

Molecular and clinical study of two myotonic dystrophy homozygotes

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EDITOR—Myotonic dystrophy (DM) is the most common form of inherited neuromuscular disease in adults and is characterised by progressive muscle wasting and myotonia. The mutation responsible for DM has been identified as the amplification of a polymorphic (CTG)_n repeat in the 3' untranslated region of a gene encoding a serine/threonine kinase (*DMPK*).¹⁻² The DM trinucleotide repeat is highly polymorphic in normal subjects, ranging from 5 to 37 CTG repeats, while 1000 or more CTG repeats have been observed in congenital DM cases.¹⁻³ It is now generally accepted that the CTG repeat length is correlated with clinical severity and the age at onset of the disease; therefore, genetic tests are essential in the monitoring and management of DM patients and their family members.⁴⁻⁶

Homozygous DM cases have very rarely been published.⁷⁻⁸ It was assumed that the homozygous state was lethal in DM or at least so severe that it would lead to intrauterine death. Cobo *et al*⁷ studied a consanguineous French-Canadian family in which two sisters were homozygous for the “at risk” haplotype but were asymptomatic and showed no evidence of DM on extensive clinical examination. Both sisters possessed two alleles with repeat sizes normally seen in minimally affected patients. Both parents were affected. Martorell *et al*⁸ described three unrelated homozygous myotonic dystrophy patients. One patient had the classical form of myotonic dystrophy and the other two were mildly affected. A remarkable feature was the mildness of the phenotype in the homozygous patients; one, for example, had late onset cataract as the only manifestation. Along with the observations of Cobo *et al*⁷ and Martorell *et al*,⁸ this led Zlotogora⁹ to conclude that in myotonic dystrophy homozygotes do not differ from heterozygotes and that, like Huntington's disease (HD), DM is a “true dominant”.

Methods

DNA was prepared from peripheral blood lymphocytes by standard procedures. PCR amplification of the CTG repeat containing region was performed with the primers described by Brook *et al*.¹ PCR products were electrophoresed on an 8% denaturing polyacrylamide gel and stained with the silver staining method. Any sample showing only one band on PCR analysis was analysed by

Southern blotting to determine whether it had an expanded DNA region too large to be detectable with the PCR conditions described. For larger CTG expansion analysis, 15 µg of genomic DNA was digested with *TaqI* restriction enzyme, electrophoresed on a 0.8% agarose gel, and hybridised with digoxigenin (DIG-11-dUTP) labelled probe. For more accurate determination of expanded CTG repeat allele sizes, genomic DNA was digested with *TaqI*, which creates a relatively short restriction fragment containing the CTG repeat region. Such fragments digested with *TaqI* range from 773 to 842 bp, indicating five to 37 repeats.

In our study, we found two patients from two unrelated families who showed homozygous alleles for myotonic dystrophy.

Case reports

In the first family, a 20 year old woman had a 10 year history of ptosis, inability to close her eyes during sleep, and difficulty in chewing. For the past five years, she had been experiencing a delay in relaxation of hand muscles after strong voluntary contraction. Her symptoms progressed gradually. She was operated on for bilateral cataracts three years ago. Her parents were first degree cousins. Her three sibs died at the ages of 6, 7, and 9 months. It was reported that an uncle of the patient was taking phenytoin for an unknown muscle disorder. Her neurological examination showed a typical facial appearance with ptosis and facial weakness. She had generalised muscle weakness (4/5 MRC scale) affecting both the proximal and distal muscles of the upper and lower limbs equally. Deep tendon reflexes were depressed. Atrophy was observed in the leg extensors and forearm muscles. She had mild frontal alopecia. Serum creatine kinase levels were within normal limits. EMG indicated myotonic discharges and “myopathic” changes. Muscle biopsy showed evidence of chronic myopathy. Bilateral ovarian cysts were detected by abdominal ultrasonography. Her electrocardiographic and echocardiographic examinations were normal.

On initial screening with PCR, we did not observe any PCR products in this patient's genomic DNA, although all other patient and control DNA showed bands as expected. However, Southern blot analysis clearly

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showed that two alleles were present in mutated form with approximately 160 and 370 CTG repeats. These results were confirmed by the molecular analysis of the parents. Clinically, the parents were not diagnosed with DM at first, but upon careful examination it was found that the mother had slight problems with relaxation of her hand muscles. The father was phenotypically normal. PCR and Southern blot analyses showed that the mother had a normal allele with five CTG repeats and a mutated allele with approximately 185 CTG repeats. The father carried a mutated allele with 60 repeats. Both maternal and paternal alleles underwent expansion during transmission to the daughter via meiotic instability.

In the second family, a 39 year old woman was first diagnosed at the age of 35 when she brought her children for examination. She recalled that she had difficulty relaxing her hands during her third and fourth pregnancies. She was most aware of this while washing clothes. After the pregnancies, she had stiffness only when lifting heavy objects. Her symptoms decreased over the years. She now has occasional difficulty with relaxation (only when she tries to break a sugar cube into two). In general, she is slow in chewing and in her motions. Her symptoms were much milder than her symptomatic children. She had mild weakness of the orbicularis oculi and sternocleidomastoid muscles. Her voice was slightly nasal. Muscle strength was normal. There was mild action myotonia of her hands, but prominent percussion myotonia of the abductor pollicis brevis, extensor carpi radialis, and tongue muscles. The rest of the examination was normal. She had mild temporal atrophy. Needle electromyography showed myotonic discharges, which were profuse in some muscles. Motor unit potentials were mildly "myopathic".

On molecular analysis using PCR, no bands were observed within the normal range; however, a 236 bp band indicating 58 repeats was clearly prominent in the agarose gel. Using Southern blotting, we observed an additional allele containing 180 CTG repeats. Two offspring of this homozygous patient, a male and female, had clinical symptoms. By Southern blot analysis, we found an allele containing approximately 500 CTG repeats in these offspring. In the other 12 year old asymptomatic child, we detected a shorter allele containing 58 CTG repeats, which was inherited from her homozygous mother. Because this child carries a shorter allele for the mutated gene, the expected age of onset of the disease is later than the other sibs.

Discussion

The exact mechanism by which (CTG)_n expansion in the 3' UTR of the *DMPK* gene causes myotonic dystrophy (DM) is unknown. Emerging studies suggest that two novel pathogenetic mechanisms may play a role. The first one is that the expanded repeats appear to cause haploinsufficiency of a neighbouring homeobox gene. The second mechanism is that the *DMPK* gene produces an abnormal RNA,

RNA homeostasis.¹⁰⁻¹⁴ Consistent with this, previous clinical and molecular studies have shown that myotonic dystrophy patients who carry the homozygously mutated allele are phenotypically indistinguishable from heterozygotes.⁷⁻⁹ Similarly, the two homozygous myotonic dystrophy patients in our study do not differ clinically from the heterozygous patients. These findings are in agreement with previous reports which suggest that DM is a "true dominant".

Special attention should be given to patients with dominantly transmitted myotonic dystrophy in genetic counselling, especially in populations with a high rate of consanguineous marriage.

- In this study, we report the clinical and molecular findings of two homozygous myotonic dystrophy patients. The first patient shows the classical form of the disease with two DM alleles with expansion sizes of 160 and 370 CTG repeats. In the second family, the homozygous patient is mildly affected with two expanded alleles (58 and 180 CTG repeats). Both homozygous cases are offspring of consanguineous marriages.
- These findings are consistent with previous reports which showed that homozygosity is not correlated with the severity of the disease. Having a homozygous form of myotonic dystrophy does not add any additional clinical symptoms to the already known phenotype of the disease.

- 1 Brook JD, McCurrach ME, Harley HG, Buckler AJ, Curch D, Aburatani H, Hunter K, Stanton VP, Thirion JP, Hudson T, Sohn R, Zemelman B, Snell RG, Rundle SA, Crow S, Davies J, Shelbourne P, Buxton J, Jones C, Juvonen V, Johnson K, Harper PS, Shaw DJ, Housman DJ. Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3' end of a transcript encoding a protein kinase family member. *Cell* 1992;68:799-808.
- 2 Mahadevan M, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Lansen G, Neville C, Narang M, Barcelo J, O'Hoy K, Leblond S, MacDonald SE, Jong PE, Vieringa B, Korneluk RG. Myotonic dystrophy mutation: an unstable CTG repeat in the 3' untranslated region of the gene. *Science* 1992;255:1253-6.
- 3 Hunter A, Tsilfidis C, Mettler G, Jacob P, Mahadevan M, Surh L, Korneluk R. The correlation of age of onset with CTG trinucleotide repeat amplification in myotonic dystrophy. *J Med Genet* 1992;29:774-9.
- 4 Hamshere MG, Harley H, Harper P, Brook DJ, Brookfield JFY. Myotonic dystrophy: the correlation of (CTG) repeat length in leucocytes with age at onset is significant only for patients with small expansions. *J Med Genet* 1999;36:59-61.
- 5 Lavedan C, Hofmann-Radvanyi H, Shelbourne P, Rabes JP, Duros C, Savoy D, Dehaupas I, Luce S, Johnson K. Myotonic dystrophy: size- and sex- dependent dynamics of CTG meiotic instability, and somatic mosaicism. *Am J Hum Genet* 1993;52:875-83.
- 6 Novelli G. CTG triple mutation and phenotype manifestations in myotonic dystrophy patients. *Biochem Med Metab Biol* 1993;50:85-92.
- 7 Cobo A, Martinez JM, Martorell L, Baiget M, Johnson K. Molecular diagnosis of homozygous myotonic dystrophy in two asymptomatic sisters. *Hum Mol Genet* 1993;2:711-15.
- 8 Martorell L, Illa I, Rosell J, Benitez J, Sedano MJ, Baiget M. Homozygous myotonic dystrophy: clinical and molecular studies of three unrelated cases. *J Med Genet* 1996;33:783-5.
- 9 Zlotogora J. Dominance and homozygosity. *Am J Med Genet* 1997;68:412-16.
- 10 Davis BM, McCurrach ME, Taneja KL, Singer RH, Housman DE. Expansion of a CUG trinucleotide repeat in the untranslated region of myotonic dystrophy protein kinase transcripts results in nuclear retention of transcripts. *Proc Natl Acad Sci USA* 1997;94:7388-93.
- 11 Korade-Mirincins Z, Babitzke P, Hoffman E. Myotonic dystrophy: molecular windows on a complex etiology. *Nucleic Acids Res* 1998;26:1363-8.

- 12 Reddy PS. The complex pathology of trinucleotide repeats. *Curr Opin Cell Biol* 1997;9:364-72.
- 13 Timchenko LT. Myotonic dystrophy: the role of RNA CUG triplet repeats. *Am J Hum Genet* 1999;64:360-4.
- 14 Wang J, Pegoraro E, Menegazzo E, Gennerelli M, Hoop RJ, Angelini C, Hoffman EP. Myotonic dystrophy: evidence for a possible dominant negative RNA mutation. *Hum Mol Genet* 1995;4:599-606.