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BRIEF REPORT — POLYMORPHISM REPORT

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An *Aci*I polymorphism in the 3′ untranslated region of the human phosphomannomutase 2 (*PMM2*) gene

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Abstract We found an *Aci*I polymorphism in the 3′ untranslated region of the phosphomannomutase 2 (*PMM2*) gene located at 16p13. A G-to-C transition at nucleotide position 96 bp downstream from the PMM2 stop codon was detected in polymerase chain reaction (PCR) products after *Aci*I digestion. The heterozygosity of the polymorphic alleles was 0.375 in a Japanese population. This polymorphism is useful for genetic analysis in patients with carbohydrate-deficient glycoprotein syndromes, of which there are four subtypes.

Key words Carbohydrate-deficient glycoprotein syndrome · Phosphomannomutase 2 · Polymorphism · *Aci*I digestion · Chromosome 16p13

Introduction

Carbohydrate-deficient glycoprotein (CDG) syndromes are autosomal recessive disorders, characterized by defective N-glycosylation of serum and cellular proteins (Jaeken et al. 1997). Four subtypes of CDG syndromes (CDG1–4) have been identified on the basis of clinical manifestations and biochemical findings in patients with CDG syndromes. At present, genes for two types of CDG syndromes have been identified: the phosphomannomutase 2 (*PMM2*) gene and the phosphomannose isomerase gene in CDG syndrome type 1 (CDG1) (Matthijs et al. 1997; Jaeken et al. 1998), and the UPD-GlcNAc: α-6-D-mannoside β-1,2-N-acetylglucosaminyltransferase 2 (*GnT2*) gene in CDG syndrome type 2 (CDG2) (Jaeken et al. 1994). The gene for PMM2, the enzyme that converts mannose 6-phosphate to mannose 1-phosphate in the synthesis of GDP-mannose, is mapped to chromosome 16p13, and misssense mutations of this gene have been identified in most patients with CDG1 (Matthijs et al. 1997, Kondo et al. 1999). In this study, we found an *Aci*I polymorphism in the 3′ untranslated region of the gene. It would be helpful for differential diagnosis by linkage analysis in patients with CDG syndromes and diseases for which the gene is located near this gene locus.

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Primers for polymerase chain reaction (PCR)

The primers were:

PMM2-F: 5′-GTGGCAATGACCTGACATC-3′
PMM2-R: 5′-GAAGTCCAGACGGCACATG-3′.

PCR conditions. PCR was performed in a volume of 25 μl, containing 200 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% of gelatin, 0.2 mM of each dNTP, 5 pmol of each primer, and 1 unit of Taq polymerase (Takara Shuzo, Tokyo, Japan) with a DNA Thermal Cycler (PJ2000; Perkin Elmer, Norwalk, CT, USA). Initial denaturation at 95°C for 5 min was followed by 30 cycles with denaturation at 95°C for 1 min, annealing at 54°C for 1 min, and extension at 72°C for 1 min, with a final extension step of 7 min at 72°C. The PCR products after digestion with a restriction enzyme, *Aci*I, were electrophoresed in a 2% agarose gel. The PCR product from the G allele was cut into two DNA fragments, of 85 base pair (bp) and 53 bp, while the C allele had a DNA bands of 138 bp in addition to a constant DNA band of 110 bp (Fig. 1). The polymorphic *Aci*I site was located to a nucleotide position 96 bp downstream from the PMM2 spot codon.

Allele frequencies. The distribution of genotypes and allele frequencies is shown in Table 1. The distribution of three genotypes did not differ significantly from the expected one

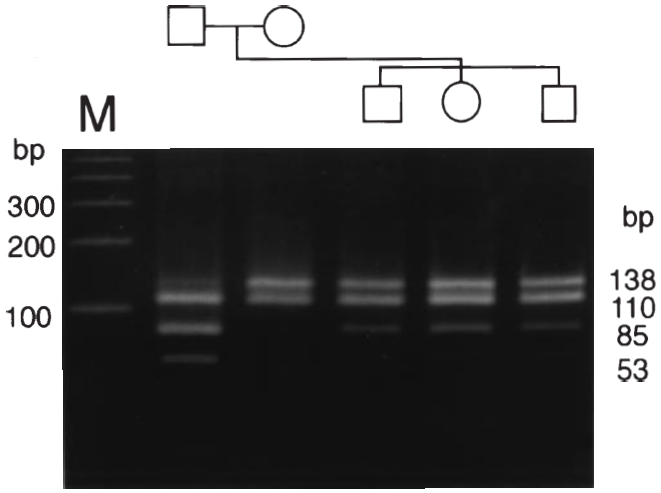

Fig. 1. Polymorphic genotypes at the 3′ untranslated region of the *PMM2* gene in a family. Genomic polymerase chain reaction (PCR) products after digestion with the restriction enzyme, *Aci*I, show three genotypes of the *PMM2* gene. The father in the family is homozygous for the G allele, which has two restriction sites in the PCR product, and has three DNA bands (110 bp, 85 bp, and 53 bp). The mother is homozygous for the C allele, resulting in the loss of one *Aci*I restriction site, and has two DNA bands (138 bp and 110 bp). Three siblings are heterozygous for these two alleles. *M*, 100-bp ladder DNA size marker

in Hardy-Weinberg equilibrium.

Chromosome localization. The human *PMM2*, gene has been assigned to chromosome 16p13.

Mendelian inheritance. Mendelian inheritance was confirmed in five families.

Table 1. Distribution of genotypes and allele frequencies of G/C polymorphic alleles in the *PMM2* gene in unrelated healthy Japanese

Genotypes	Observed no.	Expected no.
GG	39	40.5
GC	30	27.0
CC	3	4.5
Total	72	72.0

Allele frequencies; G = 0.750; C = 0.250
$\chi^2 = 1.056$; degrees of freedom (df) = 1; $P = 0.31$

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