Identification of a common nonsense mutation in Japanese patients with type I adenine phosphoribosyltransferase deficiency

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In the absence of adenine phosphoribosyltransferase (APRT) activity in man, adenine is oxidized by xanthine oxidase to the highly insoluble and nephrotoxic derivative, 2,8-dihydroxyadenine (DHA). Two phenotypic variants of APRT deficiency have been recognized (1). Type I deficiency (complete enzyme deficiency) has been observed in many different countries, but type II deficiency (complete deficiency *in vivo* but partial deficiency in cell extracts) is found only in Japan. Type II deficiency has a common ancestral origin and is the main cause (about 75%) of DHA stone formation in the Japanese (2). The majority of Type II patients is homozygous for a missense mutation in exon five and for the 2/2 *TaqI* RFLP in intron two (3), but they show two different patterns with respect to a remote *SphI* RFLP site (4). The Type II mutant alleles are designated *APRT*J* (2). Two non-Japanese brothers with Type I deficiency are compound heterozygotes for *APRT*QO* null alleles (5), but they are homozygous for the 1/1 *TaqI* pattern (6).

We have analyzed the molecular nature of the mutation in two Japanese patients, a thirteen-year-old Type I male (ASA1) from a consanguineous marriage, and a sixteen-year-old male (TAK2) who exhibits the Type II phenotype but has one *APRT*J*-type and one *APRT*QO*-type allele (Sahota *et al.*, submitted). ASA1 displays the 1/1 *TaqI* RFLP whereas TAK2 displays the 1/2 pattern. ASA1 has no detectable APRT activity in lymphoblast extracts, but TAK2 has 25% of the activity of our control range (584–633 nmol/h/mg). Cultured lymphoblasts from neither patient showed functional APRT activity as indicated by: (i) complete resistance to growth inhibition by 2,6-diaminopurine; (ii) inability to take up exogenous adenine; and (iii) lack of growth in adenine-azaserine-alanosine medium.

Genomic DNA was isolated from cultured lymphoblasts and a 2.4 kb fragment containing the coding region and the flanking sequences of the *APRT* gene amplified by the polymerase chain reaction (PCR). The PCR product was subcloned into M13 and three positive clones from each patient were completely sequenced to determine the consensus gene sequence and to correct for PCR errors. An A-to-T transversion at base 1449 was identified in ASA1. This mutation leads to the replacement of trp₉₈ (TGG) by a nonsense codon (TGA) in exon three. A silent base substitution at position 1453 (GCC to GCT, ala₉₉) was also found. In addition, C-to-T and G-to-A transitions were observed

at positions 1184 (intron two) and 1651 (intron three), respectively. The A of the ATG start codon is designated 1 with respect to our wild-type sequence (Chen *et al.*, submitted). The homozygous nature of the base substitutions at amino acid positions 98 and 99, intron polymorphisms, and the *TaqI* polymorphic site in ASA1 were confirmed by direct sequencing and *TaqI* digestion of PCR DNA. The same mutation and polymorphisms were found in the *APRT*QO* allele from patient TAK2. The other allele in this patient was of the *APRT*J* type, including the 2/2 *TaqI* RFLP pattern. The *APRT*J* allele from this patient also showed the G-to-A substitution at position 1651. We have observed this base change in the DNA from all the Japanese and non-Japanese APRT-deficient patients we have sequenced, suggesting that our wild-type sequence is a rare variant at this position.

The exon three mutation and the silent substitution identified in the *APRT*QO* allele from ASA1 and TAK2 have also been found in three other Type I Japanese patients, and the intron polymorphisms have been identified in at least one of these patients (7). This nonsense mutation has not been observed in ten non-Japanese Type I patients we have investigated so far. These findings suggest that this mutation is a common cause of Type I APRT deficiency among the Japanese and that, as for Type II deficiency, it is likely to have a common ancestral origin. More than fifty-three patients with Types I and II APRT deficiency have been diagnosed in Japan (4). It is likely that many more remain undiagnosed, possibly due to the asymptomatic nature of the defect in some patients and the occasional difficulty of distinguishing DHA from uric acid stones (1). The finding of such a large number of patients suggests that some APRT mutant alleles may confer an evolutionary advantage to homozygotes and/or heterozygotes under certain circumstances.

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