergency contacts and medical alerts. It will provide guidelines for the diagnosis and management of most frequent symptoms. Specific space will be provided to store test results and discharge notes. Patients will also be provided with a 'care card' which will include a diary to update follow-up visits and record dates of monitoring tests. *Results.* We will determine the validity and patient satisfaction of the informational booklet and care card on a limited number of patients (10) over a follow-up period of 6 months. Results of this pilot study will allow to improve and adapt the booklet and diary accordingly. *Conclusions.* A care-card for myotonic dystrophies is already available in many countries of the EC and the US with demonstrated validity. We believe that a similar management tool is mandatory in Italy too because it may provide useful information for the patients, their families and doctors and by facilitating interaction between different specialists, may result in better management and care.

P65
FROM INITIAL SYMPTOMS TO GENETIC CONFIRMATION: WHAT IS THE TIME-LAG FOR MYOTONIC DYSTROPHIES IN ITALY?

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Objective. To determine the time-lag between the onset of symptoms of myotonic dystrophy type 1 (DM1) and type 2 (DM2) and genetic diagnosis. *Background.* Although the most frequent muscular dystrophy of adulthood, myotonic dystrophies are still underdiagnosed by general practioners in Italy. *Methods.* Medical charts from 92 patients with genetically-determined DM1 and 47 patients with genetically-determined DM2 attending our Neuromuscular Center were revisited. Onset of disease and symptom at onset were determined by the patients' recollection of initial symptoms of DM or age of early-onset cataract surgery. Time lag between onset of symptoms and clinical or genetic confirmation of either DM1 or DM2 was recorded for each patient. *Results.* (i)Age at onset was 25.4 yrs±14.6 for DM1 and 37.6±8.9 yrs for DM2; (ii) Most frequent symptom at onset was myotonia for DM1 and lower limb weakness for DM2; (iii) Time-lag between onset of symptoms and genetic diagnosis was 7.2±6.3 years for DM1 and 13.1±8 years for DM2; (iv) 8/47 (17%) patients with DM2 had a misdiagnosis (progressive muscular atrophy; n=3; inclusion body myopathy n =1; non-specific myopathy n =4); (v) In 5/92 (5.4%) patients with DM1 the diagnosis followed referral from other specialists (cardiologists, n=3; pneumologists, n=2). *Conclusions.* The multisystem presentation of DM1 and DM2, the transitory nature of myotonia due to warm-up, the avoidant personality traits of many patients may justify the delayed diagnosis in Italy. Our study suggests that more information on the disease and a better interaction between the referring physicians, relatives and neuromuscular specialists may reduce the diagnostic lag and improve management and cure of these patients.

P66
DETERMINANTS OF GENETIC KNOWLEDGE IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1 (DM1)

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A recent suggestion for a more efficient delivery of care in DM1 includes a better sharing of knowledge between patients and health professionals. This study sought to identify determinants of genetic knowledge in DM1 patients. Two-hundred adult DM1 patients (79 men; M=47±12 years; M=(CTG)n 809±529) completed a structured questionnaire including 6 items pertaining to genetic knowledge. Genetic knowledge scores (GKS) ranged between 0 and 6. To evaluate the relationship between genetic knowledge and demographic, clinical, and social characteristics of DM1 patients, we performed a multiple logistic regression of high genetic knowledge (GKS≥3) with terms for age, gender, disease severity, number of CTG repeats, educational attainment, marital, and employment status. The proportion of DM1 patients with a GKS≥3 was 38%. The logistic regression analysis revealed that genetic knowledge is influenced by the level of education (p<0.001) and the employment status (p<0.05). More particularly, each additional year of education completed by DM1 patients augmented the odds of having high genetic knowledge by about 21%. Also, the odds of having high genetic knowledge for DM1 patients who have never worked are decreased by about 76% in comparison to those who currently work. It is important that health professionals involved in genetic services dedicated to DM1 patients acknowledge that genetic knowledge is influenced by the patient's educational level and employment status. Indeed, application of genetic knowledge by DM1 patients and their families may influence prevention, health promotion, and health surveillance.

P67
MYOTONIC DYSTROPHY UNLINKED TO DM1 AND DM2 MUTATIONS IN THREE SIBLINGS

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Myotonic dystrophy (DM) is the most common adult form of muscular dystrophy, and is characterized by a peculiar multisystemic involvement. DM is caused by pathogenic expansions of unstable CTG repeats in the *DMPK* gene (DM1), or CCTG repeats in the *ZNF9* gene (DM2). According to current understanding, DM pathogenesis is related to intranuclear toxicity of the mutant RNA, resulting in the impairment of alternative splicing of several genes. Only a few DM cases lacking segregation with DM1 and DM2 loci have been reported so far. We present clinical, histopathological and genetic findings in three siblings from a non-consanguineous family with a late-onset form of DM. Neuromuscular involvement was characterized by weakness and atrophy of distal, proximal, and cranial muscles, prominent clinical and electric myotonia, and a myopathic pattern with additional neurogenic features on electromyography. Muscle biopsy studies showed increased fiber size variability, nuclear clumps and neurogenic atrophy. Multisystemic involvement was represented by the occurrence of ocular cataracts in the three patients. Molecular analysis failed to detect a repeat expansion in the *DMPK* and *ZNF9* genes, excluding both DM1 and DM2 diseases. *In situ* RNA FISH on fibroblasts and muscle biopsies, using a $(CAG)_{10}$-Cy3 probe, did not reveal the presence of ribonuclear inclusions derived from the accumulation of CUG-containing expanded transcripts. We are currently studying the splicing pattern of the *IR-A/B* and *MBNL1* genes, already known to be involved in the DM1 and DM2 pathogenesis. Taken together these data will provide further information on the clinical and genetic heterogeneity of the myotonic dystrophies.

P68
SELF-REPORTED HEALTH PROBLEMS AND HEALTH HABITS IN MYOTONIC DYSTROPHY TYPE 1: A PATIENT-ORIENTED PERSPECTIVE

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Background. In myotonic dystrophy type 1 (DM1), the occurrence of several health problems besides muscle impairment is one of the most characteristic features of the disorder. From a patient-centered perspective, the symptoms associated with impairments (myotonia, abdominal pain, etc) need to be addressed not only in terms of prevalence but also in terms of self-perceived impact on the patient's daily life. *Objectives.* 1) to describe self-reported health problems in patients with adult and late-onset DM1; 2) to rate symptom's burden associated with DM1 from a patient's perspective; and 3) to assess health habits such as smoking, alcohol consumption or physical activities in this population. *Methods.* A sample of 158 adult and 42 late-onset DM1 patients completed a general health questionnaire at home. *Results.* The most important health symptoms reported by the patients are cold extremities, myotonia, loss of balance, numerous intestinal problems, stair climbing limitation and daytime sleepiness. Sixty percent (60%) of the patients are overweight or obese, 30% are regular or occasional smokers, 18% of women and 28% of males report excessive alcohol consumption and the majority (76%) is physically inactive. *Conclusion.* These evidence-based results will help clinicians to be aware and address more efficiently the DM1 patients' concerns.

P69
WHAT DO PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1 KNOW ABOUT THEIR DISORDER: THE FIRST STUDY IN BASHKORTOSTAN (RUSSIA)

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Prevalence of Myotonic Dystrophy Type 1 (DM1) in Bashkortostan is the highest in Russia (R.Magzhanov, L.Akhmadeeva, 1997-2001). All patients and their families are registered in our original database. According to our prognosis (L.Akhmadeeva, B.Veytsman, 2001-2006) the prevalence of DM1 in Bashkortostan will slowly increase in the next decades. The aim of this small study was to find out if patients with DM1 are aware of their risk to transmit the disorder to their children. We made a short questionnaire (5 questions) and mailed it (together with a prepaid envelope) to 38 patients with confirmed diagnosis of DM1. All of them were fluent in Russian (which was the language of our questionnaire) and had no dementia. We received 29 replies. The mean age of these patients was 43.6 years. Most of our patients (81%) were aware of other people with DM1 in their families. Less than a half of the responders (13 patients) knew the correct figure of theoretical risk for their children to inherit this disorder. Two patients thought that the risk was 100%, 2 patients thought it was 90%, 7 patients were sure that their children have no risk to get a mutation from them and that all of them will have no signs of DM1. Other responders mentioned figures from 10% to 40%. Almost a half of the patients (12 people) did not know that the symptoms and signs of the disorder could start earlier and could be more severe in their children. Two patients wrote that nobody talked to them about genetic risk and prognosis. Nobody mentioned a nurse as a person who explained them anything concerning inheritance of DM1.

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P70
DEVELOPMENT OF OROFACIAL FUNCTIONS IN YOUNG INDIVIDUALS WITH MYOTONIC DYSTROPHY: A RETROSPECTIVE STUDY

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This study describes the characteristics, prevalence, and development of orofacial functions in a group of children and adolescents with myotonic dystrophy type 1 (DM1). The study population consisted of 56 patients (30 males, 26 females; median age 13 years) with DM1 and 56 healthy age- and sex-matched controls. The patients represented four subgroups of DM1: severe congenital (n=18), mild congenital (n=18), childhood (n=18), and classical (n=2). Thirty-five patients and 31 controls were assessed twice at approximately a 3–4-year interval. A speech-language pathologist assessed facial expression, intelligibility, oral motor performance, and lip force, and the families answered questions about eating and drinking ability and saliva control in a questionnaire. All patients had impaired facial expression. Intelligibility was considerably reduced in 30 patients, and 6 had no speech. The majority had moderate or severe impairment of lip motility (76%), tongue motility (52%), and lip force (69%). Families reported problems with drooling (37%) and eating (52%). Intelligibility, eating and drinking ability, and saliva control improved during childhood in some patients. Facial expression deteriorated significantly, especially in patients with childhood DM1, but the progressive weakening of the orofacial muscles also manifested as reduced intelligibility and increased drooling. Deterioration of the various orofacial functions often began before puberty.

P71
MOLECULAR ANALYSIS OF A FAMILY CO-SEGREGATING MYOTONIC DYSTROPHY TYPE 1 AND CHARCOT-MARIE-TOOTH DISEASE

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Charcot-Marie-Tooth disease (CMT) is a genetically heterogeneous hereditary neuropathy affecting motor and sensory nerves of the peripheral nervous system. We are currently investigating the molecular lesion in a very unusual three-generation family in which all the affected patients co-segregate both DM1 and Charcot-Marie-Tooth disease (LOD score = 7.03). Southern blot analysis of restriction digested genomic DNA has revealed a fragment equivalent to a small CTG expansion (~200-400 repeats) at the DM1 locus in all affected members. However, an expanded allele could not be amplified by PCR. Similarly, repeat primed-PCR revealed an expanded CTG repeat at the 5'-end of the array, but was negative at the 3' end. Our working hypothesis is that a CTG expansion has been accompanied by an additional lesion such as a deletion, insertion and/or rearrangement. Such a novel mutation might modify the expression of *DMPK*, *SIX5* and/or other nearby genes and explain the unusual clinical presentation observed in this DM1/CMT family. Genotyping of flanking SNPs have failed to detect loss of heterozygosity in the immediate vicinity of the CTG repeat, but have revealed that the mutation is found on the classic DM1 haplotype. We are currently using vectorette PCR to "walk" across the chromosome to characterise the unknown flanking DNA at the 3'-end of the array, the results of which we hope will provide an insight in to the unusual mutation in these patients.

P72
AN IMPROVED METHOD FOR SOUTHERN DNA AND NORTHERN RNA BLOTTING USING A MUPID® -2 MINI-GEL ELECTROPHORESIS UNIT FOR DIAGNOSIS OF DM1 AND 2

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Polymerase chain reaction (PCR) technology has largely eliminated traditional gene analysis methods, including Southern DNA and Northern RNA blotting. On the other hand, a difference in amplification speed has become apparent between the genomic regions or genes that are easy to amplify with PCR and those that are hard to amplify. The latter include candidate areas for DM1, DM2 FSH-type muscular dystrophy, SCA10 and Unverricht-Lundborg disease, all of which are beyond the capacity of *Taq* polymerase in PCR reaction and common PCR technology is useless.

Here we present an improved method for Southern DNA and Northern RNA blotting using the Mupid®-2 Mini-Gel System. We get sharp and clear bands in Southern and Northern blotting after only 30 minutes' short gel electrophoresis even in the extremely expanded repeats in DM1 and 2 patients, instead of the several hours large gel electrophoresis of conventional methods. The high electrical voltage with a pulse-like current of the Mupid®-2 Mini-Gel System also allows reduction of the amount of formaldehyde, a harmful reagent, from the gel running buffer in RNA blotting. This minor modification of DNA and RNA blotting technique enables us to perform the complete experimental procedure more quickly, economically and easily in less space, than conventional methods.

P73
MYASTHENIC PHENOTYPE AS POSSIBLE MANIFESTATION OF MYOTONIC DYSTROPHY

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Background. Myotonic dystrophy (MD) and myasthenia gravis (MG) are two neuro-muscular disorders with different clinical and paraclinical parameters. Some symptoms are overlapped and very rare both diseases could be diagnosed in the same patient. *Aim.* To present difficulties in diagnostic procedures of MD/MG in 15 years old girl. *Case report.* According to oculo-facio-bulbar symptoms in 15 years old girl, diagnosis of MG had been determined in regional hospital. We saw the patient 3 months later and only oculo-facial symptoms were detected without positive prostigmin test, decrement at repetitive stimulation (RS) test and acetylcholine receptor antibodies (AChRc-Abs). Myotonia was found at EMG in our patient and her mother (who expressed myopathic facies and also hypothyroidism). The majority of the family of proband's mother had voice or hands problems. We supposed that MD was more correct diagnosis than MG. Immunosuppressive therapy was discontinued. However, DM1 and DM2 mutations were not detected in proband and her mother, but DM1 was found in her uncle and uncle's daughter. After one year of stable state, the proband expressed exacerbation of bulbar symptoms with positive prostigmine test but negative RS test and negative AChRc-Abs (anti-Musk Abs were not done). Bulbar symptoms reacted very well on steroid therapy. *Conclusion.* Results of genetical tests of the family of our patient were unexpected for us. Therefore a further genetical study is necessary to explain a possible genotype heterogeneity if DM1 has coexisted with MG in our patient.

P74
CLINICAL AND NEUROIMAGING FEATURES OF MYOTONIC DYSTROPHY IN CHILDHOOD

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Myotonic Dystrophy type 1(DM1) is an autosomal dominant disease with multisystemic involvement.Genetic basis is the amplification of a CTG trinucleotide repeat within the 30 untranslated region of DM1 gene on chr. 19.In a proportion of these patients (up to 13%) the disease is congenital (CDM1).We here present the clinical and neuroimaging findings of at least six patients (both males and females) affected by congenital or childhood myotonic dystrophy 60% had an unremarkable pregnancy,in others ultrasound showed polyhydramnios and clubfoot. At birth all of them showed different degrees of hypotonia and respiratory distress;50% needed assisted ventilation. For two of them diagnosis of congenital myotonic dystrophy was made in first 2 months of age,1 was diagnosed at the age of 11, after father's diagnosis. 50% were first diagnosed or screened for different diseases (mainly cerebral palsy). Only 50% had a clinically evident myotonia. All of them showed mental retardation going from borderline to medium.
The diagnosis of CDM1 may be difficult in infancy mainly due to increased risk for obstetric complications in offsprings of DM1 affected women.In our cases 50% of patients were misdiagnosed initially as cerebral palsies, mainly because of their neuroradiological picture, but also for some overlapping clinical features that are easily overlooked and can delay or hinder diagnosis of CDM1. Recent studies suggest that the hyperintensity of white matter at the posterior-superior trigone is a characteristic change in CDM1 brain, possibly of developmental origin. What is imperative for a correct diagnosis is better awareness of clinical features and attention to mother's clinical picture to formulate adequate therapeutic interventions.

P75
REDUCED OXIDATIVE STRESS MARKERS AFTER CYSTEINE DONOR ENRICHED DIETARY INTAKE IN PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

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Myotonic dystrophy type 1 (MD) is the most frequent muscular dystrophy in adulthood, and an autosomal dominant multysistem disease, characterized by myotonic phenomena, muscle weakness, with involvement of ocular, endocrine, cardiac, gastro-enteric and respiratory systems.
Although the pathogenetic mechanism is still not completely clear, an increased susceptibility to the oxidative stress and increased levels of circulating free radicals in MD suggest a role of the oxidative stress in this disease. To analyze the occurrence of oxidative stress in DM before and after 30 days of treatment with an anti-oxidant therapy (with a food-integrator cysteine donor: Prother, AFR, 10 mg/die), we assessed blood levels of the following anti-oxidant defence systems and oxidative stress markers in 14 DM patients compared to healthy controls: glutathione (GSH), advanced oxidation protein products (AOPP) and ferric reducing ability (FRAP).
Before therapy, patients blood AOPP levels were significantly higher than in controls (603.7±49.2 vs 143.4±44.7; p=0.015), and it was significantly reduced after the 30-days treatment (373.08± 23.16; p= 0.015). Blood FRAP level was higher in DM patients than in controls (1.19±0.42 vs 0.75±0.16; p=0.016), and after treatment it was unchanged (1.11±0.36). GSH level was similar in patients and controls (2.48±0.68 vs 2.28±0.22.), as before and after treatment (2.40±0.63) without statistic significances. Therefore, our results confirm the role of oxidative stress in DM and underline the beneficial effects of antioxidant cysteine-donor therapy on biochemical markers of oxidative stress in DM.

P76
DEHYDROEPIANDROSTERONE IN MYOTONIC DYSTROPHY TYPE 1

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Background. Previous uncontrolled studies suggested potential benefit from dehydroepiandrosterone (DHEA) in myotonic dystrophy type I (DM1). The current study aimed at investigating the efficacy and safety of DHEA in ambulatory DM1 patients. *Methods.* In this prospective, multicenter, randomised, double-blind trial, 75 DM1 patients were assigned to receive orally replacement dose of DHEA (100 mg/day) or pharmacological dose (400 mg/day), or placebo. Primary end point: relative change in the manual muscular testing (MMT) score from baseline to 12-week. Secondary outcome measures: changes from baseline to 12-week in quantitative muscle testing and timed functional testing, respiratory and cardiac function, and quality of life. *Results.* The median (Q1 and Q3) relative change in MMT score from baseline to 12-week was 3.10 (-0.88: 6.71), 1.90 (-2.67: 3.47) and 2.19 (0: 7.94), in the DHEA 100 mg, DHEA 400 mg and placebo group, respectively. There were no difference between placebo and combined DHEA groups (p 0.34), placebo and DHEA 100 mg (p 0.86), or placebo and DHEA 400 mg (p 0.15). There were no evidence for a difference between groups for the changes from baseline to 12-week in quantitative muscular testing, timed functional testing, respiratory or cardiac function tests or SF36. *Conclusions.* Replacement or pharmacological doses of DHEA do not improve muscle strength in ambulatory DM1 patients.

P77
ABNORMAL GLUCOSE METABOLIC DISORDER IN MYOTONIC DYSTROPHY TYPE 1 (DM1): HYPERINSULINEMIA IN DM1 WERE INHIBITED USING VOGLIBOSE

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Purpose. Insulin resistance is a well-recognized feature of DM1. Clinically, the degree of $(CTG)_n$ closely correlates with the severity of the disease. In order to identify the behavior of abnormal glucose metabolism in DM1, 75gOGTT was done. In addition, I have hit on the idea that hyperinsulinemia (hyper-IRI) of the disease might be inhibited using voglibose (vogli), which has the inhibitory actions on intestinal α-glucosidase activity and postprandial hyperglycemia. *Methods.* 1) The 75gOGTT in 70 DM1 patients was done. Shortly before and at 30, 60 and 120 min after administration of glucose, plasma samples were collected to measure both plasma glucose (PG) and insulin (IRI) levels. To determine the degree of $(CTG)_n$, Southern blot analysis was done. 2) 20 DM1 patients with hyper-IRI were chosen. All patients had 500 kcal carbohydrate rich breakfasts. Shortly before and at 15, 30, 60, 120 and 180 min after the above breakfast, blood samples were taken to measure both PG and IRI levels (vogli (-) group). In another day, one tablet of voglibose was administered in the same patients shortly before the same breakfast. Similarly, blood samples were done (vogli (+) group). *Results.* 1) From the results of OGTT, it was indicated that abnormal glucose metabolism shifted from normal pattern or hyper-IR to impaired glucose tolerance and finally to diabetes mellitus (DM) with aging, progression of muscle weakness, and elongation of $(CTG)_n$. 2) PG levels at all time points were not significant between two groups. Both IRI 15, 30 levels and total sum of IRI in vogli (+) group were significantly lower than those in vogli (-), respectively. *Conclusion.* Glucose metabolism in DM1 patients shifted from normal pattern or hyper-IRI to DM with aging, progression of muscle weakness, and elongation of $(CTG)_n$. Hyper-IRI in DM1 patients were inhibited using voglibose. In addition, administration of the above agent might prevent DM1 patients with hyper-IRI from DM.

P78
CHARACTERIZATION OF MBNL1 RNA LIGANDS AND SEARCH FOR MOLECULES DISRUPTING THE (CUG)N-MBNL1 INTERACTION

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The current model for Myotonic Dystrophy type 1 (DM1) states that most symptoms, if not all, arise from the sequestration of the MBNL1 splicing regulation factors by expanded CUG repeats. The corollary of this model is that the symptoms can be suppressed by inhibiting the interaction between MBNL1 and CUG repeats. The most important step toward a cure for DM1 is thus to find molecules able to disrupt/prevent the deleterious interaction. In order to do so, we will perform a high-throughput *in vitro* screen. The full-length MBNL1 protein (41 kDa isoform) was purified to near-homogeneity (> 95 %) with a relatively high yield (~ 0.5 mg MBNL1 per Liter of culture). As a first step, the affinity of the protein for repeats of various sequences and sizes was determined by band-shift assay. For the purpose of our screen, we chose a synthetic, rhodamine-labelled (CUG)26 RNA as the ligand for MBNL1, and fluorescence polarization to detect the interaction between the protein and the RNA. Three chemical libraries will be screened, totaling 15,000 molecules, among which 1,000 are known medicines. We will present the qualified molecules and discuss which ones would be the best candidates for the development of a drug fighting DM1 symptoms.

P79
HIGH-THROUGHPUT SCREEN OF CHEMICAL COMPOUNDS TO IDENTIFY CANDIDATES THAT RELIEVE THE NUCLEAR RETENTION OF CUG-RICH MRNA

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The current RNA-based hypothesis suggests that the retention of mutant DMPK mRNAs as nuclear foci sequesters essential proteins that normally interact with CUG triplets in other mRNAs. The disruption of these nuclear foci is an important drug target to find medications that alleviate or prevent the development of symptoms in patients with myotonic dystrophy. We have developed a tissue culture model for DM1 that will allow us to screen a comprehensive library of compounds for effectors on DMPK mRNA retention and RNA foci formation. We have applied a novel technique which allows the detection of a specific RNA in living cells by highlighting it with a fluorescent protein to the visualisation of CUG repeat mRNA foci. The reporter chosen for our DM1 model is the cDNA of β-galactosidase followed by the 3'UTR of DMPK with expanded CUG repeats (LacZ-CUG). We can monitor the nuclear export and translation of this transcript using the practical β-gal assay.
RNA-binding proteins of the Muscleblind family co-localise with the myotonic dystrophy ribonucleic inclusions. When we knockdown Mbnl1 in our DM1 model cells, the LacZ-CUG reporter transcript is more efficiently exported to the cytoplasm and translated. Mbnl1 knockdown can thus be used as a positive control in our drug screen, since it has the effects we are looking for: disruption of nuclear foci and export and translation of the reporter mRNA. Our goal is to identify small-molecule drugs that have these two activities but that don't inhibit the normal function of important splicing factors.
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P80
NON INVASIVE ASSESSMENT OF MOUSE MUSCLE VOLUME USING 3D μ-ECHOGRAPHY

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Introduction. Mouse models are now widely used for drug discovery and muscle disorder studies (e.g. myopathy). Therefore a quantitative method to determine muscle volume *in vivo* will help us for the follow-up of the muscle disease. In the present work, we report a method based on 3D ultrasound (3D-US) imaging to quantify Tibialis Anterior muscle (TA) volume with high accuracy and precision. *Methods.* Mice were anesthetized and the lower leg was scanned along the TA muscle (over a 20 mm, 0.2 mm steps) using a 3D-US high-resolution probe (spatial lateral resolution 40μm). A semi-automated segmentation algorithm was used to delineate the TA muscle in the acquired images and assess its volume.
Validation and reproducibility of the measurement was investigated in 5 mice (C57bl6 of 10 months) examined 4 times and Intra-reader was also investigated. *Results.* Mean muscle volume measured by 3D-US was 40.4±1.1 mm^3 in control mice. Statistical analysis showed an intra-reader deviation equal to or less than 2%. In addition, a high correlation between TA *muscle ex vivo* weights and the volumes obtained using 3D-US was found for the investigated mice (R>0.95). The proposed method was used to follow-up TA volume evolution in 7 normal mice from age 2 to age 7 months. We observed a mean muscle volume increase from 17.6±1.3 mm^3 to 22.5 ± 1.4 mm^3, which correspond to an increase of 28%. *Conclusion.* 3-D US imaging provided a good precision and accuracy in the measurements of muscle volume in small animal models (e.g. TA muscle). This method could be very useful for the quantification of disease progression and for evaluation of the efficacy of new therapies.

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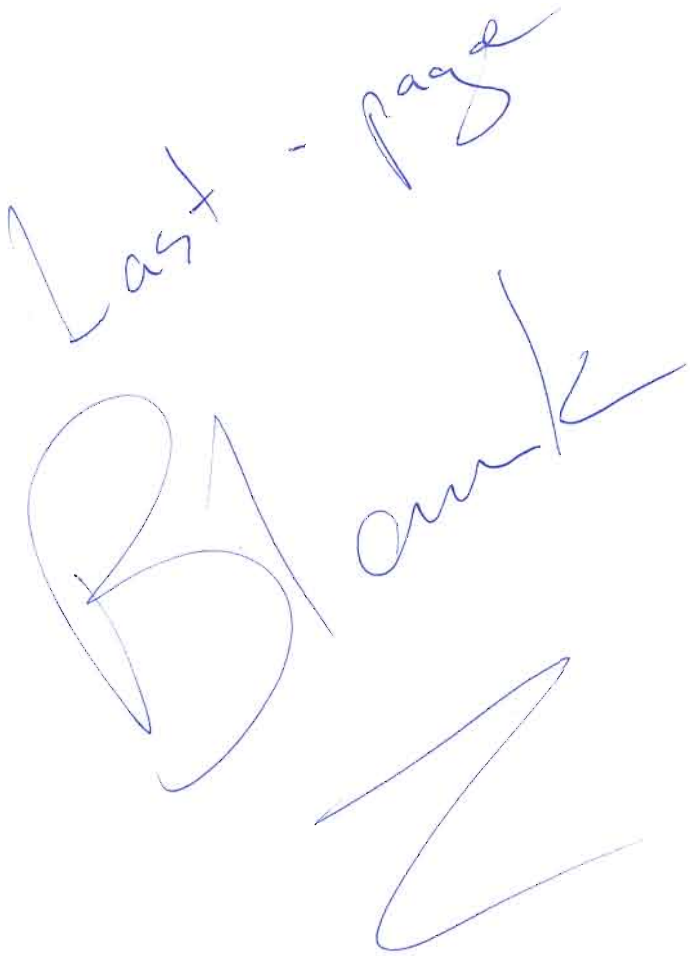
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