Contribution of molecular analyses to the estimation of the risk of congenital myotonic dystrophy

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Abstract

A molecular analysis of the maternal and child CTG repeat size and intergenerational amplification was performed in order to estimate the risk of having a child with congenital myotonic dystrophy (CMD). In a study of 124 affected mother-child pairs (42 mother-CMD and 82 mother-non-CMD) the mean maternal CTG allele in CMD cases was three times higher (700 repeats) than in non-CMD cases (236 repeats). When the maternal allele was in the 50-300 repeats range, 90% of children were non-CMD. In contrast, when the maternal allele was greater than 300 repeats, 59% inherited the congenital form. Furthermore, the risk of having a CMD child is also related to the intergenerational amplification, which was significantly greater in the mother-CMD pairs than in the mother-non-CMD pairs. Although the risk of giving birth to a CMD child always exists for affected mothers, our data show that such a risk is considerably higher if the maternal allele is greater than 300 repeats.

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Myotonic dystrophy (DM) is the most common form of adult muscular dystrophy, with an estimated incidence of 1 in 8000 in most populations.¹ DM is a multisystemic autosomal dominant disorder with highly variable expression including myotonia, progressive muscle weakness, and cataracts. The mutation underlying DM has been characterised as an expanded CTG trinucleotide repeat sequence in the 3' untranslated region of a protein kinase gene on chromosome 19q13.2-13.3 whose size correlates with the clinical severity of the disorder.²⁻⁷ The phenomenon of anticipation within families, with decreasing age of onset and increasing severity of symptoms in succeeding generations, has been documented.⁸⁻¹⁰ Furthermore, the observed intergenerational amplification of CTG repeat in the majority of the families has provided a biological basis for this phenomenon.¹¹⁻¹⁴

DM can also occur as a severe neonatal form almost exclusively transmitted by carrier mothers. The major clinical features of congenital myotonic dystrophy (CMD) are severe neonatal hypotonia, respiratory distress, facial weakness, mental retardation, and high mortality. Hydramnios, reduced fetal movements

during pregnancy, and talipes are also frequent findings.¹¹⁵ Previous studies have estimated the risk of having a congenitally affected child to be about 10 to 40%.¹⁶ A correlation between the maternal size of the CTG repeat and the frequency of CMD has been suggested.¹² We present a quantitative study of the risk for carrier mothers of having a CMD child based on their repeat size.

Patients and methods

DIAGNOSIS OF DM

Clinical diagnosis of DM was made according to the criteria of Harper¹ and the definition of the Working Group on the molecular defect in DM.¹⁷ The diagnostic criteria for CMD were hypotonia at birth, neonatal respiratory distress, mental retardation, facial weakness, and diagnosis of DM in the infant's mother or other relative.

DNA ANALYSES

Genomic DNA (5 µg) was digested with *EcoRI* and the fragments separated on a 0.6% agarose gel. Southern transfer was performed by alkali blotting onto Hybond N (Amersham) and hybridised to ³²P labelled cDNA25⁴ or pGP2.6² for 16 hours at 65°C according to standard procedures. The filters were washed to a stringency of 0.1 × SSC, 0.1% SDS, and autoradiography was for four to eight days at -80°C. PCR analysis for the detection of minimal CTG repeat expansions was performed as follows: reactions (50 µl) were set up using standard PCR conditions (50 mmol/l KCl, 1.5 mmol/l MgCl₂, 10 mmol/l Tris-HCl, pH 8.3, 200 mmol/l dNTPs, 1 µmol/l each primer, 700 ng DNA, and 1 U *Taq* DNA polymerase. Cycling conditions were as follows: 1 × (three minutes, 94°C), 30 × (one minute 30 seconds, 94°C; one minute, 67°C; two minutes, 72°C), 1 × (seven minutes, 72°C), 4°C soak. Samples were then loaded on 3.5% NUSIEVE gels and electrophoresis was performed at 120 V for two hours. The primers used, 101 and 102, have been previously described.³

CTG expansion was examined in 124 mother-child pairs affected with DM, including 42 mother-CMD and 82 mother-DM children. To compare the expansion sizes between CMD and non-CMD cases, the size of the CTG allele was also determined in a random sample of 346 typical DM (non-CMD) patients. The CTG repeat number was calculated from the expansion in kilobases estimated from auto-

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Figure 1 Estimation of the repeat number from an autoradiograph of a DM family. The DNA was digested with *EcoRI* and hybridised with the probe cDNA25 which detects the normal 9 and 10 kb alleles and the expanded CTG repeat. Lane 1: normal father with two normal alleles of 9 kb. Lane 2: mildly affected mother with cataracts; she has a normal 10 kb allele and an expanded allele of 10.5 kb (0.5 kb corresponds to 100 CTG repeats). Lane 3: asymptomatic son with a normal allele pattern of 9 and 10 kb. Lane 4: affected daughter with CMD; she presents a normal 9 kb allele and a large mutated allele of 15 kb which corresponds to 1667 CTG repeats.

Figure 2 Distribution of the CTG allele size according to the clinical status.

Area under ROC = 0.838

Figure 3 ROC curve for the estimation of the cut off point.

Table 1 Mean maternal allele size in CMD and non-CMD cases

	Mother/child pairs	No of maternal CTG repeats
CMD	42	700 (80-2333)
Non-CMD	82	236 (65-1167)

Student's *t* test $p < 0.001$.

Table 2 Distribution of the children according to the size of maternal allele, for a cut off point of 300 repeats

Maternal allele	No of CMD cases	No of non-CMD cases	Total
>300 CTG	36	25	61
<300 CTG	6	57	63
Total	42	82	124

radiographs (fig 1) where 1 kb corresponds to 333 repeats. The repeat number in smear bands was estimated from the lower limit of the smear.

STATISTICAL ANALYSIS

A comparison between the size of the maternal allele and the intergenerational amplification in CMD cases and in non-CMD cases was performed with Student's *t* test. The best cut off point regarding sensitivity and specificity was defined graphically by means of a Receiver Operating Characteristics (ROC) curve. The relative risk of having a CMD child was estimated calculating the odds ratio (OR) from a 2×2 table.

Results

In the group of 42 CMD patients, the average CTG allele size was 1496 (SD 348) repeats, with sizes ranging from 1000 to 2300 repeats. In a random sample of non-CMD patients ($n = 346$), the average allele size was 581 (SD 444), with a lower limit of 50 repeats and the largest repeat size being 2300 repeats (fig 2). An overlap exists between both groups; 21% (9/42) of CMD children showed relatively modest CTG alleles of between 1000 and 1200 repeats, while 9% (31/346) of the non-CMD patients had repeat lengths in the upper range of CMD patients. Hence, the large repeat number observed in the CMD group is not a sufficient condition for the development of CMD.

Our study showed different sized alleles in the mothers of CMD ($n = 42$) and non-CMD children ($n = 82$). The mean maternal CTG repeat size in the former group was significantly higher ($p < 0.001$, Student's *t* test) than in the latter (table 1).

A cut off point of 300 CTG repeats determined by an ROC curve was chosen to predict the occurrence of CMD cases with a maximum specificity and sensitivity. At the point of 300 CTG repeats, the sensitivity (36 CMD cases for maternal repeat size greater than 300 repeats/42 total CMD cases) was 86% and the specificity (57 non-CMD cases for maternal repeat size less than 300 repeats/82 total non-CMD cases) was 70% (table 2, fig 3). If a lower cut off point of 100 repeats is considered, the sensitivity increases to 95% but

Table 3 Intergenerational behaviour of the CTG repeat in the mother-child pairs

	CTG increase		No change in CTG		CTG decrease	
	No	Mean	No		No	
Mother-CMD	40/42 (96%)	861	1/42 (2%)		1/42 (2%)	
Mother-non-CMD	71/82 (87%)	555	10/82 (12%)		1/82 (1%)	

Student's *t* test $p < 0.001$

Figure 4 Comparison of CTG repeat lengths between mothers of CMD and non-CMD patients.

the specificity is only 48%. In contrast, for a cut off point of 500 repeats, the sensitivity decreases greatly to 55% despite a high specificity of 96%.

If the number of CMD and non-CMD cases in relation to the maternal allele are plotted, we observe that 90% (57/63) of the children are non-CMD when the maternal repeat size is less than 300 repeats. In contrast, when the size of the maternal allele is 300 CTG repeats or greater, 59% (36/61) of the children are CMD (fig 4, table 2). On the basis of our data, the estimated risk (OR) of giving birth to a CMD child is 14 times higher (95% confidence interval 4.7–41.7) for the mothers with a CTG repeat length of 300 or greater.

We have determined the intergenerational behaviour of the CTG repeat in the 124 mother-child pairs (table 3). An intergenerational increase of the CTG repeat was seen in 71/82 non-CMD pairs and in 40/42 CMD pairs. No change in the size of the expansion from mother to child was observed in 10/82 non-CMD pairs and in 1/42 CMD pairs. An intergenerational decrease was detected in 1/82 non-CMD pairs and in 1/42 CMD pairs (table 3). The mean intergenerational amplification in the mother-CMD pairs (861 repeats) is significantly greater (Student's *t* test, $p < 0.001$) than in the group of mother-non CMD pairs (555 repeats).

Discussion

The CTG repeat size in CMD children has been determined by different authors: Tsilfidis *et al.*,¹² Harley *et al.*,¹³ and Redman *et al.*¹⁴ found some congenital cases with alleles in the 500–999 repeats range and an overlap between the CMD and non-CMD patients in the 1300–2300 repeats range. In our series, all CMD patients had allele sizes greater than 1000 repeats and a similar overlap has been observed, suggesting that the repeat size is not the only

feature determining the congenital onset of the disease.

A second parameter to be taken into account is the number of maternal repeats. We have found that the mean allele size in the mothers of CMD cases is three times higher than in mothers of non-CMD cases. This finding agrees with the data of Harley *et al.*¹³ who found mean maternal repeat sizes of 600 and 220 for CMD and non-CMD children, respectively. A previous study¹² determined that the majority of mothers of non-CMD children had allele sizes smaller than 500 repeats. Our results indicate that a cut off point of 300 repeats can be defined. This cut off point is important because of its negative predictive value: for a mother having fewer than 300 repeats, the possibility of having a CMD child is about 10%. When the maternal allele is 300 repeats or greater, the possibility of predicting a CMD case is limited since only 59% of the cases are CMD. Nevertheless, at this cut off point, the estimated risk of having a CMD child for this group of mothers is 14 times greater than in mothers below 300 repeats.

Another feature involved in the risk of having a CMD child is the intergenerational behaviour of the CTG repeat. Previous studies^{12–14} have suggested that the repeat size invariably increases from mother to CMD children. However, we have observed an intergenerational increase of the CTG repeat in 96% of the mother-CMD pairs, a transmission without change in size in 2% of the mother-CMD pairs and a decrease in one pair. The latter showed the largest decrease of all our parent-child pairs. In this case, the mother had the typical features of DM and the largest number of repeats (2300 repeats) of our sample of 346 DM patients. Her son has, surprisingly, inherited a smaller allele size (1300 repeats) than his mother. This case, in which CMD is inherited despite an important contraction on transmission from mother to child, should be considered in pre-natal counselling.^{18,19}

Other studies have shown contradictory results about the intergenerational behaviour of CTG repeats. While, Tsilfidis *et al.*¹² did not identify differences in the intergenerational amplification of the mother-CMD and mother-non-CMD pairs, Redman *et al.*¹⁴ found a clear difference between the two groups. Our results are in good agreement with the latter.

Previous studies¹⁶ have shown that multi-system disease in the mother increases the risk of having a CMD child. However, the risk always exists for an affected mother, since this study has identified the existence of CMD offspring of mothers with fewer than 100 repeats and in the presence of a dramatic intergenerational contraction.

However, the data presented in this study cannot alone explain the nearly exclusive maternal inheritance of CMD. Some authors^{16,20} have suggested that CMD could arise from an “additive” pathology including an affected mother with metabolic disturbances and an affected developing fetus with a sufficiently high number of CTG repeats.

Finally, we can conclude that the risk of

giving birth to a CMD child is related to the intergenerational amplification and is considerably higher if the maternal allele is greater than 300 repeats.

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