QUEBEC 2005

WEDNESDAY 19th TO SATURDAY 22nd OCTOBER

5th INTERNATIONAL

MYOTONIC DYSTROPHY CONSORTIUM MEETING
(IDMC-5)

A MESSAGE FROM THE CHAIRS OF THE 5th IDMC-5 MEETING

Dear Friends,

It is with great pleasure that we welcome you to the magnificent city of Quebec for the 5th International Myotonic Dystrophy Consortium meeting.

Every effort was made to include, in almost all sessions, presentations of potential interest to investigators, physicians and patients, without neglecting social issues and overall the global care of our patients. As for the previous meeting in Glasgow, there were so many abstracts submitted that we were not able to fit everybody in for oral presentations. We will be having a poster session as well.

There will be numerous innovations in the course of this congress, including a special lecture on the historical aspect of myotonic dystrophy in Quebec, the participation of patients in all sessions, an interactive session between investigators, physicians and patients as well as between patients coming from different countries. A videoconference with myotonic dystrophy support group from Los Angeles will be also organized. For the first time more than 80 patients and families will be present coming from France, Belgium, Greece, Italy, the UK, Canada and the USA. We hope that this meeting will be a great success allowing us all to learn from investigators and patients' experiences.

We would like to thank all those who actively participated in the organization of this meeting, as well as our generous sponsors who made this congress possible.

While in Quebec city, we hope that you take some time to enjoy the ambiance of the most European city in North America and that you get the opportunity to walk through the old part of town with your friends and colleagues. As for the previous meeting, the timetable allows for some socializing and discussion in a more relaxed atmosphere as well. This includes a visit to the First Nations village, a tour along the New France and St-Lawrence River road. On Saturday evening, you are invited to an exclusive private evening at the « Chapelle du Petit Séminaire ».

Yours sincerely

Jack Puymirat
Chair, IDMC-5

Christopher Pearson
Co-chair IDMC-5

IDMC-5 COMMITTEES

Local Organising Committee

Christopher Pearson
Genetics & Genomic Biology
Hospital for Sick Children
Toronto
Co-Chair IDMC-5

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University of Laval
Quebec

Jean Pierre Bouchard
Department of Neurology
Hospital Enfant-Jésus
Quebec

Pascale Rousseau
MDA. Canada
Montreal

Jack Puymirat
CHU Laval Research Center
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Hunter Research Fund
Atlanta, USA

Margaret Bowler
Myotonic Dystrophy Support Group
Nottingham, UK

Claude Boulier
French Myotonic Dystrophy Support Group
Evry, France

Honorary Presidents of the IDMC-5 meeting

Mr Bernard Barataud
Vice-President of the AFM
Director of the Genethon,
Evry, France

Ms Margaret Whal
Medical and Science Editor From MDA.USA

Ms Wyn Chivers
National Executive Director of MDA.Canada

SPONSORS

The organising committee is pleased to acknowledge the support of the following organisations in funding the meeting : The AFM, MDA.USA, MDA.Canada, the Quebec Network of Applied Genetic Medicine (RMGA), Quebec Dystrophy support group, Institutes of Genetics and of Neurosciences and Mental Health of the Canadian Institutes of Health Research, Quebec minister of Health, Air Canada, Roche, Avis, Fisher Scientific, Qiagen, Sarstedt, Aetherna-Zentaris, CHU laval Research Center.

The organising committee is also pleased to acknowledge the support of the following organisations in funding travel costs : The Association Française contre les Myopathies; the Hunter Research Fund; the Aino Institute for Aging Research.

REGISTRATION

The registration Area is located in the hall of Room Place Montcalm of lower level at the hotel Loews-Concorde from 5 h 00 to 7 h 00 pm on Wednesday October 19th.

ORAL PRESENTATIONS

Each speaker will have a 7 minute period with an additionnal 3 minute for one or two questions. There will be a 30 minute period at the end of each session for discussion. Please ensure you do not overrun. The programme is packed and the session chairmen will stick rigorously to the schedule.

Please note that all presentations should be in Power Point and will be projected exclusively via a centralised server. Therefore, we remind all speakers that they must go to the Speakers Ready Room with their presentation at least one hour before the session starts.

POSTERS

Posters boards will be located in room Suzor-Côté at third level of the hotel. Please attach your poster to the appropriated numbered board as soon as possible on arriving at the meeting. Please ensure you remove your poster at the end of the final session on Saturday afternoon.

Please not that poster presenters are required to be present during the poster session on Saturday 22, 12h-14h and during the poster discussion on Saturday 22, 14h-15h30. During discussion of poster, we ask you to present one slide (on Powerpoint) which summarises your work.

LUNCHES

Lunches will be served from Thursday to Saturday in the Hotel. Lunch times are listed on the timetable overview.

Thursday : Restaurant Astral
Elevator Top floor

Friday : Buffet
Hall third level

Saturday : Buffet
Hall third level

Please note that teas, coffees and juices throughout the meeting will be served during break session.

LANGUAGE

The official language of the 5th IDMC-5 meeting is English. For the interactive session with physicians, investigators and patients, a simultaneous translation will be available to allow patients who do not speak English to participate to the session.

SOCIAL EVENTS

Opening Ceremony & Welcome Reception

The opening Ceremony will take place on Wednesday October 19th at 19 h 00 in room Place Montcalm, lower level. The ceremony will open with welcome addresses from Honorary presidents. This will be followed by a lecture on the history of myotonic dystrophy in Quebec.

The Opening Ceremony will be followed by the Welcome Reception.

Lunch at Astral restaurant at Loews Le Concorde

A special lunch will be organised at the Astral Restaurant on Thurday 20 October, at 12 h 00. This is the crowning star of the hotel : 540 feet up in the air, this revolving restaurant serves up 13 miles of stunning 360-degree views

Visit at First Nations village

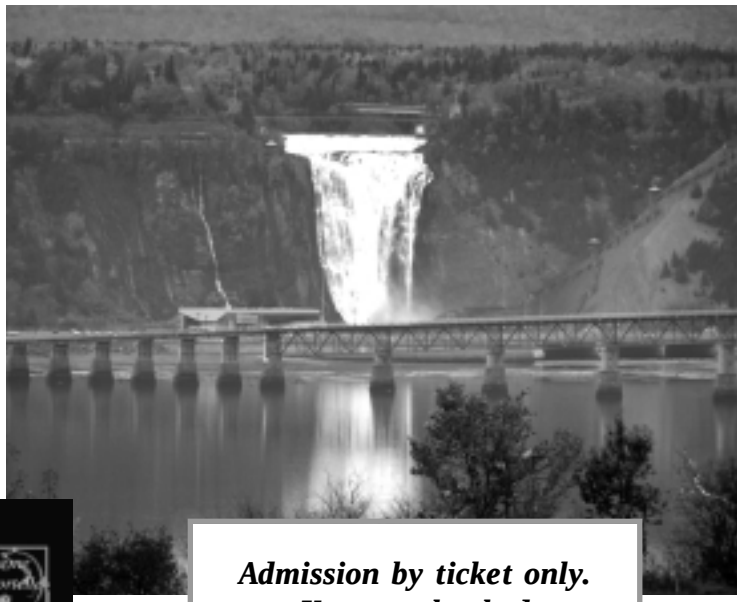
A very special evening will be organised at the First Nation village on Thursday, 20 October at 18 h 45. This visit will allow you to familiarize with songs, dance, medicine, culture, arts and crafts of the Indian Huron-Wendat Nation.

Admission by ticket only. Your pre-booked will be in your registration pack. Buses will be available at front-door.

Tour

On Friday afternoon there will be an excursion to Quebec and its environ. Following the New France, St-Lawrence River road you will visit Montmorency Falls, Cap-Tourmante, a truly peaceful haven for snow geeses and the museum of cupper Albert Gilles

Admission by ticket only. Your pre-booked will be in your registration pack.



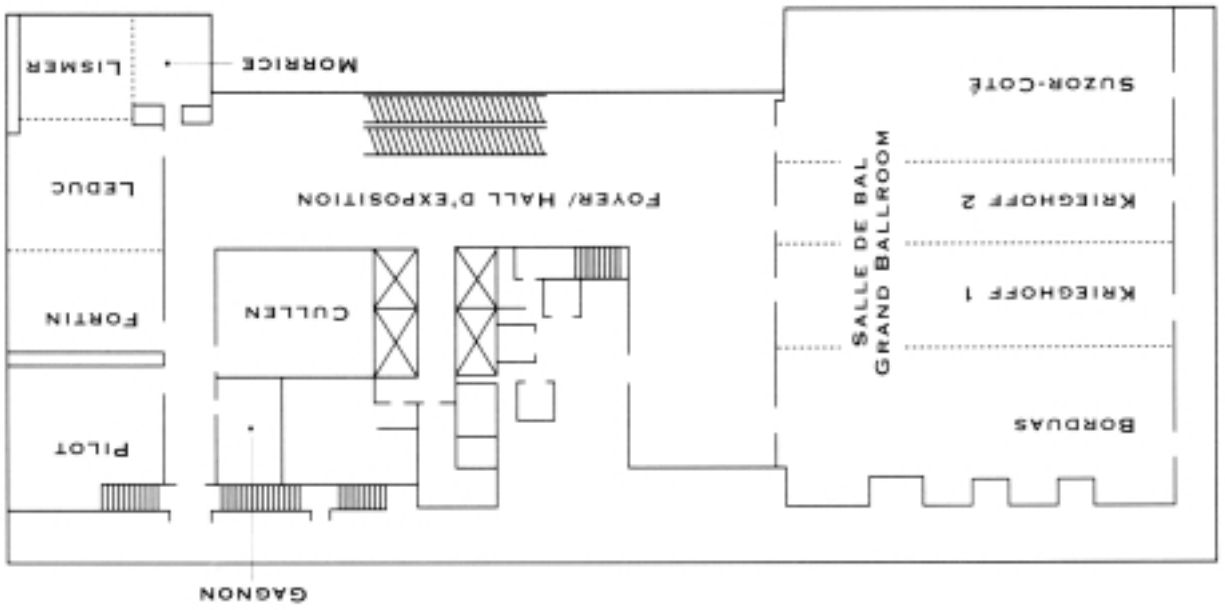
Admission by ticket only. Your pre-booked will be in your registration pack. Buses will be available at front-door.

Closing ceremony

Gala Dinner on Saturday 22 October at 20 h at the Chapel of l'Amerique française Museum. There will be a musical entertainment by Masks & Bergamasques compagny. Fantastic singers, skilled pianist and organ virtuoso perform in a show that people will remember.

Admission by ticket only. Your pre-booked will be in your registration pack. (Shuttle service) The Chapel is about 10 minutes of step from the Hotel.

PLAN



TROISIÈME ÉTAGE
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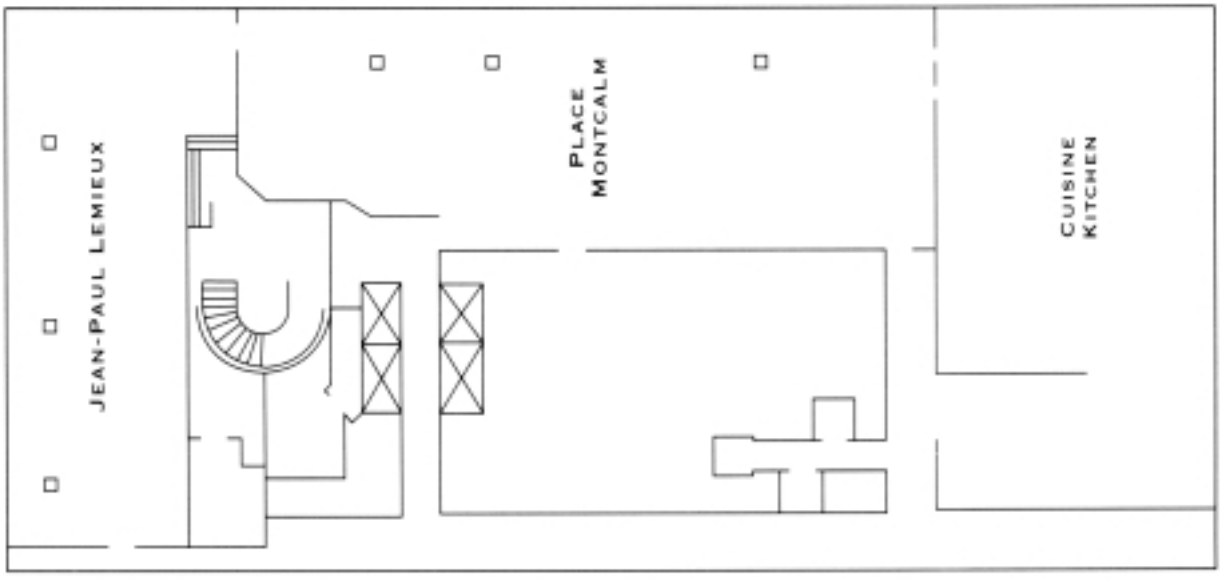


ÉTAGE INFÉRIEUR
LOWER LEVEL

Scientific Programme Wednesday October 19th

Room : Place Montcalm Lower Level

17 h - 19 h : **Registration**

19 h - 19 h 30 : **Opening by Honorary Presidents**

- Wyn Chivers, National Executive Director of MDA.Canada
- Bernard Barataud, Vice-president AFM
- Margaret Wahl, Medical and Science Editor from MDA.USA

19 h 30 - 20 h : **Special lecture**

A Historical Overview on DM1 in Northeastern Quebec

Dr Jean-Pierre L. Bouchard, MD, FRCPC
Department of Neurological Sciences
CHAUQ-Hospital Enfant-Jesus
Quebec, Canada

20 h : **Welcome Cocktail**

SESSION 1 : DNA INSTABILITY, DIAGNOSIS AND MODEL OF MYOTONIC DYSTROPHY

Session chairs : G. Gourdon and C. Pearson

8 h 30

1) Cellular factors that determine (CTG)n hypermutability in DM1
Wieringa, Be *et al*.

8 h 40

2) The behavior of triplet repeats in human embryonic stem cells
De Temmerman N. *et al*.

8 h 50

3) The expanded CTG/CAG repeat in myotonic dystrophy type 1 continues to expand in non-dividing cells
Monckton D.G. *et al*.

9 h

4) A role for DNA replication and DNA repair in CTG instability at the DM1 locus
Cleary J.D. *et al*.

9 h 10

5) Prenatal diagnosis in myotonic dystrophy type 1 (DM1) : the Spanish experience
Martorell L. *et al*.

9 h 20

6) Intergenerational contraction of the CTG repeats in large families with myotonic dystrophy.
Puymirat J. *et al*.

9 h 30

7) Assessment of *in situ* hybridization for molecular diagnosis of myotonic dystrophy type 2 (DM2) : pattern of tissue detection and new phenotype
Bassez G. *et al*.

9 h 40

8) Identification of a novel locus for myotonic dystrophy in human chromosome 16
Krahe R. *et al*.

9 h 50

9) Identification of a candidate gene for a multisystem myotonic disorder with frontotemporal dementia linked to 15q21-24 chromosome (DM3)
Bonifazi E. *et al*.

10 h -10 h 20

Tea/coffee break

10 h 20

10) Long DM1 and DM2 repeat sequences induce gross rearrangements in flanking DNA sequences
Wojciechowska M. *et al*.

10 h 30

11) Small-pool PCR study of the mutated alleles in sperm of myotonic dystrophy 1 patients
Cobo A.M. *et al*.

10 h 40

12) Somatic mosaicism and improved genotype-phenotype correlations in myotonic dystrophy type 1
Morales F. *et al*.

10 h 50

13) The laboratory abnormalities in myotonic dystrophy
Heatwole C.R. *et al*.

11 h

14) Development of, and insights from models of RNA toxicity in myotonic dystrophy (DM)
Mahadevan M. *et al*.

11 h 10

15) Inducible and tissue-specific expression of expanded CUG repeat RNA in transgenic mice using a cre-loxp approach
Wang G.S. *et al*.

11 h 20

16) A reversible multisystemic murine model of myotonic dystrophy type 2
Ranum, L.P.W. *et al*.

11 h 30 - 12 h

Discussion

12 h - 14 h

Lunch at Astral restaurant

SESSION 2 : CELLULAR AND MOLECULAR ASPECTS OF MYOTONIC DYSTROPHY

Session chairs : C Thornton and N. Ohsawa

14 h

17) Premature replicative arrest of congenital DM1 myoblasts
Furling D. *et al*.

14 h 10

18) Telomere shortening and replicative senescence in DM1 *in vitro* and *in vivo*
Xu W. *et al*.

14 h 20

19) Oxidative stress and IGF-I signaling in myotonic dystrophy type 1 (DM1) model skeletal muscle
Furutama D. *et al*.

14 h 30

20) Membrane localization of DMPK splice isoforms : differences between mouse and man
Van Herpen R. *et al*.

14 h 40

21) Expression of DMPK at the neuromuscular junction
Wheeler T.M. *et al*

14 h 50

22) Investigating RNA-mediated neuronal dysfunction in DM1 transgenic mice
Guiraud-Dogan C. *et al*.

15 h

23) Colocalization of ribonuclear inclusions and MBNL1 foci with no impairment of muscle differentiation in DM2
Meola G. *et al*.

15 h 10

24) Endoplasmic reticulum stress in DM1 muscle
Ikezoe K. *et al*.

15 h 20

25) Over-expression of SK channels increases sensitivity to calcium in DM1 lens cells
Rhodes J.D. *et al*

15 h 30 - 16 h

Tea/coffee break

16 h

26) Transcriptional profile of transgenic mice expressing an expanded CUG repeat
Osborne R.J. *et al*.

16h10

27) Dysregulation of specific gene transcripts in MBNL1 knockout brain
Swanson M. *et al*.

16h20

28) The role of protein-protein complexes in myotonic dystrophies 1 and 2
Timchenko L. *et al*.

16h30

29) Post-translational modifications of CUG-BP1 in response to CUG repeats
Kuyumcu-Martinez N.M. *et al*.

16h40

30) Analysis of candidate genes modifier for the conduction system impairment in myotonic dystrophy type 1 (DM1)
Vallo l. *et al*

16h50

31) Aberrant splicing of dystrophin and dystrobrevin in myotonic dystrophy type 1
Nakamori M. *et al*.

17h

32) Both exon 2/3 and exon 6 of tau RNAs are mis-spliced in myotonic dystrophy type 1 but differs in their tissue-specificity alteration and regulation by etr-3
Caillet-Boudin M.L. *et al*.

17h10

33) Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase in myotonic dystrophy type 1
Kimura T. *et al*.

17h20

34) The myotonic dystrophy type 2 (DM2) CCTG expansion does not alter ZnF9 expression
Ranum L.P.W. *et al*.

17 h 30 - 18 h

Discussion

18 h 45 - 22 h 30

Visit at the First Nation Village

SESSION 3 : RNA-BINDING PROTEINS

Session chairs : D Brook and M Swanson

8 h 30

35) The structural basis of myotonic dystrophy from the crystal structure of CUG repeats
Blaine H. *et al*.

8 h 40

36) Transcripts containing triplet repeat hairpins are under the surveillance of ribonuclease dicer
Krzyzosiak W.J. *et al*.

8 h 50

37) Studying nuclear foci of CUG repeat mRNA in living cells
Querido E. *et al*.

9 h

38) Muscleblind protein isoforms show differential RNA binding properties in a yeast three hybrid assay
Vicente M. *et al*.

9 h 10

39) Role of MBNL1 in DM1
Lin X. *et al*.

9 h 20

40) Elevated hnRNP-H levels contribute to abnormal IR splicing in DM1
Reddy S. *et al*.

9 h 30

41) Sequestration of a novel Zn-finger protein may cause degenerative muscle defects in DM1
Xu W. *et al*.

9 h 40

42) CUG-binding proteins, CUG repeats and Steinert myotonic dystrophy : what can we learn from *drosophila* ?
Ait-Ahmed O. *et al*.

9 h 50

43) Alternative splicing of CLC-1 chloride channel is regulated by MBNLl and CELF proteins
Kino Y. *et al*.

10 h

44) Zebrafish knock-down model for muscleblind-like 2
Machuca-Tzili L.E. *et al*.

10 h 10 -10 h 35

Discussion

10 h 35 - 10 h 50

Tea/coffee break

SESSION 4 : CLINICAL ASPECTS OF MYOTONIC DYSTROPHY

Session chairs : JP Bouchard and T Ashizawa

10 h 50

45) Analysis of annual follow-up data on 159 patients with myotonic dystrophy.
Whittaker R. *et al*.

11 h

46) Myotonic dystrophy type 1 - clinical characteristcs of patients with <100 CTG repeats
Hilton-Jones D. *et al*.

11 h 10

47) Myotonic dystrophy type 1 (DM1) with scapular involvement is genetically heterogeneous : cases with and without FSHD mutation
Eymard B. *et al*.

11 h 20

48) Electrocardiographic conduction abnormalities and sudden death in myotonic dystrophy type 1.
Groh W.J. *et al*.

11 h 30

49) CTG repeat governs the rate of decline of respiratory function in myotonic dystrophy
Bégin P. *et al*.

11 h 40

50) Differences in myotonic discharges in type 1 versus type 2 myotonic dystrophy
Logigian E. *et al*.

11 h 50

51) Non congenital paediatric myotonic dystrophy : clinical and genetic study in a series of 41 patients
Heron D. *et al*.

12 h

52) Incidence and Cohort study of congenital DM
Campbell C. *et al*.

12 h 10 - 12 h 30

Discussion

12 h 30 - 13 h 30

Lunch

13 h 30 - 19 h

Excursion

SESSION 5 : CLINICAL EVALUATION AND THERAPEUTIC ASPECTS OF MYOTONIC DYSTROPHY

Session chairs : J.W. Day and B Eymard

9 h

53) Quantitative CNS studies in adult-onset DM1 and DM2
Day J.W. *et al.*

9 h 10

54) Short-term memory test in DM1 and correlations with regional cortical atrophy
Antonini G. *et al.*

9 h 20

55) Sleep evaluation in the childhood type of myotonic dystrophy
Jacquette D. *et al.*

9 h 30

56) A sleep study of excessive daytime sleepiness in myotonic dystrophy
Laberge L. *et al.*

9 h 40

57) Myocardial contractility in myotonic dystrophy patients
Duboc D. *et al.*

9 h 50

58) Mexiletine : effective antimyotonia treatment in myotonic dystrophy type 1 (DM1)
Moxley R. *et al.*

10 h

59) Some flavonoids prevent *cis*- and *trans*- effect of expanded CTG repeats in a stable PC12 cell transformants
Furaya H. *et al.*

10 h 10

60) A potential gene therapy for myotonic dystrophy type 1
Puymirat J. *et al.*

10 h 20 -10 h 50

Tea/coffee break

10 h 50

61) A model of distribution of a mutation in DMPK gene associated with myotonic dystrophy type 1
Akhmadeeva L. *et al*.

11 h

62) Measuring health related quality of life (HRQl) with the resources of the NIH registry of myotonic dystrophy(DM) and FSHD patients and family members
Hilbert J. *et al*.

11 h 10

63) Are questionnaires an applicable investigation tool in relation to DM patients?
Schwartzbach I.

11 h 20

64) Social participation of patients with myotonic dystrophy type 1
Gagnon C. *et al*.

11 h 30

65) Parents' experiences of a diagnosis of DM : findings from a qualitative interview study
Downing C.

11 h 40-12 h 10

Discussion

12 h 10-14 h

Lunch and poster session

Room : Suzor-Côté, Third Floor

14 h - 15 h 30 : **Poster discussion**

Session chairs : Meola G. and Moxley R.

Room : Borduas, Third Floor

15 h 30 - 16 h : **Tea/coffee break**

16 h - 18 h : **INTERACTIVE SESSION WITH INVESTIGATORS, PATIENTS AND THEIR FAMILIES**
SESSION CHAIRS : P HARPER AND J PUYMIRAT

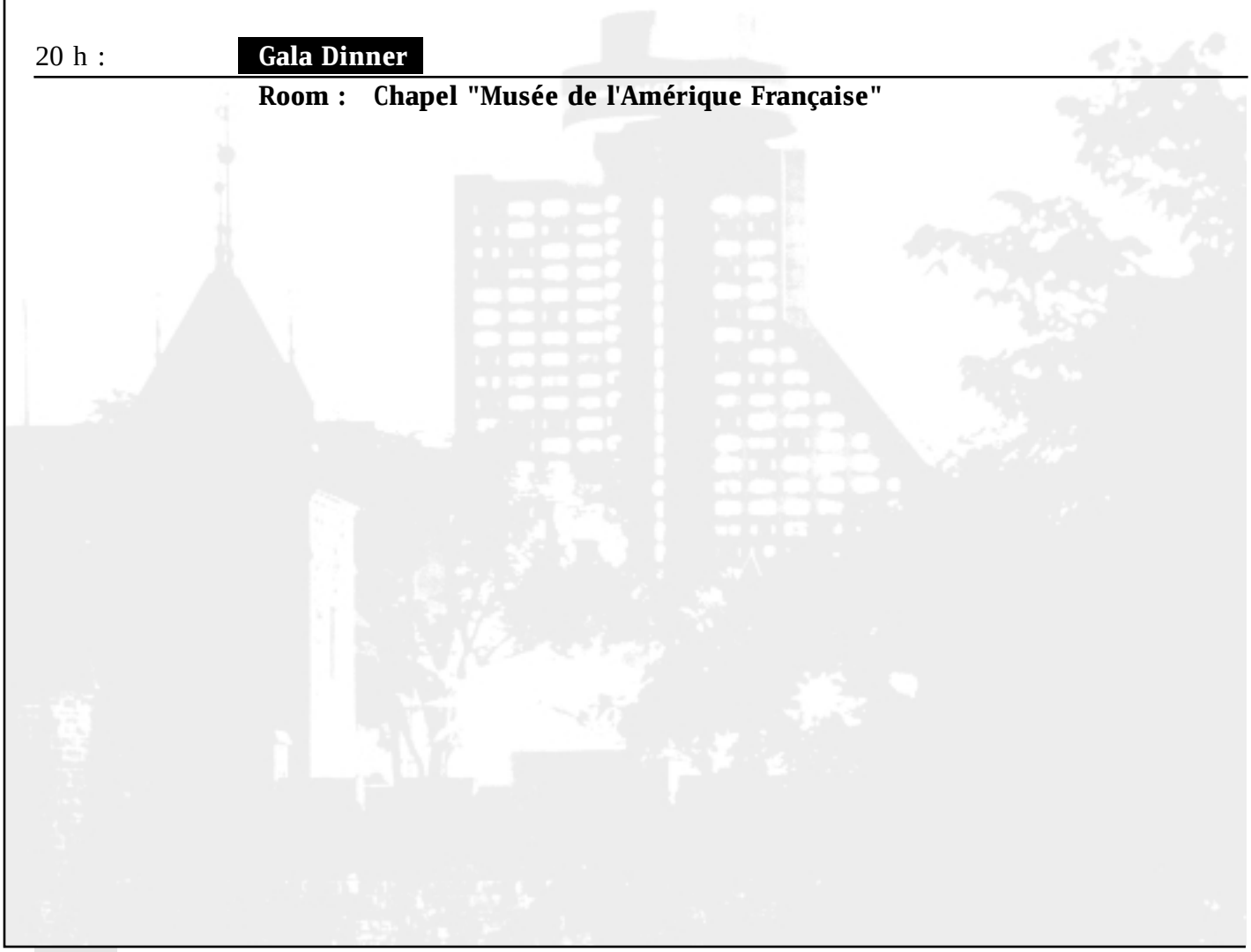
66) Myotonic Dystrophy. Patient Literature in Different Languages
Harper P.S.

Room : Borduas, Third Floor

18 h : **Film "Tomorrow Belongs to Me" presented by Jacqueline Donachie**

20 h : **Gala Dinner**

Room : Chapel "Musée de l'Amérique Française"



Friday October 21st PM

18 h 30 : **American support group organizational meeting and Buffet dinner**

Room : Borduas, Third Floor

Saturday October 22nd

11 h 00 : **Canadians Meeting**

Room : 415, 4th Floor

12 h : **Lunch**

Rooms : Krieghoff and Borduas

13 h : **Discussion workshop between participants from all over the world (Canada, France, United States, England, Italia and Greece) living with myotonic dystrophy**

Rooms : Krieghoff

15 h 30 : **Coffee break**

16 h : **Clinicians and researchers present to participants their conclusions brought by discussions with other clinicians and researches during the Consortium, followed by a period of questions**

Rooms : Krieghoff and Borduas

18 h : **Short film presentation of "Tomorrow Belongs to Me"***

Room : Krieghoff and Borduas

18 h 30 : **End of the session**

20 h : **Gala Dinner**

At the Chapel of the "Musée de l'Amérique Française"

ORAL PRESENTATIONS

CELLULAR FACTORS THAT DETERMINE (CTG)n HYPERMUTABILITY IN DM1

Van Den Broek W., Wansink D.G. and Wieringa B.

Dept. Cell Biology, NCMLS, Radboud University Medical Centre, Nijmegen
Geert Grooteplein 28, 6525 GA Nijmegen, The Netherlands

Cellular mechanisms involved in somatic (CTG)n repeat expansion during growth and ageing of DM1 patients are still ill understood. We have generated a transgenic knock-in mouse model with a (CTG)84 repeat placed at the correct position in the 3' UTR of the endogenous DMPK gene (van den Broek *et al*. (2002) Hum.Mol.Genet. 11, 191-8). Repeat instability - now expanded to (CTG)116 over successive generations – in this mouse lineage is dependent on activity of the Msh2-Msh3 DNA mismatch repair machinery, particularly prominent and progressive upon ageing in kidney, liver, stomach and small intestine, and cell-type and -state dependent. We now demonstrate that the highest degree of somatic hypermutability occurs in cells of liver and pancreas that have undergone polyploidization (change of 2n to ≥ 4 n DNA content) as the last phase of terminal differentiation. We will discuss possible mechanisms involved and present preliminary data about specific changes in the DNA repair machinery that may be associated with enhanced repeat expansion in cells with ≥ 4n DNA content. In addition, we will give an update on findings regarding the effects of endonuclease FEN-1 deficiency in early embryos with the (CTG)116 transgene.

THE BEHAVIOR OF TRIPLET REPEATS IN HUMAN EMBRYONIC STEM CELLS

De Temmerman N., Sermon K., De Rycke M., Liebaers I. and Van Steirteghem A.

VUB, Laarbeeklaan 101, 1090 Brussel, Belgium

The stability of the CTG repeat in DMPK was examined in the human embryonic stem (hES) cell line VUB03_DM1. This line was derived from a blastocyst obtained after PGD in which the affected mother carried 120 repeats. There was a generational enlargement of the repeat to 470 repeats, assumed to have been already present in the oocyte. The size of the CTG repeat was determined in cells from different colonies as well as in cells from the same colony, using a specific long-CTG PCR. In the early passages of this fast replicating cell line the repeat remained stable, showing 470 repeats in the majority of the cells analysed. At certain passages contractions (passage 4) and enlargements were present in some cells. In following passages (> 20 passages), mainly enlargements of the repeat were detected, showing an increase in repeat size in the culture ranging from 470 repeats at passage 1 to 600 and 730 repeats at passages 27 and 48, respectively. The different repeat sizes were present as well between different colonies as within a colony.

HES cells are regarded as a model to study developmental processes in preimplantation embryos, but the behaviour of the unstable CTG repeat in the hES cells of the VUB03_DM1 line resembles more the repeat instability in gametes than in cleavage embryos, in which the repeat was shown to remain stable. As described in other mitotically active tissue, the instability of the CTG repeat in hES cells is probably caused by replication slippage, although the role of repair enzymes and the effect of a prolonged culture cannot be excluded.

THE EXPANDED CTG/CAG REPEAT IN MYOTONIC DYSTROPHY TYPE 1 CONTINUES TO EXPAND IN NON-DIVIDING CELLS

Monckton D.G., Hilley J.D. and Gomes-Periera M.

Institute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK.

It is widely assumed that the mechanism of expansion of CTG/CAG repeats in myotonic dystrophy type 1 is DNA replication slippage. This model predicts that tissues/cells with higher rates of cell turnover will have higher rates of expansion. *In vivo* data from humans and transgenic mouse models shows no obvious correlation between tissue-specific expansion rates and proliferative capacity. Moreover, expansions are observed to accumulate in apparently postmitotic tissues such as skeletal muscle and brain. However, tissues are complex structures comprised of multiple cell types with differing proliferative capacities and rates of cell type-specific expansion. Previously, using a transgenic mouse cell culture model we demonstrated that tissue specific rates of expansion are conserved *in vitro*, but cannot be accounted for by differences in the rate of expansion. These data demonstrated that cell division was not sufficient to drive expansions, but left open the possibility that cell division might be required to generate expansions. In order to test this hypothesis, we have used multiple approaches to induce cell cycle arrest in our tissue culture model. Using chemical inhibitors of cell division, such as apicidin and mitomycin-C, and overexpression of cell cycle control genes, such as p16 and p21, we provide definitive proof that cell division is not necessary for expansion. Indeed rates of expansion in non-dividing cells are at least as great as, if not greater than, those in dividing cells, strongly arguing against any significant role for replication slippage in mediating expansions.

DNA REPLICATION & REPAIR IN CTG INSTABILITY AT THE DM1 LOCUS

Cleary J.D., Hagerman K., Panigrahi G.B., Lau R., Blondin M. and Pearson C.E.

Sickkids Research Institute, The University of Toronto, Toronto, Canada

In DM1 patients, CTG instability occurs in proliferating and quiescent cells, varying between tissues and developmental stage, suggesting complex mutation mechanism(s). Instability is thought to involve both the formation and aberrant processing of slipped-DNAs at the repeats in non-replicating or replicating DNA. DM1 patient fibroblasts showed spontaneous CTG expansions only in proliferating cells. Non-proliferating cells did not show CTG expansions. Treatment of cells with compounds that perturbed replication fork progression enhanced expansions-indicating a role for replication fork errors and their aberrant repair. Addressing both replication and repair we 1) determined the DNA replication profile at the DM1 chromosomal locus in DM1 patient fibroblasts in which CTG tracts were actively expanding. The DM1 locus is located within a region of abundant replication activity, with replication initiation occurring proximal to the repeat tract. We also 2) established an *in vitro* assay to determine the fidelity of slipped-DNA processing by human cell extracts. Various repair outcomes arose depending on the slipped-DNA structure. Repair outcomes could be mediated by various extracts including non-proliferating neuron-like cells, and were independent of the MSH2, MSH3, MLH1, XPF, and XPG repair proteins-suggesting they may be involved upstream of processing. Taken together these results suggest, at least for proliferating DM1 fibroblasts, that replication at the repeat tract may contribute to the formation of mutagenic DNA intermediates, and their aberrant repair may explain the spontaneous CTG expansions.

PRENATAL DIAGNOSIS IN MYOTONIC DYSTROPHY TYPE 1 (DM1) : THE SPANISH EXPERIENCE

Martorell L.[1], Cobo A.M.[2], Parra J.[3], Naudó M.[1] and Baiget M.[3]

1) Genètica Molecular, Hospital Sant Joan de Déu, Edifici Docent; C/Santa Rosa 39 08950- Barcelona, Spain
2) Genetics, Hospital Donostia, San Sebastián, Spain
3) Genetics, Hospital Sant Pau, Barcelona, Spain

Genetic counselling for myotonic dystrophy is difficult and complex, owing to the extreme variability of the disorder, in both severity and age of onset, the anticipation and the influence of the sex of the affected parent.

We have carried out 130 prenatal diagnosis(PND), most of which (70 %), the mother is the transmitting progenitor, with expansions ranging from 65 -1166 CTG. The remaining 30 % of PNDs are of paternal origin, ranging between 42-600 CTG. 48 % of PND where positive for the DM1 mutation, with expansions between 45 - 3666 CTGs in foetuses. In maternal transmissions we detected a predominance of expansions, with few contractions or no-variations. All the congenital cases are of maternal origin, and according to our data, if a female has one congenital infant, all her next affected descendants will be congenital cases. The contractions or small variations are predominant in paternal transmissions, although, the foetuses inherited alleles clearly into the pathological range.

INTERGENERATIONAL CONTRACTION OF THE CTG REPEATS IN LARGE FAMILIES WITH MYOTONIC DYSTROPHY

Puymirat J.[1], Guigère Y.[1], Lopez De Munain A.[2], Cobo A.M.[2], Bost M.[3], Ollagnon-Roman E.[4] and Vial C.[5]

1) Unit of Human Genetics & Dept of Medical Biology, CHUQ, Quebec, Canada
2) Department of Genetics & Neurology, Hospital Donostia, San Sebastian, Spain
3) Laboratory of Neurogenetics, Hospital Debrousse, Lyon, France
4) Unit of Neurogenetics, Hospital Croix Rousse, Lyon, France
5) Department of electromyography, Neurological hospital, Lyon, France

The frequency of CTG contraction during transmission from the father to the child has been estimated about 2 %, 2.8 % and 7 % in the French, Basque and French-Canadian population, respectively. In 6 of families originating from Rhone-Alpe French, French-Canadian and « Basque » DM1 population, the number of CTG repeats decreased during transmission from the father to all of their DM1 offspring's. This was associated with regression in clinical manifestations in 75 % of cases. In one of these families, two brothers with 650 and 500 repeats had all their DM1 offspring's with CTG repeat contractions, one has two children with 210 repeats each whereas the second had 2 children with 160 and 350 repeats, respectively. In all of these families, there was no case of regression associated with expansion during transmission from parent to child. These observations indicate that the paternal origin of the repeat and the presence of the repeat contraction in a sibling greatly increase the probability of the CTG repeat contraction in others siblings and that it may exist a familial paternal factor that restricts the expansion.

ASSESSMENT OF *IN SITU* HYBRIDIZATION FOR MOLECULAR DIAGNOSIS OF MYOTONIC DYSTROPHY TYPE 2 (DM2) : PATTERN OF TISSUE DETECTION AND NEW PHENOTYPE

Bassez G., Chapoy E., Bergemer A.M., Pellissier J.F., Radvanyi-Hoffman H., Gherardi R.K. and Eymard B.

Service d'Histologie - Hôpital Henri Mondor, Creteil, France

Whereas genetic diagnosis of myotonic dystrophy type 1 DM1 (DM1) is routinely available, limitations in conventional molecular genetic methods for DM2 mutation demonstration have been encountered. Recently, chromogenic *in situ* hybridization (CISH) have been reported as a new approach for the detection of DM2 expansion mutation in muscle. We evaluate the feasibility and efficiency of CISH for routine molecular diagnosis of DM2 in skeletal muscle and other tissues in frozen and paraffin embedded biopsy specimen of patients with : (1) evidenced DM2 mutation, (2) DM2 mutation excluded, (3) DM1 mutation, (4) controls with no available genetic test. All patients with identified DM2 mutation were positive by CISH, whereas no nuclear signal was observed in DM1 and normal controls. This allowed us the retrospective positive confirmation of DM2 in ancient specimen up to 17 years of age and identification of two unrelated cases with camptocormia as new phenotypic presentation. Further-more, we detected positive nuclei in cells from various tissues, including digestive tract, skin, adipocytes, and smooth muscle, and at low level in peripheral nerve and epithelial cells. Conclusion : Targeting mutant transcripts in tissue section by CISH approach seems to represent a valuable tool for molecular screening of DM2 leading to potential identification of new phenotype. Furthermore, the positive detection in nuclei of non muscle cells may offer new diagnostic opportunity.

IDENTIFICATION OF A NOVEL LOCUS FOR MYOTONIC DYSTROPHY IN HUMAN CHROMOSOME 16

Krahe R.[1], Lehmann-Horn F.[2], Jurkat-Rott K.[2], Gamez J.[3], Raskin S.[4], Schoser B.G.[5] and Bachinski L.L.[1]

1) Sect. of Cancer Genetics, Univ. of Texas M.D. Anderson Cancer Ctr., Houston, TX, USA
2) Dept. of Applied Physiology, University of Ulm, Germany
3) Dept. of Neurology, General Hospital Vall d'Hebron University, Barcelona, Spain
4) Genetics Laboratory, Hospital de Clinicas da Universidade Federal do Paraná, Brazil
5) Dept. of Neurology, Ludwig Maximilians University Munich, Germany

Myotonic dystrophy types 1 and 2 (DM1 and DM2) are caused by expansions of (CTG)n and (CCTG)n repeats in DMPK (in 19q13.3) and ZNF9 (in 3q21.3), respectively. A third DM locus was recently mapped to chromosome 15q21-q24, but the mutation remains unknown. During the course of a 10-cM genome scan to identify the DM2 locus, several families were identified that had no expansions in DMPK or ZNF9 and whose disease did not segregate with these loci. Among these families was one that showed a LOD score of >1.3 in chromosome 16. To demonstrate whether this LOD score represented a novel locus, we genotyped additional markers in chromosome 16 in this and other families segregating autosomal dominant progressive myotonic myopathy, which had also tested negative for expansions at the DM1 and DM2 loci. Using 9 such families (4 German, 4 Spanish, and 1 Brazilian), we obtained a LOD score of 3.83 in the p-arm of chromosome 16. We conducted haplotype analysis to determine the minimal region shared by these 9 families. Hypothesizing that DM in these families may be due to an expanded repeat of the type (CTG)n, (CCTG)n, or other similar sequence (xCTG)n , we interrogated all such repeats in the shared region with _4 repeat units. Repeats were examined by amplification across the repeat to check for non-Mendelian inheritance, a hallmark of expanded repeat diseases, and by a repeat-primed PCR assay (RP-PCR) to identify expansions. None of the candidate repeats showed an expansion or non-Mendelian inheritance. Since most of these families are small, each contributes only a small positive amount to the total LOD score. However, in all cases, the LOD scores observed are near the simulated ELODmax. Nevertheless, the small family size makes it impossible to exclude locus heterogeneity within this set of families, thus complicating precise localization of the linked region. We conclude that chromosome 16 harbors a locus that is responsible for DM in most of these kindreds. We are continuing the search for the causative mutation by interrogating additional repeat sequences in the regions surrounding the shared region.

IDENTIFICATION OF A CANDIDATE GENE FOR A MULTISYSTEM MYOTONIC DISORDER WITH FRONTOTEMPORAL DEMENTIA LINKED TO 15Q21-24 CHROMOSOME (DM3)

Bonifazi E., Vallo L., Contino G., Iraci R., Rinaldi F., Botta A. and Novelli G.

Genetics Unit, Tor Vergata University, Via Montpellier 1, 00133 Rome, Italy

Myotonic dystrophies (DM) are a genetically heterogeneous group of diseases, DM1 and DM2, that result respectively from dynamic mutations in the DMPK and ZNF9 genes. There are patients with characteristic DM clinical feature but wild type at loci DM1 and DM2. These cases suggest the hypothesis of another DM locus. Recently, a third locus (DM3) associated with a myotonic phenotype, was mapped to chromosome 15q21-24 (Le Ber *et al*., 2004) in large pedigree with a non-DM1 non-DM2 multisytem disorder associated with frontotemporal dementia. We identified in this region a gene, ZNF291 (GeneID : 49855) that possesses an unstable [CCTG]n repeat in the second intron. Analysis of 50 normal subjects shows the variability of the [CCTG]n repeat in ZNF291 gene between 5 and 15.

To test the ZNF291 expression in tissues involved in the DM3 pathogenesis, we used a RT-PCR based approach and we observed ZNF291 gene expression in different areas of human brain, skeletal muscle and heart. Our findings indicate that ZNF291 is a potential candidate gene for the multisystemic myotonic disorder with frontotemporal dementia phenotype.

LONG DM1 AND DM2 REPEAT SEQUENCES INDUCE GROSS REARRANGEMENTS IN FLANKING DNA SEQUENCES

Wojechowska M., Bacolla A., Dere R., Hebert M., Kosmider B., Larson J. E., Napierala M., Son L.S. and Wells R.D.

Institute of Biosciences and Technology, Texas A&M University System Health Science Center, 2121 W. Holcombe Blvd., Houston, TX 77030-3303, USA

The molecular mechanisms responsible for the genetic instabilities (expansions and contractions) of repeating tri- and tetranucleotide tracts, which are involved in the etiology of certain neurological diseases, are under investigation. Replication, recombination, and several repair mechanisms (including the involvement of DSBs) have been implicated in the bacterial and mammalian cell studies. We have recently discovered that long tracts of CTG/CAG, CCTG/CAGG, or CGG/CCG repeats cause complex rearrangements, including inversions and gross deletions, spanning several Kbp of flanking sequences. Investigations in E. coli, COS-7, and HEK-293 cells show that longer tracts (full mutations) are most potent, and the orientation of the repeats and their transcriptional status influence the mutagenic potential as well as the types of products. The locations of the breakpoints coincided with structurally contorted regions of the putative non-B DNA conformations (cruciforms, slipped structures, triplexes, tetraplexes) in these sequences. The consequences of these discoveries for DM1 and DM2 patients with full mutations remain to be elucidated but may have profound implications for the integrity of their gene products. (Supported by N.I.H, Robert A. Welch Foundation, Friedreich's Ataxia Research Alliance, and Muscular Dystrophy Association.)

SMALL-POOL PCR STUDY OF THE MUTATED ALLELES IN SPERM OF MYOTONIC DYSTROPHY1 PATIENTS

Cobo A.M.[1], Poza J.J.[2] and Lopez De Munain A.[2]

1) Genetics Unit
2) Neurology Dept. Hospital Donostia, San Sebastian 20014, Spain

The individual expansion in each DM1 patient measured in blood consists in multiple size alleles defined as somatic heterogeneity. The latter is minimal at birth and increases over time in the DM1 patients. On the other hand, DM1 males show extreme variability in the allele distribution in germline, regardless of their expansion size in blood. The increase of the expansion size in the majority of the intergenerational transmissions could be due to a selection bias in transmission, towards the highest size range of the paternal alleles.

We have analysed the expansion size in the sperm of 26 DM1 males by small-pool PCR (SP-PCR) to establish the allele distributions in DM1 male sperm and try to determine if a selection bias of the mutated alleles exists. As reported previously, a higher heterogeneity was seen in sperm than in blood. The distribution of the mutated alleles was homogeneous and no apparent excess of the upper size mutated repeats was observed except for one patient who showed more heterogeneity in blood (blood range 315-833, 315-420 in sperm). However, the major finding of our study is the high proportion of azoospermic patients (11/26, 42 %) in our series. This is observed when the expansion size in blood is ≥500 CTG except for two premutated patients. Furthermore, the upper size limit of mutated alleles present in sperm is 915 CTG in our patients, which could explain the nearly exclusive maternal transmission of the congenital form. Our findings make difficult a prediction of the severity of the affected offspring based on sperm SP-PCR results.

SOMATIC MOSAICISM AND IMPROVED GENOTYPE-PHENOTYPE CORRELATIONS IN MYOTONIC DYSTROPHY TYPE 1

Morales F., Cuenca P., Del Valle G., Brian R., Sittenfeld M. and G. Monkton D.G.

Institute of Biomedical and Life Sciences, University of Glasgow, UK

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. Importantly, a failure to account for the age-dependence and expansion-biased nature of somatic mosaicism has greatly compromised genotype-phenotype correlations. Previously, Southern blot analyses of restriction-enzyme digested genomic DNA have revealed that measured allele length typically accounts for less than 50 % of the variation in age of onset. These data leave open the possibility that other factors (genetic/environmental) might be more important in determining age of onset. In order to improve the prognostic value of genotype-phenotype relationships in DM1, we have used small pool PCR to define in detail the degree of somatic mosaicism in a cohort of Costa Rican patients. Using these data to estimate the progenitor allele length and an improved statistical analysis, we found that a logarithmic transformation of the inherited allele length accounts for >75 % of the variation in age of onset ($r=0.87$, $p<0.0001$). These data definitively establish that inherited allele length is the major modifier of the age of onset in DM1. A preliminary statistical analysis of the degree of somatic mosaicism in a given individual has revealed a complex relationship with both progenitor allele length and age at sampling. These data provide new insights in to the dynamics and mechanisms of somatic expansions and provide a basis for the quantification of individual-specific residual variation in the rate of expansion. The identification of individual-specific factors that mediate these differences is important, since these modifiers are very likely also modifiers of disease severity.

LABORATORY TEST ABNORMALITIES IN MYOTONIC DYSTROPHY

Heatwole C.R., MD, Miller J., MD PhD, Martens B. and Moxley R.T., III MD

The University of Rochester, Rochester (NY), USA, 14620

Background : Myotonic dystrophy type-1 is the most prevalent form of adult muscular dystrophy worldwide. Although well known for the classic manifestations of myotonia, weakness and early cataracts, it has broad reaching effects on multiple organ systems.

Objective : To analyze and compile the laboratory abnormalities of 126 adult myotonic dystrophy type-1 (DM1) patients.

Patients : Baseline preclinical-trial laboratory data was compiled from 126 medically healthy, mild to moderately affected, ambulatory, DM1 patients with good venous access from 12 major clinical trials between the years 1975 and 2005. These data include values for 45 different laboratory tests, and 2860 total studies.

Results : Of the 2860 laboratory studies, 470 (16 %) were outside the normal standardized parameters. Of the 45 types of laboratory tests studied in DM1 patients, 41 demonstrated abnormalities. The prevalence of an outlier laboratory value was greater than 50 % in several tests including : lactate dehydrogenase (LD), hemoglobin A1c (HgA1c), follicle stimulating hormone (FSH), and luteinizing hormone (LH) in men, and gamma-glutamyltransferase (GGT), creatine phosphokinase (CK), and hemoglobin (HB) in women.

Conclusions : There is a high frequency of abnormal laboratory values in DM1 which may provide a basis for early screening and monitoring as well as provide insight into the spectrum of tissues involved in this disease.

DEVELOPMENT OF, AND INSIGHTS FROM MODELS OF RNA TOXICITY IN MYOTONIC DYSTROPHY (DM)

Mahadevan M., Yu Q., Yadava R., Frenzel-McCardel C. and Yarasi S.

University of Virginia, Department of Pathology, 415 Lane Rd., MR5 Building, Rm. 3330, PO, USA

Myotonic dystrophy (DM) is the most common inherited neuromuscular disorder in adults. There are two types, DM1 and DM2, both being autosomal dominant disorders caused by expansions of microsatellite repeats within non-coding regions of their respective genes. DM1 is far more common; however both forms of DM are likely to share similar pathogenic mechanisms. The DM1 mutation is an expansion of a CTG triplet repeat in the 3' untranslated region (3'UTR) of the DM protein kinase (DMPK) gene. A prevailing hypothesis in the field is that many aspects of DM are caused by the expression of the mutant mRNA. DM1 and DM2 represent the first examples of toxic RNA mediated disease pathogenesis. We have already developed and characterized extensively, a myoblast cell culture model with which we were one of the first groups to clearly demonstrate the toxic effects of the mutant DMPK mRNA on muscle differentiation. To study the hypothesis further, we are trying to develop and characterize additional models of RNA toxicity for DM1. Progress and insights from ongoing efforts will be presented.

INDUCIBLE AND TISSUE-SPECIFIC EXPRESSION OF EXPANDED CUG REPEAT RNA IN TRANSGENIC MICE USING A CRE-LOXP APPROACH

Wang G.S., Bundmand D. and Cooper T.A.

Baylor College of Medicine, Houston, TX, USA

We developed a transgenic mouse model for myotonic dystrophy, Type 1 (DM1) by inducibly expression of expanded CUG RNA using a Cre-loxP approach. The transgene (EpA960) contains a CMV promoter, a floxed concatemer of the SV40 polyadenylation site, and DMPK exon 15 containing 960 interrupted CTG repeats. Expression of expanded CUG repeat RNA is induced by breeding EpA960 transgenic animals with a Cre-expressing strain. Five founder lines were obtained which express different levels of EpA transgene mRNA. To test the mouse model in heart, three EpA lines were crossed with mice expressing heart-specific and tamoxifen-inducible Cre. Bitransgenic mice expressing the highest level of EpA960 mRNA died within one week after tamoxifen administration while the two lower expressing lines remained viable. To validate that this bitransgenic mouse model, we examined genes that have been shown to undergo mis-regulated alternative splicing in individuals with DM1. Cardiac troponin T exons 4 and 5 were included in bitransgenic animals as found in DM1 cardiac tissue, whereas the inclusion was low in wild type and mock-treated animals. We found that splicing of Fragile-X-related protein 1 exons 15 and 16 reverted to an embryonic pattern in both DM1 and bitransgenic animal cardiac tissue. Nuclear RNA foci were detected in heart from bitransgenic animals by *in situ* hybridization. Histological and functional examinations are in progress. The EpA lines provide a versatile system to express mRNA containing long CUG repeats in the context of DMPK exon 15 in a variety of tissues and developmental stages.

A REVERSIBLE MULTISYSTEMIC MURINE MODEL OF MYOTONIC DYSTROPHY TYPE 2

Margolis J.M., Kang Y.L., Zu T., Day J.W. and Ranum L.P.W.

MMC 206, 420 Delaware St. SE, University of Minnesota, Minneapolis, MN 55455, USA

We previously showed that myotonic dystrophy type 2 (DM2) is caused by a large CCTG repeat expansion in intron 1 of the zinc finger protein 9 (ZNF9) gene. There is growing evidence that DM1 and DM2 are caused by an RNA gain of function mechanism in which the CUG and CCUG expansions cause the multisystemic features of these diseases by directly altering RNA processing. To investigate the role that expression of the CCUG transcripts play in the multisystemic DM phenotype, and to avoid problems such as infertility that have complicated previous systemic models of DM1, we have developed a tetracycline inducible murine model of the DM2 CCUG expansion. In our expansion lines, the expression of a CCTG repeat tract with 300 repeats is driven by a tet-responsive (TRE)/minimal CMV promoter as part of a non-coding transcript. These TRE-CCTG expansion lines have been crossed to tet-activator (tTA) mice in which the tTA is controlled by a CMV promoter. Doubly transgenic mice TRE-CCTG / CMV-tTA mice ubiquitously express CCUG expansion transcripts in the absence of doxycycline. Routine H&E staining of muscle from these doubly transgenic animals shows variation in fiber size and centrally located nuclei indicative of myopathy. Additionally, skeletal muscle shows ribonuclear inclusions and aberrant splicing of the insulin receptor (IR) by RT-PCR analysis, which has not previously been reported in mouse models of DM1. Like DM1 and DM2 patient biopsies, the predominant IR isoform in muscle from these mice excludes exon 11 which leads to a preferential expression of the insulin insensitive isoform. Further, aberrant splice patterns of cTNT are observed by RT-PCR from cardiac muscle indicating that the CCUG expansion transcripts in this model affect both cardiac and skeletal muscle. In a separate series of experiments the TRE-CCUG expansion lines described above will be crossed to two different BAC transgenic lines engineered to drive expression of the tTA using the endogenous human DMPK or ZNF9 promoters. Phenotypic characterization of these doubly transgenic mice will help determine the extent to which the clinical similarities and differences, including developmental differences between DM1 and DM2, result from differences in the temporal and spatial expression patterns of the repeat containing transcripts driven by the ZNF9 and DMPK promoters.

PREMATURE REPLICATIVE ARREST OF CONGENITAL DM1 MYOBLASTS

Furling D., Bigot A., Mouly V. and Butler-Browne G.

CNRS FRE2853, Faculté de Médecine UPMC, 105 boulevard de l'hôpital, 75013 Paris

Impairment in skeletal muscle development represents one of the main features in the congenital form of DM1 (CDM) that is associated with large CTG expansions. Using an *in vitro* model of primary satellite cell cultures isolated from the muscles of CDM fetuses, we have previously shown that the large CTG repeats affect the differentiation program of these myogenic precursor cells. Molecular alterations associated to the CTG mutation were found in this cellular model as shown by the presence of nuclear foci of DMPK mutant transcripts, splicing defects and somatic instability of the CTG expansion. We showed that the proliferative capacity of the CDM myoblasts was also significantly reduced when compared to normal cells. In human, the life span of the somatic cells is limited by a mechanism of replicative senescence. The mitotic clock determined by the telomere length was showed to play a major role in this process. However we demonstrated that the proliferative arrest of CDM myoblasts is not correlated with an excessive reduction of the telomere length since the CDM myoblasts stop dividing with larger telomeric DNA restriction fragments than control myoblasts. This result clearly indicates that the CDM myoblasts have not exhausted their proliferative capacity but have a premature replicative arrest. Activation of the cyclin-dependent kinase inhibitor p16 is another regulatory pathway of replicative senescence in some cell types. We showed that the p16 pathways is involved in cell cycle arrest of senescent human myoblasts and a similar induction of p16 was measured in CDM myoblasts with premature arrest. We suggest that a cellular stress induced by the large CTG mutation triggers a p16 dependent mechanism leading to the premature senescence in CDM myoblasts.

TELOMERE SHORTENING AND REPLICATIVE SENESCENCE IN DM1 *IN VITRO* AND *IN VIVO*

Xu W., Sarkar P. S., Gao R. and Ashizawa T.

Department of Neurology, UTMB, Galveston, TX, 77550-00874, USA

Patients with DM1 often develop clinical signs resembling accelerated aging, such as muscle weakness and wasting, insulin resistance, dilated cardiomyopathy with cardiac conduction abnormalities, chronic constipation, cataract, testicular atrophy, balding, and hearing loss. We previously showed that DM1 lymphoblastoid cell lines (LBCLs) exhibit accelerated cell-cycling and shortened lifespan in culture. To explore the relationship between the high rate of proliferation and the accelerated cellular aging, we examined the telomere length and replicative potentials of DM1 cells. DM1 LBCLs exhibited faster telomere shortening with decreased lifespan and senescence-associated morphological changes. The rate of telomere shortening correlated with the CTG repeat expansion size. DM1 fibroblasts showed a similar number of population doublings and viability as their age/sex matched controls before reaching the replicative senescence. However, DM1 fibroblasts showed significantly higher BrdU incorporation and a faster proliferation rate than the normal controls ($p<0.001$), resulting in shorter culture lifespan. Additionally, the mean telomere length in circulating leukocytes of DM1 patients was significantly shorter than that of age-matched controls ($p<0.001$), suggesting that accelerated senescence also occurs in DM1 cells *in vivo*. These *in vitro* and *in vivo* data suggest that the expansion of the CTG repeat in DM1 increases cellular turnover, leading to rapid telomere shortening with accelerated cellular senescence.

OXIDATIVE STRESS AND IGF-I SIGNALING IN MYOTONIC DYSTROPHY TYPE 1 (DM1) MODEL SKELETAL MUSCLE

Furutama D., Tanaka T., Fujita A., Fujimura C., Hirano S., Thornton C., Hanafusa T. and Ohsawa N.

1st Department of Internal Medicine, Osaka Medical College. Takatsuki, Japan

The serum concentration of dehydroepiandorosterone (DHEA) or DHEA-sulfate (DHEA-S) shows the age-related decline, although it is not clear whether they have their own anti-aging action. We have been investigating DHEA(-S) actions on skeletal muscle because we observed that DHEA(-S) improved symptoms of DM1, a kind of progeria. Recent studies revealed that oxidative stress or insulin/IGF-I signaling play roles in regulation of aging, and that DHEA(-S) is an anti-oxidant and a regulator for insulin action. These facts lead us to study the relationship among DHEA(-S), aging mechanism(s) and DM1.

Our earlier study showed that DHEA(-S) had detoxifying action through CARbeta activation, suggesting that it could prevent the "error accumulation." Because skeletal muscle is the most energy producing organ, the type 1 muscle fiber is "oxidative" and the DM1 muscle shows "type 1-predominant," we studied the DM1 model muscles, i.e. skeletal muscle of DM1 transgenic mice in which mutant gene expressed only in skeletal muscle, or the mutant DMPK overexpressing C2C12 cells, for investigating the aging mechanism(s). We found that the GSH/GSSG ratio in the muscle was lower than that in control muscle, however, anti-oxidant potential and reactive oxygen species production was not. Mutant DMPK activated FOXO signaling and affected the expression of SOD1, SOD2 and IGF-I. These findings suggested that DM1 mutation could affect the aging-related molecules and DM1 model muscle was suitable for aging research.

MEMBRANE LOCALIZATION OF DMPK SPLICE ISOFORMS : DIFFERENCES BETWEEN MOUSE AND MAN

Van Herpen R., Oude Ophuis R., Wijers M., Fransen J., Wieringa B. and Wansink D.

Nijmegen Centre for Molecular Life Sciences, RUNMC, Nijmegen, The Netherlands

Myotonic dystrophy protein kinase (DMPK) is a serine/threonine protein kinase and constitutes together with MRCK, Rho kinase and Citron kinase the DMPK kinase family. Alternative splicing results in multiple DMPK protein isoforms carrying distinct C-termini. Expression of single DMPKs in various cell types demonstrated that mouse isoform DMPK A associated specifically with membranes of the endoplasmic reticulum (ER), whereas mDMPK C located at the mitochondrial outer membrane. Unexpectedly, the corresponding human DMPK A and C proteins localized both to mitochondria. Membrane association was dependent on crucial residues in the so-called C-terminal anchors of DMPK and also on other conformational properties, which will be discussed. Expression of human DMPK A, but not DMPK C, resulted in clustering of mitochondria surrounding the nucleus, suggesting a role for DMPK in mitochondrial distribution or dynamics, fusion or fission. These and other experimental data point out that strict control of DMPK expression levels and splice isoform ratios is important for the well-being and viability of the cell.

EXPRESSION OF DMPK AT THE NEUROMUSCULAR JUNCTION

Wheeler T.M., Krym M.C. and Thornton C.A.

University of Rochester, Neuromuscular Disease Center, Rochester (NY), USA

A characteristic feature of muscle pathology in DM is the finding of fibers that are nearly devoid of cytoplasm. These severely atrophic fibers, called nuclear clumps, also are seen in denervated muscle. This observation raises the possibility that DM1 has compromised the ability of muscle fibers to establish stable innervation (i.e., "myopathic denervation"), perhaps as a result of focally accentuated RNA toxicity in myonuclei subjacent to the motor endplate. However, whether DMPK is expressed at the motor endplate is controversial. To address this question, we used fluorescent *in situ* hybridization (FISH) to examine the expression pattern of expanded repeat RNA in myonuclei subjacent to the endplate ("junctional") and distant from the endplate ("extrajunctional"). First, in HSALR transgenic mice that express skeletal actin mRNA with an expanded CUG in the 3' UTR, nuclear RNA foci are abundant in extrajunctional myonuclei but minimal to absent in junctional nuclei. Of note, HSALR mice display some histologic features similar to DM but they do not develop nuclear clumps. Next, FISH in human DM1 muscle clearly demonstrates nuclear foci in endplate nuclei, indicating that DMPK RNA is expressed by endplate nuclei. These results indicate an important difference between the HSALR transgenic model and human DM1, and show that specialization of junctional nuclei involves downregulation of genes encoding myofibrillar proteins. Finally, using a new antibody to the coiled coil region of human DMPK, studies are ongoing to investigate the relative levels of DMPK expression in different myofiber domains, including the endplate.

INVESTIGATING RNA-MEDIATED NEURONAL DYSFUNCTION IN DM1 TRANSGENIC MICE

Guiraud-Dogan C., Gomes-Pereira M., Huguet A., Sergeant N. and Gourdon G.

Inserm U383, Hôpital Necker Enfants Malades, 149 rue de Sèvres, 75015 Paris, France

Traditionally regarded as a muscle disorder, DM is also characterised by variable degrees of mental dysfunction. Mounting evidence supports a trans dominant effect of toxic RNA transcripts in disease pathogenesis. However it is not understood to which extent RNA mediated molecular pathways are responsible for the reported brain-specific disease manifestations. The DM300 transgenic mice, which carry a large DM1 repeat expansion (>300 CTG) recreate some aspects of the disease phenotype, including changes in tau protein isoforms in the brain. As in DM1 patients, triplet repeat expansion results in a similar pattern of region-specific RNA sequestration into ribonuclear foci in the brain of DM300 mice. The regional expression levels of the DMPK transgene are currently being assessed in the mouse brain. The detailed localisation of RNA foci accumulation has allowed us to select brain areas of interest to investigate neuronal dysfunction. Anatomopathological studies are in progress to assess neurodegeneration. To assess the consequences of the CTG expansion at the molecular level, the splicing of selected candidate genes, implicated in DM pathogenesis, has been studied by real-time PCR. Initial analyses of DM300 mice seem to reveal abnormal expression levels of the insulin receptor gene in a brain region-specific manner. Taking advantage of this mouse model, we aim to unravel the neuropathophysiology of DM. To this end, imaging, histological, proteomic, biochemical and electrophysiological techniques will be combined to characterise the molecular intermediates and pathways affected by the mutation. Findings will contribute to the development of therapeutic schemes.

COLOCALIZATION OF RIBONUCLEAR INCLUSIONS AND MBNL1 FOCI WITH NO IMPAIRMENT OF MUSCLE DIFFERENTIATION IN DM2

Meola G., Cardani R., Mancinelli E. and Rotondo G.

Università di Milano, Policlinico San Donato, San Donato Milanese, Milano, Italy

Myotonic dystrophy type 2 (DM2) is caused by the expansion of a CCTG tetranucleotide repeat located in the first intron of the ZNF9 gene. The mutant transcripts aggregate in multiple ribonuclear inclusions (RNI), which are thought to compromise cellular functions by interacting with essential RNA binding proteins such as muscleblind proteins (MBNLs). 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 DM2 myoblast differentiation, and whether these inclusions impair early and late muscle differentiation. Human satellite cells were isolated from the biceps brachii muscle biopsies of four DM2 genetically confirmed patients and from four subjects used as controls. By using FISH in combination with indirect immunofluorescence (IF) it appears that in DM2 cells MBNL1 nuclear foci, which represent high concentration of protein, co-localize precisely with RNI both at myoblast and myotube stage. No RNI or MBNL1 nuclear foci are present in control cells. Moreover, IF and Western Blot analysis, showed no abnormalities in the expression of different markers of muscle differentiation (MyoD, Myogenin, Myosin fast and slow) in sister pathological cultures as compared to controls. The presence of RNI colocalized with MBNL1 foci at myoblast and myotube stage seems to not impair the muscle differentiation in DM2.

ENDOPLASMIC RETICULUM STRESS IN DM1 MUSCLE

Ikezoe K.[1,2], Furuya H.[1], Nakamori M.[3], Kimura T.[3], Kanemoto S.[4], Imaizumi K.[4], Takahashi M.P.[3], Sakoda S.[3], Kira J.[2] and Fujii N.[1]

1) Department of Neurology, National Omuta Hospital, Fukuoka, Japan
2) Department of Neurology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan
3) Department of Neurology, Osaka University Graduate School of Medicine, Osaka, Japan
4) Division of Molecular and Cellular Biology, Department of Anatomy, University of Miyazaki, Miyazaki, Japan

Various abnormal conditions such as the presence of unfolded proteins and disturbed intra-cellular Ca2+ homeostasis cause endoplasmic reticulum (ER) stress. In DM1 muscle, it has been reported that the abnormal alternative splicing of mRNAs of chloride channel, ryanodine receptor, and SERCA1 takes place.

To clarify the involvement of ER stress in DM1 muscle, we examined the expression of GRP78/94, phosphorylated eIF-2alpha, and XBP-1 by immunohistochemistry in 6 DM1 muscles. In addition, we analyzed the production of mRNA of GRP78 and XBP-1 by RT-PCR in 10 DM1 muscles, for the latter is alternatively spliced under ER stress.

DM1 muscles showed the expression of GRP78/94, phosphorylated eIF-2alpha, and XBP-1, especially in sarcoplasmic masses and pyknotic nuclear clumps, which are the pathological hallmarks of DM1. The expression of these proteins tended to correlate to the expansion size of CTG repeat in the DMPK gene. The mRNA of GRP78 was significantly increased in DM1 compared with control muscles. We could detect abnormally spliced XBP-1 mRNA in several muscles of DM1, however, none in control.

These results indicate the possibility that the ER stress is caused in DM1 muscle, which results in the degeneration of muscle fiber.

OVER-EXPRESSION OF SK CHANNELS INCREASES SENSITIVITY TO CALCIUM IN DM1 LENS CELLS

Rhodes J.D., Duncan G., McAbney J.P., Monckton D.G. and Prescott A.R.

School of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, UK

Ca2+-activated K+ channels (SK3) are over-expressed in muscle of type 1 myotonic dystrophy (DM1) patients and we previously identified functional SK channels in normal human lens. Using lens cell lines derived from cataract patients, we investigated the role of SK's in DM1 related cataracts. Six cell lines were made by SV40 transformation of lens samples obtained during cataract surgery : 2 from routine cataracts and 4 from DM1 cataracts. 86Rb was used to measure K+ transport and ionomycin to stimulate internal Ca2+ in efflux experiments. CTG expansions ranging in size from 450-2950 repeats were present in all DM1 cell lines. RT-PCR showed increased expression of DMWD, and DMPK, and decreased expression of SIX5 in the DM cells. Interestingly SK3 showed a 5 fold increase in expression in the DM cells compared to the controls, although, there was no difference between the 2 cell types in the resting K+ influx or efflux kinetics. However, when intracellular Ca2+ was increased, using ionomycin, there was a greater increase in K+ efflux in the DM cells than in the controls. Significantly apamin which blocks SK channels, had no effect on the peak efflux in control cells but reduced the peak in the DM1 cells. Apamin had no effect on the protein levels, measured at the end of the experiment, in control cells but reduced the loss of protein when DM1 cells were exposed to ionomycin. These data suggest that over-expression of SK3 in DM1 could lead to an increased sensitivity to Ca2+ overload resulting in a net loss of protein and cataract.

TRANSCRIPTIONAL PROFILE OF TRANSGENIC MICE EXPRESSING AN EXPANDED CUG REPEAT

Osborne R.J., Welle S. and Thornton C.A.

University of Rochester, Department of Neurology, Rochester (NY), USA, 16642

HSALR transgenic mice expressing CUG expansion RNA in skeletal muscle have a DM-like phenotype. For several genes these mice replicate the abnormal regulation of alternative splicing seen in DM. In an attempt to identify additional pathways affected by repeat expansion RNA, we used expression microarrays to compare skeletal muscle in two independent founder lines of HSALR mice (lines 20b and 41) and in wild-type (WT) mice of the same genetic background (n=6 per group, no sample pooling). To obtain comprehensive coverage of the mouse transcriptome we used Affymetrix 430A and B chips (45,000 probe sets). These analyses identified 45 probe sets that were differentially expressed in both HSALR20b and HSALR41 versus WT comparisons ($P \leq 0.01$, fold-change ≥ 2). We postulated that some of these changes resulted from responses to myotonic discharges or nonspecific muscle injury. To eliminate these genes from consideration, we also studied adr (ClC-1 null) mice and their WT controls (n=3 per group, no sample pooling). We chose adr mice because they have severe myotonia and modest up-regulation of regeneration dependent genes. After filtering out probe sets showing similar changes in adr and HSALR mice, we were left with 24 genes that were up-regulated and 4 genes that were down-regulated in both HSALR founder lines. Of note, none of these genes have been previously implicated in DM pathogenesis. We conclude that the influence of poly(CUG) accumulation on gene expression is surprisingly restricted, even in myonuclei that have a heavy burden of repeat expansion RNA. The function of these genes suggests signaling pathways that might be involved in the DM disease process.

DYSREGULATION OF SPECIFIC GENE TRANSCRIPTS IN Mbnl1 KNOCKOUT BRAIN

Swanson M.S., Tuttle D.L. and Poulos M.G.

Department of Molecular Genetics and Microbiology, University of Florida, College of Medicine
1600 SW Archer Road, Gainesville, FL 32610-0266, USA

The RNA-mediated pathogenesis model for DM proposes that expression of mutant DMPK (DM1) or ZNF9 (DM2) genes results in the synthesis of toxic RNAs which impair the splicing activities of the muscleblind-like (MBNL) proteins during postnatal development. In support of this model, Mbnl1 isoform knockout mice develop many characteristic features of the multisystemic DM disease phenotype, including skeletal muscle myotonia, particulate cataracts and cardiac conduction abnormalities.

Although the central nervous system is also affected in DM, the effect of MBNL loss on brain structure and function has not been thoroughly investigated. To identify specific pre-mRNAs that are regulated by the CELF and MBNL protein families *in vivo*, we used a crosslinking-immunoprecipitation (CLIP) protocol. Using mouse embryos and mAb 3B1 against CUGBP1, 124 tags CLIP tags have been characterized (89 intronic, 8 exonic, 5 untranslated and 22 intergenic/ unclassified). For the intronic tags, the consensus binding site is rich in UG repeats in agreement with previous binding studies. The corresponding pre-mRNAs for 18 of the intronic tags were analyzed in wild type versus Mbnl1 knockout brains and the majority (~60 %) of these transcripts are aberrantly processed when Mbnl1 exon 3 isoforms are absent. These results support the possibility that interactions between the CELF and MBNL proteins are also required to regulate RNA processing of specific transcripts in the brain.

THE ROLE OF PROTEIN-PROTEIN COMPLEXES IN MYOTONIC DYSTROPHIES 1 AND 2

Salisbury E., Schoser B.G.H., Nguyen H., Sakai K., Agulion C., Schneider-Gold C., Timchenko N.A. and Timchenko L.T.

Baylor College of Medicine, Dep. of Medicine, Rm 536D, One Baylor Plaza, Houston, TX , USA, 77030

DM1 and DM2 are caused by an expansion of polymorphic CTG (DM1) and CCTG (DM2) repeats. We have found that, similar to CUG repeats in DM1, the expanded RNA CCUG repeats are expressed in nuclei and in cytoplasm of DM2 cells. RNA CUG and CCUG triplet repeats cause pathogenesis of DM1 and DM2 through disruption of biological functions of RNA-binding proteins. We have observed that protein levels of CUG triplet repeat binding protein, CUGBP1, are increased in DM1 and DM2 patients. Investigations of CUGBP1 transgenic mice showed that the elevation of CUGBP1 is involved in a delay of muscle development and differentiation and in muscular dystrophy. These symptoms are associated with CUGBP1-dependent increase of translation of MEF2A, C/EBP beta and p21. CUGBP1 regulates translation of these proteins through a formation of a high MW CUGBP1-eIF2 complex which consists of CUGBP1, eIF2 alpha, eIF2 beta, eIF2 gamma, CRT, eR60, grp78 and eR90. The CUGBP1-eIF2 complex specifically interacts with the 5' regions of C/EBP beta and p21 mRNAs and increases translation of these proteins. Our data suggest that, in DM2 cells, CCUG expansion affects biological functions of cytoplasmic CUGBP1-eIF2 complex as well as biological functions of high MW complexes in nuclei. These data are consistent with the hypothesis that expanded RNA CUG and CCUG repeats might destroy biological processes in DM1 and DM2 patients by disruption of multimeric protein-protein complexes.

POST-TRANSLATIONAL MODIFICATIONS OF CUG-BP1 IN RESPONSE TO CUG REPEATS

Kuyumcu-Martinez N.M. and Cooper T.A.

Baylor College of Medicine, Houston, TX, USA, 77018

There are multiple mis-splicing events in DM1, all of which inappropriately express splice variants from early developmental stages. CUG-BP1, a regulator of alternative splicing during striated muscle development, is implicated in DM1 pathogenesis. Over-expression of CUG-BP1 in mice induces a switch to embryonic splicing patterns of CUG-BP1 targets, which is also observed in individuals with DM1. We are investigating how expression of RNAs containing expanded CUG repeats induce an embryonic splicing pathway, and specifically whether expanded CUG repeats induce modifications of CUG-BP1 that affect its splicing regulation. Using transient transfections in which expression of a truncated DMPK mRNA with 960 CUG repeats induces the DM1 splicing pattern, we tested whether 960 CUG repeats induce modifications of CUG-BP1. By 2D gel analysis, we found an acidic shift of both endogenous and exogenous Flag-CUG-BP1 from nuclei of 960 CUG repeat-expressing COS M6 cells. There was no significant change in the pI of CUG-BP1or Flag-CUG-BP1 in cells expressing RNA with 0 or 960 CAG repeats. In addition, heart and skeletal muscle from newborn but not adult mice expressed acidic forms of CUG-BP1 similar to cells expressing 960 CUG repeats. Preliminary results indicate that acidic forms of CUG-BP1 are more abundant in DM1 muscle cultures as well as in hearts from transgenic mice expressing 960 CUG repeats compared to normal mouse muscle and heart, respectively. We propose that expanded CUG repeat RNA induces modifications of CUG-BP1 that are characteristic of embryonic isoforms. A similar analysis of MBNL1 in DM1and during development is ongoing.

ANALYSIS OF CANDIDATE GENES MODIFIER FOR THE CONDUCTION SYSTEM IMPAIRMENT IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

Vallo L., Bonifazi E., Rinaldi F., Contino G., Iraci R., Palladino A., Sannino P., Politano L., Botta A. and Novelli G.

Tor Vergata University-Via Montpellier 1,00133 Rome, Italy

Myotonic dystrophy type 1 (DM1) commonly includes cardiac involvement that typically occurs because of myocardial fibrosis. The myocardial fibrosis typically manifests as conduction disturbances, arrhythmias, ventricular dysfunction and sudden death. Atrio-ventricular block (AVB) is one of the most frequent conduction alteration in DM1 and it is present in 20 % of patients at baseline and 50 % at follow up. Mechanisms underlying the phenotypic heterogeneity of heart involvement in the disease are still unclear. The aim of our study is to analyse genes that could play a role in the phenotypic outcome of cardiac conduction abnormalities in DM1 patients. We selected a set of candidate genes on a functional basis : LMNA (lamin A), NK2.5 (NK2 transcription factor related, locus 5) and PRKAG2 (protein kinase, AMP-activated, γ2). Our approach consists in an association study of intragenic polymorphic variants with the AVB phenotype in genetically confirmed DM1 subjects. At this purpose, we recruited 32 DM1 patients showing AVB and 30 patients DM1-positive patients with no cardiac disease. Genotyping has been performed using a combination of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) and Taqman® real-time PCR technology. Statistical analysis did not reveal any significant association of the selected genes with the AVB in DM1. At present the characterization of other genes and the recruitment of a larger sample of affected subjects are in progress.

ABERRANT SPLICING OF DYSTROPHIN AND DYSTROBREVIN IN MYOTONIC DYSTROPHY TYPE 1

Nakamori M., Kimura T., Aoike F., Takahashi M.P. and Sakoda S.

Dept. Neurol. Osaka University, Suita, Osaka, Japan 565-0871

In myotonic dystrophy type 1 (DM1), misregulation of alternative splicing has been reported for several mRNAs. Some of them have been attributed to clinical features such as myotonia and insulin resistance. However, the cause of progressive muscle wasting, a core symptom of DM, has still been unknown. On the other hand, the abnormalities of cytoskeletal proteins have been revealed to be responsible for some forms of progressive muscular dystrophies such as dystrophinopathy. Dystrobrevin binds dystrophin and syntrophin and consists in dystrophin-associated protein complex, located at the sarcolemma. Both dystrophin and dystrobrevin have been shown to have several alternative splicing isoforms. In this study, we examined the splicing abnormalities of these cytoskeletal proteins in skeletal muscles from DM1 using RT-PCR and quantified the total amount of mRNA of dystrobrevin by semi-quantitative real time PCR.

We found significant existence of alternatively spliced isoforms of dystrophin and dystrobrevin in DM1. The splicing variant of dystrophin lacked exon 78, the region regulating hydrophobicity. The splicing variant of dystrobrevin contained exons 12 and 13, suggested syntrophin binding site. The total amount of mRNA for dystobrevin did not differ significantly.

In conclusion, we revealed aberrant splicing of dystrophin and dystrobrevin in skeletal muscles of DM1. The aberrant isoforms may be responsible to structural vulnerability of the sarcolemma or disturbance of signaling pathway and resultantly for muscle degeneration in DM1.

BOTH EXON 2/3 AND EXON 6 OF TAU RNAs ARE MIS-SPLICED IN MYOTONIC DYSTROPHY TYPE 1 BUT DIFFERS IN THEIR TISSUE-SPECIFICITY ALTERATION AND REGULATION BY ETR-3

Caillet-Boudin M.L., Leroy O., Dhaenens C.M., Schraen-Maesche S., Maurage C.A., Sablonière B., Buée L., Cooper T. and Sergeant N.

INSERM U422. Pl. Verdun. 59045 Lille cedex. France

The multisystemic feature of Myotonic dystrophy type 1 (DM1) could be explained by aberrant alternative splicing of multiple RNAs in several organs including muscles and brains. Recently, our group demonstrated a defect in Tau exon 2 and 3 splicing in DM1 brains

We first checked if DM1 splicing alterations affect tau exon 6 splicing, a minor cassette possibly inserted in tau RNAs of brain and muscle. We show a decrease in Tau exon 6 inclusion but only in DM1 brains: this alteration was tissue-specific. In contrast, increase of tau exon 2 and 3 exclusion occured in both organs. ETR-3, a CELF family member which regulates the splicing of other DM1 mis-splicing targets, favours the exclusion of exon 2/3 but not exon 6 from tau endogenous transcripts in cellular models. However, the presence of ETR-3 in normal brain suggests that its action *in vivo* on Tau splicing is more complex and ruled out the hypothesis of a DM1 Tau splicing alteration only due to a pathological expression of ETR-3. In contrast, the normal cerebral Etr-3 expression is compatible with a direct role of ETR-3 in the normal dominant brain IR exon 11 exclusion.

Altogether, these results suggest that the different splicing disregulations observed in DM1 did not result from an unique process and would involve different splicing factors according to the altered exon and tissue.

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ALTERED mRNA SPLICING OF THE SKELETAL MUSCLE RYANODINE RECEPTOR AND SARCOPLASMIC/ENDOPLASMIC RETICULUM Ca2+-ATPASE IN MYOTONIC DYSTROPHY TYPE 1

Kimura T., Nakamori M., Lueck J.D., Aoike F., Pouliquin P., Dirksen R.T., Takahashi M.P., Sakoda S. and Dulhunty A.F.

JCSMR- Australian National University, Canberra, ACT 2601, Australia

Aberrant splicing of several genes has been reported to contribute to some symptoms of myotonic dystrophy type 1 (DM1), but the cause of muscle weakness in DM1 and impaired Ca2+ homeostasis in cultured DM muscle cells is unknown. We investigated the alternative splicing of mRNAs of the skeletal muscle ryanodine receptor (RyR1) and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase (SERCA) 1 or 2. The fetal variants, ASI(-) of RyR1 and SERCA1b, were significantly increased in skeletal muscles from DM1 patients and the transgenic mouse model of DM1 (HSA-LR). In addition, a novel variant of SERCA2 was significantly decreased in DM1 patients.

Heterologous expression of ASI(-) in cultured cells showed decreased affinity for [3H]ryanodine and decreased channel activity in single channel recording, compared with wild type (ASI(+)) RyR1. In support of this, RyR1-knockout myotubes expressing ASI(-) exhibited a decreased incidence of Ca2+ oscillations during caffeine exposure compared to that observed for myotubes expressing wild type RyR1. This reduced activity could contribute to muscle weakness in DM1.

Further functional studies with synthetic peptides corresponding to the ASI region with and without exon ASI, suggested ASI(-) region may interact more tightly with other domains and produce stronger inhibition of ASI(-) RyR1.

THE MYOTONIC DYSTROPHY TYPE 2 (DM2) CCTG EXPANSION DOES NOT ALTER ZNF9 EXPRESSION

Margolis J.M., Schoser B., Mosely M.L., Day J.W. and Ranumi L.P.W.

MMC 206, 420 Delaware St. SE, University of Minnesota, Minneapolis, MN 55455, USA

We have shown that DM2 is caused by a CCTG repeat expansion (75 to > 11,000 repeats) in intron 1 of the zinc finger protein 9 (ZNF9) gene. To determine the effects of these large intronic expansions on ZNF9 expression, we performed experiments with chromosome separated hybrid cell lines and human myoblasts homozygous for the DM2 expansion. RT-PCR on chromosome separated mouse-human hybrid cell lines containing a single copy of the normal or expanded (1,000 CCTGs) human DM2 allele indicates that the CCTG expansion does not prevent allele specific splicing. Consistent with normal splicing, RNA *in situ* hybridization in myoblasts heterozygous or homozygous for the expansion show : 1) CCUG-containing ribonuclear inclusions; 2) exon 1 and exon 5 (5' and 3' of the CCTG, respectively) localize primarily to the cytoplasm; and 3) intronic sequences flanking the repeat localize to the nucleus but not to the CCUG-containing ribonuclear inclusions. Northern and western analysis using myoblasts from a homozygous DM2 patient with expansion bands of 375, 2400, 4400, and 4600 repeats show steady state levels of ZNF9 mRNA and protein levels that are comparable to controls. Similarly, western analysis of muscle biopsy tissue from individuals with various repeat expansions (2875 , 5125, 9275, and >11,000 repeats) show levels of ZNF9 protein that are comparable to controls. Surprisingly, a patient with an expansion of 75 CCTGs and minimal somatic heterogeneity has similar clinical features to patients with expansions >11,000 repeats. Our results provide strong evidence that ZNF9 expression is not affected even by large CCTG expansions, that the CCUG repeat expansion alone is found in the ribonuclear inclusions, and that although the DM2 expansions are usually much larger than those found in DM1, the pathogenic threshold may be as low as 75 CCTG repeats.

THE STRUCTURAL BASIS OF MYOTONIC DYSTROPHY FROM THE CRYSTAL STRUCTURE OF CUG REPEATS

Mooers B.H.M.[2,3], Logue J.S.[1,3] and Berglund J.A.[1,3]

1) Department of Chemistry
2) Howard Hughes Medical Institute
3) Institute of molecular Biology, 1229 University of Oregon, Eugene, Oregon, 97403-1229, USA

To better understand the molecular basis of myotonic dystrophy type 1 (DM1), or more specifically, RNA gain of function in myotonic dystrophy, we determined the 1.58 Ǻ crystal structure of an 18 basepair RNA containing six CUG repeats. The CUG repeats form antiparallel double-stranded helices which stack end-on-end in the crystal to form infinite, pseudo-continuous helices similar to the long CUG stem-loops in DM1. The CUG helix is very similar to A-form RNA. This result is striking because 1/3 of the RNA contains non-canonical basepairs. The A-form fold may be accounted for by the unusual nature of the U:U mismatches and base stacking at G:C base steps. Electrostatic and molecular surface maps give insight into the accessibility of the U:U mismatches, and how nucleic acid binding proteins may be sequestered. This structure provides the first high-resolution view of a toxic trinucleotide repeat RNA. This structure also provides the opportunity to explore structure-based approaches for therapeutic development.

TRANSCRIPTS CONTAINING TRIPLET REPEAT HAIRPINS ARE UNDER THE SURVEILLANCE OF RIBONUCLEASE DICER

Krzyzosiak W.J., Krol J. and Sobczak K.

Institute of Bioorganic Chemistry, Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland

Ribonuclease Dicer functions in cells to excise microRNAs (miRNAs) from their precursors and process long double-stranded RNAs into small interfering RNAs (siRNAs). In human cells there are hundreds of different miRNA precursors processed but little is known about other cellular substrates for Dicer. Here we show that transcripts containing long hairpin structures composed of the CNG repeats may constitute a new family of Dicer substrates. We demonstrate that the cellular levels of transcripts from several mutant genes involved in Triplet Repeat Expansion Diseases (TREDs) are under the control of Dicer. Mutant DMPK mRNA implicated in pathogenesis of myotonic dystrophy type 1 (DM1) is among these transcripts. We also postulate, that short repeat duplexes may be generated from long repeat hairpins and they may function in cells as siRNAs.

STUDYING NUCLEAR FOCI OF CUG REPEAT mRNA IN LIVING CELLS

Querido E. and Chartrand P.

Department of Biochemistry, Université de Montréal, Montréal, Qc H3C 3J7, Canada

Recent advances have shown that multiple CUG repeats in an mRNA create a toxic gain-of-function phenotype. To study the chain of events that leads to the retention of CUG triplet rich mRNAs as foci in the nucleus, we are using a novel approach that allows us to visualize the movement of a specific mRNA in living cells. We express an MS2 RNA binding protein-YFP fusion that exclusively recognizes the MS2 stem-loop RNA motif, which is incorporated as a multimer in the sequence of the mRNA of interest. This MS2-YFP protein acts as a beacon that allows the detection of this mRNA by live cell microscopy. We have created a reporter mRNA consisting of the LacZ cDNA, 24xMS2 RNA binding motifs, and the 3'UTR of DMPK containing 33 or 145 CUG repeats. When we express the 145 CUG repeat construct, we are able to visualise YFP nuclear foci of CUG rich mRNAs in live C2C12 myoblasts. Using an inducible promoter, we are measuring the kinetics of accumulation of the foci and their stability after shutting down transcription. We are filming small and large foci to describe their behaviour. We are also exploring the role of proteins like Mbnl in the formation of these foci by targeting them with short hairpin RNAs. We hope our studies using live cells will lead to novel approaches to prevent the nuclear retention of CUG repeat mRNAs and its toxic effects in myotonic dystrophy.

MUSCLEBLIND PROTEIN ISOFORMS SHOW DIFFERENTIAL RNA BINDING PROPERTIES IN A YEAST THREE HYBRID ASSAY

Vicente M., Pascual M., García-Lópes A. and Artero R.

Department of Genetics, University of Valencia, Spain, 46100

Cellular and molecular studies have shown that vertebrate Muscleblind (Mbl) proteins aberrantly bind to long CUG repeats in Myotonic Dystrophies and regulate alternative splicing of specific pre-mRNA transcripts through binding to defined consensus sequences. While vertebrate genomes contain up to five mbl homologues, the compact *Drosophila* genome contains only one. In order to cope with the developmental and biochemical functions that mbl must perform in *Drosophila*, pre-mRNA transcripts from this locus undergo a complex pattern of alternative splicing giving rise to protein isoforms MblA, B, C and D which differ extensively in their C-termini. We hypothesize that different Mbl protein isoforms perform different functions through differential binding to their molecular targets. We investigated this possibility by testing the binding capabilities of *Drosophila* Mbl isoforms, and artificial constructs, to (CUG) 480 repeats and a physiological target of Mbl (-actinin transcripts) in a yeast three hybrid system. Our preliminary results show that MblC interacts strongly with a specific region in the -actinin intron 6 while MblD binds weakly. In contrast, only MblC binds strongly to (CUG) 480. The pattern of binding to a closely related sequence is also specific. MblB interacts strongly with (CAG) 480 while MblC is a weak interactor. These results indicate that different Mbl protein isoforms perform different functions *in vivo* and suggest specific functions to a number of domains in the Mbl proteins.

ROLE OF MBNL1 IN DM1

Lin X., Mankodi A., Kanadia R., Swanson M. and Thornton C.

University of Rochester, Rochester (NY), USA 14623

In DM, expression of RNA containing an expanded CUG or CCUG repeat triggers a defect in the regulation of alternative splicing. The repeat-bearing transcripts accumulate in nuclear foci, together with proteins in the muscleblind-like (MBNL) family. To investigate the function of Mbnl1 and its role in DM, we assessed alternative splicing at different stages of muscle development in WT mice and two mouse models of DM1 : HSALR transgenic mice that overexpress CUG expansion RNA in muscle, and mice homozygous for targeted deletion of Mbnl1 exon 3. By analyzing a panel of 37 alternatively spliced exons, we found that the splicing defect in both models selectively targets exons that normally undergo a splicing transition between postnatal day 2 and 20. During this interval, MBNL1 protein redistributes from cytoplasm to nucleus in WT muscle. Blocking the access of MBNL1 to the nucleoplasm, either by expression of expanded poly(CUG) or disruption of the Mbn1 gene, causes an identical pattern of failure in these splicing transitions. For each exon that we analyzed, the splicing defect in mouse models was concordant with human DM1 and DM2. Immunoflourescence examination of DM1 and DM2 muscle showed that MBNL1 protein was recruited into nuclear foci so extensively that unbound MBNL1 in the nucleoplasm was reduced by greater than 75 %. These results suggest that symptoms of DM may have their origin in a failure to execute or maintain a set of developmentally regulated splicing transitions that are critical for postnatal remodeling of skeletal muscle. Mbnl1 has a key role in controlling these transitions, and its sequestration likely contributes to the splicing defect in DM1.

ELEVATED HNRNP H LEVELS CONTRIBUTE TO ABNORMAL IR SPLICING IN DM1

Paul S., Dansithong W., Kim D., Rossi J., Comai L. and Reddy S.

University of Southern California, Institute for Genetic Medicine, Room 240, 2250 Alcazar Street, Los Angeles, CA 90033, USA

The alternative splicing factor hnRNP H has been recently shown to bind to CUG repeat sequences. We show that steady-state hnRNP H levels are elevated in DM1 myoblasts when compared to normal myoblasts. Significantly, increased steady-state hnRNP H levels in normal myoblasts results in abnormal insulin receptor (IR) splicing, which is similar to that observed in DM1 cells and in wild type myoblasts in which either MBNL1 has been silenced or CUG-BP levels are elevated. As we show RNA-independent interaction of hnRNP H with MBNL1 these data support the hypothesis that elevated hnRNP H levels antagonize MBNL1 activity by physically interacting with MBNL1. We further observe that CUG-BP levels respond to hnRNP H dosage, suggesting that increased hnRNP H levels may contribute to elevated CUG-BP levels in DM1. Interestingly, the aberrant IR splicing pattern that results from elevated levels of CUG-BP and hnRNP H, requires normal levels of hnRNP H and CUG-BP respectively. Thus these data demonstrate that altered MBNL, CUG-BP and hnRNP H levels act in concert to set the aberrant pattern of IR splicing in DM1 myoblasts. As inactivation of MBNL1 or elevated CUG-BP or hnRNP H levels can independently establish the aberrant IR splice pattern in DM1 myoblasts, rescue experiments were carried out to determine the relative importance of these three events in abnormal IR splicing. These experiments demonstrate that MBNL loss is the primary event, while elevated CUG-BP and hnRNP H levels play a secondary role that serve to increase the severity of the IR splicing defects resulting from MBNL1 loss in DM1.

SEQUESTRATION OF A NOVEL ZN-FINGER PROTEIN MAY CAUSE DEGENERATIVE MUSCLE DEFECTS IN DM1

Xu W., Gao R., Ashizawa T. and Sarkar P.S.

Department of Neurology, UTMB, Galveston, Texas 77555-0874, USA

DM1 is characterized by the dysfunction of muscle structure and function. Delayed muscle differentiation and hypotonia are the predominant features in congenital DM1 whereas myotonia, atrophy and weakness of muscle are the features in the adult onset DM1. Mice expressing ~250 CTG repeats in skeletal muscle develop myotonia and structural defects reminiscent of DM1. Sequestration of muscle-blind proteins by expanded CUG RNA was found to be the pathogenic mechanism causing muscle myotonia in the CUG transgenic mice. Subsequent studies with muscle-blind null mice confirmed that the loss of muscle-blind function is indeed one of the important steps required to cause myotonia and structural defects of muscle. Importantly, neither Mbnl1 null mice nor the CUG transgenic mice develop dystrophic muscle defects, the hallmark features of DM1. We have identified a novel zinc-finger protein, sequestered by the expanded CUG RNA and our studies show that the novel protein is sequestered in DM1 nuclei and strongly co-localizes with the expanded CUG-RNA. These results suggest that sequestration of multiple proteins by expanded CUG RNA is most likely the primary pathogenic mechanism to inflict degenerative muscle defects in DM1.

CUG-BINDING PROTEINS, CUG REPEATS AND STEINERT MYOTONIC DYSTROPHY : WHAT CAN WE LEARN FROM *DROSOPHILA* ?

Ait-Ahmed O., Le Mee G., Capri M., Ezzeddine N. and Delaunay J.

Institut de Genetique Humaine, UPR 1142 CNRS, 141 rue de la Cardonille 34396, Montpellier, cedex 5- France

Our aim is to establish a genetically tractable system to address the questions of the mechanisms involving toxicity of CUG repeat containing RNAs in DM1 pathogenesis. This proposal is based on the evidence that the CUG binding factors are conserved between man and *Drosophila*, namely muscleblind and CUG-BP. The *Drosophila* muscleblind proteins have been shown to have orthologues in humans (Miller *et al*, Embo J (2000) 19, 4439-48) and our laboratory has shown that CUG-BP has a *Drosophila* orthologue, Bru-3 (Delaunay *et al*, Nucleic Acids Res (2004) 32, 3070-82 and unpublished data).

We present two aspects of our work :

1) The analysis of Bru-3 / CUG-BP role in *Drosophila* development. We have developed antibodies and mutants are available (gain of function and loss of function). This will allow us in particular to determine, using genetic approaches, whether muscleblind and Bru-3 are involved in the same pathway.
2) An analysis of the CTG repeat containing transgenic lines, the expression of which is inducible using the UAS-GAL 4 system. Indeed, one line expressing a (CUG)240 RNA under an eye specific promoter displays typical eye neurodegeneration. Other (CUG)240 or (CUG)480 expressing lines do not display any conspicuous effect. The molecular mechanisms that underlie these differences are being investigated.

Whether non coding expanded RNA is toxic or not for *Drosophila* remains an open question. Indeed different groups draw different conclusions.

We hope that our work on *Drosophila* will shed some light on the complex aspects of DM1.

ALTERNATIVE SPLICING OF CLC-1 CHLORIDE CHANNEL IS REGULATED BY MBNL AND CELF PROTEINS

Kino Y.W.[1], Oma Y., Sasagawa N. and Ishiura S.

Department of Life Sciences, Graduate School of Arts and Sciences, the University of Tokyo, 3-8-1, Komaba, Meguro-ku, Tokyo, 153-8902, Japan

1) RIKEN BSI, Laboratory for Structural Neuropathology, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan

Aberrantly increased inclusion of CLC-1/CLCN1 chloride channel exon 6B and/or 7A is associated with myotonia in myotonic dystrophy type 1 (DM1) and type 2 (DM2). In mouse models, inclusion of exon 7A is induced by either over-expression of CUG repeat RNAs or knockout of Mbnl1. However, the mechanism of these abnormalities is still elusive. Here we show that MBNL proteins can regulate the alternative splicing of human and mouse chloride channels (CLC-1 and Clcn1, respectively). Multiple MBNL isoforms can repress the inclusion of exon 7A. These effects are antagonized by the expression of expanded CUG repeats or some of CELF proteins. However, CUG-BP did not directly promote exon 7A inclusion, in contrast to the splicing regulation of cardiac troponin T. The splice variation of MBNL proteins correlates with their molecular properties such as splicing activity and intracellular localization. By using Clcn1 minigene, its responsive region to MBNL1 was analyzed. MBNL1 directly binds to a region around the 3' splice site flanking to exon 7A. This may lead to the inhibition of splicing *cis*-elements located in this region of Clcn1, resulting in the repression of exon 7A. These results suggest the importance of MBNL proteins in the proper expression of the chloride channel 1 as well as a possible post-transcriptional regulation of this channel by MBNL and CELF proteins.

"ZEBRAFISH KNOCK-DOWN MODEL FOR MUSCLEBLIND-LIKE 2"

Machuca-Tzili L.E. and Brook J.D.

Institute of Genetics, Queen's Medical Centre, University of Nottingham, NG7 2RD, UK

One of the models proposed to explain the molecular basis of Myotonic Dystrophy, the RNA gain of function model, suggests that the mutant RNA binds and sequesters transcription factors, depleting them from their active sites, and therefore causing disruptions in RNA processing. A family of proteins that could mediate the RNA *trans*-dominant effect is MBNL (Muscleblind-like), of which there are three members, all of them co-localise with the nuclear foci of tri and tetranucleotide repeats seen in DM1 and DM2 cells.

We have identified three homologous Mbnl genes in Zebrafish, and achieved the production of a knock-down model for Mnbl2 using morpholinos. Whole-mount *in situ* hybridization studies showed expression of Mnbl2 in the neural tube, lens, sensory neurons and other structures in different stages of development. The polymorphisms around the area where the MO should be designed were identified. Also, the gaps in the sequences of these genes were filled in by amplification and sequencing. A translation blocking gene-specific morpholino was injected into one, two, four and eight cells zebrafish embryos. The oligo was titrated to determine the effective-and-specific window of concentrations. We found that the morphants presented an overall delay in development and a mortality rate higher than the controls, as well as morphologic abnormalities, some of them being eye, heart and spinal malformations. Additionally, the survivors after four days showed abnormal "shaky" movements. A subset of embryos showed arrest of development in earlier stages, not being able to complete gastrulation, as an additional phenotype. This data contributes to the understanding of the functions of Muscleblind genes, which are believed to have a crucial role in DM pathogenesis.

ANALYSIS OF ANNUAL FOLLOW-UP DATA ON 159 PATIENTS WITH MYOTONIC DYSTROPHY

Whittaker R., Beauchamp S. and Hilton-Jones D.

Department of Clinical Neurology, Radcliffe Infirmary, Woodstock Road, Oxford. OX2 6HE, UK

Introduction : Here we present selected results of annual review data on 159 patients over an eight year period.

Demographics : 76 out of 159 were female(48 %), average age 42.7(12-75). The average length of follow up was 3.5 years.

Skeletal Muscle strength : Muscle strength was assessed on each review by the same person using the MRC Clinical Scale. Hand grip was measured by dynamometer. Average hand grip at presentation was 13.58kg (S.D. 11.187). This varied significantly from controls. The average rate of change in hand grip was a reduction of 0.69kg/yr (S.D. 0.895).

Other than hand grip, the most frequently affected movements were neck flexion and ankle dorsiflexion with 131/159 (82.5 %) and 126/159(77 %) respectively with strength 4 or less. Least affected were neck extension and hip flexion. The biggest change in MRC strength grade was observed in ankle dorsiflexion and pinch grip with no change in hip flexion or shoulder abduction.

FVC was measured on a hand-held spirometer. Average FVC was 61.2 % of predicted (S.D. 19.97). Even patients with no evidence of muscle weakness had an average VC of only 86.9 % of predicted (S.D. 16.41).

Discussion : Our results support the long-held clinical impression that neck flexion tends to be weaker than extension and that distal limb muscles are more affected than proximal. This suggests that in studies involving long-term follow up, these movements are the most useful guide to progression. Our results also emphasise the importance of regular VC measurement, even in seemingly unaffected patients.

MYOTONIC DYSTROPHY TYPE 1 - CLINICAL CHARACTERISTCS OF PATIENTS WITH <100 CTG REPEATS

Hilton-Jones D.

Radcliffe Infirmary, Oxford, UK

In general, low repeat numbers are associated with limited or absent clinical features. The purpose of the present study is to review the characteristics of subjects identified as having less than ~100 repeats, to comment on the usefulness of that information and make recommendations for future studies.

From the database of the Oxford Regional DNA service, 44 subjects with a (CTG)n of <100 repeats were identified (from the inception of the service in 1992 to the present date). At the time of abstract preparation 28 sets of medical records had been reviewed, but more data will be available in time for the meeting.

In 27/28 patients DNA testing arose because of a family history of DM1. In one case without a known family history the presenting symptoms (relating to myotonia) led to testing. 15/27 patient s had no symptoms or abnormal physical signs on clinical examination – 3 had slit-lamp changes and myotonia on EMG, but such studies were performed on only 5 patients. 2/27 patients had excessive daytime sleepiness possibly caused by DM1, but no other symptoms and no abnormal signs. 7/27 patients had symptoms and signs relating to DM1, but in none had these led to the correct diagnosis – cataracts in 7, excessive daytime sleepiness in 2. Weakness was demonstrable in only one of these 7 patients. 3/27 patients without symptoms, even on direct questioning, had abnormal physical signs relating to DM1 – balding, facial and distal weakness, myotonia and lens opacities.

Further clinical details will be provided. The need for longer term surveillance of such patients will be discussed.

8. Diagnosis and Clinical Aspects

MYOTONIC DYSTROPHY TYPE 1 (DM1) WITH SCAPULAR INVOLVEMENT IS GENETICALLY HETEROGENEOUS : CASES WITH AND WITHOUT FSHD MUTATION

Eymard B., Bassez G., Aandreux F., Laforet P., Radvanyi H. and Jeanpierre M.

Institut de Myologie - Hôpital Pitié-Salpêtrière, Paris, France

Proximal musculature is frequently spared at onset in myotonic dystrophy type 1 (DM1) and scapular winging very uncommon. We evaluate DM1 patients with early and predominant proximal muscle involvement according to the following criteria : (1)genetically confirmed DM1 mutation, (2) prominent proximal limb weakness at early stage of the disease, (3) exclusion of other neurological cause. Genetic analysis for facio-scapulo-humeral dystrophy (FSHD) and DM2 mutations were performed. 11 patients were identified (8 men, 3 women). Mean age at onset of symptoms was 28 years (10–51). DM1 phenotype included childhood-onset (n= 3), and adult form (n= 8) with various size of CTG expansion (200 to 1470 repeats). No DM2 expansion have been detected but D4Z4 fragment analysis revealed contraction under 12 repeats in 4 patients. In all cases scapular girdle was more affected than pelvic girdle. Cognitive dysfunction and other systemic manifestations did not differ from common DM1 cases. The subgroup with FSHD mutated allele did not differ regarding weakness distribution, asymmetry, and motor impairment but showed a tendency to earlier scapular winging and more severe respiratory involvement. Conclusion : Prominent scapular involvement can be observed in DM1 and may masquerade as DM2 or FSHD. Genetically, our results delineate two distinct patterns : (1) occurrence of a concurrent FSHD mutation, and (2) DM1 mutation with no D4Z4 contraction. Further studies are needed to clarify the prevalence and the role of deletion of D4Z4 repeats as a putative modifier gene in DM1.

ELECTROCARDIOGRAPHIC CONDUCTION ABNORMALITIES AND SUDDEN DEATH IN MYOTONIC DYSTROPHY TYPE 1

Groh W.J., Lowe M.R., Simmons Z., Ciafaloni E. and Pascuzzi R.M.

Indiana University, 1800 N. Capitol Ave., Rm. E406, Indianapolis, IN 46202, USA

Arrhythmias leading to sudden death are recognized as a consequence of the cardiac involvement in myotonic dystrophy type 1 (DM1). The incidence of sudden death and factors predicting it have not been ascertained in a large, non-referred DM1 population.

METHODS : The Arrhythmias in DM1 Study is a registry with a purpose of evaluating adult DM1 patients and following to determine outcomes.

RESULTS : Since March 1997, we have enrolled 401 patients at 25 U.S. MDA clinics who have a clinical and genetic diagnosis of DM1. The age at entry was 42±12 (18–78) years with 203 (50.6 %) male. The CTG repeat length was 629±386 (54–1965). The age at diagnosis was 32±14 years. Electrocardiograms were abnormal in 276 (68.8 %) with cardiac conduction abnormalities demonstrated by a prolonged PR interval or QRS duration most common. During a follow-up averaging 5.1±2.1 years, 63 patients (15.7 %) died. The cause of death was sudden arrhythmic in 21 (33.3 %), sudden non-arrhythmic in 3 (4.8 %), non-sudden cardiovascular in 4 (6.3 %), non-sudden from respiratory compromise with endstage DM1 in 22 (34.9 %), and other in 13 (20.6 %). By Cox regression analysis, a younger age at all-cause death was predicted by greater CTG length and by female gender. The risk of sudden arrhythmic death was increased in patients with more severe study entry electrocardiographic conduction abnormalities.

CONCLUSIONS : Sudden arrhythmic death is responsible for one-third of mortality in DM1 and is predicted by an increased severity of electrocardiographic abnormalities.

CTG REPEAT GOVERNS THE RATE OF DECLINE OF RESPIRATORY FUNCTION IN MYOTONIC DYSTROPHY

Begin P. and Mathieu J.

CSSS Chicoutimi - University of Montreal, 305 St-Vallier, Chicoutimi (Quebec), G7H 5H6, Canada

Respiratory muscle weakness and lung volume restriction are prominent clinical features of myotonic dystrophy (MD). We sought the rate of decline of vital capacity (VC), inspiratory and expiratory muscle strenght (MIP,MEP) in response to the trinucleotide CTG repeat. We retrospectively analysed data from 366 adult patients, of whom 210 had repeated mesurements over a mean follow-up period of 7 years. In the transversal analysis, CTG repeat was found the be significantly ($p<.001$) related to VC,MIP and MEP ($0.32<r<0.48$). A multiple analysis of all three parameters in each gender showed that an interaction factor (CTG*age) was significant, with r values up to 0.62 for VC, and to 0.37 for MIP and MEP.The longitudinal analysis showed the rate of decline of VC to be 52±6 ml (m±SEM)for males and 41±6 ml for females, and to be significantly related to CTG ($r=0.16$, $p<0.05$) while rates of MIP and MEP were not, due to a learning effect. We conclude that CTG repeat governs the rate of decline of respiratory muscle strenght and lung volume in MD.

DIFFERENCES IN MYOTONIC DISCHARGES IN TYPE 1 VERSUS TYPE 2 MYOTONIC DYSTROPHY

Logigian E., Ciafaloni E., Quinn C., Moxley R. and Thornton C.

University of Rochester, Rochester, NY (USA)

15 DM1 and 14 DM2 patients, classified by accepted clinical research criteria, prospectively underwent standardized needle EMG examination of 8 muscles : Deltoid, Biceps, Flexor carpi radialis, First dorsal interosseous (FDI), Tibialis anterior (TA), Vastus lateralis (VL), Tensor Fascia Lata (TFL), and thoracic paraspinals (TPs). Eight needle insertions/ muscle were made by experienced electromyographers blinded to DM type who recorded the presence of myotonic discharge, the type of discharge (e.g. "waxing-waning", "waning"), and its approximate duration. Overall, myotonia was more often evokable in DM1 (51 % of insertions) than DM2 (38 %) and was longer duration in DM1 (2.5 sec) than DM2 (1.9 sec), but in 2 muscles (VL, TFL), the reverse was true with myotonia found in 58 % of DM2 VL insertions (45 % in DM1) and 21 % of DM2 TFL insertions (9 % in DM1). In TPs, the % was similar (42 % DM1, 45 % DM2). The major discharge type was "waxing-waning" in DM1 (62 % of discharges), vs "waning" in DM2 (67 %). Three DM2 (21 %), but no DM1, patients had only "waning" myotonia. In both DM1 and DM2, evokable myotonia increased in a proximal to distal gradient. For example, in Deltoid, the % of insertions evoking myotonia was 33 %/27 % (DM1/DM2) vs 88 %/57 % in FDI. We conclude that in distal limb muscles, myotonia is more easily evokable in DM1 than DM2, but in proximal leg muscles, the reverse is true. In DM1, classical "waxing-waning" discharges predominate, but in DM2, less-specific and shorter-lasting "waning discharges" are the rule, making electrodiagnosis of DM more challenging.

NON CONGENITAL PAEDIATRIC MYOTONIC DYSTROPHY : CLINICAL AND GENETIC STUDY IN A SERIES OF 41 PATIENTS

Heron D., Whalen S., Mallet A., Jacquette A., Angeard N., Radvanyi H. and Eymard B.

Département de Génétique et Institut de Myologie, Paris, France

There are 2 paediatric forms of myotonic dystrophy type 1 (DM1) : the well-known severe congenital form (CMD) and the more recently described childhood-onset type. Nevertheless, the nomenclature of DM1 in children remains confusing.

We report a series of 41 patients (19 females and 22 males) aged from 6 to 18 years, presenting with a paediatric non congenital form of DM1, in order to precise its clinical and genetic characteristics. The results showed that :

1. The main clinical symptoms were slowness, hypersomnia, tiredness, dysarthria, and learning difficulties. At clinical exam, facial involvement and myotonia were frequent, but usually not recognized by the patients.
2. School disability was the most important feature in daily life, whereas neuropsychological studies performed on 36 of these patients showed full IQ averaging around 70, or more than 1 standard deviation below the normal population IQ.
3. 15/41 (36 %) patients had mild symptoms before one year of age, leading to identify an "early childhood form" of DM1
4. The sex ratio of transmitting parents was nearly one (21/20)
5. Molecular genetic studies showed CTG repeat size ranging from 200 to 1800 (mean : 679), while CTG repeat size from transmitting parent was from 65 to 1100 (mean : 380)

This report, the most important series of patients with paediatric non congenital form of DM1, confirms (1) the existence of a clinical continuum in paediatric forms of DM1 and (2) the presence of non muscular symptoms mainly learning difficulties. Several groups of these patients have been extensively studied for (1) cognitive profile (n=36), (2) reading and spelling impairments (n=23), (3) sleep evaluation (n=20) and will be presented in posters.

INCIDENCE AND COHORT STUDY OF CONGENITAL DM

Campbell C., Levin S., Jacob P., Siu V. and Venance S.

London Health Sciences Centre, UWO 800 Commissioners Rd E. London, ON, N6A 2E3 Canada

Background : Congenital Myotonic Dystrophy is the symptomatic manifestation of DM1 in a neonate. Mechanical respiratory failure, poor feeding tolerance and hypotonia are the most problematic issues. Much of the knowledge of the epidemiology, clinical features and outcome of CDM continue to be based on small, single centre case series. A national Canadian study has been initiated to address these issues. Method : The study is administered in two sections. The first part started in March 2005, administered by the Canadian Pediatric Surveillance Program, is a prospective 3 year, monthly surveillance of all Canadian pediatricians and sub-specialists to identify incident cases of CDM. A case is defined as a genetic confirmation of DM1 in any child who has neonatal respiratory dysfunction or feeding intolerance requiring admission for more than 3 days. The second section is a cohort study examining mortality, morbidity, development and quality of life in all those identified children over the first 5 years of life. Results : The results of first 6 months of the surveillance will be presented. At the current time 18 children have been reported with one child meeting the criteria. This term infant had feeding difficulties requiring admission for over one week in addition to hypotonia, but did not require ventilation. Conclusions : This study will provide a high-quality source of epidemiologic data on CDM. The clinical features and outcome measures based on a prospective cohort will help to provide valuable information for families, health care providers and research efforts.

QUANTITATIVE CNS STUDIES IN ADULT-ONSET DM1 AND DM2

Day J.W., Dalton J., Rodriguez C.P, , Wozniak J.R., Boys C., Vaurio R. , Shapiro E., Ziegler R., Lim K.O. and Ranum L.P.W.

Paul & Sheila Wellstone MDCenter, Univeristy of Minnesota, Institute of Human Genetics, Minneapolis (MN), USA, 55455

Molecular and clinical parallels of DM1 and DM2 have strongly indicated that a toxic RNA mechanism underlies the myotonia, muscular dystrophy, iridescent cataracts, hypogammaglobulinemia, testicular failure, insulin insensitivity, and other aspects of these multisystemic disorders. It remains unclear whether this RNA mechanism also underlies differences between DM1 and DM2, namely the developmental abnormalities of DM1, and CNS aspects of both diseases. To define early CNS changes in adult-onset DM1 and DM2, we studied 30-45 year old subjects with DM2 or strictly defined adult onset DM1. MRI scans were performed on 5 DM1, 3 DM2, and 8 healthy control subjects. Macrostructural differences were assessed by volumetric measures of grey matter, white matter, and CSF, which showed significantly less grey matter volume in both DM groups compared to controls. CNS microstructure was assessed with diffusion tensor imaging, quantitatively measuring white matter integrity (fractional anisotropy, FA). Inferior frontal FA was significantly lower in both DM groups compared to controls. Superior frontal region FA was significantly lower in DM1 than controls and there was a trend for lower FA in DM2. Neuropsychological tests showed a trend toward reduced working memory in both DM groups compared to controls, and toward lower IQ in DM1. Determining the CNS features common to DM1 and DM2 will help define the degenerative features caused by the toxic RNA mechanism, which can then be characterized at the cellular and molecular levels in transgenic mice and autopsy material.

SHORT-TERM MEMORY TEST IN DM1 AND CORRELATIONS WITH REGIONAL CORTICAL ATROPHY

Antonini G., Di Pasquale A., Mainero C., Morino S., Bucci E., Clemenzi A. and Caramia F.

2nd School of Medicine, University of Rome "La Sapienza", Italy

DM1 patients exhibit cortical atrophy that involves specific brain areas, which are known to be involved in cognitive processes. Voxel Based Morphometry (VBM) is a technique designed to evaluate both global and regional atrophy. Aim of this study was to evaluate short memory function in DM1 and its correlations with regional cortical cerebral atrophy studied with VBM.

Out of 22 DM1 patients (13 males and 9 females, median age 33; range 20-55 years; CTG range 96-1570) studied for cortical atrophy with VBM, 15 subjects (10 males and 5 females) were tested for short-term memory using Digit-Span test. Fifteen healthy subjects matched for sex and age were used as controls.

Fractional brain volumes (total intracranial volume, white matter volume and grey matter volume) were lower in DM1 than in controls ($p<0.005$). Regional analysis revealed local circumscribed areas of grey matter atrophy in the frontal (right superior frontal gyrus, precentral gyrus, left middle frontal gyrus), parietal (left post-central gyrus, left inferior parietal gyrus and bilateral superior parietal lobules) and temporal lobes (left superior temporal gyrus and bilateral middle temporal gyri). Digit-Span scores were significantly reduced in DM1 patients with respect to healthy controls ($p<0.05$).

VBM showed that patients with a low performance in Digit-Span had a regional cortical atrophy located in frontal lobe (Middle frontal gyrus).

SLEEP EVALUATION IN THE CHILDHOOD TYPE OF MYOTONIC DYSTROPHY

Jacquette A., Heron D., Angeard N., Devillers M., Eymard B. and Quera Selva A.

Institut de myologie Hôpital de La Salpêtrière, Paris, France

MD type1 is an autosomal dominant trinucleotide repeat disorder that shows anticipation. Several forms exist which differ in age of onset and severity. The most common is the classical adult form with muscular and polysystemic involvement. Two pediatric forms are described : The severe congenital form, and the infantile form in which constant school difficulties cause a high level of handicap.

Daytime somnolence is a prevalent complaint in the population of patients with congenital and infantile forms of MD1 but its objective assessment remains elusive. It could likely contribute to learning difficulties and if so, a treatment could be proposed. Somnolence is well documented in the adult form of MD1 where it sometimes represents a major handicap, and it is the main target of ongoing therapeutic trials.

To assess more precisely the sleep disorders in the infantile form of MD1, we started in may 2004 a study of 20 children and teenagers aged 6 to 19 years old, with infantile form of MD1, using the following tests :

1. Self evaluation of excessive sleepiness using the Epworth sleepiness scale (adapted to children by M. Lecendreux) and Conners questionnaire.
2. Actimetry : evaluation of extend and speed of motion using a wrist worn activity monitoring system.
3. 24 hour Ambulatory polysomnography
4. Multiple sleep latency test

As this study is ongoing, its initial results will be presented during the meeting. A better knowledge of this problem could provide a basis for future therapeutic trials in the infantile form of MD1.

A SLEEP STUDY OF EXCESSIVE DAYTIME SLEEPINESS IN MYOTONIC DYSTROPHY

Laberge L., Bégin P., Beaudry M., Laforte M. and Mathieu J.

Unité de recherche clinique – CSSS de Saguenay, 305 St-Vallier Saguenay (Qc), Canada

Excessive daytime sleepiness (EDS) is a prominent symptom of myotonic dystrophy (DM) but its etiology is unclear. Our goal was to clarify the link between EDS and muscular impairment, sleep fragmentation, sleep apneas and CTG repeats. Forty-three DM patients (14 men; mean age 49.7 yrs; mean CTG repeats 868.1) completed a 2-week sleep diary and underwent 2 consecutive nights of polysomnographic (PSG) recordings. Data from the 2nd night only are presented. Also, they had a multiple sleep latency test (MSLT). EDS was defined by MSLT≤8 min. Apnea-hypopnea index (AHI) was defined as the number of apneas/hypopneas per hr of sleep. Muscular impairment was categorized as mild (n=7), moderate (n=6) or severe (n=30). Mean MSLT sleep latency was 9.8 min. DM subjects without (n=24) and with EDS (n=19) did not differ relatively to their habitual bedtime, waketime and time in bed. DM subjects with EDS showed greater muscular impairment ($p<.05$), more stage 4 sleep ($p<.05$) and more sleep-onset REM periods (SOREMPs) ($p<.01$) than those without EDS. DM subjects without and with EDS did not differ as to their AHI, obstructive and central sleep apneas and CTG repeats. A high proportion of DM patients exhibit pathological daytime sleepiness according to the MSLT, considered as the gold standard of objective methods. DM patients did not have significant sleep fragmentation or sleep apneas on PSG to explain EDS. Increased SOREMPs and stage 4 sleep suggest that EDS relates mainly to a dysfunction of central sleep regulation in this patient population.

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MYOCARDIAL CONTRACTILITY IN MYOTONIC DYSTROPHY PATIENTS

Giraudeau C.[1,2], Meune C.[1], Eymard B.[3], Laforet P.[3], Wahbi K.[1], Cosnay P.[2] and Duboc D.[1]

1) Department of Cardiology, Cochin Hospital, Paris, France
2) Department of Cardiology, Trousseau Hospital, Tours, France
3) Myology Institute, Pitié-Salpétrière Hospital, Paris, France

Background : Myotonic dystrophy (MD) is associated with a high risk of sudden death; recent advances in the management include prophylactic permanent pacing and ventricular arrhythmias prevention. Ejection fraction is assumed to be preserved in MD patients, also poorly studied. Our aim was to examine left ventricular (LV) and right ventricular (RV) ejection fraction in a large cohort of MD patients with conductive system disease.

Methods : consecutive patients with genetically proven MD, previously treated with permanent pacing for conductive system disease, were included after informed consent. All patients underwent radionuclide ventriculography at rest, after red cell labeling with 99m-Tc. Their results were compared with age-sex matched controls.

Results : 86 patients were included (male 49, age 42±12 years). MD patients had reduced LVEF when compared to controls (60.5±10.2 versus 75.9±9.2, $p<0.0001$) and RVEF (39.3±9.0 versus 44.6±9.0 %, $p=0.0042$). Moreover, 7 MD patients had reduced LVEF <45 % and 22 hade reduced RVEF<35 %. LVEF and RVEF correlated together ($r=0.2$, $p=0.03$), whereas no other variable correlated with either LVEF nor RVEF.

Conclusion : Patients with MD and conductive system disturbance have frequent reduced LVEF or RVEF. EF should be investigated in these patients. However, the impact of preventive treatment (such as ACE inhibitors and/or b-blocker agents) on the prognosis of the disease remains to be determined.

MEXILETINE : EFFECTIVE ANTIMYOTONIA TREATMENT IN MYOTONIC DYSTROPHY TYPE 1 (DM1)

Martens W., Moxley III R., Logigian E., McDermott M., Thornton C., Wiegner W., Moxley IV R., Barbieri C., Blood C., Pandya S. and Dilek N.

URMC, 601 Elmwood Ave, Box 673, Rochester, NY 14642, USA

OBJECTIVE : Determine if mexiletine is safe and effective in reducing myotonia in DM1. BACKGROUND : Myotonia is an early symptom in DM1 and hampers dexterity, gait stability, speech and swallowing. A few nonrandomized trials suggest that mexiletine may be a useful treatment.

DESIGN : We performed two 7 week, double-blind, randomized,crossover trials, each having 20 ambulatory DM1 patients. All patients had delayed relaxation of grip and thenar percussion myotonia. The initial trial (mean age=45.8 yrs; range of CTG repeat size 169-885) compared placebo to 150 mg of mexiletine three times daily. The second, subsequent, higher-dose, trial (mean age=42.6 yrs; range of CTG repeat size 169-1731) compared placebo to 200 mg three times daily. Myotonia measurements were a computerized determination of the time for the isometric grip force to relax after a 3 second maximum contraction from 90 % - 5 % & 50 % - 5 % of maximum.

RESULTS : There was a significant ($p<0.05$) reduction in grip relaxation times with both 150 and 200 mg doses of mexiletine. The greatest delay and the greatest response to treatment occurred in the late phase of relaxation (50 % - 5 % of maximum grip force). Side effects were minimal for 150 and 200 mg doses of mexiletine. No ECG changes occurred during each of these studies.

CONCLUSIONS : Mexiletine at doses of 150 and 200 mg three times daily is safe and effective in DM1. Longer term trials are necessary : a) to determine if mexiletine reduces muscle pain; and, b) if mexiletine can maintain muscle strength and function.

SOME FLAVONOIDS PREVENT *CIS*- AND *TRANS*- EFFECT OF EXPANDED CTG REPEATS IN A STABLE PC12 CELL TRANSFORMANTS

Furuya H., Ikezoe K., Hayashi T.[2], Fukumaki Y.[1], Nakabeppu Y.[1], Kira J.[3] and Fujii N.

Department of Neurology, National Omuta Hospital, Fukuoka, Japan
1) Medical Institute of Bioregulation, Kyushu University and CREST, JST, Fukuoka, Japan
2) Faculty of Pharmaceutical Sciences, Toyama University, Toyama, Japan
3) Department of Neurology, Kyushu University 60, Fukuoka, Japan

To study the pathogenesis of myotonic dystrophy type 1 (DM1), we constructed a DM1 cell culture model using a PC12 neuronal cell line. The expanded 250 CTG repeat was subcloned into the 3'-UTR of the luciferase (LUC) gene yielding a stable transformant (CTG-250). We also construct a dual stable cell, which includes a renilla luciferase (RL) gene into the CTG-250 cell (CTG-250RL). The former is able to evaluate the *cis*-effect of repeat toxicity by measuring LUC activity and the latter to evaluate the *cis*- and *trans*-effect by measuring LUC and RL activity. To find agents that ameliorate the mRNA gain of function, 235 bio-flavonoids were screened with CTG-250. Screening analysis confirmed that a flavanone, and two isoflavones strongly inhibit the *cis*-effect of CTG repeats. Some of those bio-flavonoids also improve the *cis*- and *trans*-effect and cytotoxicity of expanded CTG repeats even in CTG-250RL. In conclusion, we found that some bio-flavonoids inhibit both the *cis*-, *trans*-effect and cytotoxicity, indicating that their chemical structures work to ameliorate both these toxic effects. These systems make it easy to evaluate the toxic effects of expanded CTG repeats in detail and therefore should be useful for screening other DM1 treatments for their efficacies.

A POTENTIAL GENE THERAPY FOR MYOTONIC DYSTROPHY TYPE 1

Puymirat J., Doucet G., Bélanger J., Laberge D., Beaulieu D. and Rossi J.[1]

Unit of Human Genetics, CHU Laval, 2705 Blvd Laurier, Ste-Foy, Quebec, Canada
1) Institute Beckman, City of Hope, CA

In previous work, we have demonstrated that antisense RNAs and ribozymes can target mutant DM1 transcripts and that this effect was associated with restoration in normal functions of human DM1 myoblasts, as determined by the restoration in their ability to fuse, the increase in the uptake of glucose and in the restoration in the normal splicing of insulin receptor mRNAs. To determine if antisense RNAs and ribozymes can target mutant DM1 transcripts *in vivo*, we analyzed the effect of intramuscular injection of antisense RNA and ribozymes in DM1 mice. Using AAV1, we have been able to transduce more than 80 % of skeletal muscle fibers without any toxic effect, one month after a single intramuscular injection of recombinant AAV1/museap into the tibialis anterior. In next experiments, we examined the ability of antisense RNAs and ribozymes to target mutant transcripts in DM1 mice having 91 repeats (obtained from R. Korneluk). DM1 mice received a single intramuscular injection of AAV1/antisense or AAV1/ribozyme into the tibialis anterior. One month after the injection mice were sacrificed and the levels of DM1 transcripts were analyzed by RT-PCR. The levels of DM1 transcripts were decreased by 50 % and 80 % by antisense RNAs and ribozyme, respectively. Whether these effects are associated with restoration of normal skeletal muscle functions remains to be determined. Conclusion : this is the first demonstration that antisense RNAs and ribozymes could be used for the development of a gene therapy for DM1.

A MODEL OF DISTRIBUTION OF A MUTATION IN DMPK GENE ASSOCIATED WITH MYOTONIC DYSTROPHY TYPE 1

Akhmadeeva L. and Veytsman B.

Chernyshevskogo St., 104 - 141, Ufa, 450077, Russia

Background : Myotonic Dystrophy Type 1 (DM1, OMIM #160900) is a hereditary disease, with different prevalence in different ethnic groups. The prediction of its prevalence is important for planning of health care system.

Methods : We construct a general model that accounts for differences in prevalence, number of children in families and marriage preferences for different ethnic groups. The model is applied to the current epidemiology of DM1 in the Republic of Bashkortostan (Russia).

Results : Our model predicts increase of prevalence of DM1 in the near future due to changes in the ethnic composition of the Republic.

Conclusions : We developed a general approach for predicting epidemiology of hereditary diseases in populations with mixed ethnicity.

MEASURING HEALTH RELATED QUALITY OF LIFE (HRQL) WITH THE RESOURCES OF THE NIH REGISTRY OF MYOTONIC DYSTROPHY(DM) AND FSHD PATIENTS AND FAMILY MEMBERS

Hilbert J., Donlin-Smith C., Martens W., Moxley R. III, and Registry Scientific Committee

URMC, 601 Elmwood Ave, Box 673, Rochester, NY 14642, USA

A large, well-defined population of DM patients is necessary to assess physical and psychosocial factors that affect HRQL. Validated and disease adapted instruments to measure HRQL are needed to monitor disease progression and treatment.

METHODS : We analyzed HRQL data within the Registry database and provided an overview of future research using Registry resources.

RESULTS : The Registry has enrolled 393 DM1 patients (average age 45.6 y ± 12.5; 53 % female). Average CTG size is 441 ± 321 (n=180). For the entire DM1 population, physical limitations include : myotonia (95 %), and weakness of facial (92 %), distal (89 %) and proximal (30 %) muscles. Physical therapy has occurred in 46 % of the patients, and assistive devices are used by over half the patients. The most frequent associated medical problems are gastrointestinal (GI) reflux (36 %), constipation (33 %), and pneumonia (23 %). Psychosocial manifestations include excessive sleepiness (55 %), with less than 20 % receiving treatment. DM has also affected employment in 62 % of patients through job modifications and forced disability. Depression and counseling have occurred in 29 % of patients.

CONCLUSION : Registry data indicates that myotonia, compromised employment opportunity, and GI effects are common in DM1. To further explore HRQL factors, we plan collaborative research with the NIH Patient Reported Outcome Measurement System (PROMIS), whose researchers are developing computer adaptive technology to measure HRQL.

ARE QUESTIONNAIRES AN APPLICABLE INVESTIGATION TOOL IN RELATION TO MD PATIENTS?

Schwartzbach I.

The Neuromuscular Disease Rehabilitation Center, Kongsvangs Allé 23, Århus C (DK), Denmark, 8000

A questionnaire survey was conducted (2004) among patients diagnosed with MD and registered at the Neuromuscular Disease Rehabilitation Center. This involves 64 % (173 persons) of the expected number of Danish citizens (1/20,000).

The aim of the study was to assess the validity of the patients' responses. In conjunction with dispatch of a questionnaire (Schema 1) to all 173 patients (response percentage 81 %), an additional questionnaire (Schema 2) was sent to those patients residing in Aarhus County (N=29). Not only did Schema 2 include more questions than Schema 1, but its design differed significantly as well. Responses to identical questions in the two questionnaires were compared. Only 35 % of the respondents answered both questionnaires independently, consistently and leaving no unanswered questions.

As 75 % of the patients (Schema 1) reported daily or weekly fatigue, this question was selected for closer scrutiny. 15 patients were then selected for this purpose. Individual- and focus group interviews were conducted, supplemented by gradation scales and observation. The results obtained from the various methods were then compared. This study demonstrates that this patient group experiences great difficulty in using a self-reported questionnaire; 26.4 % did not answer all the questions, 34.6 % did not answer consistently, while approximately 35 % of the responses could not be verified by means of observation. This study points out that a questionnaire survey conducted among this patient group is not viable in itself, but must be supplemented with interviews, observation, measurements, etc.

SOCIAL PARTICIPATION OF PATIENTS WITH MYOTONIC DYSTROPHY TYPE 1

Gagnon C., Noreau L. and Mathieu J.

Carrefour de santé de Jonquière, Clinique des maladies neuromusculaires, 2230 de l'Hôpital, cp 1200, Jonquière (Québec), G7X 7X2, Canada

Few studies have focussed on the impacts of myotonic dystrophy type 1 (DM1) on the accomplishment of daily activities and social roles, which is referred as social participation. The general objective of this study is to evaluate the level of social participation of patients affected by the adult and mild phenotype of DM1. The Assessment of Life Habits was administered to 200 DM1 patients (79 males; 158 adult phenotype) recruited from the registry of a neuromuscular clinic. This 77-items instrument, a person-perceived measure of social participation, assesses the performance in 12 categories of daily activities and social roles. An important disruption in the global level of social participation (total score and daily activities and social roles sub-scores) was observed for patients with the adult phenotype; their performance in the lowest quartile was lower than in a normal aging population. The global level of social participation was near normal for individuals with a mild phenotype. The level of accomplishment in most categories (8/12) including nutrition, housing, mobility, employment and recreation showed a significant lower performance in patients with the adult phenotype compared to those with a mild phenotype. The important disruption of social participation in several areas will need to be included in the evaluation and service provision for the management of DM1.

PARENTS' EXPERIENCES OF A DIAGNOSIS OF DM : FINDINGS FROM A QUALITATIVE INTERVIEW STUDY

Downing C.

Centre for Family Research, Cambridge University, Free School Lane, Cambridge CB2 3RF, England.

This paper focuses on how being diagnosed with a late-onset genetic condition such as DM impacts on parents. It draws on findings from a qualitative interview study where one parent is facing a primarily late-onset genetic disorder, which is highly variable such as Myotonic Dystrophy (DM) or which has a more clearly defined clinical course such as Huntington's disease (HD). The study deployed theoretical sampling to encompass variations for mothers and fathers in genetic risk status, disease phenotypes and parenting circumstances. This includes single parents, those parenting as couples, those who are not themselves at risk but are fostering or adopting affected children. The paper will report on how the process of diagnosis is experienced and identify issues that subsequently arise for parents and in connection with predictive testing and diagnosis for their at-risk, their affected children and their siblings. Results from the study will be fed back to families and to those who work with families in order to help them offer appropriate support.

MYOTONIC DYSTROPHY PATIENT LITERATUREIN DIFFERENT LANGUAGES

Harper P.S.(1) and Bowler M.(2)

1) Cardiff University, UK
2) Myotonic Dystrophy Support Group, Nottingham,UK

A wide range of information on myotonic dystrophy is available for patients and families as printed material and on the internet, but most of this is in English. While many professionals in non-English speaking countries will be able to use this, most patients and family members from such countries will not. This points to a need for a systematic initiative to make a full range of literature available in as many languages as possible. This can be done both by writing specific items in particular languages and by translation of existing material. There is also a need for specific information on type 2 myotonic dystrophy, now that its clinical features and complications are becoming better defined.

As an example, the small book' Myotonic Dystrophy, the Facts' is now available in English, Russian, Japanese and (shortly) German, thanks to the efforts of colleagues in the field and financial support from the Myotonic Dystrophy Support Group. The publishers (Oxford University Press) have kindly given free permission for translation and distribution in other languages, which will hopefully allow it to reach many other patients and families who are unable to utilise the English version.

There are many other items which would be of value in translation so, given that patient information is one of the most important aspects of management for myotonic dystrophy, the facilitating and, where necessary funding of this process should be a high priority for support groups and related agencies.

POSTER PRESENTATIONS

SIMPLE MATHEMATICAL MODEL OF MICROSATELLITE EXPANSIONS : WHEN SELF-REPARATION DOES NOT WORK

Veytsman B. and Akhmadeyeva L.

George Mason University, School of Computational Sciences, Fairfax, VA 22030
Bashkir State Medical University, 3 Lenina Str., Ufa, 450077, Russia

We propose a simple model of microsatellite expansion, and show that replication of DNA has an inherent self-repairing mechanism. We prove that if the probabilities of expansion and contraction during replication are equal, microsatellite expansions are always self-repairing. If these probabilities are different, self-repairing does not work any more. The mosaicism, anticipation and reverse mutation cases are discussed in the framework of the model. We explain these phenomena and provide some theoretical evidence for their properties, for example the rarity of reverse mutations. We discuss the available experimental data and compare them to our theoretical predictions.

CHEMOTHERAPEUTIC DELETION OF CTG REPEATS IN LYMPHOBLAST CELLS FROM DM1 PATIENTS

Pytlos M.J., Hashem V.I., Klysik E.A., Tsuji K., Khajavi M., Ashizawa T. and Sinden R.R.

Institute of Biosciences and Technology, TAMHSC, 2121 W Holcombe Blvd, Houston, TX 77030 USA

In myotonic dystrophy expanded repeats are biased toward further expansion upon intergenerational transmission and throughout life in somatic cells. Disease symptoms show an earlier age of onset and greater severity as the length of the triplet repeat tract increases. Most diseases exhibit progressive neurological and/or muscular degeneration that can lead to total disability and death. Given that disease severity is related to repeat tract length, reducing repeat lengths might delay the onset and reduce disease severity. We have tested the hypothesis that DNA damage, which results in subsequent repair, can lead to an increased rate of repeat deletion. We show that repeat deletion may be mediated by various chemotherapeutic agents. Three lymphoblast cell lines derived from two myotonic dystrophy type 1 patients treated with either EMS, mitomycin C, mitoxantrone, or doxorubicin, at therapeutic concentrations, accumulated deletions following treatment. Treatment with EMS frequently prevented the repeat expansion observed during growth in culture. A significant reduction of CTG repeat length by 100 - 350 (CTG)/(CAG) repeats often occurred in the cell population following treatment with these drugs. These results suggest that a chemotherapeutic approach to the reduction in triplet repeat length may provide one possible rationale to slow, stop, or reverse the progression of CTG-associated diseases.

CHARACTERIZATION OF SMALL TYPE 2 FIBRES IN MYOTONIC DYSTROPHY TYPE 2

Udd B., Vihola A., Raheem O., Haapasalo H., Paetau A., Suominen T.M., Kalimo H. and Krahe R.

Folkhälsan Genetics Institute Biomedicum, Helsinki, Finland, 000290

Muscle histopathology in myotonic dystrophy type 2 (DM2) shows frequent nuclear clump fibers, which have been considered a hallmark of neurogenic atrophy. We have previously shown that these and other extremely small fibers in DM2 are identified as type 2 fibres, expressing fast myosin heavy chain (MHCf). These atrophic type 2 fibers occur early in DM2 proximal muscles, often before clinical muscle symptoms, which is in contrast to DM1. We have investigated the origin of the small type 2 fibres in DM2 using markers for muscle regeneration, denervation and satellite cells.

Antibodies against developmental and neonatal MHC (MHCd and MHCn); adult MHCf, Serca-1 and slow MHC; quiescent satellite cell marker Pax7; myogenesis markers desmin and N-CAM. Frozen muscle biopsy sections from 20 DM2 patients, 4 DM1 patients, 4 patients with neurogenic atrophy. Most of the small type 2 fibres in both DM2 and neurogenic atrophy stained positively for MHCn, whereas only few fibers were MHCd positive. Many small fibres, including those with nuclear clumps, were N-CAM and desmin positive. Pax7 expressing satellite cells were not reduced in DM2 muscle compared to controls. The expression of MHCn but not MHCd in DM2 very small type2 fibres, together with N-CAM upregulation, suggest that the small fibres in DM2 do not result from a necrotizing process, but are rather induced by reprogramming which mimicks neurogenic mechanisms. There was no direct evidence that the very small type2 fibres would originate from satellite cells, which upon activation mature but do not fuse.

CHARACTERISATION OF MYOBLAST CELLS FROM MYOTONIC DYSTROPHY TYPE 2 PATIENTS

Hamel F., Pelletier R., Haineault C., Tarnopolsky M.[1] and Puymirat J.

CRCHUL – Laval University 2705 Boul Laurier Quebec (Quebec) G1V 4G2, Canada

1) Dept. of Medicine, Div. of Neurology – McMaster University Medical Centre 1200 Main Street West Hamilton (Ontario) L8N 3Z5, Canada

Myotonic dystrophy type 1 (DM1) and type 2 (DM2) are genetic diseases caused by non-coding repeat expansions. In DM1 patients, from 50 to 1000 CTG repeats are found in the 3' untranslated region of the DMPK gene whereas for DM2, 75 to 11000 CCTG repeats are located in the first intron of the ZNF9 gene. The repeats are transcribed along with the gene but the RNAs are not exported to the cytoplasm for translation. Instead, these stretches of RNA accumulate in the nucleus and form foci of expanded CUG or CCUG repeats. It is hypothesized that they cause *trans*-dominant effects such as aberrant RNA processing. Many aspects of DM1 pathology have been described; however DM2 cells still remain to be similarly examined. The purpose of our study was to characterize the molecular defects of myoblast cells from DM2 patients. The levels of MyoD and myogenin proteins were measured in DM2 cultured cells in comparison to normal myoblasts. Given the splicing defects that lead to diabetes in DM1 patients, we measured the ratios of two insulin receptor isoforms in DM2 and normal myoblasts. The ability of DM2 cells to differentiate and form myotubes was investigated by measuring their index of fusion. Cells from several DM2 patients were examined to see if there was a correlation between their fusion impairment and the number of repeats found at the ZNF9 locus. We also determined if culture medium that had been in contact with DM2 cells would reduce the capacity of normal myoblasts to fuse, as was previously demonstrated in our laboratory for DM1 cells. Our findings show that DM1 and DM2 myoblasts are similar on many levels but the DM2 pathology possesses its own molecular features.

FUNCTIONAL CHARACTERIZATION OF SKELETAL MUSCLES IN DM1 MICE

Vignaud A., Ferry A., Guiraud C., Gourdon G., Butler-Browne G. and Furling D.

CNRS FRE2853, Faculté de Médecine UPMC, 105 boulevard de l'hôpital, 75013 Paris

Transgenic DM1 mice, carrying the CTG expansion and producing an abnormal human DMPK mRNA with 320 repeats, has been developed by G. Gourdon's group to study Myotonic Dystrophy type 1 (DM1). These mice reproduce some features of the human disease, such as myotonia (Seznec et al. 2001). However, the functional properties of muscles from these transgenic mice have never been evaluated. In order to characterize this transgenic mouse model, and in particular the muscle function, several isometric physiological parameters related to the force production, the contraction speed and the fatigue resistance were recorded *in situ* on anesthetised animals. Hind limb muscle contractions were induced by electrical stimulation of the sciatic nerve. In the Gastrocnemius and the Tibialis anterior muscles we observed a decrease in both the muscle mass (atrophy) and in the force production (weakness) of 9 month old mice. However, nothing was observed in younger transgenic mice. The same technique was used to study the recovery of skeletal muscle after injury in these DM mice since it has been demonstrated that human satellite cells from DM1 patients that are responsible for the repair of damaged muscle fibres, have an abnormal behaviour *in vitro* (Furling et al. 2001). Preliminary results indicate that injured muscles in DM and normal mice both recover 70% of their force production 14 days after injury. However the regenerated muscles of DM mice are weaker than muscles of normal mice. Experiments are in progress to determine if this is due to a problem of regeneration. A complete functional evaluation of the DM1 mouse model is essential before it can be used for the evaluation of different therapeutic strategies for DM1.

IDENTIFICATION OF PROTEINS INTERACTING WITH MUSCLELIND PROTEINS AND DISTRIBUTION OF FOCI IN DM CELLS

Mittal S., Fardaei M., Holt, I., Morris G. and Brook J.D.

University of Nottingham, Institute of Genetics, Quennsmedical Centre, Nottingham, UK, NG7 2UH

Myotonic dystrophy, the most common form of muscular dystrophy in adults, is primarily associated with muscle weakness and myotonia. It may also produce other abnormalities such as cardiac defects, cataracts, premature balding, digestive problems, excessive sleeping, and abnormal insulin secretion.

Genetically, two DM loci have been identified DM1 and DM2. DM1 accounts for approximately 98% of all identified cases and is caused by CTG expansion in the 3' UTR of the DMPK gene located on chromosome 19q13.3. DM2 is caused by a CCTG expansion in intron1 of ZNF9 gene located on chromosome 3q.

It has been shown that in both DM1 and DM2 cells, the transcripts containing expanded repeats are retained in the nucleus and form distinct foci. A family of muscleblind proteins (MBNL1-3) have been shown to co-localise with the foci in the DM nucleus. Muscleblind proteins regulate alternative splicing and the MBNL1 knockout mouse model exhibits symptoms of DM and some of the splicing abnormalities seen in DM cells. It is hypothesised that the expanded repeats in DM cells may also sequester other RNA-binding proteins, blocking their normal activity.

We have set out to identify other proteins that interact with muscleblind and/or are sequestered by mutant RNA foci. We have used two approaches. In the first we have generated tagged proteins for use in tandem affinity purification experiments and proteomic screens. In the second we have adopted a candidate approach looking for co-localisation of speckle and paraspeckle proteins. We have used confocal microscopy to examine the distribution of nuclear foci within DM cells.

CARDIAC PHENOTYPING OF DM1 TRANSGENIC MICE USING HIGH RESOLUTION MRI

Hiba B., Gourdon G. and Janier M.

Plateforme ANIMAGE, 59 Boulevard Pinel, 69677 Bron - France

This study aimed at evaluating the contractile function in DM1 mice, a model of Steinert disease [1]. It has already been shown that these mice develop myotony and myopathy of skeletal muscle. It is of interest to evaluate the effect of the transgenic mutation on cardiac phenotype and especially contractile function.

High-resolution MRI has been applied successfully to quantify myocardial function in 3 DM1 female mice of 10 months. The quantification results were compared to those of 8 normal C57BL6 mice.

8 contiguous ECG-triggered cine images (spatial resolution : 98X98 µm2; slice thickness = 1 mm) were acquired in the short-axis orientation to cover the entire heart ventricles. End-Diastole Volume (EDV) and End-Diastole Volume (ESV) of LV were calculated as the corresponding volumes sum in each of the 8 slices. Stroke volume (SV) was calculated as EDV minus ESV, and ejection fraction (EF) was given as SV divided by EDV. Myocardial mass was defined as (EDV-ESV) multiplied by a factor of 1.05 g/cm3.

An abnormal thickness of the external wall of the left ventricle was evidenced in one of the three DM1 mice. The mean and standard deviation of the heart anatomical and functional parameters, measured in the three mice were : EDV=43.6±6.4µl; ESV=15.2±0.8µl; SV=28.4±6.7µl; EF=645%; LV-mass=85.8±16mg.

No significant difference was evidenced between the two groups. A larger number of animals should be analyzed to evaluate the existence of anatomic abnormalities of the heart and to definitively confirm the normal contractile function.

[1] Seznec et al. Hum Mol Genet. 2001 1;10(23) : 2717-26.

INHIBITION OF MUSCLE GENE EXPRESSION BY THE CHCR/MBNL3 PROTEIN

Wang E.H., Lee K.S. and Castile A.

Department of Pharmacology, University of Washington, 1959 NE Pacific St, Box 357280, Seattle, WA, 98195 USA

The goal of our research is to elucidate the function of CHCR/MBNL3 in muscle differentiation and the pathogenesis of myotonic dystrophy. CHCR colocalizes with expanded mutant RNA transcripts that accumulate in the nuclei of DM1 and DM2 cells. We have found that CHCR mRNA and protein levels decrease upon differentiation of C2C12 mouse myoblasts. Constitutive expression of CHCR in C2C12 cells antagonizes the expression of muscle specific genes. The gene expression prolifes of C2C12 cells that stably express the CHCR protein and the parental cell line were determined by DNA microarray analysis and validated by quantitative RT-PCR. These data revealed that genes involved in muscle differentiation and function, mRNA processing and cell adhesion are selectively down-regulated by CHCR expression. We are currently focusing on the IGFBP5 and other E-box containing promoters to determine the mechanism by which CHCR hinders muscle gene expression. Additional studies are being carried out to map the domain of CHCR required for its antagonistic function. Our current hypothesis is that CHCR protein levels remain elevated in DM muscle and that the continued presence of CHCR in the diseased cells contributes to the muscle weakening and wasting of myotonic dystrophy.

NEW MONOCLONAL ANTIBODIES SPECIFIC FOR EACH HUMAN MUSCLEBLIND ISOFORM SHOW THAT MBNL1 AND MBNL2 ASSOCIATE WITH RIBONUCLEAR FOCI IN DM1 AND DM2 CELLS AND THAT MBNL2 IS DOWN-REGULATED DURING MUSCLE DEVELOPMENT

Holt I., Furling D., Fardaei M., Butler-Browne G.S., Brook J.D. and Morris G.E.

Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, Shropshire. SY10 7AG. UK

Human muscleblind proteins (MBNL1, MBNL2 and MBNL3) bind preferentially to expanded RNA repeats in DM nuclei. The sequestration of muscleblind proteins is thought to prevent their normal function in the regulation of alternative splicing of pre-mRNAs. We have produced novel monoclonal antibodies specific for each muscleblind protein. They were selected for lack of cross-reaction with the other muscleblind proteins by ELISA, Western blot and immunofluorescence staining of transfected cells.

Both MBNL1 and MBNL2 colocalised with nuclear RNA foci detected by (CAG)10 and (CAGG)10 probes in DM1 and DM2 skin fibroblasts respectively. MBNL3 was not detected. The differentiation of DM1 myoblasts gave rise to a doubling in the number of foci per nucleus which may be linked to increases in DMPK mRNA during myogenesis. MBNL2 levels decreased by 75% during myogenesis but MBNL1 remained constant. Thus, MBNL1 is the dominant isoform in mature muscle which may explain why the knockout of MBNL1 alone can cause a DM phenotype in mice. The results provide evidence for distinct functional roles for the muscleblind isoforms.

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MYOTONIC DYSTROPHY (DM1) IN CHILDREN : A CONTINUOUS SPECTRUM FROM SEVERE CONGENITAL TO LATER CHILDHOOD ONSET FORM

Jacquette A., Angeard N., Radvanyi H., Eymard B. and Heron D.

Département de Génétique, Hôpital de La Salpêtrière, Paris, France

Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease with variable clinical expression, caused by an unstable (CTG)n repeat expansion in the 3'-untranslated region of the DM1 protein kinase gene. Four clinical forms have been identified : the late onset form, the classical adult form, the well-known severe congenital form and the more recently described childhood-onset type without neonatal symptoms defined as follows : age at onset between one and ten years, uneventful pre- and postnatal history and normal development within the first year of life, increasing problems such as muscle hypotonia and a variable degree of mental retardation. Nevertheless, the nomenclature of DM1 in children is confusing, and clinical experience strongly suggests that myotonic dystrophy in children is probably a continuous spectrum ranging from severely affected congenital cases, or with mild congenital features, to cases with onset in later childhood presenting with learning difficulties.

In order to characterize more precisely the paediatric forms of DM1, we present here the results of a retrospective study of 50 patients diagnosed as DM1 before 18 years. They were 26 boys and 24 girls, with a mean age of 15.2 years (from 1 to 24). Twelve had an obvious congenital form and 23 had typical childhood-onset form, whereas 15 children could not be classified according to these two types, because they presented mild signs before the age of one year. These 3 groups were compared for clinical data and genetic features. The children in the 3rd presented an intermediate severity compared with the severe CDM and mild childhood onset groups. The identification of this early childhood-onset form of DM1 is important for clinical practice, particulary for prognosis and genetic counselling.

ASSESSMENT OF NEUROMUSCULAR EXCITABILITY PROPERTIES IN MYOTONIC DYSTROPHIES : PRELIMINARY RESULTS

Boërio D., Hogrel JY., Bassez G., Creange A. and Lefaucheur JP.

Département de Myologie , Hôpital de La Salpêtrière, Paris, France

Following an action potential, the motor unit becomes first totally unexcitable : absolute refractory period (ARP) then partially unexcitable : relative refractory period (RRP). This unexcitability results from the cumulative effects of nerve, neuromuscular junction and muscle fibre refractoriness. Paired pulse techniques are commonly used to highlight this phenomenon. The evaluation of neuromuscular refractoriness remains extremely rare in muscular dystrophies. We evaluated ARP and RRP values in patients with myotonic dystrophy type 1 (DM1, n=6, including one premutation) and type 2 (DM2), and compared the results to values previously obtained in 32 healthy controls, using the paired pulse techniques. Two modalities have been performed : a supra-maximal and a sub-maximal paired pulse, depending on the intensity of the second pulse (either supra-maximal or equal to 70% of the maximal intensity). Supra-maximal paired pulse technique did not reveal any differences in ARP and RRP values in DM1, DM2, and control subjects. However, using sub-maximal technique, we found in DM1 patients significantly longer values of both ARP (3.52 ± 1.04 ms vs 1.99 ± 0.30 ms) and RRP (5.21 ± 1.90 vs 3.18 ± 0.47 ms) compared to healthy subjects. Interestingly, both the premutated DM1 (ARP : 1.8 ms, RRP : 2.78 ms) and the DM2 patients (ARP : 1.53 ms, RRP : 2.61 ms) displayed values within the normal range. These preliminary results show that neuromuscular refractoriness is markedly increased in DM1, whereas it seems preserved in DM2. Further investigations are needed to confirm whether this defect may be selective of DM1.

PROLONGED VENTILATION IN CONGENITAL DM.

Campbell C., Magalheas S., Lyttle B., Frewen T. and Levin S.

Children's Hospital of Western Ontario, UWO, 800 Commissioners Rd. E, London, ON, N6A 2E3, Canada

Background : Guidelines continue to suggest that assisted ventilation of children with congenital DM beyond thirty days is associated with high mortality and poor outcomes. The evidence on which this practice is based is limited. Health care practitioners and parents have little published information on outcomes in children receiving prolonged ventilation. This report outlines such a case.

Methods : Single case report.

Results : This 3 year old with a CTG repeat size of 2300 (E4) was born at 35 weeks gestational age. In addition to severe hypotonia he had mechanical respiratory failure requiring ventilation and tracheostomy. Other complications included shunt dependant hydrocephalus and feeding dysfunction. Family chose ventilation in the home environment. The child has never been ventilator independent, but since 8 months old has only required assisted ventilation with sleep. He has required 3-4 brief hospitalizations per year for chest infections, has scoliosis and GI dysmotility. His strength continues to improve with scores on the AIMS of 9 and GMFM of 15% (total) and 37% (2-goal total). No formal cognitive tests have been completed but the child's interests and receptive language appear near normal.

Conclusions : Ventilation duration remains the principal determinant of withdrawal of life support treatment in children with CDM. Parents and health care providers need more information regarding outcomes in children, and life satisfaction in families, choosing prolonged ventilation. This case illustrates that in CDM strength and respiratory function improve over time.

QUALITY OF LIFE IN PATIENTS WITH MYOTONIC DYSTROPHY

Laberge L., Perron M., Veillette S., Gagnon C. and Mathieu J.

Groupe ÉCOBES, Cégep de Jonquière, 3791 de la Fabrique, Jonquière (Qc), Canada

A primary aim of treatment in chronic disease is to enhance the quality of life (QOL) by reducing the impact of the disease. Our aim was to study the link between QOL and age, sex, disease severity and socioeconomic characteristics in myotonic dystrophy (DM). Two-hundred DM patients (79 men; mean age 47.0 yrs) filled the Short Form-36, for which higher scores indicate better health. Muscular impairment was rated as mild (n=40), moderate (n=36) or severe (n=124). Mean CTG repeats was 809.2. DM men have higher scores than DM women for bodily pain, general mental health and vitality ($p<.05$). Age is inversely related to general health perceptions and role limitations due to physical functioning ($p<.05$). Muscular impairment and CTG repeats are inversely related to general health perceptions, physical functioning, vitality and social functioning ($p<.05$). Higher educational level, employment status and family income are linked to higher scores in general health perceptions and physical functioning ($p<.01$). In accord with the sex difference observed in the Canadian population, DM men scored higher than DM1 women on all domains. Also, DM men with moderate and severe muscular impairment scored lower than Canadian men in physical functioning, role physical, bodily pain, general health perceptions and vitality while only DM1 women with severe muscular impairment scored lower than Canadian women on these domains. The variation of health functioning with markers of disease severity and socioeconomic disadvantage suggests that the SF-36 is sensitive to changes in DM subjects' health.

THE RELATIONSHIP BETWEEN FATIGUE AND DAYTIME SLEEPINESS IN MYOTONIC DYSTROPHY

Laberge L., Gagnon C., Bégin P. and Mathieu J.

Groupe ÉCOBES, Cégep de Jonquière, 3791 de la Fabrique, Jonquière (Qc), Canada

Daytime sleepiness and fatigue are prominent symptoms of myotonic dystrophy (DM). The two studies that have assessed their relationship suggested they constitute independent features (n=36 Rubinsztein et al. 1998; n=32 van der Werf et al 2003, JNNP). Our aim was to verify in a larger sample of patients whether these symptoms are distinct clinical entities of DM. Two-hundred DM patients (79 men; mean age 47.0 yrs) completed the Epworth Sleepiness Scale (ESS), the Daytime Sleepiness Scale (DSS), the Chalder Fatigue Scale (CFS) and the Krupp Fatigue Scale (KFS). Muscular impairment was categorized as mild (n=40), moderate (n=36) or severe (n=124). Mean CTG repeats was 809.2. Mean scores were 8.1 for ESS, 4.9 for DSS, 5.1 for CFS and 4.6 for KFS. The DSS and KFS were related to muscular impairment ($r>.29$, $p<.001$) while only the KFS was related to CTG repeat ($r=.21$, $p<.01$). The CFS and KFS correlated more highly with each other ($r=.74$, $p<.001$) than with either daytime sleepiness scale (r's between .49 and .63, $p<.001$). Conversely, the ESS and DSS correlated more highly with each other ($r=.64$, $p<.001$) than with either fatigue rating scale. There is an overlap between fatigue and daytime sleepiness in DM. Since the pathways for treating daytime sleepiness may substantially differ from those specific to fatigue, clinicians should inquire about sedentary activities, sleep schedules, and functional limitations of DM patients in an effort to separate "true" fatigue from "true" daytime sleepiness and to complement subjective rating scales by other methods of assessment.

EXCESSIVE DAYTIME SLEEPINESS IN MYOTONIC DYSTROPHY : THE HYPOCRETIN NEUROTRANSMISSION SYSTEM AND DATA FROM THE NATIONAL REGISTRY

Ciafaloni E., Mignot E., Sansone V., Hilbert J., Mc Dermott M.C. and Thornton C.

University of Rochester, Rochester (NY), USA, 14642

Excessive daytime sleepiness (EDS) is a common disabling symptom in DM1. Its pathophysiology is unclear. It is unknown if EDS is also present in DM2. Objective : To determine if a disrupted hypocretin (Hcrt) neurotransmission system is responsible for EDS in DM1.To compare severity of EDS in DM1 and DM2. Methods : The ESS from 386 DM1 and 74 DM2 patients from the National Registry of Myotonic Dystrophy and Facioscapulohumeral Muscular Dystrophy was analyzed. Polysomnograms (PSG) and Multiple Sleep Latency Tests (MSLT) were performed in 16DM1 patients.Hcrt-1 CSF levels were measured using a radioimmunoassay. Splicing of mRNA for Hcrt receptors 1 and 2 in RNA samples isolated from postmortem cerebral cortex was analyzed by RT-PCR. Results : CSF Hcrt-1 levels (pg/ml) in 38 DM1 patients (mean 277, range 176-448) did not differ from 33 controls (mean 277, range 186-382). No difference was found between patients with an ESS>10 (N=17, mean ESS 15.4, mean Hcrt-1 268) and those with an ESS<10 (N= 21, mean ESS 6.4, mean Hcrt-1 284).There was no abnormality in HcrtR1 and HcrtR2 splicing in DM1 patients. PSG showed high percentage of REM sleep (mean 30.6% +/-8.2) with 11 patients having >30% REM. ESS was greater in DM1 than FSHMD (p= 0.0001) but not in DM2 compared with FSHMD (p=0.9). No correlation was found between ESS and age, BMI, duration of disease or number of CTG repeats. Conclusions : EDS is prevalent in DM1 but not in DM2. Despite some similarity with narcolepsy, EDS in DM1 is not caused by Hcrt deficiency or defective splicing of Hcrt receptors.

DEMOGRAPHIC AND GENETIC RELATED VARIABLES TO PREDICT FULL SCALE WAIS-R IQ BASED ON THE RAVEN STANDARD PROGRESSIVE MATRICES ESTIMATION IN ADULT ONSET MYOTONIC DYSTROPHY TYPE 1

Jean S., Richer L., Gagnon C., Laberge L. and Mathieu J.

Université du Québec à Chicoutimi, Département des sciences de l'éducation et de psychologie
555, boul. de l'Université, Chicoutimi (Québec, Canada), G7H 2B1

Myotonic dystrophy type 1 (DM1) is the commonest form of adult muscular dystrophy characterized by a degenerative and invading muscles affection which evolves to atrophy, weakness and myotonia. This condition is an important feature in the evaluation of global intelligence. Classics intelligence measures, as obtained by the Wechsler Adult Intelligence Scale-R (WAIS-R), are largely dependent on the integrity of the muscular system. In counterparts, the Raven Standard Progressive Matrices (SPM) is a widely used intelligence test designed to measure nonverbal reasoning ability. A study report a significant correlation between SPM total score and WAIS-R Full Scale IQ (FSIQ) and conclude that SPM can be used as an estimate of WAIS-R FSIQ. Despite that SPM assess general intelligence without involving the muscular component, it could be valuable for clinicians to readily and reliably evaluate global intellectual level based on data found in DM1 patient medical file. 200 DM1 adults (79 men, 121 women, 20-80 years old) completed the SPM to 1) estimate WAIS-R FSIQ and 2) assess demographic (gender, age, education) and genetic ([CTG]n, muscular impairment, age of onset, parental transmission) related variables as a valuable predictors of WAIS-R FSIQ. A hierarchical multiple regression analysis showed that, when demographic variables are first entered in the equation, they accounted for a 62% of the WAIS-R FSIQ variance ($p<.001$). The addition of genetic variables accounted for an additional 4% ($p<.01$). All variables together explain 66% of the variance ($p<.001$). Multiple regression indicate that the use of commonly available data are highly valuable and accurate to assess global intelligence level and could be used as a screening method toward a comprehensive cognitive assessment.

READING AND SPELLING IMPAIRMENTS IN CHILDREN AND ADOLESCENTS WITH INFANTILE MYOTONIC DYSTROPHY

Cohen D.[1], Plaza M.[2], Gazzano O.[1], Eymard B.[3], Angeard N.[3] and Heron D.[4]

1) CHU Pitié-Salpétrière, Département de Psychiatrie de l'Enfant et de l'Adolescent, Centre Référent Langage, Paris, France
2) CNRS, Cognition Developpement, Paris, France
3) CHU Pitié-Salpétrière, Institut de Myologie, Paris, France
4) CHU Pitié-Salpétrière, Consultation de Génétique, Paris, France

This study investigated reading and spelling difficulties in subjects with the childhood form of myotonic dystrophy (DM1). Twenty three consecutive patients with childhood DM who were referred to a special clinic were assessed for reading and spelling skills (phonological processing, word identification, narrative comprehension (two tasks), information seeking in a document (TV schedule), and spelling). Reading impairments were frequent (63 to 84% of the subjects being below the level of literacy depending on the tasks), even in subjects without mental retardation (22 to 66%). All but two subjects had spelling difficulties. The severity of these learning difficulties was correlated with longer mutation size and maternal transmission, but could not be related to phonological deficit. The findings suggest that reading and spelling impairments appear to be typical of the phenotype of the childhood form of DM1 even in children with normal IQ. Children and adolescents with the childhood DM1 should systematically be assessed for reading and spelling problems and eventually treated.

Corresponding author : D. COHEN, Département de Psychiatrie de l'Enfant et de l'Adolescent, Centre Référent Langage, Hôpital Pitié-Salpétrière, 47-83, Boulevard de l'Hôpital, 75651, Paris Cedex 13, France.

Email : david.cohen@psl.ap-hop-paris.fr
Tel : 33 1 42162332 ; Fax : 33 1 42162331.

COGNITIVE PROFILE IN CHILDHOOD DM1

Angeard, N.[1], Gargiulo, M.[1], Jacquette, A.[2], Eymard, B.[1] and Heron, D.[2]

1) Institut de Myologie, Groupe Hospitalier Pitié-Salpêtrière, Paris, France
2) Département de Génétique, Groupe Hospitalier Pitié-Salpêtrière, Paris, France

Myotonic dystrophy type 1 (DM1) is an autosomal-dominant neuromuscular disease with an incidence of 1 in 8000 individuals. Four clinical forms have been identified : the late onset form, the classical form of adult, the congenital form and recently, the childhood form. Several studies have analyzed genotype-phenotype correlations with specific regard to cognitive features in adult classical form but evaluation of cognition in the childhood form has been scarcely investigated. So, we carried out a neuropsychological study on 36 patients aged from 6 to 18 years, with exclusively childhood's phenotype. Results are discussed with FIQ (Full Intelligence Quotient), VIQ (Verbal IQ) and PIQ (Non-verbal IQ) measures in terms of global versus selective cognitive impairment depending on the transmitting parent's sex and the (CTG)n repeat size. Results highlight the occurrence of 2 distinct patterns of cognitive dysfunction : (1) patients with maternal inheritance exhibited an impairment in all measures of general, verbal or non-verbal intelligence which were significantly correlated with the (CTG)n repeat size. (2) children with paternal inheritance and smallest expansion showed subnormal intelligence scores but presented selective impairment on subtests evaluating visuo-constuctive abilities and verbal working memory.

QUESTIONNAIRE STUDY

Schwartzbach I. and Rahbek J.E.S.

The Neuromuscular Disease Rehabilitation Center, Denmark

In November-December, 2004, a questionnaire survey was conducted among patients diagnosed with Dystrophia Myotonica (DM) and registered at the Neuromuscular Disease Rehabilitation Center. The center is in contact with 173 patients, which corresponds to 64% of the predictable number of DM sufferers among the Danish population (1/20,000).

The purpose of this investigation was to generate a comprehensive perception of this patient group, particularly with respect to physical capabilities, treatment and social status.

A questionnaire (questionnaire 1) was sent to all 173 patients at their home addresses; response percentage was 81%. DM patients residing in Aarhus County also received an additional questionnaire (questionnaire 2); response percentage for this questionnaire was 97%.

Responses to questionnaire 2 in particular indicate that 50% of the patients have experienced difficulty in learning to read, which corresponds to the fact that 43% of them received help in answering the questions.

Among the responses to questionnaire 1, these findings are noteworthy : 57% of DM patients over the age of 18 subsist on public benefits; 44% have been unable to complete an education beyond the compulsory nine years schooling; 42% report daily pain and 80% report fatigue-related problems; of these, 58% report fatigue on a daily basis. The questionnaire's query concerning fatigue, together with the validity of responses to the questionnaire, are investigated more closely in a consecutive study.

RESOURCES OF THE NIH REGISTRY OF MYOTONIC DYSTROPHY (DM) AND FSHD PATIENTS AND FAMILY MEMBERS

Martens W., Donlin-Smith C., Hilbert J., Moxley R. III and Registry scientific Committee

URMC, 601 Elmwood Ave, Box 673, Rochester, NY 14642, USA

No curative treatments exist for DM and only limited therapy exists for its medical complications. Databases for DM are needed to establish a more comprehensive resource of data and to facilitate research.

METHODS : The Registry contains disease-specific, psychosocial, and demographic data derived from stringently reviewed medical records and patient information forms. We summarized the Registry's enrollment data and indicated possible research opportunities.

RESULTS : We have enrolled 393 DM1, 56 DM2, and 429 FSHD patients and 109 unaffected family members. In total, the average age of enrollment is 48 y ± 15, 55% are female, and 47% have confirmed genetic testing. Annual data has been collected for 3.5 y. The RSC approved 9 research proposals to assist researchers analyze anonymous data and recruit patients.

CONCLUSIONS : Researchers have used Registry resources to study excessive sleepiness, quality of life, and wellness. Several questions remain such as: What causes excessive sleepiness? Does the genetic instability of DM increase the prevalence of cancer in patients? Are the burdens in DM similar to FSHD and other diseases? To answer these and other questions, collaboration research is underway but greater involvement from patients and investigators is vital. We encourage patients to participate actively in research supported by the Registry and assist recruitment. We also encourage investigators to recruit patients and to develop innovative approaches to use and enhance the resources of the Registry.

QUEBEC DATABASE FOR MANAGING MYOTONIC DISTROPHY DISEASE

Charland L., Lavoie J. and Puymirat J.

Centre de recherche du CHUL (CHUQ), 2705, boulevard Laurier, Ste-Foy, Qc, Canada, G1V4G2

Use of an Oracle database (DB) for managing Steinert disease data is an impressive tool for physicians and researchers. Oracle DB includes powerful tools for data mining and for web application development. For security, all communications are secured. Clinics around province can communicate with database but their nominative information are stored in a separate directory not accessible by others clinics for data confidentiality.

All patient data including familial, clinical, and genetic data, as well as molecular data can be stored on the DB. Furthermore, it's possible to automatically generate pedigree, to perform statistical analysis or to geographically localize mutations or diseases on a map of province of Quebec. The data are inserted in database via e-forms filled with a TabletPC. This DB is also used for the management of the available human biological material in the different centers of the province of Quebec.

This DB is an unique platform for the study of Steinert disease and could be also used for the management of several other genetic disorders.

This work was supported by AFM and RMGA and done in collaboration with Canada Research Chair in Territorial Decision Making.

HYPERINSULINEMIA OF MYOTONIC DYSTROPHY TYPE 1 (DM1) MIGHT BE INHIBITED USING VOGLIBOSE

Kinoshita, M.[1,2], Isoda, K.[2], Ohnuki, M.[1], Tochigi, S.[3] andHirose, K.[4]

1) Department of Neurology, Saitama Medical Center, Saitama Medical School, Japan
2) Internal Medicine, Saitama Yorii Hospital, Japan
3) Tokyo Metropolitan University, Japan
4) Internal Medicine, Ueno Hospital, Japan

Hyperinsulinemia (HI) or diabetes mellitus (DM) has been occasionally shown in DM1. I hit on the idea that HI of the disease might be inhibited using voglibose (vogli), which has the inhibitory actions on intestinal α-glucosidase activity and postprandial hyperglycemia.

11 DM1 patients with HI were chosen. After being fasted for 15 hours, all patients had 500 kcal carbohydrate rich breakfasts. Shortly before and at 30, 60, 90, 120, and 180 min after the breakfast, blood samples were taken to measure plasma glucose (PG) and insulin (IRI) levels (vogli minus group). After being fasted similarly in another day, in these patients one tablet of vogli was administered shortly before the same breakfast. Similarly, blood samples were done (vogli plus group). Southern blot analysis was done. Abnormal expanded allele sizes were shown in all patients. Both each level and total sum of PG were not significant between the two groups. IRI30 level in vogli plus group was significantly lower than that in vogli minus group, whereas another IRI levels were not significant between the two groups. Total sum of IRI in vogli plus group was significantly lower than that in vogli minus group. The values and percent of the maximum reductions in IRI levels were 112.7±71.7 μ U/ml and 60.6±13.3 %.

HI of DM1 might be inhibited using vogli and administration of that agent might prevent these patients with HI from DM.

ELECTROCARDIOGRAPHIC ABNORMALITIES IN « AQUITAINE » FRENCH POPULATION WITH MYOTONIC DYSTROPHY TYPE 1

Puymirat Etienne[1], Ferrer Xavier[2] and Lemetayer Philippe[1]

1) Service de Cardiologie, Hôpital Saint-André, Bordeaux, France
2) Service de Neurologie, CHU Haut-Lévèque, Pessac, France

Cardiac involvement in myotonic dystrophy type 1 (DM1) is well documented. The incidence of cardiac abnormalities in DM1 French population is not well known. Seventy-two (72) adult DM1 patients are followed at the neuromuscular clinic of the CHU Bordeaux. The age at diagnosis was 51 ± 15 (25-73) and the CTG repeat length was 645 ± 362 (130-11230). Electrocardiograms were abnormal in 14 (19,4%) patients. Cardiac conduction and arrhythmia defects were the most common cardiac abnormalities and were observed in 10 (13,9%) and 9 (12,5%) patients, respectively. The commonest abnormalities were prolonged PR interval and atrial fibrillation. Other changes were less common. Pacemakers and defibrillator were implanted in 4 (5,55%) and 3 (3,2%) patients, respectively. The indications of pacemakers were atrioventricular block type 2 or 3 and for one patient the presence of Adams-Stoke attacks. The indication of defibrillator was the existence of ventricular arrhythmias. There was no correlation between the CTG repeat length and the severity of cardiac abnormalities. Finally, cardiac manifestations were not restricted to specific families suggesting the absence of familial factor in the appearance of cardiac defects.

CONTACT INFORMATION

AIT-AHMED, OUNISSA (42)
Institut de génétique Humaine-CNRS
141 rue de la cardonille
Montpellier
France, 34396 cedex 5
Tel: 33 499619911
Fax: 33 499619901
E-mail: Ounissa.Ait-Ahmed@igh.cnrs.fr

AKHMADEEVA, LEILA (61)
Bashkir State Medical University
3 Lenina St.
Ufa (Bashkortostan)
Russia, 450077
Tel: +7-3472-739579
Fax: +7-3472-723751
E-mail: leila_ufa@mail.ru

ANGEARD, NATHALIE (P18)
Hôpital Pitié-Salpétrière, Institut de Myologie
47-83, Boulevard de l'Hôpital
Paris (Paris)
France, 75613
Tel: 33 1 42169656
Fax: 33 1 42163793
E-mail: nathalie.angeard@paris5.sorbonne.fr

ANTONINI, GIOVANNI (54)
Faculty of Medicine "La Sapienza" University
Via di Grottarossa 1035-1039
Rome
Italy, 00189
Tel: +390680345525
Fax: +390680345001
E-mail: Giovanni.Antonini@uniroma1.it

ASHIZAWA, TETSUO
Univ of Texas - Galveston
301 University Boulevard
John Sealy Annex 9.128
Galveston (Texas)
USA 77555-0539
Tel: 4097722466
Fax: 4097722390
E-mail: teashiza@utmb.edu

BASSEZ, GUILLAUME (7)
Service d'Histologie - Hôpital Henri Mondor
51 avenue de Lattre de Tassigny
Creteil
France, 94010 Cedex
Tel: 33 1 49 81 27 32
Fax: 33 1 49 81 27 33
E-mail: bassez@creteil.inserm.fr

BÉGIN, PAUL (49)
CSSS Chicoutimi
155 chemin de la Forêt Noire
Chicoutimi (Québec)
Canada, G7G 5G6
Tel: (418) 690-2892
Fax: (418) 549-6965
E-mail: pbegin@saglac.qc.ca

BOËRIO, DELPHINE (P11)
Institut de Myologie - Hôpital Pitié-Salpêtrière
47-83 boulevard de l'hôpital
Paris
France, 75651 Cedex 13
Tel: 33 1 42 16 58 80
Fax: 33 1 42 16 58 81
E-mail: d.boerio@myologie.chups.jussieu.fr

BONIFAZI, EMANUELA (9)
Tor Vergata University
Via Montpellier, 1
Rome (RM)
Italy, 00133
Tel: +39 06 72596078
Fax: +39 06 20427313
E-mail: emanuelabonifazi@yahoo.it

BOUCHARD, JEAN PIERRE
Département des Sciences Neurologiques
Hôpital Enfant-Jésus
Quebec, Canada
Tel : 418-649 5756
Fax 418-649 5915
Mail sylvie.bouchard2@cha.quebec.qc.ca

BOURLIER, CLAUDE
French myotonic Dystrophy support group
AFM, 1 rue de l'international
Evry, 91000 France
cbourlier@afm.genethon.fr

BOWLER, MARGARET
Myotonic Dystrophy support group
Nottingham, UK
Tel 0115 987 0080
Mail mdsg@tesco.net

BROOK, J DAVID
University of Nottingham
Institute of Genetics
Queen's Medical Centre
Nottingham
United Kingdom NG7 2UH
Tel: 00-44-(0)115-8493217
Fax: 00-44-(0)115-9709906
E-mail: david.brook@nottingham.ac.uk

CAILLET-BOUDIN, MARIE-LAURE (32)
INSERM U422
PL Verdun
Lille (Nord)
France, 59045
Tel: 00 33 320 62 20 73
Fax: 00 33 320 62 20 79
E-mail: caillet@lille.inserm.fr

CAMPBELL, CRAIG (52, P12)
Children's Hospital of Western Ontario
800 Commissioners Rd. E
London (ON)
Canada, N6A 2E3
Tel: 519.685.8332
Fax: 519.685.8350
E-mail: craig.campbell@lhsc.on.ca

CHARLAND, LUC (P21)
Unit of Human Genetics
CHU Laval, 2705 Blvd Laurier
RC-9300
Ste-Foy (Quebec)
Canada, G1V4G2
Tel: 418-654-2186
Fax: 418-654-2207
E-mail: luc.charland@crchul.ulaval.ca

CHARLET, NICOLAS
IGBMC, INSERM U596
1 Rue Laurent Fries
Illkirch (France)
France 67400
Tel: 0033 3 88 65 34 51
Fax: 0033 3 88 65 32 01
E-mail: ncharlet@titus.u-strasbg.fr

CHIVERS, WYN
Muscular Dystrophy Canada
2345 Yonge Street, Suite 900
Toronto (Ontario)
Canada M4P 2E5
Tel: (416) 488-0030 #130
Fax: (416) 488-7523
E-mail: wyn.chivers@muscle.ca

CHOUINARD, MAUD-CHRISTINE
Université du Québec à Chicoutimi
Département des sciences humaines
555, boul. de l'Université
Chicoutimi (Québec)
Canada G7H 2B1
Tel: (418) 545-5011 #5344
Fax: (418) 545-5012
E-mail: maud-christine_chouinard@uqac.ca

CHRESTIAN, NICOLAS
Université Laval, Médecine
2430 narcisse pérodeau
Québec (Québec)
Canada G1T 2J5
Tel: 418 658 2184
Fax: 418 658 2184
E-mail: chrestian_nicolas@hotmail.com

CIAFALONI, EMMA (P15)
University of Rochester
601 Elmwood Avenue.
BOX 673
Rochester (NY)
USA, 14642
Tel: 585-275-2559
Fax: 585-273-1254
E-mail: emma_ciafaloni@urmc.rochester.edu

CLEARY, JOHN (4)
The Hospital for Sick Children
101 College Street
15-312 TMDT, East Tower
Toronto (Ontario)
Canada, M5G 1L7
Tel: 416-813-8170
Fax: 416-813-7170
E-mail: john.cleary@utoronto.ca

COBO, ANA MARIA (11)
Genetics Unit, Hospital Donostia
Paseo Dr Begiristain S/N
San Sebastian (Guipozcoa)
Spain, 20014
Tel: +34 943 007301
Fax: +34 943 007112
E-mail: anacobo@chdo.osakidetza.net

COHEN, DAVID (P17)
CHU Pitié-Salpétrière, Psychiatrie de l'enfant et de l'Adolescent, Centre Référent Langage
Groupe Hospitalier Pitié-Salpétrière, 47-83, Boulevard de l'Hôpital
Paris
France, 75651
Tel: 33 1 42162332
Fax: 33 1 42162331
E-mail: david.cohen@psl.ap-hop-paris.fr

COOPER, TOM
Baylor College of Medicine
Pathology
One Baylor Plaza
Houston (TX)
USA 77030
Tel: 713-798-3141
Fax: 713-798-5838
E-mail: tcooper@bcm.edu

DAY, JOHN (53)
Univeristy of Minnesota
Institute of Human Genetics, MMC 206
420 Delaware St. SE
MInneapolis (MN)
USA, 55455
Tel: 612-625-6180
Fax: 612-625-8488
E-mail: johnday@umn.edu

DE TEMMERMAN, NELE (2)
Vrije Universiteit Brussel - Centrum Reproductive Medicine
Laarbeeklaan 101
Brussels (Brussel)
Belgium, 1090
Tel: +3224776673
Fax: +3224776692
E-mail: ndtemmer@az.vub.ac.be

DEVILLERS, MARTINE
Direction Recherche & Thérapeutiques
AFM
1 rue de l'International BP59
Evry 9100, France
Tel : 01-69-13 22 24
Fax : 01 69 13 22 22
mdevillers@afm.genethon.fr

DONACHIE, JACQUELINE
2 Clayton Terrace
Glasgow
G31 2JA
Scotland
Tel : 01 41 550 3903
jacquelinedonachie@hotmail.com

DOWNING, CLAUDIA (65)
Centre for Family Research, University of Cambridge
Free School Lane
Cambridge (Cambs)
England, CB2 3RF
Tel: 44-1223-334510
Fax: 44-1223-330574
E-mail: cd10008@cam.ac.uk

DUBOC, DENIS (57)
Department of Cardiology, Cochin Hospital
27 rue du Fg St-Jacques
Paris
France, 75014
Tel: +33(0)1 58 41 16 52
Fax: + 33 (0)1 58 41 16 05
E-mail: denis.duboc@cch.ap-hop-paris.fr

EYMARD, BRUNO (47)
Institut de Myologie - Hôpital Pitié-Salpêtrière
47-83 boulevard de l'hôpital
Paris
France, 75651 Cedex 13
Tel: 33 1 42 16 37 13
Fax: 33 1 42 16 37 93
E-mail: anne-marie.maronne@psl.ap-hop-paris.fr

FURLING, DENIS (17)
CNRS
Faculté de Médecine UPMC
105 bld de l'hopital
Paris
France, 75013
Tel: +33 1 40 77 96 91
Fax: +33 1 53 60 08 02
E-mail: furling@ext.jussieu.fr

FURUTAMA, DAISUKE (19)
Osaka Medical College
2-7 Daigaku-cho
Takatsuki (Osaka)
Japan, 569-8686
Tel: +81-72-683-1221
Fax: +81-72-683-1801
E-mail: in1003@poh.osaka-med.ac.jp

FURUYA, HIROKAZU (59)
Department of Neurology, Neuro-Muscular Center
National Omuta Hospital
Tachibana 1044-1
Omuta (Fukuoka)
Japan, 837-0911
Tel: +81-944-58-1122
Fax: +81-944-58-6804
E-mail: furuya@oomuta.hosp.go.jp;
furuya@neuro.med.kyushu-u.ac.jp

GAGNON, CYNTHIA (64)
Carrefour de santé de Jonquière
2230 rue de l'Hôpital cp 1200
Jonquière (Quebec)
Canada, G7X 7X2
Tel: 418-695-7700 2756
Fax: 418-695-7758
E-mail: cynthia.gagnon.1@ulaval.ca

GOMES-PEREIRA, MÁRIO
Inserm U383, Hôpital Necker Enfants Malades
149 rue de Sèvres
Paris (Ile de France)
France 75015
Tel: + 33 1 44 49 45 23
Fax: + 33 1 47 83 32 06
E-mail: pereira@necker.fr

GOURDON, GENEVIEVE
insermU383, Hopital Necker-Enfants Malades
149 rue de Sèvres
Paris (ile de france)
France 75015
Tel: +33 144494523
Fax: +33 147833206
E-mail: gourdon@necker.fr

GROH, WILLIAM (48)
Indiana University
1800 N. Capitol Avenue, Room E406
Indianapolis (IN)
USA, 46202
Tel: (317) 962-0102
Fax: (317) 962-0100
E-mail: wgroh@iupui.edu

GUIRAUD-DOGAN, CÉLINE (22)
Inserm U383, Hôpital Necker Enfants Malades
149 rue de Sèvres
Paris
France, 75015
Tel: + 33 1 44 49 45 23
Fax: + + 33 1 47 83 32 06
E-mail: dogan@necker.fr

HAMEL, FRÉDÉRIC (P4)
Human Genetics research center, CHUL
2705 Blvd. Laurier
RC 9300
Ste-Foy (Québec)
Canada, G1V 4G2
Tel: (418) 656-4141*47758
Fax: (418) 654-2207
E-mail: frederic.hamel@crchul.ulaval.ca

HARPER, PETER (66)
Institute of Medical Genetics
Cardiff University
Heath Park
Cardiff (Wales)
UK, CF14 4XN
Tel: 0044 29 20744057
Fax: 0044 29 20747603
E-mail: HarperPS@cf.ac.uk

HEATWOLE, CHAD (13)
The University of Rochester
174 Edgemont Road
Rochester (NY)
USA, 14620
Tel: 585-442-1942
Fax: 585-756-5189
E-mail: Chad_Heatwole@urmc.rochester.edu

HERON, DELPHINE (51, 55, P10)
HOPITAL DE LA SALPETRIERE
47-83 boulevard de l'hôpital
Paris
France, 75013
Tel: 33142163798
Fax: 33142163759
E-mail: delphine.heron@psl.aphp.fr

HIBA, BASSEM (P7)
Plateforme Animage
bât. CERMEP - Hopital Neuro-Cardiologique
59 Boulevard Pinel
Lyon (Phone)
France, 69677
Tel: 00 (33) 4 72 68 86 32
Fax: 00 (33) 4 72 68 86 10
E-mail: bassem.hiba@cermep.fr

HILBERT, JAMES (62)
University of Rochester
601 Elmwood Avenue
Box 673
Rochester (NY)
USA, 14642
Tel: 585-273-5590
Fax: 585-273-1255
E-mail: james_hilbert@urmc.rochester.edu

HILTON-JONES, DAVID (46)
Radcliffe Infirmary, Oxford, UK
Woodstock Road
Oxford (Oxon)
UK, OX2 6HE
Tel: 00-44-1865-224891
Fax: 00-44-790493
E-mail: david.hilton-jones@clneuro.ox.ac.uk

HOLT, IAN (P9)
RJAH Orthopaedic Hospital
CIND, LMARC Building,
Oswestry (Shropshire)
UK, SY10 7AG
Tel: 44-1691-404295
Fax: 44-1691-404170
E-mail: ian.holt@rjah.nhs.uk

IKEZOE, KOJI (24)
Department of Neurology, National Omuta Hospital
1044-1 Tachibana
Omuta (Fukuoka)
Japan, 837-0911
Tel: +81-944581122
Fax: +81-944586804
E-mail: ikezoe@oomuta.hosp.go.jp

ISHIURA, SHOICHI
The University of Tokyo, Dept. of Life Sci., Grad.Sch. of Arts & Sci.
3-8-1 Komaba, Meguro-ku
Tokyo (Tokyo)
Japan 153-8902
Tel: +81-3-5454-6739
Fax: +81-3-5454-6739
E-mail: cishiura@mail.ecc.u-tokyo.ac.jp

JANIER, MARC
Animage
Batiment CERMEP
59 Bd Pinel
Lyon (RA)
France 69003
Tel: +334 72 68 86 32
Fax: +334 72 68 86 10
E-mail: janier@univ-lyon1.fr

JEAN, STÉPHANE (P16)
Université du Québec à Chicoutimi
555, boul. de l'Université
Chicoutimi (Québec)
Canada, G7H 2B1
Tel: 418-545-5011 ext. 5418
Fax: 418-545-5411
E-mail: stephane_jean@uqac.ca

KIMURA, TAKASHI (33)
JCSMR Australian National University
GPO Box 334
Canberra (ACT)
Australia, 2601
Tel: +61-2-6125-8391
Fax: +61-2-6125-4761
E-mail: takashi.kimura@anu.edu.au

KINO, YOSHIHIRO (43)
RIKEN Brain Science Institute, Laboratory for Structural Neuropathology
2-1 Hirosawa,
Wako-shi (Saitama)
Japan, 351-0198
Tel: +81-48-467-9702
Fax: +81-48-462-4796
E-mail: kino@brain.riken.jp

KINOSHITA, MASANOBU (P22)
Department of Neurology, Saitama Medical Center, Saitama Medical School
1981, Tsujido, Kamoda, Kawagoe, Saitama 350-8550, Japan
Kawagoe (Saitama)
Japan, 350-8550
Tel: 81-49-228-3604
Fax: 81-49-226-8451
E-mail: mkino@saitama-med.ac.jp

KRAHE, RALF (8)
University of Texas M. D. Anderson Cancer Center
Section of Cancer Genetics
Department of Molecular Genetics
Houston (TX)
USA, 77030
Tel: (713) 834-6345
Fax: (713) 834-6319
E-mail: RKrahe@mdanderson.org

KRZYZOSIAK, WLODZIMIERZ (36)
InstitInstitute of Bioogranic Chemistry Polish Academy of Sciences
Noskowskiego 12/14
Poznan ()
Poland, 61-704
Tel: +48 61 852 85 03 ext. 136
Fax: +48 61 852 05 32
E-mail: wlodkrzy@ibch.poznan.pl

KUYUMCU-MARTINEZ, MUGE (29)
Baylor College of Medicine
One Baylor Plaza
Houston (TX)
USA, 77030
Tel: 713 798 5021
Fax: 713 798 5838
E-mail: muge@bcm.edu

LABERGE, LUC (56, P13, P14)
Groupe ÉCOBES
Cégep de Jonquière
3791, rue de la Fabrique
Jonquière (Québec)
Canada, G7X 3W1
Tel: 418-547-2191, ext. 489
Fax: 418-542-2853
E-mail: laberluc@saglac.qc.ca

LAFRAMBOISE, RACHEL
CHUL-CHUQ,CME et Universite Laval
Genetique medicale,PorteH-1336,CHUL
2705 Boul.Laurier
Ste-Foy (Quebec)
Canadn G1V 4G2
Tel: 418-656-4141ext.47130
Fax: 418-654-2780
E-mail: rachel.laframboise@crchul.ulaval.ca

LE MEE, GWENN
Institut de génétique Humaine-CNRS
141 rue de la cardonille
Montpellier (France)
France 34396 cedex 5
Tel: 33 499619911
Fax: 33 499619901
E-mail: glemee@igh.cnrs.fr

LIN, XIAOYAN (39)
University of Rochester
Box 603 URMC
601 Elmwood Ave
Rochester (New York)
USA, 14642
Tel: 585-275-4184
Fax: 585-273-1255
E-mail: xiaoyan_lin@urmc.rochester.edu

LOGIGIAN, ERIC (50)
University of Rochester Medical Center
601 Elmwood Avenue
Rochester (NY)
USA, 14642
Tel: 585-275-4568
Fax: 585-273-1254
E-mail: eric_logigian@urmc.rochester.edu

LOGUE, JEREMY (35)
University of Oregon
1229 University of Oregon
Eugene (OR)
USA, 97403-1229
Tel: +1 541-346-1576
Fax: +1 541-346-5891
E-mail: loguejeremy@yahoo.com

LOWE, MIRIAM
Indiana University
Krannert Institute of Cardiology
1800 N. Capitol
Indianapolis (IN)
USA 46202
Tel: 317.962.0080
Fax: 317.962.0068
E-mail: milowe@iupui.edu

LORD, SHANNON
Hunter Lord Funds
801 Mt Paran Road, NW
Atlanta, Georgia
USA 30327
Tel : 404-252 5451
Fax : 404-252 8356
smlord@mindspring.com

MACHUCA-TZILI, LAURA E (44)
University of Nottingham
Institute of Genetics
Queen's Medical Centre
Nottingham (Nottinghamshire)
United Kingdom, NG7 2RD
Tel: 44(0)115-924-9924 ext43919
Fax: Fax 44-(0)115-970-9906
E-mail: plxlem@nottingham.ac.uk

MAHADEVAN, MANI S. (14)
University of Virginia
415 Lane Rd., MR5 Building, RM. 3330
PO BOX 800904
Charlottesville (VA)
USA, 22936
Tel: 434-243-4816
Fax: 434-924-1545
E-mail: mahadevan@virginia.edu

MARGOLIS, JAMIE
University of Minnesota
Institute of Human Genetics MMC 206 Mayo 8206
420 Delaware
Minneapolis (MN)
USA 55455
Tel: 612-626-3521
Fax: 612-625-8488
E-mail: mali0071@umn.edu

MARTENS, WILLIAM (P20)
University of Rochester
601 Elmwood Avenue
Box 673
Rochester (NY)
USA, 14642
Tel: 585-275-2483
Fax: 585-273-1255
E-mail: bill_martens@urmc.rochester.edu

MARTORELL, LORETO (5)
Genètica Molecular, Hospital Sant Joan de Déu
Hospital Sant Joan de Déu, Edifici Docent; C/Santa Rosa 39
Barcelona
Spain, 08950
Tel: 34 93 600 9759
Fax: 34 93 600 9760
E-mail: lmartorell@hsjdbcn.org

MASANOBU, KINOSHITA
Department of Neurology Saitama Medical Center, Saitama Medical School
1981, Tsujido, Kamoda, Kawagoe, Saitama 350-8550, Japan
Kawagoe (Saitama)
Japan 350-8550
Tel: 81-49-228-3604
Fax: 81-49-226-8451
E-mail: mkino@saitama-med.ac.jp

MEOLA, GIOVANNI (23)
Universita degli Studi di Milano - Policlinico San Donato
Via Morandi, 30
San Donato Milanese (Milano)
Italy, 20097
Tel: 39-02-52774480
Fax: 39-02-5274717
E-mail: giovanni.meola@unimi.it

MITTAL, SALONI (P6)
University of Nottingham
C14, Institute of Genetics
Quennsmedical Centre
Nottingham (England)
U.K, NG7 2UH
Tel: 0115924ext-43919
Fax: 01159709906
E-mail: plxsm3@nottingham.ac.uk

MONCKTON, DARREN (3)
University of Glasgow
Institute of Biomedical and Life Sciences
Anderson College Bilding, 56 Dumbarton Road
Glasgow (Strathclyde)
UK, G11 6NU
Tel: +44 141 330 6213
Fax: +44 141 330 4878
E-mail: d.monckton@bio.gla.ac.uk

MORALES, FERNANDO (12)
Instutie of Biomedical and Life Sciences, University of Glasgow
IBLS, University of Glasgow, Anderson College complex
56 Dumbarton Road
Glasgow (Glasgow)
United Kingdom, G11 6NU
Tel: +44 141 330 6220
Fax: + 44 141 330 4878
E-mail: 0303227m@student.gla.ac.uk

MOXLEY, RICHARD (58)
University of Rochester Medical Center
601 Elmwood Avenue
Box 673
Rochester (NY)
US, 14642
Tel: 585-275-5006
Fax: 585-273-1255
E-mail: karen_berk@urmc.rochester.edu

NAKAMORI, MASAYUKI (31)
Department of neurology, Osaka university
D-4, 2-2, Yamadaoka
Suita (Osaka)
Japan, 565-0871
Tel: 81-6-6879-3571
Fax: 81-6-6879-3579
E-mail: mnakamor@neurol.med.osaka-u.ac.jp

NOVELLI, GUISEPPE
Dipartimento di Medicina di Laboratorio - Genetica Medica
Azienda Policlinico - Università di Roma "Tor Vergata" Viale Oxford 81
00133 Roma Italia
Phone +39/06/20900665 - 39/06/20900664
Fax +39/06/20900669 - 06/20427313
E.mail: novelli@med.uniroma2.it

OHSAWA, NAKAAKI
Aino Institute For Aging Research
3-9-25 Ohta
Ibaraki (Osaka)
Japan 567-0018
Tel: 81-72-621-3745
Fax: 81-72-621-3745
E-mail: ohsawa@aiar.aino.or.jp

OSBORNE, ROBERT (26)
University of Rochester
Department of Neurology, University of Rochester Medical Center
601 Elmwood Avenue Box 673
Rochester (New York)
USA, 14642
Tel: 585-273-3158
Fax: 585-273-1255
E-mail: Robert_Osborne@URMC.rochester.edu

PEARSON, CHRISTOPHER
Department of Genetics
Hospital for Sick Children
Toronto, Ont, Canada
Tel : 416-813 8256
Fax : 416-813-4931
cepearson@genet.sickkids.on.ca

PELLETIER, RICHARD
CRCHUL - Human Genetic Department
2705 Boul. Laurier
RC-9300
Quebec (QC)
Canada G1V4G2
Tel: 418-654-2186
Fax: 418-654-2207
E-mail: richard.pelletier@crchul.ulaval.ca

PUYMIRAT ETIENNE
Service de Cardiologie
Hôpital Saint-André
Bordeaux, France
e_puymirat@msn.com

PUYMIRAT, JACK (6, 60)
Unit of Human Genetics
CHU Laval, 2705 Blvd Laurier
Ste-Foy (Quebec)
Canada, G1V4G2
Tel: 418-6542186
Fax: 418-6542207
E-mail: jack.puymirat@crchul.ulaval.ca

PYTLOS, MALGORZATA (P2)
Institute of Biosciences and Technology
2121 West Holcombe Blvd
Houston (TX)
USA, 77030-3303
Tel: 713 677 7676
Fax: 713 677 7689
E-mail: mpytlos@ibt.tamhsc.edu

QUERIDO, EMMANUELLE (37)
Université de Montréal
2900 Edouard-Montpetit
Département de Biochimie, D-500
Montréal (Québec)
Canada, H3C 3J7
Tel: (514) 343-5684
Fax: (514) 343-2210
E-mail: e.querido@umontreal.ca

RANUM, LAURA (16, 34)
Univeristy of Minnesota
MMC 206
420 Delaware St. S.E.
Minneapolis (MN)
USA, 55455
Tel: 612-624-0901
Fax: 612-625-8488
E-mail: ranum001@umn.edu

REDDY, SITA (40)
University of Southern California
Room 240-Institute for Genetic Medicine,
2250 Alcazar St
Los Angeles (CA)
USA, 90033
Tel: 323-442-2457
Fax: 323-442-2764
E-mail: sitaredd@usc.edu

RHODES, JEREMY (25)
University of East Anglia
Norwich
Norwich (Norfolk)
UK, NR4 7TJ
Tel: 44 1603592252
Fax: 44 1603592250
E-mail: j.rhodes@uea.ac.uk

RICHER, LOUIS
Université du Québec à Chicoutimi
555, boul. de l'Université
Chicoutimi (Québec)
Canada G7H 2B1
Tel: 418-545-5011 ext. 5418
Fax: 418-545-5411
E-mail: Louis_Richer@uqac.ca

ROUSSEAU, PASCALE
Dystrophie musculaire Canada
1425 boul. René-Lévesque Ouest #506
Montréal (Québec)
Canada H3G 1T7
Tel: (514) 393-3522 #231
Fax: (514) 393-8113
E-mail: pascale.rousseau@muscle.ca

SARKAR, PARTHA (41)
University of Texas Medical Branch
Clay Hall - Rm. 1.134 - Department of Neurology
301 University Blvd.
Galveston (Texas)
USA, 77555-0874
Tel: (409) 747-4559
Fax: (409) 747-4562
E-mail: pssarkar@utmb.edu

SCHWARTZBACH, ISABELLE (63, P19)
The Neuromuscular Disease Rehabilitation Center
Kongsvangs Allé 23
Århus C (DK)
Denmark, 8000
Tel: +45 2265 2434
Fax: +45 8948 2238
E-mail: isch@muskelsvindfonden.dk

SWANSON, MAURICE (27)
University of Florida
Department of Molecular Genetics and Microbiology
College of Medicine, 1600 SW Archer Rd
Gainesville (FL)
USA, 32610-0266
Tel: 352.392.3082
Fax: 352.392.3133
E-mail: mswanson@ufl.edu

TAKAHASHI, MASANORI
Osaka University Graduate School of Medicine
Department of Neurology
D-4, 2-2 Yamadaoka
Suita (Osaka)
Japan 565-0871
Tel: +81-6-68793571
Fax: +81-6-68793579
E-mail: mtakahas@neurol.med.osaka-u.ac.jp

TANAKA, TOSHIFUMI
Aino Institute for Aging Research
3-9-25 ohta
Ibaraki (Osaka)
Japan 567-0018
Tel: +81-72-621-3745
Fax: +81-72-621-3745
E-mail: tanaka02@aiar.aino.or.jp

TIMCHENKO, LUBOV (28)
Baylor College of Medicine
One Baylor Plaza
Houston (TX)
US, 77030
Tel: (713) 798-6911
Fax: (713) 798-3142
E-mail: lubovt@bcm.tmc.edu

UDD, BJARNE (P3)
Department of Medical Genetics, University of Helsinki and Folkhälsan Institute of Genetics
P.O.B. 63, 00014 Helsinki, Finland
Helsinki (Fin)
Finland, 00014
Tel: +358-40-7009208
Fax: +358-6-3232888
E-mail: bjarne.udd@netikka.fi

VALLO, LAURA (30)
Tor Vergata University
Via Montpellier, 1
Rome (RM)
Italy, 00133
Tel: 39 06 72596079
Fax: 39 06 20427313
E-mail: lauravallo@yahoo.it

VEYTSMAN, BORIS (P1)
George Mason University
ITT Industries, Advanced Engineering & Sciences
761 Business Center Dr
Reston (VA)
USA, 20190
Tel: 1-703-915-2406
Fax: 1-703-438-7907
E-mail: borisv@lk.net

VICENTE, MARTA (38)
Department of Genetics, University of Valencia
Doctor Moliner, 50
Burjasot (Valencia)
Spain, 46100
Tel: 963544379
Fax: 963543029
E-mail: marta.vicente@uv.es

VIGNAUD, ALBAN (P5)
CNRS
Faculté de Medecine UPMC
105 bld de l'hopital
Paris
France, 7
Tel: +33 1 40 77 98 38
Fax: +33 1 53 60 08 02
E-mail: albanvignaud@wanadoo.fr

WANG, EDITH H. (P8)
Department of Pharmacology, University of Washington
1959 NE Pacific St
Box 357280
Seattle (WA)
USA, 98195
Tel: 206-616-5376
Fax: 206-685-3822
E-mail: ehwang@u.washington.edu

WANG, GUEY-SHIN (15)
Baylor College of Medicine
One Baylor Plaza, Dept. of Pathology, Rm 268B
Houston (TX)
USA, 77030
Tel: 7137985021
Fax: 7137985838
E-mail: Gw1@bcm.tmc.edu

WANSINK, DERICK G. (20)
Nijmegen Centre for Molecular Life Sciences
P.O. Box 9101, 6500 HB, Geert Grooteplein 26/28
Nijmegen (NL)
The Netherlands, 6525 GA
Tel: +31.24.3613664
Fax: +31.24.3615317
E-mail: r.wansink@ncmls.ru.nl

WHEELER, THURMAN (21)
University of Rochester
Department of Neurology
601 Elmwood Ave., Box 673
Rochester (NY)
U.S.A., 14642
Tel: 585-273-1456
Fax: 585-273-1255
E-mail: thurman_wheeler@urmc.rochester.edu

WHITTAKER, ROGER (45)
Department of Clinical Neurology
Radcliffe Infirmary, Woodstock Road,
Oxrord.
Oxford (Oxfordshire)
U.K., OX2 6HE
Tel: 0131 5372095
Fax: 0131 5372095
E-mail: roger.whittaker@doctors.org.uk

WIERINGA, BE (BEREND) (1)
Dept. Cell Biology, NCMLS, Radboud University Medical Centre
Geert Grooteplein 28
Nijmegen (-)
The Netherlands, 6525GA
Tel: +31-24-3614329
Fax: +31-24-3615317
E-mail: b.wieringa@ncmls.ru.nl

WOJCIECHOWSKA, MARZENA (10)
Institute of Biosciences and Technology
2121 West Holcombe Blvd.
Houston (Texas)
USA, 77030
Tel: 713-677-7656
Fax: 713-677-7689
E-mail: mwojciechowska@ibt.tamhsc.edu

XU, WEIDONG (18)
University of Texas Medical Branch
Clay Hall - Rm. 1.134 - Department of Neurology
301 University Blvd.
Galveston (TX)
USA, 77555-0874
Tel: (409) 747-4566
Fax: (409) 747-4562
E-mail: weixu@utmb.edu

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