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BRIEF REPORT—POLYMORPHISM REPORT

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Identification of a novel single base-pair polymorphism in the glutamate dehydrogenase (*GLUD1*) gene

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Abstract A novel single base-pair polymorphism, G/A at ntd 955, was identified within the coding region of the glutamate dehydrogenase gene (*GLUD1*). This polymorphism should prove useful for the study of human disorders with altered ammonia and/or blood glucose levels.

Key words Glutamate dehydrogenase (*GLUD1*) gene · Single base-pair polymorphism · Chromosome 10q

Introduction

Glutamate dehydrogenase (EC1.4.1.3) is a ubiquitously distributed mitochondrial matrix enzyme which catalyzes the reversible conversion of glutamate to α-ketoglutarate. The gene (*GLUD1*) spans about 45 kb on chromosome 10q21.1-q24.3 and is split into 13 exons (Michaelidis et al. 1993).

Unlike brain-specific glutamate dehydrogenase, deficiency of which leads to a form of neurodegenerative disease (Plaitakis et al. 1988), the role of *GLUD1* in human disease remained unknown until recently, when Stanley et al. (1998) identified activating mutations of *GLUD1* in patients with hyperinsulinism-hyperammonemia syndrome. Most of the mutations lie in the guanosine triphosphate (GTP) binding site of the gene, which is essential for the GTP-mediated allosteric inhibition of the enzyme. However, there are other mutations outside the GTP binding site, suggesting that different mutations affect the enzyme activity differently.

In a previous attempt to determine mutations in patients with hyperinsulinism-hyperammonemia syndrome, we identified a novel single base-pair polymorphism, G/A at ntd 955, within the coding region of *GLUD1* (Nakatani et al. 1988). Among the Japanese subjects studied, these two alleles showed similar gene frequency. Since this enzyme is a key enzyme connecting glutamate and energy metabolism, we believe this polymorphism should prove useful not only for the study of hyperinsulinism-hyperammonemia syndrome but also for the study of other disorders with altered ammonia and/or blood glucose levels.

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Polymerase chain reaction (PCR) conditions

Since the mutation does not alter pre-existing restriction sites of the gene, we designed a mismatched primer pair so that in the presence of the G allele the PCR products would contain an *Rsa*I restriction site.

Forward primer:
5′-CAGGGATTTGGTAATGTGGGCGT-3′
Reverse primer:
5′-CCATCAGACTCACCAACAGC-3′

PCR was performed in 5-μl reaction mixtures containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin, 200 mM of each dNTP, 5 pmol of each mismatch primer, and 0.125 units of AmpliTaq Gold DNA polymerase (Perkin-Elmer, NJ, USA). The cycling parameters consisted of initial activation at 94°C for 10 min followed by 30 cycles of denaturation at 94°C for 1 min, and annealing and extension at 53°C for 20 s. The PCR products were digested with *Rsa*I and electrophoresed on a 10% denaturing polyacrylamide gel.

Polymorphism and allele frequency

Figure 1 shows a representative result of the analysis. The allele frequencies of the polymorphisms among 33 Japanese individuals are shown in Table 1.

Table 1. Allele frequencies of the polymorphisms in 33 Japanese individuals

Allele	Frequency
G	0.59
A	0.41

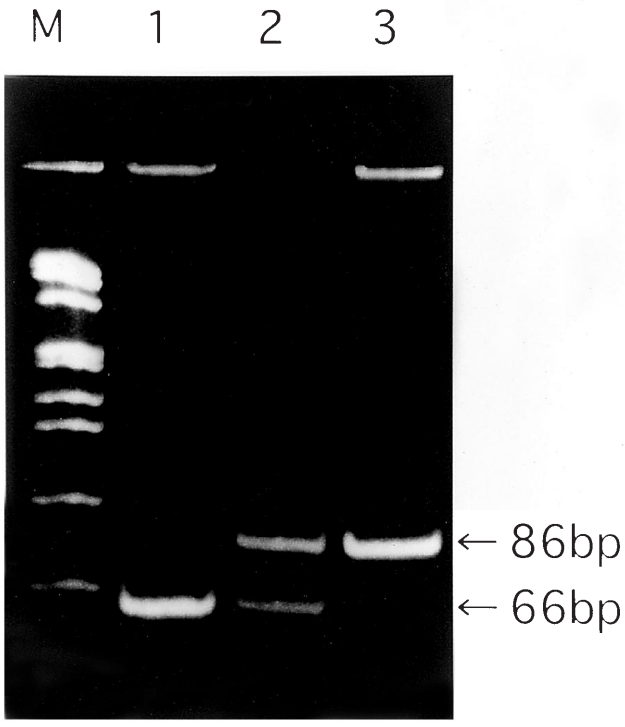

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Fig. 1. Representative result of the polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis. In the presence of the G allele, *Rsa*I digestion of the PCR products generated two fragments, 20- and 66-bp in size. With the A allele, *Rsa*I did not cleave the PCR product (86 bp). *Lane M*, ∅×174 DNA digested with *Hae*III; *lane 1*, homozygote for the A allele; *lane 2*; *lane 3*, homozygote for the G allele