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De Novo Myotonic Dystrophy Mutation in a Nigerian Kindred

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Summary

An expansion of an unstable $(CTG)_n$ trinucleotide repeat in the 3′ UTR of a gene encoding a putative serine/threonine protein kinase (*DMPK*) on human chromosome 19q13.3 has been shown to be specific for the myotonic dystrophy (DM) disease phenotype. In addition, a single haplotype composed of nine alleles within and flanking *DMPK* over a physical distance of 30 kb has been shown to be in complete linkage disequilibrium with DM. This has led to two hypotheses: (1) predisposition for $(CTG)_n$ instability results from a founder effect that occurred only once or a few times in human evolution; and (2) elements within the disease haplotype may predispose the $(CTG)_n$ repeat to instability. A detailed haplotype analysis of the DM region was conducted on a Nigerian (Yoruba) DM family, the only indigenous sub-Saharan DM case reported to date. Each affected member of this family had an expanded $(CTG)_n$ repeat in one of his or her *DMPK* alleles. However, unlike all other DM populations studied thus far, disassociation of the $(CTG)_n$ repeat expansion from other alleles of the putative predisposing haplotype was found. We conclude that the expanded $(CTG)_n$ repeat in this family is the result of an independent mutational event. Consequently, the origin of DM is unlikely to be a single mutational event, and the hypothesis that a single ancestral haplotype predisposes to repeat expansion is not compelling.

Introduction

Myotonic dystrophy (DM) is a pleiotropic, autosomal dominant disease characterized primarily by myotonia and progressive dystrophy of skeletal muscles, cardiac conduction defects, and iridescent cortical lens opacity. The clinical phenotype is highly variable, ranging from a mild late onset to a severe, mostly maternally transmitted, congenital form with polyhydroamnio, neonatal hypotonia with respiratory distress, facial diplegia, and mental retardation. Genetically, however, DM is a homogeneous disease that maps to a single locus on chromosome 19q13.3 (Whitehead et al. 1982; Stallings et al. 1988; Harper 1989). DM also shows variable penetrance and the genetic feature of anticipation, defined as the increasingly earlier onset of a usually more severe clinical phenotype through successive generations (Ashizawa et al. 1992*a*, 1992*b*; Harper et al. 1992; Shelbourne et al. 1992). DM is the most prevalent inherited adult neuromuscular disease, with an incidence of ~1:8,000 individuals in western European and North American populations (Harper 1989) and 1:18,000 in Japanese (Davies et al. 1992). Prevalence is lower in Southeast Asians (Ashizawa and Epstein 1991), and only one family case among indigenous sub-Saharan black African populations has been reported (Dada 1973). Therefore, it has been postulated that the original mutation occurred in the northern Eurasian groups, around the time of their migration out of Africa, ~92,000 years ago (Ashizawa and Epstein 1991).

The molecular basis of DM has been identified as an unstable $(CTG)_n$ trinucleotide repeat in the 3′ UTR of a gene encoding a putative serine/threonine protein kinase, designated "DM protein kinase" (*DMPK*) (Brook et al. 1992; Fu et al. 1992; Mahadevan et al. 1992). This mutation has been demonstrated in >99% of the global DM populations from very different ethnic backgrounds in Europe, North and South America, Asia, and Australia (Harley et al. 1992*b*; Mahadevan et al. 1992; Yamagata et al. 1992; Mulley et al. 1993). The size of the unstable repeat generally increases through successive generations and correlates, to a reasonable degree, with the age at onset and the severity of the disease within a given family (Ashizawa et al. 1992*a*; Aslanidis et al. 1992; Buxton et al. 1992; Harley et al. 1992*a*, 1992*b*, 1993; Hunter et al. 1992; Tsilfidis et al. 1992; Barceló et al. 1993; Novelli et al. 1993).

Strikingly, in all the DM patients studied to date, the expanded $(CTG)_n$ repeat is always associated with the 1-kb "insertion" allele of a two-allele *Alu* repeat "insertion/deletion" polymorphism, located in intron 8 of the *DMPK* gene, 5.3 kb upstream from the expanded $(CTG)_n$ repeat (Harley et al. 1992*a*; Yamagata et al. 1992; Mahadevan et al. 1993*a*, 1993*b*). (Subsequently,

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this polymorphism will be referred to as the "Alu±1kb" polymorphism; the "insertion" allele will be designated "Alu+1kb," and the "deletion" allele will be designated "Alu−1kb.") Haplotype analysis of polymorphic sites within and flanking the *DMPK* locus, spanning a physical distance of ~30 kb, showed that the most commonly observed haplotype, designated "haplotype A" (which includes the Alu+1kb allele), was exclusively associated with the DM disease phenotype and the expanded $(CTG)_n$ repeat, in >200 Caucasian DM families (Neville et al. 1994). This complete linkage disequilibrium suggests that frequent independent mutations for DM are not factors in the dissemination of this dominantly inherited disease. If new DM mutations were common, they should not be associated exclusively with the Alu+1kb allele and other alleles of haplotype A but should be present with alleles of other haplotypes, in a ratio consistent with their presence in the human population. Therefore, complete linkage disequilibrium of haplotype A alleles with the expanded $(CTG)_n$ repeat was seen as evidence for a single ancestral mutation in DM. As an alternative hypothesis, Mahadevan et al. (1993*b*) suggested that the larger Alu+1kb allele predisposes the $(CTG)_n$ repeat to instability. Recently, Imbert et al. (1993) studied the association of $(CTG)_n$ repeat alleles in a normal population to alleles of the Alu±1kb polymorphism and a (CA) repeat polymorphism 90 kb telomeric to the DM mutation. $(CTG)_5$, $(CTG)_{10}$, and $(CTG)_{19-30}$ alleles were exclusively associated with the Alu+1kb allele, whereas $(CTG)_{11-13}$ alleles were only seen in association with the Alu−1kb allele. Imbert et al. proposed a three-step model for the $(CTG)_n$ repeat expansion from $(CTG)_5$ to $(CTG)_{19-30}$ to $(CTG)_{30-50}$ to $(CTG)_{>50}$ and suggested that one or a few ancestral mutations at the repeat occurred randomly on a chromosome with the Alu+1kb allele, resulting in an apparently predisposing haplotype for DM, in which the length of the $(CTG)_n$ repeat itself is the main driving force for recurrent multistep DM mutations. The last step of the expansion has been observed recently in a Japanese DM family where a parental $(CTG)_{44}$ allele expanded to $(CTG)_{70}$ and $(CTG)_{>100}$ repeats in two sons (Yamagata et al. 1994).

We have studied a Nigerian (Yoruba) DM family, the only indigenous sub-Saharan DM case reported to date (Dada 1973). Here we report the results of a high-resolution genetic analysis of this family, within and around the *DMPK* locus. Our results suggest that this is a DM mutation that occurred independently from the Eurasian mutation and challenge the hypotheses of a single ancestral mutation and predisposing elements within the original DM haplotype.

Subjects and Methods

This study was approved by the local institutional review board.

The Nigerian DM Family

At the time of examination for this study, the proband, a Nigerian black male, was 41 years old and showed distal muscle weakness and wasting, difficulty swallowing and speaking, and memory impairment. He reported no perinatal complications, with subsequent normal growth and development until around the age of 13 years, when he noticed inability to release the trigger of an automatic rifle. Months later, the diagnosis of DM was made, based on clinical and electrophysiological studies as reported earlier (Dada 1973). The disease has progressed with increased symptomatology until the present time. On physical examination, the proband showed deficits in long-term memory, dysarthric speech, a weak and hollow voice, frontal balding, temporalis wasting, "hatchet face," facial weakness, ptosis, sternocleidomastoid atrophy, and decreased visual acuity (20/800 right, 20/200 left). His lungs were clear to auscultation, and his heart was arrhythmic, with a rate of 62 beats/min. The patient also had testicular atrophy, distal muscle weakness and atrophy in all extremities (distal greater than proximal), and percussion myotonia of the tongue and thenar eminence. Gait was wide based, with bilateral footdrop. The patient could not tandem, heel, or toe walk. Deep-tendon reflexes were diminished and symmetrical throughout.

The mother of the proband, a 68-year-old Nigerian black female, has had 11 pregnancies, with 8 live births, 2 miscarriages, and 1 placenta previa. Five of her six sons were diagnosed with DM, while her two daughters showed no signs of the disease in their 40s. On examination, she exhibited frontal balding, "hatchet face," temporalis wasting, and distal muscle weakness. She also had unequivocal percussion myotonia of her thenar eminence.

Molecular Analysis of $(CTG)_n$ Repeat Size

Genomic DNA was isolated from peripheral blood leukocytes (Miller et al. 1988). Southern-blot analysis and PCR were used to analyze the $(CTG)_n$ repeat expansion within the *DMPK* locus, as described elsewhere (Fu et al. 1992). The expansion status was determined by Southern-blot analysis of 1% agarose size-fractionated, *Eco*RI- or *Nco*I-digested genomic DNA and a [α^{32}P] dCTP–labeled genomic probe from the *DMPK* gene (pMDY1) (Sambrook et al. 1989; Fu et al. 1992). $(CTG)_n$ repeat size of the normal alleles was determined by PCR across the repeat, with flanking primers in the presence of [α^{32}P] dCTP (Ashizawa et al. 1992*a*; Fu et al. 1992). PCR products were analyzed by 6% 7 M urea denaturing PAGE. Results were visualized by autoradiography. The sizes of alleles were determined by comparison with the appropriate size markers and control samples of known size.

Typing of DM Haplotypes

The additional polymorphisms used to construct haplotypes in this study were located in and around the

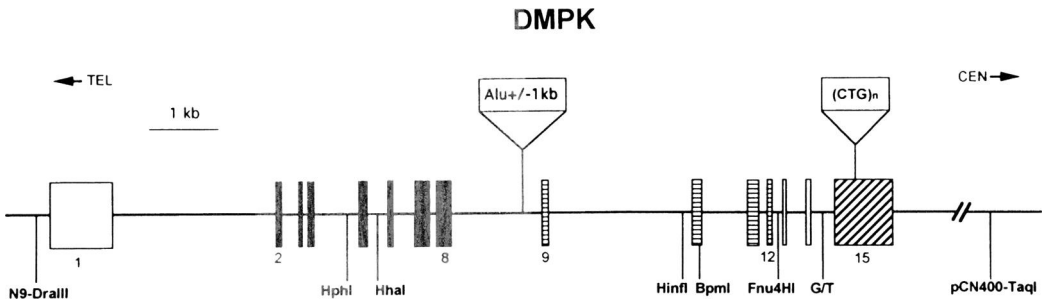

Figure 1 Location of polymorphisms within and flanking the genomic sequences of the *DMPK* locus. For each RFLP the informative restriction enzyme is indicated below the gene diagram; the DMPK-Alu±1kb polymorphism in intron 8 and the location of the unstable $(CTG)_n$ in the 3′ UTR of exon 15 are boxed above the gene diagram. The *DMPK* gene is oriented from telomere (TEL) to centromere (CEN). Exons are shown as boxes: exons 2–8 encode the putative protein kinase domain, exons 9–12 encode the coiled-coil domain, and exon 15 encodes a potential transmembrane domain.

DMPK locus (fig. 1) (Mahadevan et al. 1993*a*, 1993*b*; Sabouri et al. 1993; Neville et al. 1994). With exception of the Alu±1kb polymorphism, all other polymorphisms are RFLPs due to single nucleotide substitutions, resulting in either the formation of a new restriction site or the loss of an existing restriction site. All polymorphisms were typed by PCR. The forward (F) and reverse (R) primers used were those previously published (Mahadevan et al. 1993*a*, 1993*b*; Sabouri et al. 1993; Neville et al. 1994), except for the DMPK-*Hph*I and DMPK-*Fnu*4HI polymorphisms, for which the sequences were as follows: (1) DM-*Hph*I(F)—5′CGTCTCCGGCTGCAGCTC3′ and DM-*Hph*I(R)—5′AATCTCCGCCAGGTAGAATC3′; and (2) DM-*Fnu*4HI(F)—5′GATCATTGCAGGAGCTATG3′ and DM-*Fnu*4HI(R)—5′GTGTGCCTCTAGGTCCCG3′. PCR amplification of 200 ng genomic DNA with the appropriate primers was carried out according to a standard hot-start protocol with AmpliWax beads from Perkin Elmer: 50-μl reactions with 0.25 μM of each primer, 1 × Perkin Elmer PCR buffer (10 mM Tris-HCl pH8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% [w/v] gelatin), 200 μM dNTP mix, and 1.25 U AmpliTaq DNA polymerase (Perkin Elmer) were amplified after an initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min and the appropriate annealing and extension steps (see below) and a final extension at 72°C for 7–10 min. The annealing (A) and extension (X) conditions were 60°C/1.5 min (A) and 72°C/1 min (X) for N9-*Dra*III, 62°C/1 min (A) and 72°C/1.5 min (X) for DMPK-*Hph*I, 65°C/1 min (A) and 72°C/1 min (X) for DMPK-*Hha*I, 60°C/1.5 min (A) and 72°C/1.5 min (X) for DMPK-Alu±1kb, 62°C/1 min (A) and 72°C/1 min (X) for DMPK-*Hinf*I and DMPK-*Bpm*I, 60°C/1 min (A) and 72°C/1 min (X) for DMPK-*Fnu*4HI, and 60°C/1 min (A) and 72°C/1.5 min (X) for pCN400-*Taq*I polymorphisms. RFLPs were identified by digestion of half the PCR reaction product with the appropriate restriction enzyme, according to the manufacturer's suggestions, and were separated by agarose gel electrophoresis using 1.5%–2% SeaKem or 3% MetaPhor in 1 × Tris-acetate EDTA. Gels were stained with ethidium bromide. The sizes of alleles were determined by comparison to control samples of known genotype. When it was necessary, the gels were transferred to Hybond-N+ membranes (Amersham), which were probed with the [α^{32}P] dCTP–labeled (Megaprime kit; Amersham) uncut PCR fragment for the polymorphism of interest, in Rapid-Hyb buffer (Amersham) under high-stringency conditions according to the manufacturer's suggestions. The results were visualized by autoradiography.

Results

In 1973, Dada (Dada 1973) first reported a Nigerian (Yoruba) family with DM. To date, this still constitutes the sole reported case of DM among indigenous sub-Saharan black African populations. On reexamination at the age of 41 years, the proband, a Nigerian black male, showed the classical clinical manifestations of adult-onset DM, which had progressively worsened since the initial diagnosis at age 13 years. The proband inherited DM from his mother, a 68-year-old Nigerian black female, also with clear signs of DM.

Initial analysis for the $(CTG)_n$ repeat expansion showed that in this family DM is also associated with the expansion of the unstable repeat, as in Eurasian DM populations. In addition to the expected 8.1-kb *Nco*I fragment for the normal allele, Southern-blot analysis of *Nco*I-digested genomic DNA from peripheral blood leukocytes showed expansions for the DM allele of ~3.5 kb (≈1,170 repeats) for the proband and ~2.6 kb (≈870 repeats) in the affected mother, indicating continued intergenerational instability of the repeat (fig. 2*A*) correlating with the observed clinical anticipation. *Eco*RI digests of these DNA samples showed compatible results. The affected mother also had a normal allele composed of $(CTG)_{12}$ repeats. The unaffected father

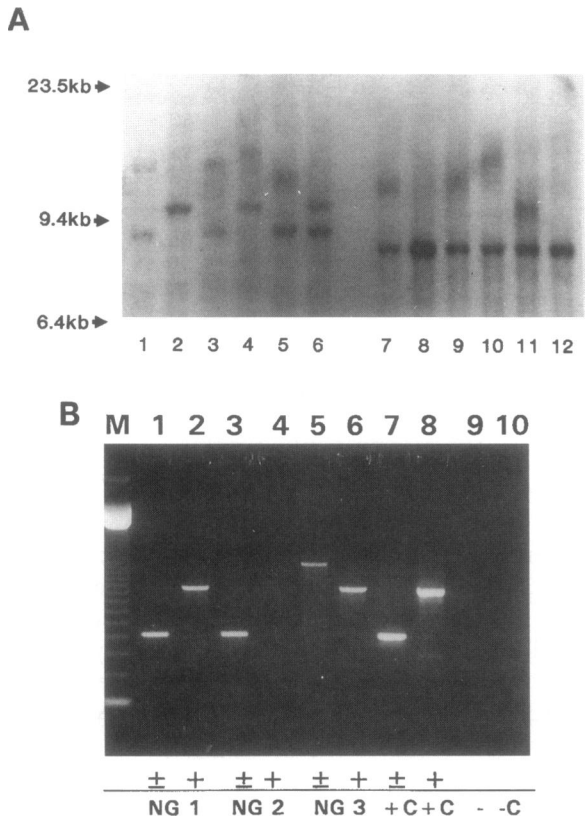

Figure 2 *A*, Southern-blot analysis of the Nigerian DM family, for $(CTG)_n$ repeat expansion in the *DMPK* gene. *Eco*RI digests (lanes 1–6) and *Nco*I digests (lanes 7–12) of the genomic DNA of the unaffected father (NG3; lanes 2 and 8), the affected mother (NG2; lanes 3 and 9), the proband (NG1; lanes 4 and 10), two DM controls (lanes 1 and 7 and lanes 5 and 11), and one normal control (lanes 6 and 12) were analyzed. The normal *Eco*RI fragments show polymorphic 10-kb and 9-kb alleles, which correspond to the Alu+1kb and Alu−1kb alleles, respectively. The normal control (lane 6) is heterozygous for the two normal *Eco*RI alleles. Enlarged fragments of ~13.5 kb and ~12.6 kb are detected for NG1 (lane 4) and NG2 (lane 3), in addition to the normal 10-kb and 9-kb alleles, respectively. NG3 shows the 10-kb allele only, without an expansion, indicating the homozygosity for this allele. The normal *Nco*I fragments show a uniform size of 8.1 kb (lanes 7–12). NG3 has a single fragment of 8.1 kb, whereas NG1 (lane 10) and NG2 (lane 9) have an expanded allele of ~11.6 kb and ~10.7 kb, respectively, in addition to the 8.1-kb normal allele. These results indicate that NG1 and NG2 have ~2.6-kb and ~3.5-kb expansions, respectively. The positions of molecular-weight markers (λ*Hin*dIII) in the gel are indicated on the left. *B*, PCR typing of the Nigerian DM family, for the DMPK-Alu±1kb polymorphism. Typing with the primer pair for both the Alu+1kb (insertion) allele (1,477 bp) and the Alu−1kb (deletion) allele (494 bp) is indicated (±), as is typing of the Alu+1kb allele alone (1,007 bp) with the internal primer that anneals to the DNA segment present only in the Alu+1kb allele (+). Lanes 7 and 8 contain the products of positive control samples for the deletion and insertion allele, respectively; lane 10 is the no-DNA control. The proband (NG1) is heterozygous. In lane 1 (± typing), the PCR preferentially amplified the deletion (Alu−1kb) allele, because of its smaller size. As a result, the insertion (Alu+1kb) allele is not visible, although the presence of the insertion allele is clearly demonstrated in lane 2 (+ typing). The affected mother (NG2) is homozygous for the Alu−1kb allele, indicated by the absence of any product in lane 4 (+ typing). The normal father (NG3) is homozygous for the Alu+1kb allele, indicated by the presence of the sole 1,477-bp fragment (the largest fragment in panel *B*) in lane 5 (± typing) as well as by the amplification of the 1,007-bp allele in lane 6 (+ typing). These results were verified by six repeated typings of different aliquots of the stock DNAs. Southern-blot analysis of *Eco*RI fragments containing this region gave consistent results (see panel *A*).

showed no enlarged fragments, being homozygous for $(CTG)_{10}$ repeats. The proband inherited one of the chromosomes 19 with a normal $(CTG)_{10}$ repeat allele from the father and inherited the DM chromosome with the unstable $(CTG)_n$ repeat from his mother.

Disassociation of the Alu+1kb allele from the expanded $(CTG)_n$ was immediately obvious, since analysis of the affected mother revealed her to be homozygous for the Alu−1kb allele. The proband was heterozygous, having received an Alu+1kb allele from his normal father, who was homozygous for that allele (fig. 2). These results were verified by five repeated typings of different aliquots of the stock DNAs. Thus, the haplotypes in this Nigerian DM family showed the same association of the $(CTG)_{10}$ allele (both paternal alleles) with the Alu+1kb allele, and of the $(CTG)_{12}$ allele (normal maternal allele) with the Alu−1kb allele, that was observed for Caucasian populations (Imbert et al. 1993; Neville et al. 1994). In contrast, the expanded $(CTG)_n$ repeats in the proband and his mother were associated with the Alu−1kb allele. This kindred, therefore, is the first reported case that violates the complete linkage disequilibrium between the expanded $(CTG)_n$ repeat and the Alu+1kb allele.

Further analysis of this family, for the other polymorphisms within and flanking the *DMPK* locus (fig. 1), was carried out to construct the respective DM-region haplotypes of each chromosome 19, summarized in the pedigree below (fig. 3) to assign each to one of the nine haplotypes that thus far have been identified for this region (Neville et al. 1994). The affected mother was homozygous for all markers studied, except for the expanded $(CTG)_n$ repeat. Both her chromosomes showed haplotype D (fig. 3). The association of haplotype D with the $(CTG)_{12}$ repeat for the normal allele in the affected mother is consistent with the results observed in Caucasians by Neville et al. (1994), indicating further that our haplotype results for the Alu±1kb polymorphism in the affected mother are internally consistent. The normal father was also homozygous for all markers. However, both of his chromosomes showed a haplotype previously not identified in Caucasians. We therefore designated this haplotype "J," adhering to and extending the DM-region haplotype-designation system put forward by Neville et al. (1994). Haplotype analysis of the proband showed that he had inherited, from his mother, the expanded $(CTG)_n$ on haplotype D and, from his father, the other chromosome with haplotype J. Haplotype A is the most common haplotype (49% frequency) in the normal Caucasian population. In con-

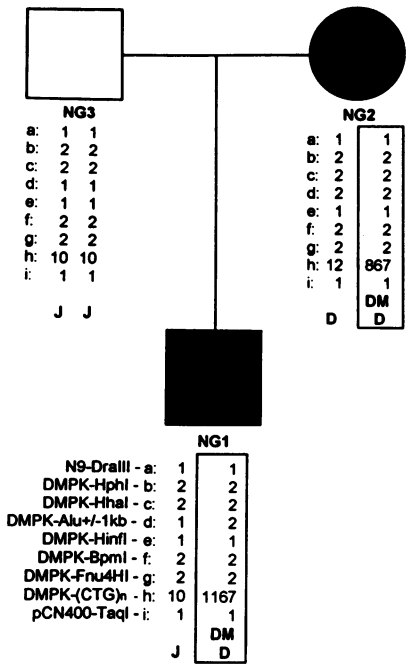

Figure 3 Pedigree of the Nigerian DM family, with respective haplotypes. The unblackened square denotes the unaffected father (NG3), the blackened circle denotes the affected mother (NG2), and the blackened square denotes the proband (NG1). The haplotype of the chromosome with the expanded $(CTG)_n$ repeat that segregates with DM in this family is boxed. The alleles at the polymorphic sites making up the haplotype, including the number of $(CTG)_n$ repeat units, are indicated. The numbers of the expanded $(CTG)_n$ repeats shown (1167 for NG1 and 867 for NG2) are approximate estimates of the average repeat sizes. The haplotype of each chromosome is indicated at the bottom of the chromosome.

trast, haplotype D is the fourth most common haplotype (frequency 8%) in Caucasians (table 1) and has never been shown to segregate with DM in Caucasians (Neville et al. 1994). Thus, this Nigerian DM family differs from Eurasian DM populations in that the expanded $(CTG)_n$ repeat correlated with DM is not associated with the Alu+1kb allele but with the Alu−1kb allele and other alleles within and flanking the *DMPK* locus conforming to haplotype D, previously not shown to segregate with the expanded $(CTG)_n$ repeat and DM.

Discussion

The Nigerian DM family analyzed in this report remains the sole identified DM case among black sub-Saharan indigenous populations, despite an organized effort to identify additional cases in this region of the world (Ashizawa and Epstein 1991). For this family we have presented genetic data indicating that the DM allele is not associated with the haplotype with which it has been shown to be in complete linkage disequilibrium in all the other human populations studied. Both Nigerian patients showed typical clinical and electrophysiological features of DM, as well as the diagnostic $(CTG)_n$ repeat enlargement in the *DMPK* locus. The affected family members also exhibited anticipation between the affected mother and the proband, with an accompanying intergenerational increase of the repeat size. Thus, the disease in this Nigerian family belongs to the classic entity of DM described in Eurasian populations.

Therefore, the question arises as to why the putative predisposing haplotype is dissassociated from the typical DM phenotype and genotype in this geographically disparate family. There are two possibilities: (1) the DM mutation in the Nigerian family has the same origin as the mutation studied worldwide, with a subsequent conversion of the background haplotype; or (2) an independent mutation occurred in the lineage of the Nigerian family, resulting in the expansion of the $(CTG)_n$ repeat. The possibility of interracial marriages with members of non-African populations was discussed with the family, but we concluded that this possibility was highly unlikely, on the basis of family history and cultural behavioral norms in their society. However, if the DM mutation in the Nigerian family has the same origin as the mutation studied worldwide, we must consider how the expanded $(CTG)_n$ mutation could be the only informative site introduced from a chromosome with haplotype A into the homologous site of a chromosome with haplotype D (or vice versa). Owing to the fact that the expanded $(CTG)_n$ repeat is flanked by different alleles in the Nigerian family, compared with those in every other DM patient studied, there would need to have been a double recombination to introduce the disease mutation from one haplotype into the other. Since the gross rate of recombination in this region of chromosome 19 has been shown (Bachinski et al. 1993; Shutler et al. 1994) to be typical of the recombination rate in the human genome as a whole (1 cM/1 Mb of physical distance/generation), with no evidence of recombination hotspots, such recombination events would be highly unlikely.

Gene conversion could possibly introduce the mutation without disturbing the flanking markers. Jeffreys et al. (1994) have demonstrated that complex gene-conversion events are associated with increased instability of a minisatellite, and a discontinuous gene-conversion event has been proposed as one of several possible mechanisms to explain a change of an expanded allele to a normal allele in one DM case (O'Hoy et al. 1993). If the condition of the family that we studied is the result of a gene-conversion event, it had to be the result of a conversion of a normal $(CTG)_n$ repeat to an expanded $(CTG)_n$ repeat on a chromosome with haplotype D. Alternatively, a chromosome of haplotype A with the expanded $(CTG)_n$ repeat could have converted with a normal chromosome of haplotype D. Indeed, Alu element–mediated gene-conversion/excision-transfer events (Mahadevan et al. 1993*b*) may explain the change at the DMPK-

Table 1

Comparison between the Nigerian DM Haplotype and Caucasian Haplotypes

Haplotype	N9-*Dra*III	DMPK-*Hph*I	DMPK-*Hha*I	DMPK-*Alu*±1 kb	DMPK-*Hin*fI	DMPK-*Bpm*I	DMPK-*Fnu*4HI	DMPK-G → T[a]	DMPK-$(CTG)_n$[b]	pCN400-*Taq*I	Haplotype Frequency
Nigerian:											
DM	1	2	2	2	1	2	2	ND	n = 867 and 1,167	1	D[c]
Normal:											
Mother	1	2	2	2	1	2	2	ND	n = 12	1	D[c]
Father	1	2	2	1	1	2	2	ND	n = 10	1	J[d]
Caucasian:											
DM	1	2	1	1	2	2	1	T	n > 50	2	100%
A	1	2	1	1	2	2	1	T	n > 5–36	2	49%
B	2	2	2	2	1	2	2	G	n = 11–14	1	27%
C	1	1	2	2	1	1	2	G	n = 11–14	1	16%
D	1	2	2	2	1	2	2	G	n = 11–14	1	8%
E	2	2	2	2	1	2	2	G	NA	2	<1%
F	1	1	2	2	1	2	2	G	NA	1	<1%
G	1	2	1	1	2	1	1	T	NA	2	<1%
H	2	2	2	2	2	2	2	G	NA	2	<1%
I	1	1	2	2	2	2	1	G	NA	1	<1%

[a] ND = not done.

[b] Data for Eurasian haplotypes are from Imbert et al. (1993) and Neville et al. (1993). NA = not applicable.

[c] Nigerian DM haplotype corresponds to haplotype D in Caucasians (Neville et al. (1993). The frequency of haplotype D in the Nigerian population has not been determined.

[d] This haplotype has not been identified in Caucasian populations (Neville et al. (1993).

Alu±1kb site in such a conversion. However, such a conversion event must have occurred at other marker sites, including the DMPK-*Hha*I, -*Hin*fII, and -*Fnu*4HI sites and the pCN400-*Taq*I site flanking the $(CTG)_n$ repeat, without conversion of the expanded repeat. In actuality the possibility of any of these improbable and/or speculative events accounting for the DM mutation in this Nigerian family is reduced to essentially zero, when one considers both the lack of DM donor chromosomes in sub-Saharan ethnic populations (Ashizawa and Epstein 1991; Goldman et al. 1994) and the frequency of such occurrences in the Eurasian populations, where analysis of >2,000 Eurasian DM chromosomes of the current generation showed no recombinations or conversions between the expanded $(CTG)_n$ repeat and the Alu±1kb polymorphism (Shaw and Harper 1992).

Finally, trivial explanations of the result (such as switching or improperly labeling samples) can also be ruled out. Since the mother is homozygous for the Alu−1kb allele and has an expansion of the $(CTG)_n$ repeat, the disassociation between the DM mutation and the insertion allele is internally controlled and therefore indisputable. The haplotype data involving nine polymorphic sites were internally consistent within this family, and the allele determination was performed at least twice for each polymorphic locus, with consistent results. Thus, we conclude that the mutation in this Nigerian DM family is an independent mutation that has not taken place within the haplotype purported to predispose for such an event.

However, despite the disassociation between the expanded $(CTG)_n$ repeat and the rest of haplotype A, it is of interest to note that the normal $(CTG)_n$ repeat alleles retain their relationship with the alleles of the Alu±1kb polymorphism, as has been reported for Caucasians (Imbert et al. 1993; Neville et al. 1994): the alleles with $(CTG)_{10}$ repeats (both paternal alleles) were associated with the Alu+1kb allele in the normal father, while the $(CTG)_{12}$ repeat allele (normal maternal allele) was associated with the Alu−1kb allele in the affected mother. If those allelic relationships were in linkage disequilibrium in the Nigerian population (yet to be determined), as they have been shown to be in Eurasians, it would imply that the independent mutation for the disease allele in the Nigerian kindred did not arise from the progression of $(CTG)_5$ to $(CTG)_{19-30}$ to $(CTG)_{30-50}$ to $(CTG)_{>50}$ alleles in the development of the expanded, unstable $(CTG)_n$, as suggested for Eurasian populations (Imbert et al. 1993). However, the complete linkage disequilibrium observed in Eurasian populations does not hold up in selected non-Eurasian populations. A disassociation between the $(CTG)_5$ allele and the Alu+1kb allele has been found in 1 of 50 Senegalese (Wolof) chromosomes (Zerylnick et al. 1995), and selected non-Eurasian populations have recently shown frequent disassociations between the $(CTG)_{11-13}$ alleles and the Alu−1kb allele (Rubinsztein et al. 1994; Zerylnick et al. 1995) (A. Goldman, personal communication). However, disassociations between the $(CTG)_5$ allele and the Alu+1kb allele have not been observed in any other ethnic populations, including Bantu-speaking South African Negroids (A. Goldman, personal communication). We propose that a pathway of $(CTG)_n$ repeat expansion resulting in DM for the lineage reported here is likely

to have involved expansions of the $(CTG)_n$ repeat from the $(CTG)_{11-13}$ alleles on a unique haplotype and that this pathway is distinct from what may have occurred in Eurasian DM populations. Indeed, $(CTG)_{21}$ and $(CTG)_{31}$ alleles have been found associated with the Alu−1kb allele in Asian populations from the Indian subcontinent and Pakistan (Rubinsztein et al. 1994). Both the absence of putatively predisposing alleles in the $(CTG)_{19-30}$ range in the Cameroonian (Bamilekes) (Novelli et al. 1994) and South African Bantu-speaking Negroids (Goldman et al. 1994) and the concordant absence of DM in sub-Saharan Africans support Imbert et al.'s (1993) progression model. Whether the current Nigerian DM haplotype has gone through the $(CTG)_{19-30}$ stage remains unknown. Further studies on haplotypes in relation to the $(CTG)_n$ repeat size and meiotic drive (Carey et al. 1994) in Yoruba and other indigenous African populations would be of interest.

Neither maintenance and expansion of the fully expanded DM $(CTG)_n$ repeat nor the DM phenotype in this Nigerian DM family appears to be dependent on haplotype A. Thus, the haplotype on chromosome 19q13.3 may be largely irrelevant, in functional terms, in the development of the $(CTG)_n$ expansion and the phenotype of DM, suggesting that the $(CTG)_n$ repeat itself is the important factor determining the genetic development of this disease (Carey et al. 1994).

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