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Characteristics of Intergenerational Contractions of the CTG Repeat in Myotonic Dystrophy

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Summary

In myotonic dystrophy (DM), the size of a CTG repeat in the DM kinase gene generally increases in successive generations with clinical evidence of anticipation. However, there have also been cases with an intergenerational contraction of the repeat. We examined 1,489 DM parent-offspring pairs, of which 95 (6.4%) showed such contractions in peripheral blood leukocytes (PBL). In 56 of the 95 pairs, clinical data allowed an analysis of their anticipation status. It is surprising that anticipation occurred in 27 (48%) of these 56 pairs, while none clearly showed a later onset of DM in the symptomatic offspring. The contraction occurred in 76 (10%) of 753 paternal transmissions and in 19 (3%) of 736 maternal transmissions. Anticipation was observed more frequently in maternal (85%) than in paternal (37%) transmissions ($P < .001$). The parental repeat size correlated with the size of intergenerational contraction ($r^2 = .50$, $P \ll .001$), and the slope of linear regression was steeper in paternal (−.62) than in maternal (−.30) transmissions ($P \ll .001$). Sixteen DM parents had multiple DM offspring with the CTG repeat contractions. This frequency was higher than the frequency expected from the probability of the repeat contractions (6.4%) and the size of DM sib population (1.54 DM offspring per DM parent, in 968 DM parents). We conclude that (1) intergenerational contraction of the CTG repeat in leukocyte DNA frequently accompanies apparent anticipation, especially when DM is maternally transmitted, and (2) the paternal origin of the repeat and the presence of the repeat contraction in a sibling increase the probability of the CTG repeat contraction.

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Introduction

Myotonic dystrophy (DM) is a pleiotropic autosomal dominant disease with the highest prevalence among inherited adult neuromuscular diseases (Harper 1989). The mutation specific for DM is an expansion of the CTG trinucleotide repeat in the 3′ UTR of a protein

kinase gene (referred to as the "DM kinase gene," or the "myotonin protein kinase gene") located in the 19q13.3 region (Aslanidis et al. 1992; Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992*a;* Mahadevan et al. 1992). The CTG repeat length is polymorphic in normal individuals, ranging from 5 to 37 repeats (Brunner et al. 1992), while DM patients exhibit expansions >50 and often up to several thousand repeats (Aslanidis et al. 1992; Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992*a;* Mahadevan et al. 1992; Shelbourne et al. 1993). Expanded CTG repeats are unstable, as evidenced by the change in length of the repeat-containing DNA fragment from one generation to another, usually increasing in size in successive generations (Ashizawa et al. 1992*a;* Harley et al. 1992*b;* Lavedan et al. 1993*b;* Mulley et al. 1993; Redman et al. 1993). This has provided a molecular basis for anticipation, a clinical phenomenon in which the disease manifests itself earlier in successive generations with increasing severity. The size of the CTG repeat generally shows an inverse correlation with the age at onset (Ashizawa et al. 1992*a;* Hunter et al. 1992; Harley et al. 1993; Lavedan et al. 1993*b;* Novelli et al. 1993*a;* Redman et al. 1993) and is particularly large in congenital DM (Tsilfidis et al. 1992; Abeliovich et al. 1993; Harley et al. 1993; Lavedan et al. 1993*b;* Novelli et al. 1993*a;* Redman et al. 1993). In patients with adult-onset DM, levels of the DM kinase mRNA derived from the disease allele and the DM kinase protein may be decreased in proportion to the expansion of the CTG repeat and the clinical severity (Fu et al. 1993; Krahe et al. 1993; Novelli et al. 1993*b*), although studies on the mRNA levels in congenital DM have shown conflicting results (Fu et al. 1993; Hofmann-Radvanyi et al. 1993; Sabouri et al. 1993). Thus, while the exact pathophysiological mechanism remains unknown, the length of the CTG repeat plays important roles in DM.

In several reports, a small number of offspring have shown a decrease in the CTG repeat size compared with their parents (Ashizawa et al. 1992*a;* Shelbourne et al. 1992, 1993; Abeliovich et al. 1993; Brunner et al. 1993*b;* Cobo et al. 1993; Harley et al. 1993; Hunter et al. 1993; Lavedan et al. 1993*a,* 1993*b;* Mulley et al. 1993; O'Hoy et al. 1993; Redman et al. 1993). In most of these cases, the DM mutation was transmitted by the father. In contrast, a large intergenerational expansion often resulted in congenital DM that occurs mostly in maternal transmissions of the disease (Tsilfidis et al. 1992; Harley et al. 1993; Redman et al. 1993). Furthermore, in paternal transmissions, the intergenerational increase of the repeat size became smaller as the paternal repeat size increased (Cobo et al. 1993; Lavedan et al. 1993*a,* 1993*b;* Mulley et al. 1993; Ashizawa et al. 1994). To arrive at a consensus among investigators from different institutions, with regard to the parameters involved in the contraction of the CTG repeat, we pooled available cases from multiple centers.

Patients and Methods

Patients

The patients included in this study were collected for either research or diagnostic purposes. In some cases, the clinical data were entirely furnished by referring physicians, especially when the samples were collected for diagnostic purposes. Altogether, we studied the CTG repeat size of 1,489 parent-child pairs of DM patients from 15 institutions in 11 countries.

Determination of the CTG Repeat Size

DNA obtained from peripheral blood leukocytes of each DM patient underwent Southern blotting and the PCR for determination of the CTG repeat size, as described elsewhere (Aslanidis et al. 1992; Brook et al. 1992; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992*a;* Mahadevan et al. 1992). In Southern blotting, DNA was digested primarily with *Eco*RI, *Bam*HI, and *Nco*I, was electrophoresed in an agarose gel, and was transferred to a nylon membrane. Radiolabeled probes containing the CTG repeat region were used to detect the restriction fragments containing the CTG repeat. For the PCR, oligonucleotide primers, which have nucleotide sequence homology with the unique sequences flanking the CTG repeat region, were used to amplify this region of the patient's DNA. The PCR was performed in the presence of radiolabeled α-dCTP, to incorporate the radioactivity into the amplified products, which were then analyzed by denaturing 6% PAGE followed by autoradiography. In some cases, the PCR products were analyzed by 2%–3% agarose gel electrophoresis with ethidium bromide staining without incorporation of radioactivity. Alternatively, the PCR products were analyzed by Southern blotting with subsequent hybridization to a radiolabeled $(CTG)_{10}$ oligonucleotide probe. The protocols slightly differed from one institution to another. However, all protocols reliably determined the size of the repeat. Samples from each DM parent-offspring pair were run on the same gel. An intergenerational change of the CTG repeat size was scored as a contraction when the average size of the

Table 1

Cases with CTG Repeat Contractions

Study Center[a]	No. of Parent-Child Pairs with CTG Repeat Contraction			No. of Parent-Child Pairs Screened for CTG Repeat Contraction		
	Father-Child Pairs	Mother-Child Pairs	Total	Father-Child Pairs	Mother-Child Pairs	Total
Barcelona[b]	15	2	17	95	89	184
Cardiff	9	2	11	64	93	157
Denver	1	3	4	7	16	23
Durham, NC	7	3	10	44	38	82
Houston[c]	7	2	9	74	66	140
Stockholm[d]	3	0	3	5	5	10
Marburg, Germany	1	4	5	17	13	30
Nijmegen[e]	9	2	11	111	61	172
Adelaide[f]	4	0	4	30	25	55
Osaka	0	0	0	41	39	80
Ottawa[g]	4	0	4	121	184	305
Paris[h]	15	0	15	87	72	159
Rome[i]	1	1	2	57	35	92
Total	76	19	95	753	736	1,489

[a] The author/investigators involved in the centers were as follows: M.B., A.M.C., and A.L. (Barcelona); H.H., D.J.S., and P.S.H. (Cardiff); W.K.S. (Denver); A.D.R. (Durham, NC); T.A. and R.G.F., Jr. (Houston); M.A. and U.G. (Stockholm); M.C.K. (Marburg, Germany); H.B. and H.S. (Nijmegen); T.M. and H.Y. (Osaka); R.G.K. and J.M.B. (Ottawa); C.J. and C.L. (Paris); and B.D. and G.N. (Rome).

[b] Some of the data have been reported elsewhere by Cobo et al. (1993).

[c] Some of the data have been reported elsewhere by Ashizawa et al. (1992*a*) and Redman et al. (1993).

[d] Some of the data have been reported elsewhere by Shelbourne et al. (1993).

[e] Some of the data have been reported elsewhere by Brunner et al. (1993*b*).

[f] Some of the data have been reported elsewhere by Mulley et al. (1993).

[g] Some of the data have been reported elsewhere by O'Hoy et al. (1993).

[h] Some of the data have been reported elsewhere by Lavedan et al. (1993*b*).

[i] Some of the data have been reported elsewhere by Novelli et al. (1993*a*).

repeat, the upper limit of the smear, and the lower limit of the smear in the offspring were smaller than those in the parent. We used the average size of the smear as the repeat size.

Results

For the 1,489 DM parent-child pairs, the CTG repeat size was smaller in the child than in the parent in 95 (6.4%) (table 1). The number of CTG repeats decreased in 19 (2.6%) of 736 maternal transmissions and in 76 (10.1%) of 753 paternal transmissions ($\chi^2 = 33.9$, df = 1, $P \ll .001$). Although the sex of the offspring appeared to be more frequently male ($n = 55$) than female ($n = 40$) in these cases, this was not statistically significant (χ^2 goodness of fit = 2.37, df = 1, $.05 < P < .1$).

Detailed clinical information was available in 56 of the 95 pairs, comprising 43 paternal and 13 maternal transmissions. In the remaining 39 pairs, the clinical information was either unavailable or not detailed enough to allow a comparison between the age at onset in the parent and that in the offspring. For the 56 pairs, anticipation was observed in 27 (48%), the age at onset in the parent and the child were similar in 3 (5%), and in 26 (46%) the child was asymptomatic at an age younger than the parent's age at onset (table 2). The ages at onset and the CTG repeat sizes of the 27 cases with anticipation were summarized (table 3). Of the 27 cases, 16 showed unambiguous contraction of the CTG repeat, without overlap of the smear sizes. When the repeat expansions were detected by Southern blotting of the PCR products with the $(CTG)_{10}$ probe, the smear tended to be particularly extensive, making precise determination of the size difficult in some cases (e.g., cases 25 and 26 in table 3). Three of the 26 cases with asymptomatic children showed reversions of the CTG repeat size to the normal range (13, 24, and 19 repeats) and have been reported elsewhere (Brunner et al.

Table 2

Anticipation and CTG Repeat Contraction

Age at Onset	No. (%) of Pairs		
	Father-Child Pairs	Mother-Child Pairs	Total
Parent > child	16 (37)	11 (85)	27 (48)
Parent = child	3 (7)	0	3 (5)
Parent < child	0	0	0
Child asymptomatic[a]	24 (56)	2 (15)	26 (46)
Total	43 (100)	13 (100)	56 (100)

NOTE.—In 56 of the 95 DM parent-child pairs, information on the age at onset allowed determination of the anticipation status, while the remaining 39 pairs failed to give clinical information clear enough to allow meaningful analyses.

[a] Cases in which the child was asymptomatic at an age younger than the age at onset in the parent.

1993*b*; O'Hoy et al. 1993). In another pair in which the ages at onset were similar, the disease of the child was milder and more slowly progressive than that of his parent (O'Hoy et al. 1993). Thus, in about half of these cases, clinical anticipation still occurred, despite the intergenerational contraction of the repeat. Two of the cases with anticipation resulted in congenital DM of the child (Cobo et al. 1993). With the CTG repeat contraction, anticipation was proportionally more frequent with maternal transmissions (11 of 13 [85%]) than with paternal transmissions (16 of 43 [37%]) (Fisher's exact test; $P < .001$) (table 2).

There was a significant correlation between the size of the parental repeat and the size of the intergenerational contraction ($r^2 = .50$, $P \ll .001$) (fig. 1). The correlation was tighter when the data were analyzed separately for paternal and maternal transmissions ($r^2 = .61$, $P \ll .001$ in paternal transmissions; $r^2 = .52$, $P < .001$ in maternal transmissions), and the slope of the regression line was significantly steeper (−.62) in the paternal transmissions than in the maternal transmissions (−.30) ($t = 7.08$, $\nu = 91$, $P \ll .001$) (fig. 1). The average ratio of the contraction size and the parental size was significantly greater in the paternal transmissions (mean ± SD = .457 ± .251, $n = 76$) than in the maternal transmissions (mean ± SD = .344 ± .145, $n = 19$) ($t = 1.875$, $\nu = 93$, $P < .05$). The maternal repeat sizes (mean ± SD = 4.0 ± 1.8 kb, $n = 19$) were significantly larger than the paternal repeat sizes (2.5 ± 1.3 kb, $n = 76$) ($t = 4.13$, $\nu = 93$, $P < .001$). There was no difference between the mean degrees of intergenerational contraction in the maternal (mean ± SD = 1.3 ± 0.75 kb) and the paternal (mean ± SD = 1.2 ± 1.1 kb) transmissions.

We also examined the 95 parent-child pairs for occurrences of the CTG repeat contractions within siblings. In 16 sib sets, involving 35 parent-to-offspring transmissions, the CTG repeats contracted in multiple siblings (31 paternal transmissions and 4 maternal transmissions) (table 4). In the remaining 60 pairs, the CTG repeat contraction occurred as a single case within the family. The observed number of sib sets in which multiple sib members had the CTG repeat contraction was greater than the number that we expected. For the 1,489 DM offspring, there were 968 DM parents. Thus, the average number of DM offspring born to a DM parent in our series was 1,489/968, or 1.54. Of the 968 DM parents, 595 had only one DM offspring, while 267, 78, 17, 8, and 3 DM parents had two, three, four, five, and six DM offspring, respectively. The probability that two of the two DM offspring of each of the 267 DM parents would have a reduced size of the CTG repeat is $.064^2$. Thus, $.064^2 \times 267$, or 1.09, sib sets are expected to show both siblings with a reduced CTG repeat size. The expected number of sib sets in which at least two of the three DM siblings have a shorter CTG repeat is $[(.064^2 \times {}_3C_2) + (.064^3 \times {}_3C_3)] \times 78$, or .98. Likewise, the expected numbers of sib sets in which multiple siblings show a CTG repeat shorter than that in the parent are 0.44, 0.35, and 0.20 for sib sets with four, five, and six DM siblings, respectively. Thus, 1.09 + 0.98 + 0.44 + 0.35 + 0.20, or 3.06, sib sets were expected to have multiple siblings with a reduced number of CTG repeat in our DM study population. Even if we deliberately overestimate the number to be 5.0 instead of 3.06, the 16 sib sets observed in our study are more than the expected number (χ^2 goodness of fit = 24.6, df = 1, $P \ll .001$).

Discussion

The increasing size of the CTG repeats in successive generations is considered to be the molecular basis for anticipation in DM (Harper et al. 1992). Clinically, anticipation has been a strikingly consistent phenomenon in a large number of DM families (Höweler et al. 1989; Ashizawa et al. 1992*b*). However, the CTG repeat size does not always increase in successive generations of DM families (Ashizawa et al. 1992*a*; Brunner et al. 1993*b*; Hunter et al. 1993; Lavedan et al. 1993*a*, 1993*b*; Mulley et al. 1993; O'Hoy et al. 1993; Redman et al. 1993; Shelbourne et al. 1993). Characteristics of these

Table 3

Cases in Which CTG Repeat Contraction Accompanied Earlier Onset in the Offspring Compared with the Parent

Case	Reporting Center	Sex (parent/child)	Size (kb) and Status of CTG Repeat		Age at Onset[a] (parent/child)
			Parent/Child[b]	Overlap[c]	
1	Barcelona	M/M	3.0/2.0	Absent	20/11
2	Barcelona	F/F	3.5/2.5	Absent	25/9
3	Barcelona	M/F	2.5/2.0	Absent	23/18
4	Barcelona	M/M	2.5/1.5	Absent	23/9
5	Barcelona	F/M	7.0/4.0	Absent	12/0
6	Cardiff	M/M	1.6/1.2	Present	20–30/13
7	Cardiff	M/M	4.6/3.3	Present	37/21
8	Cardiff	M/M	1.0/0.5	Absent	40/22
9	Cardiff	M/F	3.0/2.2	Present	20–30/10–20
10	Durham, NC	M/F	2.0/0[d]	Absent	Adult/teens
11	Durham, NC	M/M	1.6/1.1	Present	Adult/teens
12	Durham, NC	M/F	1.6/0[d]	Absent	Adult/childhood
13	Durham, NC	M/F	1.4/1.2	Present	Adult/teens
14	Durham, NC	F/F	3.5/3.1	Absent	Middle age/early adult
15	Durham, NC	F/M	5.3/3.5	Present	Adult/childhood
16	Durham, NC	F/F	5.3/4.4	Present	Adult/childhood
17	Houston	M/F	1.4/0.8	Absent	50/teens
18	Houston	M/F	1.4/0.8	Absent	Asymptomatic/adult
19	Houston	F/F	5.2/4.5	Absent	Adult/juvenile
20	Marburg, Germany	F/F	E3/E2	Absent	43/28
21	Marburg, Germany	F/F	E3/E2	Absent	35/22
22	Marburg, Germany	F/M	E3/E2	Present	25/0
23	Marburg, Germany	F/M	E3/E2	Present	27/6
24	Marburg, Germany	M/M	E3/E2	Absent	20/14
25	Nijmegen	M/F	2.0/1.5	Present	37/14
26	Nijmegen	M/M	2.0/1.5	Present	37/12
27	Rome	F/M	6.0/4.2	Absent	20/12

[a] Numbers are years; "0" denotes congenital DM.
[b] E3 and E2 are the classes of CTG repeat size corresponding to 3.0 < 4.5 kb and 1.5 < 3.0 kb, respectively (Tsilfidis et al. 1992).
[c] In smear sizes of child and parent.
[d] No detectable expansion on a Southern blot (PCR data not available), but patient was symptomatic for DM.

cases have not been systematically studied. Our study showed a CTG repeat contraction in 6.4% of 1,489 DM offspring. Approximately one half of these cases showed clinical anticipation despite the reduced CTG repeat size in the offspring. The most striking examples were the two cases in which anticipation resulted in congenital DM in the offspring with contractions of the CTG repeat. We did not observe a single case in which the age at onset of DM in the symptomatic offspring was later than the age at onset in the parent, although Harley et al. (1993) recently reported three such cases. A few exceptional cases are worth mentioning, however. In 3 of the 95 cases that have been reported elsewhere (Brunner et al. 1993*b*; O'Hoy et al. 1993), the expanded paternal CTG repeat reverted to the normal range in the offspring, and the offspring were asymptomatic at or beyond the age at disease onset in the parent. Shelbourne et al. (1992) reported another case in which there was a contraction of the DM allele back to the normal range, and they contended that such cases explain the nonpenetrance in this disorder. One of these cases showed evidence of gene conversion (O'Hoy et al. 1993).

We expected that the CTG repeat contraction would accompany a later onset of the disease in the offspring, compared with that in the parent. The mechanism that accounts for the unexpectedly frequent anticipation and for the lack of its counterpart in our cases remains

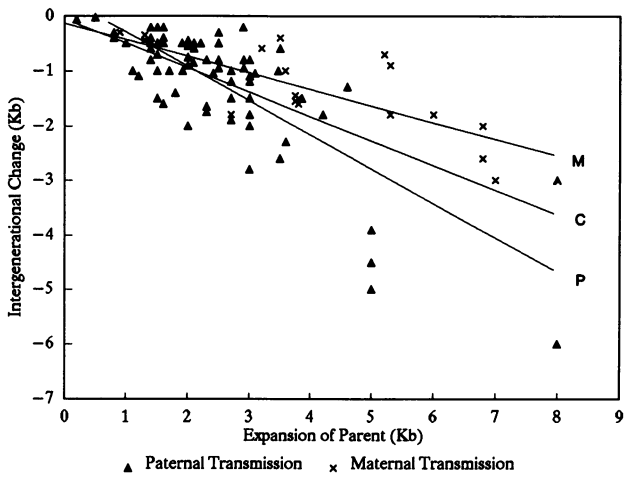

Figure 1 Relationship between the parental CTG repeat size and the size of CTG repeat contractions. The intergenerational contraction of the CTG repeat size showed significant correlation with the parental CTG repeat size (regression line "C"; $r^2 = .50$, $P \ll .001$, $n = 95$). When the correlations in paternal and maternal transmissions were separately analyzed, there was a significant correlation between the parental CTG repeat size and the size of the contraction, both in paternal transmissions (regression line "P"; slope = −.62, $r^2 = .61$, $P \ll .001$, $n = 76$) and in maternal transmissions (regression line "M"; slope = −.30, $r^2 = .52$, $P \ll .001$, $n = 19$). The slope of the regression line in paternal transmissions was steeper than that in maternal transmissions ($t = 7.08$, $P < .001$).

to be elucidated. However, we speculate that ascertainment biases and somatic mosaicism of the CTG repeat size may play an important role. Biases involved in ascertainment of the age at onset may lead to a false identification of anticipation. If the parent is not known to be a DM heterozygote at the time of symptom development but the offspring is known to be a possible mutation carrier, medical and family vigilance may result in earlier diagnosis in the offspring. An ascertainment bias may also explain the paucity of cases in which the offspring has a later age at onset than does the parent. DM patients who produce offspring tend to have a relatively late onset, with mild disease, because severely affected DM patients with an early onset generally have a shortened life span and decreased genetic fitness (Penrose 1948; Harper 1989). As a result, the age of the offspring is frequently less than the parent's age at onset, in a given study. In our series, we had 26 such cases, in which the offspring still had a potential to develop DM symptoms after reaching the parent's age at onset. Thus, ascertainment biases can explain some features of anticipation in association with the CTG repeat contraction. However, ascertainment bias cannot resolve why anticipation occurred more frequently in maternal transmissions than in paternal transmissions, among our cases with the repeat contraction. Furthermore, the observed anticipation was rather striking in many of our cases, despite the fact that the repeat contraction prompted the investigators to rigorously look for evidence of later onset in the offspring. Thus, although the ascertainment bias exists in our study, it is unlikely to totally account for the observed anticipation.

Somatic mosaicism of the CTG repeat size may also explain the frequent occurrences of anticipation and a paucity of documented cases of its counterpart in the intergenerational contractions of the CTG repeat. In virtually all studies, including this study, the age at onset was correlated with the CTG repeat size in peripheral blood leukocytes. However, the size of CTG repeat differs between the affected tissues and peripheral blood leukocytes (Anvret et al. 1993; Ashizawa et al. 1993; Lavedan et al. 1993*b*). Thus, the CTG repeat size in affected tissues may increase while the repeat size in leukocytes decreases. Comparisons of the CTG repeat sizes in affected tissues of the parent-child pairs that show the intergenerational repeat contraction in leukocytes will be critical, since, if this is not the case and clinical information is accurate, one must postulate the presence of factors other than the CTG repeat size that are sufficient, regardless of the CTG repeat size, to cause anticipation in DM. For example, in the DNA structures there may be changes associated with the transmission of the expanded CTG repeat that are cumulative in successive generations, and such structural changes may occur independent of changes in the CTG repeat size.

Among the cases with the CTG repeat contraction observed in leukocyte DNA, anticipation occurred more frequently in maternal than in paternal transmissions, suggesting that some maternal factor was involved in the pathophysiology of anticipation. Such a maternal factor may selectively promote an increase of the CTG repeat size in the skeletal muscle DNA of the offspring while the repeat size in the leukocyte DNA decreases. Alternatively, it may accelerate the disease process and make the age at onset earlier, independent of the degree of the CTG repeat expansion. The presence of such a maternal factor has been postulated in the pathogenic mechanism of congenital DM (Koch et al. 1991).

The sex of the affected parent plays an important

Table 4

CTG Repeat Contractions within DM Sibling

Sib Set	Reporting Center	Cases of DM Sibling with CTG Repeat Contractions[a]
1	Barcelona	Father (2.7 kb) to 1 son (1.2 kb) and 1 daugther (.8 kb)
2	Barcelona	Father (2.0 kb) to 3 sons (1.5, 1.2, and 1.2 kb) and 1 daughter (1.5 kb)
3	Barcelona	Father (3.0 kb) to 1 son (1.8 kb) and 1 daughter (2.0 kb)
4	Barcelona	Father (3.0 kb) to 2 daughters (1.8 and 2.0 kb)
5	Cardiff	Mother (6.8 kb) to 1 son (4.3 kb) and 1 daughter (4.8 kb)
6	Denver	Mother (1.7 kb) to 1 son (1.3 kb) and 1 daughter (1.3 kb)
7	Durham, NC	Father (8.0 kb) to 1 son (5.0 kb) and 1 daughter (2.0 kb)
8	Durham, NC	Father (1.6 kb) to 1 son (1.1 kb) and 1 daughter (0 kb[b])
9	Durham, NC	Father (5.3 kb) to 1 son (3.5 kb) and 1 daughter (4.4 kb)
10	Houston	Father (1.4 kb) to 2 sons (1.0 and .6 kb)
11	Nijmegen	Father (2.0 kb) to 1 son (1.5 kb) and 1 daughter (1.5 kb)
12	Nijmegen	Father (1.4 kb) to 2 sons (0.6 and .9 kb)
13	Ottawa	Father (1.5 kb) to 1 son (1.3 kb) and 1 daughter (1.0 kb)
14	Paris	Father (3.5 kb) to 2 daughters (2.9 and .9 kb)
15	Paris	Father (3.0 kb) to 1 son (1.9 kb) and 1 daughter (2.0 kb)
16	Stockholm	Father (5.2 kb) to 3 sons (.7, .7, and 1.3 kb)

NOTE.—There were 31 paternal and 4 maternal transmissions of the CTG repeats to sibling that had repeat-size contraction.

[a] Numbers in parentheses are the size of the CTG repeat in each patient.

[b] No detectable expansion on a Southern blot (PCR data not available), but patient was symptomatic for DM.

role in the determination of the CTG repeat size in DM offspring. Congenital DM occurs mostly with maternal transmission of the mutation, generally accompanied by a large intergenerational increase of the CTG repeat size (Tsilfidis et al. 1992; Redman et al. 1993). The size of the parental CTG repeat shows an inverse correlation with the intergenerational increase of the CTG repeat size in paternal, but not in maternal, transmission (Cobo et al. 1993; Lavedan et al. 1993*b;* Mulley et al. 1993; Ashizawa et al. 1994). As a result, the CTG repeat size is seldom >1,000 repeats in paternal transmissions (Ashizawa et al. 1994). The lack of congenital DM in paternal transmissions has been attributed to this limited expansion of the CTG repeat (Lavedan et al. 1993*a;* Mulley et al. 1993). However, males do sometimes pass on expansions that, if they were transmitted through the maternal line, would probably have led to expression of congenital DM in the child. This is further complicated by recent reports of congenital DM with a large CTG repeat of paternal origin (Fischbeck et al. 1993; Nakagawa et al., in press) and by the observations that a large expansion of a maternally transmitted CTG repeat does not always result in congenital DM (Tsilfidis et al. 1992; Abeliovich et al. 1993; Lavedan et al. 1993*b;* Novelli et al. 1993*a;* Redman et al. 1993). Nevertheless, the limited expansion of the CTG repeat in paternal transmission appears to contribute to the paucity of congenital DM cases in paternal transmissions. In our series, the intergenerational contraction of the CTG repeat occurred more frequently with paternal transmissions than with maternal transmissions. Additionally, the size of the contraction correlated with the parental repeat size, and the slope of the regression line was significantly steeper in paternal than in maternal transmissions. When the data of paternal and maternal transmissions were calculated separately, the correlation coefficient of each was greater than the overall correlation coefficient. Thus, the paternal and maternal transmissions appear to have distinct influences on the intergenerational changes of the CTG repeat size in DM. The mechanism of these parental origin effects is unknown. One speculation is that the limited expansion of the CTG repeat size around 1,000 repeats may be due to a relatively stable DNA structure at this size range. This may explain the tendency of the CTG repeat that is <1,000 repeats to expand while the repeats larger than this limit tend to contract. There may be an additional destabilizing maternal factor that allows the

CTG repeats to further expand, increasing the probability of congenital DM in the offspring. Alternatively, there may be a paternal factor that restricts the expansion and prevents the occurrence of congenital DM. Although genomic imprinting is one of the mechanisms that could explain the effect of the sex of the affected parent in DM, methylation patterns do not differ in offspring of maternal and paternal transmissions (Shaw et al. 1993; Ashizawa et al. 1994), and the DM kinase mRNA of paternal and maternal origins were equally expressed (Jansen et al. 1993).

In our patients, the mean size of the maternal CTG repeat was significantly larger than that of the paternal repeat. However, this may be due to a referral bias. Diagnostic laboratories often receive congenital DM cases in which most mothers have a relatively severe disease. In contrast, collections of DM pedigrees for research purposes tend to include an excess of asymptomatic or mildly affected grandfathers (Harper 1989; Brunner et al. 1993*a*; Harley et al. 1993). These biases, however, do not explain the predominant occurrences of the CTG repeat contractions in paternal transmissions.

Our results showed that the cases with the CTG repeat contraction clustered within the sib sets more frequently than expected. The mechanism of this phenomenon is also unknown. Further investigations of clinical and molecular characteristics unique to these siblings may provide clues to the genetic or environmental mechanisms.

We conclude that (1) the paternal origin of the repeat and the presence of the repeat contraction in a sibling increase the probability of the CTG repeat contraction and (2) apparent anticipation frequently occurs despite contractions of the CTG repeat in leukocyte DNA especially in maternal transmission of DM, while ascertainment bias contributes to this observation. These data have important clinical implications, especially in genetic counseling.

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References

Abeliovich D, Lerer I, Pashut-Lavon I, Shmueli E, Raas-Rothschild A, Frydman M (1993) Negative expansion of the myotonic dystrophy unstable sequence. Am J Hum Genet 52:1175–1181

Anvret M, Åhlberg G, Grandell U, Hedberg B, Johnson K, Edström L (1993) Larger expansions of the CTG repeat in muscle compared to lymphocytes from patients with myotonic dystrophy. Hum Mol Genet 2:1397–1400

Ashizawa T, Dubel JR, Dunne PW, Dunne CJ, Fu Y-H, Pizzuti A, Caskey CT, et al (1992*a*) Anticipation in myotonic dystrophy. II. Complex relationships between clinical findings and structure of the GCT repeat. Neurology 42:1877–1883

Ashizawa T, Dubel JR, Harati Y (1993) Somatic instability of CTG repeat in myotonic dystrophy. Neurology 43:2674–2678

Ashizawa T, Dunne CJ, Dubel JR, Perryman MB, Epstein HF, Boerwinkle E, Hejtmancik JF (1992b) Anticipation in myotonic dystrophy. I. Statistical verification based on clinical and haplotype findings. Neurology 42:1871–1877

Ashizawa T, Dunne PW, Ward PA, Seltzer WK, Richards CS (1994) Effects of the sex of myotonic dystrophy patients on the unstable triplet repeat in their affected offspring. Neurology 44:120–122

Aslanidis C, Jansen G, Amemiya C, Shutler G, Tsilfidis C, Mahadevan M, Chen C, et al (1992) Cloning of the essential myotonic dystrophy region: mapping of the putative defect. Nature 355:548–551

Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburatani H, Hunter K, et al (1992) 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 68:799–808

Brunner HG, Brüggenwirth HT, Nillesen W, Jansen G, Hamel BCJ, Hoppe RLE, de Die CEM, et al (1993*a*) Influence of sex of the transmitting parent as well as of parental allele size on the CTG expansion in myotonic dystrophy (DM). Am J Hum Genet 53:1016–1023

Brunner HG, Jansen G, Nillesen W, Nelen MR, de Die CEM, Höweler CJ, van Oost BA, et al (1993*b*) Brief report: reverse mutation in myotonic dystrophy. N Engl J Med 328:476–480

Brunner HG, Nillesen W, van Oost BA, Jansen G, Wieringa B, Ropers H-H, Smeets HJM (1992) Presymptomatic diagnosis of myotonic dystrophy. J Med Genet 29:780–784

Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, Aslanidis C, de Jong P, et al (1992) Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. Nature 355:547–548

Cobo AM, Baiget M, López de Munain A, Poza JJ, Emparanza JI, Johnson K (1993) Sex-related difference in intergenerational expansion of myotonic dystrophy gene. Lancet 341:1159–1160

Fischbeck KH, Bergoffen J, Kant J, Sladky J, McDonald-McGinn D, Zackal EH (1993) Paternal transmission of congenital myotonic dystrophy. Am J Hum Genet Suppl 53:A1157

Fu Y-H, Friedman DL, Richards S, Pearlman JA, Gibbs RA, Pizzuti A, Ashizawa T, et al (1993) Decreased expression of myotonin-protein kinase mRNA and protein in adult form of myotonic dystrophy. Science 260:235–238

Fu Y-H, Pizzuti A, Fenwick RG, King J, Rajnarayan S, Dunne PW, Dubel J, et al (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255:1256–1258

Harley HG, Brook JD, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, et al (1992*a*) Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. Nature 355:545–546

Harley HG, Rundle SA, MacMillan JC, Myring J, Brook JD, Crow S, Reardon W, et al (1993) Size of the unstable CTG repeat sequence in relation to phenotype and parental transmission in myotonic dystrophy. Am J Hum Genet 52:1164–1174

Harley HG, Rundle SA, Reardon W, Myring J, Crow S, Brook JD, Harper PS, et al (1992*b*) Unstable DNA sequence in myotonic dystrophy. Lancet 339:1125–1128

Harper PS (1989) Myotonic dystrophy, 2d ed. WB Saunders, London

Harper PS, Harley HG, Reardon W, Shaw DJ (1992) Anticipation in myotonic dystrophy: new light on an old problem. Am J Hum Genet 51:10–16

Hofmann-Radvanyi H, Lavedan C, Rabès J-P, Savoy D, Duros C, Johnson K, Junien C (1993) Myotonic dystrophy: absence of CTG enlarged transcript in congenital forms, and low expression of the normal allele. Hum Mol Genet 2:1263–1266

Höweler CJ, Busch HFM, Geraedts JPM, Niermeijer MF, Staal A (1989) Anticipation in myotonic dystrophy: fact or fiction. Brain 112:779–797

Hunter AGW, Jacob P, O'Hoy K, MacDonald I, Mettler G, Tsilfidis C, Korneluk RG (1993) Decrease in the size of the myotonic dystrophy CTG repeat during transmission from parent to child: implication for genetic counselling and genetic anticipation. Am J Med Genet 45:401–407

Hunter A, Tsilfidis C, Mettler G, Jacob P, Mahadevan M, Surh L, Korneluk R (1992) The correlation of age of onset with CTG trinucleotide repeat amplification in myotonic dystrophy. J Med Genet 29:774–779

Jansen G, Bartolomei M, Kalscherer V, Merkx G, Wormskamp N, Mariman E, Smeets D, et al (1993) No imprinting involved in the expression of DM-kinase mRNAs in mouse and human tissues. Hum Mol Genet 2:1221–1228

Koch MC, Grimm T, Harley HG, Harper PS (1991) Genetic risks for children of women with myotonic dystrophy. Am J Hum Genet 48:1084–1091

Krahe R, Ashizawa T, Dubel J, Gilbert J, Taylor H, Roses A, Siciliano MJ (1993) Evaluation of normal and mutant allele transcript levels of the myotonic dystrophy protein kinase gene in affected tissues of myotonic dystrophy patients. Am J Hum Genet Suppl 53:A1186

Lavedan C, Hofmann-Radvanyi H, Rabes JP, Roume J, Junien C (1993*a*) Different sex-dependent constraints in CTG length variation as explanation for congenital myotonic dystrophy. Lancet 341:237

Lavedan C, Hofmann-Radvanyi H, Shelbourne P, Rabes J-P, Duros C, Savoy D, Dehaupas I, et al (1993*b*) Myotonic dystrophy: size- and sex-dependent dynamics of CTG meiotic instability, and somatic mosaicism. Am J Hum Genet 52:875–883

Mahadevan M, Tsilfidis C, Sabourin L, Shotler G, Amemiya C, Jansen G, Neville C, et al (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. Science 255:1253–1255

Mulley JC, Staples A, Donnelly A, Gedeon AK, Hecht BK, Nicholson GA, Haan EA, et al (1993) Explanation for exclusive maternal origin for congenital form of myotonic dystrophy. Lancet 341:236–237

Nakagawa M, Yamada H, Higuchi I, Kaminishi Y, Miki T, Johnson K, Osame M. A case of paternally inherited congenital myotonic dystrophy. J Med Genet (in press)

Novelli G, Gennarelli M, Menegazzo E, Mostacciuolo ML, Pizzuti A, Fattorini C, Tessarolo D, et al (1993*a*) (CTG)n triplet mutation and phenotype manifestations in myotonic dystrophy patients. Biochem Med Metabol Biol 50:85–92

Novelli G, Gennarelli M, Zelano G, Pizzuti A, Fattorini C, Caskey CT, Dallapiccola B (1993*b*) Failure in detecting mRNA transcripts from the mutated allele in myotonic dystrophy muscle. Biochem Mol Biol Int 29:291–297

O'Hoy KL, Tsilfidis C, Mahadevan MS, Neville CE, Barceló J, Hunter AGW, Korneluk RG (1993) Reduction in size of the myotonic dystrophy trinucleotide repeat mutation during transmission. Science 259:809–812

Penrose LS (1948) The problem of anticipation in pedigrees of dystrophia myotonica. Ann Eugenics 14:125–232

Redman JB, Fenwick RG, Fu Y-H, Pizzuti A, Caskey CT (1993) Relationship between parental trinucleotide GCT repeat length and severity of myotonic dystrophy in offspring. JAMA 269:1960–1965

Sabouri LA, Mahadevan MS, Narang M, Lee DSC, Surh LC, Korneluk RG (1993) Effect of the myotonic dystrophy (DM) mutation on mRNA levels of the DM gene. Nature Genet 4:233–238

Shaw DJ, Chaudhary S, Rundle SA, Crow S, Brook JD,

Harper PS, Harley HG (1993) A study of DNA methylation in myotonic dystrophy. J Med Genet 30:189–192

Shelbourne P, Davies J, Buxton J, Anvret M, Blennow E, Bonduelle M, Schmedding E, et al (1993) Direct diagnosis of myotonic dystrophy with a disease-specific DNA marker. N Engl J Med 328:471–475

Shelbourne P, Winqvist R, Kunert E, Davies J, Leisti J, Thiele H, Bachmann H, et al (1992) Unstable DNA may be responsible for the incomplete penetrance of the myotonic dystrophy phenotype. Hum Mol Genet 1:467–473

Tsilfidis C, MacKenzie AE, Mettler G, Barceló J, Korneluk RG (1992) Correlation between CTG trinucleotide repeat length and frequency of severe congenital myotonic dystrophy. Nature Genet 1:192–195