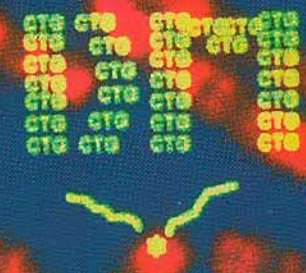
1ère conférence internationale AFM/MDA du consortium sur la dystrophie myotonique de Steinert



AFM/MDA first international myotonic dystrophy consortium conference

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

The Denver Fund For Health & Medical Research

AFM
Association Française contre les Myopathies

MDA

AFM / MDA FIRST INTERNATIONAL MYOTONIC DYSTROPHY CONSORTIUM CONFERENCE

June 30. / July 1. 1997

PREMIERE CONFERENCE INTERNATIONALE AFM / MDA DU CONSORTIUM SUR LA DYSTROPHIE MYOTONIQUE DE STEINERT

30 Juin / 1er Juillet 1997

Institut de Myologie / AFM
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WHY AN INTERNATIONAL WORKSHOP ON MYOTONIC DYSTROPHY (DM)?

1991-1997: Five years have elapsed since the discovery of this strange unstable mutation responsible for myotonic dystrophy. The list of new diseases associated with this type of mutation continues to increase, but each of them involves a different type of mechanism. However in spite of the tremendous accumulation of data, the molecular mechanisms underlying the physiopathogenesis of DM remain the most enigmatic. As quoted by K. Johnson, " we begin to realize that DM is unique amongst the mendelian inherited disorders ". However fascinating this disease can be for investigators, we must admit that it remains a terrible burden for patients for whom these scientific advances have not yet been translated in terms of slowing-down the dreadful progression of the disease or of relieving their pains.

For a long time AFM, the Association Française contre les Myopathies, has been concerned by the absence of a specific Association of DM families, who nonetheless, need better information on not only therapeutic possibilities, but also on social and psychological issues. This absence of coordination is also flagrant at the level of fundamental research. Investigators working on myotonic dystrophy molecular genetics have had the opportunity to meet through the chromosome 19 workshops and due to the organization by MDA of a satellite meeting at the annual meeting of the American Society of Human Genetics. However none of these meetings included biologists and clinicians involved in different approaches, other than genetics. With he logistic and financial support of AFM and MDA, we decided to try and fill this gap. AFM thus took charge of organization of the first International workshop on a two days scientific and medical meeting entirely devoted to DM. If this meeting lives up to its promise, the next one should be organized by MDA, next year in Tucson. We hope this meeting will light up some points whish are still dark, stimulate new scientific collaborations and help contribute to the discovery of new therapeutic approaches.

ENFIN UNE REUNION INTERNATIONALE POUR LA MALADIE DE STEINERT !

1991-1997 : Cinq années déjà se sont écoulées depuis la découverte de cette étrange mutation instable responsable de la dystrophie myotonique de Steinert. La liste des maladies associées à ce type de mutation continue de s'allonger, mais, il apparaît que des mécanismes différents sont à l'origine de ces maladies. Malgré une progression considérable dans le domaine de la connaissance, le mécanisme moléculaire physiopathologique de la dystrophie myotonique de Steinert demeure le plus énigmatique de tous. Comme l'a souligné K. Johnson, nous commençons à réaliser que la DM représente un cas unique parmi les maladies mendéliennes. Maladie certes fascinante pour les chercheurs elle n'en demeure pas moins une terrible menace pour les patients, pour lesquels ces progrès ne se traduisent pas encore par un ralentissement de la progression ou un soulagement de leurs souffrances.

L'AFM se préoccupe du soutien qu'elle peut apporter à ces patients, qui ne peuvent avoir recours à une association de familles spécifique, pour une meilleure information sur les différentes approches et aides thérapeutiques, sociales et psychologiques. Cette absence de coordination apparaissait également au niveau de la recherche. Malgré les réunions annuelles organisées par la MDA dans le cadre du congrès l'American Society of Human Genetics, ces rencontres, d'une soirée, s'adressaient aux généticiens seulement et ne permettaient pas de rassembler cliniciens et chercheurs quelle que soit l'approche utilisée. Avec le soutien non seulement logistique mais aussi financier de l'AFM et de la MDA, nous avons donc souhaité combler cette lacune. L'AFM a ainsi accepté de prendre en charge l'organisation, à Paris, du premier Workshop International à travers deux journées scientifiques et médicales entièrement consacrées à cette affection. Si cette formule s'avère efficace la MDA prendra la relève pour un deuxième workshop, cette fois à Tucson, l'an prochain. Nous espérons donc que ces journées permettront d'éclairer des points obscurs, de créér de fructueuses collaborations, d'imaginer de nouvelles approches dans le souci permanent de contribuer à l'élaboration de nouvelles thérapeutiques.

SUMMARY

SOMMAIRE

SCIENTIFIC PROGRAMME

PROGRAMME SCIENTIFIQUE

June, Monday 30.

MORNING

8.15 Registration

8.45 **C. Junien, T. Ashizawa, M.C. Lagrange**
Welcoming Introduction

9.05 **A. Roses**
Clinical Overview

9.15 **B. Wieringa**
Key Questions

INSTABILITY MECHANISMS

1 - DNA Structure, Chromatin Structure, DNA Repair

Chairpersons : R. Wells / R. Sinden

9.25 **R. Wells / R. Sinden** - Review

9.35 **Investigators' Presentations**

R. Wells	-	DNA structure and molecular mechanisms of instability
R. Sinden	-	Unusual flexible helix structure of CTG repeat
R. Sinden	-	Role of mismatch repair genes in CTG stability: *in vivo* and *in vitro* studies
J. Griffith	-	Chromatin structure of expanded triplet DNAs

10.05 **Discussion**

10.20 - Coffee Break

2 - Instability in Humans

Chairpersons : T. Ashizawa / C. Junien

10.40 **T. Ashizawa / C. Junien** - Review

10.50 **Investigators' Presentations**

L. Martorell	-	Somatic instability of (CTG)n repeat during human fetal development
M. Anvret	-	Variation of CTG repeat number of the DMPK gene in muscle tissue
T. Ashizawa	-	Instability of DM CTG repeats in cultured human cell lines
T. Ashizawa	-	Postzygotic instability of the CTG repeat in congenital myotonic dystrophy
D. Monckton	-	Male germline transmission of normal allele

11.25 **Discussion**

3 - Instability in Animal Models

Chairpersons : D. Monckton / R. Korneluck

11.40 **D. Monckton / R. Korneluck** - Review

11.50 **Investigators' Presentations**

D. Monckton	-	Hypermutable myotonic dystrophy CTG repeats in transgenic mice
G. Gourdon	-	Intergenerational somatic and germinal CTG repeat instability in mice
M. Narang	-	Transgenic Mouse Models of Myotonic Dystrophy.

12.10 **Discussion**

12.25 - Lunch

Lundi 30 Juin.

MATINEE

8h15 Accueil

8h45 **C. Junien, T. Ashizawa, M.C. Lagrange**
Introduction

9h05 **A. Roses**
Revue clinique

9h15 **B. Wieringa**
Questions clés

MECANISMES D'INSTABILITE

1 - Structure de l'ADN, structure de la Chromatine, Réparation

Modérateurs : R. Wells / R. Sinden

9h25 **R. Wells / R. Sinden -** Résumé

9h35 **Presentations**

R. Wells	-	Structure de l'ADN et mécanismes moléculaires de l'instabilité
R. Sinden	-	Structure inhabituelle en hélice des répétitions CTG
R. Sinden	-	Rôle des gènes de réparation dans l'instabilité des CTG : études *in vivo* et *in vitro*
J. Griffith	-	Structure chromatinienne des expansions de triplets

10h05 **Discussion**

10h20 - Pause

2 - Instabilité chez l'homme

Modérateurs : T. Ashizawa / C. Junien

10h40 **T. Ashizawa / C. Junien -** Résumé

10h50 **Presentations**

L. Martorell	-	Instabilité somatique des répétitions CTG pendant le développement foetal humain
M. Anvret	-	Variation des répétitions CTG dans le muscle
T. Ashizawa	-	Instabilité des répétitions CTG dans des lignées cellulaires humaines
T. Ashizawa	-	Instabilité post-zygotique des répétitions CTG dans la forme congénitale
D. Monckton	-	Transmission de l'allèle normal par la lignée germinale mâle.

11h25 **Discussion**

3 - Instabilité et modèles animaux

Modérateurs : D. Monckton / R. Korneluck

11h40 **D. Monckton / R. Korneluck** - Résumé

11h50 **Presentations**

D. Monckton	-	Répétitions CTG instables chez la souris transgénique
G. Gourdon	-	Instabilité intergénérationnelle, somatique et germinale dans la souris
M. Narang	-	Modèles de souris trangéniques pour la Dystrophie Myotonique

12h10 **Discussion**

12h25 - Déjeuner

June, Monday 30.

AFTERNOON

4. Population Genetics

Chairpersons : R. Chakraborty / K. Johnson

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

13.40 **Investigators' Presentations**

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

14.15 **Discussion**

DISEASE MECHANISMS

1 - DMPK DNA/RNA

Chairpersons : B. Wieringa / R. Korneluk

14.30 **B. Wieringa / R. Korneluk** - Review

14.40 **Investigators' Presentations**

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

16.05 **Discussion**

16.25 - Coffee Break

2 - DMPK Protein

Chairpersons : H. Epstein / B. Wieringa

16.45 **H. Epstein / B. Wieringa** - Review

16.55 **Investigators' Presentations**

- **J. Puymirat** - Characterisation of a 54-kDA human protein kinase recognized by an antiserum raised against the myotonin kinase
- **H. Epstein** - The DM protein kinase : Characterization and use of antibodies in evaluating DM in human muscle and lens
- **H. Epstein** - Studies of DM kinase action in cultured lens and muscle cells
- **S. Salvatori** - Myotonic dystrophy protein kinase is localized to the terminal cisternae of the sarcoplasmic reticulum
- **J.R. Moorman** - Phosphorylation of the Na channel inactivation gate by DM kinase
- **P. Groenen** - DMPK and Ca^{2+} homeostasis
- **F. Lehmann-Horn** - Electrophysiology of myotonic dystrophy

17.40 **Discussion**

19.30 - Dinner and Visit of the Mediaval Louvre

Lundi 30 Juin.

APRES-MIDI

4. Génétique des populations

Modérateurs : R. Chakraborty / K Johnson

13h30 **R. Chakraborty / K. Johnson** - Résumé

13h40 **Presentations**

A. Goldman	-	Analyse des haplotypes DM dans les populations africaines
R. Krahe	-	Origine et évolution des expansions CTG chez les primates
T. Miki	-	Génétique des populations des répétitions CTG
MR Passos-Bueno	-	Distorsion de ségrégation des répétitions CTG au locus DM
R. Chakraborty	-	Diversité des haplotypes du polymorphisme normal des CTG au locus DM : Implications pour l'évolution et le maintien de la fréquence de la DM

14h15 **Discussion**

MECANISMES PATHOLOGIQUES

1 - DMPK DNA/RNA

Modérateurs : B. Wieringa / R. Korneluk

14h30 **B. Wieringa / R. Korneluk** - Résumé

14h40 **Presentations**

S. Tapscott	-	Expansion des répétitions et structure locale de la chromatine
V. Funanage	-	Effets des amplifications CTG sur la structure chromatinienne et sur la maturation des ARNm DMPK dans des cellules hybrides dérivées de patients DM et CDM
P. Steinbach	-	Le gène DMPK des patients sévèremenent atteints est hyperméthylé au niveau de l'îlot CpG proche des répétitions CTG
R. Korneluk	-	Promoteur ubiquitaire et enhancer muscle-spécifique du gène DMPK
D. Brook	-	Analyse de l'expression des gènes DMPK, DMAHP et 59 dans les lignées cellulaires DM
AS. Lia	-	Expression des gènes DMPK et DMHAP pendant le développement foetal
B. Davis/R. Singer	-	L'expansion des répétitions CTG dans la région 3' UTR des transcrits DMPK entraîne une séquestration des transcrits dans le noyau
MS Swanson	-	Rôle des protéines fixant les répétitions de triplets dans la Dystrophie Myotonique
L. Timchenko	-	Altération de la phosphorylation et de la distribution intracellulaire des protéines fixant les répétitions (CUG)n et souris invalidées pour le gène DMPK
R. Sinden	-	Transcription de l'ARN polymérase II à travers les répétitions de triplets des loci humains DM et X-fragile
C. Milcarek	-	Evidence d'une perturbation au niveau ARN
C. Thornton	-	Les grandes répétitions CUG forment des structures qui interagissent avec le site de fixation de l'ARN de la PKR

16h05 **Discussion**

16h25 - Pause

2 - La protéine DMPK

Modérateurs : H. Epstein / B. Wieringa

16h45 **H. Epstein / B. Wieringa** - Résumé

16h55 **Presentations**

J. Puymirat	-	Caractérisation de la kinase humaine de 54-kDA reconnue par un anticorps dirigé contre la myotonine kinase
H. Epstein	-	Caractérisation et utilisation d'anticorps pour l'étude de la dystrophie myotonique dans le muscle et le cristallin
H. Epstein	-	Etude du rôle de la DM kinase dans des cultures de cellules du cristallin et des cellules musculaires
S. Salvatori	-	La myotonine kinase est localisée au niveau des citernes terminales du reticulum sarcoplasmique
J.R. Moorman	-	Phosphorylation des canaux Na par la DM kinase
P. Groenen	-	DMPK et Homéostasie de Calcium
F. Lehmann-Horn	-	Electrophysiologie

17h40 **Discussion**

19h30 - Diner et Visite du Louvre Médiéval

July, Tuesday 1.

MORNING

DISEASE MECHANISMS (CONTINUATION)

3 - DMAHP / 59 (N9)

Chairpersons : K. Johnson / D. Brook

8.30 **K. Johnson / D. Brook** - Review

8.40 **Investigators' Presentations**

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

9.30 **Discussion**

9.45 - Coffee Break

4 - Transgenic animal models

Chairpersons : D. Housman / B. Wieringa

10.10 **D. Housman / B. Wieringa** - Review

10.20 **Investigators' Presentations**

P. Groenen	-	Toward an animal model for DM
S. Reddy	-	Animal models for myotonic dystrophy
D. Monckton	-	Mouse models of DM
G. Gourdon	-	Transgenic models to study CTG repeat amplification consequences
M. Narang	-	Transgenic Mouse Models of Myotonic Dystrophy
J. Puymirat	-	Transplantation of myotonic dystrophy myoblasts as a potential muscular model for myotonic dystrophy

11.10 **Discussion**

5 - Clinical studies of the Disease Mechanisms

Chairperson : C. Thornton

11.30 **C. Thornton** - Review

11.40 **Investigators' Presentations**

G. Butler-Browne	-	An introduction to the development of skeletal muscle
J.P. Barbet	-	Immaturity of skeletal muscle in congenital myotonic dystrophy
B. Eymard	-	A new case of paternally inherited congenital myotonic dystrophy
M. Giacanelli	-	Magnetic resonance imaging (MRI) and genetic correlation in DM patients
D. Duboc	-	Correlation between decreased myocardial glucose phosphorylation and the DNA mutation size in myotonic dystrophy
MA. Cordoliani	-	Overexpression of the tau 55 isoform in the brain of patients with myotonic dystrophy

12.20 **Discussion**

6 - PROMM

Chairpersons : F. Lehmann-Horn / M.C. Krahe

12.35 **F. Lehmann-Horn / M.C. Krahe** - Review

12.45 **Investigators' Presentations**

M.C. Koch	-	**PROMM** - the extended German experience

12.50 **Discussion**

13.00 - Lunch

Mardi 1er Juillet

MATINEE

MECANISMES PATHOLOGIQUES (SUITE)

3 - DMAHP / 59 (N9)

Modérateurs : K. Johnson / D. Brook

8h30 **K. Johnson / D. Brook** - Résumé

8h40 **Presentations**

- **K. Johnson** - Identification et expression du gène DMAHP murin
- **S. Harris** - Identification des sites de fixation de DMAHP sur l'ADN
- **C. Thornton** - Répression en cis du gène DMAHP par la mutation DM
- **M. Gennareli** - Diminution de l'expression de DMAHP dans le cerveau
- **S. Tapscott** - Transcription du gène DMAHP à partir de l'allèle normal et de l'allèle amplifié
- **V. Funanage** - Les amplifications CTG n'interviennent pas sur l'expression des gènes DMAHP et 59 dans des cellules hybrides dérivées de patients DM et CDM
- **B. Wieringa** - Rôle de DMR-N9

9h30 **Discussion**

9h45 - Pause

4 - Modèles animaux transgéniques

Modérateurs: D. Housman / B. Wieringa

10h10 **D. Housman / B. Wieringa** - Résumé

10h20 **Presentations**

- **P. Groenen** - Vers un modèle animal de DM
- **S. Reddy** - Modèles animaux pour la Dystrophie myotonique
- **D. Monckton** - Modèles murins pour la DM
- **G. Gourdon** - Modèles trangéniques pour étudier l'influence des répétitions CTG
- **M. Narang** - Modèles transgéniques pour la Dystrophie myotonique
- **J. Puymirat** - Transplantation de myoblastes DM comme modèle potentiel de la Dystrophie myotonique

11h10 **Discussion**

5 - Etudes cliniques

Modérateur : C. Thornton

11h30 **C. Thornton** - Résumé

11h40 **Presentations**

- **G. Butler-Browne** - Introduction sur le développement du muscle squelettique
- **J.P. Barbet** - Immaturité du muscle squelettique dans la forme congénitale CDM
- **B. Eymard** - Transmission paternelle d'une forme congénitale CDM
- **M. Giacanelli** - Résonnance magnétique (MRI) et corrélation génétique chez les patients DM
- **D. Duboc** - Corrélation entre la taille de la répétition et la diminution de la phosphorylation du glucose
- **MA. Cordoliani** - Surexpression de l'isoforme tau 55 dans le cerveau de patients DM

12h20 **Discussion**

6 - PROMM

Modérateurs : F. Lehmann-Horn / M.C. Krahe

12h35 **F. Lehmann-Horn / M.C. Krahe** - Résumé

12h45 **Presentations**

M.C. Koch - **PROMM** - L'expérience allemande

12h50 **Discussion**

13h00 - Déjeuner

July, Tuesday 1.

AFTERNOON

Scientific Conclusions ; Need for Present and Future Collaborations

Chairperson : R. Korneluk

14.00 **Discussion**

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

14.20 **Data Bank**

J. Puymirat

DIAGNOSIS AND TREATMENT

1 - Treatment

Chairpersons : R.T. Moxley / T. Ashizawa

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

14.40 **Investigators' Presentations**

R.T. Moxley	-	A possible new approach to treatment in myotonic dystrophy : description of preliminary findings in a double blind randomized trial of troglitazone
M. Rogers	-	Development of a clinical protocol for use in clinical trials on ongoing assessment management in myotonic dystrophy.
N. Ohsawa	-	Therapeutic trial of dehydroepiandrostérone-sulfate (DHEA-S in DM)

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

R.T. Moxley, M. Rogers, T. Ashisawa, D. Duboc, B. Eymard, H. Epstein

- Research-deduced treatment strategies, antisense, antioxidants, free radical scavengers, trophic factors, anabolic steroids, etc.

2 - Diagnosis, Genetic Counseling, and Psychosocial Problems

Chairperson : M. Baiget

15.30 **Investigators' Presentations**

G. Novelli	-	Prenatal prediction using long PCR
M. Baiget	-	Needs for Diagnosis Guidelines for DM

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

16.00 - **Good-bye Coffee -Tea**

Mardi 1er Juillet

APRES-MIDI

Conclusion des aspects scientifiques : Collaborations présentes et futures

Modérateur : R. Korneluk

14h00 **Discussion**

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

14h20 **Banque de données**

J. Puymirat

DIAGNOSTIC ET TRAITEMENT

1 - Traitement

Modérateurs : R.T. Moxley / T. Ashizawa

14h30 **R.T. Moxley / T. Ashizawa** - Résumé

14h40 **Presentations**

R.T. Moxley - Une nouvelle approche possible pour traiter la Dystrophie myotonique : description d'une étude préliminaire en double aveugle dans un essai randomisé par la troglitazone

M. Rogers - Développement de protocoles d'essais cliniques pour la Dystrophie myotonique

N. Ohsawa - Essai thérapeutique du dehydroepiandrostérone-sulfate (DHEA-S dans la maladie de Steinert)

15h00 **Table ronde** - **Perspectives de traitement pour les maladies à triplet**

R.T. Moxley, M. Rogers, T. Ashisawa, D. Duboc, B. Eymard, H. Epstein

- Stratégies de traitement dérivées de la recherche : antisens, antioxydants, radicaux libres, facteurs trophiques, stéroïdes anabolisants, etc.

2 - Diagnostic, Conseil génétique, et Problèmes psychosociaux

Modérateur : M. Baiget

15h30 **Presentations**

G. Novelli - Diagnostic prénatal par longue PCR

M. Baiget - Recommandations pour le diagnostic DM

15h50 **C. Junien / T. Ashizawa : Conclusion et prochaine réunion du Consortium**

16h00 - **Au revoir ... autour d'un café ou thé**

June, Monday 30.
Morning

Lundi 30 Juin
Matinée

ABSTRACTS

COMMUNICATIONS

CTG/CAG STRUCTURE AND MOLECULAR MECHANISMS OF INSTABILITY.

S. Amirhaeri, A. Bacolla, R.P. Bowater, P. Chastain, E. Eichler, R. Gellibolian, D. Giedroc, J. Griffith, S. C. Harvey, A. Jaworski, S. Kang, S. Kramer, J. Larson, S. Levene, D. Nelson, K. Ohshima, B.A. Oostra, P. Parniewski, W. Rosche, M. Shimizu, R. Sinden, B.D. Stollar, Y.W. Wang, and **R.D. Wells.**
Institute of Biosciences and Technology, Texas A&M University, Texas Medical Center, 2121 W. Holcombe Blvd., Houston, Texas 77030, USA.

In an effort to understand the molecular etiology of myotonic dystrophy, we are investigating the genetic instabilities of CTG/CAG, DNA polymerase pausing and hairpin formation at these triplet repeat sequences (TRS), mismatch repair, DNA structure, and slippage of complementary strands. Expansions and deletions of CTG/CAG in E. coli are influenced by the orientation of the insert due to hairpin loop formation during replication. Comparison of the capacity of all ten triplet repeat sequences to be expanded revealed that CTG/CAG is preferred by nine fold. The CTG/CAG structure and/or its interactions with the replication enzymes is responsible for this preference. This may explain why CTG/CAG is found in virtually all of the diseases. DNA polymerases pause within long triplet repeats due to a non-B DNA structure and hence, may accentuate slippage and thus genetic instabilities. DNA hairpins are formed by primer realignment and chain elongation. An intricate relationship between E. coli growth and deletions of triplet repeats in plasmids is found; at extended growth periods, the frequencies of deletions were dramatically increased if the cells passed through stationary phase before subculturing. In addition, transcription through the repeats increases the deletion frequency. CTG/CAG is more flexible and highly writhed than random B-DNA and, thus, would be expected to act as a sink for the accumulation of superhelical density. We believe that these unusual DNA properties account, at least in part, for the slippage of complementary strands that elicits expansions and deletions.
DNA complementary strand slippage of microsatellite sequences has been frequently postulated as the mechanism responsible for a range of biological observations including DNA polymerase errors, slipped strand mispairing mutagenesis, genetic hypermutability of simple repeating sequences in mismatch repair-deficient cells related to hereditary non-polyposis colon cancer, and genetic instabilities of triplet repeat sequences associated with human neurological diseases. We will provide direct evidence for the in vivo slippage of CTG/CAG. A model will be proposed to explain the observed expansions and deletions via strand misalignment, incision or excision, followed by DNA synthesis and ligation.
These systems may enable the elucidation of the molecular mechanisms of the non-Mendelian process of expansion as related to myotonic dystrophy

Notes :

(CTG)N (CAG)N TRIPLET REPEATS ASSOCIATED WITH DM: UNUSUAL HELICAL PROPERTIES AND ALTERNATIVE DNA SECONDARY STRUCTURES.

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About twelve human genetic diseases have been associated with the expansion of CTG or CGG repeats. While the molecular etiology is unknown expansion may involve the participation of an unusual helix structure or alternative DNA structure in replications repair or recombination. DNA fragments containing (CTG)n (CAG)n (where n = 17 to 255) derived from the human myotonic dystrophy gene have up to a 20% faster-than-expected mobility in nondenaturing polyacrylamide gels. The anomalous mobility is dependent upon the number of triplet repeats, the length of the flanking DNA, and the percentage and temperature of the polyacrylamide. Applying a reptation model for electrophoresis, these results are consistent with a 20% increase in persistence length of the DNA, implying a straight helix trajectory.

We have investigated the mobility of DNA containing phased regions of curvature interspersed with (CTG)n tracts. Results demonstrate that (CTG)n sequences do not possess static curvature but that they reduced overall curvature resulting from A-tract or non-A-tract bends in an orientation independent manner. This decrease in the apparent overall curvature of A-tract bends was comparable to the effect of placing regions of increased bendability adjacent to the A-tract bends. these results and those from cyclization kinetic measurements, suggest that CTG repeats may be inherently flexible.

Using cloned fragments from the DM loci containing normal, premutation, and full mutation length of repeats, we report the first results consistent with the existence of slipped strand structures in DNA (S-DNA). These novel alternative DNA secondary structures form during reannealing of complementary strands containing (CTG)-(CAG) repeats. S-DNA maps within the repeat tracts of otherwise duplex DNA molecules and contains equal lengths of repeats. Sadness are characterized by reduced electrophoretic mobilities in polyacrylamide gels consistent with the presence of 3- or 4-way junctions. S-DNA structures are stable at physiological ionic strengths and up to temperatures of at least 55˚C. The CAG strand of S-DNA structures is preferentially sensitive to Mung Bean nuclease suggesting the presence of single stranded regions in S-DNA. For the CTG repeat length of 17, 30, and 50, S-DNA formed in 2, 39, and 70˚% of DNA molecules. The heterogeneity of S-DNA structures, as well as the propensity for formation, increased with increasing repeat length. The presence of CAT interruptions in the (CTG)n tract reduced the heterogeneity and extent S-DNA formation. The remarkable correspondence between the effect of length and the purity of the repeat tract, the extent and heterogeneity of S-DNA formation, and genetic instability in humans, suggests S-DNA may be involved in expansion leading to human disease.

Notes :

ROLE OF MISMATCH REPAIR GENES IN CTG INSTABILITY: IN VIVO AND IN VITRO STUDIES.

Phillip R. Kramer, Christopher E. Pearson, and **Richard R. Sinden.**

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Although many human genetic diseases, including myotonic dystrophy have been associated with the expansion of CTG trinucleotide repeats within the disease loci, little is known about the molecular mechanisms or the genetic control of the expansion of triplet repeats. Mutations in human mismatch repair genes are associated with the increased polymorphism of many microsatellites, including dinucleotide repeats. The effect of mutations in two mismatch repair genes on the size of trinucleotide repeats in the DM and FRAXA loci has been analyzed. PCR and Southern analysis of the triplet repeat regions of the DM and FRAXA locus in cell lines HTC116 and LoVo, which contain mutations in both alleles of the hMLH1 and hMSH2 genes, respectively, indicated that the size of the endogenous (CTG)n and (CGG)n tracts fall within the range observed in the normal population. This suggests that mutations in hMLH1] or hMSH2 do not result in the massive genetic expansion of CTG tracts to the levels observed in individuals in myotonic dystrophy.

We have studies the interaction of the hMSH2 protein with slipped strand DNA structures (S-DNA) formed from (CTC)-(CAG) trinucleotide repeats in genomic clones of the myotonic dystrophy locus containing disease relevant lengths repeats. We have also constructed heteroduplex (CTG)x (CAG)y intermediates (where x _ y), expected to arise during the expansion from normal (n=30) to premutant (n=50) repeat lengths. The heteroduplex intermediates having an excess of CTG or CAG repeats were structurally distinct and could be separated electrophoretically and studied individually. Using a band shift assay, the human mismatch repair protein hMSH2 was shown to bind to both S-DNA and the heteroduplex DNAs in a structure-specific fashion. The affinity of hMSH2 binding increased with the length of the triplet repeat. Furthermore, hMSH2 bound preferentially to the looped-out CAG repeat, implicating a strand asymmetry in hMSH2 recognition. Our results are consistent with the idea that hMSH2 could participate in trinucleotide instability, although hMSH2 is not alone responsible for massive expansion. The differential binding to the CTG and CAG strands may be important in preferential deletion or expansion of repeat.

Notes :

CHROMATIN STRUCTURE OF EXPANDED TRIPLET DNAs.

Yuh-Hwa Wang and **Jack Griffith.**

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DNAs containing expanded CTG and CCG triplet repeats have been cloned from DNAs derived from patients with Myotonic Dystrophy (DM) and the Fragile X syndrome (FraX) by R.D.Wells and colleagues. Using these plasmids, *in vitro* nucleosome reconstitution, electron microscopy, and quantitative gel retardation assays have been employed to examine the ability of these unusual repeating DNAs to form either unusually stable (hyperstable) or very unstable (hypostable) nucleosomes. We have found that DNA containing repeating CTG triplets forms hyperstable nucleosomes while repeating CCG DNA forms hypostable nucleosomes. In the case of repeating CTG triplets, once the blocks grow to a size that would encompass several nucleosomes (>400 bp or 100 repeats) then arrays of hyperstable nucleosomes would be generated. The generation of long arrays of hyperstable nucleosomes could create barriers in chromatin that would inhibit gene expression of genes in this region and such barriers may be refractory to the transit of polymerases. In the case of expanded CCG triplets a clear correlation with the nature of the Fragile X locus exists. Here, as repeating CCG triplet grow in length, a block of chromatin would appear which would be unstable, which would resist condensation into chromatin and which would be a preferred target for nucleases and enzymes which would methylate the DNA. Indeed it was observed that methylated CCG DNA resists nucleosome assembly to an even greater extent than non-methylated CCG DNA. In model experiments it was observed that a general motif of $([C/G]_3NN)_n$ to which repeating CCG belongs is commonly found in enhancer regions of human genes, and can strongly exclude nucleosome formation.

References:

Yuh-Hwa Wang, Robert Gellibolian, Miho Shimizu, Robert D. Wells, and Jack Griffith. Long CCG Triplet Repeat Blocks Exclude Nucleosomes: A Possible Mechanism for the Nature of Fragile Sites in Chromosomes. **J. Mol. Biol.** 263: 511-516. 1996.

Yuh-Hwa Wang and Jack Griffith. Methylation of Expanded CCG Triplet Repeat DNA from Fragile X Syndrome Patients Enhances Nucleosome Exclusion. **J. Biol. Chem.** 271: 22937-22940. 1996.

Yuh-Hwa Wang and Jack Griffith. The $[(G/C)_3NN]_n$ motif: a common DNA repeat that excludes nucleosomes. **Proc. Natl. Acad. Sci. USA** 93: 8863-8867. 1996.

Yuh-Hwa Wang and Jack Griffith. Expanded CTG Triplet Blocks from the Myotonic Dystrophy Gene Create the Strongest Known Natural Nucleosome Positioning Elements. **Genomics** 25: 570-574, 1995.

Yuh-Hwa Wang, Sorour Amirhaeri, Seongman Kang, Robert Wells and Jack Griffith. Preferential Nucleosome Assembly at DNA Triplet Repeats from the Myotonic Dystrophy gene. **Science** 265: 669-671, 1994.

Notes :

SOMATIC INSTABILITY OF THE MYOTONIC DYSTROPHY (CTG)n REPEAT DURING HUMAN FETAL DEVELOPMENT.

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Myotonic dystrophy is characterised by the striking level of somatic heterogeneity seen between and within tissues of the same patient, which probably accounts for a significant proportion of the pleiotrophy associated with this disorder.

We have investigated the timing of instability of myotonic dystrophy (CTG)n repeats in a series of congenitally affected fetuses and neonates.

We find that during the first trimester the repeat is apparently stable and that instability only becomes detectable during the second and third trimesters. In our series repeat instability is apparent only after 13 weeks gestational age and before 16 weeks. The appearance of heterogeneity shows some tissue specificity, with heart most commonly having the largest expansion. The degree of heterogeneity is not correlated with the initial size as gauged by chorionic villus and blood (CTG)n repeat sizes.

Notes :

VARIATION OF CTG-REPEAT NUMBER OF THE DMPK GENE IN MUSCLE TISSUE.

M. Anvret[1,2] and **T. Ansved**[2].

Departments of [1]*Clinical Genetics and* [2]*Clinical Neuroscience, Karolinska Hospital, S-171 76 Stockholm, Sweden.*

The CTG repeat number varies between 5 and 37 in lymphocytes of normal individuals, whereas myotonic dystrophy (DM) patients may have expansions from 50 to several thousand copies. Although the expansions related to DM are usually larger in muscle compared to peripheral blood, the variation in repeat number in non-dystrophic muscle is not known nor whether a premutation is more readly detected in muscle than in peripheral blood. In order to investigate this, percutaneous muscle biopsies were obtained from 86 individuals without any clinical or hispathological signs of DM. The CTG repeat number (n) was found to vary between 5 to 28 with a distribution of a major sharp peak at n=5 (27%) and a broader peak at n=8-17 (11 and 14% repectively). Alleles with 19 or more CTGs amounted to 17%. This represent a normal variation in the skeletal muscle and similar to that in peripheral blood, although there is a slight shift in the overall frequency distribution towards alleles with CTG repeats numbers in the higher range. This is of special interest since it has been suggested that CTG repeats exceding 19 may constitute a reservoir for recurrent DM mutations.

Notes :

ESTABLISHMENT OF CELL CULTURE SYSTEMS FOR STUDIES OF CTG REPEAT INSTABILITY.

William Wang[1], Gyorgyi Horvath[1], Bhavna J. Patel[1], Sukeshi Vaishnav[2], Alicia Voskova[2], C. Thomas Caskey[2,3], Darren G. Monckton[2], and **Tetsuo Ashizawa**[1]

Department of Neurology [1], Department of Molecular and Human Genetics [2], Howard Hughes Medical Institute [3], Baylor College of Medicine, Houston, Texas 77030, USA and Veterans Affairs Medical Center [1], Houston, Texas 77030, USA

Although expanded CTG repeats in the myotonic dystrophy protein kinase (DMPK) gene show both germline and somatic instability in myotonic dystrophy (DM), the mechanisms of the instablity are poorly understood. We have developed a cell culture system to study spontaneous mutation rates of DM CTG repeats. Lymphoblastoid cell lines of single cell origins were established by cloning using multiple steps of limiting dilution. After expansion to approximately 10^6 cells (equivalent to approximately 20 cell cycles), the DNAs of these cell lines were analyzed by the small pool PCR (SP-PCR) technique using primers flanking the CTG repeat region. Two types of mutations of the expanded CTG repeat alleles were detected : (1) frequent step-wise mutations resulting in alleles around the progenitor allele without expansion or contraction bias, and (2) relatively rare mutations with large changes of the CTG repeat size with a bias toward contraction. The former may represent the mechanism responsible for the somatic heterogeneity of the CTG repeat size observed in blood cells of DM patients. The latter is frequently seen in expanded CTG repeat tract introduced into prokaryotic and eukaryotic cells in culture, but rarely seen in DM patients. Transcription-based instability mechanisms have recently been postulated. To study whether transcription across the GTG repeat tract plays a role in the repeat instability, we have established HeLa cell lines stably transfected with plasmids containing an inducible promotor, the 3' region of the DMPK gene with 44-200 CTG repeats with 200 bp of flanking sequences and a CAT reporter gene. These *in vitro* experimenta systems will be useful for future studies on mechanisms involved in the regulation of the somatic stability of the CTG repeats in DM, and may facilitate identification of factors that stabilize or contract the expanded CTG repeat with potential therapeutic implications.

Notes :

POSTZYGOTIC INSTABILITY OF THE CTG REPEAT IN CONGENITAL MYOTONIC DYSTROPHY.

Lee-Jun C., Wong[1] and **Tetsuo Ashizawa**[2]

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Heterogeneity of CTG repeat size, detected as a smear of the repeat-containing DNA fragment in Southern blot analyses, has been shown to be minimal in prenatal DM samples and blood samples of newborns with congenital myotonic dystrophy (CDM). CDM neonates have also shown apparently uniforms repeat sizes with little or no smears in several different tissues. However, Lavedan et al. (1993) have shown that the CTG repeat size varied in different tissues obtained from a DM fetus of 20-week gestation. To determine when somatic instability of the expanded CTG trinucleotide repeat occurs in CDM, we examined the size and heterogeneity of the repeats in DNA of various tissues from a CDM newborn and peripheral blood leukocyte DNA from CDM infants and 10 CDM children at ages 2 to 30. Southern blot analysis of Bam HI fragments; containing the repeat region with long electrophoresis showed that a subtle size variation between tissues is already evident at birth with allele size heterogeneity. The degree of size heterogeneity correlated with the age in 15 CDM patients. These data suggest that somatic instability of the CTG repeats beging during a prenatal period and continues after birth with progressively increasing size heterogeneity in CDM patients.Two CDM patients showed a discrete bimodal distribution of the expanded allele in addition to a single normal size allele. Each peak of the expanded alleles in these CDM patients showed relatively minor heterogeneity. We speculate that the multimodal distributions of the expanded alleles may be due to somatic instability of the CTG repeat involving large repeat size changes during early embryogenesis, similar to that postulated as a mechanism for discordant repeat size and size heterogeneity in three sets of monozygotic DM twins. The two types of CTG repeat instability; i.e., (1) gradual continuous increase of the repeat size and size heterogeneity occurring throughout the life of a DM patient and (2) large repeat size changes in early embryogenesis would parallel two types of somatic instability observed in cultured lymphoblastoid cell lines from DM patients and somatic hybrids containing a DM chromosome 19.

Notes :

MALE GERMLINE TRANSMISSION OF NORMAL ALLELES.

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DM is an autosomal dominant disorder, alleles for which, due to the anticipatory process, proceed inexorably towards lethal alleles over very few generations. Since the incidence of DM is not decreasing, some mechanism must be operating to maintain a pool of pathogenic DM expansions. Thus, the origin of DM expansions remains a question very much relevant to incidence of DM. As such, we are using sensitive small pool PCR procedures in an attempt to determine the origin of DM expansions in the general population. Preliminary results suggest that normal length alleles are relatively stable during male germline transmissions with large length changes, greater than +/- 5 repeat units being extremely rare. No such large length changes, including expansions into the disease range, have been detected in the analysis of over 10,000 sperm. Moreover, these analysis also confirm normal Mendelian segregation of the two alleles at the level of the sperm in a number of unrelated males, suggesting that meiotic drive probably does not give rise to the putative segregation distortion observed for germline transmission of normal alleles.

Notes :

INTERGENERATIONAL SOMATIC AND GERMINAL CTG REPEAT INSTABILITY IN TRANSGENIC MICE

G. Gourdon[1], A.S. Lia[1], F. Radvanyi[2], C. Duros[1], M. Blanche[2], C. Saquet[1], C. Junien[1,3], H. Hofmann-Radvanyi[1,3]

1 INSERM UR 383, Hôpital Necker-Enfants Malades, Clinique Maurice Lamy, 149 rue de Sèvres, Paris, France - 2 Institut Curie, 12 rue Lhomond, Paris, France - 3 Hôpital Ambroise Paré, Boulogne, France.

In order to investigate the CTG repeat instability mechanism, involved in Myotonic Dystrophy, we have generated transgenic mice carrying a 45 kb genomic DNA fragment from the DM region cloned from DM patients DNA. Three types of mice have been obtained and contain either 20, 55 or 320 CTG. Using primers flanking the CTG repeat region, we showed by PCR experiment a moderate intergenerational, somatic and germinal instability of the 55 CTG repeat in mice (from -1 to +6 CTG). Surprisingly, germline instability was observed in sperm but not in testis suggesting that instability may occurs in spermatozoa after their formation in testis. We are investigating the precise stage of instability by analysing CTG repeat length in spermatozoa extracted from the epididymis. To gain further insight into the CTG somatic instability and to verify if it mimics the somatic instability observed in DM patients, we performed Small-Pool PCR experiments on DNA from different tissues and from mice of different ages. Pancreas, liver and kidney showed an important somatic instability compared to other tissues and this somatic instability, like in DM patients, increases with time. These results suggest that transgenic mice can be a good model to study trinucleotide repeat instability and may be useful to understand the molecular basis involved in this process. The same analysis (by PCR and Southern blot experiments) is currently underway with mice carrying 320 CTG repeat with whom we may expect larger changes in CTG size.

Work supported by AFM

Notes :

TRANSGENIC MOUSE MODELS OF MYOTONIC DYSTROPHY.

M.A. Narang and **R.G. Korneluk**

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Myotonic dystrophy (DM) is an autosomal dominant, multisystemic disorder that classically manifests as myotonia and progressive muscle weakness and wasting. The disease is associated with the expansion of a triplet (CTG) repeat, located in the 3'-untranslated region of the DM kinase (DMK) gene, such that repeat size corresponds with increased severity of disease and earlier age of onset . However, the identification of the mutation associated with DM has done little to resolve the effect of CTG expansion on DMK expression or disease determination. In an effort to clarify the effect of CTG instability, we have generated transgenic mice containing matrix attachment region (MAR) sequences over-expressing the human DMK gene ("DMK transgenics") and a DMK minigene construct 100 CTG repeats ("CTG transgenics"). MAR sequences, found at the boundaries of functional transcription units, enhance tissue-specific expression in a copy number dependent manner in transgenic mice by reducing the effect of flanking chromatin. The transgenics described here show substantial over-expression of human DMK transcript and protein in brain, skeletal muscle, tongue and eye, tissues typically affected in DM. Cryostat sections of skeletal muscle from DMK transgenic animals revealed diagnostic hallmarks of DM including sarcoplasmic masses, increased centronucleation and type I fiber atrophy. Additionally, primary myoblasts established from these mice showed reduced fusion potential indicating a delay or defect in myoblast differentiation. CTG transgenic mice showed intergenerational trinucleotide repeat instability, the degree of which correlated with increasing DM-like pathology in skeletal muscle per generation. These results indicate that over-expression of DMK in mice confers a muscle pathology similar to that observed in DM suggesting that over-expression at the RNA and/or protein level plays a role in the human disease. Furthermore, trinucleotide instability is not only possible in mouse but may allow for reproduction of the effects of genetic anticipation.

Notes :

June, Monday 30.
Afternoon

Lundi 30 Juin
Après-Midi

ABSTRACTS

COMMUNICATIONS

MYOTONIC DYSTROPHY HAPLOTYPE ANALYSIS IN AFRICAN POPULATIONS.

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In the South African (SA) populations the incidence of myotonic dystrophy (DM) varies greatly: in the Afrikaans-speaking Caucasoid people in the Northern province (formerly Northern Transvaal), one of the highest incidences has been reported (14.3/100 000) and in SA Bantu-speaking Africans no proven DM case has been described. Extensive haplotype analysis in the former revealed molecular evidence for a strong founder effect, with a single haplotype occurring on 83 % of the DM chromosomes, with the remainder sharing a common haplotype core. These molecular findings, supported by genealogical evidence, suggest that the majority of present day DM mutations in this population may have originated from a common initial founder who introduced one of the European ancestral mutations.

In an attempt to explain the low prevalence of DM in indigenous sub-Saharan people (and their descendants in the New World), the distribution of the DM CTG repeat was determined in southern African individuals and in a northeast African population. The northeast African population, represented by Ethiopian Jews, showed a high frequency of $(CTC)_{>18}$ alleles. Except for the occurrence of two chromosomes with 21 and 22 repeats in the SA Bantu-speaking Africans, large-sized alleles are present only in the Ethiopians and non-Africans. The SA Bantu-speakers and San (formarly "Bushmen") had significantly fewer large repeat length allele than the non-Aftican poputations. It has been proposed that DM chromosomes may arise from a pool of predisposing normal alleles, which serve as a source of futures DM alleles. We suggest, therefore, that the occurrence of fewer large CTG, repeats in the normal range may, in part, explain the absence of DM in southern and central Africans. Yemenits Jews, from the middle East, on the other hand, have a high frequency of $(CTG)_{>18}$ alleles and the largest size range of alleles (5-35 repeats) together with one of the highest reported incidences of DM (17/100 000).

DM-associated haplotypes consisting of four markers have been analysed in African populations. Numerous novel haplotypes (not previously described in Europeans) were found despite the occurrence of fewer large CTG repeats in sub-Saharan African populations. It thus seems likely that only a small number of these "African" chromosomes were present in the progenitors of all non-African peoples. These data provide support for the "out of Africa!" model for the origin of modern humans and suggest that the rare ancestral DM mutation event may have occurred after the migration from Africa, or in a northeast African population, hence the absence of DM in sub-Saharan Bantu-speaking Africans.

Notes :

EVOLUTION OF THE $(CTG)_N$ REPEAT EXPANSION ASSOCIATED WITH DM IN PRIMATES.

R. Krahe[1,6], E. Thaller[1], M. Shriver[2], L. Narayana[1], G. Horvath[4], B. Osuntokon[3], T. Ashizawa[4,5], and M.J. Siciliano[1].

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The expansion of the unstable (CTG)n trinucleotide repeat in the 3' UTR of the DMPK gene on human chromosome 19q13.3 is specific for the DM disease phenotype. In Caucasoids a single haplotype composed of nine alleles within and flanking DMPK over a physical distance of 30 kb has been shown to be in complete linkage disequilibrium with DM. In order to gain insights into the molecular and mutational events involved in repeat variability, a comparative evolutionary study of the DM-associated repeat and haplotype was conducted in two non-human primates (gorillas and chimpanzees) and two defined human populations (a sub-Saharan Nigerian [Yoruba] and a Caucasoid control population). Based on these data a model for the evolution of the DM-associated (CTG)n repeat was developed. (CTG)n repeat size variation was observed in all populations with sizes in chimpanzees reaching the level (26 repeats) which in humans is considered the at-risk allele range for expansion into the disease range. Sequence analysis showed that polymorphic repeat arrays consisted of perfect repeats and were not stabilized by interrupting cryptic repeats. DM haplotype analysis showed that the non-human primates were monomorphic (but different from each other) at all the sites of the DM haplotype and identified a single, previously in humans unidentified haplotype for each species. The immediate DMPK genomic region showed extensive conservation between non-human and human primates. Cladistic parsimony analysis indicated that the most common Caucasoid haplotype, the one associated with DM, is the ancestral situation. Non-DM Nigerian (Yoruba) individuals showed a considerably lower number of at-risk alleles in the 19-30 range and striking disassociation from the absolute linkage disequilibrium between certain (CTG)n repeat alleles and the alleles of a nearby Alu-insertion/deletion polymorphism observed in Caucasoids. Furthermore, at least one at-risk (CTG)n allele with 23 repeats, previously not found in other sub-Saharan populations was identified on a novel haplotype in association with an Alu-deletion allele. This observation is consistent with the identification of a single, independent DM mutation on an Alu-deletion haplotype reported by us in 1995 for a Nigerian (Yoruba) kindred, the only indigenous, sub-Saharan case identified to date. These data indicate that the DM haplotype itself is irrelevant to the propensity for repeat instability and suggest that the repeat itself may be the important factor determining repeat instability.

Notes :

POPULATION GENETICS OF THE CTG REPEAT IN JAPAN

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To find a founder chromosome of the mutated DM gene and to investigate the mechanism of the expansion mutation in the DM gene in the Japanese population, we genotyped 93 Japanese families with DM including 190 affected members and 130 unaffected members using polymorphic markers close to the (CTG)n repeat and made haplotype. Six different haplotypes were found and DM alleles were always haplotype A.
We found three families with DM in which the asymptomatic parent had been shown to be a premutation state. PCR analysis of the region spanning the CTG expansion demonstrated that three fathers of each proband had a number of repeats corresponding to premutation alleles, 44, 46 and 47 CTG alleles. Extensive clinical examination failed to demonstrate any of the symptoms of DM in these three males. Haplotype analysis of DNA markers close to the DMPK gene suggested a common ancestral DM mutation. The results strongly suggest that a few common ancestral mutations in both Caucasian and Japanese populations have originated from a haplotype A and that there is a premutation allele for DM, as predicted in a multistep model for etiology of this disorder.

Notes :

SEGREGATION DISTORTION OF THE CTG REPEATS AT THE MYOTONIC DYSTROPHY (DM) LOCUS: NEW DATA FROM DM BRAZILIAN FAMILIES.

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Myotonic dystrophy (DM) is an autosomal dominant condition caused by a (CTG)n expansion in the protein kinase (DMPK) gene locus on chromosome 19q. An intriguing question is how this disease has been maintained in the population with a relatively high prevalence, considering the rarity of new mutations. An attractive hypothesis which has been proposed to explain this observation is the occurrence of meiotic drive. Segregation distortion favoring the transmission of the larger allele both in normal individuals as well as in affected DM patients have been observed in some studies. However, controversial results regarding the origin of this distortion have been reported.

In order to address this question, we have analyzed the total number of affected versus normal individuals in 69 Brazilian DMD affected families and compared among the offspring of affected fathers versus affected mothers: a) the total proportion of affected versus normal offspring and b) the sex ratio among affected and normal descendants.

We did not observe an excess of DM patients. However, among DM patients there were more affected male than female offspring, particularly of paternal origin. These results suggested that the mutant allele is preferentially transmitted to male offspring through males. This result would give a biological explanation for the significant excess of affected males as compared to affected females observed in our sample (237 males: 174 females; $P<0.05$).

Another unexpected finding was that DM affected males have on average more children than DM mothers, although the proportion of affected patients who reproduced was similar for both sexes. In addition, the estimated relative fitness of our DM males was also slightly greater than the one observed for our normal population. Therefore, it is tempting to speculate that a slightly higher relative fertility in DM men than in the normal population associated to the observed preferential transmission of enlarged alleles to males would provide an explanation for maintaining the frequency of mutant alleles which are lost through negative selection. Other population studies will be very important to confirm this hypothesis. (supported by FAPESP, CNPq).

Notes :

GLOBAL HAPLOTYPE DIVERSITY OF NORMAL CTG POLYMORPHISM AT THE DM LOCUS: IMPLICATIONS FOR EVOLUTION AND MAINTENANCE OF MYOTONIC DYSTROPHY.

R. Chakraborty[1], Y.-X. Fu[1], A Polanski[1], D.N.Stivers1, and R. Deka[2]

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Myotonic Dystrophy (DM), an autosomal neurological disease, is caused by expanded CTG-trinucleotides located in the 3' untranslated region (UTR) of the myotonin protein kinase (DMPK) gene. World-wide data on prevalence of DM are not precise, nevertheless there are indications that DM is more common in populations of European origin (1 in 8,000), less frequent in Orientals (1 in 18,000 in Japanese), and very rare in Africans. Molecular studies also indicate that the parthways leading to expanded CTC repeats ($\geq$ 50) may have been different in different populations. To evaluate this hypothesis further, we examined haplotype distribution of CTG-repeats and the neighboring *Alu* insertion-deletion polymorphism in 802 unaffected individuals of 16 ethnycally old geographically diverse populations. In addition, we also studied 726 meiotic events in 95 nuclear families to follow transmission of normal CTC-repeats from parents to children. These analyses indicate that: (i) during female meiosis larger CTC-repeat alleles (for size $\geq$ 29 repeats) are preferentially transmitted while no segregation distortion occurs during male meiosis; (ii) $(CTG)_5$—Alu(+) appears to be the most parsimonious ancestral haplotype, from which $(CTG)_5$—Alu(-) evolved subsequently; and (iii) expansion of CTC alleles occured from $(CTG)_5$ alleles on both Alu(+) and Alu(-) backgrounds. Applications of coalescence-based simulation studies indicate that CTG-expansions are more frequent than contractions within normal size range of repeat alleles at the DMPK locus. These observations, together with a slight selective disadvantage of larger alleles, explain the world-wide haplotypic diversity at this locus, and suggest that a mutation-selection-drift equilibrium may lead to a stable frequency of DM in populations.

(Research supported by grants GM41399, GM45861 and GM58545 from the US National Institutes of Health)

Notes :

REPEAT EXPANSION AND LOCAL CHROMATIN STRUCTURE.

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The CTG repeat associated with myotonic dystrophy is in the 3-prime untranslated region of a protein kinase gene. In alleles with a normal range of CTG repeats, a nuclease hypersensitive region is present approximately 500 bp centromeric to the location of the repeat. Micrococcal nuclease studies demonstrate that the hypersensitive site is flanked by a phased array of nucleosomes, with protection covering the CTG repeat that is consistent with nucleosome positioning. Alleles with large repeat expansions (approximately 1000 repeats) do not have the region of nuclease hypersensitivity and are generally more nuclease resistant over several kilobases. These results demonstrate a specific chromatin structure in the region of the CTG repeat, implicate the CTG repeat as a possible in vivo nucleosome positioning element, and demonstrate a more condensed chromatin structure in alleles with a large repeat expansion.

Notes :

EFFECT OF CTG REPEAT EXPANSION ON CHROMATIN STRUCTURE AND PROCESSING OF *DMPK* MRNA IN HYBRID CELL LINES DERIVED FROM DM AND CDM PATIENTS.

V.L. Funanage, K.S. Singleton, R. Frisch, P. Carango, P.A. Moses, and H.G. Marks.
Alfred I. duPont Institute, Wilmington, Delaware.

Several studies have shown that the triplet repeat expansion in myotonic dystrophy (DM) leads to decreased levels of *DMPK* mRNA and protein, but the mechanism for this decreased expression is unknown. Otten and Tapscott (PNAS **92**: 5465, 1995) have recently demonstrated decreased chromatin sensitivity in the region of the repeat expansion in affected individuals and propose that this leads to decreased expression of the *DMPK* gene, perhaps by inducing premature termination of transcription or affecting mRNA splicing. We have developed a PCR-based method to assay chromatin sensitivity of this region in somatic cell hybrids carrying either normal or affected *DMPK* alleles. The highest chromatin sensitivity was exhibited by alleles with 5 repeats, and there was an inverse correlation between number of repeats and chromatin sensitivity. Somatic cell hybrids were constructed from fibroblasts of a male patient with myotonia and muscle weakness and CTG repeats of 5 and 25. The somatic cell hybrid carrying the $(CTG)_{25}$ allele had 25% of the *DMPK* mRNA levels of the hybrid with the $(CTG)_5$ allele. No mutations in either the coding region or exon/intron boundaries were found in this patient to account for this decreased expression. However, chromatin from the $(CTG)_{25}$ region showed a decreased sensitivity to digestion with *Pvu*II. Chromatin sensitivity was also analyzed in hybrids that carried CTG repeats of 133 and ~1700. These regions exhibited similar decreased sensitivities to digestion with *PvuII*. Although low levels of properly spliced *DMPK* mRNA from exons 8 and 9 were detected in these hybrid cell lines, we failed to detect the full length *DMPK* transcript that includes exons 13 and 14. Nested RT/PCR analysis of *DMPK* mRNA using primers in exons 9 and 15 showed that most of the *DMPK* transcripts expressed from the expanded alleles had spliced out exons 13 and 14, whereas full-length transcripts were expressed predominantly from the normal alleles. These results suggest that altered chromatin structure leads to a decrease in *DMPK* mRNA levels by affecting mRNA splicing and that alleles in the upper normal range for repeat number can exhibit altered chromatin structure and decreased *DMPK* mRNA levels.

Notes :

THE DMPK GENE OF SEVERELY AFFECTED MYOTONIC DYSTROPHY PATIENTS IS HYPERMETHYLATED IN A CPG ISLAND PROXIMAL TO THE EXPANDED CTG REPEAT.

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Using methylation sensitive restriction enzymes, SacII, HhaI and HpaII, we characterized the methylation pattern on the 5' side of the CTG repeat in the DMPK gene of myotonic dystrophy in normal individuals and affected patients with expansions of the repetitive sequence. The gene segment analysed corresponds to the genomic SacI/HindIII fragment carrying sequences of exons 11-15 and a large CpG island. There is constitutive methylation in intron 12 at restriction sites of SacII and HhaI localized 1,159 - 1,232 bp upstream of the CTG repeat whereas most if not all the other sites of SacII, HhaI and HpaII in the region investigated are unmethylated. In a number of young and severely affected patients, surprisingly, complete methylation of these restriction sites was found on the mutated chromosome. In most of these patients the onset of the disease was congenital. In vivo footprinting analysis gave evidence for an interaction of transcription factor Sp1 at a CAGGGCGG binding site upstream of the CTG repeat in normal genes and for a significant reduction of this interaction in cells of a fetus with hypermethylated DMPK gene.

Notes :

TRANSCRIPTION OF THE MYOTONIC DYSTROPHY KINASE (DMK) GENE IS CONTROLLED BY A HOUSEKEEPING TYPE UPSTREAM PROMOTER AND A MUSCLE SPECIFIC ENHANCER LOCATED IN THE FIRST INTRON.

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Myotonic Dystrophy (DM) is the most common inherited adult neuromuscular disorder with a global frequency of 1/8000. The genetic defect is an expanding CTG trinucleotide repeat in the 3' untranslated region (UTR) of the myotonic dystrophy kinase (DMK) gene.
We present the characterization of the *cis* regulatory elements of the DMK gene. The region 5' to the initiating ATG contains no consensus TATA or CCAAT box. We have mapped two transcriptional start sites by primer extension. Transient transfection into muscle and non-muscle cell lines of deletion constructs from this region fused to the bacterial chloramphenicol acetyl transferase (CAT) reporter gene revealed only subtle muscle specific *cis* elements. Strongest promoter activity was mapped to a proximal 189 bp fragment and gel mobility shift assays demonstrate binding of Sp1 to a GC box within this sequence. A 2 Kb fragment from the first intron of the DMK gene can stimulate reporter gene activity 4 to 6 fold in muscle cells, but not in fibroblasts. Co-transfection of a Myo D expression vector with intron 1- reporter constructs resulted in a 10 to 20 fold enhancement of transcriptional activity in fibroblasts. These data suggest that transcription of the DMK gene is controlled by a basal promoter acting in concert with a Myo D responsive enhancer located in the first intron, which together produce myoblast specific gene expression.

Notes :

ANALYSIS OF DMPK, DMAHP AND 59 EXPRESSIONS IN DM CELL LINES.

David Brook.

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Myotonic dystrophy is caused by the expansion of a trinucleotide repeat, CTG, in the 3' untranslated region of a protein kinase gene, DMPK. We set out to determine what effect this expanded repeat has on RNA processing. The sub-cellular fractionation of RNA and the separate analysis of DMPK transcripts from each allele reveals that transcripts from expanded DMPK alleles are retained within the nucleus and are absent from the cytoplasm of DM cell lines. The nuclear retention of DMPK occurs a critical threshold between 80 and 400 CTGs. Further analysis of the nuclear RNA reveals an apparent reduction in the proportion of expansion derived DMPK transcripts after polyA+ selection.

Quantitative analysis of RNA also indicates that although the level of cytoplasmic DMPK transcript is altered in DM patients, the levels of transcripts from 59 and DMAHP, two genes that immediately flank DMPK, are unaffected in DM cell lines.

Notes :

EXPRESSION OF DMPK AND DMAHP DURING FETAL DEVELOPMENT.

A-S Lia[1], Gil Butler-Browne[2], P. Barbet[3], C. Duros[1], C. Junien[1,4], G. Gourdon[1], H. Hofmann-Radvanyi[1,4]

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A consistent decrease of mRNA and protein level of DMPK has been reported in muscle and cell lines in adult patients while conflicting results were obtained with CDM samples. Expression of DMPK during embryonic and fetal development has not been examined precisely in humans and a delayed maturation has been reported in CDM muscle. We have collected DM and control fetal samples in order to determine, by total RT-PCR, the DMPK gene expression in CDM and in age-matched control during development. As different spliced forms of DMPK mRNA have been previously described (Fu et al 93, Mahadevan et al 93, Jansen et al 92), we decided to define precisely the tissue-specific expression of these spliced forms in normal and affected fetal tissues between 18 and 23 old. Preliminary data confirmed the presence of the eight spliced forms described by Fu et al (93) and the existence of the first exon described by Mahadevan et al (93) in DM and control tissues. Tissue-specific expression of these different spliced forms during development is underway.
The recently discovered DMAHP gene is expressed during murine development. A decrease of DMAHP mRNA synthetized from the mutant allele has been reported in myoblast and myotube cultures, by RT-PCR experiment. We therefore set up to study the expression of DMAHP during development in our DM and control samples. Surprisingly, RT-PCR preliminary data seem to indicate an increased expression of DMAHP mRNA during fetal development in DM muscle and heart samples. We are currently testing more samples to confirm this finding.

Work supported by AFM

Notes :

EXPANSION OF A CTG TRINUCLEOTIDE REPEAT IN THE 3'UTR OF DMPK TRANSCRIPTS RESULTS IN NUCLEAR RETENTION OF TRANSCRIPTS.

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Expansion of a CTG trinucleotide repeat in the 3' untranslated region (UTR) of myotonic dystrophy protein kinase (DMPK) induces the dominantly inherited neuromuscular disorder myotonic dystrophy (DM). Transcripts containing the expanded trinucleotide are abundant in differentiated cultured myoblasts, and they are spliced and polyadenylated normaly. However, mutant transcripts never reach the cytoplasm in these non mitotic cells ; instead, they form stable clusters that are tightly linked to the nuclear matrix, which can prevent effective biochemical purification of these transcripts. In DM patients, reduced DMPK protein levels, consequent to nuclear retention of mutant transcripts, are probably a cause of disease development. Formation of nuclear foci is a novel mechanism for preventing transcript export and effecting a loss of gene function.

Notes :

ROLES FOR TRIPLET REPEAT EXPANSION RNA-BINDING PROTEINS IN MYOTONIC DYSTROPHY.

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Myotonic dystrophy is an autosomal dominant neuromuscular disease that is associated with expansion of a $(CUG)_n$ triplet repeat in the 3'-untranslated region (3'-UTR) of the myotonin protein kinase (Mt-PK) gene. We have recently presented a model to explain the genetic dominance of this disease[1,2]. This model proposes that the Mt-PK $(CUG)_n$ expansion exerts its effect at the RNA level by modulating the binding of triplet repeat expansion RNA-binding proteins which are required for the maturation, stability and translation of specific mRNAs. Large $(CUG)_n$ expansions lead to the sequestration of $(CUG)_n$ pre-mRNA/mRNA-binding proteins on mutant Mt-PK transcripts and depletion of these proteins from wild-type Mt-PK, and possibly additional, transcripts. In support of this model, we have characterized the first $(CUG)_n$-binding protein as a novel human heterogeneous nuclear ribonucleoprotein (hnRNP), CUG-BP/hNab50[1]. HnRNPs bind to nascent RNA polymerase II transcripts, and have been suggested to play important roles in nuclear pre-mRNA splicing and polyadenylation as well as the nucleocytoplasmic export and cytoplasmic stability of mRNAs. The CUG-BP/hNab50 protein was originally isolated from HeLa cell extracts as a protein that binds $(CUG)_8$ repeats in vitro and preferentially photocrosslinks to the Mt-PK 3'-UTR[1]. In vitro photocrosslinking assays using additional Mt-PK 3'-UTR transcripts have also demonstrated that the binding of CUG-BP/hNab50 increases as the size of the $(CUG)_n$ repeat increases from 0 to 90 repeats while binding of the hnRNP C proteins decreases. We are currently investigating which step in RNA processing is primarily affected in vivo by triplet repeat expansion, and the specific roles of $(CUG)_n$-RNA binding proteins in this process.

References:

[1]Timchenko, L.T., Miller, J.W., Timchenko, N.A., DeVore, D.R., Datar, K.V., Lin, L., Roberts, R., Caskey, C.T., and Swanson, M.S. (1996). *Nucl. Acids Res.* **24**:4407-4414.

[2]Caskey, C.T., Swanson, M.S., and Timchenko, L.T. (1996). *Cold Spring Harbor Symp. Quant. Biol.* **61**: 607-614.

Notes :

ALTERED PHOSPHORYLATION AND INTRACELLULAR DISTRIBUTION OF A (CUG)n TRIPLET REPEAT RNA-BINDING PROTEIN IN MYOTONIC DYSTROPHY AND MYOTONIN PROTEIN KINASE KNOCK-OUT MICE.

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The mechanism whereby expansion of the (CUG)n repeats in the 3' untranslated region (3'-UTR) of the myotonin protein kinase (Mt-PK) gene induces myotonic dystrophy (DM) is unknown. A novel protein which specifically binds to RNA (CUG)n repeats in the 3'-UTR of Mt-PK mRNA exists in a hyperphosphorylated (CUG-BP1) and hypophosphorylated (CUG-BP2) forms. Here we present evidence that intracellular localization of CUG-BP/hNab50 is altered in patients with DM. CUG-BP1 is a major protein in tissues from normals and it is localized predominantly to the cytoplasm. In contrast, hypophosphorylated CUG-BP2 is a major protein in patients with DM, and its concentration is increased in nucleus. Similarly in heart and skeletal muscle from Mt-PK knock-out mice, CUG-BP1 is located primarily in the cytoplasm, and CUG-BP2 is observed predominantly in the nucleus. Mt-PK and CUG-BP/hNab50 interact with each other in vitro and Mt-PK phosphorylates CUG-BP in vitro suggesting that phosphorylation of CUG-BP/hNab50 by Mt-PK regulates the subcellular distribution of CUG-BP/hNab50. These results suggest that nuclear CUG-BP2 might affect processing of several mRNA's including Mt-PK mRNA in patients with DM.

Notes :

TRANSCRIPTION BY RNA POLYMERASE II THROUGH TRIPLET REPEAT-CONTAINING DNA FROM THE HUMAN MYOTONIC DYSTROPHY AND FRAGILE X LOCI.

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At the myotonic dystrophy (DM) locus, expansion of a (CTG)n segment within the 3'-untranslated region of the DM protein kinase (DMPK) gene alters mRNA production by affecting transcriptional and/or post-transcriptional processes. In an attempt to differentiate between these two possibilities, a synchronized in vitro transcription system was used to monitor the inherent ability of RNA polymerase II to transcribe DNA corresponding to normal (CTG)17 unstable (CTG)50 and affected (CTG)255 individuals, and as a control, DNA corresponding to the normal (CGG)54 fragile X repeat tact. Core RNA polymerase II efficiently transcribed all repeat units irrespective of repeat length or orientation. However, approximately 50% of polymerases transiently adopted an elongation incompetent configuration at the entrance to the CTG repeat. The dwell half-life of these complexes was $10 \leq 1$ s. Furthermore, the elongation rate within the CTG repeat was inherently slow. The average transcription rates within the CTG, CCG, CGG, and CAG tracts were 170, 250, 300, and 410 nt/min, respectively. These differences correlated with changes in the sequence-specific transient pausing pattern within the CNG repeat tracts; in general individual NAP incorporation rates were slower after incorporation of a pyrimidine. Finally, and unexpectedly, approximately 3% of the run-off transcription products generated on linear (CTG)17 and (CAG)17 templates were discrete approximately 15 nt longer-than-expected RNAs whose synthesis was inhibited by addition of transcriptional elongation factor SII. These results are discussed with respect to the intrinsic capacity of template sequence to influence transcript elongation. In addition we discuss how changes in ternary complex structure at the entrance to, and transcription rates within the CTG repeat tract might ultimately affect DMPK mRNA levels.

Notes :

MYOTONIC DYSTROPHY: EVIDENCE FOR AN RNA DISORDER.

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Myotonic Dystrophy is associated with a variable length expansion of a trinucleotide (CTG) segment within the 3' untranslated region of the DM-kinase gene; it is a dominant disorder. When a series of muscle biopsies from classical adult-onset Myotonic dystrophy patients were studied and compared to the results with both normal and myopathic control biopsies, a reduction in the level of DM kinase RNA in the total RNA fraction relative to creatine kinase RNA was found. The reductions in DM kinase RNA in the total fraction were not disease specific: myopathic controls showed similar relative decreases. Creatine kinase protein levels are known to change with physiological state of the muscle, or during degeneration/regeneration in myopathies. Isolation of polyA+ RNA from the same biopsies showed dramatic disease related reductions in DM kinase polyA+ RNA compared to both normal and myopathic controls. The disease-specific decrease in the accumulation of polyA+ DM kinase RNA to approximately 10-20% of levels seen in normal and normal myopathic muscle biopsies were greater than could be explained by loss-of-function of mutant transcript alone. Allele-specific quantitation of mutant and normal DM kinase transcripts showed that there was markedly abnormal accumulation of both normal and mutant transcripts in the polyA+ pool. We therefore hypothesize that Myotonic Dystrophy could be an RNA metabolism disorder.

It is well documented that Myotonic dystrophy patients show increased insulin resistance, perhaps as a consequence of decreased receptor number. We hypothesized that the insulin resistance could be the result of abnormal metabolism of insulin receptor RNA in patient cells brought about by the altered DM kinase RNA (dominant-negative RNA). To test this hypothesis, the expression of the insulin receptor gene in Myotonic dystrophy patient muscle biopsies was studied. Insulin receptor RNA levels were measured in both total and polyA+RNA pools, and quantitations were done relative to both dystrophin RNA and sodium channel RNA using an assay previously described (QMF-PCR). Significant reductions in insulin receptor RNA in Myotonic dystrophy muscle were found. The reductions were disease-specific, and were statistically significant in both total and polyA+ pools. Similar reductions were present when normalizing to either dystrophin or the sodium channel RNAs. Consistent with the RNA data, a disease-specific decrease in insulin receptor protein in DM patient muscle was seen. These results reinforce the concept of generalized RNA metabolism defects in Myotonic dystrophy, and offer a possible molecular mechanism for the increased insulin resistance observed in many Myotonic dystrophy patients.

In another series of experiments, insertion of $(CTG)_{50}$ in a heterologous gene resulted in decreased expression of that gene. We are attempting to determine at which stage in production the inserted CTG-repeats have an influence as a way to understand the dominant negative effect of the DM kinase RNA in muscle.

Notes :

CIS-ACTING REPRESSION OF THE DMAHP GENE BY THE DM MUTATION

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An allele-specific RT-PCR was used to analyze the relative abundance of mRNA from the DM-linked versus wild-type DMAHP allele. The assay relies on a coding sequence polymorphism which eliminates at Cac8I restriction site in exon C. Results from myoblast cultures from DM patients (n=9) show repression of the DM-linked allele which depends on the extent of the expansion. In patients with more than 200 CTG repeats, the DM-linked allele is repressed by 82 ? 9% (mean ?SD, p<0.0001) relative to mRNA levels from the opposite allele. Analysis of postmortem heart, muscle, and brain tissue from two MtD patients gave similar results. Using RNAse protection assays, DMAHP is expressed at low levels in myoblasts and muscle. We have so far been unable to detect DMAHP protein in muscle tissue by Western blot using polyclonal antisera raised against two different (amino or carboxyl terminal) fusion proteins. These antisera detect DMAHP in COS cells transfected with a cDNA construct.

Notes :

CHARACTERIZATION OF A 54-KDA HUMAN PROTEIN KINASE RECOGNIZED BY AN ANTISERUM RAISED AGAINST THE MYOTONIN KINASE.

Puymirat Jack.

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Myotonic dystrophy (DM) is one of the most prevalent dominant hereditary diseases in adults. The manifestations of the disease is caused by mutation in the length of a (CTG)-trinucleotide repeat situated in the 3' non-coding exon of a gene which encodes a serine-threonine protein kinase (DMPK). We identified a 54-kDa protein kinase in human heart and skeletal muscle by using an affinity-purified antiserum raised against a peptide sequence of the human DMPK. This protein kinase immunoprecipitated from the skeletal muscle was phosphorylated *in vivo* on tyrosine and showed a tyrosine kinase activity towards exogenous substrates. In contrast, the protein kinase immunoprecipitated from the heart was not phosphorylated on tyrosine and displayed a serine/threonine kinase activity. Both activities were attributed to the same protein based on the identity of one-dimentional peptide maps.The dephosphorylation of the skeletal muscle 54-kDa protein kinase by a purified phosphotyrosine phosphatase (PTP-1C) allowed it to phosphorylate serine/threonine residues with a concomitant decrease in its tyrosine kinase activity whereas not effect of dephosphorylation was observed on the protein kinase purified from the heart. The tyrosine dephosphorylation of the skeletal muscle 54-kDa protein kinase allowed it to phosphorylate with the highest activity the same peptide substrates as those phosphorylated by the recombinant myotonin kinase Finally, we showed that the tyrosine kinase activity observed in the skeletal muscle results from a phosphorylation of this protein kinase on tyrosine residues by a tyrosine kinase specifically expressed in this tissues. These results suggest that the 54-kDa protein kinase could be a member of the myotonin kinase family.

Notes :

CHARACTERIZATION AND USE OF ANTIBODIES IN EVALUATING DM IN HUMAN MUSCLE AND LENS.

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A monoclonal antibody to a recombinant DM protein kinase (mAb DM-1) reacts specifically with the 64kDa isoform of DM protein kinase in type I fibers in skeletal muscle, the fiber type which characteristically atrophies in the disease. Within type I fibers of normal muscle the isoform may be localized with mAb DM-1 to the triad region. In the DM disease state, the enzyme is redistributed to the pathologically characteristic peripheral sarcoplasmic masses. In markedly affected human distal myotonic muscle, the levels of the 64kDa DM kinase isoform are elevated relative to slow skeletal myosin heavy chain. These results suggest that, consistent with the dominant clinical phenotype, the localization and accumulation of the 64kDa isoform are altered in the heterozygous disease state.Lens cataracts are a prominent finding in myotonic dystrophy. DM kinase was shown to be expressed in human and bovine lenses at the RNA level and in human lenses at the protein level. Sequencing of PCR products of RNA extracted from normal human lenses demonstrated an exact match to published genomic and cDNA 3' UTR sequences. Northern blots of bovine lens RNA showed that the transcript is similar in size to the transcript detected in other tissues that are affected in myotonic dystrophy. A polyclonal antibody (DM-2) was produced against recombinant DM protein kinase in rabbits. Development of Western blots with DM-2 showed a reactive band of 67kDa. Immunofluorescent studies of formalin-fixed human lens sections detected the DM kinase in the perinuclear cytoplasm of normal human lens epithelial cells and more diffusely in superficial subcapsular cortical fibers. In contrast, the same antibody labeled the nucleus most prominently in a single DM lens.

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Notes :

STUDIES OF DM KINASE ACTION IN CULTURED LENS AND MUSCLE CELLS.

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Myotonic protein kinase localized with the endoplasmic reticulum in epithelial cells in human lenses and in the B3 human cell line. In the latter, the localization changed with mitosis and cell division. Overexpression of the active catalytic domain of myotonic protein kinase was necessary and sufficient to produce multiple kinase-containing refractile bodies up to 5_m in diameter within transiently transfected B3 cells. Control experiments included transfection with the kinase mutant K100A that expressed catalytically inactive protein but did not produce the refractile bodies and with successive truncations of the full-length holoenzyme that removed putative structural and regulatory domains until only the catalytic domain remained yet produced the bodies. Tests for apoptosis were negative. These experiments suggest the plausible hypothesis that myotonic protein kinase may function normally to regulate the assembly and disassembly of the microtubule-associated endoplasmic reticulum complex. Overexpression may lead to stabilization of the membrane structures and prevent their loss during the differentiation of lens fiber cells, thus producing cataracts. In order to test this hypothesis in vivo, complementary experiments with transgenic mice are in progress.
Myotonic dystrophy protein kinase (DMPK) is thought to be involved in myogenic differentiation, homologous to several recently-identified kinases which act as "effectors" of the GTPase Rho. We investigated the role of DMPK in transactivating skeletal alpha-actin, an SRE-containing gene encoding an important marker of early myogenesis. DMPK enhances the transcription of SkA-luc, a luciferase construct driven by the skeletal alpha-actin promoter five to six fold. The DMPK effect depends on its catalytic activity, as a kinase-deficient mutant form of DMPK does not activate transcription. In vitro, DMPK phosphorylates SRF at sites which are not targets for previously identified SRD kinases. Rho A and B also transactivate SkA-luc, and the effects of Rho and DMPK are additive. Rho-DMPK interactions and their functional consequences are under investigation.

Supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.

Notes :

THE DM PROTEIN KINASE.

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The predicted protein kinase activity of the cloned gene product of the human myotonic dystrophy locus has been experimentally verified. Affinity-purified recombinant DM protein kinase became phosphorylated itself and transphosphorylated histone H1. These activities were not present in the bacterial host cells and were exhibited by DMPK and DMPKH, recombinant proteins which contain the protein kinase domain but exhibit distinct sizes, 43 and 66 kDa, respectively. DMPKH was further purified by velocity sedimentation on sucrose gradients; both activities migrated with the recombinant protein at 41 S, consistent with discrete multimeric particles. Phosphoamino acid analysis showed that threonine (predominantly) and serine were phosphorylated in both DMPKH and histone H1. Although PKA and PKC are the known types of protein kinase with closest sequence homology to the DM protein kinase domain, purified DMPKH was inhibited by 4 mM but not 0.04-0.4 mM H7 and H8, which inhibit PKA and PKC with K_i's of 0.4-15_M. Specific inhibitors of other classes of multi functional serine/threonine protein kinases such as casein kinases I (CKI-7) and II (heparin) and calcium/calmodulin-dependent protein kinase II (KN-62) did not inhibit DMPKH. DMPKH did not phosphorylate membrane-associated phosphoproteins such as acetylcholine receptor or spectrin which are known to be substrates for PKA, PKC, and CKI and II, respectively. These experimental results suggest that the active center of the recombinant human myotonic dystrophy protein kinase may have properties distinct from the well-studied classes of serine/threonine protein kinases, in contrast to predictions based upon primary structure alone.

Supported by grants from the National Institutes of Health and the Muscular Dystrophy Association.

Notes :

MYOTONIC DYSTROPHY PROTEIN KINASE IS LOCALIZED TO THE TERMINAL CISTERNAE OF THE SARCOPLASMIC RETICULUM.

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The subcellular localization of the DM-PK in muscle tissues has been investigated at both morphological and biochemical level, by using antibodies against the DM-PK. Immunofluorescence studies and Western-blot analysis were carried out with antibodies raised against both a synthetic peptide and a recombinant fusion protein fragment specific for the DM-PK. The kinase is localized both to the surface membranes, and within the skeletal fibers in the region of the A-I band boundary. Consistent with the A-I location of the kinase is that Western-blot analysis of purified fractions from sarcoplasmic reticulum (SR) show that triads and SR terminal cisternae are immunoreactive for two DM-PK proteins of different molecular weight (85 and 54 kDa). The relative amount of these two proteins is different in relation to the muscle type, being the 85 kDa protein more evident in skeletal than in cardiac fibers. In addition, immunofluorescence studies of cardiac muscle reveal a heavy concentration of DM-PK localized to the intercalated discs, as well as a weaker reaction in the sarcoplasm.
These results taken together suggest that multiple isoforms of the DM-PK may exist and that they may be differentially located in muscle tissues.

Notes :

PHOSPHORYLATION OF THE MUSCLE NA+ CHANNEL INACTIVATION GATE BY DM KINASE: SUBSTRATE SPECIFICITY AND EFFECTS ON CHANNEL GATING.

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Background: Co-expression of DM kinase results in a reduction in the amplitude of currents through wild type skeletal muscle Na^+ channels expressed in Xenopus oocytes. A mutant Na channel which lacks the phosphorylation site in the inactivation gate is unaffected by DM kinase co-expression. To further investigate these effects, which may underlie the pathophysiological phenomenon of skeletal muscle myotonia, we have (1) tested the hypothesis that synthetic peptides corresponding to the Na^+ channel inactivation gate serve as substrates for purified DM kinase; (2) investigated the molecular mechanism for the reduction in Na^+ current amplitude using radiolabeled saxitoxin binding and single channel recording.

Methods: Synthetic 12-mer peptides were used in kinase assays with DM kinase purified from human non failing left ventricle. Oocytes expressing muscle Na^+ channels with and without co-expression of DM kinase were used for saxitoxin binding assays and for cell-attached patch clamp recordings. Full-length Na^+ channel cDNAs were constructed to replicate the mutations in the peptides, and currents were recorded through mutant channels with and without DMK co-expression.

Results: A synthetic peptide corresponding to the wild type Na^+ channel inactivation gate near the phosphorylation site was indeed a substrate for purified DM kinase. A peptide substituting alanine for serine was not a substrate, nor were peptides replacing upstream or downstream positively charged lysine residues with asparagine. The number of saxitoxin binding sites was identical in oocytes expressing muscle Na^+ channels alone or co-expressing DM kinase at a dose sufficient to reduce the Na^+ current to ten percent of the control level. DM kinase co-expression led to reduced probability of single channel opening manifest as reduced number of openings and bursts, and increased number of traces without channel activity. DM kinase co-expression had no effect on single channel conductance, open time, burst duration, or first latency. Despite the fact that mutant peptides were not substrates for DM kinase, currents through mutant channels were reduced by DM kinase co-expression to a degree greater than the suppression of currents through wild type channels. Single-channel recordings of one of the mutant channels showed that the effects of DM kinase co-expression were the same " reduced openings, bursts and increased null traces " but the effect of DMK was significantly greater than that on wild type channels.

Conclusions: (1) The Na^+ channel inactivation gate is a substrate for phosphorylation by DM kinase. Both upstream and downstream positively charged residues are important in determining substrate specificity. (2) The reduction of Na^+ current amplitude by DM kinase co-expression is not due to a reduction in the number of channels. (3) The molecular mechanism of the DM kinase reduction in Na^+ current is decreased probability of channel opening without a change in other single channel properties. We suggest that phosphorylation of the muscle Na channel inactivation gate by DM kinase reduces muscle membrane excitability.

Notes :

ELECTROPHYSIOLOGY OF MYOTONIC DYSTROPHY

Lehmann-Horn F

Department of Applied Physiology University of Ulm, Germany

The presence of clinical or electrical myotonia is a diagnostically important feature of myotonic dystrophy. Hyperexcitability of the skeletal muscle fiber membrane makes a single electrical or mechanical stimulus to cause involuntary repetitive action potentials which prolong muscle contraction and slow relaxation.
In *myotonia congenita*, characterized by warm-up phenomenon and insensitivity to potassium and cold, fiber hyperexcitability is due to a reduction of the membrane chloride conductance which is normally very high in skeletal muscles and therefore able to stabilize the resting membrane potential of the fiber. The reduced chloride conductance leads to short myotonic bursts in the electromyogram (so-called dive bombers) characterized by modulation of amplitude and frequency of the action potentials (Lehmann-Horn & Rüdel, 1996).
In *hyperkalemic periodic paralysis, paramyotonia congenita and potassium-aggravated myotonia*, a destabilization of the inactivated state of the voltage-gated muscle sodium channel is responsible for the myotonic activity. Channel reopenings generate long-lasting runs of repetitive action potentials resembling fibrillation activity (Lehtnann-Horn & Rudel, l996).
In *myotonic dystrophy*, myotonia shows warm-up (similarly to chloride channel myotonia however to a lesser degree than in myotonia congenital whereas the electrical activity is characterized by long-lasting runs which reveal waxing frequency and waning amplitudes. A reduced chloride conductance as well as a noninactiviting sodium current have been reported (Lipicky et al. 1977, Franke et al. 1990, Mounsey et al. 1995). In contrast to these reports, a calcium-activated potassium current conducted by the apamine sensitive potassium channel, normally down-regulated in mature innervated muscle fibers, has been accounted for the myotonia in myotonic dystophy (Behrens et al. l994). The results will be reviewed and new data shown.

Franke Ch, HattH, Iaizzo PA, Lehman-Horn F (1990) J Physiol 425:391-405
Lipicky RJ (1977) In: Rowland LP (ed) Pathogenesis of human muscular dystrophy. Excerpta Medica, Amsterdam pp729-738
Lehman-Horn F, Rüdel R (1996) Rev Physiol Biochem Pharmacol 128:195-268
Mounsey JP, Xu P, John JE, Horne LT, Gilbert J, Roses AD Moorman JR, (1995) J Clin Invest 95:2379-2384

Notes :

July, Tuesday 1.
Morning

Mardi 1er Juillet
Matinée

ABSTRACTS

COMMUNICATIONS

IDENTIFICATION OF AN EXPRESSION PATTERN FOR MURINE DMAHP

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Expression of the murine DMAHP gene was investigated in vivo, by the generation of mice transgenic for the DMAHP promoter and by RT-PCR analysis The transgenic mice showed expression in some of the structures associated with the central nervous system, sensory structures and the musculo-skeletal system. Expression was not observed in the retina of embryonic mice, as reported for Six5 and other members of the Six subfamily of homeobox genes. However, expression was observed in embryonic posterior lens capsules. RT-PCR analysis detected one of the DMAHP splice isoforms in a wide range of embryonic and adult tissues, in a similar pattern to that observed for DMPK. These data implicate a role for DMAHP in the pathogenesis of DM.

Notes :

IDENTIFICATION OF THE DNA BINDING TARGET of DMAHP

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DMAHP is a member of the Six subfamily of homeobox genes and is located immediately downstream of the unstable CTG repeat associated with DM. Five mammalian members of the Six subfamily are known, all of which encode another homologous domain (the Six domain) immediately upstream of the predicted homeodomain. Several members of the family have been reported to bind specifically to DNA. GSF fusion proteins of human DMAHP have been expressed, containing the Six domain, the homeodomain and both domains. These are currently being used in gel retardation assays to determine the specific DNA binding targets of DMAHP.

Notes :

LONG CUG REPEAT TRACTS IN RNA FORM STRUCTURES THAT INTERACT WITH THE DSRNA BINDING DOMAIN OF PKR.

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One proposed disease mechanism in DM is that transcripts synthesized from the mutant DMPK allele acquire a deleterious property. RNA fragments containing CUG repeat tracts ranging from 15 to 450 nucleotides were enzymatically synthesized using bacteriophage RNA polymerases. The fragments were tested for their ability to bind to the dsRNA binding domain of PKR, a dsRNA dependent protein kinase and potent regulator of translation. In gel shift assays, CUG repeat fragments larger than 60 nt bound to the dsRNA binding domain of PKR. These results suggest one factor that may contribute to reduced muscle protein synthesis and muscle wasting in DM: duplex structures formed by CUG repeat RNA may bind and activate PKR.

Notes :

REDUCED DMAHP EXPRESSION IN FRONTAL CORTEX AND CEREBELLUM OF MYOTONIC DYSTROPHY PATIENTS.

P. Amicucci*, **M. Gennareli**,* G. Zelano§, A. Corrado , G. Novelli*, B. Dallapiccola°.
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The DM locus-associated homeodomain protein gene (DMAHP) lies immediately 3" to the Mt-Pk gene, in a CpG island which contains the Mt-Pk (CTG)n repeat (Bouchet et al., Hum Mol. Genet.,4, 1919-1925, 1995). Preliminary RT-PCR data demonstrated that spliced different transcript are produced and expressed from DMAHP gene in various human tissues, including skeletal muscle, heart and brain. The expression of these isoforms, is suppressed in cis by CTG repeat expansion of DM alleles (Thorton et al., Am. J. Hum. Genet., 59, 161, 1996). We assayed the total RNA isolated from postmortem frontal cortex and cerebellum of 2 DM patients (300 - 350 CTG repeats) and 2 unaffected controls. We found a sensible reduction of DMAHP mRNA levels (80%(10% S.D.), both in frontal cortex and in cerebellum of DM patients compared to controls. GPI, GAPDH, and (b-actin mRNA levels were comparable in DM and controls. These results demonstrate that (CTG)n expansion affects the DMAHP mRNA levels in DM brain and suggest a possible dominant-negative effect of DM (CTG)n mutation on RNA metabolism of Mt-Pk neighboring mapping genes. Reduced expression of DMAHP in brain could contribute to the impairment of memory function associated with DM.

Work supported by a grant from Italian Ministry of Health

Notes :

TRANSCRIPTION OF DMAHP IN NORMAL AND EXPANDED ALLELES.

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We have previously demonstrated that a hypersensitive site a is positioned near the CTG repeat that is associated with myotonic dystrophy and that alleles with large expansions of the repeat have eliminated this hypersensitive site. We now demonstrate that this hypersensitive region contains enhancer elements. The hypersensitive site enhancer is positioned near the promoter of the DMAHP homeobox gene and the activity of the enhancer correlates with the transcription of the DMAHP gene. In cells from patients with a large expansion of the CTG repeat and elimination of the hypersensitive site, there is a 2-4 fold reduction in the steady-state levels of DMAHP mRNA relative to cells from unaffected individuals. Allele-specific RT-PCR demonstrated a large reduction in the abundance of transcripts from the expanded allele relative to the non-expanded allele. These results demonstrate that the CTG expansion alters transcription of the DMAHP gene and suggest a model in which the change in chromatin structure effected by the repeat expansion precludes transcription factor access to the regulatory regions of the DMAHP gene.

Notes :

CTG REPEAT EXPANSION DOES NOT INTERFERE WITH EXPRESSION OF EITHER *DMAHP* OR *59* MRNA IN HYBRID CELL LINES DERIVED FROM DM OR CDM PATIENTS.

V.L. Funanage, K.S. Singleton, P. Carango, P.A. Moses, R. Frisch, and H.G. Marks.
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The triplet repeat expansion in both adult-onset myotonic dystrophy (DM) and congenital DM (CDM) has been shown to result in a decreased chromatin sensitivity in the region located 3' of the CTG repeat, and nucleosomes have been demonstrated to bind strongly to CTG repeats *in vitro*. These changes in chromatin structure are believed to result in an effect on transcription of the *DMPK* gene and possibly neighboring genes. The *DMAHP* gene is located at the 3' end of *DMPK* where a large CpG island is also found. The CTG repeat lies within this CpG island, suggesting that expansion of the CTG repeat may interfere with expression of *DMAHP*. Another gene, *59*, is located at the 5' end of the *DMPK* gene and is separated from the *DMPK* gene by only 500 bp. We have utilized somatic cell hybrid cell lines constructed from either DM or CDM patients to analyze the expression of *DMPK*, *DMAHP*, and *59* mRNAs from the normal and expanded alleles. Although the expression of *DMPK* mRNA was markedly reduced from alleles with expansions of either 133 or ~1700 repeats, no inhibition of expression of either *DMAHP* or *59* mRNA was observed. A quantitative multiplex RT/PCR assay was used to measure the relative levels of *DMPK*, *DMAHP*, and *59* mRNAs in somatic cell hybrid cell lines with either normal or expanded alleles. These studies showed that the triplet repeat expansion in DM and CDM patients did not affect transcription of either *DMAHP* or *59*. Our data indicate that changes in chromatin structure at the 5' end of the *DMAHP* gene caused by large triplet repeat expansions do not affect transcriptional regulation of *DMAHP*. Thus far, the only consistently observed effect of the CTG repeat expansion is on *DMPK* gene expression, where it appears to interfere with proper splicing of *DMPK* mRNA.

Notes :

THE DMR-N9 GENE AND ITS FUNCTION.

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The DMR-N9 gene is positioned immediately upstream of the DM-PK gene with only a 1.1 kbp spacing between the poly(A) addition signals in the DMR-N9 gene and the actual transcription starts site(s) in DM-PK. From studies in knock out animals we have obtained evidence that the polyadenylation/truncation of DMR-N9 pre-mRNA is modulated by chromatin-conformational changes in the DM locus. The DMR-N9 gene product is a cytosolic WD-repeat protein with unknown function which is ubiquitously expressed at low level in all tissues, but is particularly abundant in brain and testis. Cell fractionation studies, in situ hybridisation and immunohistological studies suggest a role for this protein in spermiogenesis but its role in brain is completely elusive. In order to obtain a better understanding about its functions and candidacy for being involved in disease manifestation we are currently also studying a well conserved C.Elegans homologue for DMR-N9. We will report on the outcome of these comparative studies, which enable us to perform a more detailed cellbiological and functional analysis for this intriguing protein.

Notes :

ANIMAL MODELS FOR MYOTONIC DYSTROPHY (DM): ABNORMAL DM PROTEIN KINASE LEVELS HAVE MILD EFFECTS ON CELLULAR INTEGRITY AND Ca^{2+} RELEASE IN MOUSE MUSCLES IN VIVO AND IN VITRO.

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We have examined the effect of altered expression levels of DM-PK, which is ubiquitously expressed in all muscle cell lineages, by disrupting the endogenous DM-PK gene and overexpressing a normal human DM-PK transgene in mice and cell models. Homozygous DM-PK (-/-) mice showed minor size changes in neck muscle fibers at older age, and testicular atrophy. Animals with the highest DM-PK transgene expression showed mild hypertrophic cardiomyopathy and enhanced neonatal mortality, but both models lack other frequent DM symptoms. In mutant myotubes from these mice that were cultured in vitro DM-PK absence influenced both the basal $[Ca^{2+}]_i$ level as well as the release and sequestration of Ca^{2+} at depolarisation. Studies involving specific inhibitors of the sodium channel, the voltage-operated calcium channel and the SR Ca^{2+} release channel suggest a role of DM-PK mediated phosphorylation in the movement of gating charge and/or the activity of voltage gated channels. These results strengthen the role of DM-PK as a primary candidate for being involved in disease manifestation. The challenge for the future is to generate mouse models with expanding (CTG)n segments at cognate positions in the genome. Such models will enable us to obtain a better picture of additional multisystemic effects of DNA expansion on chromatin topology, RNA transcription and processing, and protein activity in this complex disorder.

Notes :

ANIMAL MODELS FOR MYOTONIC DYSTROPHY.

P. Sarkar1, C. I. Berul2, M.J. Aronovitz2, S. Saba2, M. Mendelsohn2, D. Housman3 and **S. Reddy1**

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The mechanism by which expansion of a CTG repeat sequence in the 3' UTR of a putative protein kinase (DMPK) results in DM pathophysiology is unknown. Current models include a partial loss of DMPK function and/or a neighboring gene or a novel gain of function of the DMPK mRNA containing the repeat expansion. To determine the possible contribution of DMPK loss to DM pathophysiology we have developed mice lacking functional DMPK (DMPK-/-). Extensive evaluation of skeletal muscle structure and function has shown that loss of DMPK results in a late onset progressive skeletal myopathy (Reddy et al. 1996 Nat. Genet. 13; 325). To determine the role DMPK in cardiac muscle structure and function we have studied the electrophysiological (EP) properties of the heart in DMPK-/- mice. In vivo EP studies have been performed via epicardial and endocardial pacing in 10 one year old DMPK-/- mice and 10 age and sex matched controls. Complete 12-lead ECG were obtained on all 20 mice. Full EP data were collected in 7 wild-type and 9 mutant mice, including sinus node recovery time, atrio-ventricular (A-V) and V-A conduction properties and atrial, A-V and ventricular effective refractory periods. On ECG, a prolonged PR interval (mean + SD = 49.5 + 9 msec) was seen in all 10 mutants compared with wild type mice (mean PR + SD = 33.2 + 5 msec $p< 0.001$). No wild type mice had a PR >35 msec, while all homozygous DMPK mutant mice had PR> 35 msec. On EP testing 4/9 knock out mice had 2o A-V block and two also developed 3o A-V block during atrial pacing. None of the wild-type mice had any A-V block. Thus DMPK-/- mice develop distinct cardiac electrophysiological abnormalities of atrioventricular conduction. DMPK+/- mice showed skeletal and cardiac muscle phenotypes intermediate to wild type and DMPK-/- mice. These results suggest that a partial loss of DMPK could contribute to DM pathology. However all features of the human disorder are not reproduced by a simple reduction of DMPK levels in mice. Phenotypic differences could be due to differences in mouse and human muscle physiology and/or other dominant effects of repeat expansion. To distinguish between these possibilities we have used an in vitro PCR amplification protocol to generate CTG repeat sequences varying from 100 bp to > 20 kilobases in length. We have stabilized up to 2.5 Kb of such sequences in bacteria. We are currently using homologous recombination to introduce CTG repeat sequences into the DMPK locus in mice. Phenotypic analysis and comparison of such mice with DMPK-/- mice should distinguish between current models for DM pathology.

Notes :

MOUSE MODELS OF DM.

Darren G. Monckton[1,2,3], Tetsuo Ashizawa[4], Mary I. Coolbaugh[2], C. Thomas Caskey[3,5], Marvin Meistrich[6] and Michael J. Siciliano[2].

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Over five years after the identification of the CTG expansion in DMPK as the primary genetic lesion in DM, we still do not have a clear understanding of how the repeat expansion leads to the complex multisystemic DM phenotype. Although much of the preliminary speculation centred on changes in the expression level of DMPK, the consensus of research suggests that DMPK expression is probably not drastically altered in DM patients. Moreover, neither transgenic mice overexpressing human DMPK nor knockout mice completely deficient for DMPK display many of the features of the DM phenotype observed in humans. As such, much attention has shifted to the potential involvement of other genes, such as DMAHP and 59, but as yet a role for these genes in DM pathogenesis is based largely on genomic location and expression patterns. More recently, results from a number of groups have implicated a role in DM pathogenesis for the DMPK message from the mutant chromosome containing an expanded CUG tract. We also speculated that part of the autosomal dominant DM phenotype might associated with a direct gain of function of the repeat and sought to test this directly using a transgenic approach. Five lines of transgenic mice that we generated with a simple non-coding expanded repeat containing transgene do not appear to display any aspect of the DM phenotype. However, second generation transgenic mice in which the same DM repeat region is put under the control of a ubiquitously expressed mammalian promoter, do display a convincing, but still only partial, DM phenotype. These results indicate that at least part of the DM phenotype is associated with a gain of function of an expanded CUG repeat containing RNA message.

Notes :

TRANSGENIC MODELS TO STUDY CTG REPEAT AMPLIFICATION CONSEQUENCES

P. Fumagalli[1], F. Radvanyi[2], H. Hofmann-Radvanyi[1,3], H. Seznec[1], O. Agbulut[4], G. Butler-Browne[4], A.S. Lia[1], M. Blanche[2], C. Duros[1], C. Junien[1,3], **G. Gourdon[1]**

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Myotonic Dystrophy is caused by the amplification of a CTG repeat located in the 3' UTR of the DMPK gene but the mechanisms involved in the pathophysiology of this affection are unknown. We have generated 3 types of transgenic mice containing 45 kb of the human genomic DM region with the N9, DMPK and DMAHP genes and either 20, 55 or 320 CTG repeat. These mice will be used i) to study the implication of the CTG repeat expansion on tissue-specific expression of the 3 DM region genes and during development ii) to verify if the expression of a 3'UTR carrying an expanded CTG could confer a dominant gain of function and could lead to a DM phenotype. Preliminary data (using semi-quantitative RT-PCR) indicate that the 3 genes contained in our construct seem correctly expressed in mice. Rnase protection assay are in progress to define more precisely the tissue-specific expression of the human N9, DMPK and DMAHP gene compared with the endogenous mouse expression and to study the influence of different repeat CTG sizes on the human transgenes expression. No phenotype has been observed yet in the mice carrying 55 CTG and histological studies are underway with the mice carrying 320 CTG. If the CUG repeat contained in the expressed DMPK 3'UTR does confer a dominant gain of function involved in DM pathology it is reasonable to speculate that the endogenous murine DMPK 3' UTR could interfere. To verify this assumption we plan to cross our mice with DMPK knock out mice.

Work supported by AFM

Notes :

TRANSGENIC MOUSE MODELS OF MYOTONIC DYSTROPHY.

M.A. Narang and **R.G. Korneluk**

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Myotonic dystrophy (DM) is an autosomal dominant, multisystemic disorder that classically manifests as myotonia and progressive muscle weakness and wasting. The disease is associated with the expansion of a triplet (CTG) repeat, located in the 3'-untranslated region of the DM kinase (DMK) gene, such that repeat size corresponds with increased severity of disease and earlier age of onset . However, the identification of the mutation associated with DM has done little to resolve the effect of CTG expansion on DMK expression or disease determination. In an effort to clarify the effect of CTG instability, we have generated transgenic mice containing matrix attachment region (MAR) sequences over-expressing the human DMK gene ("DMK transgenics") and a DMK minigene construct 100 CTG repeats ("CTG transgenics"). MAR sequences, found at the boundaries of functional transcription units, enhance tissue-specific expression in a copy number dependent manner in transgenic mice by reducing the effect of flanking chromatin. The transgenics described here show substantial over-expression of human DMK transcript and protein in brain, skeletal muscle, tongue and eye, tissues typically affected in DM. Cryostat sections of skeletal muscle from DMK transgenic animals revealed diagnostic hallmarks of DM including sarcoplasmic masses, increased centronucleation and type I fiber atrophy. Additionally, primary myoblasts established from these mice showed reduced fusion potential indicating a delay or defect in myoblast differentiation. CTG transgenic mice showed intergenerational trinucleotide repeat instability, the degree of which correlated with increasing DM-like pathology in skeletal muscle per generation. These results indicate that over-expression of DMK in mice confers a muscle pathology similar to that observed in DM suggesting that over-expression at the RNA and/or protein level plays a role in the human disease. Furthermore, trinucleotide instability is not only possible in mouse but may allow for reproduction of the effects of genetic anticipation.

Notes :

TRANSPLANTATION OF MYOTONIC DYSTROPHY MYOBLASTS AS A POTENTIAL MUSCULAR MODEL FOR MYOTONIC DYSTROPHY.

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Myotonic dystrophy (DM) is caused by mutation in the length of a $(GTG)_n$-trinucleotide situated in the 3' non-coding exon of a gene which encodes a putative serine-threonine protein kinase (DMPK). An attractive hypothesis is that the increased repeat length in patients with myotonic dystrophy causes a change in the protein kinase activity and altered phosphorylation of target proteins that leads to the varied manifestations of the disease. Unfortunately, quantitation of DMPK mRNA and protein levels in DM patient material has yielded contradictory results. To gain further insight into the biological role of DMPK and its involvment in disease manifestations, DMPK deficient mice and mice which overexpress a normal DMPK transgene were developed. These experiments have met, however, with limited success. An alternative approach to develop a muscular model for DM is the tranplantation DM myoblasts into the muscle of immunodeficient mice (SCID). In previous work we showed that transplanted human normal myoblasts participate to muscle regeneration with a high degree of efficacy even after 1 month following the transplantation (Huard et al., 1994). We therefore tested whether this approach could be used to produce DM muscle fibers. Human myoblasts from a control patient and from 3 DM patients were purified by limited dilution and by flowcytometry. The SCID mouse tibialis anterior muscles which have been irradiated and treated with notexin to damage the muscle fibers and trigger regeneration were injected with 5 million human DM myoblasts. One month following the transplantation, muscles were analyzed. Before the mice were killed, an EMG search was made for myotonic runs in muscle, using needle movements under ether anesthesia. Three of the four muscles injected with DM myoblasts showed typical myotonic runs whereas no myotonic runs were observed in muscle injected with normal myoblasts although some regenerating activities were observed in these muscles. Southern blot analysis demonstrated the presence of the CTG repeat expansion in these muscles. Preliminary results indicate the presence of several human fibers in the regenerated muscles as identified by the presence of human dystrophin. These data suggest that DM myoblasts grafted into the skeletal muscle of SCID mice could be a usefull muscle model for DM.

Notes :

AN INTRODUCTION TO THE DEVELOPMENT OF SKELETAL MUSCLE

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In man and in rodents, the development of skeletal muscle is characterized by the progressive transformation of undifferentiated fibres into mature slow and fast contracting fibres. This metabolic differentiation is accompanied by modifications in their biochemical composition and in particular of the myosin isoforms. Most limb and trunk muscles are formed from two successive generations of muscles fibres. The first generation of muscle fibres express slow twitch MHC almost as soon as they appear, which is prior to any innervation. Conversely, the second generation of muscle fibres which form around these primary generation of muscle fibres do not initially express the slow twitch MHC but they may express it secondarily. Thus, the first generation of muscle fibres gives slow fibres in the adult muscle whereas the second generation gives rise to both slow and fast fibres in the adult human muscle. The establishment of the mature phenotype depends upon the development of a mature motor innervation and of other epigenetic factors. The chronology of this maturation differs between species : in man, most muscle fibers are mature at birth whereas in rats and mice, all this muscle maturation occurs after birth.

Notes :

IMMATURY OF SKELETAL MUSCLE IN CONGENITAL MYOTONIC DYSTROPHY

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The severe congenital form of myotonic dystrophy corresponds to a very particular entity, being always maternally transmitted. This exclusive maternal transmission had previously suggested the role of environmental factors, but instead seems now to be linked to limits of transmission of the genetic defect during meiosis. Indeeds, the severe form is only found when there has been an extensive amplification resulting in the presence of more than 2000 copies of the trinucleotide repeat in the region 19q13 on chromosome 19.
Clinically, the congenital form of myotonic dystrophy presents at birth with extreme hypotonia and weakness of pharyngeal and respiratory muscles, which lead to respiratory distress and difficulties with sucking and swallowing. The mortality rate is about 50% and of those that survive there is marked mental and motor retardation. Immunocytochemical studies were carried out in a series of ten affected foetuses, using antibodies specific for markers of cell proliferation, myogenic factors, vimentin, desmin and different isoforms of the myosin heavy chains. This was completed by a biochemical analysis of the contractile proteins.
In all these affected foetuses, the skeletal muscles show essentially an arrested or delayed maturation, with abnormalities ranging from the presence of myotubes to incomplete fibre type differentiation, and a general smallness of fibres. The marked increase in numbers of central nuclei, nuclear chains, ring fibres and inflammatory changes associated with the adult form are not found. Immunocytochemical and biochemical studies demonstrated that the delay in differentiation and maturation affected both fast and slow fibers.

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Notes :

A NEW CASE OF PATERNALLY INHERITED CONGENITAL MYOTONIC DYSTROPHY.

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U 153 INSERM, Paris. Division Risler, Hôpital de la Salpêtrière. **Service de pédiatrie. Hôpital de la Salpêtrière.*Service de biochimie et génétique moléculaire. Hôpital Ambroise Paré. Boulogne.*

Case report: The patient is a 11 year old girl with mental retardation, poor facial expression, dysarthria, tent-shaped mouth and walking difficulties. She was born at 40 weeks gestation, and birth weight was 2 kg 500. She had generalized hypotonia with pes equinovarus, swallowing difficulties, hypoglycemia, and has been hospitalized during 2 months for gastric forcible feeding. All developmental milestones were delayed. Myotonic discharges were evident on the EMG. Her father, a 40 year old man, presented a typical myotonic dystrophy (DM) with hand myotonia, bilateral cataract, baldness and distal muscle weakness. A paternal first cousin, a 16 year old girl, also presented a congenital form of DM with severe hypotonia, neonatal respiratory distress, feeding difficulties, motor developmental delay and mental retardation. EMG showed myotonic discharges. The mother of this girl was 37 year and has clinical features of DM. All patients showed unstable CTG repeats on Southern Blots analysis. Copy numbers of CTG repeats are compared to clinical phenotypes.

Notes :

MAGNETIC RESONANCE IMAGING (MRI) AND GENETIC CORRELATION IN DM PATIENTS.

Abbruzzese C.°, Luna R.#, Tessarolo D.^, Costanzi Porrini S.°, Liguori M.° and **Giacanelli M.^**

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Eighteen myotonic dystrophy (DM) patients, with age ranging from 18 to 56 years and CTG expansions ranging from 100 to 1500 repeats, underwent brain magnetic resonance imaging (MRI). Patients were subdivided in two groups, one group with CTG expansion lower than 1000 and the other with CTG expansion higher than 1000 repeats. Sixteen patients showed a widespread cerebral atrophy together with multiple subcortical white matter lesions. Seven out of the sixteen patients, other than the widespread cerebral atrophy, showed a focal cerebral atrophy; the MRI findings highlighted a frontal atrophy in three subjects, parieto-occipital atrophy in two, temporal atrophy in one and cerebellar atrophy in one subject. The two subjects with parieto-occipital atrophy and the patient with temporal atrophy also showed preferential localization of the white matter subcortical lesions (characterized by areas of alterated signal in the T2 sequences weighted) in occipital lobe, particularly corresponding to the optical radiations.
All the over mentioned seven patients belonged to the group with CTG trinucleotide expansions higher than 1000 repeats. The two remaining subjects (with age <30 years) of the group didn't show any of cortical atrophy either widespread or focal while. No subcortical white matter lesions were detected. MRI patterns obtained in our study overimpose the findings described by other Authors in DM patients with CTG expansion higher than 1000 repeats. The absence of cerebral atrophy and/or white matter lesions in the two younger patients of the higher than 1000 CTG repeats group can be likely associated to the role of age in the pathogenesis of the encefalic alterations generally detected in myotonic dystrophy.

Notes :

CORRELATION BETWEEN DECREASED MYOCARDIAL GLUCOSE PHOSPHOLYLATION AND THE DNA MUTATION SIZE IN MYOTONIC DYSTROPHY

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Background Myotonic dystrophy,the most common form of adult dystrophy, has been shown to be caused by amplification of CTG triplet repeat in the 3' untranslated region of a protein kinase gene located on chromosome 19. Impaired glucose metabolism has been suggested as a possible explanation of brain and skeletal muscle involvement in this multisystem disease. We investigated whether myocardial glucose metabolism is impaired in myotonic dystrophy and whether this impairment is related to the size of the mutation.

Methods and Results: The myocardial metabolic rate for glucose (MMRGlu, $\mu molxmin^{-1}xg^{-1}$) K1 (blood-to-tissue transfer constant), k2 (tissue-to-blood transfer constant) and k3 (phosphorylation rate constant) were determined in 7 control subjects and 12 patients with myotonic dystrophy by using parametric images generated from dynamic cardiac positron emission tomography (PET) and ^{18}F-fluoro-2-deoxyglucose studies. The expansion of the CTG triplet repeats was analyzed in patients with the probe cDNA25 after EcoRI digestion. Nonparametric tests were used to compare quantitative variables between control subjects and patients. The correlations between the size of the mutation and PET parameters were studied by linear regression. MMRGlu and k3 were significantly decreased in patients compared with control subjects (0.39+0.20 versus 0.64+0.25 P=.03 and 0.09$\pm$0.07 versus 0.24+0.21 P=.03 respectively) whereas KI alid k2 were not statistically different between control subjects and patients. MMRGlu and k3 correlate inversely with the length of the CTG triplet repeat (r=-.65 alid P=.03 for MMRGlu, and r=-.85 and P=.00l for k3, respectively).

Conclusions: In myotonic dystrophy the observed reductions in MMRGlu and phosphorylation are inversely linked to the length of the mutation. This observation suggests that impaired modulation of a protein kinase involved in myocardial hexokinase activation may give a pathophysiological schema to relate the molecular defect and the abnormal myocardial metabolism in myotonic dystrophy.

Notes :

OVEREXPRESSION OF THE TAU 55 ISOFORM IN THE BRAINS OF PATIENTS WITH MYOTONIC DYSTROPHY

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Cerebral neuronal degeneration observed in neurological diseases such as Alzheimer's disease, progressive supranuclear palsy and Pick's disease is characterized by intraneuronal accumulation of filaments which the principal components are the microtubule-associated proteins Tau. These are generated by a single gene and alternative mRNA splicing at exons 2, 3, and 10, giving rise in adult human brain to six differents isoforms abnormally phosphorylated compared with normal Tau. In each disease, Tau proteins have specific profiles, indicating different post-translational mechanisms.

Myotonic dystrophy (DM) is an autosomal dominant multisystemic disorder associated with an expansion of an unstable CTG trinucleotide repeat in the 3' untranslated region of a gene encoding for a protein with a putative serine-threonine protein kinase activity. Some pathological studies have shown few neurofibrillary-like lesions in the brain from patients with DM. We performed biochemical studies with mono- and two-dimensional gel electrophoresis and Western blot analysis, using the monoclonal antibody AD2, which recognizes an abnormal phosphorylated Tau epitope located on the carboxy- terminal part of Tau proteins and different polyclonal antibodies specifically raised against the different exons 2, 3 and 10. We demonstrated (1) the overexpression, in most of the temporal areas, of the pathological Tau 55 isoform which is disease-specific and (2) that this Tau 55 protein corresponds to the fetal Tau isoform.

Using polymerase chain reaction with Southern blot analysis and immunohistologic study on DNA or tissue samples from all cortical areas, we hypothesize a link between the trinucleotide expansion, myotonic dystrophy protein kinase expression and the accumulation of pathological Tau proteins.

Notes :

PROMM - THE EXTENDED GERMAN EXPERIENCE.

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Proximal myotonic myopathy (PROMM) is a newly described autosomal dominantly inherited multisystem disorder distinct but similar to myotonic dystrophy (DM). PROMM patients have symptoms suggestive of DM, but lack the DM associated abnormal expansion of the CTG repeat in the 3' UTR of the myotonin protein kinase gene.
Since the first report about PROMM disease in 1994, we have extented our family material to about 50 German families comprising about 130 affected living individuals. All 130 patients have been personnally visited and examined. The standard test procedure for each affected comprised a neurological investigation including an EMG, testing for serum gamma-GT, CK and myotonin protein kinase associated CTG repeats. Our extensive family studies conform to the criteria for autosomal dominant inheritance of PROMM as the disease occurs in 50% of the offspring of an affected person and male to male transmission is found in our family material. Prevalence date for Germany have not been calculated yet as the disease is still not known enough among neurologists.
Our extensive clinical experience confirm the earlier described phenotype : predominantly proximal muscle weakness without atrophy, muscle pain, cataracts, occasional mild or lacking clinical myotonia, on EMG myotonic runs; elevated gamma-GT in the absence of other symptoms like gall bladder stones, elevated CK and lacking of GTG expansion.
To illustrate possible pitfalls in diagnosis an extended three-generation PROMM pedigree is presented in which the common CLCN1 mutation R894X of the skeletal muscle chloride channel segregates. Linkage analysis with markers from the long arm of chromosome 7 conclusively excluded the CLCN1 gene as a gene locus for the phenotype in this family.

Notes :

July, Tuesday 1.
Afternoon

Mardi 1er Juillet
Après-Midi

ABSTRACTS

COMMUNICATIONS

A POSSIBLE NEW APPROACH TO TREATMENT IN MYOTONIC DYSTROPHY: DESCRIPTION OF AND PRELIMINARY FINDINGS IN A DOUBLE BLIND RANDOMIZED TRIAL OF TROGLITAZONE.

RT Moxley III[1], CA Thornton[2], A Lifton[2], V Sansone[2], W Martens[2], K Manzur[2], S Pandya[2], J Wymer[2].
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ABSTRACT NOT COMMUNICATED

DEVELOPMENT OF A CLINICAL PROTOCOL FOR USE IN CLINICAL TRIALS AND ONGOING ASSESSMENT AND MANAGEMENTI IN MYOTONIC DYSTROPHY.

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We have developed a clinical protocol aimed at use in a routine clinical setting but incorporating sufficient quantifiable variables to be used in future therapeutic trials for assessment of various interventions in myotonic dystrophy.

The assessment includes quantifiable data on: a) mobility; b) motor power; c) subjective and objective swallowing; d) respiratory function; e) electrocardiographic and cardiac data.

In Cardiff we have a large muscle clinic where over 300 appointments are offered each year. More than half of the patients seen have myotonic dystrophy. These patients and families have been involved in a number of trials in the past, including a recent in depth assessment of mobility, respiratory and swallowing assessments and the recording of cardiac data. This data was too detailed to be used routinely in a busy muscle clinic so it was decided to incorporate the most informative variables into a protocol that could on the one hand address patients concerns, problems and be used for regular assessment of organs known to be affected in myotonic dystrophy ; yet on the other hand use sufficiently objective measurements to provide data for future therapeutic interventions.

Some preliminay data will be presented.

Notes :

PRENATAL PREDICTION OF MYOTONIC DYSTROPHY USING LONG DISTANCE PCR.

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The cloning of the DM gene and characterization of the DM mutation at molecular level allows the monitoring of at risk pregnancies by direct gene testing. We demonstrated that the number of CTG repeats within the DM gene, is a valuable predictor of the DM phenotype (Gennarelli et al., Am. J. Med. Genet., 65, 342-347, 1996). We monitored 10 at risk fetuses by chrorionic villus sampling (CVS), using a PCR-formatted protocol developed in our lab that allows the simultaneous identification of wild-type and large alleles (up to 4000 CTG) with a single amplification step. Six fetuses were predicted unaffected (CTG range 7-13), while other fetuses, showed a CTG in the range of 100 to 1500 CTG repeats. Two fetuses were included in the high risk class (congenital/early childhood form, CTG > 1500, score >95%), while the other two cases were included in the low risk class (minimal and late onset DM form, CTG <100, score >99%). Parents were informed on these results, and the pregnancies of the low risk predicted fetuses continued to term, while the first two fetuses were electively interrupted. At birth the two neonates did not show any obvious DM symptoms. CTG alleles were re-evaluated in lymphocyte and fibroblast DNA and complete concordance with the CVS results were obtained.
This experience demonstrates that the measurement of triplet expansion in CVS is valuable and accurate for DM prognostic assessment during the first trimester of gestation and relevant for genetic counselling of this disease. This finding has relevant implications for genetic counselling of this disease.

Work supported by a grant from Italian Ministry of Health.

Notes :

DHEA-S TREATMENT IN MYOTONIC DYSTROPHY : A PILOT STUDY

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The effects of intravenous administration of dehydroepiandrosterone sulfate (DHEA-S) preparations (200mg/day for 8 weeks) were evaluated in eleven patients with myotonic dystrophy (Myd).

In all eleven patients with MyD tested, DHEA-S treatment improved scores of activities of daily living (ADL) with increase in muscular strength significantly (from 71.9+ 5.2 to 78.5 + 5.1 in the average of ADL score and from 5.6+ 0.2 to 6.6+ 0.3 in the average of MMT). It also improved grip and percussion myotonia in eight of 11 subjects and myotonia completely disappeared in four. After the DHEA-S administration, the surface electromyogram revealed the shortening in duration of after discharge by repetitive stimuli to the median nerve in tested five cases. These effects persisted longer than two months after the session of DHEA-S treatment. All four patients with cardiac involvements (conduction block and premature beats) and three of four patients with impared glucose tolerance showed improvements after the DHEA-S treatment. Slight side effects (abdominal pain at rapid injection and slight headache) were observed in two cases.

The results of this pilot study suggests the potential therapeutic efficacy at DHEA-S preparations in MyD with minimal side effects.

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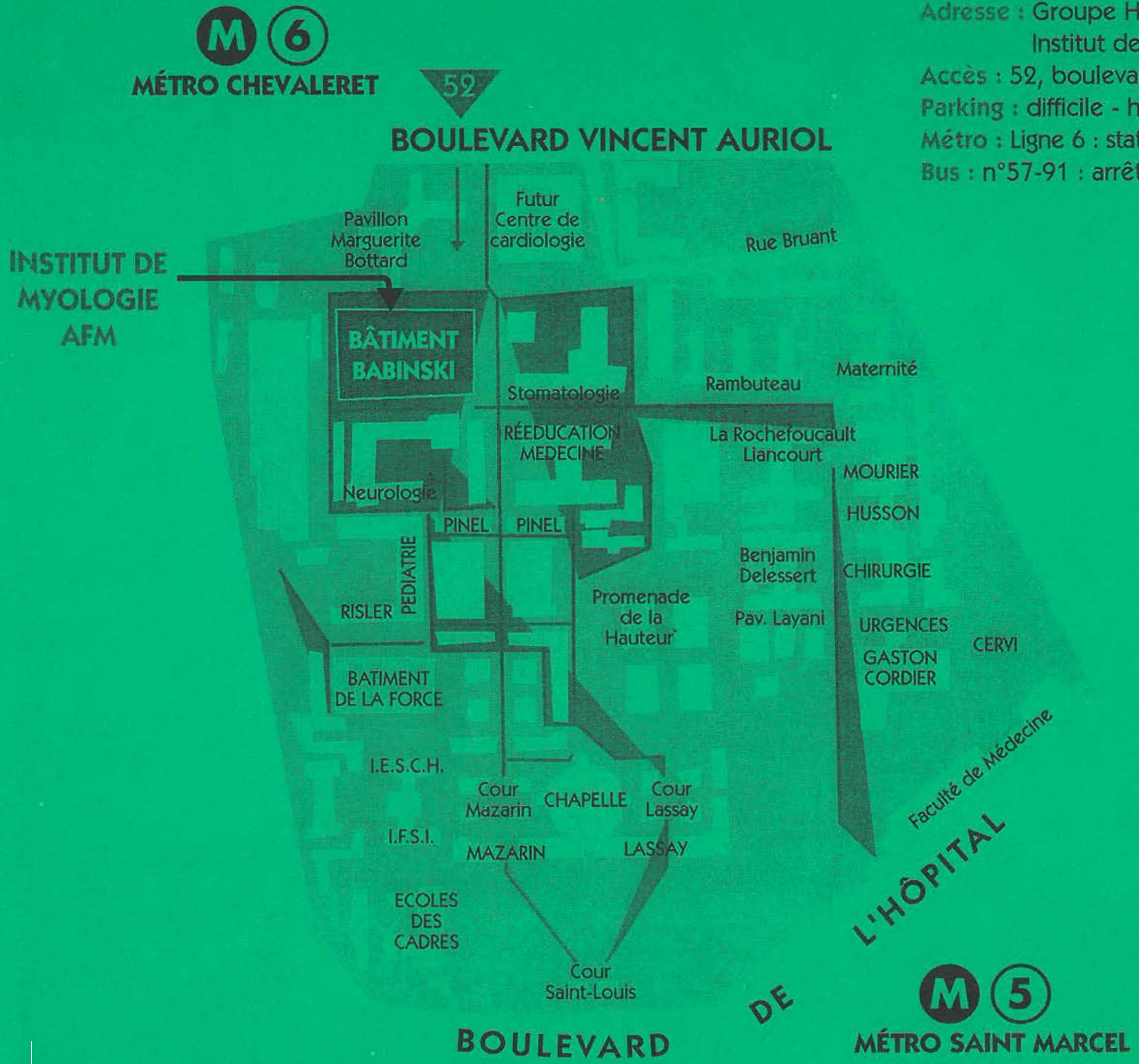

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contre les Myopathies

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