Expansion of the myotonic dystrophy gene in Italian and Spanish patients

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Abstract

Myotonic dystrophy results from expansion of a (CTG)_n repeat at the 3' untranslated region of the myotonin-protein kinase gene. We show here the genomic analysis of 322 symptomatic patients with the cDNA-25 probe detecting disease specific *EcoRI* restriction fragments. The expansion was found in the majority of Italian and Spanish patients (92%). The implications of these results for the detection of symptomatic patients in southern Europe are discussed.

(J Med Genet 1992;29:789-90)

Myotonic dystrophy (DM) is the most common inherited neuromuscular disease in adults, with an estimated world wide prevalence of 1 in 8000 persons.¹ DM segregates as an autosomal dominant mutation with marked expressivity and pleiotropic effects including myopathy, cataracts, and cardiac arrhythmias.¹ Affected families may exhibit genetic anticipation of the disease, defined by the appearance of increasing severity in successive generations.¹ The molecular basis of DM has recently been elucidated by positional cloning of DNA probes isolated from the 19q13.3 region.²⁻⁵ cDNA-25, MDY1, GB2.6, and M10M-6 probes detect disease specific restriction fragments, larger than those found in unaffected subjects. These fragments, which are variable in length and increase in size within DM families in parallel with the severity of the disease, have provided a biological basis for anticipation.^{1,6} The genomic region associated with this new allelic mutation has been characterised and found to contain a variable number of CTG repeats in the 3' untranslated region of an mRNA coding for a member of the protein kinase family, identified as myotonin-protein kinase (MT-PK).^{5,6}

We have investigated 122 DM families from Italy and Spain. A total of 322 symptomatic patients was analysed from these pedigrees. Clinical diagnosis was performed according to the criteria of Harper¹ and the definition of the working group on the molecular defect in DM.⁷ The control population consisted of 156 unrelated adult donors to the Italian Blood Transfusion Centre, Red Cross, Rome. Genomic DNA from these controls was examined with a cDNA probe (cDNA-25) which detects an *EcoRI* polymorphism² of 9 and 10 kb fragments, with a frequency of 0.58 and 0.42, respectively.

In 296 out of 322 (92%) DM patients, the

same probe detected new disease specific fragments larger than 10 kb (table). In all meioses examined, the expanded allele was cosegregating with the disease. This expansion showed intrasib variation and increased in subsequent generations. In the present set of families we found no offspring with a band corresponding to the expanded allele smaller than that observed in the parents carrying DM. The expansion was not found in 26 (8%) unrelated, minimally affected subjects, who showed low grade amplification of this region not shown by Southern blotting. In all these subjects the disease had late onset and the most consistent clinical finding was cataract. Conversely, each of their affected offspring had a large fragment, confirming the unstable nature of this mutation.^{6,8} Furthermore, low grade expansions were detected in these 26 patients using the polymerase chain reaction (PCR, data not shown). In a few patients, an extended and faint smear was observed on Southern blots, as a consequence of a high degree of somatic heterogeneity. This result has been observed previously in other DM studies⁸ and in subjects with the fragile X syndrome.⁹ No evidence of a new mutation was found in 12 pedigrees with isolated cases of DM. In fact, in each instance, the cDNA-25 probe showed that one parent carried the mutation. Since congenital DM pedigrees were excluded from this study, no consistent phenotypic difference was observed in respect to the maternal versus the paternal origin of the mutation.

Our findings compare well with those reported in other populations,^{8,10} confirming the genetic homogeneity of this disorder, as expected in view of the linkage disequilibrium between the DM mutation and flanking loci.^{11,12} In addition, the present data indicate that the majority of Italian and Spanish DM symptomatic patients could be detected using a single DNA test. Eight percent of affected patients could only be identified by PCR. These observations are relevant to genetic counselling and prenatal diagnosis of this disease.

Association of the variable *EcoRI* fragment with DM.

Alleles	Range of allele expansion (kb)	Symptomatic DM patients	
		No	%
E0	—	26	8
E1	0-2	81	25
E2	2-4	129	40
E3	> 4	86	27

— Not shown by Southern blotting.

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Received 17 June 1992.
Revised version accepted 6 July 1992.

We are indebted to our Italian and Spanish colleagues who referred the patients to us. This work was supported by grants from the Italian CNR (PF IG no 91.00037.PF99 and PF Biotecnologie e Biostrumentazione), Telethon-Italy, project 'Molecular study of the DM gene', the Muscular Dystrophy Group of Great Britain, the Muscular Dystrophy Association of America, and the Central Research Fund of the University of London.

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Editor's note

Dr Kay Davies, who edited this collection of papers on myotonic dystrophy, and who has been Molecular Genetics Editor of the *Journal of Medical Genetics* for the past six years, will step down from this position at the end of 1992. The Journal would like to take this opportunity of thanking Dr Davies for the major role she has played in helping it to develop during a time when molecular genetics has become an integral part of both research and clinical practice in medical genetics.

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