Use of LightCycler mutation analysis to detect type II adenine phosphoribosyltransferase deficiency in two patients with 2,8-dihydroxyadeninuria

Hirokazu Ikeda¹ · Tuneki Watanabe¹ · Daiske Toyama¹ · Keiichi Isoyama¹

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Abstract Recently, a number of methods have been devised for detection of mutations in the field of molecular genetics. The LightCycler system has been used for rapid PCR, while simultaneously quantifying and analyzing the amplification results. We tried to apply the LightCycler system to detect APRT*J allele mutations in two families including two children with 2,8-dihydroxyadenine urolithiasis. The first patient was a 3-year-old girl who presented with left flank pain. The second patient was a 2-year-old girl who presented with complaints of sudden dysuria. The spectrophotometric analysis of the stone fragments of both patients revealed an absorption spectrum for 2,8-DHA. We used the LightCycler system to detect APRT*J mutation. The first patient was homozygous for APRT*J/APRT*J and the second patient was compound heterozygous for APRT*J/APRT*Q0. The genetic diagnosis of APRT deficiency using this system may be useful not only as a diagnostic test for infants with known 2,8-DHA, but also as a screening of infants with a suspicion of urolithiasis. We believed that the LightCycler system still is an important means of identifying APRT*J mutation.

Keywords 2,8-Dihydroxyadeninuria · Adenine phosphoribosyltransferase deficiency · Urolithiasis · LightCycler · Child · Real-time PCR instruments

Introduction

Adenine phosphoribosyltransferase (APRT) is a purine salvage enzyme present in all human cells. APRT catalyzes the conversion of adenine to adenosine monophosphate in the presence of 5'-phosphoribosyl-1-pyrophosphate. In APRT deficiency, the substrate adenine is oxidized by xanthine dehydrogenase to the highly insoluble 2,8-dihydroxyadenine (DHA), which can precipitate in the kidney and lead to stone formation [1]. Two phenotypic variants of APRT deficiency have been recognized, based on the level of residual enzyme activity in erythrocyte lysates. Enzyme activity is virtually absent in patients with type 1 APRT deficiency, which predominantly affects whites. These patients are homozygotes or compound heterozygotes for null alleles, collectively referred to as APRT*Q0. Patients with type 2 APRT deficiency show only partial enzyme deficiency that are homozygous for the mutant allele APRT*J [2]. Both types of APRT deficiency are found in the Japanese population, but type 2 deficiency patients constitute approximately 75 % in Japan. These patients possess either one or two APRT*J alleles. This mutant allele has a missense mutation at codon 136 from TGG to TGA in exon 5 [3, 4].

Occasionally APRT deficiency in pediatric patients is misdiagnosed because the frequency of actual stone passage is very low in pediatric patients, so it is difficult to analyze the DHA urolithiasis. Analysis of the APRT gene is the most reliable diagnostic method for APRT deficiencies [1]. The major problem with conventional mutation scanning methods, however, is that it requires multiple manual steps and takes a very long time to verify detection. An online fluorescence polymerase chain reaction (PCR) detection system and HybProbe chemistry were developed for use in the LightCycler system [5]. Funato et al. reported that they had established a rapid and reliable system

✉ Hirokazu Ikeda
hiro31@mac.com

¹ Department of Pediatrics, Showa University Fujigaoka Hospital, Fujigaoka1-30, Aoba-ku, Yokohama, Kanagawa, Japan

capable of detecting mutations of the APRT gene using a LightCycler system [6].

We hereby report on two families with DHA urolithiasis using a LightCycler system to identified the APRT*J mutation.

Case report

Case 1

A 3-year-old Japanese girl was referred to our hospital in May 2008 because of left flank pain and macroscopic hematuria. She had occasionally presented with orange-colored urinary spots on her diapers since 15 months of age. Therefore, she was referred to our department this time for examination of left flank pain and hematuria. There was no family history of urolithiasis or renal disease. At the time of admission, her height was 101 cm and weight 13.4 kg. Her blood pressure on admission was 108/60 mmHg, temperature 36.8 °C, and heart rate 78 beats/min. The patient had right costovertebral angle tenderness. The rest of the physical examination was normal. Urinalysis revealed pyuria with 30–50 white blood cells (WBC)/high power field (HPF) (normal range 0–5 WBC/HPF) and round brown crystals in the urinary sediment. Blood chemistry findings were within the normal limits (Table 1).

Table 1 Results of laboratory tests conducted on admission in Case 1 and 2

	Case 1	Case 2
Urinalysis		
pH	5	7.5
Specific gravity	1.02	1.011
Protein	(±)	(1+)
Occult blood	(±)	(1+)
Urine sediment		
RBC (/HPF)	1–2	4–6
WBC (/HPF)	30–50	10–15
Cast	(–)	(–)
u-Ca/Cre (mg/mg)	0.07	0.11
Analysis of the stone fragments		
2,8-Dihydroxyadenine (%)	98	98
Others (%)		4
Hematology		
WBC (/μl)	6600	12,900
Hb (g/dl)	13.0	13.0
Plt (/μl)	34.4×10^3	29.1×10^3
Blood chemistry		
BUN (mg/dl)	15.3	10.3
UA (mg/dl)	2.5	3.2
Cre (mg/dl)	0.3	0.2

An abdominal echo examination revealed a slightly dilated right pelvis and the presence of several renal stones (Fig. 1a). A dimercaptosuccinic acid scan (DMSA) revealed normal bilateral kidneys (Fig. 1b). Following saline infusion, small stones were passed spontaneously the next day (Fig. 1c). Spectrophotometric analysis of the stone fragments revealed an absorption spectrum for 2,8-dihydroxyadenine (2,8-DHA) urolithiasis. We started treatment with allopurinol (75 mg/day), purine restricted diet, and increased fluid intake. One year after starting the allopurinol treatment, the patient had not experienced any attacks of colicky pain nor passed any urinary crystals.

Case 2

A 2-year-old Japanese girl was referred to our hospital in February 2010 because of left flank pain and sudden dysuria. From 6 months of age, she had occasionally presented with red-colored urinary spots on her diapers. A primary clinic diagnosed asymptomatic hematuria. She was hospitalized because of gradually increasing left flank pain, sudden dysuria, and passage of a large number of small stones. Upon admission to our hospital, the patient's height was 98 cm and weight 12.8 kg. Her body temperature was 37.8 °C, heart rate 72 beats per minute, respiratory rate 28 breaths per minute, and blood pressure 108/60 mmHg. She had diffuse left lower quadrant pain and tenderness of the left costovertebral angle. The blood chemistry findings were within normal limits. Urinalysis revealed pyuria with 10–15 WBC/HPF, without any casts and with 1+ proteinuria (Table 1).

An ultrasound examination showed several stones in her bladder (Fig. 2a). Renal stones were found in the left kidney. A DMSA scan indicated the presence of focal defects, particularly in the left kidney pole (Fig. 2b).

A spontaneously passed stone consisted of 2,8-DHA, as confirmed by infrared spectroscopic analysis. We performed a successful endoscopic lithectomy 1 month after admission because of continued stone passage; hence, it was impossible to extract the remaining stones with fluid infusion. Treatment with allopurinol, purine restricted diet, and increased fluid intake was then started, since then the patient has not experienced any attacks of colicky pain. Currently, the patient is 4 years old, and has shown no stones in the urinary tract.

We used the LightCycler system to detect APRT*J allele mutations. After obtaining informed consent, peripheral blood was drawn from the patients and family members. DNA was extracted from blood samples by phenol–chloroform and ethanol precipitation. Some of the APRT genes in each sample were amplified by polymerase chain reaction (PCR) using synthetic primers. These primers for APRT PCR correspond to nucleotides in the J

Fig. 1 a Abdominal ultrasound revealing a slightly dilated right pelvis and the presence of several renal stones.

b Dimercaptosuccinic acid scan revealing normal bilateral kidneys. **c** The 2,8-dihydroxyadenine stones passed spontaneously from patient 1

Fig. 2 a Abdominal ultrasound revealing several renal stones in bladder. **b** Dimercaptosuccinic acid scan revealing the presence of focal defects, particularly in the left kidney pole

regions of the APRT gene. To obtain a melting curve by monitoring the denaturation profile of the PCR product of APRT*J allele in the presence of hybridization probes, we used a LightCycler® system (Roche Molecular Biochemicals, Mannheim, Germany). Analysis of LightCycler

system was described by Funato et al. [6]. This analysis may also be used to identify the relative contributions of mutated versus wild-type sequences to signal level. For sequence-specific fluorescence detection, oligonucleotide hybridization probes coupled at the 3' end to a 5'-

Fig. 3 Genotyping of APRT*J with an allele-specific fluorescent probe by melting curve and derivative melting curve plots by the LightCycler instrument. *Trace 1* homozygous showing patient 1. *Trace 2* control showing wild type

Fig. 4 Fluorescence data for APRT*J regions from patient 2, generated by the LightCycler instrument. *Trace 1* heterozygote showing patient 2. *Trace 2* wild type showing patient 2 mother. *Trace 3* heterozygote showing patient 2 father

Fig. 5 Identification of a missense mutation in the adenine phosphoribosyltransferase (APRT) gene located on chromosome 16q24. Sequence analysis of the patient shows M1I in exon 1 and M136T in exon 5

fluorescein label and at the 5' end to 3'-LightCycler-Red 640 were used. The sequences of the two hybridization probes for the APRT*J type were LightCycler-Red 640-labeled 5'-AGGCAGCGTCGTTTCATGGTTCC-3' and fluorescence-labeled 5'-GCAGGCGGCCAGCAGCTCA-3'. Melting curve analysis with the LightCycler system was done for three variants in exon 5 at the appropriate melting temperature. These variants consisted of wild, heterozygous, and homozygous types, which appeared as a pattern of clearly separated peaks. APRT Q0 mutation was analyzed via the direct sequencing method. The results showed that patient 1 was homozygous, corresponding only to APRT*J allele, whereas both parents and her younger sister were heterozygote, containing the wild type and APRT*J allele (Fig. 3). Patient 2 was compound heterozygote, containing APRT*J, her mother was wild type, and her father was heterozygote, containing the wild type and APRT*J (Fig. 4). The APRT*Q0 mutations reported in Japanese were analyzed with the PCR-SSCP method for the patient 2, but no mutations of APRT*Q0 were detected. Subsequently, we screened the APRT gene mutation regions reported from Europe and the United States and identified a heterogeneous mutation at the start codon of APRT*Q0 from methionine to isoleucine (M1I) (Fig. 5). She was diagnosed with APRT deficiency caused by a compound heterogeneous mutation: APRT*Q0 (M1I)/APRT*J (M136T).

Discussion

In this report, we have attempted to use LightCycler system to detect APRT*J allele mutations in two pediatric patients and their families with 2,8-dihydroxyadenine urolithiasis. APRT deficiency is classified into type 1 and type 2, according to phenotype. All type 2 patients have the APRT*J allele, but only homozygotes develop clinical symptoms. About half of the reported cases of APRT deficiency involve 15-year-old patients or younger [1], but in APRT deficiency of infants, some cases cannot be diagnosed, because no excretion of calculi is observed, and DHA calculi are missed, because skill is required to judge the infrared adsorption level in calculus analysis, so it is believed that there will be a considerable number of cases in which kidney failure will be found in adulthood. In addition, even in the case of APRT deficiency, if no symptoms are apparent, analysis of urinary calculi cannot be expected to trigger diagnosis [7]. APRT gene analysis is the most reliable diagnostic method not only in cases involving infant, but also in cases involving adults [2, 8].

A DMSA scan showed different uptakes in the kidney between Case 1 and 2. Among APRT genes, APRT*Q0 is an APRT allele coding for an aberrant protein completely

devoid of APRT activity, while APRT*J is an allele coding for a protein with 25 % activity compared with normal APRT. In the Case 1 patient, the APRT gene was the APRT*J homozygote, and thus the APRT activity deficiency was only partial and 2.8 DHA-induced nephrotoxicity was mild. In the Case 2 patient, the APRT gene was a compound heterogeneous mutation: APRT*Q0/APRT*J, and thus the APRT activity was nearly absolutely absent and 2.8 DHA-induced nephrotoxicity was severe. This is presumably why the Case 2 patient presented with reduced DMSA uptake and renal atrophy in the left kidney, while DMSA uptake was normal in the Case 1 patient.

Analysis of the APRT gene is the most reliable diagnostic method for infants. There are many methods currently available for genotyping the APRT gene, including single-strand conformation polymorphism and allele-specific PCR. PCR-SSCP analysis will likely become the standard procedure for the detection of APRT genes, and it is currently the most common method of detecting APRT deficiency [2]. The major problem with the present PCR-SSCP method, however, is that it requires multiple manual steps and takes a very long time for confirmation of detection. Methods such as TaqMan chemistry and the LightCycler system can detect mutations accurately without gel electrophoresis [9, 10]. There has been a large number of reported cases of the usefulness of the LightCycler system in screening for the prevalence of the mutation of genes, which is becoming increasingly important in understanding various diseases.

Funato et al. developed a homogeneous assay using a closed tube that allowed rapid genotyping with the LightCycler system and used it to identify the APRT*J gene as homozygote or heterozygote [6]. This assay involves allele-specific analysis of probe melting curves at the completion of amplification. As demonstrated in this study, analysis of the APRT gene using the LightCycler system can provide mutation results within approximately 30 min after DNA isolation, and the results are easily and clearly viewable. In contrast with PCR-SSCP, the results obtained with the LightCycler system were consistent with those of sequencing. Genotyping with the LightCycler system is rapid and reliable, and is suitable for typing large numbers of samples. Rapid detection of APRT mutation in clinical samples can be applied to the diagnosis of APRT deficiency and may allow effective patient management to be initiated at an early stage. Genetic diagnosis of APRT deficiency using the LightCycler system may be useful not only for the diagnosis of infants with known 2,8-DHA, but also for screening of infants suspected to have urolithiasis. There are few reports using the LightCycler system to identify APRT*J mutation in diagnosis with APRT type 2 deficiency, despite it has been more than 10 years since Funato et al. demonstrated the usefulness of the

LightCycler system in this field. We believe that the LightCycler system still be an important means of diagnosing early-stage APRT deficiency because this method is reliable and rapidly demonstrates the APRT gene mutations. Furthermore, this system is ideal for detection for difference in mutations between homozygous and heterozygous genotypes as shown in Fig. 2.

Conflict of interest The authors have declared that no conflict of interest exists.

References

1. Sahota A, Tischfield J, Kamanani N, et al. Adenine phosphoribosyltransferase deficiency: 2,8-dihydroxyadenine lithiasis. In: John BS, editor. *The Metabolic and molecular basis of inherited disease*. New York: McGrawHill; 2001. p. 2571–84.
2. Fujimori S, Akaoka I, Sakamoto K, et al. Common characteristics of mutant adenine phosphoribosyltransferases from four separate Japanese families with 2,8-dihydroxyadenine urolithiasis associated with partial enzyme deficiencies. *Hum Genet*. 1985;71:171–6.
3. Kamatani N, Terai C, Kuroshima S, et al. Genetic and clinical studies on 19 families with adenine phosphoribosyltransferase deficiencies. *Hum Genet*. 1987;75:163–8.
4. Kamatani N, Hakoda M, Otsuka S, et al. Only three mutations account for almost all defective alleles causing adenine phosphoribosyltransferase deficiency in Japanese patients. *J Clin Invest*. 1992;90:130–4.
5. Mangasser-Stephan K, Tag C, Reiser A, et al. Rapid genotyping of hemochromatosis gene mutations on the LightCycler with fluorescent hybridization probes. *Clin Chem*. 1999;45:1875–8.
6. Funato T, Nishiyama Y, Ioritani N, et al. Detection of mutations in adenine phosphoribosyltransferase (APRT) deficiency using the LightCycler system. *J Clin Lab Anal*. 2000;14:274–9.
7. Arnadottir M, Laxdal T, Hardarson S, et al. Acute renal failure in a middle-aged woman with 2,8-dihydroxyadeninuria. *Nephrol Dial Transplant*. 1997;12:1985–7.
8. Terai C, Hakoda M, Yamanaka H, et al. Adenine phosphoribosyltransferase deficiency identified by urinary sediment analysis: cellular and molecular confirmation. *Clin Genet*. 1995;48:246–50.
9. Wittwer CT, Herrmann MG, Moss AA, et al. Continuous fluorescence monitoring of rapid cycle DNA amplification. *Biotechniques*. 1997;22:130–9.
10. de Kok JB, Hendriks JC, van Solinge WW, et al. Use of real-time quantitative PCR to compare DNA isolation methods. *Clin Chem*. 1998;44:2201–4.