Letters to the Editor

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Instability of the $(CTG)_n$ Repeat in Congenital Myotonic Dystrophy

To the Editor:

The mutation specific for myotonic dystrophy (DM) is an unstably expanded $(CTG)_n$ repeat in the 3′ UTR of the myotonin protein kinase gene (Brook et al. 1992; Fu et al. 1992; Mahadevan et al. 1992). Expanded $(CTG)_n$ repeats show variable size heterogeneity, as evidenced by smears of expanded alleles on Southern blots (Buxton et al. 1992; Wong et al. 1995) and by tissue-to-tissue variability of repeat size (Anvret et al. 1993; Ashizawa et al. 1993; Thornton et al. 1994). Patients with the congenital form of DM (CDM) show the most severe and unique phenotype (Harper 1989) and often have a large expansion usually >1,000 repeats (Tsilfidis et al. 1992; Ashizawa et al. 1994). Earlier, we studied 28 CDM infants and showed that none had any appreciable smears of the expanded alleles in peripheral blood leukocyte (PBL) DNA (Wong et al. 1995). Myring et al. (1992) noted that smears of the expanded $(CTG)_n$ repeat in prenatal DM samples were minimal. As to the repeat-size mosaicism between tissues, we have reported that the $(CTG)_n$ repeat of a CDM newborn with a 5.6-kb expansion appeared uniform, with little or no smearing in several different tissues (Ashizawa et al. 1993). Similarly, two DM embryos at 10 wk gestation and a CDM infant had no repeat-size variations between tissues (Hecht et al. 1993; Tachi et al. 1995). In contrast, Lavedan et al. (1993) showed that the size of the mutant repeat varied in different tissues obtained from a DM fetus at 20 wk gestation. Jansen et al. (1994) reported that a DM fetus at 12 wk gestation showed no detectable repeat-size variability between tissues, whereas subtle variability was detected in two newborn infants at 28 wk gestation and in another, full-term infant. Recently, Martorell et al. (1997) studied five DM fetuses and two DM neonates and concluded that the $(CTG)_n$ repeat–size heterogeneity between tissues becomes detectable at 13–16 wk gestation. In an attempt to reconcile these apparently conflicting data, we examined the mutant repeats in our CDM patients, using a Southern blot analysis that can detect subtle size differences of the $(CTG)_n$ repeat.

Informed consent was obtained from subjects and/or their guardians. Genomic DNA was extracted from autopsy tissues of a previously reported 3-d-old child born with CDM at 32 wk gestation (Ashizawa et al. 1993) and from PBLs of 2 CDM infants and 11 CDM subjects 2–30 years of age. Genomic DNAs were digested with *Bam*HI and were subjected to Southern blot analysis with the [^{32}P]-pMDY1 hybridization probe, as described elsewhere (Ashizawa et al. 1992; Fu et al. 1992). The *Bam*HI fragments are relatively small (1.4 kb without expansion), so that subtle differences of the $(CTG)_n$-repeat size can be detected readily when fragments are

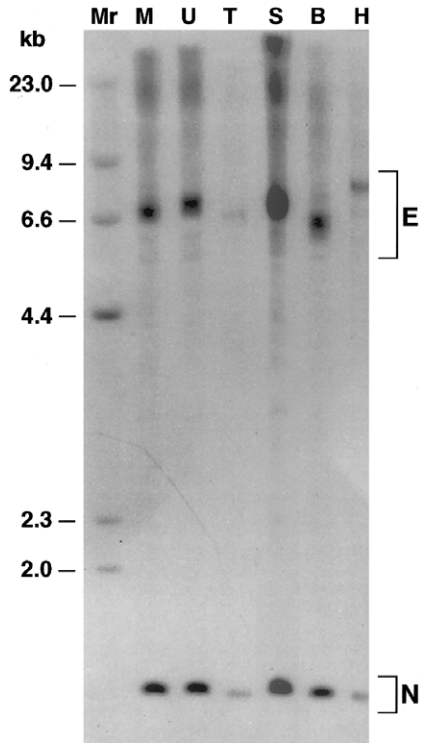

Figure 1 Southern blot analysis of $(CTG)_n$-repeat expansions in various tissues of a CDM newborn. Lane Mr, Lambda *Hin*dIII molecular-weight markers. Lanes M (muscle), U (uterus), T (thymus), S (skin), B (brain), and H (heart), Tissues from which DNA samples were obtained. E = expanded alleles; and N = normal alleles.

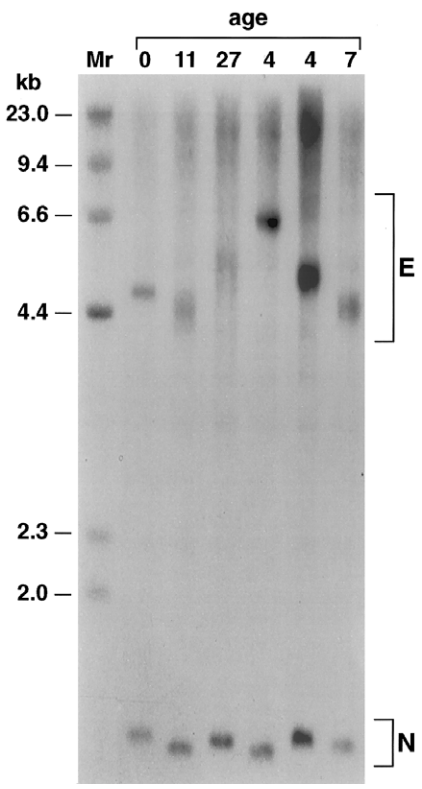

Figure 2 Southern blot analysis of $(CTG)_n$-repeat expansions in representative CDM patients of various ages. Slight size variations of the normal alleles are due to the $(CTG)_n$-repeat–length polymorphism. Lane Mr, Lambda *Hin*dIII molecular-weight markers. The numbers above the lanes show the ages of the CDM patients at the time of sample collection; "0" indicates that the patient was a newborn. E = expanded alleles; and N = normal alleles.

subjected to electrophoresis. To maximize resolution, we allowed the 1.4-kb fragment to migrate 19 cm into the gel (fig. 1). Repeat-size heterogeneity was assessed by measuring the smear size and was expressed as the mid-peak-width ratio (MWR), as described elsewhere (Wong et al. 1995).

The $(CTG)_n$-repeat size clearly differed from one tissue to another in the 3-d-old CDM infant. The largest expansion was found in the heart (5.2 kb, or ~1,730 repeats), followed by those in the uterus and skin (4.5 kb, or ~1,500 repeats), muscle (4.2 kb, or ~1,400 repeats), thymus (4.1 kb, or ~1,370 repeats), and brain (3.6 kb, or ~1,200 repeats) (fig. 1). In addition to these tissue-to-tissue differences, the mutant repeat showed size heterogeneity within a tissue, as evidenced by variable smear width of the expanded allele. The smear size was most extensive in the brain (MWR = 2.3), followed by those in the uterus (MWR = 2.0), muscle (MWR = 2.0), skin (MWR = 1.7), and heart (MWR = 1.6) and was least prominent in the thymus (MWR = 1.4) (fig. 1). There was no significant correlation between smear size MWR and repeat size. In PBL DNA of the 11 CDM subjects 2–30 years of age, the expanded *Bam*HI fragments containing the mutant $(CTG)_n$ repeat showed variable but definite smears, whereas CDM infants showed no detectable smear (fig. 2). There was a good correlation between smear size MWR and age ($r^2 = .77$, $t = 6.30$, $P < .002$) (fig. 3). However, smear size MWR showed no correlation with the repeat size.

Our data in the CDM newborn suggest that somatic mosaicism of the $(CTG)_n$-repeat size is already evident soon after birth. In the previous studies that showed minimal or nondetectable repeat-size mosaicism, *Eco*RI (Myring et al. 1992; Tachi et al. 1995) or *Nco*I (Ashizawa et al. 1993) was used in Southern blot analyses. Since these enzymes produce relatively large fragments (8–10 kb for normal-size alleles), small repeat-size differences among various tissues and subtle smears of the expanded alleles may have been missed because of limited size resolution. Thus, in most cases, somatic instability of the $(CTG)_n$ repeat does begin prenatally, presumably at <16 wk gestation, as Martorell et al. (1997) have suggested. Our data showed that the repeat size was largest in the heart and smallest in the brain. This is consistent with a previously reported pattern (Kinoshita et al. 1996; Martorell et al. 1997), suggesting the presence of tissue-specific determinant(s) of somatic instability of the $(CTG)_n$ repeat. However, in two CDM children 4 years of age who had expansion sizes of 5.1 and 3.9 kb, the smear size was greater in the latter (MWR = 1.7) than in the former (MWR = 1.2), suggesting that repeat size, age, and tissue-specific factors

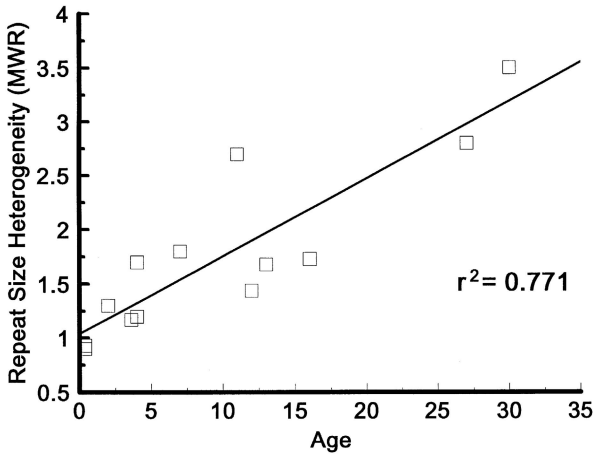

Figure 3 Correlation between size heterogeneity of expanded $(CTG)_n$-repeat alleles and ages of CDM patients. The repeat-size heterogeneity is expressed as MWR (Wong et al. 1995).

are not the only determinants for somatic instability of the $(CTG)_n$ repeat.

Heterogeneity of the $(CTG)_n$ repeat within a tissue has been seen in skeletal muscle, brain, and kidney of a fetus at 28 wk gestation, with ~2,500 repeats (Martorell et al. 1997). Interestingly, in this fetus and in our CDM infant, the tissues containing leukocytes (blood and thymus, respectively) showed little or no smearing. This is consistent with our previous observation that PBL DNA of CDM infants show no size heterogeneity of the repeat-containing *Nco*I fragments (Wong et al. 1995). In the present study, PBL DNA of two CDM infants showed no detectable repeat-size heterogeneity of the *Bam*HI fragment, suggesting that there is, indeed, no extensive somatic instability during the ontogenic development of leukocytes in the prenatal period. In contrast, our CDM patients >2 years of age showed variable but substantial smears with a strong correlation to the patient's age. Although serial samples of CDM patients are currently not available, this suggests that somatic heterogeneity of the $(CTG)_n$ repeat observed in the blood of CDM subjects is mainly a result of postnatal somatic instability. In each CDM subject with a detectable smear in PBL, the prenatal period was shorter than the postnatal life, although somatic-cell replication is expected to be far more intense during the prenatal development. Perhaps instability of mutant repeat arrays may be time dependent rather than replication dependent, in the bone-marrow stem-cell/PBL lineage. We conclude that somatic instability of the $(CTG)_n$ repeat in CDM begins prenatally in a tissue-dependent fashion and that the $(CTG)_n$-repeat-size instability within the leukocyte lineage is minimal during the prenatal development but increases with age after birth.

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Homogeneity of Kerato-Epithelin Codon 124 Mutations in Japanese Patients with Either of Two Types of Corneal Stromal Dystrophy

To the Editor:

Granular corneal dystrophy Groenouw type 1 (CDGG1; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]) is an autosomal dominant form of corneal dystrophy that is characterized by discrete deposits of gray-white material in the subepithelial and stromal layers of the cornea (Mannis et al. 1997). The precise nature and source of these deposits are unclear. Amyloid deposits have been detected in the corneas of older individuals with typical CDGG1 (Garner 1969; Akiya and Brown 1970). Lattice corneal dystrophy type 1 (CDL1; OMIM 122200 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?122200]), which is clinically distinct from CDGG1, is characterized by linear amyloid deposits in the subepithelial and stromal layers of the cornea (Mannis et al. 1997). However, granular-lattice corneal dystrophy recently has been described as having the clinical and histological features of both granular and lattice corneal dystrophies (Folberg et al. 1988, 1994; Holland et al. 1992; Rosenwasser et al. 1993). This condition has been named "Avellino corneal dystrophy" ("ACD"; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]), because most of the affected individuals could trace their origin to the Italian province of Avellino (Holland et al. 1992).

Recently, four corneal disorders with autosomal dominant traits, CDGG1, CDL1, ACD, and Reis-Bücklers corneal dystrophy (CDRB; OMIM 121900 [http://www3.ncbi.nlm.nih.gov:80/htbin-post/Omim/dispmim?121900]), have been mapped to chromosome 5q31 (Stone et al. 1994; Gregory et al. 1995; Korvatska et al. 1996; Small et al. 1996). More recently, these four corneal dystrophies have all been shown to result from mutations in the kerato-epithelin gene (Munier et al. 1997). The mutations detected in Caucasian patients in Europe were R124C, in two families with CDL1; R124H, in two families with ACD; R555W, in one family with CDGG1; and R555Q, in one family with CDRB. Each of these four mutations affects the CpG dinucleotide of an arginine codon. Kerato-epithelin genes with R124 mutations may form amyloidogenic intermediates that precipitate in the cornea.

We have demonstrated the deposition of amyloid, a feature of ACD, in corneal specimens from six of eight Japanese patients with a clinical diagnosis of CDGG1 (Konishi et al. 1997). To investigate whether the high frequency of amyloid deposition in Japanese patients with CDGG1 is attributable to a specific mutation in the kerato-epithelin gene or to other conditions, such as aging, we screened kerato-epithelin genes from Japanese patients with CDGG1, ACD, or CDL1 (primary corneal amyloidosis).

The study subjects comprised 6 Japanese patients with ACD (of whom 1, with recurrent disease, was the offspring from a consanguineous marriage), diagnosed by histological examination; 10 patients with a clinical diagnosis of CDGG1 (of whom 2, with recurrent disease, were the offspring from consanguineous marriages); and 6 patients with CDL1. None of the patients were related. The mean age ($\pm$SD) of the 6 patients with ACD was 70.5 ($\pm$14.2) years, at the time of surgery, and that of the 10 patients with CDGG1 was 50.1 ($\pm$18.0) years, at the time of their first visit to us. Individual exons (exons 4–16) of the kerato-epithelin gene were amplified from genomic DNA from each subject, by PCR with primers described elsewhere (Munier et al. 1997), and the amplification products were sequenced on both strands. We detected a homozygous R124H (np418G→A) mutation in the one ACD and two CDGG1 patients with consanguineous parents and a heterozygous R124H (np418G→A) mutation in the remaining five ACD and eight CDGG1 patients (fig. 1, *top*). Patients with the homozygous mutation experienced rapid progression of clinical manifestation and visual deterioration at an early age and required keratoplasty in the 1st decade of life. These severe corneal conditions resembled a variant of superficial granular corneal dystrophy (Haddad et al. 1977; Owend et al. 1992; Sajjadi and Javadi 1992). A heterozygous R124C (np417C→T) mutation was identified in the six patients with CDL1 (fig. 1, *bottom*). To exclude the possibility of contamination with mutated DNA in PCR or sequence-reaction mixtures, we also analyzed DNA from control subjects, at the same time as our analysis of patient DNA, and obtained normal nucleotide sequences from the controls. We also screened 40 Japanese subjects without corneal dystrophy and failed to detect any of the mutations identified in the patients.