Instability in the normal CTG repeat range at the myotonic dystrophy locus

We present a family clinically unaffected by dystrophia myotonica (DM), in whom molecular studies detected different CTG repeat numbers in sibs of one generation and also remarkable CTG repeat instability in two consecutive generations in the range of <50 CTG copies. In our family, paternal transmission (father 37 repeats, son 48 repeats) resulted in a maximum increase of 11 CTG repeats in the next generation. This increase has not previously been observed in unaffected families. The intergenerational unstable CTG repeats showed no somatic mosaicism in blood lymphocytes. CTG repeat numbers were defined by polymerase chain reaction (PCR) and a refined Southern blot protocol.

The CTG repeat region in the 3' untranslated region of the DMPK gene on 19q13.2-3 is highly variable in DM patients and also in unaffected probands. In the normal population the CTG repeat alleles are stably inherited. Because of our interest in CTG repeat allele frequencies at multiple loci, including the DM locus, in the German population we have asked families to participate in molecular testing. Here we report the results of an eight member, three generation family (fig 1A). Blood samples from subjects I.2 and III.1 were not available. All other family members donated peripheral blood for the investigation after informed consent. No signs of DM were found in the family members after a thorough neurological examination,¹ nor were there any indications of genetically determined disorders. Subject I.2 had died of lung oedema at 65 years. He had no symptoms of DM.

PCR analyses were performed with primers 101 and 102,² which flank the DM repeat. PCR products were separated on a 10%

polyacrylamide gel and visualised by ethidium bromide staining on an UV transilluminator. Automatic fragment length analysis of 5'Cy5TM fluorescently labelled PCR products was performed on ALF (ALF ExpressTM Personal DNA Sequencer, Pharmacia BioTech) using commercially available external size markers (Pharmacia BioTech) and an internal length standard (PCR product of the probe p5B1.4 containing five CTG repeats). Fragment sizes were calculated with a fragment manager software package (Pharmacia BioTech). For Southern blot analyses we used *Eco*RI, *Bgl*II, *Bam*HI, and radiolabelled probe p5B1.4.² Paternity was tested by fingerprinting.

CTG repeat numbers are shown in the pedigree (fig 1A). CTG repeat sizes were confirmed by automatic analysis of the PCR products, except for the repeat number size of the grandmother (I.1). By ALF analysis, the grandmother had 11 and 12 CTG copies. All family members of generation II had one allele with 12 CTG repeats and another allele with different CTG copy numbers: 20 × CTG (II.1), 39 × CTG (II.2), and 37 × CTG (II.3). The mother (II.4) of the child III.2 had two different common alleles: 5 × CTG and 13 × CTG. The child (III.2) had 5 and 48 CTG repeats (fig 1A).

To the best of our knowledge this is the first description of unstable transmission of CTG repeats to all unaffected sibs from the same parents. All members of generation II had more than 19 CTG repeats on this second allele. Additionally, we showed an increase of CTG copies in the third generation (III.2) at the same time. The unstable paternal transmission from father (II.3) to son (III.2) resulted in a maximum increase of 11 CTG copies. This CTG increase is distinctly smaller than usually seen in families with DM heterozygotes.

Large CTG repeats (>42 CTG repeats) are predisposed to instability⁴ and the grandchild III.2 is in this range. This fact is

very important for genetic counselling. Whether further repeat expansion will occur on transmission to the next generation, followed by the expression of the DM phenotype, remains to be seen. We are currently trying to identify additional family members such as sibs and cousins of I.2 from this pedigree. Longitudinal clinical and molecular studies of probands with critical CTG repeat numbers will be required.

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BOOK REVIEWS

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Gene Transfer in the Cardiovascular System: Experimental Approaches and Therapeutic Implications. Editor Keith L March. (£119.50.) Dordrecht, The Netherlands: Kluwer Academic Publishers Group. 1997. ISBN 0792398599.

Keith March has coordinated contributions from many established investigators in the rapidly expanding field of cardiovascular gene transfer to produce a comprehensive and up to date volume.

The book is divided into several sections, each comprising several chapters and dealing with one broad aspect of cardiovascular gene transfer. The first and largest section deals with aspects of vector systems and gives thorough accounts of retrovirus vectors, adenovirus vectors including newer "second generation" vectors, liposome mediated gene

Figure 1 (A) Family pedigree. Numbers of CTG repeats on both alleles are shown. DNA from subjects I.2 and III.1 was not investigated. (B) Restriction fragment length patterns of high resolution Southern blot analysis with endonuclease BamHI detected with DNA probe p5B1.4 in the family members. BamHI fragments were not separated on the same blot but they are of comparable resolution indicated by the PCR products.

transfer, other non-viral vectors, and antisense RNA approaches to modulated gene expression. The middle sections of the book deal with methods developed for local delivery of genetic material into cardiovascular tissues, catheter based systems for blood vessel delivery, and approaches myocardial delivery, including cell mediated gene transfer. These chapters incorporate considerable data from the individual chapter authors' own investigations. The final sections describe approaches to systemic gene expression and a chapter by the editor considering some of the biophysical aspects of vector delivery.

This book manages to encompass a remarkable amount of information on what has already become, in just a decade, a diverse field. Perhaps one criticism is that too much is included, at a risk of overwhelming the reader who is new to the field. However, the chapters are well organised and focused and the references comprehensive, so the appropriate information can be easily found and followed up. Overall, the editor and authors have produced an excellent overview of the field which combines readability, in depth information, and comprehensive coverage. *Gene Transfer in the Cardiovascular System* will prove to be an excellent guide and resource for both new and established investigators.

KEITH M CHANNON

Neuromuscular Disorders: Clinical and Molecular Genetics. Editor A E H Emery. (£75.00.) UK: John Wiley. 1998. ISBN 0-471-97817-5.

Alan Emery's latest book draws together reviews of a broad selection of neuromuscular diseases from acknowledged experts in their fields. Chapters vary in their depth and length and there seems to be no clear brief to present recent advances in any particular context. Having said that, the standard of the information presented is good and each chapter provides a good stand alone review of the current status of the various conditions covered. It would be harder, though, to go in and look for a specific answer to a counselling question as the different chapters do not present information in a standardised manner.

While a lot of the conditions described are very well covered in other publications it is probably in the rarer disorders, such as oculopharyngeal muscular dystrophy and the desminopathies, where the reviews presented are particularly clear and informative. Other chapters on rarer conditions make refreshing and challenging reading, for example, fibrodysplasia ossificans progressiva, but post polio syndrome sits unhappily with these other exclusively genetic disorders. The mitochondrial chapter presents a clear review of this complex subject.

This volume is unlikely to provide the first port of call for the geneticist with no predominant interest in neuromuscular diseases when other sources of information, and in particular OMIM, are so comprehensive at providing a quick update. It does, however, provide a readable and accessible account for those who want to take their interest further.

KATHERINE BUSHBY

Emery's Elements of Medical Genetics. 10th edition. Editors Robert F Mueller, Ian D Young. (£19.50 pb.) UK: Harcourt Brace. 1998. ISBN 0-443-05902-0.

Reviewing this book filled me with nostalgia for student days. My introduction to medical genetics was based on the 6th edition, published in 1983, and comparing that with this latest edition gave me a sense of how much the world has changed, how many breathtaking advances have been made over the last 15 years. The new edition is divided into three sections, the first on basic principles of human genetics, the second on genetics as applied to medicine, and the final one focusing on the specialty of clinical genetics. It provides a broad overview of medical genetics as it stands today, and I could not think of an important area which has been omitted. It is also up to date with, for example, sections on genomic imprinting, spectral karyotyping, and the role of HOX and Hedgehog genes in limb development. I think that this book has something for most readers. I'm sure that medical students today will find it as good an introduction to medical genetics as I did in the 1980s, giving the basics in a readable and clearly presented way, but providing enough depth for those whose interest is provoked. However, this is not just a textbook which will interest undergraduates and I would also recommend it to any more experienced readers who would like to update themselves in this fast moving field.

EVAN REID