BRIEF REPORT — POLYMORPHISM REPORT

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A novel *Sac* I RFLP in the 3' untranslated region of the *myotonin protein kinase* gene

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Abstract We found a novel *Sac* I polymorphism downstream of CTG repeats in the 3' untranslated region of the *myotonin protein kinase* (*MT-PK*) gene. A C to G transition at nucleotide 13,590 in the gene was revealed by Southern blotting and confirmed by sequencing analyses. The allelic frequency of the C:G polymorphism in 63 unrelated Japanese individuals was estimated to be 0.98: 0.02. When Southern blotting is employed in the analysis of the CTG repeat length in the *MT-PK* gene, this *Sac* I polymorphism should be taken into consideration.

Key words Myotonic dystrophy · *Myotonin protein kinase* gene · Polymorphism · *Sac* I digestion · 3'-untranslated region

Introduction

Myotonic dystrophy (DM) is an autosomal dominant multi-system disorder associated with the CTG repeat expansion within the 3' non-coding region of the *myotonin protein kinase* (*MT-PK*) gene. The *MT-PK* gene has been localized

at 19q13.3 (Tsilfidis et al. 1991). Analysis of the CTG repeat length is diagnostically important for this disorder and is usually examined by Southern blotting.

To detect the expansion of the CTG repeat, a 1.4-kb *Bam*H I genomic fragment containing the 3' end of the *MT-PK* gene, designated pM10M-6 (Brook et al. 1992) has been used as a probe for Southern blotting (Fig. 1). We have used a 0.66-kb *Pst* I/*Hinc* II subfragment (pM10M-6N) of the original *Bam*H I fragment to reduce non-specific background signals in the Southern blotting (Nakamura et al. 1996). This new probe recognized the fragment containing the unstable trinucleotide CTG repeat after digestion of genomic DNA with various restriction enzymes, such as *Bam*H I, *Bgl* I, *Eco*R I, *Hind* III, *Pst* I, and *Sac* I.

Source and description

We encountered a 63-year-old man showing neurogenic muscular atrophy and cerebellar hypoplasia but not having myotonia. Since this patient had a "hatchet face", one of the characteristic symptoms of DM, we examined the CTG repeat length of the *MT-PK* gene. Southern blot analysis with the pM10M-6N probe revealed no expansion of the CTG repeat when the genome was digested by *Bam*H I, *Bgl* I, *Eco*R I, *Hind* III, or *Pst* I. However, when the genome was digested by *Sac* I, alleles of two different sizes were detected (3.5kb [A1] and 2.0kb [A2]) (Fig. 2A). These results showed that the polymorphic *Sac* I site was located downstream of the CTG repeat within exon 15 which contained the 3' untranslated region. Sequence analysis revealed that the polymorphic site was a C to G transition at nucleotide position 13,590 (Mahadevan et al. 1993) of the *MT-PK* gene.

The genomic DNA fragment containing this polymorphic site was also amplified by the polymerase chain reaction (PCR) method with sense (base pairs 13,386 to 13,405: DM15-F: 5'-TATCGTTGGTTCGCAAAGTG-3') and antisense (base pairs 13,660 to 13,679: DM15-R: 5'-CTCAGTGCATCCAAAACGTG-3') primers. PCR reac-

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Fig. 1 Scheme showing the 3'-untranslated region of the *myotonin protein kinase* gene. Sequence analysis of proband revealed that the polymorphic site was a C to G transition at nucleotide position 13,590 of the gene and resulted in a new *Sac* I site. Restriction sites used for the Southern blotting, and the location of the original probe (pM10M-6) and newly generated probe (pM10M-6N) are also shown

Fig. 2 A Southern blotting analysis of the genomic DNA digested by *Sac* I. The pM10M-6N probe detected a 3.5-kb band in the normal control (lane 1), and two bands (3.5 kb and 2.0 kb) in the proband (lane 2). B Analysis of *Sac* I-digested polymerase chain reaction (PCR) products in a 4.0% NuSieve (FMC Corp., Chicago, IL) agarose gel stained with ethidium bromide. A *Sac* I site was found in one of the alleles from the proband (arrow), and produced fragments of 210 bp and 65 bp, together with a 275-bp undigested band from another allele (lane 1). Note that three siblings in the family were also heterozygous for this *Sac* I polymorphism (lanes 2–4). Lane M, DNA molecular weight markers

tions were performed in a total volume of 50 μ l containing 100 ng genomic DNA, 100 ng of each primer, and 0.5 mM each of dNTPs. Initial denaturation at 94°C for 5 min was followed by 30 cycles with denaturation at 94°C for 1 min, annealing at 62°C for 1 min, and extension at 72°C for 1 min. Finally, a 10-min extension step at 72°C was added. *Sac* I digestion of the amplified fragment from the patient (proband) resulted in two alleles. The allele A1 did not contain the *Sac* I site and produced an undigested fragment of 275 bp, while the allele A2 having the *Sac* I site produced two fragments, of 210 bp and 65 bp. Three siblings in the family were found to be heterozygous for the *Sac* I polymorphism and none of them had any symptoms of DM (Fig. 2B).

Polymorphism and allele frequency

We estimated the allele frequency by analysis of 126 chromosomes of 63 unrelated Japanese individuals. The allele frequency was: A1, 0.98 and A2, 0.02 and the combined observed heterozygosity in the 63 individuals with *Sac* I digestion was 0.04. Thus, when Southern blotting is employed in the analysis of the *MT-PK* gene, this *Sac* I polymorphism should be taken into consideration.

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