

Myotonic dystrophy: genetic, clinical, and molecular analysis of patients from 41 Brazilian families

Maria Rita Passos-Bueno, Antonia Cerqueira, Mariz Vainzof, Suely K Marie, Mayana Zatz

Abstract

Results of genealogical, DNA, and clinical findings in 41 families with 235 patients affected with myotonic dystrophy (DM) led to the following observations. (1) The relative proportion of affected patients among blacks is apparently lower than among whites or orientals. (2) A significant excess of males was observed. (3) The frequency of DM patients who did not reproduce was similar for males and females; however, female patients had on average 25% fewer children than male patients. (4) There was a significant intergenerational increase in the mean length of the CTG repeat which was also correlated with the severity of the phenotype. (5) No significant difference was observed in the mean size of the CTG repeat in offspring of male as compared to female transmitters. (6) With the exception of the congenital cases of maternal origin, the largest expansions were paternally inherited, but did not lead to congenital DM.

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Myotonic dystrophy (DM) is an autosomal dominant disorder, with an incidence of 1 in 7000 to 8000 births, and represents the commonest neuromuscular disorder in adulthood. Its symptoms may include muscle weakness and atrophy, myotonia, cataract, frontal baldness with a characteristic facial appearance, cardiac arrhythmias, infertility, and endocrine disorders.¹ It is characterised by an extremely variable clinical course, ranging from persons in whom the only clinical sign may be a cataract or frontal baldness to severely affected patients. In addition, there is a severe congenital form, which is frequently fatal and with rare exceptions^{2,3} is transmitted by affected mothers.⁴

An interesting feature in DM is the phenomenon of anticipation, that is, an increase in severity and earlier age of onset in successive generations.^{5,6}

Following the characterisation of the molecular defect responsible for myotonic dystrophy in 1992 by three independent groups,⁷⁻⁹ many investigations have been published worldwide. It was found that the genetic mechanism responsible for DM is an expansion of a CTG repeat in the 3' untranslated region of the myotonin protein kinase gene.¹⁰⁻¹² In normal

persons, the number of CTG repeats ranges from five to about 37 copies while in affected persons it varies between 50 to thousands of copies.^{7,10-13} The increase in size of the DNA fragment containing the repeat from one generation to another provides the molecular basis for anticipation.¹⁴⁻¹⁷

A relationship between the size of the CTG repeat and disease severity has been reported in several studies,^{16,18-20} although there is an overlap between clinical groups.^{14,16-19}

Other features which have been investigated include: the influence of the sex of the transmitting parent as well as of parental allele size on the CTG expansion^{19,20}; the proportion of affected male versus female patients in multigeneration families²⁰; the mechanism and the frequency of intergenerational CTG contraction²¹; the association between the presence of smear and the patients' age as well as the CTG repeat size.¹⁷⁻¹⁹ All these investigations have been done in DM families from Europe, United States, Canada, or Japan but apparently none has been reported so far for the population of South America.

The purpose of the present study is to report results of genealogical, DNA, and clinical findings in 235 DM patients, belonging to 41 Brazilian families of different racial backgrounds, with the following purposes; (1) to verify if the relative frequency of affected DM families from different racial backgrounds (white, black, oriental) is in accordance with the frequency of each of these racial groups in our population; (2) to investigate the sex ratio and the relative reproductive performance of DM males as compared to DM females from multigeneration families; (3) to correlate the size of the CTG repeat from leucocyte DNA with the phenotype in patients with different degrees of clinical severity; (4) to analyse the influence of sex as well as the size of the parental CTG repeat in the CTG expansion of affected offspring; (5) to estimate the frequency of contraction of the CTG repeat in this sample of families.

Patients and methods

A total of 235 DM patients, belonging to 41 families, have been ascertained at the Centro de Miopatias, Departamento de Biologia, Universidade de São Paulo during the last 10 years. Of them, 111 patients (aged 1 to 78 years) and 120 normal relatives had their DNA analysed.

Based on the severity of the phenotype and

Centro de Miopatias,
Departamento de
Biologia, Instituto de
Bióciências,
Universidade de São
Paulo, Rua do Matão
277, São Paulo, SP
Brazil 05508-900
M R Passos-Bueno
A Cerqueira
M Vainzof
M Zatz

Departamento de
Neurologia, Hospital
das Clínicas,
Universidade de São
Paulo, São Paulo,
Brazil
S K Marie

Correspondence to:
Dr Zatz.

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age of onset, patients were classified into four groups¹⁹: (A) the mildest form, in which the only clinical sign may be cataract or frontal baldness, with little or no muscle involvement; (B) the classical form, with onset in adolescence or early adult life, characterised by myotonia and progressive muscle weakness; (C) the rare congenital severe form, with early onset, generalised muscular hypoplasia, and mental retardation, which is almost invariably transmitted through carrier mothers; (D) the early childhood form, which shows some features comparable to those seen in the congenital form, but with no documented abnormality at the time of birth.

DNA was extracted from whole blood according to the method of Miller *et al.*²² Molecular analyses were performed according to methods described previously.^{7,10} The DNA was digested with *EcoRI* and electrophoresed in a 0.7% agarose gel. In all cases that showed small or undetectable expansions, DNA analysis was repeated after digestion with *PstI*.¹⁹

For estimating the size of the repeat the most prominent band was taken into consideration or the average size of the smear.²¹ Samples from all affected members belonging to each family were run on the same gel. All molecular results are expressed in terms of kilobases (kb) of additional DNA.

Statistical analysis included χ^2 analysis and the usual paired and unpaired tests for comparing means (*t* tests and one way analysis of variance).

Results

Clinical and DNA findings are summarised in the table.

ANALYSIS OF PEDIGREES

The affected patients belonged to 41 unrelated families of the following racial background: 35 white, three black, and three oriental (Japanese).

Analysis of the sex ratio showed an excess of DM males ($n=153$) as compared to DM females ($n=82$), which was statistically significant ($p<0.001$). This included patients clinically affected as well as non-manifesting transmitters of the DM mutation from the 41 families. A statistically significant ($p<0.01$) disproportion between the sexes was also observed among the probands (29 males: 12 females). When the analysis was repeated without the probands, there were still significantly more male ($n=124$) than female ($n=70$) patients ($p<0.01$). However, the sex ratio among unaffected adult relatives (107 males and 98 females) did not differ from 1:1.

In order to compare the reproductive performance between affected males and females we considered the total number of children born to DM males versus those born to female DM patients who were older than 25. It was observed that of 110 DM males, 36 did not reproduce and the remaining had a total of 250 children (250/110, average 2.27 per affected male). On the other hand, of 68 DM females,

22 did not reproduce and the remainder had a total of 116 children (116/68, average 1.70 per affected female). Although DM males had on average more children than DM females, the distribution of the mean number of children per affected patient did not differ significantly between the two sexes ($p>0.05$).

ANALYSIS OF THE CTG REPEAT

Considering all affected patients, the size of the repeat varied from 0.1 to 9.0 kb with no statistically significant difference between sexes (mean = 1.6 kb, SD 1.3, $n=62$ for males; mean = 2.0 kb, SD 1.7, $n=49$, for females; $p>0.05$) (fig 1).

In 13 patients (seven males and six females), the CTG repeats were very small or undetectable (less than 0.5 kb) following *EcoRI* digestion but were confirmed with *PstI*. The largest CTG repeats (ranging from 5.0 to 5.2 kb) were observed in three congenital cases (fig 2), in four patients from group D who were mentally retarded (3.4, 4.0, 5.0, and 5.3 kb, respectively), and in three female patients (including two sisters) classified in group B, who had severe muscle weakness (3.5, 5.0, and 9 kb, respectively).

Five children (aged 1 to 14) who had inherited the DM allele from their affected parent (two mothers and three fathers), with repeats ranging from 0.2 to 1.0 kb, were clinically asymptomatic.

Comparison of the CTG repeat size among the four clinical groups of patients (A to D) showed that, on average, the size of the repeat was larger in the groups of more severely affected patients, although there was an overlap between them (table). The mean size of the CTG repeat was similar in patients from group C and group D, but the number of persons was too small for statistical analysis.

A wider variability in the size of the CTG repeat (from 0.2 to 9.0 kb) was observed among patients classified as classical DM (group B). Interestingly, in this group, the mean size of the CTG repeat was significantly greater ($p<0.05$) among affected females (2.3 kb, SD 1.6, $n=32$) than among affected males (1.6 kb, SD 1.0, $n=45$).

SIZE OF REPEAT AND SEX OF TRANSMITTING PARENT

Analysis of 48 pairs of affected parent/child showed a highly significant intergenerational increase in the mean size of the CTG repeat (mean = 1.46 kb, SD 0.20, $p<0.001$).

No statistically significant difference between the CTG expansion in maternally as compared to paternally transmitted alleles ($p>0.05$) was observed (for 33 pairs of affected DM fathers/offspring: mean = 1.39 kb, SD 1.17; for 15 pairs of affected DM mothers/offspring: mean = 1.63 kb, SD 1.24).

Interestingly, although not statistically significant, the analysis of the distribution of the CTG intergenerational expansion according to the sex of the parent showed a higher proportion of fathers (68%) than mothers (47%)

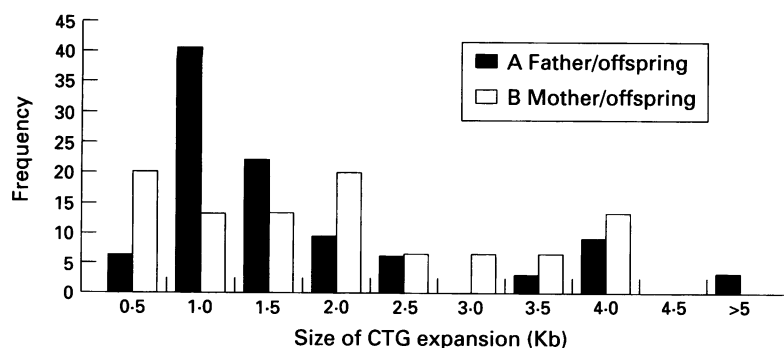


Figure 1 Comparison of the frequency of intergenerational CTG expansion in paternally transmitted alleles ($n=33$) (A) and in maternally transmitted alleles ($n=15$) (B).

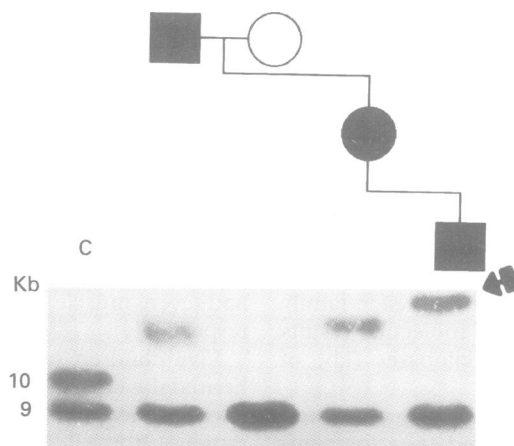


Figure 2 Example of a family with intergenerational CTG expansion and clinical anticipation, resulting in a male with congenital DM (see arrow).

transmitting small expansions (up to 1.5 kb) (fig 1). In order to assess if the lack of significance is because of the sample size it would be important to verify if this difference is maintained in a larger sample.

A contraction in the size of the trinucleotide repeat was observed in only one affected female (fig 3). The abnormal allele was transmitted by her DM father, who had a normal son and three affected daughters, one of whom had a child with the severe congenital form.

Discussion

RACIAL DISTRIBUTION

The incidence of DM has been estimated as 1 in 8000 in western European and North American populations¹ and as 1 in 20 000 in Japan.²³ However, there are apparently no reports of DM in African populations.²⁴

The relatively low frequency of DM families of black racial background (three of 41) in the

Clinical and DNA findings in 111 DM patients

Clinical classification	No of patients in each group	Age range (y)	Mean size of the CTG repeat (kb)*
Group A	27	36-76	0.49 SD 0.35
Group B	77	17-66	1.88 SD 1.32
Group C	3	1-10	5.20 SD 0.00
Group D	4	8-15	4.08 SD 1.49

*Analysis of variance among the four groups: $F=25.19$, $p<0.001$

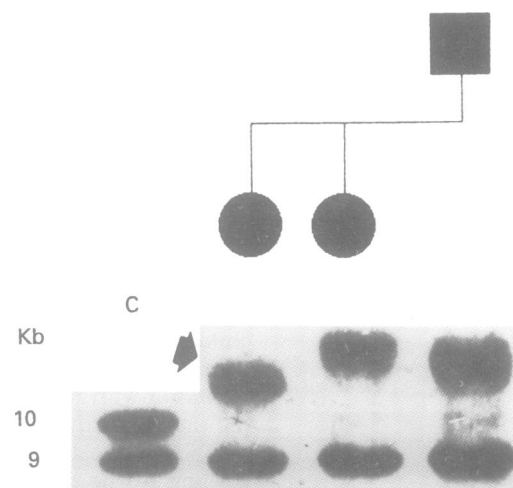


Figure 3 Intergenerational CTG contraction in a female who inherited the DM allele from her father (arrow). Her affected sister had an expansion.

present sample attracted our attention, since the population in the city of São Paulo is made up of about 50% white, 40% black, and 10% oriental (mostly of Japanese ancestry). Therefore we would expect a proportional distribution of these racial groups among the DM families ascertained. One possibility that we cannot rule out would be a bias in ascertainment, although, in our experience of 761 families with Duchenne muscular dystrophy, the proportion of affected patients from different racial backgrounds is in accordance with that expected (unpublished observations).

On the other hand, several independent studies suggest that DM is a consequence of one or a few ancestral mutations.^{7,19,23} If the ancestral mutation in the DM gene had occurred after divergence of the black from other racial groups, we would expect to find this condition among blacks only through miscegenation, which would explain its relatively lower incidence in our population. One possible explanation is that the DM gene would be more stable in this racial group, that is, less prone to expansion. The recent report of Goldman *et al*,²⁴ in which they show that non-DM South African blacks have a significantly lower frequency of large CTG repeat alleles than in populations in which the disease occurs relatively frequently, supports this hypothesis.

PROPORTION OF MALE VERSUS FEMALE AFFECTED PATIENTS

The significantly greater proportion of affected male patients in the families ascertained is in accordance with the recent report of Brunner *et al*²⁰ and other previous studies,²⁵⁻²⁸ who observed a disproportionate excess of males in their families. However, in the study of Brunner *et al*²⁰ the disproportionate excess of males was more evident in the last asymptomatic generation while in the present report such disproportion was found in all generations, including the index cases. We did not observe a significant difference in the proportion of patients classified as asymptomatic or minimally affected among males as compared to

DM females (45/153 = 28.1% for males; 21/82 = 25.6% for females; $p > 0.05$).

We have no explanation for the excess of affected DM males except for ascertainment bias, that is, families in which the proband is a male or with multiple affected males would be more often referred to genetic services because: (1) owing to the early frontal baldness, males more often have the characteristic facial appearance of DM at an earlier age than females; (interestingly this has been observed by us among affected sibs who carry the same size CTG repeat in leucocyte DNA); (2) many physicians still believe that muscular dystrophy is restricted to males.

SIZE OF THE CTG REPEAT IN SUCCESSIVE GENERATIONS

As reported in numerous publications,^{19,29} we also observed a significant increase in the average size of the CTG repeat in successive generations, which constitutes the biological explanation for anticipation. There was also a relationship between the size of the CTG repeat and severity of the phenotype in affected patients. Although there was some overlap in the mean CTG size among the four clinical groups, the larger ones were observed in patients from groups C and D (congenital and early childhood) and in three females who were classified in group B who had severe muscle weakness.

In the present sample, the mean size of the CTG repeat did not differ significantly in the offspring of affected mothers as compared to the offspring of affected fathers, in accordance with Harley *et al.*¹⁹ No statistically significant difference between the average repeat expansion for maternally and paternally inherited alleles was also reported by Redman *et al.*,^{14,17} but, contrary to our data, only when the congenital cases were excluded.

The severe congenital form of DM has been associated with large CTG expansions which are transmitted almost exclusively through the maternal line. However, interestingly, in the present sample, excluding the three congenital cases, the largest CTG repeats (ranging from 3.4 to 9 kb) were paternally transmitted in five of six unrelated families. Such expansions were found in four children in group D (who were also mentally retarded) as well as in three females (including two sisters) who had the adult form but with severe muscular weakness. In four families the leucocyte DNA of the transmitting father could be analysed: three of them had CTG repeat sizes ranging from 0.2 to 0.9 kb and one had a repeat size of 1.3 kb. In the fifth family with paternal inheritance, in which the female proband had the largest expansion in the present study (9 kb), the transmitting father had only cataract of late onset. Paternal transmission of large expansions very rarely leads to congenital DM.²³ However, such expansions maternally transmitted would probably result in the severe form.

A greater tendency to initial instability in male meiosis has been previously suggested.¹⁹ Such increased instability associated with male

transmission could reflect the larger number of cells divisions in spermatogenesis than in oogenesis or that expansion is predominantly a postzygotic event which would be influenced differentially by maternal or paternal genetic imprinting.²⁰

An intergenerational decrease in the size of the fragment, more often of paternal origin, has been reported in a small proportion of cases.^{14,19-21,30-32} The mechanism of this phenomenon is still unknown, and several hypotheses such as meiotic instability, gene conversion, or deletion of the expanded repeat have been suggested.³¹⁻³³

The only contraction of the CTG repeat in the present sample was observed in an affected female and was transmitted, as the majority of the previously reported cases, through the paternal line. Since the affected father had late onset of clinical symptoms and the affected daughter is still young, it was not possible to assess if her clinical picture will be milder than the one observed in her DM father. However, interestingly, recent results of a large multicentre study have shown that in approximately 50% of the cases there was clinical anticipation despite the contraction of the CTG repeat size in the leucocytes of the offspring.²¹ Although this could not be assessed in the present series, we observed in one pair of affected mother/son that although both had the same size of the CTG repeat (1.5 kb), the mother was only minimally affected while her son had a severe phenotype with early onset. This observation supports the hypothesis that some maternal factor may be involved in the pathophysiology of anticipation.²¹

RELATIVE MALE VERSUS FEMALE FERTILITY AND CONGENITAL MYOTONIC DYSTROPHY

The three congenital cases (two males and one female), belonging to two unrelated families, were transmitted through an affected mother, who had inherited the DM gene, in both cases, from their minimally affected father. Although the sample was small, an excess of transmitting grandfathers has also been reported for congenital cases by Harley *et al.*¹⁹

A hypothesis to explain the predominantly maternal transmission of congenital cases is that large expansions would have a detrimental effect on male fertility but apparently not on female fertility.^{19,34,35} In a study of 101 kindreds, Harley *et al.*¹⁹ found only four males in which the repeat size was greater than 2.0 kb.

Interestingly, when we compared the proportions of patients older than 25 who had not reproduced, they were very similar for both sexes: 36/110 or 32.7% among males and 22/68 or 32.3% among females. In addition, the analysis of the mean size of the CTG repeat in 23 males and 15 females who had not reproduced (23 males and 15 females) did not differ significantly between sexes (mean = 1.83 kb, SD 1.23 for males and 2.5 kb, SD 2.1 for females; $p > 0.05$).

It is important to bear in mind that these expansions were assessed in leucocyte DNA and might be different in germline tissues.³³

However, our data suggest that although the size of the CTG repeats might not be a direct cause of sterility in females there is reduced fertility in both sexes, probably associated with other factors such as the severity of the condition.

Conclusions

In summary, the results of the present study led to the following observations. (1) The relative proportion of affected DM families among 41 pedigrees was apparently lower in black than white or oriental populations suggesting that the DM mutation might be rarer in this racial group. (2) A significant excess of affected males was observed in our families including the probands. (3) The distribution in the number of children and the frequency of DM patients who did not reproduce was similar for males and females; however, female patients had on average 25% fewer children than male patients. (4) There was a significant intergenerational increase in the mean length of the CTG repeat which was also correlated with the severity of the phenotype. (5) No significant difference was observed in the mean size of the CTG repeat in the offspring of male transmitters as compared to female transmitters. (6) With the exception of the congenital cases of maternal origin, the largest expansions were paternally inherited, but did not lead to congenital DM. (7) Only one CTG contraction of paternal origin was observed in a female from a three generation family.

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