Human Adenine Phosphoribosyltransferase

Identification of Allelic Mutations at the Nucleotide Level as a Cause of Complete Deficiency of the Enzyme

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Abstract

This study reports the first demonstration of specific mutations leading to human adenine phosphoribosyltransferase (APRT) deficiency. The molecular basis of the deficiency was investigated by determining the sequence of both alleles of a patient with a complete deficiency in APRT activity. A trinucleotide deletion, corresponding to phenylalanine on the deduced amino acid sequence, was confirmed on one allele. A single nucleotide insertion, immediately adjacent to the splice site at the 5′ end of the fourth intervening sequence, was confirmed on the other allele. This insertion lead to aberrant splicing, as was demonstrated by the absence of exon 4 in the complementary DNA sequence and by altered RNase mapping analysis of the abnormal messenger RNA.

Introduction

The first report of adenine phosphoribosyltransferase (APRT)[1] deficiency by Kelley et al. (1) described four members of one family over three generations. These subjects were shown to be heterozygous for APRT deficiency and had no symptoms attributable to the enzyme defect. Subsequently, more than 20 affected families have been studied by groups in Asia, Australia, Europe, and North America and the incidence of heterozygosity at this locus has been estimated to be from 0.42 to 1.21%, with the highest occurrence reported in Japan (2–5).

A homozygous deficiency of APRT was first reported by Cartier et al. (6) in a patient who passed a 2,8-dihydroxyadenine urinary stone. Since then, over 30 patients with 2,8-dihydroxyadenine urolithiasis associated with APRT deficiency have been reported in Austria, Belgium, Canada, England, France, Japan (reviewed in reference 7), and the United States (8). More than 20 of these cases have been found in Japan (reviewed in reference 5). All families studied to date have exhibited a familial pattern consistent with an autosomal recessive mode of inheritance.

APRT is a purine salvage enzyme which catalyzes the conversion of adenine to 5′-AMP in the presence of 5-phosphoribosyl-1-pyrophosphate (5-PRPP) and magnesium. Adenine supplied from diet, and from the polyamine pathway, is converted to AMP in the reaction catalyzed by APRT. In subjects with APRT deficiency, adenine is not salvaged to AMP and the only metabolic pathway available for adenine is through its oxidation to 8-hydroxyadenine and subsequently to 2,8-dihydroxyadenine in reactions catalyzed by xanthine oxidase (9). Both of these products are relatively insoluble in urine and thus precipitate, leading to the formation of urinary stones in many affected patients (10).

APRT has recently been the focus of considerable investigation at the molecular level. Human APRT has been purified and sequenced (11, 12). It is a dimer composed of two identical subunits. The subunit has 179 amino acid residues with a calculated molecular weight of 19,481. The normal human APRT gene has also been cloned and sequenced (Hidaka, Y., S. A. Tarlé, T. E. O'Toole, W. N. Kelley, and T. D. Palella, submitted for publication) (13). The human APRT gene is ~ 2.5 kilobase (kb) in length and consists of five exons. The coding region on the complementary DNA (cDNA) is 540 nucleotides in length, which corresponds to 180 residues on the deduced amino acid sequence including the NH_2-terminal methionine, which is cleaved as the protein is processed (Hidaka, Y., S. A. Tarlé, T. E. O'Toole, W. N. Kelley, and T. D. Palella, submitted for publication). The APRT gene is located on chromosome 16 (14).

The molecular basis of the APRT-deficient state, however, has not been clarified. Attempts to study the mutant APRT protein have been hampered significantly since none of the homozygous-deficient subjects identified to date have had enough mutant protein present to allow purification and sequencing (15, 16). The APRT protein present in the heterozygotes studied appears to be only the product of the normal allele. While a restriction fragment length polymorphism has been identified in genomic DNAs from APRT-deficient families (17), no studies have been carried out to define directly the nature of the mutations leading to APRT deficiency.

In this study we report the isolation, characterization, and sequence of mutant APRT genes using genomic DNA and cDNA from the lymphoblast cell line derived from a patient with complete APRT deficiency. The nucleotide sequence was compared with that of normal APRT, and revealed different mutations on each allele that are responsible for severe APRT deficiency in this family.

Methods

Materials. Restriction enzymes were purchased from New England Biolabs, Beverly, MA, Bethesda Research Laboratories, Gaithersburg, MD, or International Biotechnologies, Inc., New Haven, CT. T4 DNA ligase was from New England Biolabs. Nucleotide reagents and Klenow fragment were from Pharmacia Fine Chemicals, Piscataway,

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1. *Abbreviations used in this paper:* APRT, adenine phosphoribosyltransferase; IVS, intervening sequence.

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NJ. Lambda gt10 and gt11 arms and packaging extract were from Vector Cloning Systems, San Diego, CA. Ribonuclease inhibitor, SP6 RNA polymerase, DNase, and pGEM-3blue plasmid were from Promega Biotech, Madison, WI. RNaseA and RNaseT1 were from Sigma Chemical Co., St. Louis, MO. [α-^{32}P]dCTP, [α-^{32}P]GTP, and [α-^{35}S]-dATP were from New England Nuclear, Boston, MA. Nitrocellulose filters were from Millipore Corp., Bedford, MA.

Cell lines. The transformed lymphoblast cell lines 904 and 905 were derived from two brothers who are completely deficient in APRT activity as first described by Van Acker et al. (18). There was no consanguinity in the family. These cell lines were generous gifts from Dr. Seegmiller, University of California, San Diego, La Jolla, CA. A lymphoblast cell line with normal APRT activity, WIL2, was obtained from Human Genetic Mutant Cell Repository (Camden, NJ) and used as a normal control. All cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum. Radioisotopic assays of APRT activity in lymphoblast extracts were performed as described (16).

Northern blot analysis. Total cytoplasmic RNA was extracted from cultured lymphoblasts lysed in 6 M guanidium isothiocyanate, 5 mM sodium citrate, pH 7.0, 0.5% sodium sarcosyl, and 0.1 M β-mercaptoethanol according to the modified method of Chirgwin et al. (19). Poly(A)$^+$ RNA was isolated by oligo(dT) column chromatography (20). The enriched poly(A)$^+$ RNA (5 μg) from lymphoblasts was electrophoresed in an 0.8% agarose–formaldehyde gel and blot transferred to a nitrocellulose filter (21). The filter was probed with a 0.54-kb Bam HI fragment of normal APRT cDNA and a 0.95-kb Pst I fragment of the HPRT (hypoxanthine phosphoribosyltransferase) cDNA that were labeled by random oligonucleotide priming (22) using [α-^{32}P]dCTP.

cDNA cloning. The cDNA was synthesized from the poly(A)$^+$ RNA extracted from cell line 904 by the method of Okayama and Berg (23), as modified by Gubler and Hoffman (24), blunt-ended with T4 polymerase, and ligated with Eco RI linkers. After digestion with Eco RI, the cDNA was separated from linkers and small DNAs by Sepharose CL-4B column chromatography. The cDNA was ligated with lambda gt11 arms and packaged (25). Using probes prepared from a 0.54-kb fragment isolated from human APRT cDNA, 200,000 plaques were screened by standard procedure (26).

Genomic DNA cloning. Total genomic DNA was extracted from lymphoblasts (26) and digested with Bam HI before being electrophoresed on an 0.8% agarose gel. The DNA banding at the 2–2.5-kb size range was excised and extracted from the gel, blunt-ended, ligated with Eco RI linkers, and cloned into lambda gt10. Screening was performed on 50,000 plaques.

Sequencing. The inserted cDNAs and genomic DNAs of positive clones were recloned into pUC18, and then cloned into M13 for sequencing (27). The DNA nucleotide sequence was determined by the method of Sanger et al. (28) using [α-^{35}S]dATP.

Construction of plasmids. For the synthesis of anti–messenger RNA (mRNA) probes, two plasmids, pGP1 and pGS1, were constructed. To obtain pGP1, the 331-basepair (bp) Pst I fragment, which consists of a 308-bp normal APRT cDNA from position 133 to 440 and a 23-bp poly(G) tail, was cut out from pT02 (normal APRT cDNA cloned into pBR322) and cloned into pGEM-3blue plasmid, which had an SP6 promoter. pGS1 was constructed by cloning the 746-bp Sma I fragment of the normal APRT genomic DNA into the plasmid pGEM-3blue. The Sma I fragment consists of the 5′ flanking region, exon 1, and part of intervening sequence (IVS) 1.

RNase mapping analysis. The anti-mRNA probe was synthesized from the plasmid pGP1, and pGS1 linearized by digestion of Eco RI according to manufacturer's recommendations, with the substitution of 2.5 mCi/ml of [α-^{32}P]GTP (800 Ci/mmol) in the reaction mixture in place of [α-^{32}P]CTP. After DNase digestion the synthesized RNA was isolated, ethanol precipitated, and resuspended in water to a final concentration of 5×10^5 cpm/μl. 5×10^5 cpm of labeled RNA probe was hybridized to 50 μg of total cytoplasmic RNA in 30 μl of 80% formamide, 40 mM piperazine-*N*-*N*′-bis(2-ethane sulfonic acid) (PIPES), pH 6.7, 0.4 M NaCl, and 1 mM EDTA at 42°C for 16 h. The RNA was digested at 30°C for 1 h by the addition of 300 μl of RNase buffer containing 40 μg/ml RNaseA, 2 μg/ml RNaseT1, 10 mM Tris-HCl, pH 7.5, 5 mM EDTA, and 300 mM NaCl. Two ethanol precipitations were performed. The RNase-digested RNA was then electrophoresed in a 6% denaturing polyacrylamide gel and autoradiographed.

Results

APRT activity and the level of immunoreactive APRT protein in hemolysate of patient 904 were < 1% of normal control values (15). The APRT assay on extracts of the lymphoblast cell line (904) derived from this patient also revealed < 1% of normal activity. The 904 lymphoblast cells remained viable in medium containing 100 μM 6-methylpurine, an adenine analogue that is toxic to APRT$^+$ cells but not to APRT$^-$ cells. These results confirmed that the patient, as well as the lymphoblast cell line derived from him, are virtually completely deficient in APRT enzyme activity.

Southern blot analysis of Bam HI digested genomic DNA of 904 cells identified only one band of 2.2 kb in length, which is similar to the Bam HI fragment of normal APRT genomic DNA (13, 17) (data not shown). Thus, no large deletion or insertion was expected in the 904 genomic DNA.

The Northern blot analysis showed that the APRT mRNA is present in the 904 cell line (Fig. 1). Since the size of the mRNA from 904 showed no gross difference from normal APRT, a major deletion or insertion in the mRNA was considered unlikely. This technique allows detection of a difference in mRNA size of 200 nucleotides or greater.

The presence of APRT mRNA in the 904 lymphoblast cell line enabled the construction of a cDNA library from cytoplasmic RNA. Three positive clones were obtained and recloned into pUC18. They were designated p44, p46, and p68. Their lengths were 0.63, 0.8, and 0.6 kb, respectively, and the entire sequences of all three cDNA clones were determined. Since p44 and p46 showed the same deletion in the nucleotide sequence, and p68 showed a different abnormality, we concluded that p44 and p46 were derived from one allele, desig-

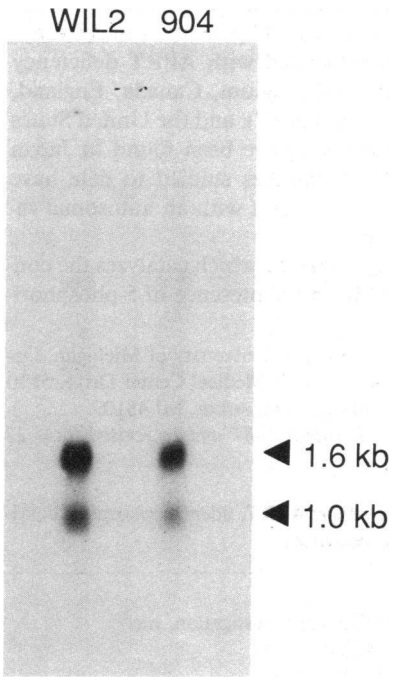


Figure 1. Northern blot analysis of poly (A)$^+$ RNAs from WIL2 and 904 cells. Approximate sizes are shown in kilobases. The 1.6-kb band reflects HPRT mRNA and the 1.0-kb band reflects APRT mRNA.

nated allele I, and that p68 was derived from the alternative allele, designated allele II. In addition, the 2.2-kb Bam HI genomic fragment of the 904 APRT gene was cloned. Two positive plaques were identified. The DNA inserts were re-cloned into pUC18 and designated genomic clones, p9B7 and p9B12. The entire sequence of both clones were determined, revealing that p9B7 represented allele I and p9B12 represented allele II.

Diagrams of cDNA and genomic clones of allele I are shown in Fig. 2. A deletion of a trinucleotide was found in both p44 and p46 (allele I) when it was compared with the normal APRT cDNA sequence (Fig. 3 *A*). In the resulting nucleotide sequence, the deletion of a phenylalanine in the protein may be predicted. The remainder of the sequence for both clones was found to be completely identical to normal APRT cDNA. The existence of the trinucleotide deletion in allele I was confirmed in the genomic clone; the identical trinucleotide deletion was found in p9B7 at the same position as noted in p44 and p46. No other abnormalities were found in p9B7 when compared with the sequence of the normal APRT genomic DNA.

Diagrams of cDNA and genomic clones of allele II are also shown in Fig. 2. Allele II was represented by cDNA clone p68

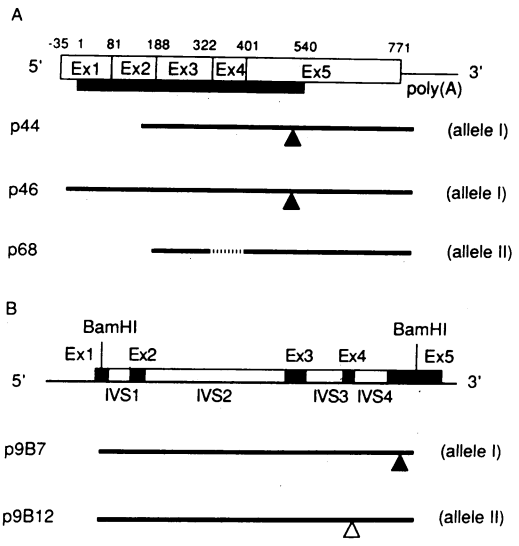


Figure 2. Diagrams of cDNA clones and genomic DNA clones from alleles I and II. (*A*) Normal APRT mRNA is represented by the combination of exons, each of which is designated by an open box, with exon 1 abbreviated as Ex1. A poly(A) tail is shown as a solid line. The coding region is denoted as a solid box, beginning at nucleotide +1 and ending at +540. Numbers over the exons denote the beginning of each exon, the coding region, and the poly(A) tail, except for the number 540, which represents the end of the coding region, and the number 771, which indicates the poly(A) addition site. The three cDNA clones of 904 mRNA, p44, p46, and p68, are represented by a solid line. Solid triangles in p44 and p46 derived from allele I show the position of the trinucleotide deletion. The dotted line in the cDNA clone, p68, derived from allele II, shows the deleted exon 4. (*B*) Normal APRT genomic DNA is represented by five exons (Ex) and four IVS, designated by solid boxes and open boxes, respectively. Two Bam HI sites shown were used for cutting out the APRT genomic DNA. The solid triangle in the genomic DNA clone, p9B7, derived from allele I, shows the position of the trinucleotide deletion. The position of the T insertion in IVS 4 is shown by an open triangle in the genomic DNA clone, p9B12, derived from allele II.

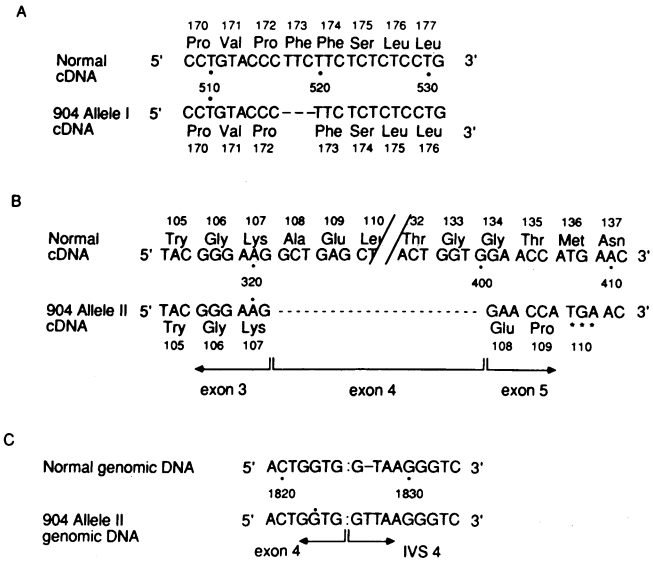
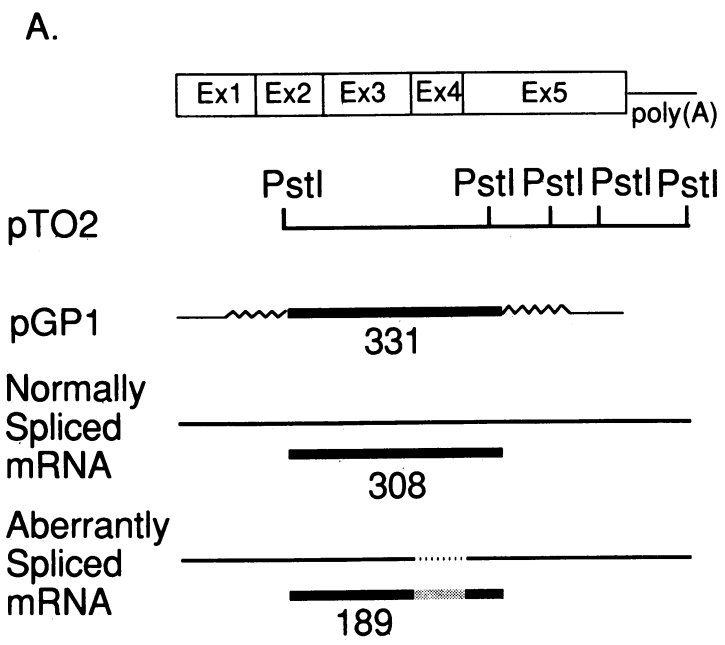


Figure 3. The nucleotide sequence of the mutated region of allele I and allele II. (*A*) The sequences from the normal and the 904 allele I are compared for the region including the trinucleotide deletion. Amino acid residues are numbered from the start codon of the open reading frame along with the nucleotide numbers. The two sequences are aligned based on the TTC deletion. Dashes represent deleted nucleotides. (*B*) The sequence of the cDNA clone, p68, from 904 is compared with that of normal. Dashes represent deleted nucleotides. The position of premature termination generated in 904 is indicated by ***. Amino acid residues are numbered from the start codon of the open reading frame, along with the nucleotide numbers. (*C*) The nucleotide sequences of the junction of exon 4 and IVS 4 of the normal and 904 genomic DNA clone, p9B12, are illustrated. Numbers denote the nucleotide positions, starting from the first nucleotide in the start codon of the reading frame.

and genomic clone p9B12. A deletion of 79 nucleotides was identified in p68, which spanned from position 322 to 400 (Fig. 3 *B*). This deletion began exactly at the beginning of exon 4 and ended at the end of exon 4, indicating that the entire exon 4 was missing from the mRNA transcribed from allele II. The genomic DNA clone, p9B12, was sequenced. There were no abnormalities except for the insertion of a single nucleotide, T, in the area immediately adjacent to the splice site at the 5′ end of IVS 4 between positions 1826 (G) and 1827 (T), or positions 1827 (T) and 1828 (A) (Fig. 3 *C*). The trinucleotide deletion found in allele I was not present in p68 or p9B12 (allele II).

To further examine APRT mRNA, RNase mapping analysis using a riboprobe was performed. The RNA probe was synthesized from the 331-bp Pst I fragment, which consists of a 308-bp normal APRT cDNA from position 133 to 440, and a 23-bp poly(G) tail. This probe was hybridized to the total cytoplasmic RNA from the normal and the mutant cell lines (904 and 905). The normal APRT sequence produced a band 308 nucleotides in length. The putative 79-nucleotide deletion, starting at position 322 on the mRNA, would be expected to produce a novel 189-nucleotide band (Fig. 4 *A*). As shown, both 904 and 905 had bands of 308 and 189 nucleotides (Fig. 4 *B*). The existence of the mRNA with exon 4 missing was confirmed by this experiment. A faint band of approximately 270 nucleotides in length was found in the lane WIL2 in addition to a 308-nucleotide band. This faint band observed is unlikely



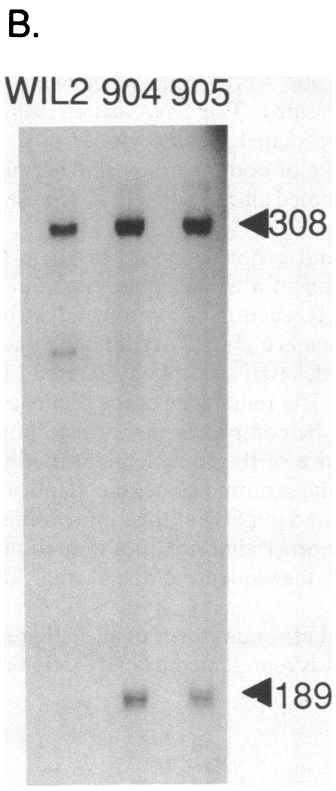


Figure 4. RNase mapping analysis for the region of exon 4 of mRNA. (*A*) pT02 is a normal APRT cDNA cloned into the Pst I site of pBR322. The two Pst I sites at the ends of pT02 are not internal sites but the cloning sites in pBR322. Plasmid pGP1 was constructed from pGEM-3blue and the 331-bp Pst I fragment of pT02, which consists of a 308-bp cDNA from position 133 to 440 and a 23-bp poly(G) tail. This fragment includes the entire exon 4. The dotted line in aberrantly spliced mRNA represents the missing exon 4 in 904 mRNA. Probe RNA was synthesized from the pGP1 and hybridized to mRNA, then digested with RNaseA and RNaseT1. Digestion protected RNA probes are designated by thick solid lines with the size under the line. A stippled line shows the unprotected part of the RNA probe that should be digested with RNase. Hybridization to 904 mRNA should generate a novel 189-nucleotide RNA from allele II as well as the normal 308-nucleotide fully protected RNA from allele I. (*B*) RNase mapping analysis of mRNA from the WIL2, 904, and 905 cell lines was performed, utilizing the pGP1-derived RNA probe. The labeled RNA probe protected from digestion by hybridization to mRNA was separated on a 6% denaturing polyacrylamide gel and autoradiographed.

to be caused by a nucleotide change because fainter bands were also observed in 904 and 905 at the same position on the longer exposed autoradiograph. These bands in WIL2, 904, and 905 were reproducible. These results suggest that the RNA probe might cross-hybridize to non-APRT RNA, which existed in these three cell lines. Further experiments will be necessary to prove this observation.

Discussion

We have studied two related patients previously reported to have complete deficiency in APRT (16). Since the gene encoding APRT is on an autosome (chromosome 16), examination of both alleles was necessary. We expected that the subjects might be compound heterozygotes with different mutations in each allele because of the common prevalence of altered APRT alleles and the absence of consanguinity in this family (18). We sequenced cDNA and genomic DNA from each of the two alleles of the 904 lymphoblast cell line and selected confirmatory studies were conducted using DNA from the 905 lymphoblast cell line. The RNA mapping analysis of the 5′ region of exon 1 (which the sequence of cDNA, p68, and the genomic DNA, p9B7 and p9B12, lacks) indicated that the sequence of exon 1 of 904, including both alleles, is identical to that of normal (data not shown).

The trinucleotide deletion in the cDNA sequence corresponding to allele I was confirmed by sequencing the genomic DNA of the allele, which showed the same deletion. Although the exact location of the trinucleotide deletion is indeterminate (Fig. 5), the resulting nucleotide sequence indicates a deletion of a single amino acid, phenylalanine, in the protein product. The entire coding sequence of allele I revealed no other change in the deduced amino acid sequence. Therefore, we conclude that the trinucleotide deletion is responsible for the abnormal protein encoded by allele I. The phenylalanine deletion would presumably alter the secondary or tertiary structure of the protein product, which would result in enzyme dysfunction and/or increased lability, thus accounting for the absence of enzyme protein or activity.

Multiple base deletions such as this have been shown in studies of α-thalassemia and β-thalassemia. There are reports of a pentanucleotide deletion in α-thalassemia (29) and a dinucleotide (30), a tetranucleotide (31), and a 25-nucleotide deletion (32) in β-thalassemias. The region of the trinucleotide deletion consists of only T's and C's, and there are three double-tandem repeats of a trinucleotide. Due to the presence of the tandem repeats, as shown in Fig. 5, the precise location of the trinucleotide deletion could have been at one of any six positions: 2170, 2171, 2172, 2173, 2174, or 2175.

In a pentanucleotide deletion of α-thalassemia and a tetranucleotide deletion of β-thalassemia, short repeats can be

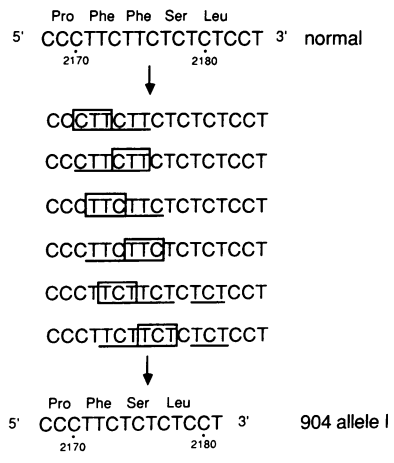


Figure 5. The nucleotide sequence of the T,C-rich region where the trinucleotide deletion occurred. At the top, the normal sequence of the APRT gene is shown with the amino acid sequence over the nucleotide sequence. Numbers below the nucleotide sequence denote the nucleotide positions in the genomic DNA, starting from the first nucleotide in the start codon of the reading frame. In the middle, the six possible deletions are shown in boxes. The repeated trinucleotides are underlined. At the bottom, the nucleotide sequence of 904 allele I is shown with the amino acid sequence above and the nucleotide positions in the genomic cDNA below.

identified within or flanking the deletion. The examination of the human β-like globin genes and their flanking sequences showed that short, direct repeats might be involved in the generation of many deletions, which included one or two of the repeats (33). A remarkable similarity to the trinucleotide deletion of 904 is found in *Escherichia coli,* where the pattern of the deletions and insertions of a tetranucleotide accounts for two-thirds of spontaneous mutations in the *lac I* gene of *E. coli* (34). The model of slipped mispairing during DNA replication has been proposed as the explanation of these deletions and insertions. The trinucleotide deletion of 904 may have been promoted by short tandem repeats through the slipped mispairing mechanism during DNA replication. This could provide a plausible mechanism to account for the relatively frequent occurrence of human APRT mutations.

The cDNA sequence of the p68 clone from allele II showed that exon 4 was entirely deleted from the mRNA. This exon 4 deletion from the mRNA would generate a frameshift, and produce a termination codon at the position of the new codon 110. This premature termination would, in turn, cause a short product of 109 amino acid residues, which would presumably be nonfunctional or unstable, consistent with the absence of detectable protein or enzyme activity.

A series of alternative approaches were designed to confirm this finding on allele II. RNase mapping analysis in the vicinity of exon 4 demonstrated the predicted novel 189-nucleotide band, confirming this deletion in both APRT-deficient siblings. In the genomic DNA clone of this allele, p9B12, exon 4 was conserved as expected. The RNase mapping analysis for the 5′ region of mRNA showed no detectable nucleotide changes in the area, and the remainder of the sequence was normal, except for a single nucleotide insertion, T, at the 5′ splice region of IVS 4. Thus, the T insertion may be responsible for the exon 4 deletion on mRNA. RNase mapping analysis showed the existence of mRNA transcribed from both allele I and allele II. The mRNA from allele II with the exon deletion is shorter by 76 nucleotides as compared with the mRNA from allele I, a difference which cannot be resolved on standard agarose gel electrophoresis.

Consensus sequences conserved within 5′ and 3′ slice regions at the junction of an exon and an IVS have been described (35, 36) [AG:GT($^{G}_{A}$)AGT in the 5′ splice region, $(Py)_{11}$NCAG:G in the 3′ splice region. The colon (:) denotes a cleavage site]. The 5′ splice site consensus sequence and the exon 4–IVS 4 junction of normal APRT gene show two nucleotide deviations, two bases upstream, and six bases downstream from the cleavage site (TG:GTAAGG). These two nucleotide substitutions do not appear to affect normal splicing. The identical sequence to that of the normal APRT gene has been reported at the 5′ splice region of IVS 5 of the chick ovalbumin gene (37).

The T insertion on allele II of 904 alters two additional nucleotides within the 5′ splice region of IVS 4, three bases and five bases downstream from the cleavage site (TG:GTTAAG). Based on the finding of a deletion of exon 4 in the mRNA, these two base changes appear to abolish the normally functioning splice signal and result in the aberrant splicing of allele II. It cannot be excluded that normally spliced mRNA might be also transcribed from allele II. However, the abnormally spliced mRNA lacking exon 4 seems not to be a minor transcript from the RNase mapping analysis. Incorrect splicing caused by nucleotide changes at various positions within the 5′ splice region have been reported. Three different mutations in β-thalassemia, occurring at positions 1, 5, or 6 of the 5′ splice region of IVS 1 of β-globin gene, abolished or reduced normal splicing and gave rise to alternative splicing (38). Aebi et al. examined 24 artificial point mutations of 12 positions within the 5′ and 3′ splice regions of the rabbit β-globin gene (39). Point mutations at seven out of nine positions of the 5′ splice region of IVS 2 caused anomalous splicing in vitro leading to exon 2 deletion. These in vitro results are similar to our finding of aberrant splicing in the 904 APRT mRNA on allele II. In vivo, however, the same point mutations did not affect splicing, except for those at the first and the second positions. The relationship of splice site mutations and mode of splicing does not seem to be uniform.

Recently, splicing has been shown to occur within a multicomponent splice complex consisting of RNAs and proteins (40, 41). According to the above process, the defective 5′ splice signal in IVS 4 of 904 somehow forces the multicomponent splice complex to select the distal 3′ splice site of IVS 4 instead of the normally chosen proximal 3′ splice site of IVS 3, resulting in the joining of exons 3 and 5 and the deletion of the IVS 3–exon 4–IVS 4 complex. Similar splicing patterns with an exon missing has been observed in β-thalassemia and phenylketonuria. In β-thalassemia, a single nucleotide substitution at the first position at the 5′ splice region in IVS 2 of the β-globin gene generated normal mRNA and abnormal mRNA lacking exon 2 (42). In phenylketonuria, a base change within the 5′ splice region in IVS 12 in mRNA of the phenylalanine hydroxylase gene resulted in the deletion of exon 12 (43, 44). Thus, the abnormality of the 5′ splice region in IVS somehow influences the selection of the 3′ splice site.

It is still unknown how the precise splice site is selected. To elucidate the process by which the mutation in the 5′ splice region causes the aberrant splicing of 904, further RNA processing studies in vitro and in vivo are indicated. This mutated 5′ splice region of 904 may prove an excellent candidate in the investigation of the mechanisms of splicing.

These studies represent the first direct demonstration of

mutations leading to APRT deficiency in humans. The demonstration of two different mutant alleles was anticipated because of the high frequency of mutant alleles in the population, although their complex nature was not. The finding of two different mutations does establish that the subjects of this study are compound heterozygotes and not homozygotes for APRT deficiency. Finally, we are hopeful that studies such as these will eventually help us not only to understand the molecular pathology of human APRT deficiency but also to define the mechanism(s) responsible for the very common occurrence of mutant alleles for APRT in the human population.

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