

## 2,8-DIHYDROXYADENINE UROLITHIASIS DUE TO PARTIAL DEFICIT IN ADENINE PHOSPHORIBOSYLTRANSFERASE : A CASE REPORT

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Inherited metabolic diseases resulting in urolithiasis secondary to urinary excretion of insoluble substances are rare but often present as urinary obstruction of renal insufficiency. We herein report a case of partial adenine phosphoribosyltransferase deficiency associated with 2,8-dihydroxyadenine urolithiasis. In family members the propositus and his younger brother are homozygotes for defective APRT genes, and who exhibits the type II phenotype designated APRT\*J (Japanese type).

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**Key words:** 2,8-dihydroxyadenine (2,8-DHA), Adenine phosphoribosyltransferase (APRT)

### INTRODUCTION

Adenine is normally converted into adenine monophosphate (AMP) by adenine phosphoribosyltransferase (APRT), a salvage enzyme of purine metabolism. In the absence of the APRT, adenine becomes available for oxidation by xanthine oxidase the 8-hydroxy- and 2,8-dihydroxyadenine (2,8-DHA). 2,8-DHA is an extremely insoluble compound with a solubility in water of only 1 to 3 mg per liter 50 times less soluble than uric acid. Overexcretion of 2,8-DHA in the urine results in the formation of urinary stones. The genotype of this metabolic disease is inherited by an autosomal recessive mode, and only homozygotes for APRT deficiency do manifest any associated biochemical or clinical abnormalities, or excrete any adenine metabolites in the urine. A larger number of Japanese patients with APRT partial deficiency have been reported<sup>1)</sup>, which may contribute to the overwhelming increase with those in European countries and the United States.

We recently experienced a case of proved APRT partial deficiency by determining

APRT enzyme activity in which a 2,8-DHA stone was identified in the chemical analysis on crystals in the urine sediment and spontaneously excreted stone. In family members the propositus and his younger brother were homozygotes for defective APRT, suggesting a possibility of stone formation.

### CASE REPORT

An 8-year-old boy was found to have proteinuria and positive occult blood in the urine by a school mass examination in July 1977. Hypertension was also found in the pediatric department. Complete blood count was normal. Other laboratory investigations including blood urea nitrogen, creatinine and uric acid were within normal limits. However, he had hyperphosphatemia (4.4~6.2 mg/dl) and low complement value ( $\beta_2$ : 56~65 mg/dl, CH50 19~25 U/ml). Plasma renin activity and aldosterone level were both normal. Urinalysis revealed a trace of protein and no red blood cells, and sediment contained numerous crystals. The patient was followed at the Department of Pediatrics until he was 15 years old. In March 1984,

an X-ray study showed right hydronephrosis and hydroureter with a filling defect and without opacities just above the ureter-vesical junction (Fig. 1). Subsequently retrograde pyelography was performed, then ureteral catheter progressed easily upward and a thumb-sized filling defect was demonstrated in the region. Urine cytology was negative. Under the preoperative diagnosis of radiolucent calculi or ureteral tumor a surgical exploration was attempted in July 1984. Operative findings disclosed the incarceration of stone accompanying tissue granulation in the ureteral lumen. A stone was light brown and friable. The stone analysis performed at that time indicated a composition of ammonium urate.

The postoperative course was uneventful. An excretory urography (IVP) and ultrasonic tomography showed no change in degree of hydronephrosis, while hydroureter was improved distinctly. Urinalysis revealed persistent crystalluria even after surgery. So-called round crystals were seen in the urinary sediment on optical microscopy (Fig. 2) and polarized light

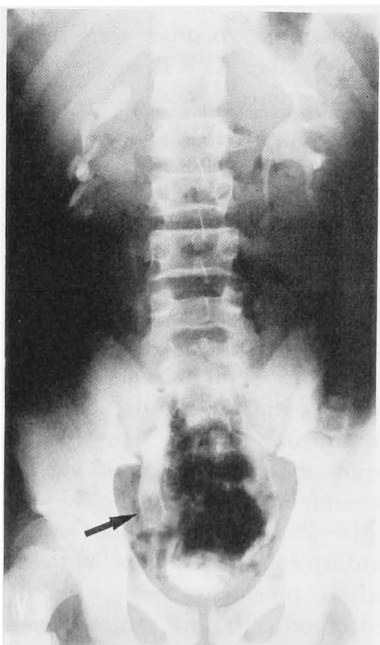


Fig. 1. Excretory urogram shows right hydronephrosis and hydroureter with a filling defect (arrow).

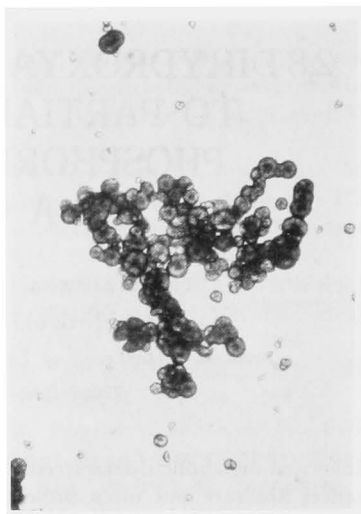


Fig. 2. So-called round crystals are seen in the urinary sediment on optical microscopy (original magnification,  $\times 100$ ).

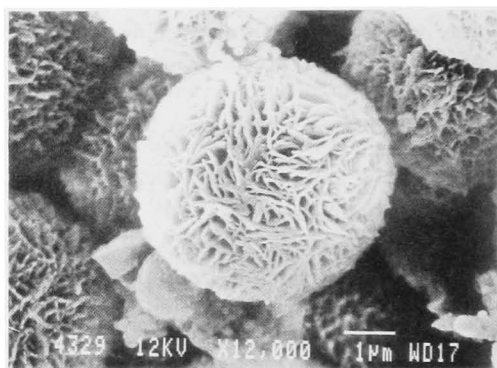


Fig. 3. Scanning electron microscopy shows aegagropilia-like appearance.

showed a fan-shaped appearance. Scanning electron microscopy revealed "aegagropilia-like appearance" (Fig. 3). The patient passed spontaneously an asymptomatic urinary stone in October, 1988. Spectrophotometric analysis revealed an absorption spectrum for 2,8-DHA. High pressure liquid chromatography (HPLC) of the urine sample demonstrated a compound identical with 2,8-DHA.

APRT enzyme activity in erythrocyte lysates was 0.033 nmol/min/mg protein and corresponded to 8.4% of the control activity, so that the diagnosis of APRT partial deficiency was made. Although hemolysate APRT activities for the patients and fami-

Table 1. APRT activity and genotype in families

Individuals	Sensitivity <sup>a)</sup> (6-metP)	Genotype	APRT activity	Possibility of stone formation
Propositus	Resistant	APRT * J/APRT * J	0.033 (8.4%) <sup>b)</sup>	(+)
Father	Sensitive	APRT * I/APRT * J	0.152 (39.4%)	(-)
Mother	Sensitive	APRT * I/APRT * J	0.144 (37.3%)	(-)
Brother	Resistant	APRT * J/APRT * J	0.034 ( 8.9%)	(+)
Grandmother	Sensitive	APRT * I/APRT * I	0.171 (44.2%)	(-)
Uncle	Sensitive	APRT * I/APRT * J	0.080 (20.6%)	(-)
Aunt	Sensitive	APRT * I/APRT * I	0.435 (113.0%)	(-)

a) determined by testing the resistance of viable T cells to adenine analog, 6-methylpurine

b) n mol/min/mg protein (per cent control activity).

Table 2. Genotype of APRT deficiency

Genotype <sup>a)</sup>	APRT activity	Sensitivity	Stone formation	Trait
I/I	100 %	Sensitive	Impossible	Normal
QO/QO	0 %	Resistant	Possible	Complete, homozygous
I/QO	25 %	Sensitive	Impossible	Complete, heterozygous
J/J	25 %	Resistant	Possible	Japanese type, homozygous
I/J	50 %	Sensitive	Impossible	Japanese type, heterozygous
J/QO <sup>b)</sup>	5 %	Resistant	Possible	Mixed

a) alleles' abbreviation: I = APRT\* QO, J = APRT\*J

b) most recently, APRT\* QO allele has been found in a Japanese patient (Sahota, et al (12))

ly members were lower than the mean value (Table 2), the reduction of the enzyme activity may have occurred, during the transportation of the blood samples. Functional APRT activity determined by using viable T cells was resistant to adenine analogues. The genotype indicated by molecular hybridization using patient DNA was a homozygote of the Japanese type APRT deficiency. In family members the propositus and his younger brother demonstrated gross deficiency of the enzyme APRT consistent with the homozygous trait, while others were heterozygote or normal (Table 1). A possibility of causing DHA urolithiasis was thus restricted to the propositus and his brother.

Treatment with a low purine diet (150 mg/day) and allopurinol (200~300 mg/day) was then started. Within a few weeks, the crystals disappeared from the urinary sediment, although a left renal stone was found before allopurinol treatment.

The patient's clinical condition remained excellent. Thereafter, a mixture of sodium citrate and potassium citrate in doses of 2.0~3.0 g, given orally, have been used to reduced stone growth. Consequently, a left renal stone was completely dissolved using this regimen. In the meantime, round crystals corresponding to 2,8-DHA were seen in the urine from his younger brother. The same doses of allopurinol have been used successfully in preventing new stone formation in his brother.

#### DISCUSSION

Debray et al.<sup>2)</sup> and Simmonds et al.<sup>3)</sup> reported the first individuals homozygous for APRT deficiency and associated with 2,8-DHA stones. Many cases have been found especially in Japan<sup>4)</sup> accounting for more than half of the total number reported in the world. Biochemically as well as at a molecular level, this disease has been fully elucidated by the works of Fujimori et

al.<sup>5)</sup> and Hidaka et al.<sup>6)</sup>

In patients with DHA urolithiasis round brown crystals can be observed in the urine, and may vary in size and shape depending on the time of sampling, then divided into three groups according to the size seen on the optical microscope as follows: a) small crystals (below 3  $\mu\text{m}$  in diameter), b) medium crystals (3~10  $\mu\text{m}$  in diameter), c) large crystals (10~20  $\mu\text{m}$  or 25  $\mu\text{m}$  and more in diameter). Round brown crystals in the urinary sediment should be subject of further examination, so that early detection of 2,8-DHA urolithiasis and renal injury secondary to acute crystal nephropathy may be possible.

The stone analysis of spontaneously excreted or surgically removed calculi is commonly performed by infrared spectrophotometry, and X-ray diffraction of HPLC will identify the proper components.

Two phenotypic variants of APRT deficiency have been recognized. 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. The type II mutant alleles are designated APRT\*J.

APRT enzyme activity in erythrocyte lysates of heterozygotes of complete deficiency is approximately 25% of control activity, and erythrocyte lysates of homozygotes of complete deficiency have been almost zero per cent<sup>7)</sup>. With regard to APRT deficiency of Japanese type (APRT\*J), the corresponding values are 25 or 50% of the control activity. Therefore, it is difficult to distinguish homozygosity from heterozygosity of Japanese. The alternative method demonstrates that by testing the resistance of viable T cells to adenine analogues 6 methylpurine or 2,6-diaminopurine, all the individuals whose T cells are resistant to the adenine analogues are homozygotes for the APRT alleles. Our patients with the Japanese type APRT deficiencies have the genotype APRT\*J/APRT\*J.

Kamatani and associates<sup>1)</sup> postulate that

the difference in the percentage of partial APRT deficiencies between Japanese and Caucasian patients with DHA urolithiasis can no longer be considered incidental, because all Caucasian patients have been completely deficient in hemolysate APRT activities, while 76% of the Japanese patients were only partially deficient. The incidence of 2,8-dihydroxyadenine lithiasis has been reported to be apparently high in Japan, because of the wide distribution of the unique mutant gene APRT\*J that was created many years ago in a Japanese ancestor<sup>8)</sup>.

The combination of xanthine oxidase inhibitor allopurinol and low purine diet has proved effective. In most cases, a dose of 10 mg/kg daily has eliminated the insoluble 2,8-DHA metabolite<sup>9)</sup>. Simonds<sup>10)</sup> recommended allopurinol should be decreased to 5 mg/kg daily in patients with renal failure to prevent the accumulation of oxypurinol.

It is said that alkalization of the urine commonly used in treatment of uric acid stones is ineffective in 2,8-DHA in the physiologic pH range<sup>11)</sup>. Based on our experience, long term treatment with a mixture of sodium citrate and potassium citrate can be expected to reduce stone growth and the incidence of stone recurrence in patients with residual stone disease.

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## 和文抄録

## Adenine phosphoribosyltransferase 部分欠損による 2,8-dihydroxyadenine 結石症例

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近年, 2,8-dihydroxyadenine (以下 2,8-DHA と略す) を主成分とする尿路結石が注目されている。2,8-DHA は adenine の代謝産物であるがプリン体サルベージ酵素の一つである adenine phosphoribosyltransferase (APRT と略す) の欠損症では尿中に多量に排泄され, この物質が不溶性であることから結石が形成されると考えられている。

今回, 尿沈渣中の結晶および自然排石された結石分析の結果, 2,8-DHA 結石と判明し, APRT 活性の測定より APRT 部分欠損症, 遺伝子型は日本人型と診断した22歳, 男性の症例を経験した。なお, 血縁者の中ではT細胞による判定で本人の他には弟がホモ接合体で尿路結石形成の可能性が示唆された。他の家族はヘテロ接合体または正常と判定された。allopurinol 300 mg/日の投与と低プリン食 (150 mg/日) を指導し, 尿中の結晶は消失した。

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