

KIDNEY BIOPSY TEACHING CASE

Decreased Kidney Function and Crystal Deposition in the Tubules After Kidney Transplant

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Adenine phosphoribosyltransferase (APRT) deficiency is an autosomal recessive purine enzyme defect that results in the inability to utilize adenine, which consequently is oxidized by xanthine dehydrogenase to 2,8-dihydroxyadenine (2,8-DHA), an extremely insoluble substance eventually leading to crystalluria, nephrolithiasis, and kidney injury. We describe a case of APRT deficiency not diagnosed until the evaluation of a poorly functioning kidney transplant in a 67-year-old white woman. After the transplant, there was delayed transplant function, urine specimens showed crystals with unusual appearance, and the transplant biopsy specimen showed intratubular obstruction by crystals identified as 2,8-DHA using infrared spectroscopy. APRT enzymatic activity was undetectable in red blood cell lysates, and analysis of the *APRT* gene showed 1 heterozygous sequence variant, a duplication of T at position 1832. The patient was treated with allopurinol, 300 mg/d, and transplant function progressively normalized. Because patients with undiagnosed APRT deficiency who undergo kidney transplant may risk losing the transplant because of an otherwise treatable disease, increased physician awareness may hasten the diagnosis and limit the morbidity associated with this disease.

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INDEX WORDS: Adenine phosphoribosyltransferase (APRT) deficiency; crystal nephropathy; renal biopsy; renal transplant; crystalluria; urinary sediment.

Around the world, registries still report an unacceptably high prevalence of undiagnosed causes of end-stage kidney failure, ranging from 15% to >50%. It is likely that genetically determined chronic kidney disease accounts for a large fraction of these cases.¹⁻³ Obviously, undiagnosed kidney disease in native kidneys negatively affects the chances of having a successful kidney transplant, and such failures are even more difficult to accept in the case of undetected, but treatable, diseases. We describe the case of a kidney transplant recipient with a metabolic deficiency that was not diagnosed until the evaluation of a poorly functioning transplant.

CASE REPORT

Clinical History and Initial Laboratory Data

A 67-year-old white woman underwent transplant of a kidney from a 73-year-old deceased donor in December 2007. From childhood, the patient had experienced repeated renal colic with recurrent episodes of spontaneous elimination of small kidney stones, which never were examined. Family history was negative except for hypertension. Chronic kidney disease of unknown cause with small kidneys was diagnosed when she was 63 years old during an evaluation for hypertension, and maintenance dialysis therapy for end-stage renal disease attributed to nephroangiosclerosis was started 2 years later.

Donor kidney biopsies performed at the time of transplant showed a normal parenchymal pattern with mild interstitial and vascular damage. After transplant, there was delayed transplant function, with serum creatinine level never decreasing to <3.5 mg/dL (<309 μ mol/L; estimated glomerular filtration rate [eGFR], 13.9 mL/min/1.73 m² [0.23 mL/s/1.73 m²] assessed using the 4-variable Modification of Diet in

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Renal Disease [MDRD] Study equation) and a subsequent further increase in serum creatinine level to 5 mg/dL ($442 \mu\text{mol/L}$; eGFR , $9.2 \text{ mL/min/1.73 m}^2$ [$0.15 \text{ mL/s/1.73 m}^2$]). Kidney ultrasound was normal, and urine specimens showed specific density of 1.015, pH 6, trace protein and hemoglobin, rare red blood cells, and a few crystals lacking diagnostic features.

Kidney Biopsy

Transplant kidney biopsy performed 3 weeks after surgery showed normal glomerular, interstitial, and vascular morphologic patterns, but intratubular obstruction by crystals of unknown type. These crystals were absent in the initial donor biopsy specimens; von Kossa stain was negative, indicating the absence of phosphate. Despite aggressive intravenous fluid administration and use of furosemide, transplant function did not improve. A second transplant kidney biopsy therefore was performed 2 weeks later, again showing many brown and irregular needle-shaped intratubular crystals (Fig 1A) that were refractile when examined under polarized light (Fig 1B).

Diagnosis

The final diagnosis of acute kidney injury caused by intratubular obstruction by 2,8-dihydroxyadenine (2,8-DHA) crystals was rendered after crystal analysis, quantitative enzymatic assay for adenine phosphoribosyltransferase (APRT) activity in erythrocytes, and analysis of the *APRT* gene.

Crystal Analysis

Repeated examination of the urinary sediment using phase contrast microscopy showed the presence of unusual crystals. Some crystals appeared as round particles with dark edges, whereas others had an irregular shape and size. Moreover, although some crystals were free in urine, isolated or in clusters, others were embedded within the matrix of casts, a finding that unequivocally showed their tubular origin. Using polarized light, the crystals showed a strong polychromatic birefringence (Fig 2). Phase contrast investigation gave conflicting results. The round particles (Fig 2A) might have been identified as 2,8-DHA crystals, but this possibility was ruled out because of their birefringence (Fig 2B) and the lack of “Maltese cross” appearance typical of 2,8-DHA crystals.

The definitive confirmation that the crystals were composed of 2,8-DHA came from results of infrared spectroscopy performed on a sample of filtered and dried urine sediment and a fragment of tissue from the kidney biopsy specimen. These findings are consistent with previous studies that have shown that 2,8-DHA crystals may have unusual morphologic features, especially in patients with impaired kidney function.⁴⁻⁶

Determination of APRT Enzymatic Activity

APRT enzymatic activity was measured in red blood cell lysates using radiolabeled ^{14}C -adenine in a chromatographic assay as described previously.^{7,8} This assay showed no detectable activity.

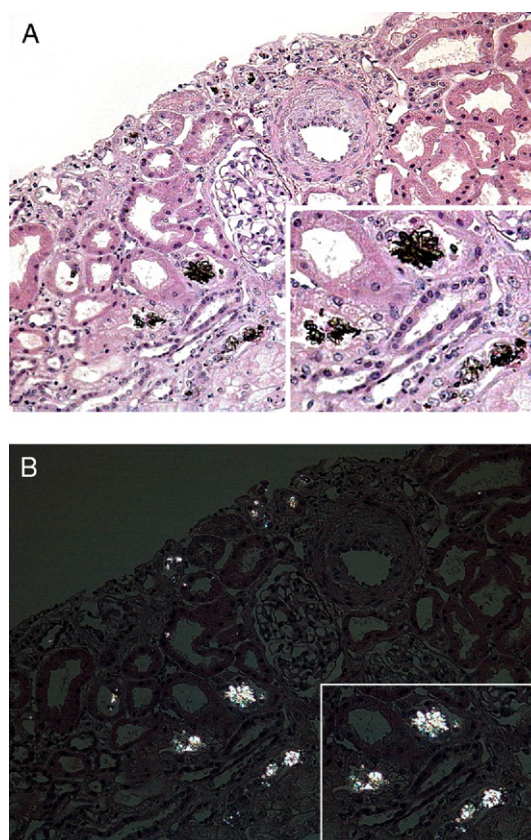


Figure 1. Light microscopy of the kidney transplant biopsy specimen. (A) Cortical tissue includes a glomerulus, middle-sized artery, and several tubules. Round brown crystals are visible in tubular cells and occlude the tubular lumen (original magnification, $\times 200$). Inset, higher magnification shows the needle-shaped features of the crystals (original magnification, $\times 400$). (B) The same tissue area under polarized light (original magnification, $\times 200$). The crystals (at higher magnification in the inset) are birefringent (original magnification, $\times 400$).

Genetic Analysis

Genetic analysis was performed after written informed consent was obtained from the patient. All coding regions and intron/exon junctions of the *APRT* gene were amplified using polymerase chain reaction from genomic DNA and sequenced directly using the polymerase chain reaction primers (full information, including primer sequences, is provided in Item S1, provided as online supplementary material available with this article at www.ajkd.org). Sequences were compared with the theoretical sequence of the *APRT* gene using Serial Cloner software (Serial Basics, serialbasics.free.fr), with GenBank accession number NG_008013.1 used as the sequence reference, except that numbering is based on the A of the initiation codon being position 1.

This analysis showed only 1 heterozygous sequence variant, a duplication of T at position 1832 in the genomic DNA. This position is the second nucleotide of intervening se-

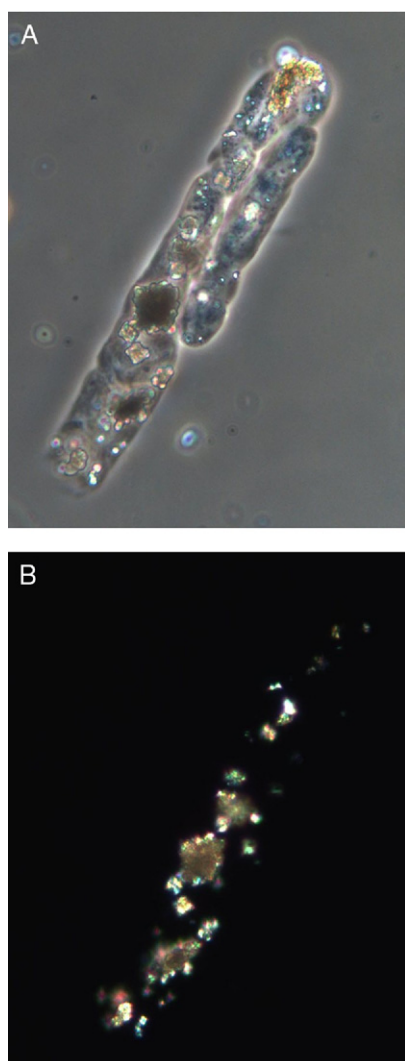


Figure 2. A cast containing many crystals of 2,8-dihydroxyadenine with different shape and size. (A) Phase contrast microscopy, (B) polarized light (for all images, original magnification, $\times 400$).

quence 4. As described by Hidaka et al,⁹ this extra T disrupts the consensus sequence of the 5' splice site at the junction of exon 4 and intron 4, resulting in deletion of exon 4 in messenger RNA, premature termination at amino acid 110, and a truncated protein of 109 amino acids instead of 180.

Clinical Follow-up

The patient was treated with allopurinol, 300 mg/d, and a low-purine diet. Her kidney function eventually improved, and 1 year after treatment, she is now free from renal colic, stones, and infections, with a serum creatinine level stable at 1.9 mg/dL (168 $\mu\text{mol/L}$; eGFR, 28 mL/min/1.73 m² [0.47 mL/s/1.73 m²]).

DISCUSSION

APRT deficiency is an autosomal recessive disorder of purine metabolism. Normally, APRT (EC [Enzyme Commission] classification code 2.4.2.7) catalyzes the conversion of adenine to adenosine monophosphate using phosphoribosylpyrophosphate (PRPP) as a cosubstrate (Fig 3). An inherited deficiency of this salvage enzyme results in an inability to utilize adenine, which then is oxidized by xanthine dehydrogenase through an 8-hydroxy intermediate to 2,8-DHA. Because 2,8-DHA is extremely insoluble, its excretion by the kidney into urine can lead to crystalluria and the formation of urinary stones. Furthermore, 2,8-DHA crystals exert direct toxic effects on renal tubular and interstitial cells, eventually leading to end-stage renal failure.^{4-6,10} The mechanisms involved in tubular toxicity are not well understood. Experimental studies showed extensive tubular dilation, inflammation, necrosis, and fibrosis in APRT-deficient mice developing 2,8-DHA nephrolithiasis.¹¹ Complementary DNA microarrays further showed that DHA crystals stimulate the expression of specific genes in kidney epithelial cells, leading to the induction of adhesion molecules and PDGFB (platelet-derived growth factor β polypeptide). These molecules then affect cell-cell or cell-matrix interactions and/or alter the actin cytoskeleton and may ultimately contribute to crystal-induced kidney injury, with a pathway similar to that of calcium oxalate crystals.^{12,13}

The enzymatic defect is related to mutations in a single gene located on chromosome 16q24. There are 2 common allelic variants that differ in the level of residual APRT activity: type I, mainly observed in white patients, in which enzyme activity is virtually undetectable, and type II, found only in Japanese patients, in which APRT activity is present, but strikingly decreased because of a low affinity of the enzyme for PRPP.¹⁴⁻¹⁸

In our patient, there was no measurable APRT activity and the allelic variant was consistent with type I APRT deficiency. However, because the patient is only heterozygous for this allele, we infer that she is heterozygous for 2 different mutations, 1 of which we did not detect. Analysis of the promoter of the *APRT* gene likely would be necessary to find the second mutation.

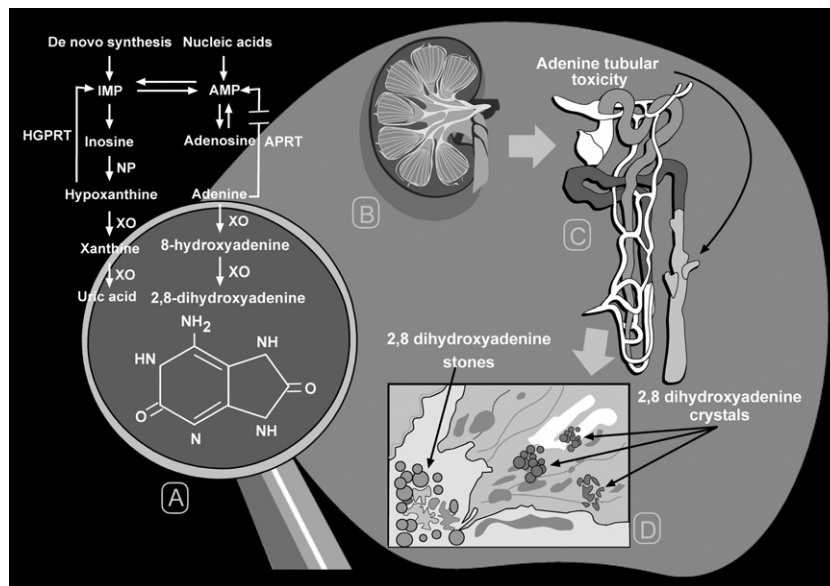


Figure 3. Mechanisms of tubular damage by toxic adenine compounds. (A) Metabolic pathways of purines, (B-D) sites of intrarenal adenine toxicity, crystals, and stone formations. Abbreviations: AMP, adenosine monophosphate; APRT, adenine phosphoribosyltransferase; ATP, adenosine triphosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine monophosphate; NP, nucleoside phosphorylase; XO, xanthine oxidase. Figure courtesy of Federico Roggero.

It has been reported that the frequency of heterozygosity for APRT deficiency is 0.4%-1.2% in the general population, which suggests a frequency for homozygosity of approximately 1 in 250,000 to 1 in 33,000. However, it also has been stressed that the number of observed cases is lower than expected.¹⁹⁻²⁴ Clinical symptoms may include renal colic, hematuria, dysuria, kidney stones, urinary tract infection, acute kidney injury due to crystal-induced nephropathy, and chronic kidney disease. However, in as many as 15% of individuals with homozygous defects in *APRT*, disease presentation may be so subtle that the signs and symptoms are easily overlooked during the indolent progression to end-stage renal disease. As a result, patients may present with recurrent urinary lithiasis of undetermined composition²⁵⁻³¹ and may remain undiagnosed until the condition is detected by the kidney transplant

biopsy (Table 1). Because patients who undergo kidney transplant belong to the subgroup of cases with severe phenotypic expression, it is not surprising that the disease is underdiagnosed.³²

Of note, even when a kidney biopsy is performed, it is difficult to differentiate 2,8-DHA crystals deposited in kidney tissue from calcium oxalate monohydrate crystals on the basis of morphologic appearance and polarization alone. However, 2,8-DHA deposits often may appear as rings composed of numerous crystals with a radial organization (sample images from this case are provided in Fig S1, available as online supplementary material associated with this article at www.ajkd.org), a feature not observed in the case of calcium oxalate crystal deposits, even in patients with primary hyperoxaluria type I. Another characteristic of DHA crystals is that they often are very small ($<3 \mu\text{m}$), even if they

Table 1. Cases in Which APRT Deficiency Was First Diagnosed After Kidney Transplant

Year	First Author	Country	Follow-up	Reference
1988	Glicklich D	United States	Worsening graft function	25
1994	Gagnè ER	Canada	Transplant loss	26
1996	deJong DJ	The Netherlands	Transplant loss	27
1998	Brown HA	Canada	Transplant loss	28
2001	Benedetto B	United States	Improved after diagnosis/treatment	29
2004	Cassidy MJ	United Kingdom	Improved after diagnosis/treatment	30
2004	Eller P	Austria	Three graft losses	31
2009	Stratta P	Italy	Improved after diagnosis/treatment	Present case

can form large aggregates. In contrast, calcium oxalate monohydrate crystals may be large, either isolated or grouped as aggregates. Of note, specific staining may be used to distinguish calcium salts (alizerin red stain), phosphate-containing deposits (von Kossa staining), or non-calcium-containing crystals, such as 2,8-DHA or drug crystals. When doubt remains, it is wise to confirm the precise identity of the crystals using infrared microscopy.^{8,13}

After detection of 2,8-DHA crystals using infrared analysis of urinary sediment or spectrophotometric analysis of biopsy material, the diagnosis is confirmed by assaying red blood cell lysate for APRT activity and using genetic analysis. However, because crystallization of 2,8-DHA and subsequent kidney damage may easily be prevented with the xanthine dehydrogenase inhibitor allopurinol, early detection of the disease is critical. Although >20 years have elapsed since the first published case of a patient with APRT deficiency diagnosed after kidney transplant, the diagnosis of APRT deficiency as a cause of end-stage renal failure in dialysis and kidney transplant registries still is largely overlooked and has not significantly increased in recent years. Our case emphasizes that patients with undiagnosed APRT deficiency who undergo kidney transplant may risk losing their transplant because of an otherwise treatable disease.

Increased physician awareness may hasten diagnosis and limit the morbidity associated with this disease. Even in the complete absence of symptoms, 2,8-DHA crystals are present from birth in urine in all individuals with homozygous defects in *APRT*. Thus, the first simple step is careful and repeated urine examination in case of crystalluria that is either atypical or similar to urate crystals, especially in the absence of detectable abnormalities of serum and urine uric acid concentrations.³³⁻³⁵ A complete diagnosis of APRT deficiency needs sophisticated technologies, but clinical suspicion is within everybody's reach.

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SUPPLEMENTARY MATERIALS

Item S1: Genetic analysis methodology.

Figure S1: Crystals of 2,8-dihydroxyadenine (DHA).

Note: The supplementary material accompanying this article (doi:10.1053/j.ajkd.2009.12.028) is available at www.ajkd.org.

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