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CORRESPONDENCE

Myotonic dystrophy type 2 is rare in the Japanese population

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Myotonic dystrophy (DM) is the most common form of adult-onset muscular dystrophy and is characterized by autosomal dominant progressive myopathy, myotonia and multiorgan involvement. There are two distinct entities currently known: DM type 1 (DM1) and type 2 (DM2). DM2 is caused by the expansion of a tetranucleotide CCTG repeat in the first intron of the zinc finger protein 9 (*ZNF9*) gene on chromosome 3q21,[1] whereas DM1 is caused by a CTG repeat expansion in the 3′-untranslated region of the dystrophia myotonica-protein kinase gene (*DMPK*).[2] In the normal allele for *ZNF9*, the repeat sequence is a complex motif with an overall configuration of $(TG)_n(TCTG)_n(CCTG)_n$. The number of CCTG repeats is <30 in the normal allele, with interruptions by GCTG and/or TCTG motifs, and this allele is stably transmitted from one generation to the next.[1,3] However, in the expanded allele only the CCTG tract elongates and no GCTG and TCTG interruptions occur. The expanded *ZNF9* allele is extremely unstable and the size is highly variable, ranging from 75 to 11 000 repeats, with a mean of 5000 CCTG repeats. This unprecedented repeat size and somatic heterogeneity make the molecular diagnosis of DM2 difficult, and explain why the expansion yields variable clinical phenotypes.[4]

To date, DM2 mutations have been identified predominantly in European Caucasians.[3,5] Although a small number of DM2 mutations have been reported in non-European populations, including families in Morocco, Algeria, Lebanon, Afghanistan and Sri Lanka,[6,7] all reported that DM2 patients had been considered to originate from a single common founder because they shared an identical haplotype.[3,5,7] However, in 2008 we identified the first case of DM2 in an East-Asian population, in a Japanese patient with a disease haplotype distinct from that shared among Caucasians, indicating that DM2 exists in non-Caucasian populations and that there may have been separate founders.[8]

Thus, it was of interest to determine the frequency of DM2 in non-Caucasian populations. We studied a Japanese population for the presence of the DM2 mutation. We included both patients with clinically and/or electrically confirmed myotonia in which the DM1 mutation had been excluded and patients with the limb-girdle muscular dystrophy (LGMD) phenotype, because DM2 is generally proximal dominant[4] and the phenotype often lacks myotonia,[9] similar to LGMD, a heterogenous group of muscle disorders for which >60% of the genetic causes have remained undisclosed in Japan (Y.K. Hayashi *et al.*, unpublished data). It has been currently reported that the frequency of the DM2 mutation is more than DM1 in the European population:[10] 1 in 1830 in the general Finnish population, 1 in 988 Finnish patients with non-myotonic neuromuscular diseases and 1 in 93 Italian patients with undetermined non-myotonic proximal myopathy or asymptomatic hyperCKemia. Both the Finnish and Italian population are expected to be a relatively representative European population with regard to the DM2 mutation, because of a single European founder haplotype.[3,5,7]

Genomic DNA was extracted from blood leukocytes or muscle biopsy samples according to the standard protocols. The CCTG repeat size was determined by PCR, using primers flanking the repeat. When a single allele was amplified, Southern blot analysis using *Eco*RI or repeat-primed PCR specific for the DM2 expansion[1,4] was performed to distinguish homozygosity from heterozygosity involving a large CCTG expansion. All subjects included in this study gave informed consent and the protocol was approved by the Ethical Committee of Okayama University, Nagoya University and the National Center of Neurology and Psychiatry. In total, we studied 153 unrelated patients. In all, 34 were myotonic patients without the DM1 mutation and 119 showed a LGMD phenotype without identified LGMD mutations. Clinical

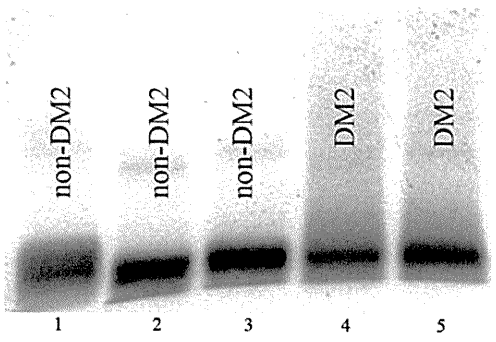

Figure 1 Repeat-primed PCR analysis. Expanded CCTG repeats in the two DM2 patients (Caucasian and Japanese DM2[9] in lanes 4 and 5, respectively) are detected as a continuous characteristic smear of products at higher molecular weight than those in non-DM2 patients (3 different individuals from the 11 patients showing a single allele by PCR amplification of the DM2 repeat in lanes 1–3).

information was assessed based on records provided by the physicians.

We identified 295 alleles ranging in length from 180 to 258 bp by PCR amplification of the DM2 repeat. Heterozygosity was identified in 142 individuals (0.93). In the remaining 11 samples showing a single allele, Southern blot or repeat-primed PCR analysis showed no expanded CCTG repeats (Figure 1), indicating that all of them are homozygous for a single allele. Thus, in our extensive survey, no DM2-related CCTG expansion was detected.

Most DM patients in Japan have been considered to have DM1 (NIH Genetics Home Reference, http://ghr.nlm.nih.gov/condition/myotonic-dystrophy). Our study confirms that DM2 is an extremely rare cause of myotonic and/or LGMD patients in Japan. Although the spectrum of clinical presentation of DM2 is variable and only one Japanese DM2 patient has been reported to date, our data have important implications concerning the indications for genetic testing and counseling for DM2 in East-Asian populations. The origin of most DM2 mutations is estimated to be 200–540 generations ago in Europe, and DM2 has since spread into several European populations.[3] The rarity of DM2 in East-Asian populations may be because of a lack of founder effects or extinction of DM2 by genetic drift or selective causes.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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