IDMC-4

4th International Myotonic Dystrophy Consortium Meeting

Thursday 10th to Saturday 12th
April 2003
Glasgow University
Glasgow, Scotland

4th International Myotonic Dystrophy Consortium Meeting

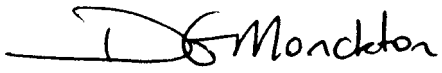
Welcome to the 4th International Myotonic Dystrophy Consortium meeting here in Glasgow. Following on from previous highly successful get-togethers in Kyoto (2001), Chapel Hill (1999) and Paris (1997), this meeting has a lot to live up to. But, from the abstracts submitted, it looks like we are in for an exciting few days learning about the latest breakthroughs in myotonic dystrophy research. Indeed, there were so many abstracts submitted, that for the first time we haven't been able to fit everybody in for an oral presentation and we will be having a poster session as well. In doing so, we have also reserved a bit more time for discussion at the end of each session and hope that this period can be used to think about how the various pieces of work fit together. All in all, this format will give us more time for thinking aloud about what we need to determine next, and discussing what lies ahead, in this exciting and fast moving field of research.

As at previous meetings though, the timetable allows for some socialising and discussion in a more relaxed atmosphere as well. This will include the opportunity to sample the Scottish countryside and one of its most sought after products, uisge beath, 'the water of life' or whisky, as you probably know it. In addition, there will be the chance to try out your moves on the dance floor at the ceilidh on Friday evening. Whilst on Saturday evening we will be the guests of the Lord Provost of Glasgow at a Civic Reception he will be hosting for us at the Kelvingrove Art Gallery and Museum.

It is with great pleasure that I also welcome, not only the scientists and clinicians who are actively researching in this area, but also the patients and families who will be attending a joint session of the meeting on Saturday afternoon, as well as the social events on Friday and Saturday evenings. This session has been made possible by the simultaneous hosting in Glasgow of the Myotonic Dystrophy Support Group's AGM. This is the first time that all the interested parties have got together at one of the IDMC meetings and we hope it will be a big success allowing us all to learn from each others experiences.

Finally, it just remains for me to say a big thank you to everyone whose hard work has made this meeting possible, to our generous sponsors and to you all for coming here and contributing to what I am sure will be another great IDMC meeting.

Slàinte mhath!



Darren Monckton
Chair, IDMC4

Cover design. The front cover incorporates elements associated with the DNA expansions at the myotonic dystrophy type 1 and type 2 loci, Glasgow University and Hunter family tartan. The Hunter family provided collections and donations that made possible the establishment of the Hunterian Museum and Art Gallery within the University of Glasgow in the late 1700s. It is a striking coincidence that some 200 years later, one of the Hunter family descendents, Shannon Lord, will be attending IDMC4 here at the University of Glasgow to describe her experiences of living with myotonic dystrophy and the establishment of the Hunter Research Fund. The shaking hands also depicted on the cover represent not only the simple test often used by clinicians to detect myotonia, but more importantly symbolises the interactions that we hope to generate between clinicians, scientists, patients and families here at IDMC4. The cover was designed and prepared by Chris Hoke and Scott Thompson with help from Shannon Lord and Darren Monckton.

IDMC4 Committees

Local Organising Committee

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Radcliffe Infirmary
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Paris

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Pharmacia Corp.
Chicago

Shannon M. Lord
Hunter Research Fund
Atlanta

Nakaaki Ohsawa
Aino institute for aging research
Ibaraki

Sponsorship

The organising committee is pleased to acknowledge the support of the following organisations in funding the meeting: GlaxoSmithKline; Pharmacia Corp; The Robertson Trust; The Genetics Society; The Myotonic Dystrophy Support Group; The University of Glasgow; Glasgow City Council; and the Muscular Dystrophy Campaign.

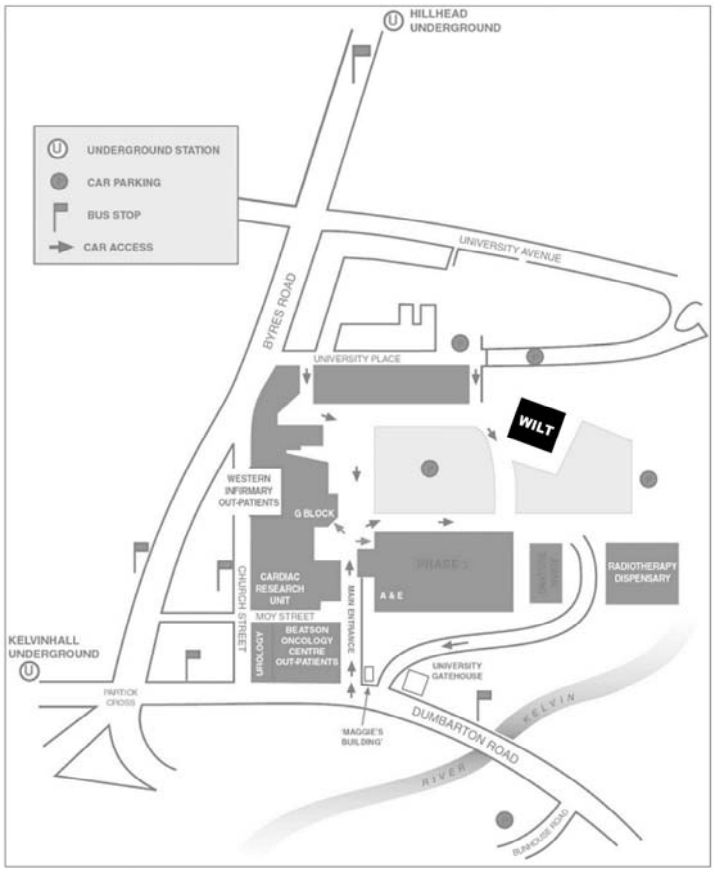
The organising committee is also pleased to acknowledge the support of the following organisations in funding travel costs: The Hunter Research Fund; the Aino Institute for Aging Research and the Association Française contre les Myopathies.

Acknowledgements

The organisers would like to thank all those who have contributed toward the planning and organisation of IDMC4 including: Jane Kelly, Claire McCulloch, Sabrina Sharma, Bronwyn Syed, Alexis Stevenson, John McAbney, Graham Hamilton and Katherine Allen from the Institute of Biomedical and Life Sciences, University of Glasgow; Claire Harper from the University of Glasgow Conference and Visitor Services; Ellen Thompson from Glasgow University Hospitality Services; Raymond Dixon and Lynne Aikman from The Print Unit of Glasgow and Strathclyde Universities; Chris Hoke, Scott Thompson and Susan Greco from Fusion Designworks; Lorna McClelland and Lorna Clarkson from the Greater Glasgow Tourist Board; Allen Roses from GlaxoSmithKline; Keith Johnson from Pharmacia Corp; Helen Sang and Jayne Richards from the Genetics Society; James the janitor for the WILT; The City of Glasgow's Lord & Lady Provost; Prestige Tours; Alison Adams from Encore Catering; Neil Drover; RocknReel ceilidh disco band; Harpbeat; Jenny Versnel from The Muscular Dystrophy Campaign; The Grosvenor Hotel; Maragret Bowler from the Myotonic Dystrophy Support Group; Anne Theriault and Betty O'Hare from Yorkhill Hospital. Our sincerest apologies if we have missed anybody out.

Registration

Registration will be in the foyer of the Western Infirmary Lecture Theatre (WILT) from 8.00 am on Thursday 10th April. Tea, coffee and biscuits will be available. Please arrive in good time so that we may start the meeting promptly at 9.00 am.



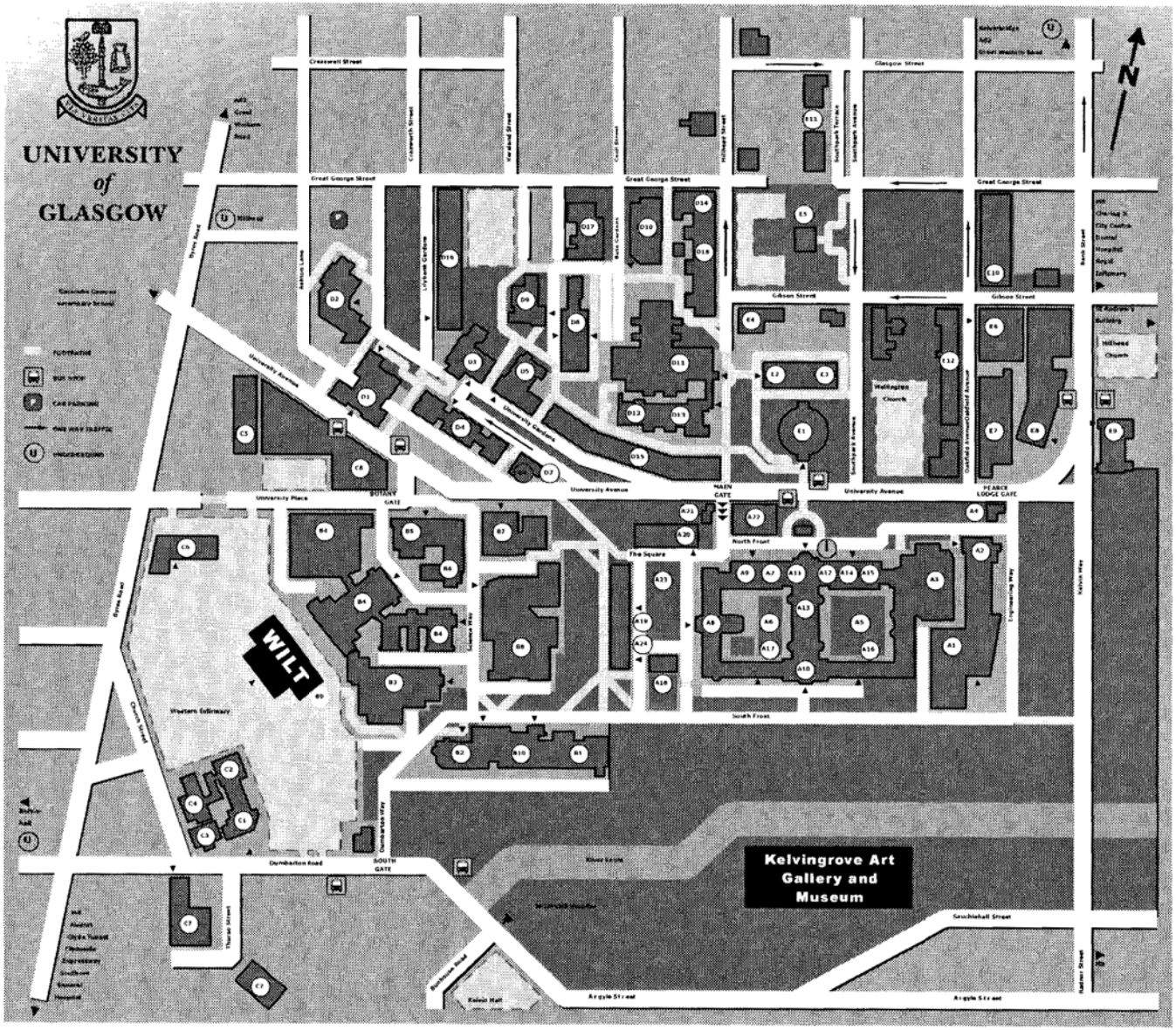
How to get to the Western Infirmary Lecture Theatre

The Western Infirmary Lecture Theatre is located in the rear car cark of the Western Infirmary Hospital. Access is from Dumbarton Road (see maps on following pages).

By foot. From Dumbarton Road proceed up the hill around the west side of the main hospital building toward the rear. The WILT is located in the north east corner of the rear car park.

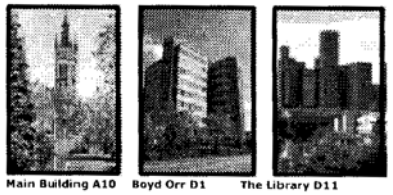
By taxi. Ask the taxi driver to take you to the rear car park of the Western Infirmary.

By car. Limited car parking for disabled visitors only is available on the Western Infirmary site. There is no parking within the University grounds. On street car parking is available in the surrounding area, but the area is very busy and only limited spaces will be available. You are advised to use public transport or walk.



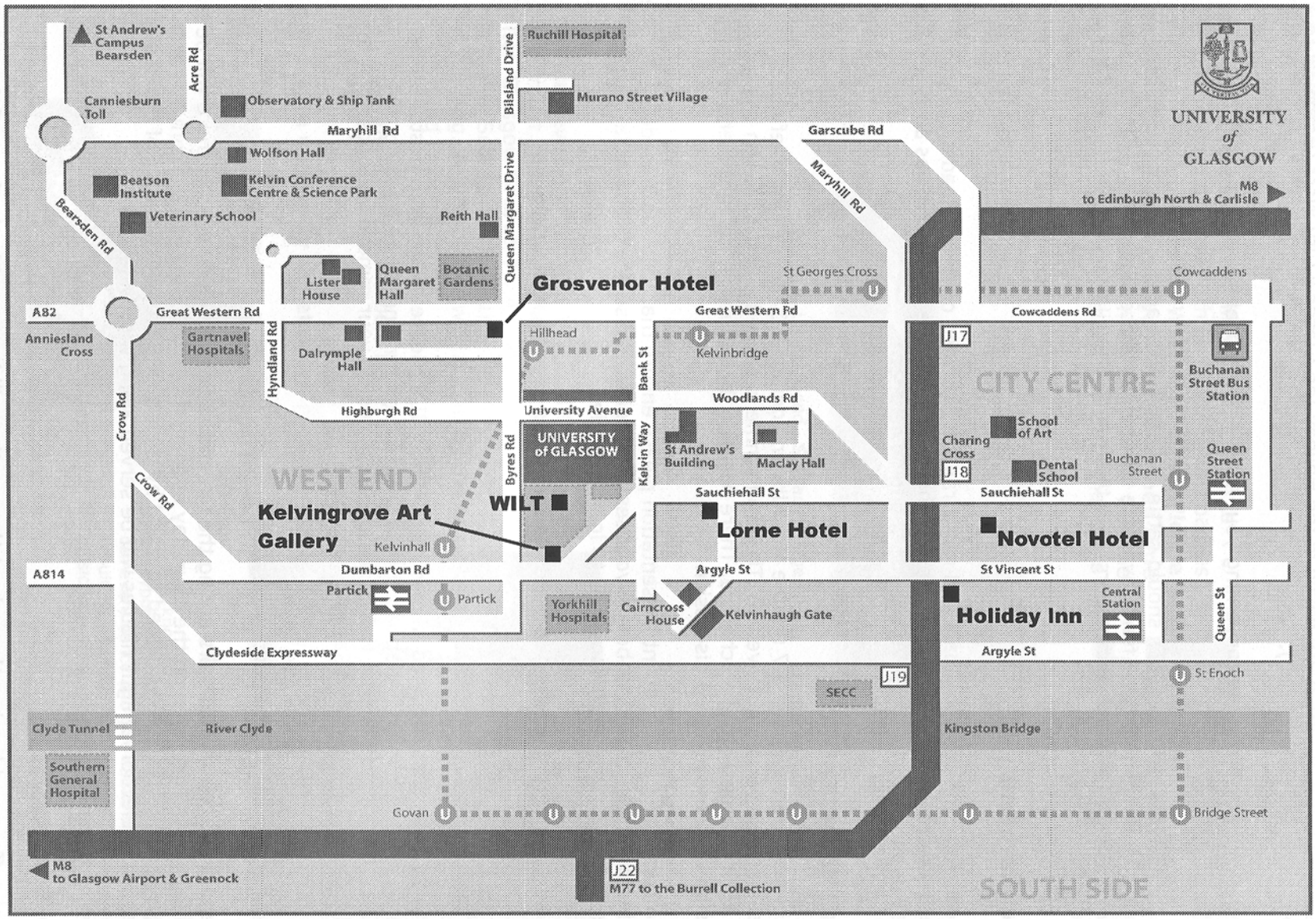
Key to buildings

Accommodation Service	D14	Library	D11
Adam Smith Building	D8	Lilybank Gardens	D16
Anderson College	C1	Lilybank House	D9
Archive Services	C7	The Mackintosh House	D13
Joseph Black Building	B4	Main Building	A10
Bower Building	B7	Main Gatehouse	A21
Boyd Orr Building	D1	Mathematics Building	D4
Bute Hall	A13	Medical School	C8
Bute Gardens	D17	John McIntyre Building	A22
Careers Service	D15	Modern Languages	D5
Catholic Chaplaincy	E11	Oakfield Ave	E12
Chapel	A8	Officers' Training Corps	B5
Chaplaincy Centre	A24	Pearce Lodge	A4
College Club	A9	Pontecorvo Building	C3
Computing Service	A2	Principal's Lodging	A18
Concert Hall	A12	Queen Margaret Union	D3
Conference & Visitor Services	A20	Rankine Building	E7
Davidson Building	B1	Reading Room	E1
Dining Rooms	A9	Refectory	E3
East Quadrangle	A5	Registry: Enquiry Office	A17
Estates & Buildings	B6	Robertson Building	C2
Florentine House	E4	Senate Room	A17
Gardiner Institute	C6	Student Recruitment & Admissions Service	A20
George Service House	D7	George Service House	D7
Gilmorehill Centre	E9	Adam Smith Building	D8
Graham Kerr Building	B3	Southpark House	E5
Gregory Building	D2	The Square	A23
Henry Jones Building	E10	Stair Building	A19
Hetherington Building	D10	Stevenson Building	E6
Hetherington House	D6	Thomson Building	A3
Hillhead St Terrace	D18	University Gardens	D15
The Hub	E2	University Offices	A16
Hunter Halls	A14	University Union	E8
Hunterian Art Gallery	D12	Virology Institute	C4
Hunterian Museum	A15	Visitor Centre	A11
James Watt Building	A1	James Watt Building	A1
John McIntyre Building	A22	West Medical Building	B2
Joseph Black Building	B4	West Quadrangle	A6
Kelvin Building	B8	Western Lecture Theatre	B9
Kelvin Gallery	A7	Wolfson Building	B10
Graham Kerr Building	B3	Zoology Museum	B3



Main Building A10 | Boyd Orr D1 | The Library D11

The Western Infirmiry Lecture Theather (WILT) and the Kelvingrove Art Gallery are highlighted in black.



The Western Infirmary lecture Theatre (WILT), Kelvingrove Art Gallery, Grosvenor Hotel, Novotel Hotel, Lorne Hotel and Holiday Inn are highlighted in black.

Accommodation

The conference hotels are the Kelvin Park Lorne Hotel (923 Sauchiehall Street, Tel: 0141 314 9955) and the Novotel Hotel (181 Pitt Street, Tel: 0141 222 2775). The Myotonic Dystrophy Support Group are based in the Holiday Inn Glasgow City West (Bothwell Street, Tel: 0870 400 9032).

If you have any problems concerning your hotel accommodation, please deal with the hotel concerned directly.

Please note that all attendees should settle their own hotel accounts on checking out. There may be some limited funds available for support of accommodation costs of presenting authors (oral and posters), but this will only available after the close of the meeting.

Local amenities

A good selection of shops, bars and restaurants are located on Byres Road and along Great Western Road to the north west of the University. The city centre has an extensive array of shops, including several large shopping centres.

Oral presentations

Each presenter will have a 7 minute slot with an additional 3 minutes for brief questions. Please ensure you do not overrun. The programme is packed and the session chairmen will stick rigorously to the schedule. There will be a 30 minute period at the end of each session for more involved discussion.

If you are using a PowerPoint presentation please ensure that your file is loaded onto one of the meetings computers before your session begins. Please see Graham Hamilton (Macintosh) or Douglas Wilcox (PC) to load your file.

If you are using 35mm slides please see John McAbney to obtain a carousel.

Posters

Poster boards will be located in the foyer of the WILT. Please attach your poster to the appropriately numbered board as soon as possible on arriving at the meeting. Velcro stickers will be available for this purpose. Please ensure your remove your poster at the end of the final session on Saturday afternoon.

Cheese and wine poster session

In addition to viewing posters during the tea and coffee and lunch breaks, there will be a dedicated poster session on the Thursday evening from 5.00 pm until 7.00 pm. Presenting authors are requested to stand by their posters for the duration of the poster session. A selection of light nibbles will be served along with wine.

Hospitality

Teas, coffees and lunches throughout the meeting will be served in the foyer of the WILT.

Excursion

On Friday afternoon there will be an excursion to Glengoyne Distillery and Loch Lomond. Two coaches will depart from outside the WILT at 1.00 pm. Please make sure you have had your lunch in good time and are ready to go. The three coaches will visit the venues in alternate order and will return to the hotels at around 6.30 pm.

Glengoyne Distillery

Loch Lomond - Scotland's largest loch

Ceilidh

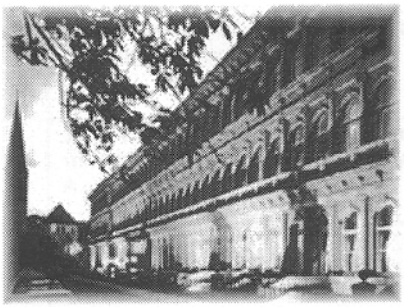
On Friday evening (11th April), from 8.00 pm till midnight, there will be a ceilidh in the Hilton Glasgow Grosvenor. A ceilidh is a traditional Scottish social gathering with music and dancing. The festivities will be lead by the ceilidh band Rock'n'Reelin.

For refreshments there will be a buffet supper and a cash bar.

Admission will be by ticket only.

Rock'n'Reelin

"We know from our experience that not everybody is sure of what to do, therefore we call every dance and give demonstrations. This results in a band of highly professional trained musicians who guarantee a full dance floor and a memorable night."



The Hilton Glasgow Grosvenor, Great Western Road.
Tel: 0141 339 8811.

Bring and buy sale

The Myotonic Dystrophy Support Group will be running a 'bring and buy sale' during the joint session on Saturday 12th April. This is an informal sale to raise money for myotonic dystrophy research. The MDSG would be delighted if attendees could bring an inexpensive item to donate to the sale. If the item were representative of the area that you come from that would be great.

Civic reception and conference dinner

The Kelvingrove Art Gallery and Museum is world renowned for the quality of its international art collection which includes Impressionists and Italian and Dutch Renaissance paintings. Without question the Art Gallery houses one of Scotland's finest civic art collections. Delegates will have an opportunity to view part of the collection following the meal, if they wish.

A Civic Reception for the delegates of IDMC4 will be held in Glasgow Kelvingrove Art Gallery, West Balcony from 7.00 pm until 8.00 pm on Saturday 12th April. This event is hosted by the Lord Provost of Glasgow, Mr Alex Mosson.

A Scottish Buffet Dinner will then be held in the Centre Hall from 8.00 pm until 11.00 pm. 'Harpbeat', a trio of musicians playing flute, fiddle and clarsach (Celtic harp) will entertain us throughout the evening.

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

Programme

Thursday 10th April AM

8.00 am	**Registration with tea/coffee**
9.00 am	**Welcome – *Meeting chair: Darren G. Monckton***
	Session 1 – Diagnostics and DNA instability in myotonic dystrophy ***Session chair: Genevieve Gourdon***
9.10 am	**1) Origins of the myotonic dystrophy mutation: worldwide study in the CEPH-Human Genome Diversity Project** Hidehisa Yamagata* *et al.*
9.20 am	**2) Possible *de novo* CTG repeat expansion in DM protein kinase gene of a patient with isolated focal cardiomyopathy** Daisuke Furutama* *et al.*
9.30 am	**3) CTG repeat instability timing during transgenic mice spermatogenesis** Cédric Savouret* *et al.*
9.40 am	**4) Role of mismatch-repair proteins and Fen-1 exonuclease in somatic expansion behaviour of the $(CTG)_n$-repeat in cellular and animal models for DM1** Marcel R. Nelen, … … and Bé Wieringa*
9.50 am	**5) DNA double-strand breaks induce instability of CTG•CAG repeats related to myotonic dystrophy in an orientation dependent manner in *Escherichia coli*** Micheal L. Hebert* and Robert D. Wells
10.00 am	**6) Could a change in direction of DNA replication make the repeat unstable?** Celia H. Burgoyne* *et al.*
10.10 am	**7) Replication inhibitors modulate instability of an expanded CTG tract at the DM1 disease locus in human cells** Zhi Yang, … … and Christopher E. Pearson*
10.20 am	**8) Chemotherapeutically induced deletion of expanded (CAG)•(CTG) repeats** Richard R. Sinden* *et al.*
10.30 am	**Tea/coffee break**
11.00 am	**9) Chemically induced modification of DNA dynamics: towards chemogenetherapy in the repeat expansion disorders** Mário Gomes-Pereira* *et al.*
11.10 am	**10) High-resolution marker analysis of the region flanking the DM2-$(CCTG)_n$ expansion reveals a single shared haplotype among mutation carriers** L.L. Bachinski* *et al.*
11.20 pm	**11) Molecular genetics of the DM2 CCTG expansion** Laura P.W. Ranum* *et al.*
11.30 pm	**12) Triplet and quadruplet repeat-primed PCR in DM1 and DM2 diagnosis: detection of unusual mutations** David J. Cockburn* *et al.*
11.40 pm	**13) A new method for routine molecular diagnosis in myotonic dystrophy type 2 using chromogenic *in situ* hybridization (CISH)** A. Vihola, … … and B. Udd*
11.50 pm	**Discussion**
12.20 pm	**Lunch**

Thursday 10th April PM

	Session 2 – Neighbouring gene effects and cell biology in myotonic dystrophy ***Session chair: Keith Johnson***
2.00 pm	**14) Impaired muscle development in congenital myotonic dystrophy** Denis Furling* *et al.*
2.10 pm	**15) Expression of myogenic regulatory factors in myotonic dystrophy myoblasts** Caroline Haineault, … … and Jack Puymirat*
2.20 pm	**16) A molecular genetic analysis of muscle differentiation in *Drosophila melanogaster*** Michael V. Taylor* *et al.*
2.30 pm	**17) The role of the *Drosophila SIX5* homologue, *D-Six4*, in embryonic muscle development** Ivan B. N. Clark* *et al.*
2.40 pm	**18) A comparative whole genome approach to study SIX5 function and its contribution to the DM1 phenotype** Graham M. Hamilton* *et al.*
2.50 pm	**19) DM1 and DM2 transcriptome profiling reveals global gene expression changes** Ralf Krahe* *et al.*
3.00 pm	**20) Six5-target genes and DM1 symptoms** Shigeru Sato* *et al.*
3.10 pm	**21) *SIX5* target genes: functional links between *SIX5* and DM1 symptoms** Rami A. Jarjour, … … and Keith J. Johnson*
3.20 pm	**Tea/coffee break**
3.40 pm	**22) Problems with antibodies** Glenn E Morris* *et al.*
3.50 pm	**23) Characterization of potential DMPK substrates in a cellular blebbing model.** James D. Waring* *et al.*
4.00 pm	**24) Alternative splicing controls DMPK structure, enzymatic activity and subcellular localization** Derick G. Wansink* *et al.*
4.10 pm	**25) C-terminal targeting-motifs direct DMPK to the endoplasmic reticulum or mitochondrial outer membrane** René E.M.A. van Herpen* *et al.*
4.20 pm	**26) Towards the understanding of the myotonic dystrophy type 2 pathogenesis** Emanuela Bonifazi* *et al.*
4.30 pm	**Discussion**
5.00 pm to 7.00 pm	**Poster session with cheese and wine**

Friday 11th April

	Session 3 – Toxic RNA in myotonic dystrophy ***Session chair: David Brook***
9.00 am	**27) Foci formation and the disruption of splicing by CUG-repeat RNA are separable events** Thai H. Ho* *et al.*
9.10 am	**28) Gene expression profiles of mouse C2C12 cells which stably express human DMPK with 160 CTG repeat** Noboru Sasagawa* *et al.*
9.20 am	**29) Inhibition of myogenesis in transgenic mice expressing the human *DMPK* 3' UTR** C.J. Storbeck* *et al.*
9.30 am	**30) Mouse muscleblind gene knockout models for myotonic dystrophy** Rahul N. Kanadia* *et al.*
9.40 am	**31) Ribonuclear inclusions in the central nervous system in myotonic dystrophy type 1** Hong Jiang … … and Charles A. Thornton*
9.50 am	**32) Analysis of alternative splicing in muscleblind deficient *Drosophila*** Helena Thorpe* *et al.*
10.00 am	**33) A *Drosophila* model of myotonic dystrophy** Jonathan M. Houseley* *et al.*
10.10 am	**34) RNA mechanism for myotonic dystrophy 1: elevation of CUGBP1 disorders myogenesis through the disruption of MEF2A and p21 pathways** Roma Patel … … and Lubov T. Timchenko*
10.20 am	**Tea/coffee break**
10.50 am	**35) Aberrant splicing of the ryanodine receptor in myotonic dystrophy** Takashi Kimura* *et al.*
11.00 am	**36) Developmental roles of the muscleblind and CUG-BP homologues in *C. elegans*** Aidyl-Gonzalez-Serricho* *et al.*
11.10 am	**37) Insights from a C2C12 myoblast model of the effects of the mutant DMPK 3'UTR RNA on myogenic differentiation** Jeffrey D. Amack … … and Mani S. Mahadevan*
11.20 pm	**38) Molecular pathogenesis of the DM2 CCTG Expansion** Laura P.W. Ranum* *et al.*
11.30 pm	**Discussion**
12.00 pm	**Lunch**
1.00 pm to 6.00 pm	**Excursion - Glengoyne Distillery and Loch Lomond**
8.00 pm to 11.00 pm	**Ceilidh - Grosvenor Hotel**

Saturday 12th April AM

	Session 4 – Clinical and therapeutic aspects of myotonic dystrophy ***Session chair: Peter Harper***
9.00 am	**39) Somnolence in DM1: cause, assessment and management** David Hilton-Jones* *et al.*
9.10 am	**40) Neuropsychiatric, neuropsychological and neuroimaging studies in myotonic dystrophy type 1: significance and clinical correlates?** G. Meola* *et al.*
9.20 am	**41) Educational and psychological group observations of children and juveniles with congenital myotonic dystrophy** Jes Rahbek* *et al.*
9.30 am	**42) A peculiar neurofibrillary degeneration distribution in one DM2 brain: a case report** Claude-Alain Maurage, Nicolas Sergeant* *et al.*
9.40 am	**43) Testosterone and diurnal rhythmicity of leptin, TNF-alpha, and TNF-II receptor in insulin-resistant myotonic dystrophy patients** Åsa Johansson* *et al.*
9.50 am	**44) Heart involvement in young myotonic dystrophy type 1 patients** G. Bassez* *et al.*
10.00 am	**45) Steinert's disease (DM1) with predominant proximal involvement** Frédéric Andreux … … and Bruno Eymard*
10.10 am	**46) Clinical and molecular features of myotonic dystrophy type 2** John W. Day* *et al.*
10.20 am	**Tea/coffee break**
10.50 am	**47) Is this myotonic dystrophy type 3?** Robert McWilliam* and Ed Tobias
11.00 am	**48) Identification of problems with extended activities of daily living in people with myotonic dystrophy** Margaret F. Phillips*
11.10 am	**49) Parenting in the face of a late-onset genetic disorder: a comparative study of parents facing either myotonic dystrophy (DM) or Huntington disease (HD)** Claudia Downing*
11.20 pm	**50) A patient held "Care Card" to improve the management of myotonic dystrophy** Douglas E Wilcox* *et al.*
11.30 pm	**Discussion**
12.00 pm	**Lunch**

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Saturday 12th April PM

	Session 5 – All together now – patient, scientist, clinician and family gathering ***Session chair: Darren Monckton***
2.00 pm	**51) My family has myotonic dystrophy** Shannon Lord
2.20 pm	**52) Recent scientific advances in myotonic dystrophy** David Brook
2.40 pm	**53) Living with myotonic dystrophy** Margaret Bowler
3.00 pm	**Tea/coffee**
3.40 pm	**54) One doctor, two sisters and a professor** Jacqueline Donachie
4.00 pm	**55) Towards treatments for myotonic dystrophy** Tetsuo Ashizawa
4.20 pm	**Panel discussion**
7.00 pm to 10.30 pm	**Civic reception and Scottish buffet – Kelvingrove Art Gallery**

Abstracts

Oral presentations

1) Origins of the myotonic dystrophy mutation: worldwide study in the CEPH-Human Genome Diversity Project

Hidehisa Yamagata*[1], Satoru Akiyama[2], Yusen Chen[3], Ikuko Kondo[1] and Tetsuro Miki[3]

[1]Department of Medical Genetics, Ehime University School of Medicine.

[2]Faculty of Health Science, Ehime University School of Medicine.

[3]Department of Geriatric Medicine, Ehime University School of Medicine, Shigenobu-cho, Onsengun, Ehime 791-0295, Japan.

Although it is believed that a major ancient mutation underlying DM1 has originated from reservoir pool (CTG19-37), the molecular mechanisms underlying repeat instability are not well understood. Population-based study will provide a clue for the origins of DM1 mutations and the evolution of modern humans. We previously reported that Japanese DM1 alleles were always haplotype A and DM1 alleles had not originated from CTG5. Here we examined the association between CTG repeat length and DM1 locus polymorphisms in normal individuals from 51 ethnically diverse human populations in 21 countries (CEPH-HGDP).

We determined CTG-repeat number in 1064 CEPH-HGDP individuals using ABI PRISM 310 Gene Scan and examined 8 SNPs at the 20kb DMPK-SIX5 region using PCR-RFLP methods. As for CTG repeat distributions, the results were similar to those reported by Tishkoff et al.(1998). Some populations showed trimodal distribution (CTG5, 6-17, 18-37) and others did not. Frequencies of CTG5, 11-13, >19 were grouped. As expected, DM1 rare populations (sub-Saharan African, China etc) had no CTG>19 repeats. As for Amerindians (Karitiana, Maya etc), a major genetic bottleneck from Asia to the Americas was seen.

The SNPs in promoter region of DMPK showed an evenly distributed pattern when normal alleles were grouped according to CTG repeat length. Even for CTG>20 alleles there were different haplotypes according to SIX5 locus SNPs. It suggests that CTG>20 alleles are also comparatively stable and not all >20 repeat allele will increase its size in successive generations. Common DM1 founder chromosome region was postulated to be within about 17kb including CTG repeat. Our data suggest founder chromosomes rather than predisposing alleles.

2) Possible *de novo* CTG repeat expansion in DM protein kinase gene of a patient with isolated focal cardiomyopathy

Daisuke Furutama*[1], Nobuyuki Negoro[1], Fumio Terasaki[2], Kuniko Tsuji-Matsuyama[1], Masaaki Hoshiga[1], Tadashi Ishihara[1], Toshifumi Tanaka[3], Takumi Ito[4], Toshiaki Hanafusa[1] and Nakaaki Ohsawa[3]

[1]First Department of Internal Medicine, Osaka Medical College, 2-7 daigaku-cho, Takatsuki 569-8686, Japan.

[2]Third Department of Internal Medicine, Osaka Medical College, 2-7 daigaku-cho, Takatsuki 569-8686, Japan.

[3]Aino institute for aging research, 3-9-25 Ohta, Ibaraki 567-0018, Japan.

[4]Deaprtment of Internal Medicine, Seikeikai hospital, 4-2-10 Kohryonaka-machi, Sakai 590-0024, Japan.

It has been believed that triplet repeats of "normal" length are stable and new pathological expansion could not developed. Here we report an unusual and possible *de novo* CTG repeat expansion in myotonic dystrophy protein kinase (DMPK) gene especially in heart of a patient with "idiopathic" cardiomyopathy.

A case report: A 73 year-old Japanese woman, who had been implanted with a permanent cardiac pacemaker for complete atrioventricular block, was admitted to our hospital because of congestive heart failure. She had no family history of myotonic dystrophy type 1 (DM1). Imaging and pathological studies revealed multiple focal fatty degeneration of cardiac muscle. She was diagnosed as idiopathic cardiomyopathy. However, in addition to cardiomyopathy, she had cardiac conduction block, cataracts, and three episodes of fetal loss of unknown cause, all of which were common symptoms in DM1. Then, we examined this patient for DM1. Neurological and electrophysiological studies showed no DM1 signs including myotonia. Although the genomic Southern blot and conventional PCR analysis showed only normal alleles with 12 CTG repeats in her peripheral blood mononuclear cells (PBMNC), the small pool PCR (SP-PCR) technique revealed that there were expanded alleles, with more than 50 CTG repeats, in only a fraction of cells. Surprisingly, the expanded alleles were more frequently detected in the biopsied cardiac muscle than in the PBMNC.

Our results suggest *de novo* CTG repeat expansion in only a fraction of cells and possible DM1-associated cardiomyopathy in this patient. It could be clinically important to add DM1 to the list of differential diagnose for "idiopathic" cardiomyopathy, and we believe that it would stimulate further research about genetic background, i.e. *de novo* DM1 mutation, in patients with cardiomyopathy, cataract or other isolated DM1 symptoms.

3) CTG repeat instability timing during transgenic mice spermatogenesis

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In order to understand the molecular mechanisms underlying the CTG repeat instability occurring in DM1, we used our transgenic mice model carrying 45 kb of the human DM1 region with >350 CTG (line DM300-328). As observed in patients, the DM300-328 mice showed a bias towards expansions (>86%) over generations, with sex- and size-dependence. Somatic instability towards expansions is also reproduced. By breeding these mice with DNA repair KO mice to assay the potential role of DNA repair on instability, we also observed that *MSH2*-/- mice showed no more expansions, neither over generations nor in tissues. However, the absence of Msh2 did not stabilize the repeat, and in sharp contrast with what was observed with other mice models, a strong bias towards contractions (>90%) was obtained in these mice. This demonstrates that if expansions require Msh2 to occur, contractions are probably generated through an Msh2-independant pathway. While no influence of double-strand break repair genes *RAD54* and *DNA-PKcs* on instability could be noted, our data show that Rad52 may be involved in the range of expansions.

So far, it was mainly thought that germinal instability in the transmitting parent accounts for intergenerational instability. However, careful analysis of expansions and contractions between generations with regards to the parents and kindred *MSH2* genotypes demonstrated that a second instability event takes place just after fertilization. We will present our current work focusing on the timing of intergenerational instability, especially during spermatogenesis. We confirmed by single-cell PCR that CTG instability in sperm increases over lifetime in the DM300-328 mice. In order to see if instability is produced in spermatozoa or before during spermatogenesis, we sorted the different types of germinal cells from transgenic mice spermatogenesis by flow cytometry. A double labelling was used to define precise sorting regions, both against cell DNA content and against a mitochondrial membrane protein whose repartition and expression change throughout spermatogenesis. The length of the CTG repeat in each fraction was analyzed by normal and single-cell PCR. We show that a high level of repeat length mosaicism is already present in spermatogonia, thus at the very beginning of spermatogenesis, and preliminary results suggest that the mosaicism in spermatozoa is comparable with that in spermatogonia. This demonstrates that germinal instability takes place very early during the spermatogenesis process, in spermatogonia. The same experiments performed with *MSH2*-/- males showed that contractions are also produced in spermatogonia.

4) Role of mismatch-repair proteins and Fen-1 exonuclease in somatic expansion behaviour of the $(CTG)_n$-repeat in cellular and animal models for DM1

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Somatic expansion of the $(CTG)_n$-repeat is thought to be the underlying mechanism involved in the variability and progression of disease manifestation in myotonic dystrophy type 1 patients. Both cis- and trans-acting factors may play a role in the mutational process.

We have produced mice carrying "humanized" myotonic dystrophy protein kinase (*DMPK*) alleles with a $(CTG)_{84}$-repeat, inserted at the correct position into the endogenous *DM1* locus (Van den Broek et al. (2002) Hum. Mol. Genet. 11:191-198). During breeding over ten generations the repeat length has now expanded to $(CTG)_{106}$, indicating a marginal instability during intergenerational segregation. Somatic expansion in these mice occurs in various tissues, is most conspicuous in kidney, stomach, small intestine and liver, and prominent after an age of 6 months. We have demonstrated earlier that recognition by the DNA mismatch repair (MMR) machinery is an important parameter in repeat instability. Deficiency for the Msh3 MMR-protein has a marked stabilizing effect on the rate of repeat mutation, whereas deficiency for the Msh6 MMR-protein increases instability. Cell type remains an important determinant as demonstrated by tissue fractionation experiments and a high degree of instability in a spontaneously occurring liver tumour in $(CTG)_n/Msh6^{-/-}$ mice. When genomic amplification of the *Dhfr/Msh3* locus was used to remodel the composition of MMR repair complexes in *in vitro* cultured $(CTG)_{84}$-ES cells, we found a conspicuous high degree of repeat instability (now mainly contractions!). These data support an important role for *Msh3* (MutSβ) involvement in repeat instability.

Finally, $(CTG)_{84}$-ES cells were also used to test the effects of allelic loss (via genetic inactivation) of the Fen-1 "flap" exonuclease, a candidate protein for being involved in $(CTG)_n$-repeat expansion via its role in Okazaki fragment processing. Initial tests in chimaeric animals produced with these cells do not support a clear role for Fen-1 in repeat instability, but until now effects of complete Fen-1 deficiency could not be tested due to embryonic lethality.

Taken combined, our results suggest that DNA repair and/or recombination, rather than replication proper are central processes in DM1 trinucleotide instability.

5) DNA double-strand breaks induce instability of CTG•CAG repeats related to myotonic dystrophy in an orientation dependent manner in *Escherichia coli*

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This laboratory has investigated the molecular mechanisms of genetic instabilities of CTG•CAG repeat tracts with respect to myotonic dystrophy. Prior studies revealed the involvement of replication, repair (methyl-directed mismatch repair, nucleotide excision repair, and DNA polymerase III proofreading), and recombination in this process. The role of non-B DNA secondary conformations (slipped structures) in the slippage of the complimentary strands resulting in primer realignment has been implicated in most models of instability.

We have recently investigated the influence of double-strand breaks (DSBs) in the CTG•CAG repeat sequence on its genetic instabilities (expansions and deletions). Plasmids containing 43 or 70 CTG•CAG repeats were linearised at a unique site *in vitro* near the centre of the repeats and transformed into parental, RecA dependent homologous recombination deficient, or RecBC exonuclease deficient *Escherichia coli*. DSBs increased deletion of the repeating sequence. The repeat was further destabilized in the absence of RecA or RecBC. The orientation of the insert relative to the unidirectional ColE1 origin of replication affected the amount of instability generated during the repair of the DSB. When the CTG strand was the template for lagging strand synthesis, instability was increased, most markedly in the $recA^-$ strain. Experiments with the $(CTG•CAG)_{70}$ insert showed more instability than studies with the TRS insert containing 43 repeats. Hence, ColE1 origin-dependent replication was involved during the repair of the DSB. We hypothesize an important role of DNA secondary structures in the replication-repair unpaired intermediates.

6) Could a change in direction of DNA replication make the repeat unstable?

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The length of the CTG repeat expansion is observed to increase in successive generations and also to vary between different tissues within an individual. There is a general positive correlation between the length of the repeat tract and disease severity. However, the factors responsible for germline instability and somatic instability are largely unknown. This study aimed to characterise the timing and direction of DNA replication around the *DMPK* locus in DM1 and control cell lines, to establish why repeat expansions become unstable initially and why they are more unstable in some tissues. This would test the hypothesis that repeat expansion causes a slowing or block to DNA replication in the normal direction, leading to a switch in the direction of replication, which is in turn responsible for accelerated repeat length instability.

Myotonic and control lymphoblastoid cells have been synchronised for passage through S-phase by arrest at the G1/S border and subsequent release, and their position in S-phase confirmed by flow cytometry. DNA has been extracted at time points throughout replication, for relative copy number analysis using Multiplex Amplifiable Probe Hybridisation (MAPH). This technique uses a set of identically linkered probes for genomic loci to assay copy number after hybridisation to genomic DNA, with the signal from any one probe being proportional to the copy number of its target site in the sample. Comparison of probes for the *DMPK* region, with control probes for known early and late replicating loci, will enable the timing and direction of replication to be determined.

7) Replication inhibitors modulate instability of an expanded CTG tract at the DM1 disease locus in human cells

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Gene-specific (CTG)•(CAG) repeat expansion is associated with at least 12 human diseases, including myotonic dystrophy (DM1). Most of our understanding of trinucleotide instability is from non-human models which have presented mixed results, supporting replication errors or processes independent of cell division. Nevertheless, the mechanism occurring at any of the disease loci in patient cells is poorly understood. Using DM1 patient-derived fibroblasts, we have shown that spontaneous expansion of the diseased (CTG)216 allele occurred in proliferating but not quiescent cells. Furthermore, cells were treated with agents known to alter DNA synthesis but not to directly damage DNA. Inhibiting replication initiation with mimosine had no effect upon instability. Inhibiting both leading and lagging strand synthesis with aphidicolin, or blocking only lagging strand synthesis with emetine significantly enhanced CTG expansions. Strikingly, only the expanded DM1 allele was altered, leaving the normal allele, (CTG)12 and other repeat loci unaffected. Standard and small-pool PCR revealed that inhibitors enhanced 3-fold the magnitude of short expansions in most cells, while 11-25% of cells experienced gains of 122-170 repeats. Our results support a role for the perturbation of replication fork dynamics in DM1 CTG expansions within patient fibroblasts. This is the first report that repeat length alterations *specific* to a disease allele can be modulated by exogenously added compounds. The identification of compounds that *specifically* alter repeat instability at a disease locus provides insight for research aimed at developing pharmacological agents that would intervene with the mutation process to slow pathogenesis in man.

8) Chemotherapeutically induced deletion of expanded (CAG)•(CTG) repeats

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The number of neurodegenerative disorders associated with the expansion of DNA repeats, currently about eighteen, continues to increase as additional diseases caused by this novel type of mutation are identified. Typically, as is the case for myotonic dystrophy, expanded repeats are biased toward further expansion upon intergenerational transmission, and 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. At present, no treatment exists correcting or preventing the genetic basis of any repeat disease. Given that the severity of these diseases is related to repeat tract length, reducing repeat lengths might delay the onset and reduce disease severity.

We have tested the hypothesis that the introduction of damage into DNA, which results in subsequent repair events, can lead to an increased rate of repeat deletion. Applying a sensitive genetic assay in *Escherichia coli* we have demonstrated that certain DNA damaging agents, including EMS, ENU, UV light, and anticancer agents mitomycin C, cisplatin, and X-rays increase the rate of deletion of (CTG)•(CAG) repeats in a length and orientation dependent fashion. In addition, oxidative damage to DNA also increases the deletion rate of repeats. 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 these diseases.

We have tested this rationale for repeat deletion on human lymphoblast DM1 cell lines containing expanded triplet repeats by treating cells with some of these and other agents. Following treatment and growth, in general, the continued expansion observed in DM1 cells in culture appears to be prevented and in many cases large deletions in the repeats are observed. 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.

9) Chemically induced modification of DNA dynamics: towards chemogenetherapy in the repeat expansion disorders

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Myotonic dystrophy type 1 (DM1) is an autosomal dominant disease caused by the expansion of a CTG repeat in the 3' untranslated region of the *myotonic dystrophy protein kinase* (*DMPK*) gene. Similarly to other trinucleotide repeat conditions, such as Huntington disease, fragile X syndrome and Friedreich ataxia, expanded *DM1* alleles exhibit several distinctive features associated with DNA instability. In particular, somatic mosaicism of repeat length is often prominent and tends to be age-dependent, tissue-specific and expansion-biased. These properties most likely contribute towards the tissue specificity and progressive nature of the disease symptoms.

Despite the common features associated with the DNA dynamics, pathology in triplet repeat disorders is mediated by a variety of complex and unrelated molecular routes. Thus, therapies aimed at the downstream effects of the repeat expansion will be largely disease-specific. On the other hand, therapies targeted directly at the repeat mutation may have general utility in these disorders. Specifically, strategies that result in suppression of somatic repeat expansion would be expected to be of therapeutic benefit, whilst reversion of the expanded mutant repeat to within the range observed in the general population would be predicted to be curative.

To this end we have determined that the dynamics of expanded triplet repeats may be modified by chemical treatments in a tissue culture model of unstable DNA. Cell lines established from transgenic animals, carrying a randomly integrated CAG•CTG repeat derived from the human *DM1* locus, have been treated with a variety of chemicals and repeat size variability assessed by sensitive small pool PCR techniques. Reagents that result in both acceleration and deceleration of the rate of expansion have been identified, establishing that drug-induced modification of DNA dynamics, *chemogenetherapy*, presents a possible new route to treatment in these disorders.

10) High-resolution marker analysis of the region flanking the DM2-$(CCTG)_n$ expansion reveals a single shared haplotype among mutation carriers

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Expansion of the $(CCTG)_n$ repeat in ZNF9 causes PROMM/DM2. To understand the evolution of this mutation, we determined if a unique haplotype segregated with the expansion. We sampled 14 unrelated Caucasian families from Finland (3), Italy (3), the USA (3), France (2), Spain (1), Switzerland (1) and the UK (1) and genotyped 47 affected individuals and 16 normal individuals along with 22 index cases from the same populations. As controls we included 108 members from 11 families and 10 unrelated individuals from matching ethnic backgrounds. We genotyped 27 microsatellite markers over ~11 Mb flanking the DM2 locus. Evidence of extensive allele sharing was seen in the Finnish families (Northern haplotype) over ~1.8 Mb centromeric and ~200 kb telomeric to the expansion. A subregion of this haplotype was shared with 1 USA and 1 UK family. Four families (1 Swiss, 1 French, 2 Italian) shared a different haplotype over ~600 kb (Southern haplotype). The other 4 families shared alleles with either haplotype only 200 kb telomeric to the expansion. These data suggested a single or a few founding mutations. To refine the haplotype(s) flanking the mutation, BAC clones containing ZNF9 were identified, their sequences assembled and divided into four bins based on overlap. We identified and interrogated 22 SNPs, present in our BAC contig and verified by STS-content mapping. Using GeneHunter we characterized 13 independent phase-known chromosomes containing the DM2 expansion and 137 phase-known normal chromosomes. Of the 22 SNPs, only 6 showed variation. We observed a total of 6 different normal haplotypes. The most common was present in 126 of 137 (91.9%). All 13 independent DM2 chromosomes were invariant for all markers over ~114 kb surrounding the mutation and shared a single haplotype identical to the most common normal haplotype. This is reminiscent of the situation in DM1. It is noteworthy that for most of the SNPs studied, little variation was observed over a distance of ~200 kb surrounding ZNF9, suggesting a highly conserved haplotype block. Taken together, these data suggest a single founding mutation in Caucasian DM2 patients

11) Molecular genetics of the DM2 CCTG expansion

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We recently demonstrated that myotonic dystrophy type 2 is caused by a CCTG expansion in intron 1 of the zinc finger protein 9 (*ZNF9*) gene. Using a panel of 74 DM2 families, we have identified two conserved haplotypes spanning at least 425 kb. Additional markers near the DM2 repeat could not distinguish if the two haplotypes are truly distinct or if they converge close to the mutation. In either case, our data indicate that a founder haplotype(s) is responsible for the predominant Northern European ancestry of DM2. The DM2 repeat tract contains the complex repeat motif (TG)n(TCTG)n (CCTG)n. The CCTG portion of the repeat tract is interrupted on normal alleles and expands on affected alleles. To gain insight into possible functions of the repeat tract we looked for evolutionary conservation. Variations of the repeat motif and flanking sequences within intron 1 are conserved between human, chimp, gorilla, mouse and rat suggesting a conserved function.

12) Triplet and quadruplet repeat-primed PCR in DM1 and DM2 diagnosis: detection of unusual mutations

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We have introduced triplet repeat primed-PCR (TP-PCR) into our testing protocol for DM1, and developed an analogous test, quadruplet repeat primed-PCR (QP-PCR) for the detection of CCTG expansion mutations in DM2 (PROMM). These assays use one primer which anneals to unique sequence adjacent to the repeat motif; a second one anneals within the (CTG)n or (CCTG)n motif itself; and a third stabilises the amplification of a 3 or 4bp ladder, characteristic of an expansion mutation.

Since introducing these tests in the diagnostic setting, we have analysed 107 DM1 and 34 DM2 referrals. Among these samples, 42 DM1 and 5 DM2 patients showed typical expansions. The tests have proved to be powerful and rapid alternatives to Southern blot analysis, enhancing our diagnostic service.

The power of the TP-PCR and QP-PCR assays has been demonstrated further by the identification of three atypical expansions in this period. And we have investigated these unusual cases further by exploiting the flexibility of the assays, employing modifications to the basic design.

The first atypical case is a DM1 expansion in a 50 year-old patient where TP-PCR revealed a discontinuous pattern indicative of regular interruptions. Further studies by Southern blot analysis, a modified TP-PCR and partial sequencing showed that the expansion is at least 1.5kb long, and contains a CCG triplet every 8-11 repeats, at least on the proximal side. To our knowledge, this is the first punctuated DM1 expansion to be described.

The second case is a 30 year old man, clinically affected and from a known DM1 family. He showed a normal pattern on TP-PCR, even though another affected family member showed the 3bp ladder typical for an expansion. Southern blot analysis revealed two abnormally sized bands, indicative of a *de novo* rearrangement. A modified TP-PCR designed specifically for the distal side of the (CTG)n repeat, demonstrated that an expansion is present, thereby suggesting that the rearrangement disrupts only the proximal side. To our knowledge, this is the first described example of a rearrangement associated with a DM1 CTG expansion.

The third case is a referral for DM2 investigations where an unusually faint 4bp ladder was detected by the standard QP-PCR assay. A modified assay was designed, and results revealed that an expansion is present, but that it does not consist of a pure (CCTG)n repeat. This is currently being investigated further.

In conclusion, TP-PCR and QP-PCR are rapid and reliable alternatives to Southern blot analysis for detection of expansion mutations. They may reveal additional features associated with the expansions, and may be adapted readily for further investigations. These methods have enabled us to detect and elucidate the first cases of interruptions and rearrangement associated with DM1 and DM2 expansions.

13) A new method for routine molecular diagnosis in myotonic dystrophy type 2 using chromogenic *in situ* hybridization (CISH)

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The final diagnosis of myotonic dystrophy type 2 (DM2) is based on molecular genetic verification of the underlying (CCTG)n expansion mutation on chromosome 3q21. Clinical diagnosis prior to molecular genetic confirmation is a challenge for the neurologist. We have previously shown that DM2 displays preferential fiber type 2 atrophy in contrast to fiber type 1 atrophy in DM1, and that nuclear clump fibers occur at very early stages of the disease in biopsies of proximal muscles. The accumulating RNA product of the expansion mutation is possible to detect as ribonuclear inclusions by fluorescent in situ hybridization (FISH). We have used the muscle biopsies of 8 DM2 mutation verified patients and of DM1 and healthy controls to determine if the mutation is possible to detect also by using a chromogenic in situ hybridization (CISH) technique which is more convenient for routine diagnosis in the pathology departments. We applied both antisense (CAGG)8 and sense (CCTG)8 oligonucleotides for hybridization and a polymeric commercial amplification kit for detection of the chromogenic label. Both direct DM2 mutation detection as a single dot per nucleus on the leading DNA strand when hybridizing with the sense oligos, and the RNA accumulation as ribonuclear inclusions using the antisense oligos were easily identified in all 8 DM2 patient samples. The method worked also on old paraffin embedded muscle tissue. No chromogenic signal was found in the control samples. This CISH method is an extension of the the routine histopathological test panel used for diagnostic work-up of muscle biopsy samples and could be used as the molecular genetic verification of the mutation and the diagnosis in DM2 patients.

14) Impaired muscle development in congenital myotonic dystrophy

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Impairment in skeletal muscle development represents one of the main features in the congenital form of DM1 (CDM). A delay in the maturation of the skeletal muscle occurs in CDM foetuses with large CTG repeats (1000 and more). Moreover muscles from the CDM foetuses are frequently atrophic and there is a reduction in the number of satellite cells suggesting that there may be a defect in satellite cells with large CTG expansions.

To follow the maturation of normal and CDM foetal muscles, we analysed the pattern of expression of myosin heavy chain (MyHC) isoforms in muscles from foetuses aged between 16- and 35 weeks of development. We showed a persistence of embryonic and foetal MyHC and a dramatic reduction in the amount of slow MyHC at the end of gestation in CDM foetuses carrying large CTG expansions (1800 to 3700). The abnormal muscle development in CDM foetuses is characterised by a delay in maturation and an almost total absence of the secondary generation of slow muscle fibres. In parallel, we have measured the level of DMPK protein during muscle development. The level of DMPK increases dramatically between 9 and 16 weeks of development and remains high throughout the remaining gestation period. The amount of DMPK protein in CDM skeletal muscle was reduced to 57% of control levels. Since the DMPK protein was found in both fast and slow fibres, it's seems to be unlikely that the reduced DMPK protein level is involved in the delayed slow fibre maturation in CDM. In addition, we have shown that abnormal splicing of specific mRNAs occurs in CDM fœtal muscles. We showed that the splicing of the insulin receptor mRNA is altered and we also demonstrated a novel aberrant alternative splicing in the myotubularin-related gene 1 (MTMR1) mRNA. In fact this MTMR1 transcript is muscle-specific and is specifically induced during myogenesis. We found a striking reduction in the level of this muscle-specific isoform and the appearance of an abnormal MTMR1 transcript in differentiated CDM muscle cells and muscle tissue. These results suggest that MTMR1 could play a role in muscle formation and could be implicated in the abnormal CDM muscle phenotype.

15) Expression of myogenic regulatory factors in myotonic dystrophy myoblasts

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One characteristic feature of the congenital form of myotonic dystrophy (CDM1) is a delay of skeletal muscle development. Little is known about the mechanisms by which the DM1 mutation causes the delay in muscle differentiation. The data available suggest that the delay in muscle differentiation may be caused either by decreased levels in myogenin or myoD or p21 expression.

To clarify the potential role of these factors in DM1 muscle pathogenesis, we examined the expression of myogenin, myoD and p21 in normal and six DM1 human myoblasts with different expansions (from 750 to 3700) by Western blot analysis and we correlate the levels of these factors with the ability of DM1 myoblasts to fuse.

We found an excellent correlation between the loss of the ability of DM1 myoblasts to fuse and the length of the expansion in 5 of the 6 DM1 myoblast cell line tested. However one DM1 myoblast cell line with 2800 repeats differentiate as normal myoblasts. Myogenin is expressed by DM1 cells which differentiate and its expression is completely abolished in DM myoblasts which have lost their ability to fuse (DM1 myoblasts with more than 3,000 repeats). In contrast p21 and myoD mRNAs are expressed by DM1 cells and their levels are not correlated with the lose of the ability of DM1 myoblasts to fuse.

Conclusion: 0ur data demonstrate that the lose of the ability of DM1 myoblasts to fuse is correlated with decreased levels in myogenin but not in myoD and p21 gene expression.

16) A molecular genetic analysis of muscle differentiation in *Drosophila melanogaster*

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We study muscle development in the fruit fly, *Drosophila melanogaster*. Our work is likely to be significant for human muscle biology. This is suggested both by the finding that 60% of *Drosophila* genes have human counterparts and by the general conservation of molecular mechanisms of muscle differentiation between *Drosophila* and other species. Moreover, 77% of human "disease genes" match sequences in the *Drosophila* genome. This includes genes implicated in myotonic dystrophy.

A great deal is known about the patterning mechanisms that establish the basic animal body plan and then govern its subsequent subdivision. However, there is a major gap in our knowledge of what happens next. How do these populations of cells then differentiate to produce functional tissues, like muscle?

This process is complex involving many different genes, only some of which have been characterised. Progress therefore requires first identification and then analysis of the remaining genes. We therefore undertook a systematic molecular screen using subtractive hybridisation. From this we isolated cDNA clones for a population of genes that are specifically expressed during muscle differentiation. This cDNA population is a valuable resource for analysing unexplored features of muscle development.

Our current work centres on the analysis of a small group of genes from this screen together with an ongoing study of the function of Dmef2, which is a pivotal regulator of muscle differentiation. Initial studies on the function of two of these genes indicate that they encode novel nuclear proteins / transcriptional regulators and that they have roles in muscle differentiation. One may direct muscle migration and/or attachment. The second inhibits muscle differentiation and interacts genetically with Dmef2. When over-expressed in the developing myogenic mesoderm it results in almost no myosin positive muscle fibres being produced. Our results suggest that it functions in a molecular switch that regulates muscle differentiation.

17) The role of the *Drosophila SIX5* homologue, *D-Six4*, in embryonic muscle development

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D-Six4 is the closest *Drosophila melanogaster* homologue of *SIX5*, the homeodomain gene located downstream of *DMPK* at the human type 1 myotonic dystrophy (*DM1*) locus. Expansion of the *DMPK* CTG(n) repeat results in reduced *SIX5* expression, which is likely to be a contributing factor in DM1 pathology. *Drosophila* embryos homozygous for EMS-induced alleles of *D-Six4* show defects in gonad and muscle development. Wild-type larvae show a stereotyped pattern of 30 muscles per hemisegment, each muscle being formed from the fusion of a single founder cell with a number of fusion competent myoblasts. Founder cells express a distinct set of transcription factors, encoded by the muscle identity genes, which determine the eventual size, shape, position and innervation of the muscle. In *D-Six4* mutant embryos, large numbers of unfused cells indicate a defect in myoblast fusion. Numerous muscles are absent, misshapen or mislocalised. In contrast to other mutations affecting myoblast fusion, we observe that in *D-Six4* mutants certain groups of muscles are consistently affected more severely than others. The muscles of the pharynx are always absent in embryos homozygous for the most severe allele, probably a functional null. In contrast we observe no visible defects in embryonic cardiac and visceral muscles. Somatic muscles are affected to varying degrees, the laterally located muscles being consistently more abnormal than more dorsal and more ventral muscles. We suggest that *D-Six4* may regulate expression of some of the muscle identity genes, leading to defects in the specification of particular founder lineages. We are testing this hypothesis by visualising specific founders and their precursors in *D-Six4* mutant embryos.

18) A comparative whole genome approach to study SIX5 function and its contribution to the DM1 phenotype

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Myotonic dystrophy type 1 (DM1) is characterised by a number of pathologies including muscle defects and testicular atrophy. The mutation, a CTG triplet repeat expansion, is within the 3' untranslated region of a protein kinase gene *DMPK* and the promoter region of a homeobox gene, *SIX5*. The mechanism by which the mutation causes the disease phenotype has yet to be elucidated, but the *SIX5* transcript has been shown to be reduced in DM patients. *SIX5* is a member of the *Six* family of genes, named after the first member found in *Drosophila melanogaster*, *sine oculis*. We have shown that *D-Six4* is the closest *Drosophila* homologue of *SIX5* and have characterised the wild type expression pattern. Mutations in *D-Six4* have revealed its role in mesoderm, gonad, head and central nervous system patterning in the developing embryo. Moreover, adult males with a defective *D-Six4* gene exhibit testicular atrophy. The defects in *D-Six4* mutant flies suggest that human *SIX5* should be strongly considered as responsible for the muscle wasting and testicular atrophy of adult phenotypes in DM1 and the distinctive phenotype of infants with the congenital form of DM1.

We are in the process of studying the changes in RNA transcription levels between these mutant fly lines and wild type flies by microarray analysis. This will identify the genes that are directly and indirectly controlled by D-Six4. Preliminary results indicate that a range of genes are differentially expressed in the *Drosophila* mutants. The majority of the differentially expressed genes were of unknown function (>60%), the remaining known genes had a variety of functions including DNA/RNA binding activities, transcription factors, protease activity, protease inhibition and cytochrome p450. When these data from the transcription profile have been analysed and confirmed by RT-PCR it will be compared with results obtained from similar analyses carried out with DM1 patient tissue. We envisage that this type of approach will greatly enhance our knowledge of the disease pathology.

19) DM1 and DM2 transcriptome profiling reveals global gene expression changes

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The myotonic dystrophies (DM) are now collectively recognized as a clinically and genetically heterogeneous group of neuromuscular disorders, characterized by autosomal dominant inheritance, muscular dystrophy, myotonia, and multi-system involvement. For DM1, the variable phenotype and anticipation have been linked to a (CTG)n repeat expansion in *DMPK* in 19q13.3. For DM2, the variable phenotype has been linked to a (CCTG)n repeat expansion in *ZNF9* in 3q21.3. In both DM1 and DM2, expression of the mutant alleles containing (CUG)n and (CCUG)n expansions, respectively, leads to the formation and accumulation of mutant RNA transcripts in ribonuclear inclusions. For DM1, transcription of the mutant repeat and accumulation in ribonuclear inclusions appears to be both necessary and sufficient to cause disease (RNA gain-of-function model). Ribonuclear inclusions have been shown to bind various *trans*-acting factors involved in splicing, resulting – directly or indirectly – in the aberrant splicing of at least five gene products (*CTNT*, *IR*, *MAPT*, *CLCN1* and *MTMR1*). Given the similar pathophysiological manifestations of DM1 and DM2, we hypothesized that they share a common pathological pathway(s). To test these hypotheses, we used gene expression profiling with microarrays. We globally compared expression in skeletal muscle biopsies of normal, DM1 and DM2 individuals. Comparison of expression profiles of a pool of 10 normal skeletal muscle biopsies with DM1 (6) and DM2 (4) biopsies showed considerable overlap in the genes down-regulated among DM1 and DM2 patients and dysregulation of several functional gene categories, including muscle, calcium channel, DNA repair, ribosomal proteins, RNA binding and proteasome. Numerous skeletal muscle-specific genes (e.g., myosin heavy chain, β-tropomyosin, desmin, several troponins and skeletal muscle sodium channel 1) were specifically affected. Similar changes were seen *in vitro* with primary myoblast cultures established from skeletal muscle of two DM1 patients for either total RNA or nuclear and cytoplasmic fractions separated. Expression profiling of nuclear and cytoplasmic fractions of DM1 myoblasts that displayed an inability to differentiate into multinucleated myotubes identified sets of genes down-regulated in both fractions (e.g., β-tropomyosin) and down-regulated in only the cytoplasmic fraction (e.g., troponin I, the slow-twitch/type-I fibre skeletal muscle isoform; myomesin2, a titin-associated protein). Taken together, these data suggest a global *trans*-effect of the transcribed expansion on the DM1 transcriptome. As the DM1 and DM2 cellular pathologies show considerable overlap, expression profiling will provide new and valuable insights into the pathology of DM.

20) Six5-target genes and DM1 symptoms

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The CTG repeat expansion located in the 3' untranslated region of DMPK causes suppression of the expression of *SIX5*, the neighbouring homeobox gene. *SIX5* is expressed tissues affected by DM1 and mice deficient for *Six5* display several DM1-related defects such as cataracts and hypogonadism. To elucidate the mechanisms by which *SIX5* haploinsufficiency contributes to the development of DM1 symptoms, we have previously established an experimental system to identify downstream target genes whose expression is regulated by SIX5. In this study, we sought to identify SIX5-target genes in the lens to define how *SIX5* deficiency causes cataracts. Overexpression of constitutively active Six5 (VP16-Six5) in cultured human lens epithelial cells upregulated (>2-fold) the expression of 227 genes out of 12,814 genes examined. Importantly, the following genes were identified as potential targets: 1) 5 genes strongly implicated in cataractogenesis and 2) >10 genes encoding proteins important for the maintenance of lens transparency (ion-channels and transporters). We are currently investigating the expression pattern of these genes in *Six5*-deficient animals.

21) *SIX5* target genes: functional links between *SIX5* and DM1 symptoms

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We report the identification of the DNA binding site of SIX5, using random oligonucleotide selection and bacterially expressed recombinant GST-SIX+HD (a GST fusion protein of the SIX- and homeo- domains of SIX5). Using bioinformatic screens of the human genome we have identified gene sequences containing the SIX5 binding site which indicate likely candidates for regulation by SIX5. Gel shift assays showed that recombinant GST-SIX+HD binds to these sequences which were identified in the following genes: dopamine receptor gene (*DRD5*), calcium-activated potassium channel gene (*SK3*), *myogenin* (*MYOG*), *DOCK180* and the insulin receptor gene. In addition, we have shown that GST-SIX+HD binds to intron 1 of *ZNF9*. These data suggest a link in the molecular pathology of DM1 and DM2 and might explain some of the overlap in phenotypes.

22) Problems with antibodies

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Although useful information about the subcellular behaviour of proteins can be obtained by transfection of cultured cells with corresponding cDNAs, specific antibodies are essential to understand the behaviour of endogenous protein in normal and pathological states. This presentation will summarize the experience of our laboratory in producing antibodies against human DMPK, Six5 and, more recently, CUG-binding proteins.
The main problem with DMPK was cross-reaction with other proteins, including proteins more abundant than DMPK itself. This was partly due to other cyclic-AMP dependent kinases, such as MRCK, with significant sequence homology with DMPK. It was partly solved by avoiding the catalytic domain as immunogen, but only finally solved by the use of a panel of monoclonal antibodies (mAbs) against several different DMPK epitopes. A second problem that DMPK may share with other globular enzymes is the protein conformation requirement for antibody binding; thus, mAbs that bind denatured protein may not bind native protein and vice-versa. This problem can make immunolocalisation difficult.

Six5, a nuclear transcription factor, is also potentially subject to cross-reaction because its homeodomain and Six domain sequences encoded by exon A are closely homologous to similar sequences in five other proteins in the Six family. However, we have produced panels of mAbs against exon A and exon B and have shown by epitope mapping that they all recognise sequences outside the shared domains. Six5, however, is much less abundant than DMPK and it is difficult to immunolocalise endogenous Six 5 in tissues (although it is easily detected on western blots). Another problem with nuclear proteins is epitope masking where epitopes are rendered inaccessible to antibody by the association of other proteins and nucleic acids with the antigen. This problem is especially significant where antigen mobility is limited.

It seems likely that similar problems will be encountered with CUG-binding proteins. Thus, CUGBP-1 is a member of a large family of related proteins (Brunol) with high sequence homology in their C-terminal domains and three separate genes for human muscleblind have been identified (MBLL, MBNL and MBXL). Unlike Six5, they are found in the cytoplasm as well as in the nucleus. Preliminary antibody studies will be presented.

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23) Characterization of potential DMPK substrates in a cellular blebbing model

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Current studies have reached a consensus revolving around the importance of the metabolism of mutant transcripts in the pathology of myotonic dystrophy types I and II. However, a depletion of DMPK due to retarded processing of mutant transcripts has been proposed to contribute to certain symptoms of DM1 in a tissue-specific manner, such as cardiac conduction defects. Little is currently known regarding the role of DMPK in the metabolism or structure of affected tissues. Elucidation of its activities would allow us to determine the effects its loss would have upon key sites of expression, such as cardiac intercalated discs.

DMPK is most closely related to a number of kinases that are regulated by the Rho subfamily of p21 GTPases. These kinases modulate cytoskeletal structure during a diverse range of processes, primarily by the stimulation of actomyosin contractility through the phosphorylation of the myosin regulatory light chain (MRLC). For example, the related rho-kinase (ROK) both directly phosphorylates MRLC and indirectly stimulates this through the phosphorylation and consequent inhibition of myosin phosphatase. Furthermore, DMPK has recently been shown to strongly stimulate cytoplasmic blebbing (in the absence of cell death) in cultured cells, an activity also believed to depend upon MRLC phosphorylation, implying that DMPK shares this substrate specificity. We have tested this in a cellular model of blebbing and found that MRLC phosphorylation at relevant sites was unaltered, despite that fact that the majority of the cells were dramatically affected. We have also tested two other substrates of ROK involved in the regulation of cytoskeletal structure, the ezrin-radixin-moesin family and cofilin. Again, we found these to be unaffected. These surprising results suggest that DMPK may have a mode of action distinct from its related kinases, perhaps consistent with its divergent C-terminal structure. As cultured cells which express DMPK also "round up" concomitant with blebbing, indicative of detachment from the substratum, we are currently examining the possibility that DMPK modulates focal adhesion formation.

24) Alternative splicing controls DMPK structure, enzymatic activity and subcellular localization

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Transcripts of the *DMPK* gene are subject to constitutive and regulated (cell-type specific) modes of alternative splicing, which are conserved between man and mouse. A down-regulation of DMPK protein levels, in addition to an imbalance in the DMPK splice isoform profile, is thought to play a role in aspects of the pathophysiology of DM1 that cannot be easily explained with dominant-negative interference by expanded mRNA, like heart arrythmia, CNS features like hypersomnia, or congenital DM1. We have investigated how structural subdomains determine biochemical and cell biological properties of DMPK splice isoforms.

Mouse *DMPK* cDNAs encoding the six major splice isoforms, including several mutants, were cloned and individually expressed in COS-1 cells. A newly developed kinase assay (using individual peptides from a peptide library as substrate) revealed that all DMPK isoforms are Lys/Arg-directed kinases, which is in line with the fact that DMPK is a member of the Rho kinase/PKB family. Individual DMPK isoforms displayed comparable transphosphorylation activity and sequence preference for peptide substrates. However, DMPK autophosphorylation and phosphorylation of MYPT1 (myosin phosphatase targeting subunit), as putative *in vivo* target of DMPK, were dependent on presence or absence of an alternatively spliced VSGGG-motif and the nature of the C-terminus (3 different variants). In-gel effects of the VSGGG-motif on the migration behaviour of full-length kinase provide evidence for a model in which this motif mediates 3-D-conformational changes in DMPK isoforms. Finally, the three different C-termini endow DMPK with the ability to bind exclusively to either ER or mitochondria, or adopt a cytosolic location (*see for more detail Abstract van Herpen et al.*).

We hypothesize that DMPK splice isoforms have cell-type and location-dependent substrate specificities with a role in cytoarchitectural-organellar dynamics or ion homeostasis.

25) C-terminal targeting-motifs direct DMPK to the endoplasmic reticulum or mitochondrial outer membrane

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The genetic mutation causing myotonic dystrophy type 1 is a (CTG)n-trinucleotide repeat expansion in the 3'-UTR of the myotonic dystrophy protein kinase (*DMPK*) gene. Cell-type specific alternative splicing of *DMPK* transcripts results in six DMPK splice isoforms with different C-termini, in man as well as in mouse. DMPK isoforms can have a hydrophobic tail (Tail 1, isoforms A/B), a less hydrophobic tail (Tail 2, isoforms C/D) or a truncated tail (Tail 3, isoforms E/F). We have investigated the influence of DMPK C-termini on subcellular localization and show that different C-termini direct DMPK to specific subcellular locations.

Confocal laser scanning microscopy and immuno-electron microscopy (EM) studies were performed on Neuro2A cells transfected with cDNAs encoding single DMPK splice isoforms. Using organellar markers, colocalization of Tail 1-containing isoforms was found with the endoplasmic reticulum (ER) and the nuclear envelope. Presence of Tail 2 resulted in a mitochondrial localization. Immuno-EM analysis showed that Tail 2 directs DMPK to the mitochondrial outer membrane. DMPK isoforms containing Tail 3 exhibited a cytosolic localization. Using DMPK deletion constructs and YFP fusion proteins we could demonstrate that the ultimate 47 amino acids of Tail 1 form the minimal region required for targeting to the endomembrane system. Likewise, the final 45 amino acids of Tail 2 conferred mitochondrial targeting properties to DMPK, but here correct localization was dependent on presence of the internal alpha-helical coiled-coil domain. The mitochondrial localization was found to be saturable, suggesting a receptor-type interaction. The relationship of DMPK targeting motifs with motifs in other known ER-mitochondrial resident proteins will be discussed.

We hypothesize that the partitioning behaviour of DMPK isoforms may specify their role in regulation of ion-homeostasis or ER-mitochondrial dynamics.

26) Towards the understanding of the myotonic dystrophy type 2 pathogenesis

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Myotonic dystrophy type 2 (DM2, MIM #602668) is a dominant inherited disorder with multisystemic clinical features stickling similar to adult-onset myotonic dystrophy type 1 (DM1, MIM #160900). The mutation responsible for DM2 is an untranslated CCTG expansion in intron 1 of the *ZNF9* gene on chromosome 3q21.3. This gene encodes for a small, highly conserved, protein of 19kDa whose biological function and role in the disease pathogenesis are still unclear.

As part of a multidisciplinary project focused on the understanding of the DM2 molecular basis, we developed a rapid genetic test for DM2 mutation which combines a long-PCR and Southern blot protocol to reveal expanded *ZNF9* alleles. This protocol allowed us to detect expanded alleles up 15 Kb and to reveal somatic heterogeneity. Using this protocol we analyzed 20 DM1-negative patients and identified the DM2 mutation in 12 of them. This result confirm the existence of at least another locus involved in DM phenotype. To analyze the subcellular localization and the expression of the ZNF9 protein, we have performed immunofluorescence (IF) experiments on human and rat skeletal muscle. In longitudinally sectioned myofibres of both species, IF reactivity for ZNF9 showed a regular transverse banding pattern throughout the fibre width. The transverse bands width were similar to sarcomeric I-bands, and showed in some instances a beaded appearance. In double IF experiments observed by confocal microscopy, ZNF9 and the sarcoplasmic reticulum (SR) ca/Mg ATPase (SERCA1) localized to the same transverse elements, but the two signals did not show a superimposition in merged images. These data indicate that in skeletal myofibres, ZNF9 localized to I-band associated elements, other than the SR terminal cisternae. Such a distribution does not match exactly to the one observed for DMPK, the protein product of the DM1 gene, therefore suggesting different functions for these proteins.

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27) Foci formation and the disruption of splicing by CUG-repeat RNA are separable events

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The RNA gain of function hypothesis proposes that the expression of expanded repeats leads to a disruption of normal splicing of a subset of pre-mRNAs. Two proteins, CUG binding protein (CUG-BP) and muscleblind (MBNL1), are thought to be involved in the pathogenesis of DM1. MBNL1 colocalises with the expanded repeats in the nuclear foci. CUG-BP binds short CUG motifs and regulates splicing of three pre-mRNAs known to be mis-regulated in DM1 striated muscle. The splicing pattern observed in DM1 patients can be reproduced in normal cells by expression of CTG repeats or CUG-BP. Mutation of CUG-BP binding sites block the effects of repeats or CUG-BP. Interestingly, CUG-BP is not detected in the nuclear foci containing the expanded CUG repeats. Thus, there seems to be a paradox between MBNL and CUG-BP: CUG-BP is functionally linked with the aberrant regulation of splicing but not physically associated with foci while MBNL is physically linked with the foci but its function is unknown.

Using a transient transfection assay, we have studied the relationship between the formation of nuclear foci and the disruption of normal splicing regulation of target genes. Two pre-mRNAs that are affected by the CUG repeats in DM1 striated muscle are cardiac troponin T (cTNT) and the insulin receptor (IR). Coexpression of a DMPK transgene containing 960 CUG repeats with a cTNT or IR minigene in COS cells reproduces the splicing pattern observed for both genes in DM1 (increased exon inclusion for cTNT and increased exon skipping for IR). The identical minigene containing 960 CAG repeats has little to no effect on cTNT or IR splicing as does a DMPK minigene with no repeats. *In situ* hybridization with Cy3-labeled peptide nucleic acid (PNA) probes demonstrates that both CUG and CAG repeats form RNA foci.

Since the expanded repeats in DM1 patients colocalise with MBNL1, 960 CUG or 960 CAG repeats were cotransfected with a GFP-MBNL1 fusion protein. Unexpectedly, both CUG and CAG repeats recruit GFP-MBNL1 into foci. Furthermore, GFP-MBNL2 and GFP-MBNL3 are also recruited to CUG and CAG foci. Similar amounts of RNA and foci formation were formed by CUG and CAG repeats. The same relationship between foci formation and altered splicing regulation was observed in C2C12 myoblasts and HeLa cells, suggesting that the effect of CUG repeats and foci formation is not cell line specific.

In summary, the formation and number of foci does not correlate with the aberrant regulation of alternative splicing of two known DM1 targets (cTNT and IR). The nuclear foci formed by CUG and CAG repeats can recruit GFP-MBNL1, but only CUG repeats reproduce the splicing pattern observed in DM1.

28) Gene expression profiles of mouse C2C12 cells which stably express human DMPK with 160 CTG repeat

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The molecular basis of Myotonic Dystrophy (DM) is explained for the expansion of CTG triplet repeat located in the 3'-untranslated region (3'-UTR) of DM protein kinase (DMPK). Our stable cell lines (mouse C2C12) expressing human DMPK with 160 CTG triplet repeat shows several phenotypical change; slow differentiation, higher sensitivity to an oxidative stress, and so on. To investigate the gene expression profile of this cell, we performed Northern analysis by using several genes as probes. We observed that the expression level of *EXP* and MBLL mRNA increased in the cell with 160 CTG repeat, compared to the cell with 5 CTG repeat.

On the other hand, our DNA microarray analysis revealed that many genes are differently expressed in the C2C12 cells with DMPK/long CTG repeat. Especially, we confirmed that gene expression of the secretory carrier membrane protein 3 was increased, while endosulfine alpha was decreased by Northern analysis.

Moreover, we further examined promoter regions of genes which showed marked expression levels by DNA microarray. The gene group having low expression level had an AP-1 region with a significant percentage. On the other hand, SRF, ICSbf and myosin-specific sequence were mainly observed in the high expression gene group. These results may favour an "RNA gain of function" pathway caused by the expansion of CTG triplet repeat.

29) Inhibition of myogenesis in transgenic mice expressing the human *DMPK* 3' UTR

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Myotonic Dystrophy (DM) is a multisystemic disorder caused by expanding CTG or CCTG repeat sequences within the *DMPK* or *ZNF9* genes respectively. DM results in delayed muscle development, muscle weakness and wasting, cardiac conduction abnormalities, cognitive defects and eye abnormalities such as cataracts. One theory explaining the molecular basis of DM is a dominant gain of function of these repeat sequences within their respective mRNA molecules. Recent evidence from cell culture and mouse models suggests that the CUG repeats alone, although required, may not be sufficient for the entire phenotypic spectrum of the disease and that additional *DMPK* 3' UTR sequence may be required for muscle wasting in particular. Here we report that expression of the *DMPK* 3' UTR utilizing endogenous regulatory sequences with a wild type complement of CUG repeats (11) or mutant number of CUG repeats (91) can result in abberant and delayed muscle development *in vivo*. In addition, adult transgenic animals with 91 CUG repeats display muscle atrophy at 3 months of age. When myoblasts from both 11 and 91 CUG repeat transgenic mice are cultured, both display reduced fusion potential with a greater reduction observed in the 91 CUG repeat myoblast cultures. Taken together these data indicate that expression of wild type numbers of CUG repeat sequences in conjunction with the *DMPK* 3' UTR is sufficient to interfere with muscle development *in vivo* and that inclusion of additional repeats abrogates the condition.

30) Mouse muscleblind gene knockout models for myotonic dystrophy

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We have proposed a toxic RNA model for the myotonic dystrophies in which transcription of mutant *DMPK* and *ZNF9* genes results in the synthesis of dsCUG and dsCCUG RNAs that are sequestered by members of the human muscleblind-like (MBNL) protein family. Interactions between mutant *DMPK* and *ZNF9* gene transcripts and the muscleblind proteins results in the formation of aberrant ribonucleoprotein complexes in the nucleus and the corresponding loss of normal MBNL protein functions.

To test this model, we compared the expression of mouse *Mbnl* and *Dmpk* genes both in adult tissues and during embryonic development and subsequently generated mouse *Mbnl* knockout lines to examine the physiological effects resulting from specific loss of Mbnl proteins. The mouse genome contains three *Mbnl* genes (*Mbnl1*, *Mbnl2/Mbll*, *Mbnl3/Mbxl/Mblx/Chcr*) related to the *Drosophila mindmelt/muscleblind* (*mm/mbl*) gene. Based on our expression analysis, we selected *Mbnl1* for our initial knockout studies. We will report preliminary behavioural, biochemical and physiological characterization of *Mbnl1* knockout mice and describe the relevance of our findings to the toxic RNA model.

31) Ribonuclear inclusions in the central nervous system in myotonic dystrophy type 1

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Manifestations of DM in skeletal muscle result from toxicity of RNA produced from the mutant gene. These repeat expansion RNAs accumulate in the myonucleus, forming ribonuclear inclusions (RIs). Do RIs also form in the central nervous system? We used fluorescence in situ hybridization and immunofluorescence to examine postmortem brain tissue obtained from patients with DM1 (n=7) or controls (Huntington disease, n=2; Alzheimer disease, n=2; no neurologic disease, n=4). RIs were not observed in any region of the CNS in controls. By contrast, RIs were found in many neuronal populations in every patient with DM1. In cerebral cortex, RIs were present in >85% of neurons and were distributed throughout all cortical layers and in all lobes. RIs were also abundant in hippocampus, thalamus, substantia nigra, and brainstem tegmentum. In the cerebellum, RIs were observed only in Purkinje cells and were smaller and less intensely fluorescent than in cerebral cortex. Small RIs were also present in oligodendrocytes of the subcortical and deep hemispheric white matter. Of note, RIs in cerebral cortical neurons were larger, more numerous, and more intensely fluorescent than in skeletal muscle from the same patient. Muscleblind proteins were redistributed into RIs in neurons. Analyses of other RNA binding proteins are in progress and will be presented. These studies show that mutant DMPK mRNA is expressed in neurons and could be a factor in producing the CNS manifestations of DM1.

32) Analysis of alternative splicing in muscleblind deficient *Drosophila*

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The human homologues of the *drosophila* gene *Muscleblind* have been implicated in the pathogenesis of myotonic dystrophy due to the fact that the proteins they encode co-localise with expanded repeats in DM cell lines. It has also been seen that mutant flies lacking muscleblind show disruption of their z-band organisation, a feature also seen in Myotonic Dystrophy patients.

There is an increasing amount of evidence to suggest that disrupted alternative splicing of certain genes may be a key mechanism underlying the pathophysiology of Myotonic Dystophy. Aberrant splicing has been reported in several genes including cTNT, Insulin Receptor, Tau and more recently ClC-1 chloride channel protein.

It has been suggested that the sequestration of muscleblind proteins may represent a bystander effect, not related to DM pathophysiology. In order to examine the link between muscleblind sequestration in DM and the observed alternative splicing defects we have examined alternative splicing in mutant flies lacking muscleblind. In particular our interest has focussed on genes encoding proteins involved in the z-band structure, concentrating initially on alpha-actinin.

Several *muscleblind* knockout flies are available, the majority are lethal at embryonic stage 17, or hatching. The genomic organisation of *drosophila* alpha-actinin was established from database searches and alternatively spliced forms identified. PCR primers were designed to amplify the different splice-forms. RT-PCR was performed on *muscleblind* (-/-) and *muscleblind* (+/+) control flies and the products analysed by restriction digest and sequencing. Our initial data supports the theory that loss of muscleblind leads to an alteration in the splicing pattern of alpha-actinin.

33) A *Drosophila* model of myotonic dystrophy

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Myotonic dystrophy is a relatively common genetic disease, but its mode of action has until recently remained elusive. It is caused by an expansion of CTG repeats in the 3' untranslated region of the *DMPK* gene; this is unusual since the mutation does not directly effect the structure or function of a protein. Therefore, the mutation must act through a DNA or an RNA based mechanism (or both), and the prospect of a mutation asserting its effects through RNA is unusual to say the least. Nevertheless, it has recently been demonstrated that transgenic mice expressing expanded CTG repeats in an unrelated gene's mRNA exhibit symptoms resembling those of the human disease, albeit to a lesser extent. Just as in patients, mutant mRNA forms aggregates within muscle cell nuclei and muscle structure is perturbed. It is also known that in human cells, expression of mRNA containing expanded CUG repeats is sufficient to cause certain other mRNAs to be mis-spliced, presumably accounting for the observed physiological effects. To model myotonic dystrophy in *Drosophila*, we have established transgenic lines expressing expanded CTG repeats in the 3' untranslated region of a *GFP* marker. Surprisingly, flies expressing the expanded repeats show no adverse phenotype, but the expanded repeat RNA does form nuclear foci, although not in all cells in which it is expressed. We have also noticed that a significant proportion of the expanded repeat RNA contains less repeats than the DNA, although it is not known whether this effect occurs in humans.

34) RNA mechanism for myotonic dystrophy 1: elevation of CUGBP1 disorders myogenesis through the disruption of MEF2A and p21 pathways

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Myotonic Dystrophy 1 (DM1) is caused by expansion of CTG trinucleotide repeats within the 3' UTR of DMPK gene. Accumulation of long RNA CUG repeats in DM1 patients increases levels of a CUG binding protein, CUGBP1. To examine the role of the un-programmed elevation of CUGBP1 in DM1 pathology, we generated transgenic mice that overexpress CUGBP1 mainly in skeletal muscle. Analyses of CUGBP1 transgenic mice showed that genetically un-programmed elevation of CUGBP1 in mice disrupts myogenesis. The severity of muscle disruptions correlates with the levels of CUGBP1 elevation. Mutant mice with the levels of CUGBP1 comparable to patients with congenital DM1 show significant delay in muscle development. CUGBP1 transgenic mice with the levels of CUGBP1 similar to adult form of DM1 develop muscular dystrophy. Examination of key regulators of muscle differentiation showed that protein levels of MEF2A and p21 are misregulated in skeletal muscle of CUGBP1 transgenic mice and in DM1 patients. These data suggest that the "un-programmed" elevation of CUGBP1 increases p21 and MEF2A levels at wrong time of development leading to muscle deficiency.

Investigation of molecular mechanisms by which CUGBP1 increases expression of p21 and MEF2A showed that CUGBP1 interacts with MEF2A and p21 mRNAs and increases translation of these proteins. In cell culture models, expression of CUGBP1 is induced during myoblast differentiation, which leads to the up-regulation of p21 and MEF2. In DM1 cells with elevated levels of RNA CUG repeats, protein levels of CUGBP1 and intracellular localization of CUGBP1 are altered. These alterations cause a reduction of protein levels of p21, MEF2A and myogenin. Taken together, data from transgenic animals and from tissue culture models support the hypothesis that the elevation of CUGBP1 is, at least in part, responsible for skeletal muscle pathology in DM1 patients.

35) Aberrant splicing of the ryanodine receptor in myotonic dystrophy

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It was reported that mRNAs for chloride channel, cardiac troponin T, insulin receptor and myotubularin-related 1 were aberrantly spliced in muscles from myotonic dystrophy (DM). In all cases, the developmentally regulated splice switch that involves a choice between two or more alternative isoforms is skewed, resulting in preferential expression of the isoform that is usually expressed in immature or non-skeletal muscle tissues. We previously reported the induction of the mRNA of small-conductance Ca^{2+}-actvated K^{+} (*SK3*) channel that is usually expressed in immature fibres. Taken together, we postulate that there is a maturation-related abnormality in DM that explains the abnormal splicing and transcription. Based on this hypothesis, we investigated the splicing of two candidate mRNAs, which are developmentally regulated.

We used 28 muscle specimens; 10 from DM1, 5 from ALS, 5 from PM, 2 from limb-girdle muscular dystrophy and 6 from normal control. Myotubes cultured from 2 muscle specimens; 1 from DM and 1 from normal control, were also used. We examined splicing pattern of insulin receptor, ryanodine receptor of skeletal muscle type (RyR1) and β-dystroglycan, using RT-PCR. The total amount of RyR3 mRNA, which is expressed in immature muscles, were also quantified.

We found a significant increase of an alternatively spliced isoform of RyR1. The alternative splicing results in the insertion of 5 amino acids at modulatory region of the receptor and the isoform is normally expressed in undifferentiated muscles. The other splicing isoform of RyR1 was not significantly altered. The splicing of β-dystroglycan and the total amount of mRNA for RyR1 and RyR3 did not differ significantly.

The increase of an immature isoform of RyR1, supports our hypothesis of maturation-related abnormality in DM. Furthermore, RyR1 channel is a major calcium release pathway from sarcoplasmic reticulum and regulate contraction of skeletal muscle. It is possible that the aberrant isoform may be responsible for muscular degeneration of DM, although functional studies for this isoform are needed,

36) Developmental roles of the muscleblind and CUG-BP homologues in *C. elegans*

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Loss of function of MBNL, MBLL and MBXL, the Muscleblind family of RNA-binding proteins and increased activity of CUG-BP, the prototype of the CELF family of RNA-binding proteins, have been hypothesized to contribute to DM pathophysiology. In this study we have used the nematode, C. elegans, to study the developmental roles of these proteins.

A gene showing 58% homology with the Muscleblind family of proteins is located on the *Caenorhabditis elegans'* cosmid K02H8. K02H8 is found on the long arm of the X chromosome in *C. elegans'*. We silenced *K02H8.1* by designing siRNAs directed to the second exon of *K02H8.1* whose amino acid sequence is homologous to the first Zinc knuckle in MBNL and MBLL: **TLEVCR**. We also directed silencing to the 1st exon of *K02H8.1* that does not show homology to any of muscleblinds, in order to confirm single gene silencing with the same reproducible "null" phenotype compared to the siRNA of the second exon of *K02H8.1*. We injected 1 µg of K02H8.1 siRNA into gonads of wild type adult hermaphrodites. The next generation was viewed at the plate level for any unusual phenotypes and then viewed in more detail under Nomarski. At the plate level, we saw that at least 15-20% worms in which KO2H8.1, were silenced were lethargic and small. Under closer inspection, we viewed young larvae undergoing necrosis of the head neurons and ventral/dorsal nerve cord but the motor movements were normal until the neurons were totally destroyed at about larval stage 4. Embryonic arrests were also seen. Currently, we are making [K02H8.1::gfp] constructs by fusing a 4 kb genomic fragment upstream of the first exon of K02H8.1 to sequences encoding a green fluorescent protein (GFP) in order to determine its expression pattern of KO2H8.1. We are also injecting K02H8.1 siRNA into different mutant background labeled with GFP to decipher when and where during development KO2H8 is required.

etr-1 encodes a muscle-specific ELAV-type RNA-binding protein, whose homologues include Drosophila ELAV and human CUG-BP . *etr-1* function is needed for embryonic muscle development, suggesting that *etr-1's* homology to CUG-BP (implicated in myotonic dystrophy) echoes conserved roles in development (Milne et al., 1999). We have replicated the gene silencing experiments done by Milne et al. resulting in muscle defects and embryonic lethality. In complimentary studies we have over-expressed *etr-1* by injecting *etr-1 coda* in to a wild type background. Over-expression of *etr-1* results in failure to hatch. Those worms that did hatch did not have a posterior and demonstrated extra cuticle in around the head. The data obtained demonstrate that the Muscleblind and CUG-BP proteins control important developmental pathways and misregulation of these proteins could contribute to the development of one or more features of DM pathophysiology.

37) Insights from a C2C12 myoblast model of the effects of the mutant DMPK 3'UTR RNA on myogenic differentiation

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Over the past several years, we have established a cell culture model to study the effects of the *DMPK* 3'UTR using C2C12 mouse myoblasts. To establish this model, the human *DMPK* 3'UTR sequence containing either a pathogenic $(CUG)_{200}$ repeat tract or a wild-type $(CUG)_5$ tract was fused to a reporter gene (*lacZ or GFP*) and expressed in C2C12 cells. C2C12 are muscle precursor cells originally isolated from injured adult mouse muscle that have proven an excellent system to study myogenic differentiation. When cultured appropriately, the C2C12 cells, exit the cell cycle and form multinucleated myotubes. Using this model, we have shown that transcripts containing the mutant DMPK 3'UTR aggregate into nuclear foci and also block myogenic differentiation. This differentiation defect may represent muscle development abnormalities found in congenital DM patients and/or point to defects in muscle regeneration, which could contribute to muscle wasting in adult patients.

Using our cell culture model, we have previously shown that the programmed up-regulation of *myogenin* and *p21* is severely blunted in cells expressing the mutant DMPK 3'UTR, suggesting defects in the early steps of myogenic differentiation. MyoD, a transcription factor required for myoblast differentiation during muscle regeneration, is thought to be required at these early stages. We now show that MyoD protein levels are compromised in cells that express the mutant DMPK 3'UTR transcripts and that this reduction correlates with blunted E-box (MyoD binding site) mediated gene expression at time points critical for initiating differentiation. We show that this effect occurs at a post-transcriptional stage. Importantly, restoring MyoD levels rescues this defect. We conclude that mutant DMPK 3'UTR transcripts disrupt myoblast differentiation by reducing MyoD levels below a threshold required to activate the differentiation program and suggest that this may be relevant to muscle defects in congenital DM and muscle regeneration in adult DM.

38) Molecular pathogenesis of the DM2 CCTG expansion

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The DM2 expansion is larger than previously reported microsatellite expansions with CCTGs that can exceed 44 kb (mean ~20 kb). Similar to DM1, RNA containing the repeat accumulates as nuclear foci. Because haploinsufficiency has been suggested to play a role in DM1, we performed a series of experiments to determine if the DM2 expansion interferes with normal *ZNF9* transcription and to define what portion of the *ZNF9* transcript is present in the nuclear foci. Consistent with normal splicing, RNA *in situ* hybridization in affected muscle and fibroblasts indicate that transcripts containing exon 1 (5' of the expansion) or exon 5 (3' of the expansion) localize to the cytoplasm but do not co-localize with the CCUG-containing nuclear foci. Probes to sites distributed throughout intron 1 detect transcripts as small punctate signals that are evenly dispersed throughout the nucleus, but that do not co-localize to the much larger nuclear CCUG containing foci. The effects of the CCTG expansion on *ZNF9* expression were independently evaluated using monoallelic mouse-human cell lines containing either a normal or an expanded (~1000 repeats) human DM2 allele. RT-PCR between exons 2 and 1 amplified spliced human transcripts from cells containing either the normal or the expanded allele and Northern analysis showed comparable levels of human *ZNF9* mRNA with no evidence of unprocessed transcripts. Similarly, equal transcript levels were detected by Northern analysis in diploid fibroblasts from normal and affected individuals. Consistent with a gain rather than a loss of function model, our results demonstrate that the DM2 expansion does not prevent transcription or splicing of *ZNF9*, the repeat-containing nuclear foci do not contain intron 1 or other portions of the transcript, and the DM2 expansion does not reduce allele specific or overall *ZNF9* mRNA levels.

39) Somnolence in DM1: cause, assessment and management

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Somnolence (or excessive-daytime sleepiness) is an extremely common, but under recognised, symptom in DM 1. It can be a major cause of morbidity for the patient, and a source of considerable irritation for other family members. It may be multifactorial in origin. Recent research has indicated that it is potentially treatable.

Cause Evidence will be presented to suggest that it is mainly central in origin, relating to underlying cerebral dysfunction. In some patients, there may be a contribution from sleep fragmentation associated with sleep apnoea.

Assessment Although the clinical history of somnolence may be clear it is helpful to have objective and quantitative measures of daytime sleepiness. This is important for clinical trials, but is also useful in everyday practice to help assess response to intervention. The Epworth Sleepiness Scale is widely used, in large part because of its simplicity. Objective assessments of sleepiness include the multiple sleep latency test (MSLT), the maintenance of wakefulness test (MWT), and modifications of the MWT. Recent studies in DM 1 have suggested that each of these standard measures have limitations when applied to DM.

It is important to exclude sleep apnoea. Formal overnight sleep studies in hospital are expensive and inconvenient for the patient. More limited overnight oximetry, using a home oximeter, is satisfactory to exclude major abnormality.

Management If troublesome daytime somnolence is identified, then further assessment is indicated. Routine "sleep hygiene" advice should be given. Sleep apnoea should be excluded. Then, it is appropriate for a trial of modafinil.

Since Damian's *et al* (2001) open study of modafinil, which suggested substantial benefit, two double-blind studies have been reported. The findings of the Oxford study (Talbot *et al* 2003) will be presented and compared with the North American (MacDonald *et al* 2002) study. The findings of the French study are awaited.

40) Neuropsychiatric, neuropsychological and neuroimaging studies in myotonic dystrophy type 1: significance and clinical correlates?

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Background. In a previous study we demonstrated that patients with DM1 had a selective visual-spatial impairment on neuropsychological tests which correlated with hypoperfusion in frontal and temporal poles determined by PET scans more than with white matter hyperintense lesions observed on brain MRI.

Aims: On the basis of our previous PET studies the aims of our study are: (i) to further investigate into frontal lobe function to determine whether there is a typical cognitive or behavioural trait in DM1; (ii) To correlate neuropsychological test results with brain MRI findings; (iii)To explore the social consequences of this brain involvement.

Methods: Twenty-one patients with moderately severe DM-1 (500-700 CTGn)(age range: 25-62. mean age 44.1 ± 9.5; 8 women and 13 men) from 11 unrelated families underwent neuropsychiatric interviews, neuropsychological tests selective for frontal lobe function, brain CT or MRI scans. Results were compared to 20 age, sex and education-matched controls (age range: 25-62. mean age 44.3 ± 8.7; 9 women and 11 men). **Results**: (i) Neuropsychiatric interviews demonstrated an avoidant trait personality disorder in DM1 compared to controls ($p < 0.05$). (ii) Neuropsychological tests: DM1 patients had significantly lower scores on tests of frontal lobe function (Tower of London Test, Coloured Word Stroop Test, Wisconsin Card Sorting Test) compared to controls ($p < 0.001$). (iii) Neuroimaging demonstrated: mild to moderate general cerebral atrophy and temporal cortical atrophy in 9 of 21 patients. Small non-confluent, diffuse white matter hyperintense lesions were present in 5 of 21 patients while in 3 patients these lesions were greater than 2 cm in some areas. In only 1, 57 year-old woman, the lesions were confluent and greater than 2 cm.

Conclusions: (i) The low scores on those tests selective for frontal lobe function suggest a frontal disexecutive syndrome is present in these patients. (ii) All patients show an avoidant personality trait disorder. (iii) There seems to be no correlation between the neuropsychological test scores and the degree of cerebral atrophy and white matter hyperintense lesions. Cognitive and behavioural limitations associated with this condition adversely affect the patients in their educational and employment levels independently of the degree of muscle weakness or myotonia. In general there was a lower educational level compared to the general population. Eighteen of 21 patients were reluctant to make new friends, carry out new activities or take personal risks. Fourteen of 21 patients were employees with no responsibility or decision-making positions. The remainder were unemployed and showed no concern over being unemployed.

Significance: Awareness that the brain is involved in DM1 is helpful in understanding why some patients with myotonic dystrophy may not function as well as would be expected from their physical disabilities. In addition, knowledge that the avoidant trait disorder is a feature of DM1, is helpful in planning medical visits and examinations on a regular basis, because these patients are reluctant to seek medical attention. Timely intervention on potentially fatal cardiac arrhythmias or respiratory problems has obvious therapeutic and prognostic implications.

41) Educational and psychological group observations of children and juveniles with congenital myotonic dystrophy

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There is a lack of systematic knowledge of the behavioural features in children and juveniles with congenital myotonic dystrophy. Generally the patient group continues to be regarded as mentally retarded without any specific studies have been carried out to describe in details the mental traits. Consequently nurseries and schools treat the group according to this undifferentiated idea of retardation.

We have identified 16 patients diagnosed with congenital myotonic dystrophy and aged 3-18 years. The patients took part in three courses organized and guided by a team from the Institute of Neuromuscular Diseases composed of a doctor, an educationalist and a psychologist. The main purpose was to observe behavioural features and assess possible unmet needs of the patient group. Courses lasted 10, 3 and 3 days respectively.

The patients seem to have a substantial demand for physical contact with other people. At the same time they require their own personal "space". They have difficulties in adjusting to shifting situations, and generally they are not able to interpret expressions of other people. They lack initiative as well as capabilities to plan and to judge a situation when needed. They perceive concretely and seem to learn best by use of the body. Most participants expressed a wish to learn more about the rules of being together.

We have reason to believe that these patients clearly constitute their own entity in a behavioural perspective. Studies, preferably multi centre studies, are needed to define feasible and appropriate educational, psychological and medicosocial support and interventions.

42) A peculiar neurofibrillary degeneration distribution in one DM2 brain: a case report

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Neurofibrillary degeneration (NFD) is a neuropathological hallmark of more than 22 neurological disorders, referred to as Tauopathies. NFD is characterised by the intraneuronal accumulation of pathological fibrils made of abnormally modified tau proteins. In the human brain, tau proteins consist of six isoforms generated by alternative splicing. We have previously shown that NFD was present in the brain tissue from DM1 affected individuals and that a reduced number of tau isoforms was expressed, thus suggesting that tau pathology occurs in DM1. Herein, we report the presence of tau pathology in one genetically confirmed DM2 affected patient.

The brain was obtained at autopsy of a 71 year-old man, who had never been affected by cognitive decline or Parkinsonism. Formalin-fixed brain tissue was investigated for NFD by the mean of Gallyas silver staining and immunohistochemistry. Tau pathology, consisting of neurofibrillary tangles and neuropil threads, was observed in the neocortex (polymodal), in the allocortex (hippocampus), and in the midbrain (periaqueducal grey substance, occulomotor nuclei, locus niger, and locus ceruleus). Nor pigment neither neuron loss of both nuclei were observed. Intriguingly, tau-immunoreactive neurites were observed in the anterior horn of the spinal cord. Multiple neuropathological markers were analysed to ascertain that the tau pathology was not overlapping with other neurodegenerative disorders. Nor amyloid deposition, as in Alzheimer's disease, neither alpha-synuclein, the main component of Lewy bodies and Lewy neurites, were detected. Cystatin labelling was normal. In contrast, ubiquitin-positive, intranuclear inclusions, reminiscent of Marinesco bodies, were observed in many pigmented neurons in the locus niger.

In conclusion, tau pathology is also observed in DM2. The topographic distribution of the tau pathology closely resembles that of the fives DM1 individuals previously examined. Neuropathological examination strongly suggests that this tau pathology does not result from cerebral ageing or other neurological causative factors, as Alzheimer's disease, Parkinson disease or Lewy body dementia. Further analyses would be necessary to determine whether the tau isoform expression is affected similarly to that of DM1 individuals. Nevertheless, our results suggest that tau pathology and neurodegeneration occur in DM2 patients.

43) Testosterone and diurnal rhythmicity of leptin, TNF-alpha, and TNF-II receptor in insulin-resistant myotonic dystrophy patients

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The metabolic syndrome consists of a cluster of factors including insulin resistance, impaired glucose tolerance, hypertension, central obesity, and hypertriglyceridemia. Insulin resistance is also a cardinal feature of DM1. Like other patients with the metabolic syndrome, DM1 patients have an increased body fat mass and increased circulating triglyceride levels. However, glucose intolerance is unusual and blood pressure is not increased. We have previously shown disturbed cortisol regulation, impaired fibrinolysis and altered secretion of cytokines. A baseline hyperleptinemia has been shown but so far the 24h leptin profile has not been established.

Leptin is mainly produced by adipose tissue and circulating levels are closely related to fat mass. Leptin secretion has been suggested to be modified by e g TNF and testosterone. TNF has also been attributed an important position in the pathogenesis of insulin resistance. Testosterone may contribute to the distinct gender differences in leptin secretion. Importantly, testosterone levels are markedly decreased in DM1 males. We investigated the leptin and TNF systems in relation to testosterone in insulin resistant DM1 subjects.

Forty-two DM1 subjects (20 women and 22 men) and 50 controls participated. Body composition was measured and circulating levels of leptin, TNF-alpha, TNFR-II, insulin, testosterone and lipids were measured in fasting morning samples. Nine patients and controls participated in diurnal sampling. The number of CTG repeats was analysed.

Basal as well as median 24 h levels of leptin and TNFR-II were significantly increased in DM1 patients, independent of body fat mass. This was associated with higher insulin and lower testosterone levels in DM1 patients. The genetic defect was related to leptin and TNFR-II levels.

Hyperleptinemia in DM1 is clearly linked to the concomitant hypogonadism. The genetic defect may directly or indirectly contribute to increased leptin levels. Increased exposure of cytokines may contribute to insulin resistance and other hormonal disturbances in DM1.

44) Heart involvement in young myotonic dystrophy type 1 patients

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Cardiac involvement is a well-known crucial prognostic factor in adults DM1 patients and the occurrence of cardiac symptoms in young affected individuals is usually considered as uncommon. However, there is little available data concerning cardiac findings for children and teenagers. It is also unclear whether severe cardiac signs may be encountered as an isolated inaugural symptom in DM1 adolescents, or constantly combined with other significant clinical DM1 signs. Cardiac evaluation in young DM1 patients (<18 year old) may be of great interest for both follow-up and management of cardiac features in childhood type-DM1, and may add useful information for genetic counselling for asymptomatic individuals at risk under the age of 18. In a retrospective study of DM1 patients, we analysed cases with severe cardiac involvement identified before the age of 18. Age at onset, neuromuscular and systemic clinical features, results of detailed cardiac investigations, severity, follow-up, and number of (CTG)n triplet repeats were investigated. Severe heart involvement was identified in 9 young individuals between the age of 12 and 18. Two subgroups can be differentiated: cardiac signs occurring in previously diagnosed childhood or congenital type of DM1, and severe cardiac events in DM1 teenagers with no or mild clinical signs of the disease. In the first group (n=5), cardiac abnormalities were sudden death in 3 boys (aged 14, 17 and 18); recurrent ventricular tachycardia episodes during dancing in a 12 year old girl; and a worsening intraventricular conduction defect in a 15 year old girl, requiring pace-maker at the age of 22. In the second group (n=4), a 16 year old asymptomatic boy presented with cardiac arrest after running with no recurrence after implantable cardiac defibrillator; a 14 year old boy with mild distal muscle weakness had atrial fibrillation; recurrent paroxysmal atrial flutter was diagnosed in a clinically unaffected 13 year old girl; and an atrioventricular block with an HV=70 ms was diagnosed in a 16 year old boy. Sport/exercise triggering was found in 5 patients. A large variation of CTG expansion was observed (from 250 to 1200 repeats), and no particular range of CTG expansion appeared predictive. Severe heart involvement may occur early in the course of DM1 in two different manners: (i) in congenital/childhood type affected patients, as well as (ii) in clinically asymptomatic adolescents with only minor muscle signs. Rarely, cardiac manifestation may be the first isolated clinical sign inaugurating DM1. Exercise seems to be a prominent triggering factor. These data may be helpful for accurate cardiac management during infancy/adolescence in young DM1 individuals.

45) Steinert's disease (DM1) with predominant proximal involvement

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Myotonic Dystrophy type 1 (DM1), Steinert's disease, typically involves the distal muscles of the extremities. The proximal musculature is only later affected. Scapulae, shoulder girdle, and pectoral muscle are frequently spared. On the contrary, a prominent and early proximal involvement characterizes the proximal myotonic myopathy (PROMM), the main clinical phenotype related to the DM2 mutation. We report Steinert's disease affected patient with a predominant involvement of proximal musculature mimicking PROMM and even facio scapulo humeral dystrophy. Six patients (4 men, 2 women) had a genetically confirmed DM1 mutation (expansion of CTG triplets ranging from 466 to 1470). At first examination, all patients showed an prominent proximal upper limb weakness with a severe and progressive limitation of arm's elevation and abduction. The muscle weakness was initially asymmetric for 4 patients mimicking FSH. Five patients had scapular winging which was unilateral in one. In one patient scapular winging was only observed after 6 years of follow-up. All patients had facial and axial involvement. Clinical myotonia was marked in 3 patients, mild in 2 patients and absent in one. Mean age at onset was 28 years (10-51). First signs were myotonia (3/6), dysarthria and mental retardation (1), scapular winging since childhood (1), swallowing impairment (1). In Conclusion we describe here six observations of DM1 affected patients with unusual proximal musculature involvement. Thus, prominent proximal involvement in a myotonic dystrophy, if it is usually suggestive of PROMM, may also be found in molecularly proven Steinert's disease.

46) Clinical and molecular features of myotonic dystrophy type 2

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To better define the pathophysiology of myotonic dystrophy we have studied families with the characteristic multisystemic features but without the DM1 expansion on chromosome 19. We have compared clinical, histological and molecular features of genetically diagnosed subjects with DM2, which we recently reported is caused by a CCTG repeat expansion in intron 1 of *ZNF9*.

Due to the repeat tract's unprecedented size (mean ~5000 CCTGs) and somatic instability, expansions were detectable by Southern analysis in only 80% of known carriers. We developed a repeat assay that increased the molecular detection rate to 99%. Among all genetically diagnosed adults, 90% had electrical myotonia, 82% weakness, 61% cataracts, 23% diabetes and 3% cardiomyopathy. The majority of tested subjects had insulin resistance, hypogammaglobulinemia and testicular failure. Only 30% of the positive samples had single sizeable expansions by Southern analysis; 70% showed multiple bands or smears. Among individuals with single expansions, repeat size did not correlate with age of disease onset, age of onset of weakness or age at initial cataract extraction, but did correlate with age when blood was drawn. In parent child pairs in which both had single sizeable bands on Southern analysis, the children had a repeat size that on average was 17kb (-4250 CCTGs) less than their parents. Offspring had earlier age of onset than parents (mean=-13 years, n=79), but lack of correlation between repeat length and disease severity. as well as the apparent intergenerational shortening of repeat length, prevented clear interpretation of this observation. All muscle biopsies had ribonuclear inclusions, even those rare cases with normal histology. We have no definite examples of DM3; some families have the cardinal features of myotonic dystrophy without either DM1 or DM2 expansions, but all features may not truly co-segregate in affected individuals.

DM2 is present in a large number of families of Northern European ancestry. Clinically, DM2 has the multisystemic features of adult-onset DM1, but no congenital form has been identified. .The clinical features common to DM1 and DM2 are presumably caused by CUG and CCUG expansions in RNA. Additional investigations are needed to determine whether the clinical differences of DM1 and DM2 are also caused by deleterious effects of the repeat expansions in RNA, or whether additional pathogenic mechanisms are involved.

47) Is this myotonic dystrophy type 3?

Robert McWilliam*[1] and Ed Tobias[2]

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We describe a four generation pedigree in whom at least seven (possibly ten) members presented with congenital cataract. These individuals also express a constellation of additional features, highly suggestive of myotonic dystrophy. Dominant inheritance appears likely and there may be a degree of anticipation. Molecular genetic studies have failed to demonstrate a mutation in or linkage to either the DM1 or DM2 gene.

The proband, a 12 year old boy, was referred to the Neurogenetic Clinic following his second cataract operation. He also has bilateral ptosis, mild learning disability and chronic constipation. Both his mother and maternal aunt had congenital cataracts and have myopathic facies with mild distal weakness and myotonia. Both have experienced delayed recovery from anaesthesia and the aunt has recurrent tachy – arrhythmias. The mother has longstanding faecal incontinence. Family members from earlier generations who have had congenital cataract also have a myopathic facial appearance but no other symptoms.

48) Identification of problems with extended activities of daily living in people with myotonic dystrophy

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People with myotonic dystrophy have difficulty with several extended activities of daily living (EADL) and so may benefit from interventions directed at problem areas. This was a retrospective audit of the use of a specific EADL questionnaire in patients attending a muscle clinic in a department of rehabilitation medicine. The questionnaire was a self report instrument which is undergoing validation, and is derived from the Nottingham Extended Activities of Daily Living Index (NEADL). It covers 48 aspects of EADL, each being graded from 'alone easily' through 'alone with difficulty' and ' with help' to 'not at all', and scored 3 to 0. Patients were asked to complete it whilst in the waiting room at the time of their appointment. 21 of 23 patients so requested completed the questionnaire. Median age was 36, range 17 to 67. Total score ranged from 42 to 144, median 126. There was a ceiling effect (5 patients with maximum score). There were only 2 items which no patients had difficulty with ('open the front door to visitors' and 'read a newspaper or book'), and these items might have been difficult in a more disabled population. Five items were found difficult by over 50% of the group: 'walking over uneven ground, 'doing the housework', 'using items e.g. scissors, screwdrivers', managing the garden', and 'driving a car'. A further 22 items were found to be difficult by 25 – 50% of the group. 7 patients reported falls within the last few weeks.
Therefore this tool does recognise difficulties with EADL in myotonic dystrophy. Some of these problems could be recognised from scales already in common use in DM, such as those in the Cardiff protocol, but others would not be. In addition to research use it could be used as a means of identifying areas where intervention may benefit patients. A formal prospective study of reliability and validity would assist in modifying the tool for this population. A related question concerning the scale would be regarding the sensitivity of a self report tool in DM. In addition this tool, by its nature, will only recognise problems that are apparent at the level of activity limitation (disability) and further questions regarding participation restriction (handicap) may also be of use in both research and for clinical use.

49) Parenting in the face of a late-onset genetic disorder: a comparative study of parents facing either myotonic dystrophy (DM) or Huntington disease (HD)

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Late-onset disorders typically manifest in mid-life making it likely that people will have had had to make decisions about childbearing and know of their risk (whether or not they have had genetic tests) before developing any overt symptoms. Thus, in addition to concerns about whether any child will inherit the disorder, they face uncertainty about whether the at-risk parent will be able to sustain a parenting role.

This study deploys a model of responsibility generated in my doctoral research (Downing, 2002) to *first*, compare ethical dilemmas associated with having genetic testing, becoming a parent and parenting across two genetic disorders, DM and HD. Both disorders affect men and women making it possible to *second*, compare ethical issues across conditions around fathering and mothering in recognition that parenting is experienced differently by men and women. Since the introduction in 1987 of predictive testing for HD and in 1992 for MMD an increasing number of men and women are now living with the *certain* knowledge of their gene positive status but they still represent a small proportion of the total at-risk population. The *third* aim of the study is to focus specifically on the ethical dilemmas that arise in connection with mothering and fathering when risks for these two conditions are transformed into certainties. This is being achieved by comparing accounts from those having and not having predictive testing.

Participants are being recruited to the qualitative in-depth interview study through support groups and clinical genetics departments. By theoretically sampling from initial respondents it become possible to select for interview within each disorder group mothers and fathers who are either mildly affected, have tested positive, or are still at-risk and their partners, who are not themselves at-risk. The study also has sought to include those parenting under different conditions, such as couples, single parents, and step-parents. Parenting concerns are also likely to vary according to the age of the dependent child or children. Participants are also therefore being selected to ensure that within each group some will have only young children (under six years of age) and others will have older children (up to sixteen years, or still dependent in that they are affected by juvenile forms of the disorders).

Interviews with parents are identifying the nature of ethical concerns that genetic information raises for them in their interactions with each other, their children, and professionals such as lawyers, social workers, community health workers and genetic counsellors. Some preliminary findings from the first set of interviews will be presented. Findings from this study will eventually be disseminated to both families and professionals. It is envisaged that the study will inform counselling being offered to those who are considering testing and identify ongoing counselling and support needs of parents who have tested positive for a late-onset genetic disorder.

Downing, C (2002) Reproductive decision-making in families at risk for Huntington's disease: perceptions of responsibility. Unpublished PhD thesis. Centre for Family Research, University of Cambridge, Cambridge.

50) A patient held “Care Card” to improve the management of myotonic dystrophy

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Although myotonic dystrophy (DM) is a multi-system disorder, many UK patients do not have a physician who takes an overall view of their management. Care may be uncoordinated and delivered by staff who are not expert in DM. Management pathways for DM have been developed but are available only in muscle centres. At the Scottish Muscle Network meeting in 2000, the Myotonic Dystrophy Support Group (MDSG) challenged us: "What are you going to do about our members who do not attend special clinics?" The MDSG supported a meeting of 33 UK specialists. The result was the patient held “Care Card” (www.gla.ac.uk/muscle/dm.htm). Printed on A4 it folds to fit a small plastic wallet. The Care Card has a plain language list of ways DM may affect health and a summary about main risks. There are also personal and specialist contact details. The reverse lists specialists that can help and has a diary for clinic attendances and recommended screening procedures. There is also a web address for an anaesthetic protocol. Patients are encouraged to carry the Care Card and show it at clinics. We have been monitoring its introduction in a number of ways. Patients seem to like them and worldwide, over 4,000 have now been downloaded from our website. It is also available on a number of other websites. 126 professionals have contacted us, all have been positive; all found the Care Card helpful, often suggesting improvements and requesting copies for their patients or for local versions. A parallel study has found that in Scotland DM patients feel better informed than other muscular dystrophy patients they also feel more involved in their own management. We are auditing the effect of the card in a group of 360 patients and their affected relatives who have been identified from clinical and DNA diagnostic records. We are comparing a number of outcomes in card users and non-users, such as the time since the last ECG.

51) My family has myotonic dystrophy

Shannon Lord

The Hunter Research Fund, Atlanta, Georgia, USA.

As the daughter of a career Naval officer, Shannon lived in several places in the continental United States and Hawaii before her father retired in Mobile, Alabama when she was fourteen. As a child, life's challenges came relatively easy for her in academics, athletics and socialization. She and her four siblings adapted easily to each move, though they always required lots of sleep and struggled to wake up in the morning.

After graduating from college with a Bachelor of Arts in English, she worked, traveled, and lived in France and Switzerland before getting married in 1971. Since then she has worked as a teacher, artist, writer, and fundraiser for The Hunter Fund for myotonic dystrophy research, but most of all as a parent advocate for her two sons with DM. As is so often the case, she learned that she, herself, has DM only after her older son was diagnosed thirteen years ago. "Basically," she says, "my sons, with childhood-onset, have been my career, and a very challenging one at that." Alas, many of ***their*** life challenges --- unlike her own --- have come with enormous struggle and difficulty. Shannon has lived in Atlanta, Georgia for over thirty years with her architect husband, Larry, whose design interest and specialty is medical research laboratories.

She will present her family's varied responses to their genetic disorder: one son's response with acceptance, compliance, and contentment; the other's with anger, defiance, and self-loathing; her husband's with excessive work and community involvement; and her own with initial depression followed by acceptance that she could not "fix" her children and herself. She ultimately developed a compelling need to "do" something to support research efforts through fundraising, writing, and cheering from the sidelines.

For her sons with childhood onset, the most challenging symptoms to date have been the cognitive and behavioral aspects for which there are few conclusive studies and treatments. As a family, they fly by the seat of their pants, through trial and error with medication, "tough love,"and behavior modification, with limited results, especially with her younger son.
She endorses the shaking hands (on the cover of the abstracts), symbolizing physicians, scientists, and patients working together to find a treatment for this sad and debilitating disease.

52) Recent scientific advances in myotonic dystrophy

David Brook

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

David Brook obtained his BSc degree in Genetics and Cell Biology from the University of Manchester and his PhD from the University of Edinburgh. He worked as a postdoc in Peter Harper's Institute for Medical Genetics in Cardiff for six years, and subsequently at MIT for three years. In 1992, he was appointed to the post of Senior Lecturer at the University of Nottingham and was promoted to Professor in 1995. He became Head of the Institute of Genetics at Nottingham in 2000.

Recent years have seen many significant advances in our understanding of the molecular basis of myotonic dystrophy including the factors that regulate the unusual genetics of the disorder and give rise to the symptoms observed. David will discuss recent progress, including a summary of the new data presented earlier in the meeting.

53) Living with myotonic dystrophy

Margaret Anne Bowler (nee Jones)

The Myotonic Dystrophy Support Group, Nottingham, UK.

Margaret was born 1st February 1937 in Nottingham, UK. Her father was a brick maker and her mother a dress maker (those skills not passed on to Margaret). She has one brother. She trained as a State Registered Nurse and State Certified Midwife at the Nottingham City Hospital and furthered her career by being a Community Midwife in her home area for 40 years. She is an active member of the Parish Church, having been Church Warden for several years, and Guide captain of the church guide company for 40years, and was a Guide Commissioner prior to retirement.

She has four children, her daughter is a teacher, her eldest son is a Consultant Anaesthetist (Cardiff), her second son a director of his building firm. Her third son has myotonic dystrophy, the only sibling to be affected.

Her introduction to myotonic dystrophy was the diagnosis of husband and son in 1985. Her son was 12 years old and her husband 42 years old. Her husband's cataracts had been removed several years before. His occupation was a cartographer and he worked at Nottingham University. Her son had everyday living difficulties, including clumsiness, soiling and difficulty in writing. She had been blamed for his emotional state (soiling). Nine years passed until the diagnosis was made, the fight for survival mentally, had been a great strain on mother and child. Immediate physical relief was felt by mother on the announcement of a diagnosis, not her fault after all!

One year later she visited Prof. Peter Harper in Cardiff with her son and husband. The visit gave confidence, someone else knew about myotonic dystrophy.

She will try to share with you experiences that people have shared with her since she started the Myotonic Dystrophy Support Group in 1989. For a few years prior to this contacts had been made with other families by advertising in the press and radio. This did help the isolation experienced with the family having a rare condition.

2003 will be the Myotonic Dystrophy Support Groups' 14th Annual Conference, and the Charity is honoured at being asked to join the IDMC4 meeting in Glasgow.

MDSG is affiliated to the Muscular Dystrophy Campaign, Long term Medical Alliance, Genetic Interest Group, National Council for Voluntary Organisations, Scottish Muscle Centre and Self Help Nottingham.

Margaret has Spoken to over 1000 families via the telephone.

The Myotonic Dystrophy Support Group has 1300 on the mailing list, including 22 different countries. There are 14 Trustees, 7 Medical Advisors and 23 Contact Families. MDSG is a Registered Charity.

She has been given 20 minutes to share with you a little of 'Living with myotonic dystrophy'. She can only give you a taster.... you have to live with myotonic dystrophy to know how and whom it affects.

54) One doctor, two sisters and a professor

Jacqueline Donachie

Artist, Glasgow, UK.

Jacqueline Donachie was born in 1969 in Glasgow, Scotland. She studied in the Environmental Art Department of Glasgow School of Art, where she gained a BA (Hons) in Fine Art in 1991, and later won a Fulbright Award to study on the Masters Programme at Hunter College in New York (1995-96), before finally completing her MFA at Edinburgh College of Art in 1998. Since 1991 she has been exhibiting widely, both nationally and internationally, and has recently completed solo exhibitions in France, Denmark and Sweden. In 2001 she was the Henry Moore Sculpture Fellow at Spike Island in Bristol, and in 2002 was a guest of IASPIS, the International Artists' Studio Programme in Stockholm, Sweden. She continues to live and work in Glasgow, where she lives with her husband and 2 sons

Alongside her work for exhibitions and public commissions Donachie has also published a series of artists' books, begun in 1994 with *Part Edit*, produced to coincide with her first solo exhibition at Tramway in Glasgow. She is currently working on the 6th book in the series, to be published later this year. The fifth book in the series, *DM*, was produced as the result of a collaboration between herself and Dr Darren Monckton and Professor Keith Johnson of the Division of Molecular Genetics at the University of Glasgow. The collaboration was instigated by Donachie following the diagnosis of myotonic dystrophy in her own family, first discovered on the birth of her sister Susan's second child who was born congenitally affected in May 1999. Since then the gene has been traced to Susan and her other son, Fraser, as well as her father, brother and several cousins in the family. In 2001 the partnership successfully applied for a research award from the Sciart Consortium, an initiative of the Wellcome Trust set up to encourage collaborations between scientists and artists. This award enabled Donachie to spend several months resident within the Division looking at how the department operated, and funded a visit by the trio, accompanied by Donachie's sister Susan, to the Montreal and Saguenay regions of Quebec, Canada to look at the exceptionally high founder effect in the area. It was this extremely moving journey that is described in part in DM, and that will provide the basis for her presentation to IDMC4.

Relevant Facts

DM
Published by the University of Glasgow
ISBN 0 85261 764 X
Funded by a Research Award from the Sciart Consortium,
with additional support from COPUS, the UK Science Communication Partnership.

The *Sciart Consortium* no longer exists, but has been replaced by a new **sciart** award scheme, funded directly by the Wellcome Trust. The £1 million pound scheme will award grants for a series of projects over the next two years to further support and encourage innovative arts projects investigating biomedical science and its cultural and social contexts. Jacqueline Donachie and Dr. Darren Monckton hope to apply to this new scheme in 2003 for a major production award, to enable them to continue their collaboration.

55) Towards treatments for myotonic dystrophy

Tetsuo Ashizawa

Department of Neurology, The University of Texas Medical Branch, Galveston, Texas, USA.

Dr. Ashizawa was born in Tokyo, Japan on September 11th, 1948. In 1973, he received his medical degree from Keio University School of Medicine in Tokyo, Japan. He had his internship at Allegheny General Hospital in Pittsburgh, Pennsylvania and graduated from neurology residency at Baylor College of Medicine in 1978. After one year of a postdoctoral clinical fellowship in neuromuscular disease and a two years research fellowship in neurochemistry, he joined the faculty at Baylor in 1981. He became a full professor at Baylor in 1997, and about a year ago he moved to The University of Texas Medical Branch at Galveston, Texas, where he is Professor and Chairman of the Neurology Department. Dr Ashizawa will discuss current treatments and possible future therapies of myotonic dystrophy.

Current drug treatments of myotonic dystrophy type 1 (DM1) are aimed to alleviate symptoms such as myotonia, daytime sleepiness, psychological symptoms, and some of the cardiac complications. Surgical treatments are for cataracts, gall stone, and jaw and teeth problems. Pacemaker implantation often saves lives in patients who have severe cardiac conduction block. When anesthesia must be used, specific precautions are necessary to minimize risks for breathing complications. Although these "symptomatic" treatments are important, no treatments are available to improve the disease itself by intervening in the mechanism of the disease. Researchers are trying to develop these treatments by two approaches: one is discovery based and the other is hypothesis based. The former starts with a discovery of a potentially effective treatment. The efficacy of the treatment must be confirmed by rigorous clinical trials, and the mechanism of action is then explored. Drugs such as DHEA-S, myotrophin, and CoQ10 may fall into this category. The latter is based on the understanding of the disease mechanism. In DM1, there may be multiple mechanisms accounting for different sets of symptoms. Researchers are working on each mechanism to develop innovative treatments including those that shorten the CTG repeats in DNA and CUG repeats in RNA, and ones that correct the consequences of the repeat expansion mutation.

Abstracts

Poster presentations

P1) Clinical and molecular analysis of myotonic dystrophy in Uruguay

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Myotonic dystrophy type I (DM1) is the most common muscular dystrophy of adult life, with a world-wide incidence of 1 in 8.000 adults. This autosomal dominant multisystemic disorder is clinically variable. Due to the variability of this disease a confirmation of the clinical diagnosis was desirable.

The Institute of Neurology and The Genetics Department, Faculty of Medicine, started in 1998 the first molecular study of myotonic dystrophy in Uruguay. Twelve unrelated patients and 20 at risk relatives were clinically examined and the CTG repeat length determined. Patients showed a wide variation in the range and severity of muscular and systemic signs, with a high prevalence of thyroid abnormalities. The clinical diagnosis was confirmed by molecular analyses in 31/32 cases. Southern blot analysis showed large expansions ranging from 130 to 3610 CTG repeats in 17 patients, while PCR detected 4 carriers of small expansions in the low end of disease range, one being carrier of a premutation. SP-PCR was done in the carrier of a premutation allele of 45 repeats to investigate the risk of DM1 to his offspring. A expansion bias was detected predicting that individuals inheriting the expanded allele would be at high risk of developing the late onset form of the disease. A patient with clinical diagnosis of myotonic dystrophy but negative for DM1 mutation was analysed by PCR for DM2 locus, and resulted negative for this mutation.

The molecular diagnosis by PCR and Southern blot was optimised and an area of research for this pathology in our country was started. Among the patients studied so far no significant correlation between the number of CTG repeats and the clinical manifestations was found. However, a tendency could be observed within some families. The lack of correlation was probably due to the small number of patients examined and the confounding effects of age dependent somatic mosaicism on measured allele length.

P2) Molecular and genetic studies of the DM1 mutation in Costa Rica

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In order to improve the clinical management and genetic counselling offered to patients and their relatives, we established the molecular diagnosis of DM1 in Costa Rica, studied the intergenerational behaviour of the CTG repeat and the transmission ratio of the DM1 allele. The clinical diagnosis of DM1 was confirmed through molecular diagnosis using Southern blot and PCR. We studied 126 patients from 30 different families. The mutation was present in 21 of 30 families, the other families were expansion negative and affected by other myotonic conditions. The size of the mutation presents a positive and a negative correlation with the severity of the symptoms and with the age of onset respectively. Transmission of the DM1 mutation is sex and size-dependent among the Costa Rican patients as worldwide described. The DM1 allele was transmitted in 49%, so that there is not preferential transmission of the DM1 allele, however, it is preferentially transmitted in the firsts generations from carriers males. The more offspring a male with a few CTG repeats has, the more affected offspring he will have. According to this and other results, the transmission ratio of the DM1 allele could depend on the number of offspring, the sex of the initial transmitting parent, its place in the generation, the fertility and mechanism of instability between sexes, the size of the mutation of the transmitting parent and most likely, some inherent and unknown events occurring during gametogenesis which are probably different between sexes. Molecular diagnosis must be used as a tool for a proper clinical classification of the patients. The adequate clinical approach, since there is no treatment so far, should include, besides a multidisciplinary clinical management, prevention through genetic counselling based on the exact molecular diagnosis of the carriers.

P3) Myotonic dystrophy type 2: preliminary results in the Spanish population

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The aim of this study is the analysis of the CCTG unstable locus in the ZNF9 gene which causes myotonic dystrophy type 2 (DM2).

Since the beginning in 1988, of myotonic dystrophy studies in our genetics unit until now, we have analysed the CTG repeat expansion causing DM1 in 1200 families suspicious of having Steinert disease. In 290 of these families the DM1 molecular analysis was negative. We have reviewed the clinical data and in 105 families the diagnosis agreed with a myotonic dystrophy syndrome. Nine of these families have a PROMM diagnosis.

We started conducting the PCR analysis of the DM2 locus in the families with negative molecular diagnosis of DM1 (at this point 38 families) and in the nine families with PROMM diagnosis.

The PCR has confirmed the DM2 diagnosis in five of the nine families with clinical diagnosis of PROMM, whereas only one of the families gave a negative result. The analysis for the three remaining families is still in course.

We have cloned and sequenced some alleles to analyse the complex tract and to determine the normal range for the DM2.

Our preliminary data in the study of DM2 in that population will be presented.

P4) How common is the DM2 mutation as the cause of clinical myotonic dystrophy?

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Myotonic Dystrophy is usually the result of a CTG repeat expansion in the 3' untranslated region of the DMPK gene on chromosome 19q13.3 (DM1). Recently, a second mutation (DM2), a CCTG expansion in intron 1 of the ZNF9 gene on chromosome 3q21, has been found to be responsible for some cases of myotonic dystrophy, as well as for the allied clinical disorder Proximal Myotonic Myopathy (PROMM). We have previously found only one family with the DM2 mutation in a research series of 95 myotonic dystrophy families

In a series of 205 diagnostic referrals to the Cardiff laboratory between 1992 and June 2000, 101(49.3%) were positive for the DM1 expansion. Of the 104, DM1 negative patients, 40, unrelated, adult patients, with no family history of the DM1 mutation, referred from either Neurologists or Geneticists, were analysed using a combination of direct PCR and QP-PCR for the CCTG expansion at the 3q21 locus. Three patients were positive, suggesting a frequency of around 1.5% for the DM2 mutation in all cases of clinically suspected myotonic dystrophy in Wales. All three cases with the DM2 mutation showed clinically atypical features, suggesting that analysis of the DM2 mutation in UK patients will be of greatest value in suspected cases of myotonic dystrophy that are atypical or where the DM1 mutation has proved normal.

P5) Quantification of the degree of mutation length variability in Huntington's disease patients: buccal cell analysis

Nicola J. Veitch*, John P. McAbney, Peggy Ennis, The Venezuelan Collaborative Huntington's Disease Research Group, Peggy F. Shelbourne and Darren G. Monckton

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A number of neurodegenerative disorders have been associated with the expansion of DNA triplet repeats, including Huntington's disease, myotonic dystrophy, spinocerebellar ataxia and fragile X syndrome. Huntington's disease (HD) is associated with an unstable trinucleotide CAG repeat expansion within exon 1 of the *HD* gene. Germline repeat instability at the *HD* locus leads to mutation sizes in HD patients that vary between 37-86 repeat units in most cases. Although the trinucleotide repeat length is inversely correlated to the age at onset of HD in patients, there is still a great deal of variation between individuals. Interestingly, very large increases in mutation length have been observed in the striatum of HD patients with some cells carrying more than 1000 CAG repeats. This pattern of mutation length variability suggests that somatic mosaicism of the repeat length may contribute to the tissue-specificity and progressive nature of HD pathogenesis. Genetic modifiers of mutation length instability may therefore modify the age at onset of the disease.

The aim of the project is to quantify mutation length variability in buccal cells and determine if repeat length and age at sampling are modifiers of somatic instability. Buccal cells from 770 individuals were obtained from a Venezuelan community with a high prevalence of HD. Genotyping was performed by sizing fluorescent PCR products on a denaturing polyacrylamide gel. The mutation profiles of the samples were obtained by determining the CAG repeat length on individual mutant alleles using small pool and single molecule PCR assays. The resulting information will help evaluate whether buccal cell tissue can be used as a surrogate tissue for brain in mutation instability studies as well as clarifying the relationship between repeat instability and the age of onset of disease symptoms. This study will also inform future studies aimed at identifying genetic modifiers of somatic instability in triplet repeat diseases.

P6) Investigating *cis*-acting factors affecting the stability of trinucleotide repeats

Helen E. James*, Graham J.R. Brock and Darren G. Monckton

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Large expansions of trinucleotide repeats have been identified as causing a growing number of different human diseases, including myotonic dystrophy type 1 (DM1) and Huntington's disease (HD). The repeats are genetically unstable both in somatic and germline cells, with longer repeats associated with more severe forms of disease.

Levels of somatic mosaicism vary between different tissue types - in DM1 patients the repeat is found to be longer in muscle cells than circulating lymphocytes, while *Hdh* knock-in mice show high levels of somatic instability in the striatum, the major affected tissue in HD. Differences in stability are also seen between different loci in humans, for example the spinocerebellar ataxia type 3 (SCA3) locus is relatively stable, the DM1 locus far less so.

The mechanism for the expansion of these repeats is not understood, but is affected by both *cis* and *trans*-acting factors. The Dmt mouse lines contain a transgene derived from the human DM1 repeat and its flanking sequence. The stability of the repeat varies between lines and tissue types, being most unstable in Dmt-D and very stable in Dmt-E. Currently we are investigating the insertion site of the transgene in Dmt-D, using bioinformatic and molecular biology approaches, in order to identify its exact location within the genome. Further research will compare the different lines, and attempt to identify the *cis*-acting factors which affect the stability of the repeat at different locations in the genome.

P7) The effect of altering the ratio of MutSα to MutSβ on trinucleotide repeat instability *in vitro*

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The expansion of simple sequence repeats has been identified as the cause of a growing number of hereditary human diseases, including myotonic dystrophy type 1 (DM1). Disease-associated alleles, in which the repeat has expanded above a threshold number of repeats, are extremely unstable in both the germline and soma, and show a heavy bias towards further expansion. While germline instability provides a molecular basis for the phenomenon of 'anticipation', the multi-systemic nature of these progressive disorders may be explained by the age-dependent, tissue-specific somatic instability that is observed. In *Dmt*-D transgenic mice, which carry a single copy of an expanded CAG•CTG repeat, the dynamic nature of the somatic instability observed in humans is reconstituted. *In vitro* cell cultures from a variety of tissues isolated from these mice have been established, with cultured kidney cells showing the greatest levels of instability at this locus.

Currently, little is known about the mutational mechanisms underlying trinucleotide repeat instability, or the factors governing which tissues are affected. However, recent studies have suggested the involvement of the mismatch repair (MMR) system, as the stability of expanded trinucleotide repeats is enhanced in mice deficient for Msh2, Msh3 or Pms2, indicating that these proteins facilitate repeat expansion events. Conversely, mice deficient for Msh6 show a significant increase in repeat instability, implying that the presence of this component constrains mutation. MSH2 interacts with either MSH6 or MSH3 to form the heterodimers MutSα and MutSβ respectively. In mammals, the ratio of MutSα to MutSβ is approximately 6:1, however, treatment of mammalian cell cultures with the folate antagonist methotrexate has been found to alter this ratio dramatically in favour of MutSβ. This is due to co-amplification of the *MSH3* gene alongside the *DFHR* gene, the product of which confers resistance to methotrexate. In order to further investigate the roles of MutSα and MutSβ in trinucleotide repeat instability, several subclones derived from a *Dmt*-D kidney cell line will be treated with methotrexate to amplify the *Msh3* gene. The levels of repeat instability will be assayed over both time and number of population doublings and compared to control cultures to determine the effect of altering the ratio of Msh3 to Msh6.

P8) *Cis*-acting modifiers of regional repeat mosaicism in the brains of SCA7 transgenic mice

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Spinocerebellar ataxia type 7 (SCA7) is an autosomal dominant cerebellar ataxia caused by a CAG repeat expansion in the ataxin-7 gene. In humans the disease is characterised by high levels of intergenerational repeat instability. Indeed, the SCA7 repeat is the most unstable of those associated with polyglutamine tract expansions.

In order to analyse the molecular basis of the repeat instability, Libby *et al*., generated lines of transgenic mice with either a SCA7 cDNA construct or a 13.5kb genomic fragment, both inserts containing 92 CAG repeats. This study concluded that genomic context drives CAG repeat instability, as those mice carrying the genomic fragment displayed marked intergenerational repeat instability, while mice with the cDNA construct showed very little. An analysis of somatic mosaicism revealed that this too was similarly modified by flanking sequences.

We have gone onto investigate age dependence and regional variation in somatic mosaicism in the brains of these animals. Our findings concluded that, like previous studies on these mice, that the transgenic line containing the 13.5kb genomic fragment displays marked instability, whilst the line carrying the cDNA construct remains relatively stable. Nonetheless, low level instability is detected in older cDNA construct mice. Mosaicism is similarly age dependent in the genomic fragment mice, with very large expansion observed in older animals. Significant variation also exists between different brain regions with a noticeably higher rate of expansion observed in the cerebellum than in other transgenic models. However, the precise patterns of regional mosaicism differ between lines. Thus, although the human genomic fragment use is capable of driving high levels of instability, the precise pattern appears to be further modified by the integration site, confirming a role for high order sequence effects.

P9) Investigating somatic instability in a transgenic mouse model of Huntington's disease

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Like many of the disease-associated trinucleotide repeats, the myotonic dystrophy (DM) type 1 CTG repeat undergoes germline and somatic instability. The CTG repeat displays an age dependent, expansion biased and tissue specific pattern of somatic instability. It is believed that somatic instability in many of the trinucleotide disorders may contribute to the tissue specificity and progressive nature of these diseases. In the case of DM, the primary affected tissue, muscle, has previously been shown to contain repeats bearing large expansions when compared with blood from DM patients. High levels of repeat length variation in skeletal muscle, kidney and liver have also been described in a transgenic mouse model carrying an expanded CTG repeat in the 3' UTR of the *DMPK* gene.

The patterns of somatic instability in a transgenic mouse model of Huntington's disease (HD) were investigated in this study. HD is a progressive neurodegenerative disorder caused by the expansion of a (CAG)n repeat in the first exon of the *HD* gene over ~36 repeats. Expression of the expanded allele leads to the expansion of a polyglutamine stretch in the product of the *HD* gene, huntingtin. The HD CAG repeat also undergoes tissue specific somatic instability, but the striatum and cortex are the tissues that display the greatest levels of instability. The largest expansions appear to be confined to the regions most susceptible to HD pathology, therefore it has been suggested that there may be a link between somatic instability and HD neuropathology.

The R6/2 line used in this study carries a mutant form of the first exon of the *HD* gene containing an expanded CAG tract of ~230 repeats. The R6/2 mice display some pathological features reminiscent of HD, but appear to suffer a particularly severe disease phenotype when compared with knock-in HD models. The patterns of cell loss in these mice also do not appear to correlate well with the patterns of selective pathology observed in human tissue. These observations may be related to the longer polyglutamine tract present in the R6/2 mice compared with those found in HD patients. Small pool PCR (SP-PCR) analyses were used to assess the behaviour of the HD repeats in various tissues from 19 week old R6/2 mice. The HD repeat was found to be unstable in a wide range of peripheral and CNS tissues. There are notable differences between the tissue-specific mutation profiles but aspects of these profiles differ from those obtained from knock-in HD models and HD patients. In addition, very high levels of instability were observed in these mice, which is somewhat surprising given their age.

P10) Differential fibre type myonuclei internalisation in DM1 and DM2

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Light microscopic myopathological findings in both type 1 (DM1) and type 2 (DM2) myotonic dystrophies share common alterations, including increased fibre size variation and numerous internally placed myonuclei sometimes forming nuclear chains or nuclear clumps in highly atrophic fibres. Ringed fibres and subsarcolemmal masses are mainly encountered in DM1. Consequently, PROMM/DM2 muscle biopsy features have been reported to be similar to those of DM1 with no specific findings. Recently, we together with our collaborators demonstrated a preferential type 2 fibre atrophy in PROMM/DM2, the smallest fibres being mostly identified by the fast MHC isoform immunohistochemical analysis, and the absence of type 1 fibre atrophy compared to DM1. Both disorders are characterized by ribonuclear inclusions, but the comparative distribution of internal nuclei in DM1 and DM2 human muscles has not yet been evaluated. Deltoid muscle biopsies from 6 age-matched patients with mutation confirmed DM1 (n=3) or DM2 (n=3) have been studied by morphometric analysis after fast MHC/emerin double immunolabelling in order to quantify the number of internally placed myonuclei in type 1 and type 2 muscle fibres, respectively. The overall proportion of muscle fibres with internal nuclei was 29.3% in DM1 and 36% in DM2 muscle sections, regardless of the fibre type. In DM1, the mean percentage of internal myonuclei was 49.6 +/- 13.6 in type 1 fibres and 9.6 +/- 3.1 in type 2 fibres. Inversely, the results were 17.3 +/- 15.0 in type 1 fibres and 60.7 +/- 32.7 in type 2 fibres in PROMM/DM2 cases. In each sample the difference in the fibre type distribution of central nuclei was significant ($p<0.0001$), indicative of a marked preferential nuclear accumulation in type 1 fibres in DM1 and in type 2 fibres in DM2. As a new finding we present a preferential and differential skeletal muscle fibre type nuclear centralisation in DM1 and DM2. The results further strengthen the early involvement of type 2 fibres in PROMM/DM2 pathophysiology and emphasize distinctive histopathological features of DM1 and DM2. Furthermore, we suggest that distinct fibre type isoforms may be differentially affected by the respective mutant RNAs of both types of myotonic dystrophy. We speculate that the differential fibre type involvement in DM1 and DM2 is the result of potential defect(s) affecting the alternative splicing of a gene(s) or isoform(s) expressed in type 2 fibres.

P11) The human lens expresses calcium activated SK channels linking cataract with myotonic dystrophy

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Apamin-sensitive, calcium-activated SK potassium channels have been implicated in myotonic dystrophy (MD) and schizophrenia, and both conditions carry an increased risk of cataract. In MD a reduction in anterior epithelial cell density has been observed. Therefore, the presence and functional activity of SK channels were investigated in the human lens.

The expression of all 3 members of the SK channel family was investigated by TaqMan RT-PCR using human primers for SK1, SK2 and SK3. The functional activity was investigated following G-protein and tyrosine kinase receptor activation using electrophysiological and calcium-imaging techniques. Lens voltage was monitored by inserting a single electrode into the intact human lens, perifused with artificial aqueous humour, and changes in intracellular calcium were recorded simultaneously after fura-2 incorporation. Lens epithelial cell growth was quantified by imaging cell cover of the posterior capsule in an *in vitro* capsular bag model.

Expression of all 3 SK family members was detected in both anterior and equatorial lens epithelial cells. Application of either G-protein (*e.g.* ATP, 100 μM) or tyrosine kinase (EGF, 10 ng/ml) receptor agonists induce a rapid hyperpolarisation of lens voltage which was accompanied by a parallel increase in intracellular calcium. Application of the calcium ionophore ionomycin also induced a rapid hyperpolarisation. All of the hyperpolarising responses were abolished by apamin (10 nM) and trifluoperazine (100 μM). The hyperpolarising responses were accentuated by the SK channel activator 1-EBIO. The growth of epithelial cells on the posterior capsule was inhibited by apamin.

This study shows that SK channels are an integral part of the G-protein and tyrosine kinase calcium signalling mechanisms in the human lens and inhibiting their activation leads to a decline in cell function. This helps to explain why a change in their activity, whether by disrupted gene expression (*e.g.* in MD) or by drug intervention (*e.g.* in schizophrenia), can lead to cataract.

P12) Biochemical properties of native forms of DMPK

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Most of data referring to physico-chemical and biochemical properties of DMPK, including its catalytic activity arise from studies carried out on human recombinant proteins. We show here some preliminary results on the native forms of DMPK.

As a starting point, we have raised several peptide-specific anti-DMPK antibodies directed against different domains of the protein. We found that CD1 and TA1, which are directed against epitopes located within the catalytic site and the C-terminal domain, respectively, were immunoreactive against two proteins of 85 kDa and 72 kDa which are lacking in homozygous DMPK-deficient mice. Similar results were obtained with commercially available anti-DMPK antibodies (Zymed Laboratories inc., CA). Another antibody (CC1) is directed against an epitope of the coiled-coil region, not completely shared between human and mouse sequences. The reactivity of CC1 against the two proteins in mice over-expressing DMPK has been previously demonstrated. In rat striated muscles, we found that CD1, CC1 and TA1 reacted with the same proteins demonstrating that they are *bona fide* genuine forms of native DMPK.

By using these anti-DMPK antibodies in Western blot analysis, we found that both 85 kDa and 72 kDa proteins were easily released from muscle after homogenization. However, a significant amount of the 85 kDa was more firmly bound to the membranes, from where it was removed after alkali treatment. Taking advantage of these solubility properties, we have obtained by conventional chromatographic procedures a fraction enriched in DMPK.

The high-speed supernatant and the chromatographic fraction have been used for binding and activity assays. The results suggest that: a) a soluble low molecular weight component may be bound in vitro from partially purified DMPK, b) a big molecular complex of about 250 kDa is present in the high-speed supernatant, as shown by non denaturing electrophoresis, c) a thiol-inducible basophilic activity seems to be maintained after the first purification step toward specific peptides.

P13) Role of Wilms Tumor 1 (WT1) protein in myotonic dystrophy

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Previously we established clonal lymphoblastoid cell lines (LBCLs) prepared from DM1 patients to study the somatic instability of the DM1 CTG repeat. We showed that larger CTG repeat expansion had a growth advantage over those with smaller expansions in culture. We attributed this growth advantage to a decrease in the expression of the cell cycle inhibitor, $p21^{WAF1}$. However, the mechanisms by which $p21^{WAF1}$ expression is decreased in DM1 cells with large CTG repeats are unknown.

The *Wilms Tumor 1* (*WT1*) gene encodes a zinc-finger transcription factor that has high affinity towards CG- and TC-rich sequences. WT1 regulates the transcription of numerous genes that are vital for the regulation of cell growth, apoptosis, and differentiation, including $p21^{WAF1}$. Furthermore, the expression of WT1 protein is essential for the development of the heart, male and female gonads, and retina, which are organs that are often affected by the disease in DM1 patients. We hypothesize that a decrease in the expression/function of WT1 protein is part of the disease-causing mechanism in DM1. We used the 313 LBCL cell lines that have different CTG repeat sizes to investigate the role of WT1 protein on the expression of $p21^{WAF1}$ and the regulation of cell growth and apoptosis in DM1 cells.

Western blot was used to study WT1 protein expression in DM1 LBCLs with 1150-CTGs (313-1), 770-CTGs (313-15), and 550-CTGs (313-2). Similar levels of WT1 protein expression were found in LBCLs with 770-CTGs and 550-CTGs. However, a 2-fold decrease in WT1 protein expression was found in LBCLs containing the 1150-CTGs, thus correlating decreased WT1 protein level with decreased $p21^{WAF1}$ level. To determine whether WT1 protein has a role in regulating the expression of $p21^{WAF1}$ protein, antisense oligodeoxynucleotides (oligos) specific for the translation initiation site of the *WT1 mRNA* were used to downregulate WT1 expression. We found that downregulation of WT1 protein expression led to a decrease in $p21^{WAF1}$ protein level. When LBCLs with 1150-CTGs (313-1) were incubated with WT1 antisense oligos for 3 days, an increase in cell growth was observed. However, when the incubation of 313-1 cells with WT1 antisense oligos was prolonged to 6 days, cell growth was inhibited and cells appear to be dying. These data suggest that WT1 plays an important role in regulating the cell growth and/or cell death of DM1 cells, possibly by regulating $p21^{WAF1}$ expression.

P14) Identification and characterisation of *CG3800*, the *Drosophila* homologue of *ZNF9*, the gene involved in myotonic dystrophy type 2

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ZNF9 is the only known human member of the Cellular Nucleic Acid Binding Protein (CNBP) family; distinguished by their seven closely linked CCHC zinc knuckle motifs similar to those seen in retroviral nucleic acid binding proteins. CNBP has a putative role in regulating *β-myosin heavy chain* and *c-myc*, as well as translational control of L4 ribosomal proteins. Expansion of a CCTG repeat element in intron one of *ZNF9* has been shown to cause myotonic dystrophy type 2 (DM2), a late onset disorder characterised by myotonia, myopathy, cataracts, gonadal atrophy and cardiac conduction defects. In order to gain insight into *ZNF9* function, we are characterising its homologue in the genetically tractable model organism, *Drosophila melanogaster.* Bioinformatic analysis of the *Drosophila* genome identified the as yet uncharacterised *CG3800* as the most likely homologue of *ZNF9;* and indicated the gene is contained in one exon and comprises of only six CCHC zinc knuckles. BLAST searches identified putative homologues in mosquito, *Anopheles gambiae*, and silkworm, *Bombyx mori*, that also have six CCHC motifs. This indicates a rearrangement of CNBP between insects and vertebrates. RT-PCR using RNA from various stages of *Drosophila* development indicate *CG3800* is expressed at all stages in the life cycle, except early embryo. *In-situ* hybridisation of whole mount embryos show that *CG3800* expression is ubiquitous; at low level in most tissues but much higher in brain, gut and segment muscle precursors from development stage 11. Additional studies will look at *CG3800* mutant phenotypes to attempt to clarify its function.

P15) A soluble factor produced by myotonic dystrophy myoblasts inhibits myogenic differentiation

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One characteristic of myotonic dystrophy (DM1) is the presence of a congenital form (CDM1) which differs from the adult form in that the characteristic features of the skeletal muscle is a delay of development. To date little is known about the mechanisms by which the expansion causes the delay of CDM1 skeletal muscle development.
Using primary DM1 human myoblast cultures with different expansions (from 750 to 3700), we showed that the ability of DM1 myoblasts to fuse is inversely proportional to the length of the expansion. We also showed that conditioned media by DM1 myoblasts block the fusion of normal human myoblasts and this effect was directly correlated with the loss of the ability of DM1 myoblast to fuse. These data suggest that the loss of DM1 myoblasts to fuse may be caused by a soluble factor secreted in the culture medium. Our efforts were then toward the identification of this soluble factor produced by DM1 myoblasts. The first potential candidate tested was BMP-4 since it was shown to inhibit terminal differentiation of myoblastic progenitor cells. We showed that recombinant BMP-4 blocks the fusion of normal myoblasts in a dose-dependent manner. The levels of BMP-4 RNAs were undetectable or very low in normal myoblasts and in DM1 myoblasts with 750 repeats and, its expression increased by 8-fold in DM1 myoblasts with 1200 repeats. However, the levels of BMP-4 was not found to be increased in 3 other DM1 myoblasts which have lost their ability to fuse. These data indicate that there is not a clear correlation between BMP-4 mRNA in DM1 cells and their ability to fuse. The BMP-4 protein is regulated by the binding of its antagonist, Noggin and the balance between noggin and BMP-4 proteins is essential for BMP-4 activity. We measured the levels of Noggin mRNA and we observed that Noggin expression is increased only in DM1 myoblasts which have retained their ability to fuse. Furthermore, the ability of DM1 myoblasts to fuse is directly correlated with the levels of Noggin mRNAs in all of DM1 myoblast cell lines. Finally, we have shown that treatment of DM1 myoblasts with exogenous noggin is capable to force DM1 myoblasts to fuse.
The DM1 mutation induced a misregulation of Noggin and BMP-4 signalling pathway, both proteins being involved in the differentiation of myoblasts. Our data also suggest that another soluble factor produced by DM1 myoblasts is involved in the fusion defect of myoblasts with large expansions.

P16) Changes in extractability of DMPK during muscle development

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Expression of DMPK (myotonic dystrophy protein kinase) is important for DM1 because the expanded CUG repeats in this disease are carried by DMPK transcripts in the nucleus. Reduction of DMPK expression levels is a potential approach to therapy. DMPK is expressed mainly in skeletal and cardiac muscle. Related proteins that are more widely expressed include MRCK and an unidentified cross-reacting protein (CRP), both of which are recognised by the anti-DMPK mAb, MANDM1 (Lam, Pham, Nguyen thi Man and Morris, *Hum Mol Genet*, **9** (2000) 2167-2173).

DMPK increases during human myoblast differentiation in culture and even more dramatically between 9 and 16 days of human foetal development (Furling, Lam, Agbulut, Butler-Browne and Morris, Amer. J. Pathol. March 2003). Simple biochemical fractionation can distinguish between soluble cytosolic proteins, proteins associated with membranes (Triton X-100 extractable) and insoluble matrix proteins that include many nuclear and myofibrillar proteins.

In differentiating myotubes in culture, most DMPK was easily extractable in low-salt buffer, consistent with a mainly cytosolic protein. In adult skeletal muscle, neither DMPK nor MRCK were extracted by low-salt buffers, although CRP did appear in the cytosolic fraction. DMPK remained insoluble when pellets were extracted sequentially with Triton X-100 (to extract membranes), up to 2M NaCl (to dissolve myofibres) or buffers up to pH 12 (to dissociate complexes). In a sample of 24-week foetal human muscle, CRP was cytosolic, but both DMPK and MRCK were non-extractable with low-salt buffers, with or without Triton X-100.

The results suggest that a change in the subcellular localisation of DMPK occurs during later stages of human muscle development.

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P17) The cellular localisation of DMPK

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DM1 and DM2 are caused by dominant negative effects of expanded CUG or CCUG repeats in the nucleus. Expression of DMPK (myotonic dystrophy protein kinase) remains vitally important for DM1, however, since the expanded CUG repeats are carried by DMPK transcripts in the nucleus. Reduction of DMPK expression levels is a potential approach to therapy.

We have investigated the cellular localisation of DMPK in transfected COS-7 cells. Three DMPK cDNA fragments were cloned into the mammalian expression plasmid, pcDNA4. The cloned sequences contained exons 2 to 15 (full-length, except for exon 1), exons 2 to 12 ("catalytic + coil domains") and exons 9 to 15 ("coil + tail domains").

After transfection (48h), cells were fixed with 50:50 acetone/methanol and expressed protein was detected with anti-XPress tag antibody. Transfected "full-length" DMPK appeared to form aggregates in the cytoplasm of the COS cells, possibly an artefact of over-expression. Transfection with either "kinase + coil" or with the "coil + tail" constructs produced a generally even and intense expression staining of the cytoplasm with less intense staining in the nuclear compartment. Aggregation of over-expressed "full length" DMPK appears to require both the tail and catalytic domains.

To examine the same transfected cells using monoclonal antibodies (mAbs) against the catalytic or coil domains of DMPK, the cells were fixed with formalin and permeabilised with Triton X-100. As before, cytoplasmic aggregates were observed using anti-DMPK mAbs on cells transfected with "full length" DMPK. However, use of anti-DMPK mAbs on cells transfected with either kinase + coil or with coil + tail domains showed a stronger nuclear localisation than the anti-XPress antibody in acetone/methanol fixed cells. The results suggest that some DMPK is localised in the nucleus, although the apparent distribution depends on the method of fixation

To determine whether the distribution of transfected DMPK reflected the endogenous distribution, cultured human foetal muscle cells were formalin-fixed and incubated with anti-DMPK mAbs. DMPK was observed in both cytosolic and nuclear compartments.

Supported by the MDC and, more recently, by AFM.

P18) Glucose tolerance and insulinemia in transgenic mice carrying expanded CTG repeats

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Glucose intolerance, associated with an hyperinsulinemia in response to a glucose load. is frequently observed in myotonic dystrophy type 1 and type 2 patients. Although the insulin resistance mechanisms remained unclear until recently, several data demonstrated alterations in the insulin receptor (IR) function. A lot of common symptoms between DM1 and DM2 are probably resulting from a *trans*-dominant gain of function of RNA containing CUG or CCUG expansions, leading to splicing defects of other RNA. The switch from the normally predominant IR isoform to the lower-signalling isoform, observed in DM patients, could explain glucose intolerance and decreased insulin sensitivity.

In order to verify if these abnormalities could be modelled in transgenic mice, we investigated glucose tolerance and insulinemia in transgenic mice carrying the human DMPK gene with >350 CTG repeat. We previously showed that the human DMPK transgene was expressed in mice with a similar expression pattern than in human although at a lower level. As in DM1 patients, expanded DMPK RNA are retained in the nucleus and homozygous mice showed myotonia, myopathy and alteration of the tau isoforms in brain, in concordance with the *trans*-dominant effect of the DM1 mutation. We performed glucose-tolerance tests in mice carrying >350 CTG repeats or normal repeats of 20 CTG and in non transgenic mice. Mice were tested after a 16 hour fast. Glucose and insulin levels in blood were measured before and after intraperitoneal injection of glucose. Results and abnormalities observed in the transgenic mice will be presented and discussed.

P19) An *in vitro* model of myotonic dystrophy

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Molecularly DM1 is associated with the expansion of a CTG repeat in the 3' UTR of the *DM protein kinase* (*DMPK*) gene. How this expansion leads to the DM1 phenotype is still unclear but evidence is gathering for a gain of function model in which the repeat expansion within the *DMPK* RNA renders it toxic to downstream cellular processes. Indeed, in DM patient cells foci of aggregated expanded *DMPK* RNA are present which are not seen in control samples. These foci co-localise with CUG binding proteins MBNL; MBLL and MBXL, but have not been associated with a phenotype.

Although it doesn't co-localise with foci, CUG binding protein (CUGBP1) -a regulator of RNA processing, is known to have increased activity and levels in DM1 patient cells. This alteration can also be induced *in vitro* by expression of an expanded repeat and furthermore leads to aberrant splicing directly related to the phenotype: so far the *chloride channel* (myotonia), *insulin receptor* (diabetes), *tau protein* (dementia) and *cardiac troponin T* (reduced cardiac function) genes have been identified.

We believe that major aspects of DM pathology, in a variety of affected tissues are triggered by toxic RNA foci and CUG-BP mediated mis-splicing. To test this theory we are creating a mouse model using the cre-lox system to achieve conditional expression of an expanded CUG array. Expression can be spatial or temporal. Here we can delineate each facet of the complicated multisystemic phenotype *in vivo*. Temporal control will allow the study of the earliest molecular events —which are likely to be the most critical in DM pathogenesis.

Cre is a site-specific recombinase which recognises a DNA sequence termed loxP, and excises DNA situated between two of these sites. In the transgene, we have an EGFP reporter gene with the CUG repeat array in the 3'UTR. It is only expressed after Cre mediated excision of a 'stuffer' fragment flanked by lox P sites. The 'stuffer' region of DNA contains an SV40 polyadenylation to eliminate downstream gene expression. Our analyses have revealed that in the presence of the stuffer fragment no reporter gene expression can be detected. After the mouse model has been generated, expression of the CUG array is instigated by genetic breeding with a Cre-expressing mouse. It is the Cre mouse which defines tissue specificity and temporal expression. The original transgenic line may be bred with many different cre-expressing lines. Control may be achieved temporally using cre mice with inducible promoters such as the Mx1 promoter. This promoter is silent in healthy mice, but can be induced to high levels of transcription by administration of interferon alpha, interferon beta, or synthetic double-stranded RNA.

To date the constructs have been tested in HeLa and Cos7 cells by co-transfection of a cre-expressing plasmid. The 250 repeat construct forms foci, whereas the control with 5 repeats does not. We have generated the control mouse and pronuclear injections are ongoing with the 250 repeat.

P20) Three proteins, MBNL, MBLL and MBXL, co-localize *in vivo* with nuclear foci of expanded-repeat transcripts in DM1 and DM2 cells

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Myotonic dystrophy is a complex neuromuscular disorder associated with DNA expansion mutations in two different genes. In DM1 a CTG repeat in the 3'-untranslated region of DMPK is expanded, whereas in DM2 an intronic CCTG expansion occurs in the gene ZNF9. Transcripts containing expanded repeats form foci in the nuclei of DM1 and DM2 cells. Recent work using antibodies has shown that proteins related to *Drosophila* muscleblind co-localize with repeat foci in DM1 and DM2 cells. We show that rather than there being a single human muscleblind gene producing multiple proteins through alternative splicing, there are in fact three different muscleblind genes, MBNL, MBLL and MBXL, which map to chromosomes 3, 13 and X, respectively, and which show extensive alternative splicing. Two of the genes, *MBNL* and *MBLL*, are expressed in many adult tissues whereas MBXL is expressed predominantly in the placenta. Green fluorescent protein-tagged versions of MBNL, MBLL and MBXL co-localize with nuclear foci in DM1 and DM2 cells, suggesting that all three proteins may play a role in DM pathophysiology.

P21) *Cis*-effect and cytotoxicity of a reporter gene containing an expanded CTG repeat in a stable transfectant of PC12 neuronal cell

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Objective: The expansion of an unstable CTG triplet repeats in the 3'-untranslated region of the DM protein kinase mRNA is known to be responsible for myotonic dystrophy type 1(DM1). To understand the pathogenesis of DM1 in detail, we have established stable transfectant of PC12 cell (a rat neuronal cell line) and detected the cytotoxicity of expanded repeats containing LUC mRNA (Ex-mRNA).

Materials and Methods: A stable cell culture system expressing the reporter gene luciferase (LUC) alone or fused to the 250 CTG repeats (CTG250) were established using *p*CAGGS*neo* vector and PC12 cell. These cells were cultured with or without nerve growth factor (NGF) in serum-free medium as an oxidative stress model. Following several days of culture, cell cytotoxicity was detected by an LDH cytotoxicity detection kit (Takara, Co.), counting the number of cells and a LUC assay after neuronal differentiation. Cell apoptosis and cytoskeletal gene expression was confirmed by detection of the activated form of caspase-3 or several neuronal marker proteins by Western blotting.

Results: The number of CTG250 cells was significantly decreased comparing with LUC after replating those cells maintained in serum-free media with NGF. We probed for the active form of caspase-3 by Western blotting indicating that the cytotoxicity of Ex-mRNA causes apoptosis. Western blotting also showed low expression ß-tubulin in CTG250.

Discussion: PC12 cells show frequent cell division and the nuclear membrane disappears at intervals until NGF is added to the culture medium. Once NGF is added to the medium, the cells start differentiation that results in formation of a permanent nuclear membrane. In the undifferentiated condition, there are no differences in cell number and cytotoxicity between CTG250 and LUC, while in differentiated condition, decreased cell number, LDH activity and LUC activity were seen in the CTG250 cell line, indicating that Ex-mRNA is toxic to the neuronal cell and causes apoptosis. Our data also shows that the CTG-repeat expanded construct has a negative *trans*-effect on expression of other cytoskeletal genes. In addition to a *cis*-effect, a *trans*-effect was also observed even in neuronal cultured cells. We assume that the Ex-mRNA facilitate neurodegeneration in the CNS in DM, resulting in tauopathy.

P22) Comparative analysis of RNA repeat-binding proteins

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Recent studies have suggested that RNA-binding proteins are involved in the pathogenesis of myotonic dystrophy with the expanded CUG/CCUG repeat. First, we analysed the RNA-binding specificity of three such proteins, CUG-BP, EXP/MBNL, PKR. For this purpose, we used yeast three-hybrid system in which an interaction *in vivo* between repeat RNA and protein can be detected by the activity of reporter gene.

CUG-BP was shown to interact strongly with sequences containing UG/GU dinucleotide repeat. However, its binding to CUG and CCUG was not reproduced in this system. Although abnormal splicing events caused by altered expression of CUG-BP have been suggested, CUG-BP does not appear to be a direct target of expanded repeats. PKR, known as a double-stranded RNA-binding protein, also showed little affinity to these repeats. In contrast, EXP distinctly interacted with both CUG and CCUG repeats, suggesting that this protein is directly involved in the repeat expansion in the pathogenesis of both DM1 and DM2.

Deletion analysis of EXP indicated the splice variation in the affinity to the RNA repeat, reflecting the composition of the zinc finger motifs. From the results of yeast three-hybrid analysis, we deduced the target sequence of EXP. Based on this sequence, we listed several candidate targets of EXP by database search. Identification of the target genes of EXP is important because if the molecular function of EXP is disturbed by the expanded repeat, the innate target of EXP might also be affected and this would lead to the pathogenesis of myotonic dystrophy. We are now trying to detect the binding of EXP to one of the candidate genes by *in vitro* experiments.

P23) RNA-based gene therapies for myotonic dystrophy type 1

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The main obstacle for developing a successful gene therapy approach for myotonic dystrophy is specific targeting of the mutant DMPK transcripts sequestered in the nucleus. Many of the molecular and cellular disruptions seen in the disease are believed to be related to the presence of the large CUG expansion on the RNA. Here we assess whether different RNA-based gene therapies such as antisense RNAs and ribozymes could prove effective in DM1.

In order to specifically target mutant DMPK transcripts, we developed a vector system employing a modified tRNA-met promoter capable of producing high levels of DMPK-specific hammerhead ribozymes in the nucleus. Accessible ribozyme target sites were identified in 3'UTR of the DMPK mRNA thus allowing targeting of most DMPK splicing isoforms. Ribozymes transcribed with this expression system are retained in the cell nucleus because of mutations blocking RNA maturation and nuclear export. Cleavage of DMPK transcripts by the ribozymes generates a 5'-hydroxyl group rendering the RNA suitable for degradation. Here we report 50% and 63% reductions of endogenous wild type and mutant DMPK transcripts respectively. Destruction of DMPK mRNA correlates with a reduction in nuclear foci number and intensity and also with partial restoration of type B insulin receptor mRNA expression in DM1 myoblasts with 750 CTG repeats. Our results thus indicate that destruction of mutant DMPK mRNA is possible and may prove necessary to restore normal cell functions in DM1 myoblasts.

P24) An RNA gain of function for CUG-repeats disrupts regulation of alternative splicing

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A major component of DM pathogenesis involves disruption of nuclear RNA processing due to expression of RNA transcripts from the expanded allele. We have previously identified three genes which undergo aberrant regulation of alternative splicing in DM striated muscle: cardiac troponin T (cTNT), insulin receptor (IR), and the muscle-specific chloride channel (ClC-1). Aberrant splicing regulation results in expression of natural mRNAs at inappropriate times or in inappropriate tissues. Mis-regulated alternative splicing of IR and ClC-1 is linked to insulin resistance and myotonia in DM1, respectively. The mis-regulated pre-mRNAs are natural targets of CUG-BP, a known splicing regulator and one of a family of six related CELF proteins. The splicing patterns for all three genes reflect increased CUG-BP activity consistent with increased levels of CUG-BP detected in the relevant DM tissues. It is unclear what fraction of alternative splicing is regulated by CELF proteins and, by extension, what fraction of pre-mRNA alternative splicing is disrupted in DM. We have analyzed additional alternative splicing events in tissues from unaffected individuals and DM1 patients and have found additional genes whose splicing regulation is affected (including tau and FXR1) and genes not affected (including amyloid precursor protein and clathrin light chain). Splicing of the clathrin light alternative exon is not significantly affected by overexpression of CUG-BP consistent with the absence of aberrant regulation in DM1 tissue. We plan to expand this analysis using technologies to assay large numbers of alternative splicing events.

P25) The myotonic dystrophy mutation sensitizes myoblasts to apoptosis

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The pathogenic mechanism for DM is unknown, however, mounting evidence suggests a dominant gain of function conferred by CUG repeat expansions upon mutant transcripts. We have used stable C2C12 mouse myoblast lines to model the effects of this gain-of-function in muscle. A *GFP* reporter gene was expressed alone or was fused to the *DMPK* 3' UTR containing either 11 or 99 CTG repeats. We found that transgene expression levels were approximately ten fold higher in the control and 11 CTG repeat cell lines compared with the 99 CTG repeat lines, suggesting a negative selection upon clones expressing high levels of longer repeats. Consistent with this idea, 99 repeat lines were more sensitive to staurosporine-induced apoptosis than cell lines with 11 CTG repeats. Similarly, DM patient amniocytes and myoblasts were more susceptible to staurosporine-induced apoptosis than control cells. Furthermore, DM myoblasts also showed higher than normal levels of the apoptosis that is normally associated with myogenic differentiation, suggesting again a heightened sensitivity to apoptosis, which is triggered by cell cycle withdrawal.

One candidate mediator of these effects is the double stranded RNA-activated protein kinase (PKR), which induces apoptosis and determines myoblast cell fate upon differentiation. PKR was activated preferentially by the *DMPK* 3' UTR containing 99 CTG repeats compared with that containing 11 CTG repeats or by a control UTR. PKR protein levels were elevated in myoblasts expressing the *DMPK* 3' UTR with 11 or 99 CTG repeats but not in cells expressing a control UTR. Taken together, these data demonstrate that a possible pathogenic mechanism for DM is a lowered apoptotic threshold in part through activation of PKR, resulting in delayed myogenesis and muscle loss.

P26) Development and analyses of mice expressing expanded CUG repeat tracts in the heart

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DM1 is a multi-system disorder characterized by cardiac disease. Heart disease in DM1 manifests primarily as conduction disorders with cardiac hypertrophy and dilated cardiomyopathy being observed at lower frequency. We have previously shown that decreased levels of Dmpk result in cardiac conduction defects. To test if expression of expanded (CUG)n repeats in heart result in cardiac defects, we have developed novel strains of mice expressing 0.7 and 1.2 kb CTG tracts in cardiac muscle. In these experiments expanded CTG tracts were cloned in the 3' UTR of ß-galactosidase gene driven by myosin heavy chain promoter. Structure-function analyses of these mice will be presented.

P27) Expression of $(CUG)_n$ RNA in skeletal muscle lead to decreased tetanic force development and mitochondrial abnormalities

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Myotonic dystrophy type1 (DM1) is an autosomal dominant multi-system disorder associated with a CTG repeat expansion located in the 3' untranslated region of DMPK and upstream of a homeo-domain encoding gene, SIX5. Skeletal muscle is prominently affected in DM1. DM1 skeletal muscle defects include myotonia, muscle weakness and wasting. Ultra-structural abnormalities in mitochondria, the Z line and in the sarcoplasmic reticulum are features observed in DM1 muscles. Previous studies by Mankodi et al (Science, 289,1769, 2000) have shown that expression of an □-actin cassette containing a 0.7 kb tract of CUG repeats in skeletal muscle leads to myotonia, muscle fibre loss and the formation of central nuclei. To test if expression of various lengths and dosage of (CUG)n repeats in muscle result in other muscle abnormalities, we have developed mice expressing various lengths of CUG repeats in skeletal muscle. In these experiments expanded CTG tracts were cloned in the 3' UTR of □-galactosidase gene under the transcriptional control of the human □-actin promoter. Mice expressing CUG RNA show as myotonia, as previously reported by Mankodi et al. Significantly, we demonstrate a 30-40% reduction of muscle tetanic force generation. Electron microscopic analysis show swollen cristae and mitochondrial loss. The details of the various analyses will be presented.

P28) Assessing neurological disability in myotonic dystrophy

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Myotonic dystrophy (MD) is an inherited multi-systemic disease. Although myotonia, muscle weakness and wasting are characteristic features of the disease, neurological disability in MD is often due to somnolence, fatigue and related behavioural changes. Unlike other progressive neurological disorders, there is no agreed assessment scale in MD to monitor disease progression and disability. No specific treatment is yet available to modify the natural history of neurological disease in MD.

An ongoing prospective pilot study in Glasgow is currently assessing a cohort of genetically confirmed MD patients longitudinally in order to identify appropriate markers for disease severity and neurological disability in MD. Recruited patients have periodic assessments for 2 years. At the predetermined assessment points during this period, the patients fill in previously validated self-completed questionnaires for daily activities, range of symptoms and quality of life in addition to their physical examination and annual electrocardiogram. Proton magnetic resonance spectroscopy scans of brain are also carried out in the regions of interest that are known to correlate with the severity of MD gene expression. Muscle mass in the arms and legs are measured by using dual X-ray energy absorptiometry to objectively identify limb muscle wasting over time.

Cumulative longitudinal data in these patients are expected to provide an opportunity to develop and validate the first unified MD disease severity rating scale. This scale will be essential not only for disease prognosis but also to test the efficacy of specific therapeutic interventions in limiting progression of neurological disability.

P29) The relationship between myotonic dystrophy type 1 and tumours

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Myotonic dystrophy type 1 (DM 1) is an autosomal dominant disorder characterized by a wide variety of clinical features. The mutation responsible for DM1 is an unstable expansion of a CTG triplet repeat ((CTG) $_n$) in the 3' untranslated region of a gene encoding DM protein kinase (DMPK). Genetically, it has been speculated that the DMPK gene with normal (CTG) $_n$ length may act as a tumor suppressor. Clinically, it has been often reported that a variety of benign and malignant tumours are associated with DM1. We found a woman's patient with DM 1 associated with an ovarian cancer and a man's patient with DM 1 accompanied by laryngeal and renal cell carcinomas previously. We therefore investigated whether tumours that accompany DM1 are incidental or disease-related.

The subjects included 55 patients with DM1, 27 men and 28 women (mean age 46.2±10.9). We examined whether they were affected with tumours, using medical history, cervical and abdominal echography, and abdominal CT. Southern blot analysis was done as described previously to determine the $(CTG)_n$ lengths. Briefly, the genomic DNAs extracted from the leukocytes were digested with the restriction enzymes of Eco R I, then hybridized with a ^{32}P-labeled cDNA25 probe

Abnormal expanded alleles were exhibited in all patients. Those allelic sizes ranged from 9.9 to 18.0 kb. Ovarian cyst, myoma uteri, and endometriosis were present in 21 (77.8 %), 12 (44.4 %), and 3 (11.1 %) of 27 women's patients, respectively. Adenomatous goiter in the thyroid gland and parotid gland tumor were found in 22 (7.3 %), 3 (11.1 %) of 55 patients, respectively. In addition, an ovarian cancer was found in one women's patient, and laryngeal and renal cell carcinomas in one man's patient. Abnormal expanded allelic sizes in the leukocytes obtained from the former patient and the latter were 15.4 and 9.9 kb, respectively

Ovarian cyst and myoma uteri might be associated with DM 1 because the frequency of each tumor was relatively great. However, it was unclear the relationship between the (CTG) $_n$ length and tumours.

P30) Database for myotonic dystrophies (DM1 and DM2): general principles and description

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An international database dedicated to myotonic dystrophies is a very useful tool for promoting clinical research. The main objective of such a database is to collect the clinical and paraclinical data from a large group of type 1 and type 2 DM patients in order to develop clinical research in the field of myotonic dystrophies. We present here the main elements of the database, which include two complementary sections. The first part is an inclusion document collecting the following basic information: identification, elements for diagnosis, clinical history, clinical evaluation of neuromuscular and systemic signs, professional and social consequences of the disease. The second part is an annual follow-up section reporting the recent clinical events and an actualised clinical evaluation. The clinical research objectives derived from the database will be: 1) to compare the main features of both diseases in patients from different countries. In particular, the study will compare the DM1 genetically homogenous Quebec population to the genetically heterogeneous European patients 2) to identify severity factors (weakness, cardiac and respiratory involvement, swallowing disturbances, intellectual impairment) and to study their potential interrelations; 3) to study mortality and morbidity; 4) to precise genotype/phenotype correlations; 5) to compare DM1 and DM2 features; and 6) to select patients for future therapeutic trials.

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See you all then!

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