Missense mutation in the adenine phosphoribosyltransferase gene causing 2,8-dihydroxyadenine urolithiasis

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Received December 14, 1993; Revised and Accepted March 4, 1994

Adenine phosphoribosyltransferase (APRT) catalyzes the synthesis of AMP from adenine and 5-phosphoribosyl-1-pyrophosphate. Deficiency of APRT can lead to an autosomal recessive disorder called 2,8-dihydroxyadenine (2,8-DHA) urolithiasis (1). The APRT gene is located at 16q24.3. A number of point mutations and small deletions and insertions have been identified in the APRT gene as a cause of 2,8-DHA urolithiasis (2). Here we report a missense mutation (CTG-to-CCG, L110P) in APRT in a patient with 2,8-DHA urolithiasis from Newfoundland. A younger sister has the same mutation but she is disease-free.

SF had an attack of renal colic with hematuria in 1981 at the age of 42 (3). She underwent a right pyelolithotomy and a small calculus, identified as 2,8-DHA, was removed from the renal pelvis. She has been on allopurinol (600 mg/day) for the past 13 years without any further evidence of urolithiasis. A younger sister (BB), then aged 23, excreted 2,8-DHA and adenine in the urine but there was no radiologic evidence to suggest stone disease. She has been on allopurinol (300 mg/day) without ever having urolithiasis. In hemolysates, both SF and BB exhibited <1% of normal APRT activity and <1% of cross-reacting material to anti-APRT antibodies (4). The parents, four of the 12 siblings in the SF sibship, and all four children of SF, had enzyme activity in the heterozygous range. Consanguinity has been denied by the family.

Transformed lymphoblast cell lines, designated GM07290 and GM07291, respectively, were established from blood samples from BB and SF, and they have been deposited with the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ). The family lives in various parts of Newfoundland, often long distances from St Johns, and it has not been possible to obtain blood samples from the parents and siblings of SF and BB, or the children of SF.

DNA was isolated from cultured lymphoblasts from SF and BB, and it was digested with SphI, transferred to a GeneScreen Plus membrane, and probed with the 2.2 kb BamHI fragment of the APRT gene (5). A 2.4 kb genomic fragment containing the entire coding region and the flanking sequences of APRT was amplified by PCR (6). PCR-amplified DNA was digested with TaqI, electrophoresed on an agarose gel, and then stained with ethidium bromide (5). PCR-amplified DNA from SF was subcloned into M13mp18 and sequenced completely (6). Selected regions of PCR DNA from SF and BB were also sequenced directly.

SF and BB were homozygous for the 8 kb SphI RFLP (Fig. 1) and for the 1.25 kb TaqI RFLP. The 1.25 kb fragment seen in TaqI digests of PCR-amplified DNA is equivalent to the 2.1 kb fragment seen in genomic digests (7). The polymorphic SphI site is outside APRT and is 2.8 kb upstream of the polymorphic TaqI site, which is located within intron 2 of APRT (6). A T-to-C transition was found at position 1759 in several clones from SF (Fig. 2). The APRT sequence is numbered with respect to the A of the ATG initiation codon as base 1 (6). The base change was confirmed by direct sequencing of PCR-amplified DNA from SF and from BB. No other sequence changes were found in the 2.4 kb fragment. The observed change is expected to lead to a leu-to-pro substitution in codon 110 (CTG-to-CCG, L110P) in exon 4.

The above data suggested that the sisters were homozygous for a mutant APRT allele, but the possibility of hemizygosity could not be ruled out. In an attempt to resolve this question in this, and other APRT-deficient families, we have initiated a search

Figure 1. An SphI Southern blot. Lane 4 is DNA from SF. Lanes 1–3 and 5 are DNAs from other APRT-deficient patients. The fragment size for BB (not shown) was identical to that for SF.

Figure 2. A part of a DNA sequencing gel showing the T-to-C substitution in APRT at position 1759. The wild type sequence is on the left and the mutant sequence is on the right.

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for markers flanking APRT that can be assayed by PCR. Fourteen such markers (6 upstream of the gene and 8 downstream) have been identified (Boyadjiev et al., unpublished data). The sizes of the upstream and downstream regions examined were 1.2 kb and 1.8 kb, respectively. DNA from one of the sisters (BB) was analyzed for six of the downstream markers, but she was not heterozygous for any of these markers. DNA from BB was then analyzed using three microsatellite markers for chromosome 16 (D16S303, D16S305, and D16S308) flanking APRT (8), and she was heterozygous for all three markers (data not shown). D16S303 is 3 cM distal to APRT, D16S305 is 5 cM proximal to APRT, and D16S308 is close to the centromere (8). BB thus has both copies of chromosome 16q and she and her sister are most likely to be homozygous for a region including and flanking APRT. The origin of the mutant allele is not known, but the family ancestors most likely came from southern England in the 1700s.

The loss of APRT activity, and the resulting 2,8-DHA urolithiasis in SF, indicate that the observed base change is a mutation and not a polymorphism. BB has the same mutation and excretes 2,8-DHA and adenine in the urine, but she is disease-free. Analysis of urine samples from many APRT-deficient families has shown that the excretion of 2,8-DHA and adenine occurs only in individuals homozygous for APRT deficiency, and not in heterozygotes or in normals (1). The one published exception to this is an APRT heterozygote from Japan (9). This individual has a mutation in one APRT allele but he has been reported to have passed 2,8-DHA calculi. SF has a severe clinical phenotype and BB, with the same mutation, has no clinical phenotype. This phenomenon has also been observed in a number of other APRT-deficient families (1).

Two APRT mutations were first identified in a family with 2,8-DHA urolithiasis from Belgium (10). Since then, 14 mutations have been identified in APRT-deficient patients of non-Japanese origin (2). Four mutations have been described in Japanese patients, and three of these account for 96% of the mutant APRT alleles in that population (11). Nine of the 18 APRT mutations are located in exon 3 and two mutational hot spots have been identified, one in this exon and one at the intron 4 splice donor site (12). Twelve mutations, including the mutation described here, are due to single base pair changes in DNA (3 transversions and 9 transitions). Point mutations are thus the major cause of mutation in APRT. The other six mutations included three small insertions (1–4 base pairs) and three small deletions (2–7 base pairs) (2).

ACKNOWLEDGEMENTS

We thank SF and BB for providing blood samples for this study. This work was supported by NIH grant DK38185.

REFERENCES