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# A Mutant Allele Common to the Type I Adenine Phosphoribosyltransferase Deficiency in Japanese Subjects

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## Summary

**Adenine phosphoribosyltransferase (APRT) deficiency is a genetic disorder which causes 2,8-dihydroxyadenine urolithiasis. The estimated incidence of heterozygosity in Caucasian and Japanese populations is 1%. Mutant alleles responsible for the disease have been classified as *APRT*Q0* (type I) and *APRT** (type II). In our previous study, we demonstrated in *APRT*J* a single common base change which accounts for 70% of the Japanese mutants. The present report describes the analysis of an *APRT*Q0* mutation in Japanese subjects. Two nucleotide substitutions common to all seven affected alleles from four unrelated subjects (three homozygotes and a heterozygote) were identified: G→A at nucleotide position 1453 and C→T at 1456. The G→A altered the amino acid $Trp^{98}$ to a stop codon. The C→T did not alter $Ala^{99}$. These point mutations were demonstrated by sequence analysis of polymerase chain reaction (PCR)–amplified genomic DNA and cDNA. The G→A change at 1453 results in the elimination of a *Pfl*MI site in the APRT gene. *Pfl*MI digests, which were used to confirm the G→A transition, can be useful in screening for this specific mutation.**

## Introduction

Adenine phosphoribosyltransferase (APRT) is a purine salvage enzyme which is widely distributed in tissues. Deficiency of the enzyme results in the accumulation of both adenine and its relatively insoluble derivative 2,8-dihydroxyadenine (2,8-DHA), which can cause nephrolithiasis (Simmons et al. 1989). The APRT gene encodes for 180 amino acids and is located on chromosome 16 (Tischfield and Ruddle 1974). Its complete nucleotide sequence has been determined (Broderick et al. 1987, Hidaka et al. 1987*b*). Deficiency of the enzyme is inherited in an autosomal recessive mode. Since the first description of APRT deficiency in a heterozygote (Kelley et al. 1968), more than 60 homozygotes have been reported (Simmons et al. 1989). APRT deficiency is a genetic disorder, with an estimated allelic frequency at approximately 1% in the Caucasian (Simmons et al. 1989) and Japanese populations (Kamatani et al. 1987, 1988).

At the present time, two types of APRT deficiency have been described (Simmons et al. 1989). Type I (*APRT*Q0*) is characterized by no detectable APRT activity. Type II (*APRT*J*) demonstrates both an altered $K_m$ value for phosphoribosylpyrophosphate (PRPP) and significant APRT activity in in vitro assays of cellular lysates (Fujimori et al. 1985, Kamatani et al. 1987).

Thus far, the type II deficiency has been found exclusively in Japanese subjects. These patients are considered homozygotes with *APRT*J/APRT*J* or compound heterozygotes with *APRT*J/APRT*Q0*. This type accounts for 80% of the deficiencies in this population (Kamatani et al. 1987, 1988). The nucleotide change responsible for *APRT*J*'s characteristic phenotype has been determined (Hidaka et al. 1988). The present report supported the possibility that these $K_m$ mutants have a common progenitor.

In contrast, type I deficiency, to which all the Cauca-

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sian and 20% of the Japanese subjects have been assigned, represents a heterogeneous collection of mutations. Protein studies in Caucasian subjects have demonstrated that these mutations are heterogeneous (Simmons et al. 1989). Thus far, two different mutant alleles have been established from one "Caucasian" family (Hidaka et al. 1987*a*).

In the present report we describe in Japanese subjects an *APRT**Q0* (type I) mutation at the nucleotide level. We examine and demonstrate a mutant allele common to four unrelated subjects.

## Material and Methods

### *Cell Lines*

Cell lines WR10, WR15, WR128, and WR158, from four unrelated Japanese subjects, were established from the transformation, by the Epstein Barr virus, of peripheral B lymphocytes (Kamatani et al. 1987). Records show that the families of the subjects have long resided on three separate islands (Kyushu, Shikoku, and Honshu) in Japan. With routine activity assays, homozygous type I (*APRT**Q0*) deficiency was found in WR15, WR128, and WR158 (O'Toole et al. 1983). WR10 was derived from the mother of a patient with type I deficiency (Nobori et al. 1986). APRT assays on these cell lines were performed according to a method described elsewhere (O'Toole et al. 1983).

### *PCR Amplification*

The polymerase chain reaction (PCR) (Mullis and Faloona 1987; Saiki et al. 1988) was used to amplify the APRT gene. An automated DNA thermocycler and *Taq* polymerase were used (Perkin Elmer–Cetus, Norwalk, CT). All primers were obtained from the University of Michigan Oligonucleotide Synthesis Facility. Because of the low yield of amplified DNA, PCR reaction conditions were modified by the addition of 7-deaza-2′-deoxyguanosine-5′-triphosphate (C7-deaza-dGTP; Pharmacia, Piscataway, NJ) (McConlogue et al. 1988). Thirty cycles of PCR were performed, although annealing and extension conditions differed as noted in table 1.

Amplification of the entire 2.4-kb APRT gene from genomic DNA of WR15 required denaturation at 94°C for 1 min 15 s, followed by an extension step at 72°C for 6 min. Primer AP17 and primer AP22 were used. Partial APRT gene amplification was also performed on WR10, WR15, WR128, WR158, and WR173 (a normal cell line with normal APRT activity) by using primers AP03 and AP12. These amplifications required denaturations at 94°C for 1 min 15 s, annealing at 55°C for 1 min, and extensions at 72°C for 2 min.

Total cytoplasmic RNA, extracted from cultured WR15 B lymphoblasts, were used in the preparation of first-strand cDNA (Gubler and Hoffman 1983). These cDNAs were amplified using primers AP22 and AP01 (Davidson et al. 1989). The thermoprofile was 30 cycles of 94°C for 1 min 15 s, and 72°C for 3 min.

### *Cloning*

The amplified APRT gene of WR15 was isolated from 0.8% low-melting-point agarose, was purified, and was digested with *Eco*RI. The purified digestion product was then ligated into λ-gt10 (Promega, Madison, WI). After phage plaques were screened, 40 APRT positive clones were obtained. Five of these were subcloned into pGEM 3Zf plasmids (Promega, Madison, WI) and designated 1, 2, 3, 4, or 5. These clones were subcloned into the M13 vector for sequencing.

### *DNA Sequencing*

Single-stranded DNA, derived from the M13 or pGEM clones, was sequenced using the Sanger et al. (1977) method, [α-$^{35}$S] dATP, and PCR primers or M13 universal primer. Direct sequencing of the genomic DNA fragments, amplified with AP03 and AP12, was

**Table 1**

**PCR Primers**

| Primer | Nucleotide Position | Sequence[a] |
|---|---|---|
| AP 01 | −34 to −4 | 5′-CGCTCGGGCTGCCGCTGGCTCTTCGCACGCG-3′ |
| AP 03 | 1405 to 1423 | 5′-CTGCGTGCTCATCCGAAAG-3′ |
| AP 12 | 1880 to 1864 (antisense strand) | 5′-CACCAGGCCCTTGGAGC-3′ |
| AP 17 | −163 to −134 | 5′-<u>GAATTG</u>ATGGCGCCTAGGAGTCCATGAAATACGG-3′ |
| AP 22 | 2250 to 2218 (antisense strand) | 5′-<u>GAATTC</u>CTGGGATCCAGCTGGAGATGTTGGGCTGG-3′ |

[a] *Eco*RI sites (underlined) were attached to primers.

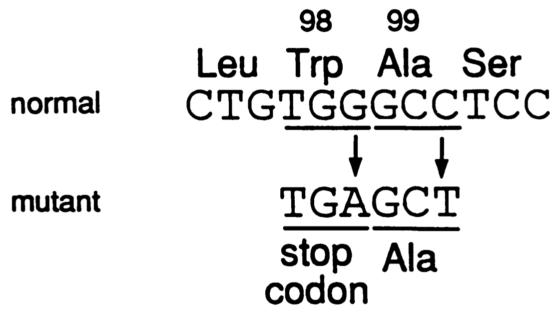


**Figure 1** Nucleotide changes in exon 3, shown with their corresponding amino acids. Direct sequencing showed that the homozygotes WR15, WR158, and WR128 had the mutant sequences, whereas the heterozygote WR10 had both the normal sequence and the mutant sequence.

performed using AP03 as the sequencing primer. The sequencing of amplified cDNA used AP03 and AP22. Primer end labeling and sequencing were performed according to methods described elsewhere (Davidson et al. 1989).

*Southern Blot*

Five micrograms of genomic DNA were digested with 50–100 units of each restriction enzyme, were electrophoresed, and were transferred onto nylon filters (Zeta probe; Bio-Rad, Richmond, CA). These were probed with an APRT genomic DNA probe, which had been radiolabeled by random priming (Sambrook et al. 1989).

*RNase Mapping*

The 331-bp antisense-mRNA probe pGP1, which spans from the 3′ end of exon 2 to the 5′ end of exon 5, was labeled with [α-$^{32}$P]GTP. Total cytoplasmic RNA (10–50 μg from each cell line) was hybridized with the probe and was RNase digested, electrophoresed and autoradiographed according to methods described elsewhere (Hidaka et al. 1987*a*).

## Results

Southern blot analysis of WR15 genomic DNA digested with *Bam*HI, *Bgl*II, *Cla*I, or *Taq*I, showed banding patterns identical to that of normal controls (data not shown). These data indicated that there was no major deletion or insertion in WR15.

Of the five clones that were analyzed, clones 1 and 2 were sequenced over the entire 2.4-kb APRT gene. For clones 3–5, 1,000 bases of sequence data, covering exon 3 and exon 5 and parts of IVS 2 and IVS 3, were obtained. Sequencing data were compared with the normal APRT sequence (Hidaka et al. 1987*b*). Four nucleotide substitutions were identified in all five clones. These were a G→A substitution at position 1453 (exon3), a C→T at 1456 (exon3), a C→T at 1187 (IVS 2), and a G→A at 1654 (IVS 3). There is evidence that the point mutations found on IVS 2 and IVS 3 may be polymorphic sites, as these were independently observed in WR128 (Wu 1990). Seven separate nucleotide changes were observed in the intronic sequence of the clones, but these mutations were unique to each clone (data not shown). These unique nucleotide changes appear to be artifacts, which occurred during either PCR or cloning. The three nucleotide transitions that were found in all of the clones were confirmed by the direct sequencing of amplified genomic DNA of WR15 (fig. 1; IVS data not shown). The nucleotide change at position 1187 (IVS2) was not examined by direct sequencing. Amplified cDNA was also directly sequenced. The two point mutations in exon 3 were confirmed.

Genomic DNA from WR10 (heterozygote) and WR158 (homozygote) were amplified by PCR using AP03 and AP12 and then directly sequenced. WR158 was shown to have the same two point mutations in exon 3. The autoradiograph of WR10's sequence showed two bands, at nucleotide positions 1453 and 1456 (not shown). The normal and mutation bands were both represented on the autoradiograph. Both alleles were amplified and had been sequenced simultaneously. These point mutations were not found on the amplified genomic DNA from normal cells (fig. 1) and thereby ruled out the possibility of systematic PCR artifacts.

The mutation at position 1453, which would generate a stop codon, alters one of the *Pfl*MI restriction-enzyme sites within the APRT gene (fig. 2). The genomic

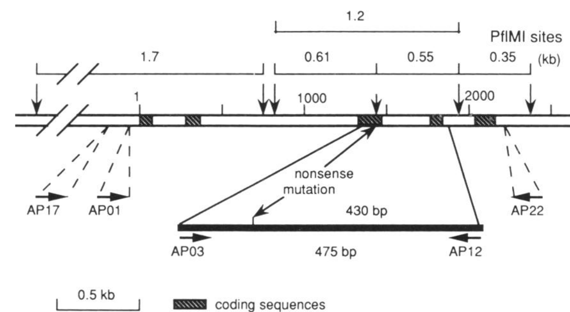


**Figure 2** Restriction map of APRT gene, PCR primer sites, and mutation site. Restriction sites of *Pfl*MI (↓), PCR primers, (→, ←), and the mutation site that generates the stop codon (*) are shown, including the coding and intervening sequences.

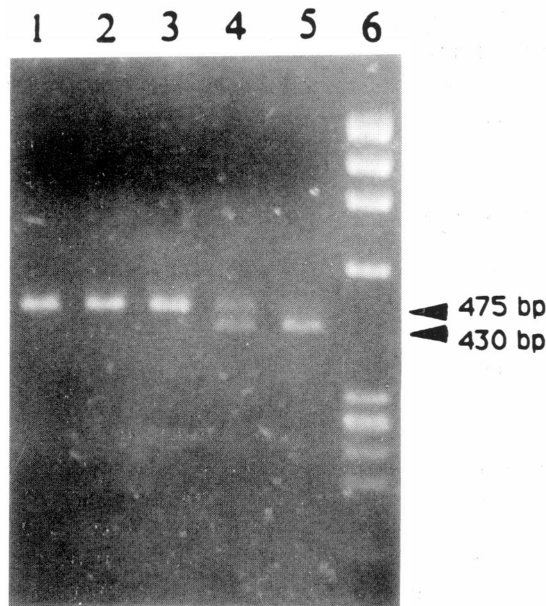


**Figure 3** Genomic DNA amplified by PCR with AP03 and AP12. The resulting 475-bp PCR product was purified, digested, and electrophoresed. The mutant alleles remained undigested, while the normal alleles produced 430-bp and 45-bp (invisible) bands. Lane 1, WR15(homozygote). Lane 2, WR128(homozygote). Lane 3, WR158(homozygote). Lane 4, WR10(heterozygote). Lane 5, Normal. Lane 6, Φx174 digested with *Hae*III.

DNAs from the cell lines WR10, WR15, WR128, and WR158 were amplified with AP03 and AP12. The resulting 475-bp fragment was digested with *Pfl*MI, electrophoresed, and ethidium bromide stained. The staining produced discrete bands which enabled the discrimination between homozygotes with the mutation in each allele and the heterozygote with one normal and one mutated allele (fig. 3). Southern analysis of *Pfl*MI digestion of genomic DNA confirmed the above results (not shown).

RNase mapping analysis showed reduced intensity of mRNA bands of WR15, WR128, and WR158. When compared with the normal cell line WR173, the intensity was estimated to be one tenth of normal. The band intensity of the heterozygote WR10 was near normal. The predicted cleavage bands were not detected (data not shown).

## Discussion

RNase mapping, for the identification of the base substitutions, proved to be inconclusive. However, the quantity of APRT-mRNA in the mutant cells was found to be reduced. Our study showed that the mutant gene had no abnormality in the 5′ noncoding sequences up to −133. This covers the region necessary for the expression of the APRT gene, according to previous reports (Stambrook et al. 1984; Broderick et al. 1987). A normal size mutant cDNA was synthesized using oligo-dT as a primer, followed by PCR amplification. It has been reported that premature termination by nonsense mutations might cause rapid mRNA decay (Brawerman 1989). It is still possible that the nucleotide changes existing outside our area of investigation result in the reduction of the mRNA level, but this decrease cannot explain the complete APRT deficiency. In a previous study, no cross-reacting material (CRM) was present in the cell extracts from WR15 (Kamatani et al. 1989). This did not exclude the presence of a truncated peptide, since the antibody used in that study detects the amino acid sequence that would be missing in the mutant protein. The predicted mutant protein would lack the putative PRPP binding region (Broderick et al. 1987) and thus would be nonfunctional.

We found that the use of C7-deaza-dGTP, as suggested by McConlogue et al. (1989), greatly improved the amplification yield, although it was still relatively low. The relatively low efficiency of our PCR amplification ($10^3$) for the entire 2.4-kb APRT gene might be explained by the GC-rich sequences found in the 5′ region of the gene (Broderick et al. 1987; Hidaka et al. 1987*b*). Nevertheless, sufficient amounts of amplified DNA were obtained, which enabled faster construction of the genomic DNA clones than is possible with conventional methods.

It has been reported that errors in polymerization by *Taq* polymerase occur at an approximate rate of $2 \times 10^{-4}$/base/duplication (Saiki et al. 1988; Keohavong and Thilley 1989). Therefore, one would predict 6 errors/kb for each 30-cycle amplification. The error rate on the APRT genomic DNA, after sequencing of 8,000 bases, was found to be lower. This difference could be attributed in part to differences in the template sequence or to the low amplification efficiency of our PCR. The effective amplification might have been lower than 30 cycles. It has been found that accidental errors made during amplification can be averaged out and may be undetected when direct-sequencing methods are used (Saiki et al. 1988). We could observe base differences between the two alleles of the heterozygote WR10 by direct sequencing, assuring authenticity of the sequences.

This mutant allele can easily be identified by the PCR amplification of the APRT gene, followed by digestion

with *Pfl*MI. Further studies should be able to determine more accurate frequencies of this mutant allele in various populations of Japan and other Asian countries. According to the data on the type I deficiency in a previous report (Kamatani et al. 1988), the frequency at which the mutant allele presented here occurs among the Japanese population is expected to be high.

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