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Negative Expansion of the Myotonic Dystrophy Unstable Sequence

Dvorah Abeliovich,* Israela Lerer,* Iris Pashut-Lavon,* Esther Shmueli,*
Annick Raas-Rothschild,† and Moshe Frydman†

*Department of Human Genetics, Hadassah University Hospital, Jerusalem; and †Institute of Genetics, Sheba Medical Center, Tel Hashomer, Israel

Summary

We have analyzed the unstable fragment of the myotonic dystrophy (DM) gene in a pregnancy at 50% risk for DM. The affected father in this family had a 3.0-kb expansion of the DM unstable region. The fetus inherited the mutated gene, but with an expansion of 0.5 kb. This case represented a counseling problem in light of the absence of data concerning "negative expansion." Analysis of the DM gene in 17 families with 72 affected individuals revealed four more cases of negative expansions, all of them in paternal transmissions. The possible significance of this finding is discussed.

Introduction

Myotonic dystrophy (DM) is an autosomal dominant disorder, the most common of adult-type muscular dystrophies (Harper 1989). DM is a multisystem disorder with marked variability of expression, in both age at onset and clinical severity. Anticipation—namely, increased severity through the generations—is a unique feature of DM.

The DM gene was recently cloned, and an unstable region of the gene was detected in DM patients (Aslanidis et al. 1992; Buxton et al. 1992; Harley et al. 1992*a*). The unstable fragment was located at the 3′ end of the untranslated region of the gene, because of a variable number of the trinucleotide CTG repeat (Brook et al. 1992; Fu et al. 1992; Mahadevan et al. 1992). The CTG repeat number is expanded in DM while transmitted from parent to child, as well as in mitotic divisions resulting in somatic heterogeneity. An approximate correlation was demonstrated between the degree of expansion and clinical severity (Harley et al. 1992*b;* Hunter et al. 1992; Suthers et al. 1992; Tsilfidis et al. 1992).

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Address for correspondence and reprints: Dvorah Abeliovich, Department of Human Genetics, Hadassah University Hospital, Ein Kerem, Jerusalem 91120, Israel.
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Detection of the unstable fragment provides a direct tool for the diagnosis of DM carriers, regardless of their clinical status and family history. We have been using this approach in current diagnoses of DM and have analyzed all the DM patients who were previously analyzed by linkage of RFLPs closely linked to the DM locus. Our findings are presented.

Material and Methods

DNA extraction from whole-blood samples, genomic digests, and Southern blots were performed according to standard techniques. Linkage analysis was performed using DNA probes which are listed in table 1. The unstable region of the DM gene was analyzed by Southern hybridization with the cDNA probe CA25 (Buxton et al. 1992) and the DNA probe pM10M-6 (Brook et al. 1992). The fragment sizes were measured from *Hin*dIII- or *Eco*RI-digested DNA; in these conditions most of the bands were distinct. In cases where smear was detected, the range of the fragment size is given. Small-size fragments were measured from *Bam*HI-restricted DNA. Amplification of the CTG repeat region was done according to the method of Mahadevan et al. (1992) using primers 406 and 409. The PCR products were electrophoresed in 4% 3:1 NuSieve agarose gel, stained by ethidium bromide, and visualized under UV light.

Fibroblast cultures of the abortus were established in

Table 1

RFLPs Associated with the DM Locus

Locus	RFLPs	Reference
BCL3	α1.4P/*Ban*I	Korneluk et al. 1989
APO CII	*Apo*CII/*Ban*I	Frossard et al. 1985
APO CII	*Apo*CII/*Ava*II	Korneluk et al. 1987
CKMM	CKM/*Taq*I	Perryman et al. 1988
CKMM	CKM/*Nco*I	Perryman et al. 1988
D19S63	pD10/*Pst*I	Brook et al. 1990
DM	CA25/*Eco*RI or *Hin*dIII	Buxton et al. 1992

F10 medium with 20% FCS. After the formation of a monolayer in the primary culture flask, 10 passages (dilution 1 in 3 each) were performed. The fibroblasts were collected, and the DNA was extracted using the phenol:chloroform method.

Case Report

Family CD (fig. 1) was brought to our attention after the birth of a baby girl (III-I) who suffered from symptoms which suggested the diagnosis of congenital DM (CDM). The baby was born at term by cesarean section, because of footling presentation. Apgar score was 8 at 1 min and 9 at 5 min. Severe hypotonia, narrow palpebral fissure, limited-opening mouth, and retrognathia with hypotonic facies were noted. She had feeding problems in her first weeks of life. Ophthalmology and cardiac evaluations were normal. The baby was born to a 31-year-old mother who suffered from 5 years of infertility, and the present pregnancy was induced by pergonal and chorigen. The mother (II-2) was found to have clinical myotonia and characteristic EMG findings; her brother (II-3) at the age of 28 years had clinical myotonia. The grandparents (I-1 and I-2) had no clinical signs of myotonia, but the grandfather (I-2) and his mother (not shown in the pedigree) had surgery for bilateral cataract. The sister-in-law (II-4) was pregnant and asked for first-trimester prenatal diagnosis by chorionic villus sampling (CVS).

Results

DNA Analysis

The first DNA analysis of this family was performed before the cloning of the DM gene. Linkage analysis (fig. 1) revealed that the mother and her brother (II-2 and II-3) shared the same paternal haplotype but had received different maternal haplotypes, indicating that it was their father (I-2) who transmitted the DM gene to both his children. Analysis of the unstable region of the gene confirmed this conclusion (fig. 2). The expansion size in the grandfather (I-2) was 250 bp. Individual II-2 had an expansion of 2.5 kb, and II-3 had an expansion of 3.0 kb. The baby (III-1) had an expansion of 5.0 kb. The fetus, in the CVS analysis, inherited the DM-bearing chromosome, as was demonstrated by the RFLP CKM/*Nco*I. The size of the expansion mutation in the chorionic villi was 0.5 kb, 2.5 kb smaller than that of his father. The couple decided to terminate the pregnancy, and fetal tissues were available for confirmation of the diagnosis. The expansion size was found to be 0.5 kb in fetal brain, lung, and muscle. In the cultured fibroblasts after about 40 divisions, the size of the unstable fragment was 0.6 kb (fig. 2*D*).

The DM Unstable Region in Family Studies

We have since analyzed 72 individuals, affected or carriers, of 17 DM families. In all of them the DM expansion mutation was associated with the disease. The clinical status of the DM patients was classified according to the method of Harley et al. (1992*b*). In figure 3 the correlation between the fragment size and

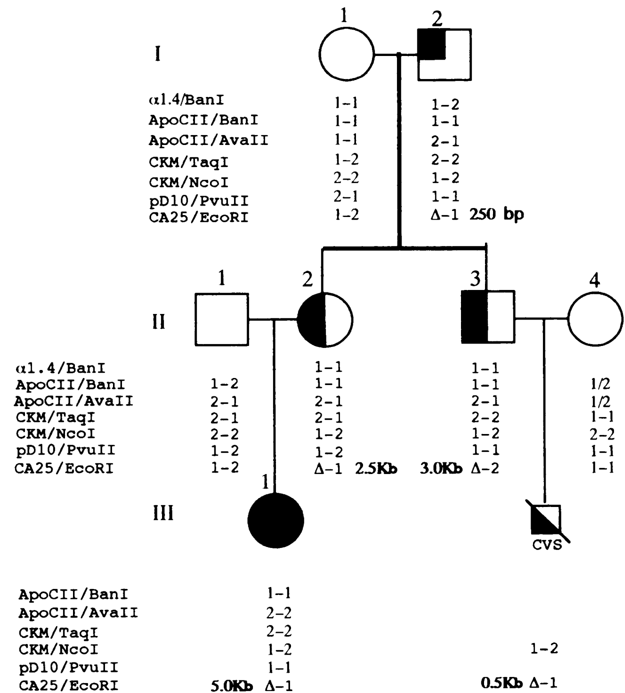

Figure 1 Pedigree of family CD. ◧ = mildly affected; ◐ and ◧ = classic DM; ● = CDM; and ◪ = DM carrier with unknown clinical status.

the clinical status is presented. The mean fragment size of the unstable fragment was in direct correlation with the clinical severity, but overlapping between the various groups exists. In this sample of families, there were 15 mothers who transmitted their DM gene to 23 offspring, and there were 15 fathers who transmitted the DM gene to 30 offspring. Among the 23 maternal offspring there were 8 with congenital DM, 8 with early-childhood-onset DM, 2 with classic DM, and 4 who were carriers yet asymptomatic. One case was a fetus diagnosed prenatally. The majority (17/27) of offspring of affected fathers had classic DM, 3 had the mild disease, 7 were young carriers yet asymptomatic, and 3 additional cases were fetuses diagnosed prenatally; none of the paternal offspring had severe disease. The sizes of the unstable fragments in the parents and in their offspring are given in tables 2 and 3. Cases with overlapping fragment sizes between parent and child were not included in our analysis. In all the maternal transmissions the unstable fragment increased in size; in the paternal transmissions the unstable fragment decreased in size in five cases and did not change (zero expansion) in three cases. Two of the cases with reduced fragment size were in fetuses diagnosed by CVS; the other three cases were found in adults (two brothers in family 5 and one individual in family 11) who had the same clinical status as did their fathers.

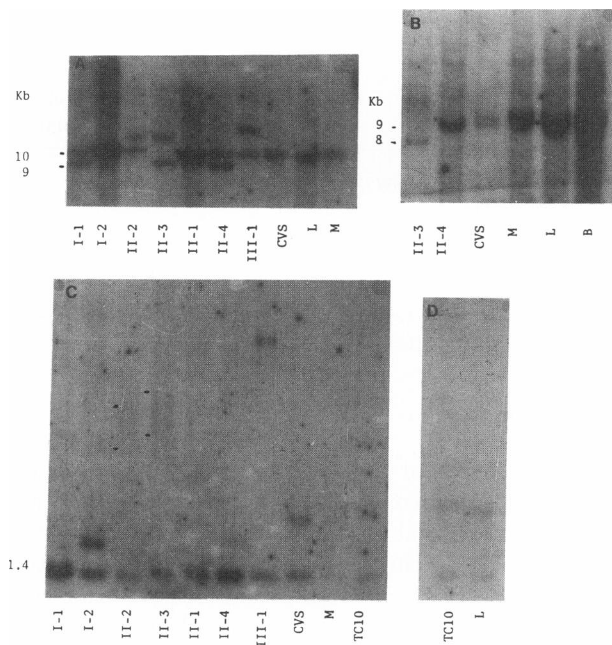

Figure 2 Southern blot analysis of members of family CD. Pedigree symbols are according to the pedigree in fig. 1. TC10 = tissue culture at passage 10; and M, L, and B = fetal tissues from muscle, lung, and brain, respectively. Genomic DNA was digested with *Eco*RI (*A*), *Hin*dIII (*B*), and *Bam*HI (*C* and *D*). Hybridization was performed with the DNA probe pM10-6.

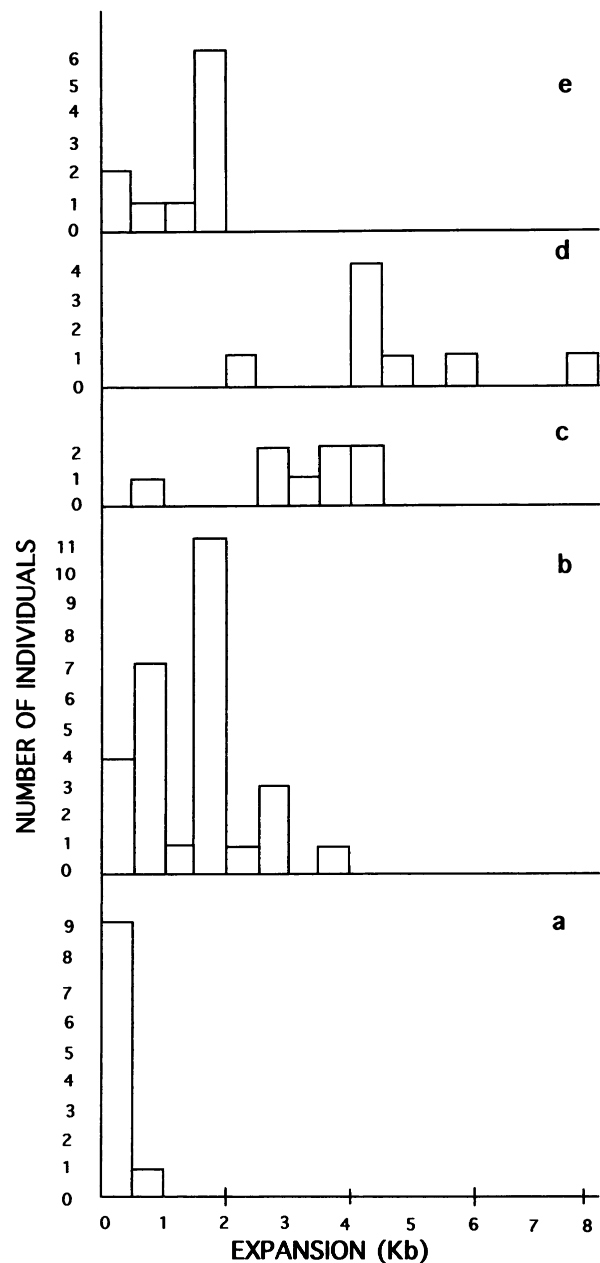

Figure 3 Size of the unstable fragments in the various clinical groups: mild disease (*a*), classic DM (*b*), early-childhood onset (*c*), congenital DM (*d*), and young carriers without symptoms (*e*).

Table 2

Maternal Transmissions of DM Unstable Fragment

	Mother		Offspring			
Family	Clinical Status[a]	Fragment Size (kb)	Sex	Clinical Status[a]	Fragment Size (kb)	Expansion (kb)
1	1	.2	M	2	.6	.4
	2	1.5	F	3	4.5	3.0
			F	3	4.5	3.0
	2	2.0	M	CVS	3.5	1.5
2	2	2.0	M	4	6.0	4.0
			M	4	4.5	2.5
6	1	.1	M	2	.4	.4
			F	3	1.0	1.0
7	2	3.0	M	3	4.0	1.0
	2	2.0	F	3	4.0	2.0
			M	3	3.0	1.0
9	2	4.0	M	4	8.0	4.0
10	2	2.5	F	4	5.0	2.5
11	2	2.0	M	4	4.0	2.0
12	2	2.0	F	4	4.0	2.0
13	2	2.0	M	3	3.5	1.5
15	2	1.0	M	5	2.0	1.0
			M	5	2.0	1.0
16	1	.2	M	4	4.0	3.8
			F	3	3.0	2.8
			M	5	2.0	1.8
			M	5	2.0	1.8
17	1	1.0	M	4	2.5	1.5
Mean		1.69			3.39	1.98
Range		.1–4.0			.4–8.0	.4–4.0

[a] 1 = Mild disease; 2 = classic DM; 3 = early-childhood DM; 4 = congenital DM; and 5 = young carriers without symptoms.

Among the cases with zero expansions, one individual (family 5) was born to a mildly affected father, and he presented with a cataract (mild disease) at the age of 40 years. Another individual was born to a classic DM father (family 1); she was still asymptomatic at the age of 20 years. The third case (family 4) was a fetus diagnosed at amniocentesis.

Mitotic Instability of the Expansion Mutation

Somatic heterogeneity of the unstable fragment was noted in most of the patients and could be best illustrated in Southern hybridization of *Bam*HI genomic digest and pM10-6 as the DNA probe (Brook et al. 1992). In this condition the size of the normal restricted fragment is 1.4 kb, and small variations in size were observed as a smear. Two classes of patients had relatively stable fragments (which appeared as a sharp band in the autoradiograms): adults with an expansion mutation of <500 bp (e.g., individual I-2 in fig. 2C) and babies or fetuses regardless of their fragment size (individual III-I in fig. 2C).

Discussion

The discovery of both the expansion mutation in DM and the approximate direct correlation between the size of the unstable fragment and the severity of the disease provides a clue to the anticipation phenomenon (Harper et al. 1992). It also provides a direct means for the diagnosis of gene carriers and could be used as a prognostic predictor. However, the overlapping between the fragment size and the various clinical groups may mislead the clinical interpretation. Another obstacle in the clinical interpretation of the molecular data is the cases who inherited the DM gene from their parents, but with reduced fragment size. In the prenatal

Table 3

Paternal Transmissions of DM Unstable Fragment

	Father		Offspring			
Family	Clinical Status[a]	Fragment Size (kb)	Sex	Clinical Status[a]	Fragment Size (kb)	Expansion[b] (kb)
1	2	.2	F	2	2.0	1.8
	2	.5	M	2	2.0	1.5
			F	5	.5	.0
3	1	.2	M	2	.8	.7
			M	2	.4	.2
			F	2	1.0	.8
	2	.8	F	5	2.0	1.2
			F	5	1.2	.4
			F	5	1.2	.4
4	1	.5	F	2	2.0	1.5
			M	2	2.0	1.5
			M	2	.5–2.0	?
	2	.5–2.0	F	5	1.0	?
				Amnio	.5	.0
5	1	.4	M	2	1.0	.6
			M	1	.4	.0
			M	1	.2	−.20
			M	1	.15	−.25
	1	.4	F	5	2.0	1.6
			M	5	.5	.1
			M	2	1.0	.6
	1	.2	F	2	.8	.6
7	2	.3	F	2	3.0	2.7
			F	2	2.0	1.9
8	2	2.0	F	CVS	1.0	−1.0
10	1	.25	F	2	2.5	2.25
			M	2	3.0	2.75
	2	3.0	M	CVS	.5	−2.5
11	2	3.0	F	2	2.0	−1.0
14	1	.2	F	2	2.0	1.8
Mean		0.85			1.33	.71
Range		.2–3.0			.15–3.0	−2.5–2.75

[a] As in table 2.
[b] Underlined nos. denote cases with negative expansion.

diagnosis which we described, we faced a counseling dilemma because of the absence of clinical data in similar cases. While delivering the results of the prenatal diagnosis to the parents, we had two main concerns: (*a*) to what extent the chorionic villi represent the fetus itself and (*b*) whether "negative expansion" means a better prognosis. The family, who did not want to take any risk, decided to terminate the pregnancy. We analyzed the abortus tissues (muscle, brain, and lung) and could confirm the finding in the chorionic villi. Since all tissues had a fragment of the same size, we concluded that the reduction in the fragment size is not a consequence of postzygotic, somatic instability, unless it took place in the very early stage of the embryo formation. It is possible that the 0.5-kb expansion mutation represented the mutation which existed in the paternal gonads and that the 3.0-kb unstable fragment which was identified in DNA sample of the father evolved somatically. Alternatively, it is possible that the same mechanism which brings about the expansion can work in the reverse manner, to reduce the fragment size, or else the negative expansion is a unique event. At present, we cannot discriminate between these alternatives. As to the prognosis of the fetus, we could a priori ex-

clude congenital DM, since the transmitting parent was the father (Koch et al. 1991), but we could not predict whether it would likely be better than that of the father. It should be mentioned here that in none of our families was an offspring with a milder disease than his parent brought to our attention.

Somatic instability of the DM unstable fragment of the fetus was demonstrated in cultured fibroblasts. After 10 passages the size of the unstable fragment increased from 0.5 kb to 0.6 kb. If the rate of expansion in vivo is similar to that in tissue culture, then the somatic instability could mask the initial size of the fragment in the zygote. Somatic heterogeneity in vivo could explain the overlapping in the fragment sizes of the different clinical groups, especially in adults with classical DM. This conclusion is based on the age and the size dependence of the somatic heterogeneity; fragments <500 bp tend to be homogeneous, whereas, in babies or fetuses, even fragments of 5 kb were homogeneous. Since most of the DNA analyses were performed on DNA samples which were isolated from lymphocytes, it might be that this tissue does not represent the affected organ.

In a combined analysis of our sample of families, we grouped the patients according to the transmitting parent. The mean fragment size of the fathers was 0.85 kb, and that of the mothers was 1.69 kb. This difference may be understood in terms of ascertainment bias toward mothers of severely affected offspring. The expansion size in paternal transmission reached a maximum of 3.0 kb; in maternal transmission the expansion size reached 8 kb. The most striking difference was the "negative expansion" which was found in five offspring (in addition to three offspring with zero expansion) among 30 paternal transmissions but in none of 23 maternal transmissions. The unstable fragment did not increase in size in any of the fetuses of paternal transmission; in the only fetus of maternal transmission the fragment size expanded from 2.0 kb to 3.5 kb. In this respect, our study is different from those of Harley et al. (1992*b*) and Tsifidis et al. (1992), although the latter studies analyzed larger samples of families. Recently, other laboratories have reported data in line with our observation—namely, negative expansion exclusively in association with paternal transmission (Shelbourne et al. 1992; Lavedan et al. 1993; Mulley et al. 1993). However, the clinical data are not yet sufficient to draw any conclusion concerning the consequences of this negative expansion.

Our observations regarding (*a*) the age and the size dependence of the somatic heterogeneity and (*b*) the negative or zero expansion in paternal transmission preferentially found in fetuses led us to propose the following hypothesis: the expansion of the DM unstable fragment in paternal transmission is mainly a somatic, postzygotic event, and the initial size of the unstable fragment in the zygote is limited either by (1) its size in the paternal gonads or, if meiotic expansion occurs in the male, (2) because there is a selective advantage for sperm with the shortest fragments. It is suggested that in maternal transmission the expansion is mainly a meiotic event which may be followed by postzygotic mitotic instability. The gender-dependent difference in the expansion may explain the maternal inheritance of CDM, by the presence of the fully developed expansion mutation in the very early stages of the embryonal development. However, the exclusivity of maternal inheritance of CDM may be a hint of additional factors which play a role in determining the clinical symptoms, such as genomic imprinting of the DM gene or related genes.

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