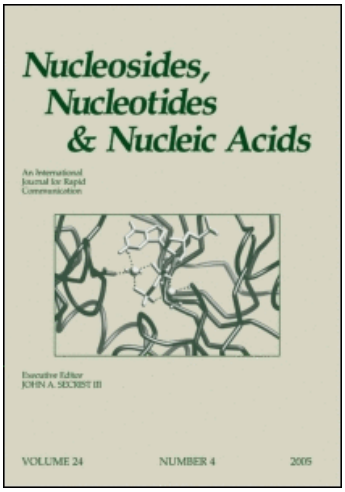

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Identification of Two Novel Mutations in Adenine Phosphoribosyltransferase Gene in Patients with 2,8-Dihydroxyadenine Urolithiasis

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ABSTRACT

Five mutations in the adenine phosphoribosyltransferase (APRT) gene have been described in Japanese patients with APRT deficiency. We investigated the APRT gene from three patients with APRT deficiency and two novel mutations, G133D and V84M, were determined.

Key Words: Adenine; Phosphoribosyl transferase; 2,8-Dihydroxyadenine.

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INTRODUCTION

Adenine phosphoribosyltransferase (APRT, EC2.4.2.7) is a purine salvage enzyme that converts adenine into AMP. Germline mutations in this gene in humans cause APRT deficiency and homozygotes develop 2,8-dihydroxyadenine (2,8-DHA) urolithiasis.[1] The largest number of patients has been found in Japan. Therefore, a total of five mutations has been identified in Japanese patients.[2] However, the mutation leading to loss of APRT activity remains to be determined in several alleles. In this report, we describe analysis of genomic DNA from three Japanese patients with APRT deficiency. This study allows to identify two novel missense mutations in the *APRT* gene.

PATIENTS AND METHODS

Patient A was a 72-year-old male and has been passing a number of urolithiasis since he was twenty years old. He suffered from chronic renal failure. Patient B was a 43-year-old male. Examinations showed hydronephrosis of the left kidney due to ureteral stones and shock wave lithotripsy was done. Patient C was a 52-year-old male and was passed a urolithiasis. An ultrasonographic examination demonstrated many small stones in both kidneys. All of the three patients were diagnosed to have 2,8-DHA urolithiasis by infrared spectroscopy. A diagnosis of homozygosity for APRT deficiency was demonstrated by resistance of proliferating T cells to 2,6-diaminopurine.[3]

Genomic DNA was extracted from blood. Nucleotide −392 to 2458 of the genomic human APRT gene (the A of the initiation codon ATG was counted as 1) was fully amplified in five fragments and sequenced by an automated DNA sequencer.

RESULTS

Sequence analysis of the PCR fragments showed that Patient A was homozygous for G to A transition at the nucleotide position 1359 (R67Q). The base substitution found in Patient A alters Ava I site and generates Sty I site within the amplified genomic region (Fig. 1).

Patient B was homozygous for G to A transition at the nucleotide position 1831 (G133D) which was confirmed by digestion of amplified DNA with Bsr I (Fig. 2).

In Patient C, two kinds of mutation were detected by sequencing analysis; G to A transition at nucleotide position 1409 (W98X) and G to A transition at nucleotide position 1453 (V84M). W98X mutation was determined as described elsewhere[4] (data not shown) and V84M mutations were confirmed by digestion of the PCR product with BsiHKA I and Sph I (Fig. 3). LDSUPPORT which was a computer software based on expectation–maximization algorithm was applied to estimate the diplotype configuration of Patient C[5] and it was confirmed that the Patient C was compound heterozygous for W98X and V84M.

Among 93 control DNA samples, the three mutations were not detected, suggesting that these are not a common polymorphism.

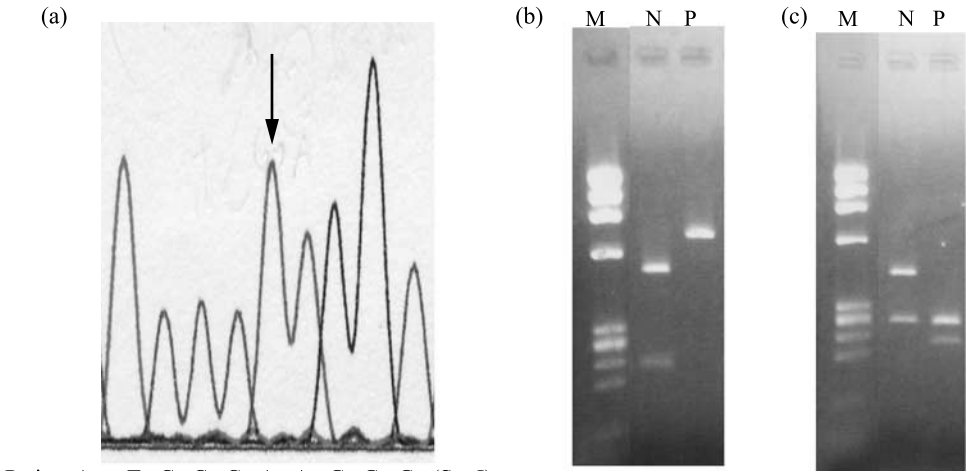

Figure 1. Sequence analysis of Patient A showed G to A transition at the nucleotide position 1359 (a). The amplified DNA was subjected to digest with *Ava I* (b) and *Sty I* (c). The PCR product for Patient A were resistant to cleavage with Ava I and was cleaved with Sty I, indicating Patient A was homozygous for the mutation. (M: molecular marker, phiX174 cleaved with *Hae III*, N: normal control, P: Patient A.)

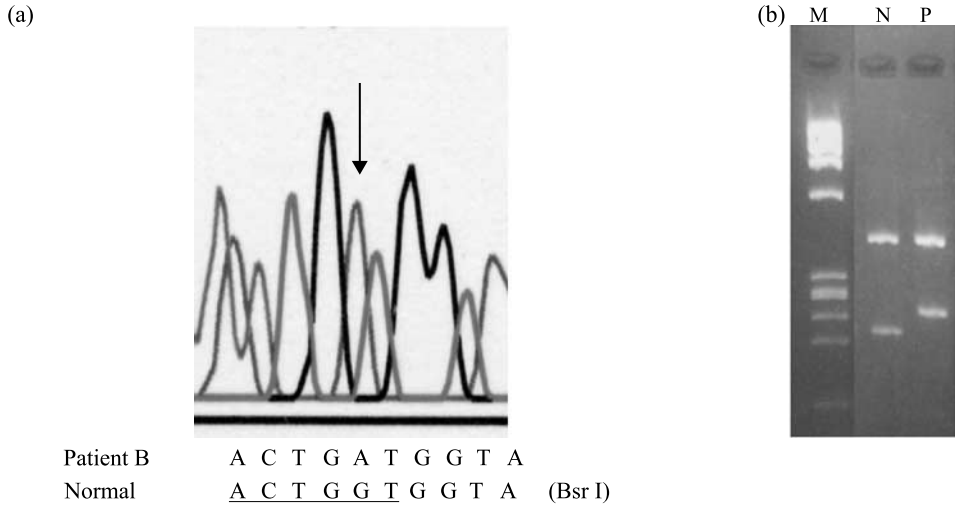

Figure 2. Sequence analysis of Patient B showed G to A transition at the nucleotide position 1831 (a). The PCR product was subjected to digest with *Bsr I* (b). The PCR product for Patient B was resistant to cleavage, indicating Patient B was homozygous for the mutation. (M: molecular marker, phiX174 cleaved with *Hae III*, N: normal control, P: Patient B.)

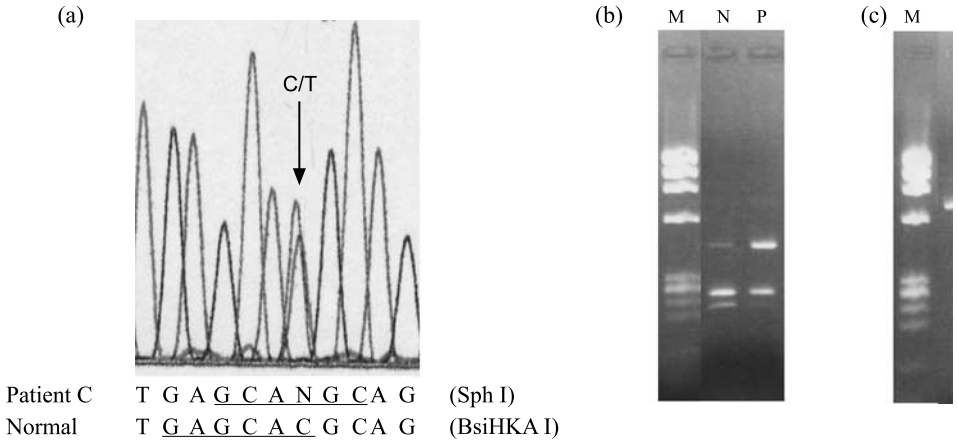

Figure 3. Two mutations were revealed by sequence analysis in Patient C. G to A transition at nucleotide position 1453 was determined according to Ref. [4]. G to A transition at nucleotide position 1409 (a) were confirmed by digestion of amplified DNA with *BsiHKA I* (b) and *Sph I* (c), indicating Patient C was heterozygous for the mutation. (M: molecular marker, phiX174 cleaved with *Hae III*, N: normal control, P: Patient C.)

DISCUSSION

In this study, R67Q, G133D and V84M mutations were detected at APRT locus in Japanese patients with APRT deficiency. Among them, R67Q mutation has already been reported in USA.[2] Thus, G133D and V84M are novel mutations. Although experiments with expression of the mutant enzymes are in progress, the crystal structure of APRT will give some information about the functional significance of these mutations. The crystal structure of human APRT has not been determined, but the structures of some microorganisms have been reported. R67 is one of the conserved amino acid[6] and the equivalent *G. lamblia* APRT residue, R63, is suggested to be involved in hydrogen bonds with adenine and pyrophosphate.[7] G133 is also one of the conserved amino acid, and the equivalent *G. lamblia* APRT residue, G130, is located in the 5′-phosphate binding loop.[7] V84 is not a conserved amino acid, but is supposed to be located near the dimer interface.[6] These findings suggest that the three mutations, R67Q, G133D and V84M, may affect the enzymatic activity.

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