Adenine Phosphoribosyltransferase Deficiency

Prevalence and Clinical Outcomes

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UNIVERSITY OF ICELAND
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Adenínfosfóríbósýltransferasa-skortur

Algengi og afdrif sjúklinga

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Ágrip

Adenínfosfóríbósýltransferasa (APRT)-skortur er sjaldgæfur galli í efnaskiptum adeníns sem erfist víkjandi og leiðir til myndunar mikls magns af 2,8-díhýdroxýadeníns (DHA) og útskilnaðar þess í þvagi, en efnið er mjög torleyst og veldur bæði nýrnasteinum og kristallanýrnameini sem leiðir til langvinns nýrnasjúkdóms (LNS) og jafnvel lokastigsnýrnabilunar (LSNB). Röng sjúkdómsgreining eða alvarleg greiningartöf veldur því að sjúklingar fá oft ekki rétta meðferð í tíma. Meðferð með xanþínoxidóredúktasa (XOR)-hemlunum allópurínóli og febuxóstati getur dregið verulega úr afleiðingum sjúkdómsins. Takmarkaðar leiðbeiningar um meðferð APRT-skorts eru fyrirliggjandi. Þá skortir upplýsingar um algengi APRT-skorts, framrás sjúkdómsins, útskilnað DHA í þvagi og svörun við lyfjameðferð.

Markmið með þessari ritgerð voru að meta algengi APRT-skorts, kanna ástæður fyrir rangri greiningu og meta framrás nýrnasjúkdómsins, framvindu eftir ígræðslu nýra og áhrif þess að hefja meðferð með XOR-hemli snemma, einkum í æsku, á sjúkdómsbyrði. Einnig að beita nýrri aðferð sem byggir á háhraðavökvaskilju tengdri tvöföldum massagreini til að rannsaka útskilnað DHA í þvagi hjá sjúklingum með APRT-skort og bera þá saman við arfbera og heilbrigða einstaklinga til að meta næmi og sértæki aðferðarinnar til sjúkdómsgreiningar. Loks var eitt markmiða að skoða áhrif breytilegs magns púrína í fæðu og meðferðar með allópúrínóli og febúxóstati á útskilnað DHA í þvagi.

Í grein I var reynt að hafa upp á öllum þekktum tilfellum APRT-skorts og meinvaldandi breytileikum í APRT-geninu með heimildaleit í margvíslegum gagnagrunnum sem aðgengilegir eru á Internetinu. Þá var leitað að öllum þekktum meinvaldandi stökkbreytingum í lýðgrunduðum arfgerðarsöfnum og tíðni arfhreinna einstaklinga reiknuð út frá lögmáli Hardy og Weinberg. Greinar II-V fjölluðu um þýðisrannsóknir sem byggðar voru á gögnum er safnað var frá þátttakendum í APRT Deficiency Registry, en það er alþjóðleg skrá yfir sjúklinga með APRT-skort sem tilheyrir Rare Kidney Stone Consortium. Fjórum sjúklingum til viðbótar var vísað til þátttöku í rannsókninni frá stofnunum í Frakklandi og Ástralíu. Í greinum II, VI og VII voru gögn úr APRT Deficiency Registry og pöruð lífsýni úr lífsýnabanka skrárinnar notuð til rannsóknar á útskilnaði DHA í þvagi sem mældur var með háhraðavökvaskilju tengdri tvöföldum massagreini. Lýsandi tölfræði var beitt og hefðbundnar tölfræðilegar aðferðir notaðar við samanburð hópa. Fylgnigreining var gerð með fylgnistuðlum Pearsons eða Spearmans (r).

Alls fundust 438 tifelli af APRT-skorti um allan heim, þar af 250 í Japan, 37 á Íslandi, 33 í Frakklandi og 26 í Bandaríkjunum, ásamt 62 meinvaldandi breytileikum í APRT-geninu. Algengi APRT-skorts virðist mun hærra á Íslandi og í Japan en í öðrum löndum, líklega vegna landnemastökkbreytinga. Aðrar stökkbreytingar reyndust mjög fátíðar en tíðni þeirra bendir þó til að sjúkdómurinn sé víða algengari en fjöldi greindra tilfella gefur til kynna. Töf á sjúkdómsgreiningu frá upphafi einkenna var algeng og var miðgildi (spönn) tímalengdar 7,5 (0,4-47,9) ár. Helmingur sjúklinga sem taldir voru hafa APRT-skort á grunni steinagreiningar reyndist hins vegar ekki hafa sjúkdóminn. Við greiningu voru 55% sjúklinga með nýrnasteina en 38% höfðu LNS á stigi 3-5 og þriðjungur bráðan nýrnaskaða (BNS). Enginn þeirra sjúklinga sem hóf meðferð með XOR-hemli á barnsaldri greindist með LNS á stigi 3 eða hærra. Sjúklingar sem fengu meðferð með XOR-hemli fyrir nýraígræðslu höfðu miðgildi reiknaðs gaukulsíunarhraða (r-GSH) 61,3 (24,0-90,0) ml/mín./1,73 m^2 tveimur árum eftir ígræðsluaðgerðina, samanborið við 16,2 (10,0-39,0) ml/mín./1,73 m^2 hjá þeim sem ekki hófu lyfjameðferð fyrir ígræðslu (p=0,009). DHA-útskilnaður í þvagi var mikill en breytilegur hjá sjúklingum með APRT-skort en DHA fannst hvorki í þvagsýnum frá arfblendnum né heilbrigðum einstaklingum. Aðferðin var því bæði 100% næm og sértæk til greiningar á APRT-skorti. Fylgnistuðullinn milli 24 klukkustunda DHA-útskilnaðar og DHA/kreatínín-hlutfalls í fyrsta morgunþvagi var 0,84 (p <0,001). Engin áhrif af neyslu púrína á DHA-útskilnað í þvagi komu fram, en meðferð með allópúrínóli 400 mg á dag og febúxóstati 80 mg daglega dró marktækt úr útskilnaði DHA.

APRT-skortur er sjaldgæfur sjúkdómur sem virðist þó vera vangreindur sums staðar í heiminum. Nýrnasteinar eru algengasta birtingarmynd sjúkdómsins en BNS og ágengur LNS án sögu um nýrnasteina eru algengari en áður var talið. Ágengur LNS er ein helsta afleiðing APRT-skorts og leiðir oft til LSNB. Sé meðferð með XOR-hemli hafin snemma tekst að varðveita nýrnastarfsemi og koma í veg fyrir endurkomu DHA-kristallanýrnameins í ígræddum nýrum. Ný aðferð til mælingar á DHA í þvagi virðist vera mjög nákvæm við greiningu á APRT-skorti, auk þess að vera gagnleg til mats á virkni lyfjameðferðar. Rannsóknirnar sem fjallað er um í þessari ritgerð hafa aukið verulega við þekkingu á APRT-skorti og munu vonandi leiða til bættrar útkomu fyrir einstaklinga með þennan alvarlega sjúkdóm.

Lykilorð: 2,8-Díhýdroxýadenínmiga, nýrnasteinar, langvinnur nýrnasjúkdómur, nýraígræðsla, xanþínoxidóredúktasa-hemlar

Abstract

Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive disorder of adenine metabolism that results in the generation and renal excretion of large amounts of the poorly soluble 2,8-dihydroxyadenine (DHA), causing kidney stones and crystal nephropathy which can progress to end-stage kidney disease (ESKD). Treatment with the xanthine oxidoreductase (XOR) inhibitors allopurinol and febuxostat is beneficial but has been underutilized due to missed or delayed diagnosis and guidance for clinical management has been lacking. Information is scarce on the true prevalence of APRT deficiency, the course of the disease, the characteristics of DHA excretion and the response to treatment.

The aims of this thesis were to estimate the prevalence of APRT deficiency, examine the reasons for missed diagnosis and study long-term renal outcomes, the course following kidney transplantation and effect of timely intervention, particularly when initiated in childhood. Furthermore, to use a novel UPLC-MS/MS assay to study the urinary excretion of DHA in APRT deficiency patients, heterozygotes and healthy control subjects, to test the sensitivity and specificity of the assay for diagnosis of the disorder and to examine the effect of dietary purine modification and treatment with allopurinol and febuxostat on urinary DHA excretion.

In Paper I, all reported and registered cases of APRT deficiency and pathogenic *APRT* mutations were identified through a search of multiple online sources. Population-based genomic databases were searched for all known disease-causing mutations and the frequency of homozygous genotypes calculated assuming the Hardy-Weinberg equilibrium. The studies described in Papers II-V were based on observational data collected from participants enrolled in the APRT Deficiency Registry of the Rare Kidney Stone Consortium. Four additional patients were referred to the research program from institutions in France and Australia. In Papers II, VI and VII, data and matching biosamples from the APRT Deficiency Registry and Biobank were used for the analysis of urinary DHA excretion using the UPLC-MS/MS assay. Descriptive statistics were used to present the data and groups were compared employing conventional statistical methods. Correlation analysis was performed using Pearson´s or Spearman´s correlation coefficients (r).

A total of 438 cases of APRT deficiency were identified worldwide, including 250 in Japan, 37 in Iceland, 33 in France and 26 in the US, together

with 62 pathogenic mutations in the *APRT* gene. The prevalence of APRT deficiency appears to be much higher in Iceland and Japan than in other populations, clearly due to a founder effect. Other mutations were rare, but their frequency nevertheless suggests that the disease may be more common in some areas than reflected by the number of reported cases. The median (range) delay between the first symptoms and diagnosis of the disease was 7.5 (0.4-47.9) years. Half of the patients referred to the APRT Deficiency Research Program with stone analysis suggesting DHA had been incorrectly diagnosed with the disorder. Nephrolithiasis was the most common clinical manifestation, observed in 55% of patients at diagnosis, while 38% had chronic kidney disease (CKD) stages 3-5 and one-third had acute kidney injury (AKI). No patients who initiated XOR inhibitor treatment in childhood progressed to CKD stage 3 or above. Patients receiving XOR inhibitor treatment prior to kidney transplantation had a median (range) eGFR of 61.3 (24.0–90.0) mL/min/1.73 m^2 at two years post-transplant compared with 16.2 (10.0–39.0) mL/min/1.73 m^2 in those who did not initiate drug treatment pre-transplant (p=0.009). Urinary DHA excretion was high but variable in patients with APRT deficiency, whereas DHA was not detected in urine samples from heterozygotes and healthy individuals. Hence, the urinary assay yielded 100% sensitivity and specificity for the diagnosis of the disease. The correlation coefficient between 24-h urinary DHA excretion and the DHA-to-creatinine ratio in first morning void urine samples was 0.84 ($p<0.001$). No effect of dietary purine intake on urinary DHA excretion was observed, while treatment with allopurinol 400 mg/day and febuxostat 80 mg/day significantly reduced DHA excretion.

APRT deficiency is a rare disease but appears to be underdiagnosed in some areas of the world. Nephrolithiasis was found to be the most common clinical manifestation, though progressive CKD and AKI are more frequent than previously reported. Indeed, advanced CKD is a major feature of APRT deficiency, commonly resulting in ESKD. Timely initiation of XOR inhibitor therapy preserves kidney function and prevents recurrence of DHA nephropathy in kidney allografts. The novel UPLC-MS/MS urinary DHA assay appears very accurate in the diagnosis of APRT deficiency, in addition to being useful for monitoring of pharmacotherapy. The work presented in this thesis has significantly advanced the field of APRT deficiency and will hopefully lead to improved outcomes for patients affected by this serious disease.

Keywords: 2,8-Dihydroxyadeninuria, kidney stones, chronic kidney disease, kidney transplantation, xanthine oxidoreductase inhibitors.

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Contents

List of abbreviations

ACMG	American College of Medical Genetics and Genomics
ADA	Adenine deaminase
AKI	Acute kidney injury
AMP	Adenine monophosphate
APRT	Adenine phosphoribosyltransferase
ATR	Attenuated total reflection
ATR-FTIR	Attenuated total reflection fourier transform-infrared spectroscopy
BP	Base pair
BSA	Body surface area
CKD	Chronic kidney disease
CKiD	Chronic Kidney Disease in Children
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
cMAF	Cumulative minor allele frequency
DHA	2,8-Dihydroxyadenine
DNA	Deoxyribonucleic acid
eGFR	Estimated glomerular filtration rate
ESKD	End-stage kidney disease
ESWL	Extracorporeal shock-wave lithotripsy
FTIR	Fourier transform-infrared spectroscopy
GFR	Glomerular filtration rate
GMP	Guanosine monophosphate
gnomAD	Genome Aggregation Database
H&E	Hematoxylin and eosin
HGVD	Human Genetic Variation Database

HPLC	High-performance liquid chromatography (HPLC)
HPLC-MS/MS	High-performance liquid chromatography-tandem mass spectrometry
HPRT	Hypoxanthine phosphoribosyltransferase
IMP	Inosine monophosphate
KDIGO	Kidney Disease: Improving Global Outcome
KOVA	Korean Variant Archive
LUH	Landspitali–The National University Hospital
MAF	Minor allele frequency
MCT	Mercury cadmium telluride
mRNA	Messenger ribonucleic acid
NLRP3	Nucleotide-binding domain (NOD)-like receptor protein 3
OMIM	Online Mendelian Inheritance in Man
PCR	Polymerase chain reaction
PRP	Purine nucleoside phosphorylase
PRPP	5-phosphoribosyl-1-pyrophosphate
RBC	Red blood cells
RKSC	Rare Kidney Stone Consortium
RRT	Renal replacement therapy
SCr	Serum creatinine
UK	United Kingdom
UPLC-MS/MS	Ultra-performance liquid chromatography–tandem mass spectrometry
US	United States
XMP	Xanthine monophosphate
XOR	Xanthine oxidoreductase

List of figures

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List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals:

I. Runolfsdottir HL, Sulem P, Sayer JA, Indridason OS, Edvardsson VO, Palsson R. Allele frequency of variants reported to cause adenine phosphoribosyltransferase deficiency. *European Journal of Human Genetics*. In revision.

II. Runolfsdottir HL, Goldfarb DS, Sayer JS, Michael M, Ketteridge D, Rich P, Edvardsson VO, Palsson R (2019). Are conventional stone analysis techniques reliable for the identification of 2,8-dihydroxyadenine kidney stones? A case series. *Urolithiasis*. 48(4):337-344.

III. Runolfsdottir HL, Palsson R, Agustsdottir IM, Indridason OS, Edvardsson VO (2016). Kidney disease in adenine phosphoribosyltransferase deficiency. *Am J Kidney Dis*. 67(3):431-438.

IV. Runolfsdottir HL, Palsson R, Agustsdottir IM, Indridason OS, Edvardsson VO (2018). Long-term renal outcome of APRT deficiency presenting in childhood. *Pediatr Nephrol.* 34 (3):435-442.

V. Runolfsdottir HL, Palsson R, Agustsdottir IM, Indridason OS, Li Jennifer, Dao Myriam, Knebelmann B, Milliner D, Edvardsson VO (2019). Kidney transplant outcomes in patients with adenine phosphoribosyltransferase deficiency. *Transplantation.* In press.

VI. Runolfsdottir HL, Palsson R, Thorsteinsdottir UA, Indridason OS, Agustsdottir IM, Oddsdottir GS, Thorsteinsdottir M, Edvardsson VO (2019). Urinary 2,8-dihydroxyadenine excretion in health and disease. *Mol Genet Metab*. 128:144-150.

VII. Edvardsson VO, Runolfsdottir HL, Thorsteinsdottir UA, Agustsdottir IM, Oddsdottir GS, Eiriksson F, Goldfarb DS, Thorsteinsdottir M, Palsson R (2018). Comparison of the effect of allopurinol and febuxostat on urinary 2,8-dihydroxyadenine excretion in patients with APRT deficiency: a clinical trial. *Eur J Intern Med.* 48:75-79.

In addition, some unpublished data are presented.

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Declaration of contribution

The doctoral candidate, Hrafnhildur Linnet Runólfsdóttir, was involved in designing the doctoral project and studies I-III. The doctoral candidate participated in data collection and entry into the APRT Deficiency Registry, performed all analyses and drafted and critically revised the manuscripts of Papers I-VI.

For study IV, presented in Paper VII, the doctoral candidate assisted with the data collection and performed all statistical analyses. VÖE designed the study and drafted the paper.

The doctoral candidate wrote this thesis under the guidance of her supervisor and doctoral committee.

1 Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (Online Mendelian Inheritance in Man, OMIM 102600, 614723) is a rare autosomal recessive disorder of adenine metabolism that results in the generation and renal excretion of large amounts of the poorly soluble 2,8-dihydroxyadenine (DHA), causing kidney stones and crystal nephropathy. A significant proportion of patients experience progressive chronic kidney disease (CKD) and even end-stage kidney disease (ESKD), on the basis of crystal nephropathy, especially in the absence of effective pharmacotherapy (Bollee et al., 2010; Edvardsson, Palsson, Olafsson, Hjaltadottir, & Laxdal, 2001). William Kelley and coworkers first described partial deficiency of the APRT enzyme in 4 asymptomatic subjects in 1968 (Kelley, Levy, Rosenbloom, Henderson, & Seegmiller, 1968), and the first case of complete APRT enzyme deficiency was reported by Cartier five years later in a young child with DHA nephrolithiasis (Cartier, Hamet, & Hamburger, 1974).

Radiolucent kidney stones are the most commonly reported presenting feature of APRT deficiency, typically occurring during adulthood, followed by CKD (Edvardsson, Palsson, & Sahota, 2012). A small subset of patients are asymptomatic at the time of diagnosis. Unfortunately, the disorder is often first recognized in the setting of ESKD or when allograft failure occurs due to disease recurrence after kidney transplantation. Consequently, several published reports have described unfavorable outcomes of kidney transplantation in APRT deficiency (Bertram, Broecker, Lehner, & Schwarz, 2010; de Jong et al., 1996; Eller et al., 2004; Gagne, Deland, Daudon, Noel, & Nawar, 1994; Kaartinen et al., 2014). Treatment with the xanthine oxidoreductase (XOR; xanthine dehydrogenase/oxidase) inhibitors allopurinol and febuxostat effectively prevents stone formation and may even improve kidney function in some cases (Bollee et al., 2010).

Approximately 300 cases of APRT deficiency have been reported worldwide, though only a handful come from large nations such as the United Kingdom (UK) and the United States (US). Based on reports of measured enzyme activity in healthy populations, the heterozygote frequency may be in the range of 0.4–1.2% (Johnson, Gordon, & Emmerson, 1977; Srivastava, Villacorte, & Beutler, 1972). Albeit limited data, the number of cases worldwide would be expected to be much greater than previously reported.

Thus, lack of awareness of the disorder among clinicians and pathologists is concerning and may contribute to the low number of reported cases.

Most physicians may not see a case of APRT deficiency in their lifetime, even nephrologists practicing at institutions where the disorder is well recognized. Importantly, research of rare metabolic disorders is hampered by the small case number, including studies of epidemiology and therapeutic interventions. The focus of the studies presented in this thesis was to ascertain the prevalence of APRT deficiency by identifying all known cases worldwide and using genomic data to examine the frequency of pathogenic mutations as well as to study the clinical manifestations and outcomes of the kidney disease and the effect of both pharmacotherapy and dietary interventions.

1.1 Adenine metabolism

Purines are nitrogen bases found in nucleic acids (Blanco & Blanco, 2017). Purines are also structural components of several coenzymes, take part in cellular signaling and modulate energy by driving biochemical reactions. There are two purine nucleobases, adenine and guanine, both of which participate in the formation of RNA and DNA (Kamatani, Jinnah, Hennekam, & van Kuilenburg, 2014). Nucleotide synthesis occurs via two routes: de novo synthesis which primarily takes place in the liver and through the more energy-saving salvage pathways. The latter occurs through the conversion of purine nucleotides back to ribonucleotides by the respective phosphoribosyltransferases, mainly in extrahepatic tissues. De novo synthesis generally yields adequate supply and purines, which are available through dietary sources are mostly converted to uric acid by XOR (Bhagavan & Ha, 2015).

1.1.1 Adenine phosphoribosyltransferase

Adenine phosphoribosyltransferase (APRT) is a cytoplasmic enzyme expressed in all tissues that is involved in the purine salvage pathway. Human APRT was purified from erythrocytes in 1979 (Holden, Meredith, & Kelley, 1979) and the amino acid sequence of erythrocyte APRT determined in 1986 (Wilson et al., 1986). In healthy individuals, the APRT enzyme activity can be measured in red cell lysates by chromatographic assay, using radiolabeled ^{14}C-adenine, and ranges from 16-32 nmol/hr/mg hemoglobin in healthy individuals (Cartier et al., 1974). The mature enzyme is a homodimer with each subunit composed of 179 residues with a calculated molecular weight of 19,481. A comparative analysis of mouse and human APRT

revealed an 82% homology. Like other purine phosphoribosyltransferases, APRT catalyzes the reversible transfer of a phosphoribosyl group from phosphoribosylpyrophosphate (PRPP) to a purine base, possessing a core domain composed of a 4- or 5-stranded parallel β sheet flanked by 3–4 α-helices (Craig & Eakin, 2000). The C-terminal ends of the β sheets of the core domains form the floor of the active site and a poorly conserved hood domain contributes residues that complete the active site and participate in substrate binding. Silva and coworkers generated the three-dimensional structure of recombinant APRT expressed in E. coli, crystallized in complex with adenosine monophosphate (AMP) (Silva, Silva, Iulek, & Thiemann, 2004). The structure of APRT is composed of nine beta-strands and six alpha-helices, and the active site pocket opened slightly to accommodate the AMP product. The core of APRT is similar to that of other phosphoribosyltransferases, although the adenine-binding domain differs substantially.

APRT catalyzes the synthesis of 5´-adenosine monophosphate from adenine and 5-phosphoribosyl-1-pyrophosphate, and thereby recycles adenine into to the nucleic acid metabolism pathway (Sahota, Tischfield, Kamatani, & Simmonds, 2001). As a result, adenine is nearly undetectable in both urine and plasma in healthy individuals (Sahota, 2001). In the absence of functional APRT, adenine is oxidized by XOR to the poorly soluble DHA, via 8-hydroxyadenine as a major intermediate (Figure 1). Differences between individuals in plasma XOR activity have been reported, which may contribute to variable generation of DHA (Watanabe et al., 2019).

1.1.1.1 *The APRT gene*

The human *APRT* gene, first described by Broderick et al. in 1987, is located on the long (q) arm of chromosome 16 at position 24.3 (16q24.3). Approximately 2.6 kb long (Figure 2), the gene encodes a 180 amino-acid protein, containing 540 base-pairs in the protein-coding region (Broderick et al., 1987). The gene contains 5 exons and 4 introns. The promoter region of the human *APRT* gene has five GC boxes that are potential binding sites for the Sp1 transcription factor. However, the 'TATA' and 'CCAAT' boxes are lacking (Broderick et al., 1987).

1.2 Adenine phosphoribosyltransferase deficiency

1.2.1 Pathobiology of 2,8-dihydroxyadenine nephrolithiasis and nephropathy

Renal excretion of the poorly soluble DHA is generally marked in patients with APRT deficiency, resulting in high urinary supersaturation and precipitation of DHA in the kidneys in the form of crystals. Solute concentration and varying ability to supersaturate the urine may contribute to urine crystallization, and crystal aggregation that eventually leads to kidney stone formation (Sahota, 2001). DHA crystals also precipitate within the renal tubules and cause severe injury to the renal tubular cells (Bollee et al., 2010; Fye et al., 1993; Kamatani, Terai, Kuroshima, Nishioka, & Mikanagi, 1987). The solubility of DHA does not vary greatly within the physiological range of urine pH.

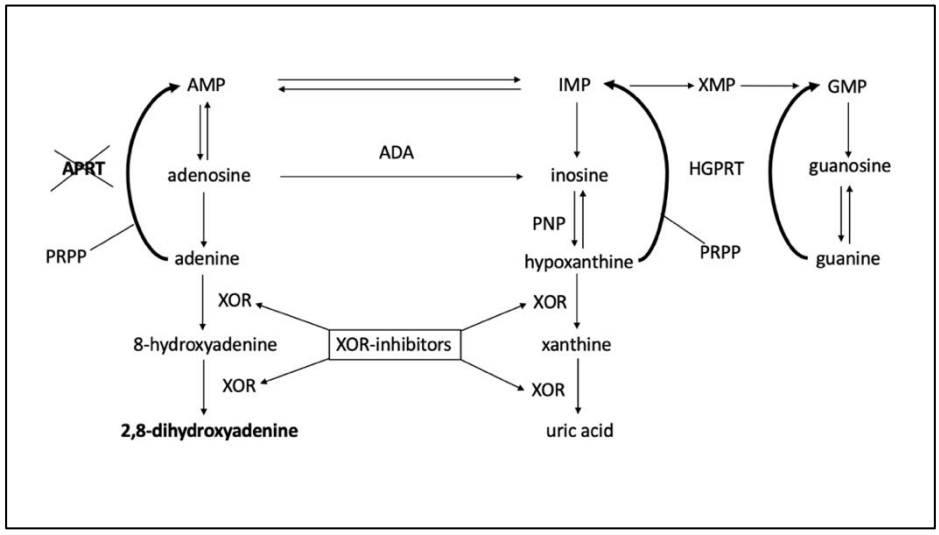


Figure 1. Overview of adenine metabolism**.** When APRT is nonfunctional, adenine is oxidized by XOR to 2,8-dihydroxyadenine via 8-hydroxyadenine. Abbreviations: ADA, adenosine deaminase; AMP, adenosine monophosphate; APRT, adenine phosphoribosyltransferase, GMP, guanosine monophosphate; HPRT, hypoxanthine phosphoribosyltransferase; IMP, inosine monophosphate; PNP, purine nucleoside phosphorylase; PRPP, 5-phosphoribosyl-1-pyrophosphate; XMP, xanthine monophosphate; XOR, xanthine oxidoreductase.

The mechanisms by which DHA crystal deposition in the kidney leads to stone formation and irreversible tubular atrophy and interstitial fibrosis have long been poorly understood. For decades, kidney stone research has centered on the formation, growth, aggregation and retention of crystals, with

solute supersaturation as the driving force (Coe, Evan, & Worcester, 2005). Experimental animal models have shed some light on the pathologic features and time course of the kidney disease. The *Aprt*$^{-/-}$ mouse was shown to exhibit all of the characteristics of the human disease (Engle et al., 1996). The APRT-deficient mice excrete adenine and DHA and develop crystalluria, kidney stones and the characteristic progressive chronic tubulointerstitial nephropathy (Stockelman et al., 1998). Twelve-week-old homozygous null males had an average GFR about half that of healthy animals, while female APRT-deficient mice were much less severely affected than males. Tubular obstruction caused by crystal plugs has traditionally been thought to be the principal pathophysiologic mechanism of kidney injury in APRT deficiency, but in recent years, the focus has shifted to the role of inflammation. The importance of inflammatory mechanisms in the pathogenesis of crystal-induced injury is highlighted by the discovery of nucleotide-binding domain (NOD)-like receptor protein 3 (NLRP3) inflammasome-mediated immune activation upon crystal uptake into intracellular lysosomes (Mulay et al., 2013). The NLRP3 inflammasome is an intracellular signaling complex that regulates the innate immune activity, most commonly through secretion of the proinflammatory cytokines IL-1β and IL-18 which induce the recruitment and activation of leukocytes. The NLRP3 inflammasome has been shown to trigger inflammation and acute kidney injury (AKI) in oxalate nephropathy (Knauf et al., 2013; Mulay et al., 2013). The same pathobiological mechanism appears to be instrumental in DHA nephropathy, based on work by a German investigator team who showed that inhibition of NLRP3 inflammasome activation strongly attenuated kidney fibrosis in murine models of crystal nephropathy induced by diets rich in adenine or oxalate (Ludwig-Portugall et al., 2016). These findings hold promise for the development of novel therapeutic intervention for DHA nephropathy and other crystal-induced kidney diseases.

1.2.2 Molecular genetics

The mode of inheritance in APRT deficiency is autosomal recessive, in which the affected individuals receive a mutated gene from both parents. Siblings have a 50% chance of being heterozygous carriers who have partial enzyme deficiency without any metabolic derangements or clinical abnormalities. A number of patients have been born to consanguinous parents, and a recent report from the UK identified a high number of South Asian patients with such a history (Balasubramaniam et al., 2016).

Approximately 40 functionally significant human *APRT* mutations have been reported to date in all ethnic groups (Edvardsson et al., 2012). Patients are either homozygous or compound heterozygous for pathogenic mutations, the majority of which are single nucleotide changes and small deletions. Three mutations seem to be most frequent; a missense mutation in exon 5, replacing threonine for methionine (c.407T>C, p.(Met136Thr)), substituting the phosphoribosylpyrophosphate (PPRP) binding site, is the most commonly reported mutation in Japan, described in 79% of Japanese patients with APRT deficiency (Kamatani et al., 1989). The most commonly reported mutation found in European patients, namely in France, a single T-insertion at the splice donor site of intron 4 (c.400+2dupT) results in a truncated protein (Bollee et al., 2010). A missense mutation in exon 3, where aspartic acid is replaced by valine (c.194A>T, p.(Asp65Val)), accounts for all known cases of APRT deficiency in Iceland (Edvardsson et al., 2001).

Genetic analysis includes single-gene sequencing of PCR-amplified DNA, which has revealed a pathogenic mutation in roughly 90% of cases (Edvardsson et al., 2012). No genotype-phenotype correlations have been identified as all reported pathogenic variants in homozygotes and compound heterozygote carriers of pathogenic *APRT* mutations result in abolished enzyme function (Bollee et al., 2010; Edvardsson et al., 2001; Harambat, Bollee, Daudon, Ceballos-Picot, & Bensman, 2012).

1.2.3 Epidemiology

Only a few studies have been conducted on the prevalence of this rare disorder. In Japan, an estimated heterozygous carrier frequency of >1.2% was based on kidney stone analysis at a large clinical laboratory. Based on these numbers, the expected prevalence of APRT deficiency would be 1:27,000 (Kamatani et al., 1987). The most common mutation, c.400+2dupT, was found to be carried by 2 of 204 (0.98%) healthy newborn chromosomes screened at the Necker Hospital in Paris, France (Bollee et al., 2010). In Iceland, 23 patients homozygous for the p.(Asp65Val) variant have been reported, suggesting a prevalence of roughly 1:15,000 in the Icelandic population (Edvardsson et al., 2001). In the white population, a heterozygote carrier frequency of 0.4-1.1% was reported based on analysis of APRT enzyme activity in healthy subjects (Fox, 1977; Johnson et al., 1977; Srivastava et al., 1972). According to these observations, the prevalence is estimated to be 1:50,000-1:100,000 individuals. Based on those figures, there should at least be 3000 cases in the United States and close to 700 cases in the United Kingdom. The relatively large number of cases in Iceland,

France and Japan likely reflect a founder effect in these populations although the aforementioned prevalence numbers indicate that the disorder may also be underdiagnosed. However, in a study performed at the Mayo Clinic in Rochester, MN, the composition of 43,545 kidney stones was analysed, finding only a single stone composed of DHA (Lieske et al., 2014).

1.3 Clinical characteristics

The most common clinical manifestations of APRT deficiency are radiolucent kidney stones and CKD secondary to crystal nephropathy, even in the absence of a past history of kidney stones. At least 15% of adult patients have reached ESKD at the time of presentation (Bollee et al., 2010; Edvardsson et al., 2001). A number of patients are asymptomatic at the time of diagnosis but are at a risk for developing progressive CKD if left untreated. Indeed, the reported age at onset of the disorder and clinical manifestations are quite variable and factors that may influence the phenotype are incompletely understood. In many cases, the disorder has only been recognized and diagnosed following kidney transplantation (Benedetto et al., 2001; Cassidy, McCulloch, Fairbanks, & Simmonds, 2004; S. H. Nasr et al., 2010; Zaidan et al., 2014). Therefore, APRT deficiency should be considered in all patients with radiolucent urinary tract calculi, including those with presumed uric acid stones, or CKD of unknown cause in children and young or even middle-age adults (Bollee et al., 2010; Edvardsson et al., 2012). Finally, macroscopic crystalluria presenting as reddish-brown diaper stains is a rather frequently reported finding in infants and young children (Edvardsson et al., 2012). DHA crystals can usually be found when microscopic examination of urine specimens from patients with APRT deficiency is performed by trained professionals (Edvardsson et al., 2012).

1.3.1 Kidney stone disease

Urolithiasis is the most commonly reported manifestation of APRT deficiency and can develop at any age. The stones may be recurrent, even in patients receiving pharmacotherapy (Bollee et al., 2010). DHA stones typically have a rough surface and appear reddish-brown when wet, but the color becomes greyish-white when dry (Figure 2). As the stones are radiolucent, they may be confused with those comprised of uric acid or other purines such as xanthine. Their composition is typically 100% DHA, although cases of nephrolithiasis have been reported that contain other constituents, such as calcium (Kamatani et al., 1987; Yagisawa, Yamazaki, Toma, & Kamatani, 1999).

Figure 2. 2,8-Dihydroxyadenine (DHA) kidney stone. The stone measures 7 mm in largest diameter and the color is grey which is typical for dry DHA stones, whereas wet stones are usually reddish brown.

1.3.2 Chronic kidney disease

CKD is defined as abnormalitites of kidney structure or function present for at least three consecutive months, recognized by detecting markers of kidney damage, including proteinuria, histological abnormalities and pathologic imaging findings, or reduced glomerular filtration rate (GFR) < 60 mL/min/1.73 m^2 (Levey et al., 2005). CKD is classified into six stages based on the severity level as described in Table 1. The final stage, ESKD, represents the need for renal replacement therapy (RRT) to sustain life. Glomerular filtration can be evaluated by directly measuring the clearance of exogenous substances that are eliminated solely by filtration including inulin, iothalamate, iohexol and ^{51}Cr-EDTA, though these methods can be both cumbersome and expensive (Soveri et al., 2014). Several equations have been derived to estimate glomerular filtration rate (eGFR) as a measure of kidney function which is widely used, both in research and clinical practice. The eGFR is most commonly based on levels of serum creatinine (SCr) as it is readily available and inexpensive. The use of SCr as a biomarker for kidney filtration may be misleading as it has several limitations, including influence of muscle mass, reflected by age, sex and ethnicity. Cystatin C, which is less influenced by muscle mass may be used for glomerular filtration

rate estimation in situations when SCr may be unreliable for kidney function assessment.

Table 1. KDIGO classification of CKD based on estimated glomerular filtration rate.

CKD stage	eGFR (mL/min/1.73m^2)
Stage 1	>90
Stage 2	60-89
Stage 3a	45-60
Stage 3b	30-44
Stage 4	15-29
Stage 5	<15

Abbreviations: KDIGO, Kidney Disease: Improving Global Outcomes; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate.

Very few studies have focused on the progression of kidney function and long-term outcomes of patients with APRT deficiency. In the largest cohort study reported to date from an APRT deficiency research group in France, roughly one-third of the 53 patients had CKD stage 3-5 at the time of diagnosis, almost half of whom had progressed to ESKD. As only a handful of cases of biopsy-proven DHA crystal nephropathy have been reported worldwide, the true prevalence may be significantly underestimated since one-third of patients with APRT deficiency have CKD stage 3 or above at diagnosis, and only a small fraction of CKD patients undergo kidney biopsy prior to onset of dialysis or kidney transplantation (Bollee et al., 2010). Most cases of APRT deficiency have been described in patients with recurrent DHA nephrolithiasis (Bollee et al., 2012), while a few cases have been reported without a history of kidney stones (Arnadottir, Laxdal, Hardarson, & Asmundsson, 1997; Brown, 1998; Samih H. Nasr et al., 2010).

1.3.2.1 *Histopathological features of 2,8-dihydroxyadenine crystal nephropathy*

The renal lesion observed in patients with APRT deficiency is a chronic DHA crystal-induced tubulointerstitial nephropathy (Figure 3) (Bollee et al., 2010; Edvardsson et al., 2012; S. H. Nasr et al., 2010). Histologic examination shows extensive DHA crystal deposits within tubular lumens, inside tubular

epithelial cells and in the interstitial space. Significant inflammatory cell infiltration is generally present as is variable degree of renal scarring and glomerulosclerosis. On examination of hematoxylin and eosin (H&E)–stained kidney sections, the crystal material has a brownish appearance and the crystals are shaped as needles, rods or rhomboids, and are strongly birefringent when viewed under polarized light. Fourier-transformed infrared microscopy is an important tool that can be used for identifying the composition of crystal deposits in renal tissue.

Renal histological examination will invariably reveal DHA crystal nephropathy in patients with APRT deficiency and CKD or acute allograft dysfunction. However, the histopathologic manifestations of DHA nephropathy are similar to other types of crystal nephropathies. When observed, it is important to ascertain the nature of such deposits since several metabolic conditions can cause crystal nephropathy. DHA nephropathy has for example been falsely diagnosed as primary hyperoxaluria (S. H. Nasr et al., 2010). Commonly used drugs such as methotrexate and valaciclovir may also cause crystal deposits in the kidneys.

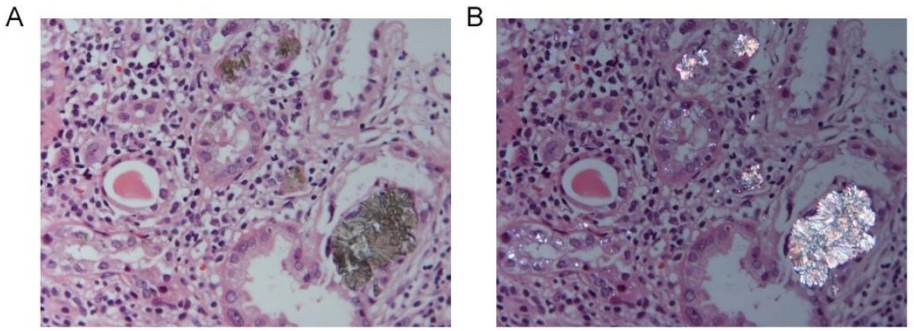


Figure 3. Photomicrograph of a kidney biopsy specimen revealing 2,8-dihydroxyadenine crystals. Crystals are observed within the tubular lumen and interstitium under (A) conventional and (B) polarized light microscopy.

1.3.3 Extrarenal manifestations

Although the APRT enzyme is widely distributed in human tissue, no extrarenal manifestations of the disorder have been confirmed. Interestingly, two Belgian brothers with APRT deficiency were reported in 1984 describing findings of corneal dystrophy believed to be caused by crystal deposition (Neetens, Van Acker, & Marien, 1986). Since then, no further reports have

concerning ocular manifestations have been published.

1.3.4 Asymptomatic patients

As many as 15% of patients with APRT deficiency may be asymptomatic at diagnosis according to published reports (Bollee et al., 2010). However, patients may present with clinical manifestations of the disorder at any age, from infancy to adulthood, even as late as in the fifth or sixth decades of life. It has been speculated that asymptomatic patients diagnosed through family screening simply may not yet have developed signs or symptoms of the disorder. Interestingly, cases have been described where untreated homozygous siblings of symptomatic patients had not developed symptoms or signs of the disease, reportedly at an old age (Kamatani et al., 1987).

1.4 Diagnosis

The diagnosis of APRT deficiency should be considered in patients with recurrent radiolucent kidney stones, in unexplained CKD and in the setting of protracted allograft dysfunction following kidney transplantation.

Features highly suggestive of the disorder include the typical urinary DHA crystals seen on urine microscopy; the identification of DHA as a kidney stone component; and the characteristic renal histopathological findings of crystal nephropathy. The diagnosis of APRT deficiency is made by demonstrating abolished enzyme APRT activity in cell lysates or the identification of biallelic pathogenic mutations in the *APRT* gene (Figure 4).

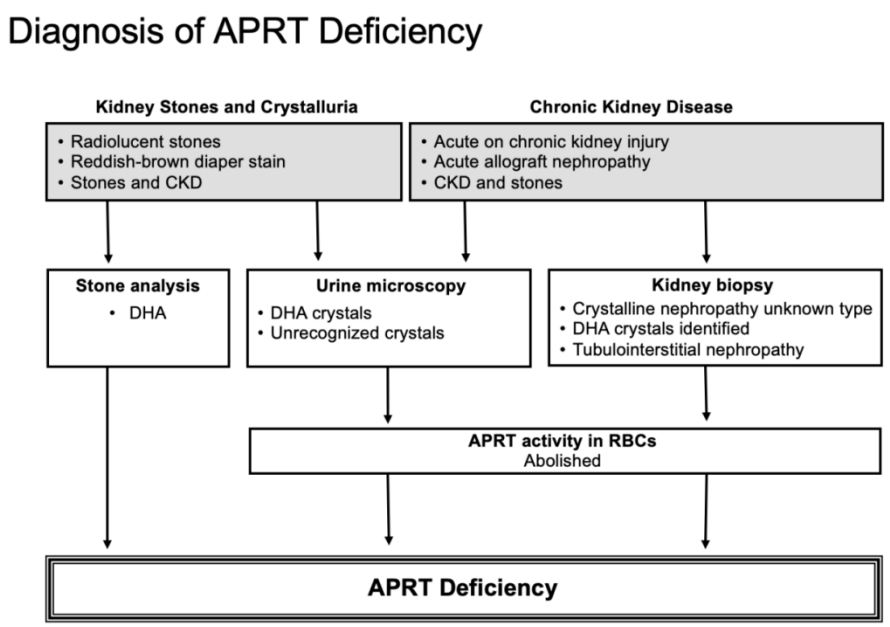


Figure 4. Diagnostic algorithm for adenine phosphoribosyltransferase (APRT) deficiency. Adapted and reprinted with permission from Springer Nature ((Edvardsson et al., 2013) Abbreviations: CKD, chronic kidney disease; DHA, 2,8-dihydroxyadenine; RBC´s, red blood cells.

1.4.1 Urine microscopy

Urine microscopy is a simple, noninvasive test that often yields important information, but is currently not as widely used as in the past. In recent years, automated urine sediment analysis has increasingly replaced urine microscopy, employing an instrument which unfortunately may fail to identify urine crystals. For diagnostic purposes, manual or automated urine microscopy has not proven to be reliable as the crystals are often overlooked or misidentified. On light microscopy of the urine sediment, DHA crystals are reddish-brown and typically round with a central density, radiating spicules and often feature a dark outline (Figure 5). When viewed under polarized light, small and medium-sized DHA crystals have a characteristic Maltese cross pattern which is not observed for larger cystals as they are impermeable to light (Sahota, 2001). Crystalluria may be decreased or diminished in patients with advanced CKD (Bollee et al., 2010). When identified, DHA crystals are highly suggestive of the disorder and call for a definitive diagnostic procedure. The higher concentration of DHA crystals in first morning void urine samples renders these specimens preferable for microscopic evalutation and the crystals can be quantified by counting the

number per volume unit (Daudon & Jungers, 2004b). Assessment of crystalluria is also used for monitoring effect of treatment (Ceballos-Picot et al., 2014)

A B

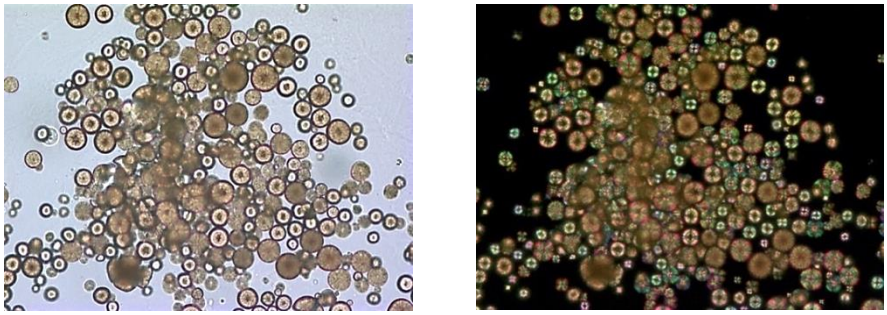


Figure 5. Urinary 2,8-dihydroxyadenine crystals. (A) The characeristic medium-sized crystals are brown with a dark outline and central spicules. (B) The same field viewed with polarized light microscopy shows that the small- and medium-sized crystals appear yellow and produce a central Maltese cross pattern (A, B: original magnification, x1000).

1.4.2 Analysis of kidney stone composition

Stone analysis using infrared or ultraviolet spectrophotometry has been considered a gold standard method for the diagnosis of DHA lithiasis (Ceballos-Picot et al., 2014; Edvardsson et al., 2013). X-ray crystallography may also correctly identify DHA in stone materials. By contrast, biochemical kidney stone analysis is not longer recommended as it does not differentiate between DHA and uric acid. Kidney stones in patients with APRT deficiency are typically composed of pure DHA, though rare cases have been reported where stone analysis has demonstrated DHA calculi mixed with other components such as calcium phosphate or oxalate. Even then, the DHA has been greater than 96%. False-positive stone results have rarely been reported (Kamatani et al., 1987).

1.4.3 Adenine phosphoribosyltransferase enzyme activity

The diagnois of APRT deficiency is usually made be demonstrating abolished APRT enzyme activity. All patients with APRT deficiency carry biallelic pathogenic mutations in the *APRT* gene and have no detectable APRT activity *in vivo*.

Two types of APRT deficiency have, however, been described based on the level of enzyme activity *in vitro* (Deng et al., 2001). In type I deficiency, no enzyme activity is detected, neither in intact cells nor cell lysates. Type I

deficiency has been found in patients from all ethnicities who are homozygotes or compound heterozygotes for null alleles in the *APRT* gene, classified as APRT*Q0. Heterozygotes for the null alleles are found to have reduced enzyme function in type 1 deficiency, between 10% and 50% (Ceballos-Picot et al., 2014). Type II deficiency is characterized by complete lack of enzyme function in intact cells, but partial deficiency in cell lysates. Type II deficiency, which is mainly found in Japanese patients is caused by a missense mutation on exon 5, p.(Met136Thr) (Kamatani et al., 1987), classified as APRT*J. It appears to result in a reduced affinity for phosphoribosylpyrophosphate (PRPP) and less sensitivity towards its stabilizing effects. The enzyme activity in vitro is less than 30% for individuals with biallelic mutations while heterozygote carriers have been found to have more than 50% of normal values (Kamatani et al., 1987). The biochemical implications and clinical manifestations of the disease are the same in type I and II deficiency.

1.4.4 Genetic testing

Confirmed homozygosity or compound heterozygosity for pathogenic *APRT* mutations is diagnostic of APRT deficiency (Edvardsson et al., 2012). Sequencing of PCR-amplified DNA has become more readily available in recent years and the small size of the *APRT* gene simplifies the testing.

1.4.5 Kidney biopsy

A kidney biopsy is a procedure performed to obtain a small tissue sample for microscopic evaluation, most often for diagnostic or monitoring purposes. Indications for percutaneous kidney biopsy vary between institutions but are predominantly performed in the setting of suspected glomerular disorders and unexplained AKI. Although invasive, the procedure carries a relatively low risk of complications, the most common of which are bleeding events, either accumulation of blood surrounding the kidney (perinephric hematoma) or bleeding into the collecting system causing hematuria (Hogan, Mocanu, & Berns, 2016). A recent systematic review revealed that only 0.9% of 9474 kidney biopsies required red blood cell transfusion (Corapi, Chen, Balk, & Gordon, 2012). Renal histological findings consistent with DHA crystal nephropathy are highly suggestive of the disorder.

1.4.6 Measurement of urine 2,8-dihydroxyadenine

Several methods have been developed to quantify urine DHA, though none are currently used routinely in clinical practice (Table 2). The high-

performance liquid chromatography (HPLC) was described in 1980 (Ericson, Groth, Niklasson, & de Verdier, 1980), and the method was subsequently coupled to a multichannel ultraviolet detector as described in 1987 (Kojima, Nishina, Kitamura, Kamatani, & Nishioka, 1987). This was followed by alternative procedures, including capillary electrophoresis (Adam, Friedecky, Fairbanks, Sevcik, & Bartak, 1999; Sevcik, Adam, & Mazacova, 1996; Wessel, Lanvers, Fruend, & Hempel, 2000b). In 2006, a method was described using HPLC coupled to tandem mass spectrometry (HPLC-MS/MS) (Hartmann et al., 2006).

Table 2. Reported methods for measuring urine DHA concentration.

Reference	Method	Patient samples (no)	DHA
Van Acker et al. (1977)		1	0.251 mmol/24 h
Kojima et al. (1989)	HPLC	5	54 µmol/mmol Cr
			32.3 µmol/mmol Cr
			26.1 µmol/mmol Cr
			44.4 µmol/mmol Cr
			13.4 µmol/mmol Cr
Wessel et al. (2000)	Capillary electrophoresis	1	481 µM/24 h
Mac-Way et al. (2008)	HPLC	1	111 µmol/mmol Cr

Abbreviations: DHA, 2,8-dihydroxyadenine; HPLC, high performance liquid chromatography; Cr, creatinine

A novel urinary assay based on ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) for quantification of DHA, adenine and other purine metabolites was recently reported (Thorsteinsdottir et al., 2016). The method includes an isotopically labelled internal standard for absolute urinary DHA quantification. Instrumentation consisted of Quattro PremierTM XE tandem quadrupole mass spectrometer coupled to an ACQUITY UPLC (Waters Corporation, Milford, MA). The coefficient of variation for different urine aliquots was <5%, indicating a robust sampling method. The lower limit of quantification of the method was 100 ng/mL for DHA. The intra-day accuracy and precision CVs were well within +/-15% limits. The finding of DHA in urine samples is suggestive of APRT deficiency while the accuracy for diagnosis of the disorder is currently not known.

1.4.7 Family screening

As the pattern of inheritance in APRT deficiency is autosomal recessive, screening siblings of affected individuals is recommended, irrespective of whether they are symptomatic or not (Edvardsson et al., 2012). Early diagnosis is particularly important as effective pharmacologic treatment is readily available.

1.5 Treatment and outcome

1.5.1 Pharmacotherapy

Treatment of APRT deficiency with allopurinol, which inhibits XOR and thereby blocks the formation of DHA from adenine (Figure 1), may prevent kidney stone formation and the development of CKD in patients with APRT deficiency. In patients with CKD, stabilisation and even improved kidney function has previoulsy been demonstrated in some cases (Bollee et al., 2010; Edvardsson et al., 2001). All patients diagnosed with the disorder should, therefore, initiate treatment without delay, regardless of their clinical symptoms.

Allopurinol is generally well-tolerated in patients with APRT deficiency. Allopurinol in the dose of 5-10 mg/kg/day in children and 200-300 mg/day in adults (maximum daily dose of 600-800 mg/day) has been found to reduce DHA crystalluria, the number of clinical kidney stone episodes, the development of CKD due to renal crystal deposition and progression to kidney failure (Bollee et al., 2010; Edvardsson et al., 2001). The more recently introduced XOR inhibitor febuxostat provides an alternative treatment option for patients who are allergic to or intolerant of allopurinol, although no dosing recommendations currently exist (Becker, Schumacher, Wortmann, MacDonald, Palo, et al., 2005) for pharmacotherapy in APRT deficiency. Treatment with alkalizing agents is not recommended as DHA remains insoluble within the range of physiological urinary pH (Sahota, 2001).

The current practice is to monitor the effectiveness of pharmacotherapy by periodic urine microscopy where the absence of urinary DHA crystals is considered indicative of adequate treatment. However, some patients have continued to form stones despite a major decrease in or even disappearance of urinary crystals, while others remain free of stones and do not show evidence of CKD progression despite persistent crystalluria. Therefore, a more sensitive and reliable method to guide treatment is needed. The recently introduced UPLC-MS/MS urinary assay appears to be a promising option that requires further study.

1.5.2 Dietary modifications

High fluid intake is a standard approach in the care of patients with kidney stone disease and is supported by evidence (Borghi et al., 1996). Patients with APRT deficiency have been advised to increase their fluid consumption up to a minimum of 2.0-2.5 litres per day (M. L. Irène Ceballos-Picot, Lionel Mockel, Véronique Droin, Michel Daudon, Mohamad Zaidin, Jérôme Harambat and Guillaume, Bollée, 2014). Purine-restricted diet is often recommended for patients with the disorder in addition to a treatment with an XOR inhibitor. However, evidence supporting that approach is lacking. The only published study suggesting a potential beneficial effect of limited purine intake on urinary DHA excretion is based on a single study that included only one patient and one healthy control subject (Simmonds, Van Acker, Cameron, & McBurney, 1977). Therefore, the effect of varied dietary purine intake clearly warrants further study, especially for patients unable to tolerate XOR inhibitor therapy.

1.5.3 Surgical management of kidney stone disease

Surgical treatment of DHA stones is the same as for other forms of nephrolithiasis. The choice of interventional technique is based on the size and location of the stone(s). Extracorporeal shock wave lithotripsy (ESWL) is a minimally invasive procedure and has been shown to be an effective method and well tolerated (Coupris, Champion, Duverne, Varlet, & Ratajczak, 1989), using high-energy shock waves to break stones into small pieces for easier passage. In more complex cases where the size or position of the stone does not allow the use of ESWL, invasive procedures such as flexible ureteroscopy, laser lithotripsy or percutenaous nephrolithotomy may be required.

1.5.4 Kidney transplantation

In patients with ESKD due to DHA nephropathy who have undergone kidney transplantation, numerous cases of early disease recurrence with poor allograft outcomes have been reported (Table 3). Notably, the majority of patients with failed grafts were first diagnosed with APRT deficiency post-transplant resulting in significant delay in institution of pharmacotherapy (Benedetto et al., 2001; Bollee et al., 2010; Cassidy et al., 2004; de Jong et al., 1996; Gagne et al., 1994; Sharma, Moritz, & Markowitz, 2012; Zaidan et al., 2014). As kidney transplantation does not correct the underlying enzyme deficiency, the institution of effective pharmacotherapy prior to transplantation may be critical for the prevention of disease recurrence in these patients.

1.5 Knowledge gaps

APRT deficiency is a rare disease and both epidemiological and clinical information are based on case reports and case series comprising small samples. Therefore, the current understanding of the true prevalence, disease course and the effect of medical treatment is limited. The value and accuracy of available laboratory methods, including urine microscopy, kidney stone analysis and the recently developed UPLC-MS/MS DHA urinary assay for diagnosis and therapeutic monitoring of APRT deficiency is not known. The studies undertaken for this thesis were designed to fill in these knowledge gaps.

Table 3. Reported cases of kidney transplantation in patients with APRT deficiency

Case report	Year	Case (no)	XOR inhibitor pre-transplant	Delayed graft function	Outcome
Benedetto et al.	2001	1	No	No	Chronic graft dysfunction
Bertram et al.	2010	1	Yes	Yes	Chronic graft dysfunction
Bollée et al.	2010	5	No	NA	Graft loss: 2 Chronic graft dysfunction: 3
Brilland et al.	2015	1	No	Yes	Chronic graft dysfunction
Brown	1998	1	No	Yes	Graft loss
Cassidy et al	2004	1	No	No	Chronic graft dysfunction
De Jong et al	1996	1	No	Yes	Graft loss
Eller et al.	2004	1	Tx 1: No	Tx 1: No	Graft loss
			Tx 2: No	Tx 2: No	Graft loss
			Tx 3: Yes	Tx 3: Yes	Chronic graft dysfunction / graft loss
			Tx 4: No	Tx 4: Yes	Chronic graft dysfunction
Gagné et al	1994	1	No	No	Graft loss
Glicklich et al.	1988	1	No	No	NA
Kaartinen et al.	2014	1	Tx 1: No	No	Graft loss
			Tx 2: No	Yes	Graft loss
Nasr et al.	2010	1	No	No	Graft loss
		1	No	No	Graft loss
		2	Yes	No	Expired with functioning

					graft
		3	No	Yes	Chronic graft dysfunction
Sharma et al.	2012	1	No	Yes	Chronic graft dysfunction

Abbreviations: APRT, adenine phosphoribosyltransferase; NA, not available; Tx, transplant; XOR, xanthine oxidoreductase.

2 Aims

The overall aim of the work described in this thesis was to study the epidemiology of APRT deficiency and long-term renal outcomes. Specific aims were as follows:

1. **To determine the prevalence of APRT deficiency**
   i. To identify all known cases using published reports, registry data and other resources (Paper I).
   ii. To leverage public genomic data to estimate the prevalence of APRT deficiency in various geographic and ethnic populations (Paper I).
   iii. To identify reasons for missed and false-positive diagnoses of the disorder (Paper II).
2. **To determine clinical characteristics and outcomes of APRT deficiency**
   i. In an observational cohort study using data from the APRT Deficiency Registry of the RKSC (Paper III),
   ii. In an observational cohort study focused on patients presenting with clinical manifestations <18 years of age, using data from the APRT Deficiency Registry of the RKSC (Paper IV).
   iii. To examine kidney allograft function, survival rates and predictors of allograft outcomes (Paper V).
3. **To study the clinical applicability of a novel UPLC-MS/MS assay for the measurement of urinary DHA excretion**
   i. To ddetermine the range of urinary DHA excretion for homozygotes, heterozygotes and healthy non-carriers (Paper VI).
   ii. Assess the correlation of 24-h urinary DHA excretion and the DHA-to-Cr ratio in random urine samples (Paper VI).
   iii. To test the sensitivity and specificity of the UPLC-MS/MS assay for the diagnosis of APRT deficiency (Paper VI).
   iv. Study the effect of dietary purine intake on urinary DHA excretion.
4. **To evaluate the applicability of a novel UPLC-MS/MS urinary DHA assay in the monitoring of pharmacotherapy of APRT deficiency.**
   i. Perform a clinical trial comparing the effect of allopurinol and febuxostat on urinary DHA excretion in patients with APRT deficiency (Paper VII).

3 Materials and methods

3.1 Ethical approvals

Appropriate ethical approvals were obtained for the studies in this thesis in accordance to the Declarations of Helsinki and Istanbul. The studies were approved by the Icelandic Data Protection Authority, the Icelandic National Bioethics committee (NBC-072 and 13-115-S1) and the Icelandic Medicines Agency (EudraCT No. 2013-00975-33). The Clinical Trial is registered at www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT02752633). Informed consent was obtained from all study participants.

3.2 Study design and populations

This thesis is based on seven studies presented in the papers listed on page xvii, as well as a report on known cases of APRT deficiency and a dietary study that is also described herein. For Paper I, a comprehensive search for patients with APRT deficiency was conducted using web-based resources, such as the National Library of Medicine, and through the APRT Deficiency Registry and personal communication. Available exome and whole-genome sequencing data from various populations worldwide were used to estimate the prevalence of APRT deficiency. The studies in Papers II-VI were observational cohort studies and Paper VII was a clinical trial based on a population of patients enrolled in the APRT Deficiency Registry of the RKSC or referred to the research group between 2010 and 2019. Data were collected both retrospectively and prospectively. As analyses were performed at different time points during the ten-year study period and recruitment was continuously ongoing, the number of patients varies between the published papers. An overview of the methods for Papers I-VII is shown in Table 4.

Table 4. Study populations and methods of Papers I-VII

	Population	Study period	Number of participants	Outcome measures
Paper I	Confirmed cases and public genomic databases worldwide	2017-2019	-	Number of known cases worldwide and number and frequency of pathogenic variants in population databases
Paper II	APRT Deficiency Registry / patients referred to the APRT Research Program	2001-2018	17	False positive diagnosis of APRT deficiency and results of kidney stone analysis
Paper III	APRT Deficiency Registry	1949-2015	53	Long-term renal outcomes in patients with APRT deficiency, kidney stone events and development of CKD
Paper IV	APRT Deficiency Registry	1950-2017	21	Long-term renal outcomes in patients who present <18 years of age, kidney stone events and development of CKD
Paper V	APRT Deficiency Registry / patients referred to the APRT Research Program	1968- 2019	17	Renal allograft function and survival, risk factors and disease recurrence
Paper VI	APRT Deficiency Registry	2010-2018	47	Urinary DHA excretion in patients with APRT deficiency, heterozygous carriers of pathogenic variants and healthy controls.
Paper VII	APRT Deficiency Registry	2013-2015	8	Urinary DHA excretion in patients receiving treatment with allopurinol and febuxostat

Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease; DHA, 2,8-dihydroxyadenine.

3.3 Data sources and collection

3.3.1 Adenine Phosphoribosyltransferase Deficiency Registry and Biobank of the Rare Kidney Stone Consortium

The APRT Deficiency Registry and Biobank of the Rare Kidney Stone Consortium were established in 2009 at LUH to collect data and biosamples from patients with the disorder, both retrospectively and prospectively. As of September 1st, 2019, 63 patients from 8 countries were enrolled, most of whom are from Iceland (n=34). The remaining 29 patients are from the US (n=17), Italy (n=3), India (n=3), Austria (n=2), United Kingdom (n=2), Norway (n=1) and New Zealand (n=1). The diagnosis of APRT deficiency was confirmed by determination of biallelic pathogenic *APRT* mutations or absent APRT enzyme activity in red blood cell lysates.

At enrollment, patients were asked to complete a questionnaire focusing on clinical manifestations at presentation, diagnosis and latest medical follow-up. A retrospective review of available medical records included a search for age at onset of symptoms and at diagnosis, clinical features such as stone events, AKI, CKD or ESKD, and potential extrarenal features such as eye complaints; results of blood and urine testing, imaging results, kidney biopsies and kidney stone analyses; results of urinary metabolic risk factor assessment for kidney stones when available; surgical treatment of kidney stones; information on pharmacotherapy for APRT deficiency and a complete medication list; and results of previous *APRT* genotyping and APRT enzyme activity measurements for the purpose of diagnosis of the disorder. The information was added to the Registry. Data were also collected at yearly intervals following enrollment of Icelandic as well as international participants, providing medical updates and biosamples in an effort to characterize the clinical features of the disease and describe its natural history.

The RKSC APRT Deficiency Biobank has collected biosamples throughout the 10 years of study from all Icelandic registry patients and some of the foreign patients. Samples collected include spot urine samples (first morning void and random samples), 24-h urine samples, blood and plasma samples and kidney stone material when available. The 24-h urine collection bottles were inverted 3 times immediately before aliquoting and storage in the APRT Deficiency Biobank. Genomic DNA was stored at 4°C and plasma and urine samples at -80°C. Biosamples were also collected from heterozygote carriers of pathogenic mutations and healthy individuals. Samples from patients enrolled in the clinical trial and dietary study were also stored in the biobank. The biobank is located at LUH and the samples were analysed at the clinical

laboratories, with the exception of the urine DHA and adenine measurements which were performed at the bioanalytical laboratories of ArcticMass in Reykjavik, Iceland.

3.3.2 Landspitali–The National University Hospital of Iceland electronic medical record system

Clinical data were collected from medical records of patients who had been seen at the LUH in Iceland, both Icelandic patients and visiting international participants. The data included the age at presentation and diagnosis of APRT deficiency; information on kidney stone events, surgical treatment, lower urinary tract symptoms and results of kidney stone analysis; AKI episodes; stage of CKD; results of imaging studies and kidney biopsies; XOR inhibitor treatment; RRT; and cause of death. Also, measurements of serum serum Cr (SCr); urine studies such as microscopic assessment of DHA crystals; *APRT* genotype; and APRT enzyme activity.

3.3.3 Pathogenic *APRT* variants and genomic databases

To identify cases of patients with APRT deficiency, three different search strategies were used:

1. Medical literature and databases. A web-based search and assessement of reported mutations in cases of APRT deficiency were performed using PubMed, the Human Gene Mutation Database (HGMD)(Stenson et al., 2012), OMIM® and ClinVar (Landrum et al., 2016) through November 2019.
2. Expanded web search. The full text of published articles, conference abstracts and book chapters identified using PubMed® (https://pubmed.ncbi.nlm.nih.gov/) and Google® (http://www.google.com) with the terms „APRT deficiency", “adenine phosphoribosyltransferase deficiency”, "2,8-dihydroxyadenine", "2,8-dihydroxyadeninuria" and “2,8-DHA” were also assessed.
3. APRT Deficiency Registry. Pathogenic variants were identified through personal communication and in the APRT Deficiency Registry of the Rare Kidney Stone Consortium (RKSC, http://www.rarekidneystones.org). The RKSC was established in 2009 to study hereditary causes of kidney stone disorders and the APRT deficiency research group maintains a private database with unidentifiable clinical information on participating individuals. As of September 2019, 63 patients from 8 countries were enrolled

in the Registry, 56 of whom had undergone genetic testing with identification of biallelic pathogenic *APRT* variants.

Cases confirmed by APRT enzyme analysis and/ or genetic testing, without disclosing the pathogenic variant were included.

In order to determine the diagnosis APRT deficiency, the clinical, biochemical and molecular diagnosis were assessed. The set of all identified pathogenic variants is presented in the Appendix (Paper 1, Supplemental Table 1).

The set of reported pathogenic variants was looked for, as described above, in multiple public exome and whole-genome databases to examine the occurrence of such mutations. In particular, the individual and cumulative frequency of these disease-causing mutations were reviewed. Such information can be used to estimate the expected prevalence of the disorder in specific geographic and ethnic groups.

Six genomic databases were used, in all containing information on sequence variation for over 300,000 individuals. Overall, these databases cover a variety of geographic and ethnic origin.

(1) The database at deCODE genetics includes whole-genome sequencing data from 53,964 Icelanders, 3,153 Swedes, 8,831 Danes, 2,920 Norwegians and 1,365 Irish individuals enrolled in various studies (Gudbjartsson et al., 2015). (2 and 3) Two open-access databases containing information on individuals within the United Kingdom (UK) were explored; the UK Biobank project, a large prospective cohort study with exome sequencing of approximately 50,000 individuals (by 2019) (Sudlow et al., 2015) and the 100,000 Genomes Project which includes whole-genome sequencing data from 63,737 patients with rare diseases from across the UK. (4) The Genome Aggregation Database (gnomAD) browser (version 2.1.1) (Karczewski et al., 2019) includes whole-genome and exome data from 141,456 unrelated individuals, sequenced as a part of various disease-specific and population genetic studies of diverse ethnicity and geography. (5) The Human Genetic Variation Database of Japan (Higasa et al., 2016; Narahara et al., 2014) (HGVD, n=1,208), which contains exome sequencing data from 1,208 individuals and genotyping information for common variants from 3,248, and (6) the Korean Variant Archive (Lee et al., 2017) (KOVA, n=1,055). The minor allele frequency (MAF) of each variant among different ancestries was exctracted.

3.3.4 Clinical trial comparing allopurinol and febxostat in the treatment of adenine phosphoribosyltransferase deficiency (Paper VII)

Nine patients enrolled in the APRT Deficiency Registry were enrolled in the clinical trial to examine the effect of allopurinol and febuxostat on urinary DHA excretion. Eight patients completed the study. The intervention schedule is presented in Table 5.

Following a 7-day washout period, the consenting participants were prescribed 400 mg daily of allopurinol for 14 days. Febuxostat 80 mg/day for 14 days was prescribed following a second 7-day washout period. The interventions were not randomized between patients due to the relatively short half-life of both of the study drugs (febuxostat 5-8 hours; oxypurinol, the active allopurinol metabolite, 15 hours). The urine samples, both 24-h and first morning void urine specimens, were collected at baseline and following the allopurinol and febuxostat treatment periods (days 7, 21 and 42). The samples were returned to the laboratory on the same days the 24-h collections were completed, days 8, 22 and 43.

Table 5. Study and intervention schedule of participating patients

Protocol activity	**First visit**	**Wash-out period 1**	**Allopurinol (400 mg/day)**	**Wash-out period 2**	**Febuxostat (80 mg/day)**
	Day 1	Days 1-7	Days 8-21	Days 22-28	Days 29-42
Physical examination	X				
Serum creatinine measurement	X				
24-h urine samples		Day 7	Day 21	NA	Day 42
First morning urine sample collected		Day 7	Day 21	NA	Day 42
Adverse events			Continuously monitored throughout		

Abbreviations: NA, not available.

No dietary interventions were included in the study, but participants were asked to keep a food record and adhere to the same diet for the duration of the study. All patients were advised to return to their regular allopurinol dosing regimens at the end of the study period.

3.3.5 Study on the effect of diet on urinary 2,8-dihydroxyadenine excretion

Four patients enrolled in the APRT Deficiency Registry not receiving treatment with an XOR inhibitor participated in the study on the effect of dietary purine modification on DHA urinary excretion. Each participant first underwent an evaluation of urinary DHA excretion on a baseline habitual dietary intake, then on a purine-restricted diet, and lastly during a dietary purine challenge.

In the first study phase, while on their baseline habitual dietary intake, participants were asked to keep a 3-day food record and collect two consecutive 24-h urine samples on days 2 and 3. During the second phase, participants were asked to follow weight maintenance moderately purine-restricted weight maintenance diet for 5 days (avoiding only purine-rich meat and fish, bakers and brewer's yeast supplements high-fructose corn syrup and alcohol; and restrict intake of moderate meat and fish purine sources) and to keep a 3-day food record on days 3–5 and collect two consecutive 24-h urine samples on days 4 and 5. In the last phase, study subjects were asked to a add known amount of high-purine sources to same moderately purine-restricted weight maintenance diet for 3 days and collect two consecutive 24-h urine samples on days 2 and 3. The patients were asked to keep a food record for the duration of the study for dietary monitoring.

3.3.6 Other data sources

Several patients referred to the research program were not included in the APRT Deficiency Registry. Referring institutions include the Necker Hospital in Paris, France, and the Westmead Hospital in Sydney, Australia (Paper V). Patients believed to have APRT deficiency based on results of kidney stone analysis were referred for diagnostic testing from Texas Children's Hospital in Houston, Texas; Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK, Women's and Children's Hospital, Adelaide, Australia and the NY Harbor VA Medical Center, New York, NY, USA. These patients, who did not have the disorder, were included in Paper II.

Four heterozygotes for pathogenic *APRT* mutations, all of whom are parents of patients enrolled in the Registry, and 10 healthy volunteers donated blood and urine samples, both 24-h collections and single-void

morning samples, for study purposes which were stored in the APRT Deficiency Biobank. None of the participants were treated with an XOR inhibitor or other drugs affecting the excretion of uric acid. Clinical data collected for the purpose of the study, were limited to their age, sex, height, weight and blood pressure.

3.4 Definitions

3.4.1 Acute kidney injury

Acute kidney injury (AKI) in both native kidneys and kidney allografts was defined according to the SCr component of the KDIGO criteria as an increase in SCr of ≥ 26.5 μmol/L (≥ 0.3 mg/dL) within 48 h or ≥ 1.5 times baseline, presumed to have occurred over seven days (Kidney Disease Improving Global Outcomes: (KDIGO) Acute Kidney Injury Work Group, 2012).

3.4.2 Chronic kidney disease and estimated glomerular filtration rate

Because of the structural abnormality caused by chronic crystal deposition, all patients were assumed to have CKD. The Kidney Disease; Improving Global Outcomes (KDIGO) classification system was used to stage CKD (Kidney Disease: Improving Global Outcomes (KDIGO) CKD Work Group, 2013). The staging of CKD was based on annual eGFR values derived from the lowest available SCr measurement in each calendar year. SCr values obtained during episodes of AKI were excluded.

In Papers II-VII, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation was used to calculate eGFR in adults (Levey et al., 2009).

The CKD-EPI equation:

eGFR (mL/min/1.73 m²) = 141 x $\min(S_{Cr}/\kappa, 1)^{\alpha}$ x $\max(S_{Cr}/\kappa, 1)^{-1.209}$ x 0.993^{Age} x 1.018 (if female) x 1.159 (if black)

The modified Schwartz (CKiD) equation was used for patients below age 18 years (Schwartz et al., 2009).

Schwartz equation:

eGFR (mL/min/1.73 m²) = k × (height in cm/SCr)

k = 0.413 when SCr is in mg/dL

k = 36.5 when SCr is in μmol/L

Height measurements were extrapolated based on data points from patients´ growth charts, when otherwise unavailable. Non-standardized values of SCr were reduced by 5% before calculation of eGFR (Skali et al., 2011). Patients receiving dialysis were assigned an eGFR of 10 mL/min/1.73 m^2.

3.4.3 Other definitions

Kidney stones were considered symptomatic when stone passage or abdominal pain associated with hematuria was reported and/or a urinary tract stone was confirmed with an imaging study. When identified by imaging without reports of symptoms, the kidney stones were considered asymptomatic. Stone recurrence was defined as the detection of a stone in a patient with a history of kidney stones who has previously been shown to be stone-free by imaging.

Delayed graft function following kidney transplantation was defined as the need for dialysis in the first week post-transplant and graft loss was defined as return to dialysis or re-transplantation.

3.5 Laboratory testing

3.5.1 Quantification of urinary 2,8-dihydroxyadenine

The UPLC-MS/MS assay recently developed by our research group was used for measurement of urine DHA and adenine (Thorsteinsdottir et al., 2016). Urine samples were diluted 1:15 with 10 mM NH_4OH prior to analysis. Finally, isotopically-labeled DHA internal standard was added and the samples then blended for 3 minutes, centrifuged for 10 min at 3100 rpm and 4°C and subsequently injected into the UPLC-MS/MS system. Using this method, DHA was quantifiable between 100 ng/mL and 5000 ng/mL, while the lower limit of detection was 20 ng/mL. The 24-h urinary DHA and adenine excretion (mg/24 h) was measured and the urine DHA- and adenine-to-Cr ratios (mg/mmol) in first morning-void urine samples were calculated (Papers II, VI and VII).

3.5.2 Genetic testing

A method for the screening of *APRT* mutations was developed at the Department of Genetics and Molecular Medicine at LUH. Primers were selected using the Primer3 program to amplify five separate PCR products. This strategy generated readable sequences of all coding regions, 15 bp upstream of 3´ splice sites and 10 bp downstream of 5´ splice sites. All

missense and nonsense mutations involving the coding sequence as well as splice site mutations are detected with this method. Optimization was successful with a dimethyl sulfoxide adjuvant as judged by a single band for each PCR product on an agarose gel electrophoresis. Sequencing of these products yielded excellent results with minimal background, consistent with high-quality determination of the complete coding sequence and splice sequences in the human *APRT*. Finally, a second-tier mutation screening strategy was developed, including a qPCR, in order to detect duplications and deletions using assay reagents from ThermoFisher.

3.5.3 Kidney stone analysis

To guide the evaluation of kidney stone spectra, attenuated total reflection-Fourier transform infrared (ATR-FTIR) reference spectra were developed in the Glynn Laboratory of Bioenergetics at University College London under the direction of Prof. Peter Rich. Reference spectra were generated for DHA, supplied by Santa Cruz Biotechnology (cat. no. sc-498575), and the following compounds: hydroxyapatite (Sigma H-0252; calcium hydroxide phosphate, dried at pH 6.8 with 1mM phosphate), solid urea, ammonium acid urate, sodium hydrogen urate and uric acid. The chemicals were purchased from Sigma Aldrich Chemical Company, with the exception of ammonium acid urate which was synthesised by Prof. Rich. A Bruker IFS 66/S spectrometer, fitted with a liquid nitrogen-cooled MCT-A detector and a silicon ATR microprism (3 mm diameter; 3 reflections; DuraSampl*IR* II, SensIR/Smith Detection) was used to record the ATR-FTIR spectra. Frequencies quoted have an accuracy to ±1 cm^{-1}. Sample spectra were recorded *versus* a background spectrum of the clean prism surface.

A kidney stone sample was available from one patient in the APRT Deficiency Biobank and was analysed to produce an ATR-FTIR spectrum. The original infrared spectra of kidney stone specimens from three patients were available for examination and these were compared to the recorded DHA reference spectra.

3.6 Statistical considerations

Data were entered into a standardised Excel (Microsoft Corp., Redmond, WA, USA) data sheet in all studies. Statistical analyses in Papers I-V and VII were performed using SPSS (IBM SPSS Statistics version 21.0, 2012, Armonk, NY, USA) and R (R Foundation for Statistical Computing, Vienna, Austria; version 3.3.3) in Paper VI. The survival analysis in Paper V was performed using STATA.

In Paper I, The Hardy-Weinberg equilibrium principle ($p^2+2pq+q^2=1$) was used to calculate the expected genotypic frequencies; for heterozygotes (2pq) and homozygotes (p^2) using the minor and total allele counts; where p is equal to the cumulative minor allele frequency (cMAF) of pathogenic mutations and q is (1-p). To determine the cumulative allele frequency of any of the reported *APRT* variants (cMAF), we used the sum of the allele frequencies of individual variants in databases of genome and exome sequences available for a given geographic or ethnic group.

Descriptive statistics were reported as number (%) and median (range) in Papers II-VII. Continuous variables were compared between groups using the Mann–Whitney U-test (also known as the Wilcoxon-Mann-Whitney test). The Wilcoxon signed-rank test was used to compare variables for each subject at different time points. The Chi-square test was employed to compare categorical variables when the minimum expected number was >5, and the Fisher's exact test in other cases. A p-value of <.05 was considered statistically significant.

In Paper V, a Kaplan-Meier analysis was used to estimate death-censored allograft survival, comparing the group of patients who received XOR inhibitor treatment pior to kidney transplantation to those who were not receiving such treatment using the log-rank test.

Slopes of eGFR and staging of CKD were based on annual eGFR values derived from the lowest available SCr measurement in each calendar year, after excluding all SCr values obtained during episodes of AKI (Paper III and IV). When receiving RRT, either hemodialysis or kidney transplantation, patients were assigned an eGFR of 10 mL/min/1.73 m^2. Slopes and eGFR trajectory lines were calculated by fitting a linear regression line through available eGFR values (Paper IV).

For presentation of urinary DHA excretion, the data were expressed as 24-h urine DHA (mg/24 h) and DHA-to-creatinine ratio (mg/mmol) in first morning void urine samples (Papers II, VI and VII). In Paper VI, the mean urine DHA values were used to calculate the median 24-h DHA excretion for participants with multiple urine samples available. Spearman's (r_s) and Pearson's (r_p) correlation coefficients were used to assess the correlation between 24-h urinary DHA excretion and both the first morning-void and random urine DHA-to-Cr ratio and DHA crystalluria, as well as weight, body surface area (BSA) and eGFR. The sensitivity and specificity of the novel UPLC-MS/MS urine DHA assay for the diagnosis of APRT deficiency was examined by comparing the urine DHA concentration in samples from patients and healthy controls.

4 Results

This thesis is based on seven papers listed on page XIX. All reported cases of APRT deficiency, known pathogenic variants and their frequency in different populations were explored. Furthermore, the clinical manifestations and long-term kidney outcomes in patients with APRT deficiency, outcomes of kidney transplantation and kidney allograft survival were examined. Urinary DHA excretion in patients with APRT deficiency, heterozygous carriers of known pathogenic *APRT* mutations and heatlhy wild-type individuals was studied as well as the effect of diet on urinary DHA excretion. Lastly, a clinical trial was carried out to determine the effect of treatment with allopurinol and febuxostat on urinary DHA excretion.

4.1 The prevalence of adenine phosphoribosyltransferase deficiency

4.1.1 Assessment of prevalence based on published reports, registry data and other sources

In total, 438 cases from 33 countries were identified worldwide (Table 6), the majority coming from Japan, France and Iceland. Based on the number of cases known to have been diagnosed in these countries, the prevalence is 1:9740 in Iceland, 1:506,533 in Japan, 1:1,976,201 in France, 1: 2,258,006 in the UK and 1:12,700,326 in the United States.

Table 6. Known cases of APRT deficiency worldwide

Country	Number of known cases
USA	26
Canada	4
Iceland	37
Finland	1
Norway	1
Netherlands	1
UK	30

Hungary	1
Turkey	1
Greece	1
France	33
Germany	3
Spain	7
Italy	9
Belgium	2
Austria	2
Poland	2
Portugal	1
Czech Republic	1
Bermuda	1
Martinique	1
Morocco	1
Senegal	1
Kuweit	1
United Arab Emirates	1
Pakistan	1
Iraq	1
India	9
Japan	250
China	1
Singapore	1
Australia	4
New Zealand	1
Unknown	1

Abbreviations: APRT, adenine phosphoribosyltransferase.

4.1.2 Prevalence estimation using whole-exome and whole-genome sequencing data (Paper I)

A total of 62 pathogenic mutations in the *APRT* gene were identified. These mutations have been observed in homozygous or compound heterozygous patients with APRT deficiency who were identified in any of the three sources: (1) Medical literature and databases; (2) expanded web search; and (3) specialized APRT deficiency registry. Of the 438 APRT deficiency

patients worldwide, 359 (81.9%) had received a molecular diagnosis to our knowledge. Thirty-nine variants were detected in single affected individuals, and the remaining twenty-three were observed in two or more cases, 9 of which were observed in 5 or more cases. We counted 28 missense, variants and the remaining 35 variants correspond to nonsense variants, indels and frameshifting, splicing or start lost variants.

In addition to the 62 mutations, we noted two variants that were reported in heterozygous individuals with partial enzyme deficiency; a missense mutation, c.266G>A (p.(Arg89Gln)), from Australia and the c.346G>A (p.(Ala116Thr)) missense mutation from China. Neither of the two variants have been found in confirmed cases of APRT deficiency, and therefore they were not include in our set of 62 pathogenic variants.

Of the 62 pathogenic *APRT* variants, 57 had already been reported in the literature. Five novel variants were discovered through our APRT Deficiency Research Program; A C-to-G substitution (c.81-3C>G) in intron 1 was identified in a homozygous state in 2 siblings in the US and a boy in Italy. A C-to-T substitution (c.58C>T, p.(Pro20Ser)) in exon 1 was found in a homozygous patient in the UK. Two compound heterozygous patients from the US had a mutation that has not been previously described, in addition to one reported mutation. One of these two had a frameshift mutation (c.23dupT p.(Val9Glyfs*2)), while the other had a missense mutation (c.264G>T, p.(Lys88Asn)) in exon 3. The fifth novel mutation was identified in a patient from India who was referred to the APRT Deficiency Research Program but had already been found to be a compound heterozygote during diagnostic testing at his home institution, carrying a missense mutation in exon 3 (c.227C>T, p.(Ala76Val)).

All known cases harboring the three most common mutations causing APRT deficiency, are presented in Table 7. The number of reported cases and the frequencies of these three mutations in the different ethnical and geographic groups of the available databases are summarized. These 3 mutations were observed in 510 (71.0%) of the 718 alleles.

Table 7. The frequency of the three most common pathogenic *APRT* variants among confirmed cases and in population genomic databases.

	Identified cases		Genomic databases		
	Homozygotes	**Compound heterozygotes**	**Allele count**	**Allele number**	**Allele frequency (%)**
c.407T>C (p.(Met136Thr))					
Japan	126	44			
Japan (HGVD)			3	2326	0.13
USA	1				
KOVA (Korea)			1	1898	0.05
gnomAD					
East Asian			1	18360	0.005447
African			0	16760	0.000
Ashkenazi Jewish			0	9956	0.000
European (Finnish)			0	20398	0.000
European (Non-Finnish)			0	112090	0.000
Latino			0	34518	0.000
Other			0	6066	0.000
South Asian			0	30590	0.000
c.194A>T (p.(Asp65Val))					
Iceland	37				
Iceland (deCODE)			1299	107928	1.2
Sweden (deCODE)			1	6306	0.016
Denmark (deCODE)			1	17662	0.006
UK		1			
UK (100,000 Genomes Project)			1	127474	0.0008
Spain	1				
France		1			

Australia	1			
gnomAD				
European (Non-Finnish)		1	124598	0.0008026
Estonian		1	4768	0.02097
African		0	24072	0.000
Ashkenazi Jewish		0	10156	0.000
East Asian		0	19736	0.000
European (Finnish)		0	24188	0.000
Latino		0	35102	0.000
Other		0	7032	0.000
South Asian		0	30262	0.000
c.400+2dupT				
Europe	13	23		
UK (Biobank)		22	1000000	0.022
UK (100,000 Genomes Project)		4	127474	0.003
Ireland (deCODE)		5	2730	0.18315018
Denmark (deCODE)		4	17662	0.02264749
USA	6			
Australia	1			
Unknown	1			
gnomAD				
European (Non-Finnish)		27	128838	0.02096
Southern European		5	11592	0.04313
Other non-Finnish European		10	32948	0.03035
North-Western European		11	50708	0.02169
Swedish		1	26100	0.003831

African	1	24940	0.004010
South Asian	1	30614	0.003266
Latino	1	35430	0.002822
Ashkenazi Jewish	0	10350	0.000
East Asian	0	19942	0.000
European (Finnish)	0	24890	0.000
Other	0	7280	0.000

Abbreviations: APRT, adenine phosphoribosyltransferase; gnomAD, Genome Aggregation Database; HGVD, Human Genomic Variant Database; KOVA, Korean Variant Archive.

Fourty-one case of APRT deficiency carrying p.(Asp65Val) have been reported. Of these, 37 patients are from Iceland, all of whom were found to be homozygous. One patient from Spain was homozygous for the same mutation and 3 other patients were compound heterozygotes; 1 from France, 1 from the UK and 1 from Australia. On whole-genome sequencing of 53,964 Icelanders present in the deCODE database, we observed 1,299 alleles of the variant in the *APRT* gene. The minor allele frequency (MAF) was 1.2%. Icelanders have ancestries originating from Scandinavia and the British Islands. Out of 14,904 Scandinavian individuals with whole-genome sequening data at deCODE, only two heterozygotes carrying the p.(Asp65Val) mutation were identified, and one single heterozygote was discovered in the UK. The very low frequency outside Iceland is consistent with p.(Asp65Val) being a founder mutation in the Icelandic population.

The T-to-C missense mutation at codon 136 in exon 5 (p.(Met136Thr)) has been described in 171 patients of Japanese descent, all but one of whom were published in the literature. The additional case was identified in the APRT Deficiency Registry in a Japanese patient living in the US. This pathogenic variant was also found in the Human Genetic Variation Database (HGVD) in Japanese cohorts from Tohoku University (Alt/Ref: 1/76, MAF 1.3%) and the University of Tokyo (Alt/Ref: 2/666, MAF 0.3%). This geographic distribution is consistent with a previous report (Kamatani et al., 1996). In the Korean Variant Archive (KOVA), the MAF of this variant was 0.05% (1/1898). The variant was not detected in other genomic databases. It is noteworthy that the number of Japanese within the gnomAD database is

only 150. This mutation was not detected outside of Japan or Korea despite a very large sample size.

A T insertion at the splice donor site in intron 4 (c.400+2dupT) was found in a total of 41 cases, all of European descent. The mutation has been identified in 13 homozygotes and 23 compound heterozygotes from Europe, including France (n=29), Germany, Austria, Belgium, Italy, Poland. The variant has also been found in patients in the US (n=6) and Australia (n=1). Consistently, the variant was present in European populations in the population databases, both gnomAD and deCODE. The highest frequency were observed among Irish (5 carriers; MAF 0.18%) and Southern-European (MAF 0.043%).

The presence and frequency of the 62 pathogenic *APRT* mutations were searched for in the available databases, by ethnic and geographic group. Out of the 62 pathogenic mutations, 29 were observed in the scrutinized databases. Known APRT deficiency patients carrying these mutations accounted for 564 of the 718 alleles with a molecular diagnosis.

The three most common mutations in terms of case numbers are quoted above. Two of these are clearly indicative of a founder effect, the Icelandic and Japanese mutations, both of which had a very low frequency in public databases. The third mutation showed a distribution of cases and of copies in population databases from multiple European countries (c.400+2dupT), and was the pathogenic variant that most frequently occured in the public databases. Besides the aforementioned three variants, the most commoly reported mutations were p.(Trp98Ter) in 24 Japanese cases and p.(Phe174del) observed in 7 cases from European coutries and the US.

Table 8. Pathogenic APRT variants detected in the deCODE database

No.	Reference sequence	Position (Build 38)	Location	Base change	Amino acid change	Mutation	Alt/Ref	MAF (%)	Country of origin
1	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1299/107928	1.2	Iceland
2	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	4/107928	0.003706	Iceland
3	NM_000485.2	88810141	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	1/107928	0.000927	Iceland
4	NM_000485.2	88810141	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	5/17662	0.028309	Denmark
5	NG_008013.1	88810067	Intron 4	c.400+2dupT	-	Indel	4/17662	0.02264749	Denmark
6	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	2/17662	0.01132374	Denmark
7	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1/17662	0.00566187	Denmark
8	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	1/6306	0.015857913	Sweden
9	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1/6306	0.015857913	Sweden
10	NM_000485.2	88809717	Exon 5	c.521_523del	p.(Phe174del)	Deletion	1/6306	0.01585713	Sweden
11	NG_008013.1	88810067	Intron 4	c.400+2dupT	-	Indel	5/2730	0.18315018	Ireland
12	NM_000485.2	88809700	Exon 5	c.541T>C	p.(*181Argext*?)	Stop lost	2/2730	0.07326007	Ireland
13	NM_000485.2	88810494	Exon 3	c.250G>A	p.(Val84Met)	Missense	2/5840	0.0342465	Norway
14	NM_000485.2	88809717	Exon 5	c.521_523del	p.(Phe174del)	Deletion	1/5840	0.0171232876	Norway

Abbreviations: APRT, adenine phosphoribosyltransferase; MAF, minor allele frequency.

Iceland is the country where the highest fraction of the population has been sequenced (around 1 in 6 Icelanders). In addition to p.Asp65Val discussed above, 5 individuals heterozygous for 2 other known pathogenic *APRT* mutations were discovered in the deCODE database comprising 53,964 Icelanders (Table 8). Of these, the c.1A>C (p.(Met1?)) nonsense variant, previously reported in patients from Hungary and France, was observed in 4 individuals with a MAF of 0.004%.

To determine the cumulative MAF (cMAF) of any of the reported *APRT* mutations, the sum of allele frequency of individual pathogenic variants was used. Given that APRT deficiency is an autosomal recessive disease, an affected individual is expected to carry two copies of pathogenic mutations. Using the Hardy-Weinberg principle, the cumulative allele frequency of the p.(Asp65Val) mutation in the Icelandic population was 1.2% with a predicted frequency of homozygotes of 1 in 6840 (Table 9). In the Scandinavian countries (Denmark, Sweden and Norway), the cumulative allele frequency of any of the pathogenic variants from 14,904 individuals was stable at around 0.05%. This would correspond to an expected number of homozygous individuals of about 1 in 4 million individuals, or about 5 expected cases for a total population of 20 million subjects. Notably, no cases of APRT deficiency in European subjects in these three countries have been reported. In Ireland the cumulative allele frequency of pathogenic mutations observed was 0.26% (i.e. 7 out of 2730 alleles); this was represented by 5 copies of c.400+2dupT and a single copy of two other pathogenic mutations. In the Irish population, the expected frequency of individuals homozygous for pathogenic mutations would be 1 in 152,100, corresponding to an expected number of homozygotes of about 35 subjects given the size of the country. To our knowledge, no cases of APRT deficiency have been reported in Ireland, potentially consistent with lack of reporting cases that do exist or missed diagnosis.

The overall frequency of the reported pathogenic mutations in gnomAD was similar among the European (Non-Finnish) population, with a calculated allelic frequency of 1:2046 (63 copies out of 128,838 alleles). Frequencies of pathogenic variants in Latinos and East Asians was similar as Europeans in gnomAD, whereas other groups had lower frequencies.

Table 9. Cumulative allele frequencies of pathogenic variants observed in population databases

Popluation databases	Allele count (Alt/Ref)	Allele frequency (%)	Expected homozygous frequency (1 in)	Expected heterozygous frequency (1 in)
gnomAD				
African	9/24940	0.0360	7,679,057	1386
Latino	11/35430	0.0508	3,712,044	964
Ashkenazi Jewish	1/10350	0.0097		
East Asian	9/19946	0.0451	4,911,641	1109
Finnish	1/24920	0.0080		
European (Non-Finnish)	63/128828	0.0489	4,181,571	1023
South Asian	5/30614	0.0163	37,488,680	3062
UK Biobank				
All	77/1000000	0.071	1,686,625	650
100,000 Genomes Project				
All	11/127474	0.0086	134,294,386	5795
deCODE				
Iceland	1304/107928	1.2082	6,840	42
Denmark	12/17662	0.0678	2,166,293	736
Sweden	3/6306	0.0476	4,418,404	1052
Norway	3/5840	0.0514	3,789,511	974
Ireland	7/2730	0.18315	298,116	274
HGVD	3/2326	0.1289	601,142	388

Abbreviations: APRT, adenine phosphoribosyltransferase; gnomAD, Genome Aggregation Database; HGVD, Human Genome Variant Database.

4.1.3 Reasons for missed diagnosis

It is noteworthy that the estimated prevalence of APRT deficiency is higher in most populations than reflected by the number of reported cases. Therefore, factors that might contribute to missed diagnosis were examined. Of the 63 patients enrolled in the APRT Deficiency Registry, 40 patients experienced a median diagnostic delay of 5.47 (0.42-47.9) years after their first symptomatic stone event or detection of reduced kidney function. Reasons for missed diagnosis included misidentification of DHA kidney stones as uric acid calculi in 6 patients and confusion of renal histopathological findings with other forms of crystal nephropathy in 6 patients. The urine DHA crystals were not correctly identified in 18 cases (Papers III and IV).

4.1.4 Missed diagnosis based on stone analysis (Paper II)

Seventeen patients were referred to the APRT Deficiency Research Program at LUH after analysis of kidney stone composition demonstrated DHA. In 14 of the cases, the testing was carried out using infrared spectroscopy (Table 10). The diagnosis of APRT deficiency was confirmed by analysis of APRT enzyme activity and/or genetic testing in 7 out of 14 patients, all of whom had kidney stones composed of 100% DHA. The stone analysis spectra could not be retrieved for any of these 7 cases. However, analysis of a kidney stone sample obtained from Case 13 (Table 10) revealed an Fourier-transform infrared spectrum which corresponded closely to that of the pure crystalline DHA, indicating that it was indeed an essentially pure (100%) DHA stone (Figure 6). In 7 cases, the diagnosis of APRT deficiency made by stone analysis was rejected by confirmatory testing (Table 10).

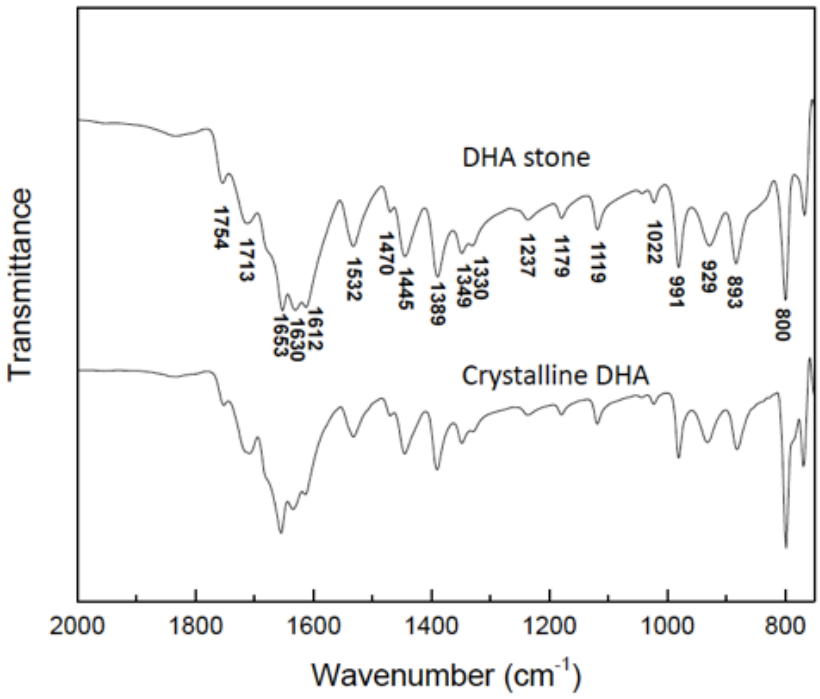


Figure 6. Comparison of the fingerprint region of FTIR spectra of a 2,8-dihydroxyadenine (DHA) stone and pure crystalline DHA. Abbreviations: FTIR, Fourier transform infrared.

Table 10. Clinical characteristics of patients referred due to a DHA kidney stone identified by FTIR spectroscopy analysis

Case	Sex	Country	Age at stone analysis (years)	Stone events (number)	SCr at last follow-up (µmol/L)	Stone analysis method	Proportion of DHA in stone material (original report)	Urine DHA/Cr ratio (mg/mmol)	Genetic testing	APRT enzyme analysis
1	Female	US	34	1	NA	FTIR	100%	BLQ	NA	Normal
2	Male	US	58	4	88	FTIR	100%	NA	Normal	NA
3	Male	UK	28	1	70	FTIR	12%	BLQ	NA	Normal
4	Female	UK	45	1	95	FTIR	64%	BLQ	Benign variant	NA
5	Male	South Africa	22	1	88	FTIR	60%	BLQ	Normal	Normal
6	Male	South Africa	26	1	80	FTIR	30%	BLQ	Normal	Normal
7	Female	Australia	11	NA	NA	FTIR	Trace	BLQ	Normal	Normal
8	Male	US	37	5	153	FTIR	100%	NA	Pathogenic biallelic variants	No enzyme function
9	Female	US	22	7	106	FTIR	100%	14.9	Pathogenic biallelic variants	NA
10	Female	US	47	6	72	FTIR	100%	NA	Pathogenic biallelic variants	No enzyme function
11	Male	US	30	2	71	FTIR	100%	BLQ*	NA	No enzyme function
12	Female	India	2	2	35	FTIR	100%	26.3	Pathogenic biallelic variants	No enzyme function
13	Male	Italy	2	2	49	FTIR	100%	2.5*	Pathogenic biallelic variants	No enzyme function
14	Female	UK	55	4	69	FTIR	100%	37.2	Pathogenic biallelic variants	NA

*On treatment with a xanthine oxidoreductase inhibitor. Abbreviations: APRT, adenine phosphoribosyltransferase; BLQ, below limits of quantification; Cr, creatinine; DHA, 2,8-dihydroxyadenine; FTIR, Fourier-transform infrared; NA: not available; SCr, serum creatinine.

4.2 Clinical characteristics and outcomes of adenine phosphoribosyltransferase deficiency

4.2.1 Kidney disease in adenine phosphoribosyltransferase deficiency (Paper III)

In Paper III, the clinical characteristics and kidney outcomes in 53 patients enrolled in the APRT Deficiency Registry before November 2014 are reported. At the time of diagnosis, 29 patients had experienced kidney stones and 20 had progressed to CKD stages 3-5 (Table 11), with advanced disease being a more commonly observed presentation among adults than children (Figure 7). Nine patients were found to have advanced CKD (stages 3-5) without a history of kidney stones.

Table 11. Clinical characteristics at the diagnosis of APRT deficiency in 53 patients from the APRT Deficiency Registry

Characteristics	Value
Female sex	30 (57)
Median (range), y	37.0 (0.6-67.9)
<18 y	14 (26)
≥18 y	39 (74)
Kidney stones	29 (55)
Chronic kidney disease, stages 3-5	20 (38)
Renal replacement therapy	8 (15)
Acute kidney injury	16 (30)
Lower urinary tract symptoms	15 (28)
Reddish-brown diaper stains in infancy	11 (21)
DHA crystalluria	34 (64)
Asymptomatic	5 (9)
Family screening	3
Incidental finding	2

Abbreviations: APRT, adenine phosphoribosyltransferase; DHA, 2,8-dihydroxyadenine.

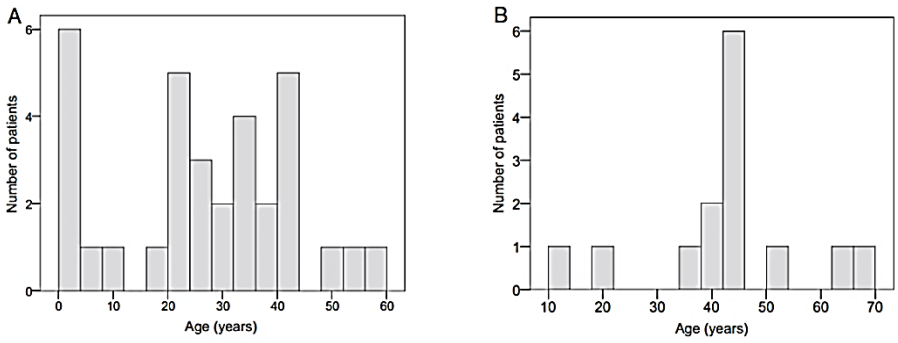


Figure 7. Age distribution at major clinical events in patients with APRT deficiency. **(**A) at the first kidney stone event and (B) at the detection of CKD stage 5. Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease.

At the time of diagnosis, 29 patients had suffered 80 symptomatic stone events, 20 of whom had their first stone episode 10.5 (0.8-47.9) years before the diagnosis of APRT deficiency, which in only 9 patients was made during the initial stone event. The clinical course and management of all 33 patients who eventually developed kidney stones is outlined in Table 12.

Two of the 16 patients who suffered AKI before the diagnosis of APRT deficiency required transient hemodialysis and 2 additional affected individuals developed AKI at a later stage that responded well to conservative treatment. AKI occurred in 18 patients during the course of follow-up. Of these, 10 progressed to CKD stages 3-5 at a median of 1.6 (0.3-29.5) years after the AKI event. Seven patients had AKI due to biopsy-proven crystal nephropathy while AKI was caused by urinary tract obstruction from stones in 9 patients and volume depletion in two.

Table 12. Kidney stone disease in patients with APRT deficiency

Variable	Value
Number of patients with kidney stones	33 (62)
At time of diagnosis	29 (55)
During follow-up	4 (8)
Age	
At first kidney stone event, years	26.4 (0.3-56.4)
≥ 18 years at first episode	26 (49)
Kidney stones before diagnosis of APRT deficiency	20 (38)
Delay from first clinical stone event to diagnosis, years	10.5 (0.8-47.9)
Kidney stone recurrence	18 (34)
Off XOR inhibitor treatment	2 (4)
On XOR inhibitor treatment	16 (30)
Allopurinol dosage, mg	200 (100-600)
Kidney stone events	
1	14 at diagnosis; 7 during follow-up
2-3	10 at diagnosis; 8 during follow-up
4-5	2 at diagnosis; 2 during follow-up
> 5	3 at diagnosis; 0 during follow-up
Asymptomatic stones	5 at diagnosis; 2 during follow-up
Urologic procedures	
Extracorporeal shockwave lithotripsy	4 at diagnosis; 6 during follow-up
Endoscopic surgery	6 at diagnosis; 9 during follow-up
Open or percutaneous surgery	4 at diagnosis; 3 during follow-up

Abbreviations: APRT, adenine phosphoribosyltransferase; XOR, xanthine oxidoreductase.

Twenty patients had developed CKD stages 3-5 at the time of diagnosis at the median age of 44.5 (11.9-67.9) years (Table 11), and 2 patients experienced CKD stages 3-5 during follow-up. Six patients initiated RRT 3.8 (1.1-7.4) years before the diagnosis of APRT deficiency, 4 of whom experienced disease recurrence in a kidney allograft. Twelve of the 14 patients who reached CKD stage 5 had initiated RRT during the follow-up period. At diagnosis, 7 of the 33 Icelandic patients had developed CKD stages 3-5 compared with 13 patients (65%) from other countries (p=0.001; Figure 8).

Table 13. Stages of CKD and RRT in patients with APRT deficiency

	At diagnosis	At last follow-up
CKD stage		
1	19	19
2	14	12
3a	2	2
3b	2	3
4	5	3
5	11	14
RRT		
Functioning transplant	3	7
Dialysis	5	2

Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease; RRT, renal replacement therapy.

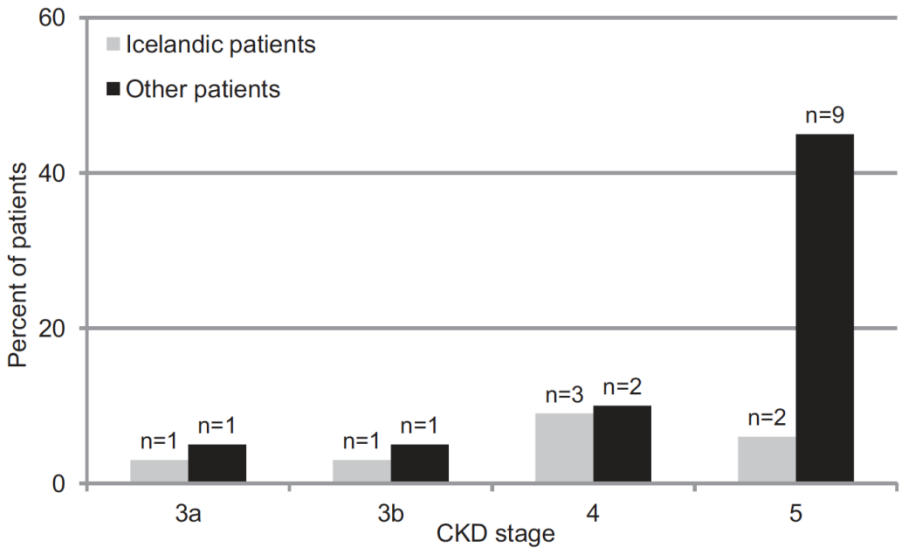


Figure 8. CKD stages 3-5 at time of diagnosis in patients with APRT deficiency from Iceland and other countries. Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease.

The 53 patients reported in Paper III were diagnosed with APRT deficiency at the median age of 37.0 (0.6-67.9) years. Thirty-seven of these

individuals had a delay in diagnosis, a median of 7.5 (0.4-47.9) years after the first symptomatic stone event or detection of elevated SCr (see 4.1.3). The diagnosis of APRT deficiency was first suggested by detection of urine DHA crystals in 34 cases, by histological findings of crystal nephropathy in 9, and kidney stone analysis in 8 cases.

Additionally, review of autopsy findings, which were consistent with DHA crystal nephropathy, lead to the diagnosis in 2 cases. Diagnosis of the disorder was confirmed by absence of APRT enzyme activity in red cell lysates (n=11) and/or identification of biallelic pathogenic mutations (n=46).

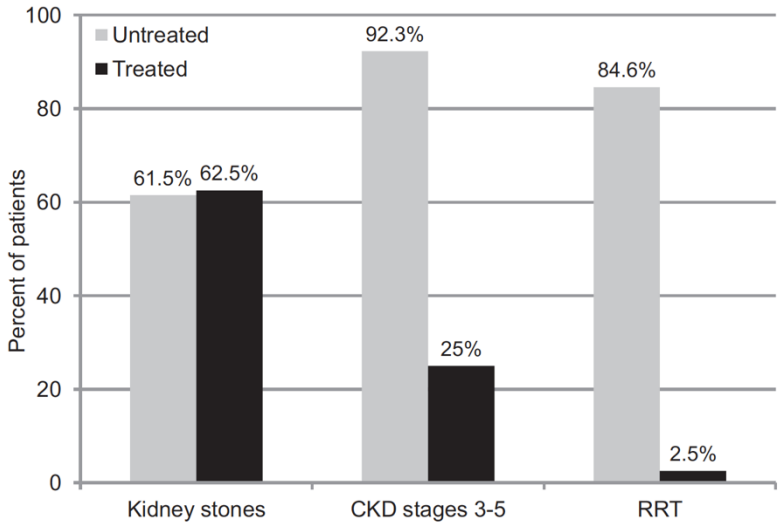
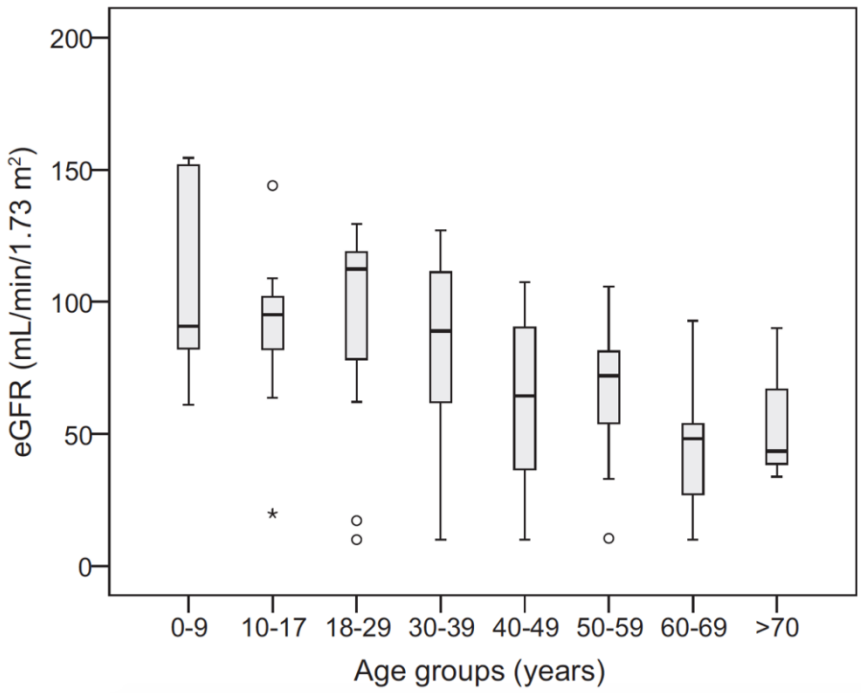


Figure 9. Kidney stones, CKD stages 3-5 and initiation of RRT in patients with APRT deficiency who did and did not receive treatment with an XOR inhibitor. Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease; RRT, renal replacement therapy; XOR, xanthine oxidoreductase.

None of the 11 patients who developed stage 5 CKD prior to diagnosis had received XOR inhibitor treatment. The clinical features of patients who received allopurinol treatment prior to onset of RRT and those who did not are presented in Figure 9. Thirty-eight adult patients initiated allopurinol treatment at age 42.2 (20.5-62.8) years in a daily dose of 100 mg (n=7), 150 mg (n=3), 200 mg (n=21) or 300 mg (n=7). Treatment with allopurinol was initiated at 3.3 (0.6-16.6) years of age in 14 children at a daily dose of 8 (3-11) mg/kg or a total dose of 100 (25-200) mg. Seven patients had to discontinue allopurinol after 7.1 (0.4-16.4) years of treatment because of presumed adverse reactions including itching, hair loss and severe ocular

symptoms, such as pain, photosensitivity and blurred vision. All 7 patients were subsequently prescribed febuxostat 80 mg/day once the drug became available. Febuxostat was later discontinued in 2 patients due to severely blurred vision and punctate keratitis in one patient and ocular dryness in the other. None of the 12 minimally symptomatic patients who began XOR inhibitor therapy at the age of 7.5 (1.0-39.2) years developed kidney stones,



AKI or CKD stages 3-5.

Figure 10. Box plot of estimated glomerular filtration rate (eGFR) in different age groups of patients with adenine phosphoribosyltransferase deficiency. Patients contributed data to every age group for which they had a serum creatinine value available, and for each individual the mean of all eGFRs in any given age group was used. Patients receiving renal replacement therapy were assigned an eGFR of 10 mL/min/1.73 m^2.

Eighteen patients (33.9%) experienced 35 clinical stone events while on allopurinol treatment (Table 12), most frequently at a daily dose of 300 mg (n=8). One patient with CKD stage 3b, one with CKD stage 4 and another with CKD stage 5 experienced transient improvement in kidney function after initiation of XOR inhibitor treatment. Nevertheless, 2 additional patients developed CKD stage 3-5 CKD during the follow-up period (Table 13).

The fifty-three patients had a median eGFR of 68 (3-165) mL/min/1.73 m^2 at diagnosis, including patients who presented with AKI, and 73 (10-163) mL/min/1.73 m^2 at latest follow-up. An eGFR boxplot for different age groups showed a progressive decline in median eGFR, particularly after the age of 40 years (Figure 10). The median eGFR slope was -0.38 (-21.99 to 1.42) mL/min/year in patients treated with XOR inhibitor prior to the development of CKD stage 5 and -5.74 (-75.8 to -0.10) in those who did not receive such treatment (p=0.001). Seven patients had serial SCr measurements available before and after the initiation of XOR inhibitor therapy, exhibiting a median eGFR slope of -3.01 (-14.43 to 0.92) and 1.76 (-0.7 to 13.50) mL/min/year, respectively (p=0.04).

4.2.2 Long-term kidney outcomes of adenine phosphoribosyltransferase deficiency presenting in childhood (Paper IV)

The importance of disease awareness, early diagnosis and treatment were evident in Paper III, leading to the examination of the disease course in the pediatric population with APRT deficiency (Paper IV). The characteristics of the 21 patients who presented with clinical features of APRT deficiency or were diagnosed with the disorder before age 18 years are presented in Table 14. The median age at presentation was 1.6 (0.2-16.5) years. The most common presenting features in these young individuals were reddish-brown diaper spots in 62% of patients and kidney stones in 52%. Eleven patients had their first symptomatic stone event at the median age of 3.4 (0.3-6.9) years. Three patients had AKI due to obstructive stone disease, one of whom required transient hemodialysis. Of the 4 patients who had progressed to CKD stages 3-5 at diagnosis, one had initiated RRT for ESKD.

The patients who presented with symptoms of APRT deficiency in childhood had clinical findings strongly suggestive of the diagnosis, such as urine DHA crystals (n=18) and stone analysis revealing DHA (n=2). The diagnosis of APRT deficiency was confirmed in all cases by identification of biallelic pathogenic *APRT* mutations (n=20) and/or absent APRT activity (n=4).

Table 14. Clinical characteristics of children (n=21) with APRT deficiency at the time of first presentation

Females, n (%)	**12 (57)**	
	Diagnosis at age <18 years	**Diagnosis at age ≥18 years**
Number of patients	15 (71)	6 (29)
Age at first presentation, years	1.5 (0.2-16.5)	4.4 (0.5-7.1)
Age at diagnosis, years	2.5 (0.6-16.5)	35.5 (20.5-42.4)
Diagnostic delay, years	1.2 (0.6-10.4)	29.2 (20.1-39.2)
Kidney stones	7 (46.7)	4 (66.7)
Chronic kidney disease stages 3-5	0	0
Acute kidney injury	2 (13.3)	1 (16.7)
Reddish-brown diaper stain in infancy	12 (80.0)	1 (16.7)
Asymptomatic crystalluria	2 (13.3)	1 (16.7)

Abbreviations: APRT, adenine phosphoribosyltransferase.

The median age was 4.8 (0.6-42.4) years at the time of diagnosis of APRT deficiency. Thirteen patients experienced a diagnostic delay of 10.4 (0.6-39.2) years after their first stone event (n=10) and/or detection of diaper stains (n=3). In 8 patients, urine DHA crystals were misidentified and 2 patients were presumed to have uric acid stones.

Treatment with allopurinol was prescribed to 14 children in the daily dose of 100 (25-200) mg or 6.0 (3.0-20.8) mg/kg. Of the 14 patients, 7 had developed 8 kidney stone events when XOR inhibitor treatment was started at the age of 2.6 (0.6-16.5) years. After 18.9 (1.7-31.5) years of drug treatment, one additional patient had experienced an incident kidney stone and one had suffered stone recurrence, bringing the total number of stone events to 10. Stone removal procedures were required in 5 cases. One patient underwent unilateral nephrectomy due to non-function of the kidney as a result of irreversible damage caused by stone obstruction. Four patients experienced AKI that was associated with volume depletion in 2 cases, obstructive kidney stone in 1 case and unknown cause in 1 individual. None of the 14 patients who received allopurinol treatment in childhood had developed CKD stages 3-5 at the time of last follow-up. Five of these patients did not develop any clinical events.

Six patients first received allopurinol therapy as adults, in a daily dose of 200 (100-300) mg. Five patients had developed 24 kidney stone events at the age of 29.8 (20.5-42.4) years, when they were diagnosed with the disorder and XOR inhibitor treatment was started. After 11.2 (4.2-19.6) years of pharmacotherapy, stone recurrence had occurred in all 5 of these patients, bringing the total number of stone events to 35. Four stone removal interventions were carried out in 2 patients and 1 patient underwent a partial nephrectomy in adulthood due to severe kidney damage caused by stone obstruction. Six episodes of AKI were observed in 3 patients, 4 of which were due to obstructive kidney stones and 1 was caused by a biopsy-proven crystal nephropathy. At last follow-up, 2 of the 3 patients who suffered AKI had progressed to CKD stages 3-5, one had stage 3a while the other had reached stage 5 CKD requiring RRT. A third patient progressed to CKD stage 3b without a known history of AKI. At last follow-up, 5 of the 6 patients who initiated treatment with an XOR inhibitor as adults were receiving allopurinol in a daily dose of 300 (200-400) mg. Febuxostat 80 mg/day had been prescribed in 1 patient as allopurinol therapy was discontinued due to an adverse reaction (pruritus). The clinical manifestations of the 20 patients who presented with symptoms of APRT deficiency in childhood and received treatment with allopurinol prior to the development of ESKD, are summarized in Figure 11.

The eGFR in the 21 patients who did not have ESKD at disease presentation was 83 (35-165) mL/min/1.73 m^2, despite the inclusion of several patients with AKI. Eighteen patients who had two or more SCr values available were included in the assessment of the evolution of kidney function. At last follow-up, the eGFR was 114 (70-163) mL/min/1.73 m^2 and the median eGFR slope (n=12) was 0.04 (range, -5.28 to 2.79) mL/min/1.73 m^2 per year in the patients who initiated XOR inhibitor treatment in childhood. As these 14 patients only had SCr values available after starting pharmacotherapy, the potential effect of XOR inhibitor treatment on kidney function could not be evaluated. For the 6 patients who initiated drug treatment as adults, the eGFR was 62 (10-103) mL/min/1.73 m^2 at last follow-up, and the median eGFR slope (n=6) was -0.47 (-1.32 to 0.38) mL/min/1.73 m^2 per year. Four of these six patients had SCr values available before and after onset of pharmacotherapy, all of whom displayed improvement of kidney function following initiation of treatment. The median eGFR slope was -0.47 (-1.23 to -0.26) before starting therapy and 0.85 (0.29 to 5.13) mL/min/1.73 m^2 per year after treatment initiation. The results for both patient groups are depicted in Figure 12.

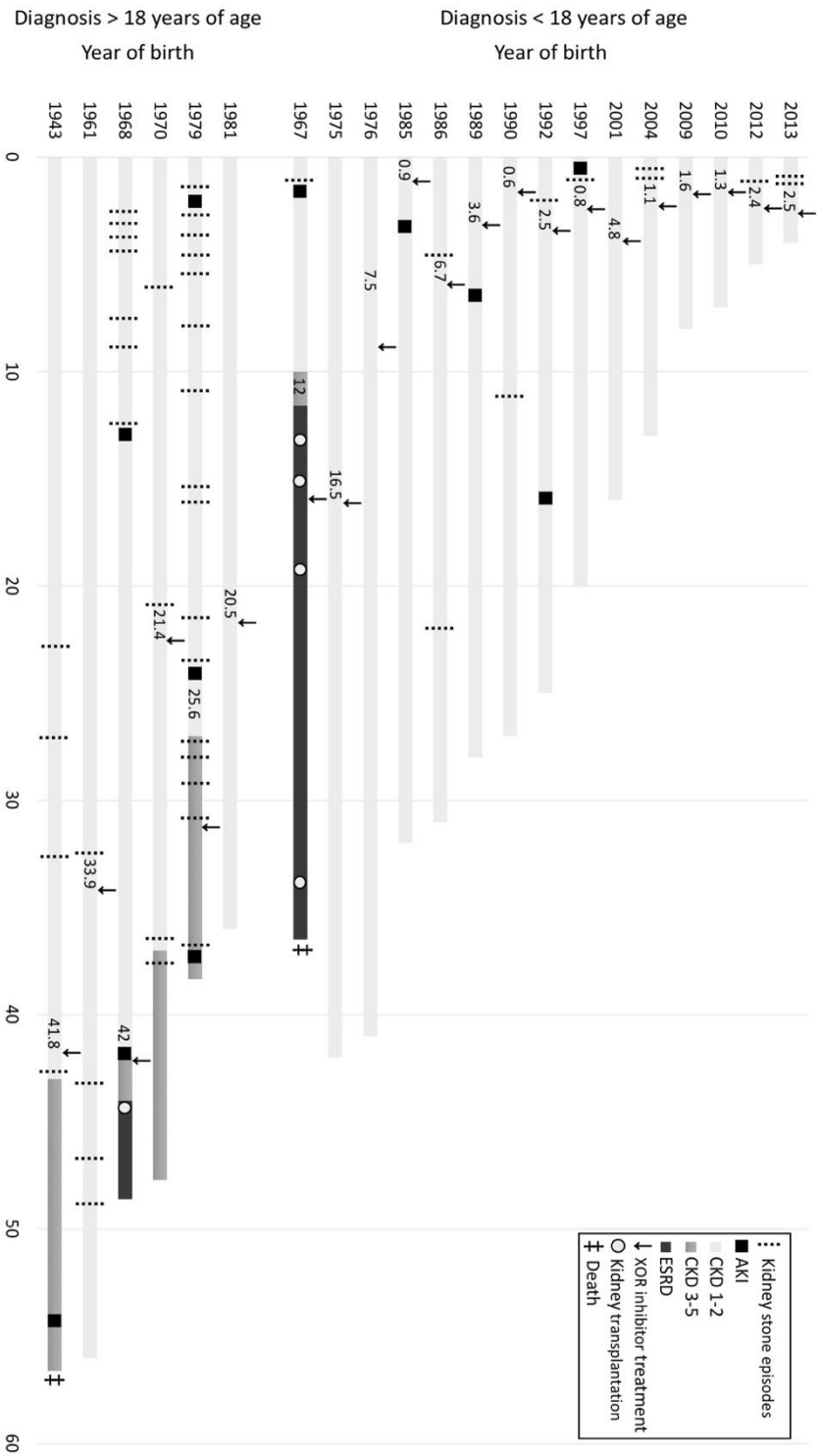


Figure 11. Clinical manifestations up to last follow-up in patients with APRT deficiency. The patients are stratified by age at diagnosis before or after the age of 18 years. Abbreviations: AKI, acute kidney injury; APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease; ESRD, end-stage renal disease; XOR, xanthine oxidoreductase.

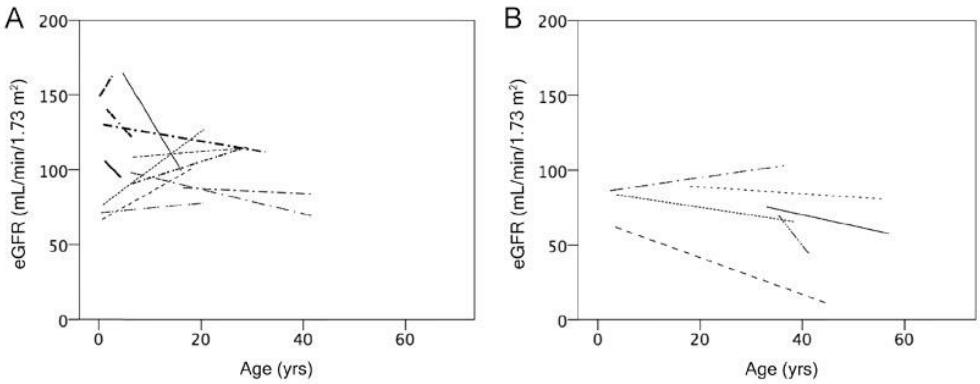


Figure 12. Temporal changes in estimated glomerular filtration rate (eGFR) in patients with APRT deficiency. Patients receiving treatment with XOR inhibitor before the age of 18 years (Panel A, n=12) and in those who first started drug therapy as adults (Panel B, n=6). eGFR trajectory lines were created by fitting a linear regression line through all available eGFR values for each patient, excluding AKI episodes. Abbreviations: AKI, acute kidney inury; APRT, adenine phosphoribosyltransferase; XOR, xanthine oxidoreductase.

4.2.3 Kidney transplant outcomes in patients with adenine phosphoribosyltransferase deficiency (Paper V)

The findings presented in Papers III and IV provide evidence for the importance of XOR inhibitor treatment for long-term renal outcomes in patients with APRT deficiency. In this study, the clinical course of 17 patients who had undergone kidney transplantation was examined, especially with regard to XOR inhibitor treatment status at time of transplant surgery. Thirteen of the 17 patients were enrolled in the APRT Deficiency Registry while the other four were referred from their treating physicians, 2 from Paris, France, and 2 from Sydney, Australia. Their clinical characteristics at diagnosis of APRT deficiency are shown in Table 15. The median age at diagnosis was 44.5 (11.9–67.9) years, by which time 13 of the 17 patients (76%) had initiated RRT for ESKD. Fifteen patients experienced a delay in diagnosis of 7.8 (1.1–47.9) years. APRT deficiency was confirmed in 11 patients (65%) prior to the first kidney transplantation; at diagnosis 1 of the 11 patients had CKD stage 3a, 3 had reached CKD stage 4 or 5 and 7 were already on hemodialysis. The diagnosis of APRT deficiency was suggested by detection of DHA crystals on urine microscopy in 2 of these 11 cases, kidney stone analysis indicating DHA as a stone component in 1 case, and by renal histological findings of crystal nephropathy in 5 individuals. Three patients were diagnosed through family screening of index cases, 2 of whom had a personal history of kidney stone disease.

Table 15. Clinical characteristics at diagnosis of APRT deficiency in patients who underwent kidney transplantation

Patient	Sex	History of kidney stones	Age at diagnosis (y)	Diagnostic delay (y)	Kidney function (eGFR, mL/min/1.73m²)	Age at kidney biopsy (y)	Original native kidney biopsy findings
1	M	No	62	1.1	ESKD	62	DHA crystal nephropathy, global glomerularsclerosis (21 of 45 glomeruli); severe interstitial fibrosis and arteriosclerosis
2	F	No	43	5.1	ESKD	38	Crystals thought to be consistent with primary hyperoxaluria
3	M	Yes	43	11.1	ESKD		NA
4	F	Yes	68	47.9	ESKD		NA
5	F	No	52	7.5	ESKD		NA
6	F	No	52	6.0	ESKD	45	Interstitial inflammation with inflammatory infiltrate; refractory golden-brown crystalline material seen
7	F	Yes	59	24.0	ESKD		NA
8	M	Yes	12	10.4	ESKD		NA
9	F	Yes	49	7.8	9	42	Tubulointerstitial fibrosis; presumed calcium oxalate crystal deposits
10	M	Yes	42	39.2	17	42	DHA crystals; interstitial inflammation
11	F	Yes	45	1.4	ESKD		NA
12	F	No	21	0	ESKD	21	Tubulointerstitial nephritis with extensive calcium oxalate deposits
13	M	No	40	4.7	ESKD	35	Chronic interstitial nephritis; crystals seen but not identified
14	F	Yes	24	4.0	54		NA
15	M	Yes	50	20.0	ESKD	50	Small number of scattered tubules contain intraluminal polarizable crystals believed to be calcium oxalate
16	M	No	43	0.2	ESKD	42	DHA crystals with advanced glomerulosclerosis, tubular atrophy and interstitial fibrosis
17	M	Yes	52	20.0	6		NA

Abbreviations: APRT, adenine phosphoribosyltransferase; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; NA, not available.

Table 16. Pharmacotherapy of patients with APRT deficiency and outcomes of kidney transplantation

Patient	Treatment before Tx	Age at RRT (y)	Graft number	Age at Tx (y)	Type of donor	Treatment with XOR inhibitor	Outcome	Last follow-up (y after Tx)	Latest eGFR (mL/min/1.73 m²)
1	No	62	1	65	DD	Febuxostat 40 mg/day from 3 wk post-Tx	Recurrence of DHA nephropathy 3 wk post-Tx; functioning graft	1.6	25
2	No	38	1	39	DD	None	Graft lost due to recurrence of DHA nephropathy 1 mo post-Tx	0.1	ESKD
	No	39	2	42	DD	Allopurinol 200 mg/day from 6 mo post-Tx	Recurrence of DHA nephropathy 3 d post-Tx and allograft failure at 13 mo post-Tx; died while on dialysis	1.1	ESKD
3	No	42	1	43	DD	Allopurinol 300 mg/day from 4 mo post-Tx	Died with a functioning graft	0.5	41
4	No	65	1	67	DD	Allopurinol 150 mg/day from 3 wk post-Tx	Functioning graft	6.5	28
5	No	45	1	46	DD	Allopurinol 150 mg/day from 3 y post-Tx	Died with a functioning graft	5.4	29
6	No	50	1	51	DD	Allopurinol 300 mg/day from 5 wk post-Tx, later febuxostat 80 mg/day	Recurrence of DHA nephropathy 24 d post-Tx; lost after 19 mo	1.7	AKI/dialysis
7	No	44	1	58	LRD	Allopurinol 300 mg/day from 2 mo post-Tx	Functioning graft	9.7	37
8	No	12	1	15	DD	None	Graft lost 18 mo post-Tx	1.5	ESKD
	No	16	2	17	DD	Allopurinol 300 mg/day from d 28 post-Tx	Recurrence of DHA nephropathy 1 mo post-Tx, graft lost after 3.4 y	3.4	ESKD
	Yes	20	3	21	DD	Allopurinol 300 mg/day	Chronic allograft failure	7.8	ESKD
	No	27	4	35	DD	Allopurinol 150 mg twice-a-week, from d 36 post-Tx	Recurrence of DHA nephropathy 1 mo post-Tx; died with a functioning graft	1.4	15
9	Yes	54	1	56	DD	Allopurinol 300 mg/day for 7 y pre-Tx, subsequently 600 mg/day	Functioning graft	13.3	50

10	Yes	46	1	46	LUD	Allopurinol 400 mg/day for 3 y pre-Tx	Functioning graft	4.4	71
11	Yes	43	1	47	DD	Allopurinol 300 mg/day for 2 y pre-Tx	Functioning graft	4.4	45
12	Yes	21	1	22	LRD	Allopurinol 300 mg/day 1 y pre-Tx, later also febuxostat 120 mg/day	Graft lost due to recurrence of DHA nephropathy 5 y post-Tx	5.2	ESKD
	Yes	28	2	29	LUD	Allopurinol 600 mg/day and febuxostat 120 mg/day	Functioning graft	1.8	69
13	Yes	36	1	41	LRD	Allopurinol 200 mg/day for 1 mo pre- Tx, subsequently 400 mg/day	Died with a functioning graft	0.3	39
14	Yes	41	1	41	DD	Allopurinol for 15 y pre-Tx, subsequently 600 mg/day	Functioning graft	3.9	80
15	Yes	50	1	53	LUD	Allopurinol for 2 y pre-Tx, subsequently 600 mg/day	Functioning graft	2.8	65
16	Yes	42	1	47	DD	Allopurinol 150 mg/day for 4 y pre-Tx, subsequently 300 mg/day and febuxostat 40 mg/day	Recurrence of DHA nephropathy 10 d post-Tx; functioning graft	1.0	60
17	Yes	66	1	66	DD	Allopurinol 200 mg/day for 12 y pre-Tx, subsequently 300 mg/day	Functioning graft	2.1	82

Abbreviations: APRT, adenine phosphoribosyltransferase; DD, deceased donor; LRD, living-related donor; LUD, living-unrelated donor; ESKD: end-stage kidney disease; RRT, renal replacement therapy; Tx, kidney transplantation; XOR, xanthine oxidoreductase. The shaded area denotes allografts where xanthine oxidoreductase treatment was not initiated prior to kidney transplantation.

In 6 patients, the diagnosis of APRT deficiency diagnosis was not made until after kidney transplantation, which in 5 cases occurred following their first transplant and in one individual after the failure of two kidney allografts. The presumed causes of ESKD before transplantation in these 6 patients were primary hyperoxaluria (n=2), chronic interstitial nephritis (n=2), kidney stone disease (n=1) and CKD of unknown causes (n=1).

The 17 patients received a total of 22 kidney allografts, the first transplant at the median age of 47.2 (14.9–67.0) years, 1.8 (0.7–13.5) years after reaching ESKD in 14 cases (Table 16). Kidney transplantation was preemptive in 3 patients.

Ten patients received allopurinol in the daily dose of 200 (100–300) mg for 2.6 (0.1–15.6) years prior to the transplantation of 11 kidney allografts (Table 16). Delayed graft function was observed in 3 allografts, and AKI in 4, arising within the first post-transplant week in 2 cases. Eight allografts in 7 patients were biopsied post-transplant (Table 17). Disease recurrence was noted in 3 allografts, at 10 days, 8 weeks and 4 months post-transplant (Figure 13). One of these 3 patients, 1 (patient No. 16) was taking allopurinol 150 mg/day at the time of the biopsy, while the remaining 2 allografts were from the same patient (No. 12) who was prescribed allopurinol 300–600 mg/day before and following both transplants. However, a significant medication non-adherence following the first transplant was acknowledged. Renal histological findings suggested acute rejection in 2 cases, immediately post-transplant in 1 case and 1 month following the transplant surgery in the other. One individual (No. 13) died with a functioning allograft 4 months following transplantation from bacterial sepsis associated with peritonitis, and 1 graft (patient No.8) was lost approximately 8 years post-transplant, with an allograft biopsy being consistent with chronic allograft nephropathy. No DHA crystals were detected.

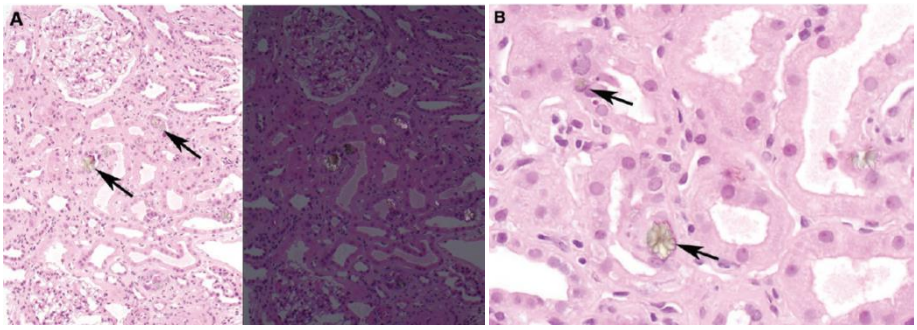


Figure 13. Recurrent 2,8-dihydroxyadenine (DHA) nephropathy in a kidney allograft biopsy (patient number 12, Table 10). A). Hematoxylin and eosin stained section shows numerous brown DHA crystals (arrows) within the tubular lumens and tubular epithelial cell cytoplasm (left panel). Upon examination under polarized light, these crystals are strongly birefringent (right panel). B). High magnification (600x) of the hematoxylin and eosin stained section, depicts small brown crystals (arrows) within the tubular epithelial cytoplasm.

Table 17. Clinical features and renal histopathological findings at the time of kidney allograft biopsy in patients with APRT deficiency

Patient	Allograft	Delayed graft function	Time from Tx, (months)	Treatment with XOR inhibitor	Renal histolopathogical findings	eGFR (mL/min/1.73 m^2)
1	1	No	0.75	Febuxostat 40 mg/day	Numerous Intratubular DHA crystal deposits	23
	1	No	0.75	None	Extensive crystal deposits	Dialysis
			0.03	None	Acute tubular necrosis; no crystals	6
			0.1	None	Intratubular crystal deposits	25
2	2	No	4	None	Extensive crystal deposits within the interstitium	29
			5	None	Mild focal interstitial fibrosis and tubular atrophy associated with mild inflammation; polarizable intraluminal crystals in several tubules	20
			12	Allopurinol 200 mg/day	Minimal interstitial fibrosis and focal tubular atrophy; occasional intratubular crystals	17
3	1	No	3.5	None	Intratubular crystal deposits	40
			4	None	Subjectively more crystals within the tubules	-
4	1	No	0.75	None	Diffuse intratubular crystals identified as DHA	8
			36	None	Intratubular crystals assumed to be uric acid	20
5	1	No	37	None	Diffuse crystal nephropathy with tubulointerstitial inflammatory infiltrates; crystals thought to be uric acid	10
			39	Allopurinol 150 mg/day	Persistence of intratubular brown crystals	23
			0.1	None	No crystals detected	Dialysis
			0.75	None	Crystal deposits presumed to be oxalate	24
6	1	Yes	1.2	Allopurinol 300 mg/day	Crystal deposits suspected to be DHA	26
			2.1	Allopurinol 300 mg/day	DHA crystals identified	43
			12	Febuxostat 80 mg/day	Crystals present (15%); severe tubular atrophy and interstitial fibrosis (>50%)	48
7	1	No	2	Allopurinol 300 mg/day	DHA crystals present	52
			12	Allopurinol 300 mg/day	No crystals detected	47
8	2	Yes	1	None	Multiple strongly birefringent DHA crystals in tubules	-
			12	Allopurinol 300 mg/day	Crystal deposits within tubules and interstitium	-

			19	Allopurinol 300 mg/day	No crystals detected	-
	3	Yes	1	Allopurinol 300 mg/day	No crystal detected	-
			0.25	None	No crystals detected	-
	4	No	1.2	None	Intratubular crystal deposits	-
			2	Allopurinol 150 mgx 2/week	Multiple deposits of highly birefringent crystals	11
9	1	Yes	0.25	Allopurinol 300 mg/day	Acute tubular necrosis; no crystals	-
			0.75	Allopurinol 600 mg/day*	-	51
			4	Allopurinol 300 mg/day*	Extensive DHA crystal deposits	20
	1	No	5	Allopurinol 450 mg/day*	20-30% reduction in DHA crystals	20
12			12	Allopurinol 300 mg/day, Febuxostat 80 mg/day*	Some reduction in DHA crystals	28
			24	Allopurinol 300 mg/day, Febuxostat 80 mg/day*	Rare crystals within the interstitium	24
			36	Allopurinol 300 mg/day, Febuxostat 80 mg/day*	>100 crystals within the parenchyma	23
	2	No	2	Allopurinol 300 mg/day, Febuxostat 80 mg/day	Rare intratubular DHA crystal deposits	69
13	1	No	3	Allopurinol 400 mg/day	No crystals detected	39
			4	Allopurinol 600 mg/day	-	50
15	1	No	12	Allopurinol 600 mg/day	-	64
			0.3	Allopurinol 150 mg/day	DHA crystals in ~30% of tubules	Dialysis
			0.6	Allopurinol 300 mg/day	DHA crystals in ~40% of tubules	Dialysis
16	1	Yes	2	Allopurinol 600 mg/day	Subjectively fewer DHA crystals	33
			12	Allopurinol 300 mg/day, Febuxostat 80 mg/day	Scant DHA crystals	45
17	1	No	3	Allopurinol 300 mg/day	No crystals detected	99

The shaded area denotes allografts where XOR inhibitor treatment was not initiated prior to kidney transplantation.

*Reported non-adherence to XOR inhibitor treatment. Abbreviations: APRT, adenine phosphoribosyltransferase; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; Tx, kidney transplantation.

Eight patients did not receive XOR inhibitor therapy prior to transplantation of 11 kidney allografts (Table 16). The XOR inhibitor treatment was initiated at a median of 0.1 (0.1–2.9) years post-transplant in 9 of these allografts. Graft function was delayed in 2 allografts, both from deceased donors, and AKI was observed in 7 transplants. Ten of the allografts were biopsied revealing recurrence of DHA nephropathy in all of them (Table 17), which was significantly more common than in those receiving XOR inhibitor treatment pre-transplant (p=0.004; Table 18). Two patients lost 4 allografts due to disease recurrence, 1.3 (0.1–3.4) years post-transplant. The patients were both untreated before and after their first transplant, but initiated treatment with allopurinol 200 mg/day and 300 mg/day at 1 and 7 months following the second transplant, respectively. Five patients who initiated pharmacotherapy post-transplant had persistent biopsy proven DHA allograft nephropathy, 4 of whom were treated with allopurinol in doses ranging from 150 mg twice a week to 300 mg daily. Three patients died with a functioning graft (Table 16); one patient (No. 3) from miliary tuberculosis at 5 months post-transplant, 1 (No. 5) from breast cancer 5.8 years post-transplant and the third one (No. 8) from sepsis 1.4 years following a fourth kidney transplant. One patient (No. 2), who had lost two allografts due to disease recurrence, expired while on hemodialysis 8 years after the second transplant. Another patient (No. 6) developed acute liver failure 1.5 years post-transplant, suspected to be drug-induced, though the offending agent was not identified. The patient died while on hemodialysis for AKI 2 months later, in the setting of multiorgan failure.

Table 18. Allograft outcomes in patients who initiated XOR inhibitor treatment with allopurinol or febuxostat prior to kidney transplantation compared with those who did not receive such treatment until post-transplant, or not at all.

	No XOR inhibitor therapy pre-transplant	XOR inhibitor therapy pre-transplant	p-value
Number of patients	8	10	
Number of grafts	11	11	
Age at transplant, years	42.8 (14.9–67.0)	45.5 (20.7–66.2)	0.974
Delayed graft function	2	3	1.0
Post-transplant acute kidney injury	7	4	0.395
eGFR, mL/min/1.73 m^2			
At 6 months	24.9 (9.6–53.3) [9 grafts]	61.5 (22.5–93) [10 grafts]	0.003
At 12 months	27.5 (10.0–67.5) [9 grafts]	64.8 (28–93.8) [10 grafts]	0.035
At 2 years	16.2 (10.0–39.0) [6 grafts]	61.3 (24.0–90.0) [8 grafts]	0.009
Biopsy-proven recurrence of DHA nephropathy	10	3	0.004
Graft loss due to recurrence of DHA nephropathy	4	1	0.31
Death	5	1	0.043
Death with a functioning graft	3	1	0.275

Data are presented as median (range). Abbreviations: AKI, acute kidney injury; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; XOR, xanthine oxidoreductase.

The median eGFR at 6 months post-transplant was 61.5 (22.5–93.0) mL/min/1.73 m^2 in patients who received XOR inhibitor therapy pre-transplant, while it was 24.9 (9.6–53.3) mL/min/1.73 m^2 in those who did not receive such treatment prior to transplantation (p=0.003; Table 18). Similarly, the graft function was greater in the XOR inhibitor-treated group at 2 years post-transplant, with a median eGFR of 61.3 (24.0–90.0) mL/min/1.73 m^2 compared with 16.2 (10.0–39.0) mL/min/1.73 m^2 in those who had not received XOR inhibitor treatment prior to transplantation (p=0.009; Table 18). At 2 years post-transplant, the allograft survival was 91% in the group receiving XOR inhibitor treatment pre-transplant versus 55% in the untreated group. However, the difference in allograft survival did not reach statistical significance (p=0.16; Figure 14). In general, patients receiving higher allopurinol doses appeared to be less likely to experience disease recurrence (Tables 16 and 17).

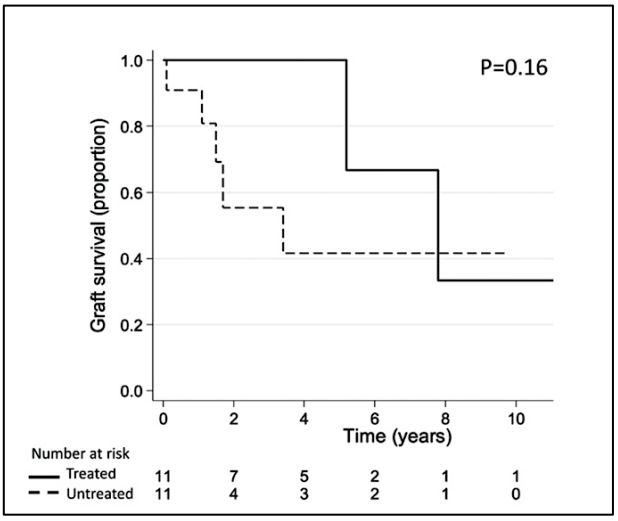


Figure 14. Survival of kidney allografts in patients who received treatment with an XOR inhibitor pre-transplant (solid line) and those who did not (broken line). Kaplan-Meier method, sensoring at death, log-rank test used for comparison.

4.3 Urinary 2,8-dihydroxyadenine excretion in patients with adenine phosphoribosyltransferase deficiency, heterozygotes and healthy control subjects (Paper VI)

The diagnosis of APRT deficiency has been shown to be challenging as the DHA crystals in both urine and renal tissue specimens are often misidentified or overlooked. The newly developed UPLC-MS/MS assay was used to measure DHA in urine samples from 33 patients (22 women) in the APRT Deficiency Registry and from from 4 heterozygotes and 10 healthy individuals (Paper VI). Twenty-one of the patients had a history of kidney stones, 11 had experienced AKI and 6 were asymptomatic. One patient had CKD stage 3a at the time of urine collection, whereas another had reached CKD stage 5 with an eGFR of 14 mL/min/1.73 m^2. All other patients had eGFR >60 mL/min/1.73 m^2.

For the analysis of DHA excretion, 31 timed urine collections from 14 patients were available. The patients were either untreated or had paused therapy prior to the collection period for the purpose of the study. The median 24-h urinary DHA excretion was 138 (64–292) mg in the patient samples. No DHA was detected in the 24-h collections and first morning-void urine samples from the 4 heterozygotes and 10 healthy individuals participating in the study (Table 19).

Table 19. Urinary 2,8-dihydroxyadenine (DHA) excretion in individuals with APRT deficiency, heterozygotes and healthy non-carriers in 24-h and first morning void urine samples.

	Homozygotes	Heterozygotes	Healthy non-carriers
Number of participants	19	4	10
Age, years	35.4 (16.1-67.0)	47.4 (36.6-56.6)	25.0 (23.5-30.1)
eGFR, mL/min/1.73 m^2	101 (14-131)	94 (55-100)	106 (78-125)
Weight, kg	79.4 (52–112)	77.2 (62–116)	72.4 (58–93)
BSA, m^2	1.9 (1.5–2.4)	1.9 (1.7–2.5)	1.8 (1.7–2.2)
24-hour urine samples			
Number of participants	14	4	10
Number of samples	31	4	10
DHA/24 h, mg	138.2 (63.5–291.5)	BLQ*	BLQ*
Urine creatinine, mmol/kg/24 h	0.15 (0.10-0.20)	0.18 (0.15-0.24)	0.15 (0.10-0.21)
First morning void urine samples			
Number of participants	19	4	10
Number of samples	44	4	10
DHA/Cr, mg/mmol	12.7 (3.8–37.2)	BLQ*	BLQ*

*p<0.005. Data are displayed as median (range). Abbreviations: APRT, adenine phosphoribosyltransferase; DHA/Cr, DHA-to-creatinine ratio; BSA: body surface area; eGFR: estimated glomerular filtration rate; BLQ, Below limit of quantification (<100 ng/mL).

Men had a median urinary DHA excretion of 233 (95-289) mg/24 h, which was significantly greater than in women, in whom the excretion was129 (64-291) mg/24 h (p=0.03; Figure 15A). No association was observed between the urinary DHA excretion and age (rs=− 0.332, p=.068), weight (rs=0.36, p=.203; Fig. 15B), BSA (rs=0.36, p=.208; Fig. 15C) or eGFR (rs=0.35, p=.227; Fig. 15D). Five untreated patients had multiple urine collections at different time points available (Figure 16), demonstrating marked intra-individual variability in DHA excretion.

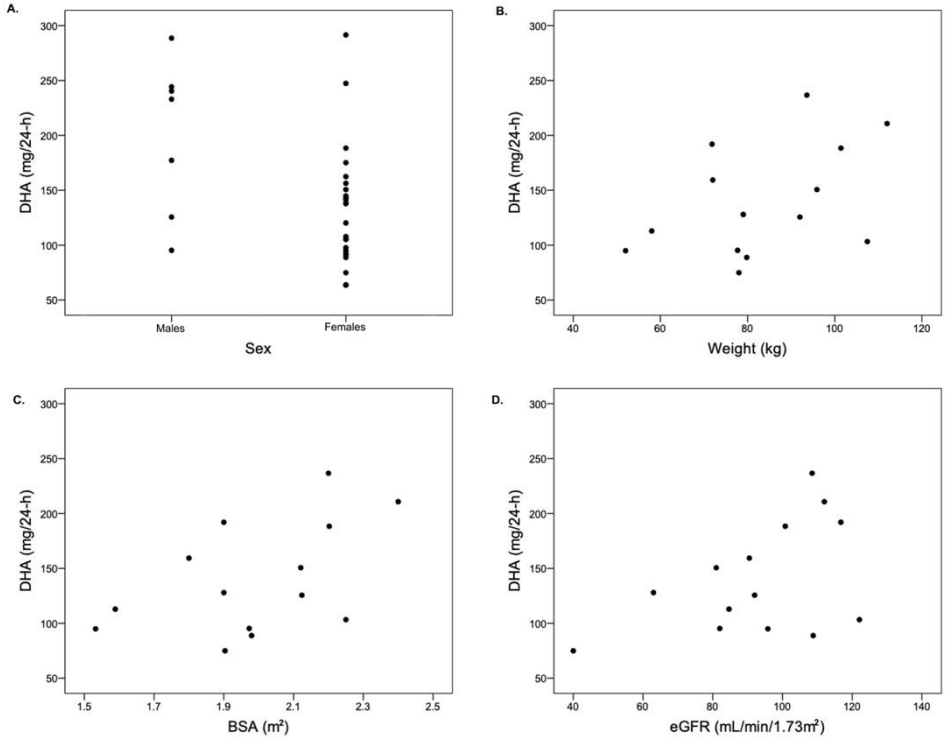


Figure 15. Correlation between 24-hour urine 2,8-dihydroxyadenine (DHA) (mg/24 h) and other factors in patients not treated with an XOR inhibitor. Weight (kg) (A), body surface area (BSA, m^2) (B), estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) (C), and sex (D).

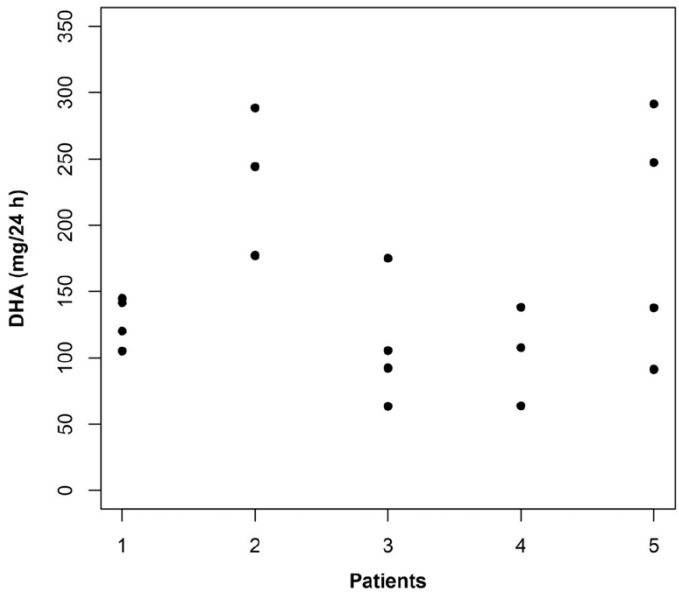


Figure 16. Twenty-four hour urinary 2,8-dihydroxyadenine (DHA) excretion in five untreated patients with adenine phosphoribosyltransferase (APRT) deficiency in urine samples obtained at different points in time.

The DHA-to-creatinine ratio was determined in 44 first morning void urine samples, available from 19 untreated patients, and 24 random urine samples from 4 untreated patients, yielding a median of 12.7 (3.8–37.2) mg/mmol and 15.5 (10.4–19.3) mg/mmol, respectively. A total of 121 first morning void urine specimens were available from 33 patients while they were receiving XOR inhibitor treatment. No DHA was detected in 44 of these samples (from 21 patients), whereas the DHA-to-creatinine ratio was 4.5 (0.4–24.8) mg/mmol in 77 samples (from 25 patients).

Thirty-six paired timed and first morning void urine samples from the same 24-h period from 11 patients on (n=17) and off (n=19) XOR inhibitor treatment were used to assess the correlation between the DHA-to-creatinine ratio in first- morning void urine specimens and 24-h urinary excretion (Figure 17). The correlation was highly significant when calculated before (r_s = 0.78, r_p = 0.71, p <0.001) and after removing 3 outliers (r_s = 0.84, r_p = 0.89, p <0.001).

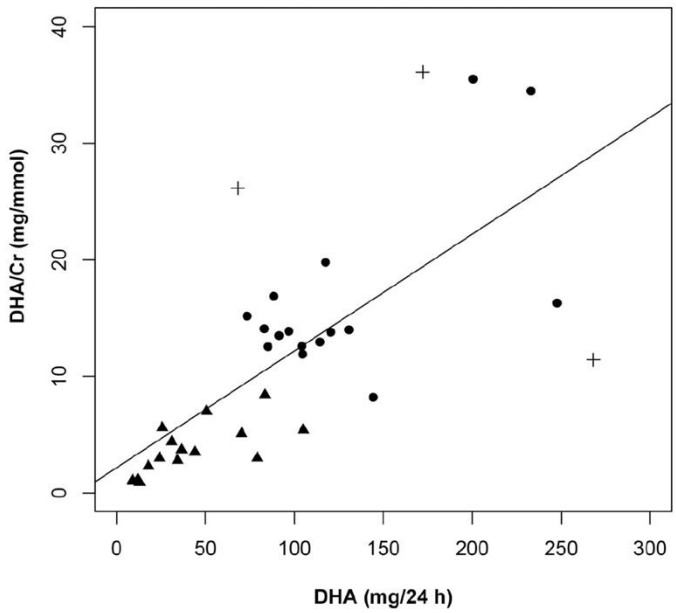


Figure 17. Scatterplot of 24-hour urinary 2,8-dihydroxyadenine excretion versus the DHA-to-creatinine (DHA/Cr) ratio in first morning void urine samples. Data represent treated (▲) and untreated (•) patients. *Outliers excluded from correlation analysis.

Microscopic DHA crystalluria strongly correlated with 24-h urinary DHA excretion (r_s=0.823, p <0.001) in 91 collections from patients off (n=31) and on (n=60) pharmacotherapy (Figure 18). In 16 of 40 urine samples where no crystals were observed, obtained from patients treated with 300-400 mg/day of allopurinol, the DHA excretion ranged from 19-88 mg/24 h. The remaining 24 specimens had DHA concentration below the detection limit and were from patients treated with allopurinol 300-600 mg/day or febuxostat in the daily dose of 80 mg.

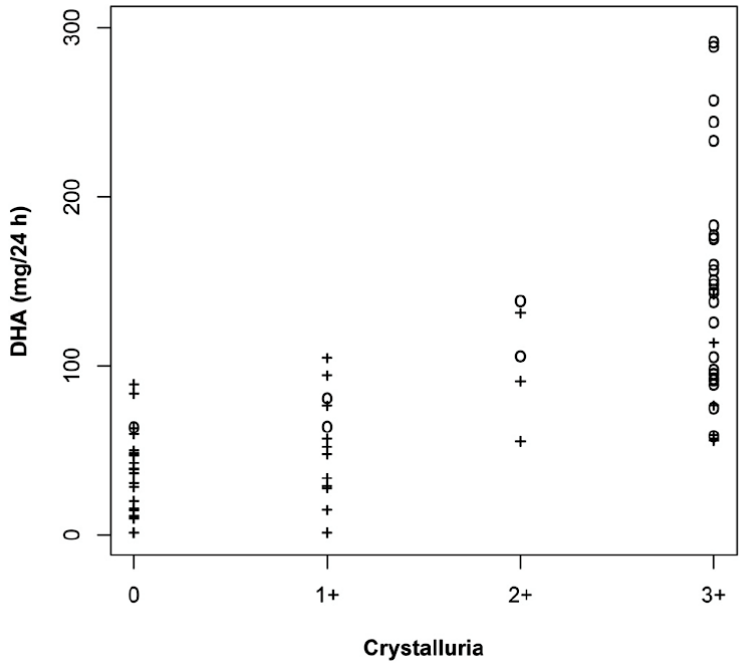


Figure 18. Urinary 2,8-dihydroxyadenine (DHA) excretion (mg/24 h) by microscopic DHA crystalluria in treated (+) and untreated patients (∘).

4.3.1 The effect of dietary purine intake on urinary 2,8-dihydroxyadenine excretion

Four patients completed this the study. The participants underwent measurment of urinary DHA excretion while consuming their ususal diet, followed by a purine-restricted diet, and lastly during a dietary purine challenge. The 24-h urinary DHA excretion is shown in Table 20. The median 24-h urinary DHA excretion was 128.6 (107.2-160.1) mg on a self-selected diet; 133.5 (102.6-159.6) mg after 3 days of restricted purine intake and 135.8 (114.4-188.4) mg following 3 days on a purine-enriched diet. Therefore, no relationship was detected between dietary purine intake and DHA excretion in these four patients.

Table 20. Effect of varied dietary purine intake on 24 hour urinary 2,8-dihydroxyadenine excretion

		Patient 1	Patient 2	Patient 3	Patient 4
Age (years)		34	35	52	62
eGFR (mL/min/1.73 m^2)		109	63	99	83
Self-selected diet	Urine DHA/24 h (mg)	114.6	128	222.1	127.1
	Urine creatinine (mmol/kg/day)	0.16	0.12	0.16	0.17
Purine-restricted diet	DHA/24 h (mg)	109.7	127.4	205.9	146.6
	Urine creatinine (mmol/kg/day)	0.16	0.11	0.16	0.14
Purine-enriched diet	DHA/24 h (mg)	152.9	122.9	261.4	110.8
	Urine creatinine (mmol/kg/day)	0.15	0.11	0.17	0.25

Abbreviations: eGFR, estimated glomerular filtration rate.

4.3.2 Comparison of the effect of allopurinol and febuxostat on urinary 2,8-dihydroxyadenine excretion (Paper VII)

The XOR inhibitors allopurinol and febuxostat act by decreasing synthesis of DHA, reducing crystalluria and kidney stone formation and ameliorating the crystal nephropathy in patients with APRT deficiency. Due to the inability to accurately quantify urinary DHA excretion, the dosing of these drugs has been empiric and guided by the extent of microscopic DHA crystalluria and clinical course. The novel UPLC-MS/MS method provided the possibility to measure urine DHA and thus examine the effect of treatment on DHA excretion. An open-label, single-center, crossover, non-randomized clinical trial was conducted to compare the effect of treatment with the XOR inhibitors allopurinol and febuxostat on urinary DHA excretion as described in Paper VII. Eight patients enrolled in the APRT Deficiency Registry completed the study, and the characteristics of these patients are presented in Table 21.

Table 21. Clinical characteristics of participants in the study of the effect of allopurinol and febuxostat on urinary 2,8-dihydroxyadenine excretion

Patient	Age (y)	Sex	eGFR (ml/min/1.73 m^2)	Age at diagnosis (y)	Major clinical feature
1	33	Female	103	0.5	Crystalluria
2	61	Male	90	23	Asymptomatic
3	28	Male	103	0.8	Kidney stones
4	38	Female	87	3.3	LUTS
5	52	Male	99	24.2	Kidney stones
6	62	Female	83	33.1	Kidney stones
7	67	Female	37	52.2	CKD
8	56	Male	80	32.9	Kidney stones

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; LUTS, lower urinary tract symptoms.

The 8 participants had a baseline 24-h urinary DHA excretion of 116 (75–289) mg when not receiving XOR inhibitor treatment. Following allopurinol therapy for 14 days, the DHA excretion decreased to 45 (13-112) mg. All 8 participants had DHA excretion below the lower limit of quantification (100 ng/mL) after 14 days of febuxostat therapy (P=0.036); the median urinary DHA excretion was 13 (10-13) mg/24 h in 4 participants, while it was below detectable limits (<20 ng/mL) in the other 4 (P=0.036) (Table 21). In first morning void samples, the baseline urine DHA-to-Cr ratio was 16.1 (8.2-34.5) mg/mmol and decreased to 5.3 (1.1-8.4) mg/mmol following treatment with allopurinol (P=0.036). On treatment with febuxostat, the urine DHA-to-Cr ratio was below the lower limit of quantification in all participants (P=0.036). The median DHA-to-Cr ratio was 1.0 (0.8-1.1) mg/mmol in 4 patients (Table 22).

Table 22. Twenty-four hour urinary 2,8-dihydroxyadenine excretion at baseline and at the conclusion of allopurinol and febuxostat therapy

Patient	Baseline	Allopurinol	Febuxostat
	DHA/24 h (mg)	DHA/24 h (mg)	DHA/24 h (mg)
1	89	32	13*
2	126	54	13*
3	233	112	10*
4	151	35	ND
5	289	90	13*
6	106	27	ND
7	75	13*	ND
8	95	75	ND

*Below limit of quantification (<100 ng/mL). ND, not detectable (limit of detection< 20 ng/mL).

The urinary adenine excretion was 32 (18-46) mg/24 h at baseline, compared with 96 (26-139) mg/24 h on allopurinol treatment and 112 (54-158) mg/24 h after two weeks of febuxostat therapy. The plasma uric acid concentration decreased from 257 (180-454) µmol/L at baseline to 179 (131-295) µmol/L following allopurinol therapy and was 137 (88-221) µmol/L after treatment with febuxostat. No adverse events were reported during the study period.

After resuming allopurinol therapy in the daily dose of 400 mg, the DHA excretion was re-evaluated in 6 of the 8 participants, a median of 6 (2-12) months following the study completion. The median 24-h urinary DHA excretion in these 6 patients was 33 (9-88) mg after completing the study compared to 45 (13-112) mg while taking allopurinol during the study. In the first morning void urine samples, the median urine DHA-to-Cr ratio was 3.0 (1.0-3.7) mg/mmol after study completion and 5.3 (1.1-8.4) mg/mmol during the study (Table 24).

Table 23. 2,8-Dihydroxyadenine-to-creatinine ratio (DHA/Cr) in first morning void urine samples at baseline and at the completion of allopurinol and febuxostat treatment periods.

Patient	Baseline	Allopurinol	Febuxostat
	DHA/Cr (mg/mmol)	DHA/Cr (mg/mmol)	DHA/Cr (mg/mmol)
1	12.5	4.4	0.9*
2	22.8	7.0	ND
3	34.5	5.4	1.1*
4	8.2	2.8	0.8*
5	17.4	8.4	1.1*
6	13.9	5.6	ND
7	15.2	1.1*	ND
8	16.9	5.1	ND

* Below limit of quantification (<100 ng/mL). ND, not detectable (limit of detection <20 ng/mL).

Table 24. Twenty-four hour (24-h) 2,8-dihydroxyadenine excretion and DHA-to-creatinine ratio (DHA/Cr) in first morning void urine samples on allopurinol therapy (400 mg/day) during and following the study.

Patient	Allopurinol[a]		Allopurinol[b]	
	DHA/24 h (mg)	DHA/Cr (mg/mmol)	DHA/24 h (mg)	DHA/Cr (mg/mmol)
2	54	7.0	47	3.5
4	35	2.8	27	3.0
5	90	8.4	88	3.0
6	27	5.6	18[c]	2.3
7	13[c]	1.1[c]	9[c]	1.0[c]
8	75	5.1	39[c]	3.7

ND, not detectable (limit of detection<20 ng/mL). a Allopurinol treatment period, b during allopurinol treatment following study completion, c Below limit of quantification (<100 ng/mL).

5 Discussion

The studies described in this thesis have provided important insights into the rare disorder, APRT deficiency. Firstly, the number of identified cases and mutations in the *APRT* gene exceeds what has previously been reported. The relatively high number of patients in Japan and Iceland is undoubtedly explained by founder mutations and the prevalence of APRT deficiency in other populations seems to be much lower, although the disorder may still be underdiagnosed. The diagnosis is commonly overlooked, mostly due to the misidentification of DHA crystals, both in urine and kidney biopsy specimens. The diagnosis of the disorder should always be confirmed by demonstrating absent APRT enzyme activity or pathogenic variants in both alleles of the *APRT* gene.

While APRT deficiency can lead to severe recurrent kidney stone disease and/or kidney failure, a variable clinical presentation was observed in the studies presented herein. Only one-third of the population had signs or symptoms of the disorder in childhood. The most commonly observed clinical manifestation was nephrolithiasis, but a significant proportion of patients had progressive CKD in the absence of nephrolithiasis, eventually requiring dialysis or kidney transplantation. Moreover, AKI was more common than has been previously reported. Patients who progressed to kidney failure almost invariably had not received XOR inhibitor treatment, whereas timely pharmacotherapy seemed to prevent decline in kidney function. This was particularly apparent in patients presenting in childhood who initiated drug treatment early, none of whom had CKD stages 3-5 at the end of the follow-up period. The importance of pharmacotherapy was also highlighted in the study of kidney transplant recipients where the absence of timely treatment with allopurinol or febuxostat resulted in poor allograft outcomes. Interestingly, the recurrence of DHA nephropathy in the allograft occurs very early in the post-transplant period in untreated or inadequately treated patients. Patients treated with an XOR inhibitor pre-transplant had more favourable allograft outcomes.

In the study of urinary DHA excretion using the UPLC-MS/MS assay, DHA was detected in all patients who were not receiving XOR inhibitor treatment whereas it was undetectable in the urine of heterozygotes or normal controls. These findings are consistent with 100% sensitivity and specificity of the

UPLC-MS/MS method for the diagnosis of APRT deficiency. A clinical trial comparing the XOR inhibitors allopurinol and febuxostat, showed that while both agents markedly suppressed the urinary DHA excretion, febuxostat was more effective in the doses tested. Urinary DHA excretion remained detectable despite conventional doses of allopurinol, suggesting that higher doses may be required for adequate control of 2,8-dihydroxyadeninuria. Finally, a modification of dietary purine intake did not affect urinary DHA excretion in patients with the disorder.

5.1 Prevalence

More than half of the 438 cases of APRT deficiency identified worldwide are from Japan, the majority of which are due to a founder mutation. Interestingly, very few cases have been reported from other Asian countries and heavily populated continents such as North-America and Africa. No cases have to our knowledge been diagnosed in South-America. This would suggest that pathogenic mutations have a distinct geographical distribution or that the disease may be underdiagnosed in some areas. In order to clarify the ambiguity regarding the prevalence of APRT deficiency, a search for pathogenic mutations in genomic databases worldwide was undertaken.

The recent introduction of high-throughput sequencing technologies has facilitated the creation of large databases of human genomic information, generating possibilities to estimate carrier frequencies using large population-based datasets. Twenty-seven of the 62 known pathogenic mutations were identified in large genomic population databases and were assessed for the individual and cumulative frequencies of the minor alleles. The 3 most commonly reported mutations, p.(Asp65Val), p.(Met136Thr) and c.400+2dupT, were observed in 58% of the reported cases with a molecular diagnosis. The high carrier rate of the missense mutation, p.(Asp65Val), of 1.2% in the Icelandic population clearly represents a founder effect as is the case with the missense mutation, p.(Met136Thr), in the Japanese population. Not surprisingly, analysis of the large Icelandic genomic data disclosed a number of additional undiagnosed homozygous individuals in the Icelandic population. Interestingly, 2 other pathogenic variants were identified among Icelanders in the deCODE genomic database. As would be expected, the two founder mutations in Iceland and Japan are very rare outside of their resepective countries. In particular, when assessing the frequency of the Icelandic founder mutation (present in a heterozygous state in 1 out of 42 Icelanders) in large datasets from their ancestral population in Scandinavia

and UK, we found that the mutation is approximatevly 100 times less common in these locations.

The c.400+2dupT mutation was observed in APRT deficiency cases from many countries in Europe, the US and Australia, as well as in most of the pubic databases used in the studies in Paper I. In a report from a French cohort, this mutation was detected in 54% of the patient population, all of whom originated from metropolitan France, as well as in one Italian family with an allele frequency of 39/98 in that cohort (M. L. Irène Ceballos-Picot, Lionel Mockel, Véronique Droin, Michel Daudon, Mohamad Zaidin, Jérôme Harambat and Guillaume Bollée, 2014). The same investigator group also detected an allele frequency of 2/204 (MAF 0.98%) for the mutation in newborn screening (Irène Ceballos-Picot & Bollée, 2014), suggesting a higher prevalence in certain areas. However, physicians practicing in countries other than Japan, France and Iceland are unlikely to encounter a single case of APRT deficiency during their career.

Using the MAF for the most commonly reported mutations to calculate the inferred prevalence, yielded as many as 50 cases in Iceland homozygous for p.(Asp65Val) and 200 cases in Japan homozygous for p.(Met136Thr), which is higher than the number of cases we identified in both populations. A previous study assessing the frequency of p.(Met136Thr) observed an allele frequency of 7/1910 among Japanese and 2/756 among Koreans, whereas it was absent in samples from Taiwanese individuals. In the same study, the geographical dristibution in Japan was determined to be rather uniform (Kamatani et al., 1996). In the European (non-Finnish) population, the cumulative MAF was 0.05%, indicating there may be roughly 160 cases. Interestingly, the cumulative MAF in the Irish population was quite high. No cases have been reported in Ireland to the best of our knowledge, which indicates that the disease may be underrecognized and underdiagnosed. In the UK, however, the allele frequency observed in the population databases was quite low. Even the cumulative count did not indicate that one might expect to find any cases in the UK population, when 30 have in fact been diagnosed. This might reflect selection bias in the genetic databases or the effect of immigrants as 10 of the 20 patients recently reported from the UK were of South Asian descent, some of whom were from consanguineous families (Balasubramaniam et al., 2016).

Two mutations, p.(Ala116Thr) (Chen & Schumacher, 2009) and p.(Arg89Gln) (Sahota AS, 2001), have been reported in individuals with decreased APRT enzyme function that is consistent with the heterozygous

carrier state of other pathogenic *APRT* mutations. Nevertheless, it remains unclear whether these two variants are truly pathogenic as no individuals homozygous for these mutations have been observed. Both variants had high allele frequencies in the databases analyzed, especially among the Asian populations. If proven to be disease-causing, there could be somewhere between 10,000-15,000 cases in South Asia based on the calculations, though only a few have been reported. However, it is possible that the mutations have variable expressivity in homozygotes, causing a milder phenotype or no phenotype at all.

Other variants with a high allele count in the gnomAD database, none of which have been determined in patients with APRT deficiency, were examined (Paper 1, Supplemental Table 5). Notably, the p.(Gln121Arg) variant, listed as probably damaging, was quite common among the African population, with an MAF 0.5% and 2 homozygous individuals among the study subjects. To date, only two cases of APRT deficiency have been reported on the continent of Africa, 1 in Senegal and 1 in Morocco. The discovery of this mutation or any uncharacterized novel mutation would require testing in a functional assay to demonstrate markedly decreased or absent enzyme activity.

In a study on primary hyperoxaluria, another rare monogenic cause of nephrolithiasis and CKD, estimation of allele frequency based on both known pathogenic mutations and predicted pathogenic alleles using publicly available whole-exome sequencing data indicated a significantly higher prevalence of the disorder than has previously been reported (Hopp et al., 2015)

The interpretation of previously unknown rare variants is associated with several important challenges, including the accuracy of variant calling, identification of pathogenic variants, and interpretation of low-penetrant variants. The American College of Medical Genetic and Genomics (ACMG) and the Association for Molecular Pathology (AMP) have issued joint guidelines to support a standardized approach to variant classification (Richards et al., 2015). Variants with much higher allele frequencies than would be expected should be carefully interpreted. Correct annotation of genetic variants is important in clinical practice as misinterpretation may lead to incorrect genetic diagnosis and harmful and/or ineffective treatment.

Publicly available genomic datasets are still scarce and the inclusion of certain ethnic groups is very limited or does not exist. Additional work is required in order to characterize the prevalence of APRT deficiency

worldwide. Access to genomic information from high-risk groups, namely patients with nephrolithiasis and/or unexplained CKD at a relatively young age, might become available in the future.

5.1.1 Misdiagnosis

The studies on pathogenic mutations suggest that the few cases reported worldwide may be related to a lack of recognition of the disorder. Only a few reports have examined the cause for misdiagnosis in patients with APRT deficiency. A number of patients described in Papers III, IV and V suffered a delay in diagnosis, including those in which APRT deficiency was only first diagnosed following kidney transplantation in the setting of severe allograft dysfunction. Similiar delays in diagnosis and treatment have been observed in other kidney transplant case series (Zaidan et al., 2014). Approximately one-third of the cohort of patients reported by the French group had impaired kidney function at the time of diagnosis when half of them had reached ESKD. This was considerably lower than reported in Paper III, as a delay in diagnosis occurred in 70% of the patients, ranging up to 50 years.

Misinterpretation of kidney biopsy findings contributed to the diagnostic delay observed in at least 7 cases described in Papers III and V. When the histological examination of renal tissue reveals a crystal-associated tubulointerstitial lesion, DHA nephropathy should always be considered (Arnadottir et al., 1997; Edvardsson et al., 2013; Samih H. Nasr et al., 2010). However, correct indentification of the crystals can be problematic as both DHA and oxalate crystals are positively birefringent and deposit in the interstitium and tubular lumina. Indeed, cases have been reported where DHA nephropathy has been confused with oxalate nephropathy in kidney allografts (Samih H. Nasr et al., 2010).

Erroneous interpretation of kidney stone analysis results appears to be a source of both false positive, and as false negative diagnosis of DHA stones and APRT deficiency. In Paper II, most of the patients appeared to be erroneously diagnosed with APRT deficiency as a result of incorrect interpretation of the infrared spectra. Although, the infrared spectrum of DHA is very specific and should allow for definitive identification of DHA when performed by trained laboratory personnel (Daudon & Jungers, 2004a), less well trained operators can erroneously attribute spectra of other materials such as uric acid and other stone components to DHA, as was clearly demonstrated in Paper II. It is important for clinicians caring for patients with rare kidney stone disorders to be familiar with the potential misidentification of DHA stones from infrared spectra.

The increased use of automated urinalysis threatens the ability to correctly identify DHA crystals on urine microscopy. This is highlighted in the cohort of patients from Iceland where a significant proportion of the cases were diagnosed with the disorder following identification of urinary DHA crystals, likely due to the competence and familiarity of the laboratory staff. Since APRT deficiency is a preventable cause of progressive CKD, strategies to increase awareness among clinicians and pathologists are important. The disorder should always be considered in the differential diagnosis of radiolucent kidney stones, unexplained CKD in children and young or middle-aged adults, and in cases of kidney allograft dysfunction of unclear etiology. While the diagnosis of APRT deficiency may be suggested by the identification of DHA crystals in urine or renal tissue samples, the diagnosis should be confirmed using APRT enzyme function analysis or determination of biallelic pathogenic mutations in the *APRT* gene. Due to increasing availability and markedly reduced cost, genetic testing is becoming the favoured diagnostic method for APRT deficiency in developed countries. Furthermore, the *APRT* gene should be included in high-throughput next-generation sequencing panels for rare types of CKD and kidney stone disease (Hill & Sayer, 2019)

5.2 Clinical presentation and disease course

5.2.1 Kidney stone disease

Kidney stones are a common feature of APRT deficiency, with just over half of the patients reported in Paper III already affected at the time of diagnosis. This is somewhat less than reported in a previous study from France where 90% of the 40 patients had experienced kidney stones at diagnosis (Bollee et al., 2010). In Paper IV, almost 60% of our pediatric APRT deficiency cases had kidney stones at the time of diagnosis, while 80% of the patients reported by the French investigators had a symptomatic stone event at presentation of the disorder. The higher proportion of patients with kidney stones as the presenting feature in the French cohort may reflect the strength of the stone analysis laboratory at the Necker Hospital in Paris. Failure to identify other less apparent manifestations of APRT deficiency in that study, such as diaper stains, lower urinary tract symptoms and asymptomatic crystalluria may also have played a role, as well as the warmer climate in France (Fakheri & Goldfarb, 2011).

It is striking, however, that recurrent stone events were observed more frequently in the cohort presented in Paper III, compared to the work of

others, despite the use of similar doses of allopurinol (Bollee et al., 2010; Harambat et al., 2012). The longer median observation period and the relative completeness and high quality of data obtained at scheduled annual follow-up visits for a large proportion of the cohort in Paper III, likely increased the likelihood of identifying stone events. XOR inhibitor treatment adherence may also have played a role.

5.2.2 Acute kidney injury

One-third of the cases reported in Paper III experienced episodes of AKI, which previously has only rarely been reported for patients with APRT deficiency. The abundance of longitudinal SCr values available for the patient cohort and long follow-up time likely allowed for the detection of AKI episodes, possibly explaining the relatively high frequency observed in our study population. In comparison, only 1 case of AKI was reported in the aforementioned French cohort (Bollee et al., 2010). While all of the patients recovered kidney function to a degree, these findings suggest that AKI may contribute to disease progression. Indeed, the patients who developed AKI more frequently experienced progressive CKD, in line with the well-known relationship between AKI and CKD in the general population (Coca, Singanamala, & Parikh, 2012)

In Paper IV, AKI caused by bilateral obstructive stone disease was the presenting feature of three pediatric patients, which has also been described by the French APRT Deficiency Research Group (Harambat et al., 2012). However, AKI was also more commonly observed in the APRT Deficiency Registry cohort during the follow-up period than previously described (Papers III and IV). The proportion of patients with AKI at diagnosis reported by the French investigators may have been underrecognized in light of the large number of their patients who experienced a marked improvement in kidney function during the first months of allopurinol therapy (Harambat et al., 2012).

5.2.3 Chronic kidney disease

In the cohort of patients reported in Paper III, 42% eventually progressed to CKD stages 3-5 which is similar to that observed in the French study (Bollee et al., 2010). The absence of kidney stones in many of the patients with CKD stages 3-5 is noteworthy as stone disease has generally been considered the characteristic feature of APRT deficiency. Interestingly, the prevalence of advanced CKD or kidney failure was much lower in the Icelandic patients compared with those from other countries. As the number of reported cases in those countries is very low, the data suggest a lack of awareness of APRT

deficiency among nephrologists and renal pathologists as a cause of crystal nephropathy and CKD. While crystal nephropathies, which are associated with inflammation and fibrosis, may lead to progressive kidney damage, the pathogenesis of crystal-induced injury in humans remains elusive. The best characterized mediator of crystal-induced inflammation is the intracellular NLRP3 inflammasome which has been shown to cause direct injury to tubular cells, tubulointerstitial inflammation and kidney failure in both oxalate and DHA nephropathy (Knauf et al., 2013; Ludwig-Portugall et al., 2016; Mulay, Evan, & Anders, 2014).

None of the pediatric patients reported in Paper IV, who were treated with an XOR inhibitor from an early age, progressed to CKD stage 3 or above. However, two-thirds of those who did not start pharmacotherapy until reaching adulthood, usually due to a severe diagnostic delay, had a much less favorable kidney outcome. The French group has reported similar findings, as none of their patients diagnosed in childhood had developed advanced CKD (Harambat et al., 2012). Although their study period and follow-up time was considerably shorter than in Paper IV, this finding underscores the importance of early initiation of treatment.

The high-quality longitudinal data, including the abundance of SCr values available in the APRT Deficiency Registry, allowed for a detailed analysis of the evolution of kidney function over time. The group of patients commencing treatment with allopurinol in childhood demonstrated an initial improvement in eGFR, followed by preservation of kidney function which was within normal limits at the end of the study period.

5.3 Urinary 2,8-dihydroxyadenine and adenine excretion

The novel work presented in Paper VI showed that all patients with APRT deficiency had high, albeit variable, urinary DHA excretion that was significantly greater in men than women. There was a strong correlation between DHA excretion in timed urine samples and the DHA-to-Cr ratio in first morning void urine specimens. Importantly, DHA was not detectable in urine samples from heterozygotes, healthy individuals and many patients on XOR inhibitor therapy.

A wide range of urinary DHA excretion was demonstrated in APRT deficiency patients who were not receiving XOR inhibitor treatment. As the reported intra- and inter-day variability in the accuracy of quantification associated with the UPLC-MS/MS urinary DHA assay is within ±15% (Thorsteinsdottir et al., 2016), it does not explain the large variability in

urinary DHA excretion observed in the patient cohort. A more likely explanation is variability in dietary or systemic adenine load and/or the rate at which adenine is converted to DHA by XOR. Moreover, multiple types of human intestinal bacterial taxa use APRT to metabolize adenine to AMP through the adenine and adenosine salvage pathways (Dush, Sikela, Khan, Tischfield, & Stambrook, 1985; Hershey & Taylor, 1986). Therefore, modulation of adenine metabolism by the gut microbiome, affecting the amount of adenine available for intestinal absorption, may have contributed to the variable urinary DHA excretion observed between study subjects. High-purine diet may also increase systemic adenine load although dietary studies in humans are almost non-existent (see below). Finally, significant inter-individual differences have recently been reported in plasma XOR activity in humans (Watanabe et al., 2018), which might be a plausible mechanism for the variability in renal DHA excretion observed.

No obvious explanation exists for the significantly higher urinary DHA excretion observed in men compared with women, a finding that has not been reported previously. The larger muscle mass in men may have contributed to some of the differences observed, although we did not find any significant variability in DHA excretion when correlated with BSA. The generally larger food portions consumed by men may also have contributed to the sex difference, although there are no data to support this notion. Interestingly, there are reports showing differences in the intestinal microbiome between the sexes (Dush et al., 1985), a fact that may have implications for the variation in DHA excretion observed between men and women. The small sample size may have amplified any errors associated with urine sampling, such as inaccuracy in the documentation of collection time, which may have falsely increased the DHA excretion. Additionally, also due to the small sample size, the difference in urinary DHA excretion between the sexes may simply have occurred by chance.

The intra-individual variability noted in the DHA excretion could also be affected by sampling or measurement errors, while temporal changes in systemic adenine load, and less likely XOR activity, cannot be excluded. Although no correlation between urinary DHA excretion and eGFR was found, it is important to note that only one untreated patient had severe renal dysfunction at the time of urine sampling.

Urinary DHA excretion rates have previously only been published in a few cases of APRT deficiency and the reported values in both random void (Kojima et al., 1987) and timed (Simmonds et al., 1977; Wessel, Lanvers,

Fruend, & Hempel, 2000a) urine specimens, have been at the very low end or markedly below the range observed in Paper VI. While this may be explained by dietary factors or other influences mentioned earlier, methodological differences in the DHA assays used likely play a role.

The close correlation between 24-h urinary DHA excretion and the DHA-to-Cr ratio in first morning void urine samples in Paper VI is an important finding. Based on these results, the determination of 24-h urinary DHA excretion can be replaced with DHA-to-Cr ratio in first morning void urine samples for monitoring the effect of pharmacotherapy and treatment adherence, both in the clinic and in clinical research studies. Assessment of the excretion of various urinary biomarkers has been challenging through the years, due in part to the tedious nature of 24-h urine sampling which has rendered sample collections subject to poor compliance and error. Hence, the measurement of solute-to-Cr ratio in random urine samples has been increasingly used for quantification of renal excretion. The best example is first morning void urine albumin-to-Cr ratio which has been shown to correlate well with 24-h urinary albumin excretion, the correlation coefficient being over 0.8 in most reports (Price, Newall, & Boyd, 2005), as in the present study.

A strong correlation was also observed between 24-h urinary DHA excretion and DHA crystalluria, currently widely used for therapeutic monitoring. While absence of crystalluria mostly correlated with unquantifiable amounts of DHA, it is noteworthy that potentially significant urinary DHA excretion was observed in several timed urine collections despite the absence of microscopic crystalluria. This finding suggests that DHA crystalluria is not reliable enough for assessment of the effectiveness of XOR inhibitor therapy. It is currently unknown whether persistent low levels of DHA excretion enhances the risk of kidney disease progression.

A more rapid method than currently available for screening and diagnosis of this rare and frequently underdiagnosed disease would be of great value. Since DHA was undetectable in the urine of heterozygotes and healthy control subjects and abundant in untreated patients, the novel UPLC-MS/MS assay appears to be highly accurate in identifying patients with APRT deficiency. Indeed, the findings presented in this thesis suggest that detection of any urine DHA using this assay can be considered diagnostic of APRT deficiency in individuals not treated with an XOR inhibitor.

5.4 Management

5.4.1 Pharmacotherapy and clinical outcomes

As previously reported (Bollee et al., 2010; Edvardsson et al., 2001), treatment with an XOR inhibitor, primarily allopurinol, clearly stabilized or improved kidney function in the studies presented in Papers III and IV whereas eGFR invariably declined in untreated cases. Indeed, allopurinol treatment preserved kidney function and prevented stone formation for decades in a subgroup of patients with minimal or no symptoms at diagnosis. The frequent development of advanced CKD and ESKD in patients not receiving XOR inhibitor treatment underscores the importance of timely diagnosis and pharmacotherapy.

The dose of the XOR inhibitors allopurinol and febuxostat required to adequately reduce urinary DHA excretion has not been defined. The adverse outcomes reported in Paper III suggest a need for higher doses of allopurinol than have generally been used, perhaps in the range of 600-800 mg daily. In the past, it has been recommended to avoid using allopurinol doses above 200-300 mg per day in patients with CKD based on a report of increased risk of allopurinol hypersensitivity syndrome (Hande, Noone, & Stone, 1984). Later studies have contradicted this notion (Thurston, Phillips, & Bourg, 2013) and the benefits of higher doses in transplant recipients were emphasized in Paper V. However, it is recommended to begin with a low dose as recent work has shown that higher allopurinol starting doses may increase the risk of allopurinol hypersensitivity syndrome (Stamp et al., 2012).

Adverse reactions that led to discontinuation of XOR inhibitor therapy, such as eye pain, photophobia and blurry vision (Paper III) have not been previously reported for these drugs (Faruque et al., 2013; Paisansinsup, Breitenstein, & Schousboe, 2013). Two cases of corneal dystrophy were reported in Belgium in 1986 and the authors concluded that corneal crystal deposition was a probable cause of the findings, although this was not histologically confirmed (Neetens et al., 1986). No other reports of ocular manifestations in patients with APRT deficiency exist and no involvement of other organ systems outside of the kidneys and urinary tract has been demonstrated in APRT deficiency. However, the frequent reports of eye symptoms included in this thesis warrant further investigation to determine the etiology of the eye complaints.

5.4.2 Pharmacotherapy and urinary 2,8-dihydroxyadenine excretion

The clinical trial reported in Paper VII is the only existing study designed to compare the effect of allopurinol and febuxostat on the urinary DHA excretion in patients with APRT deficiency. The more powerful reduction in urinary DHA excretion observed during febuxostat treatment may simply reflect more effective XOR inhibition compared with allopurinol in the doses prescribed. However, dose-equivalence studies comparing febuxostat and allopurinol for lowering urinary DHA excretion are not available. Consequently, it is possible that higher doses of allopurinol might have led to a more complete inhibition of DHA generation and should be studied in this patient population. Doses of allopurinol up to 800 mg/day have been approved for the treatment of gout by the US Food and Drug Administration and the European Medicines Agency but have not been tested in a systematic way. Studies in gout patients have shown that febuxostat in a dose of 80-120 mg daily resulted in a greater proportion of patients achieving serum uric acid levels <357 µmol/L, compared with allopurinol 300 mg daily (Becker, Schumacher, Wortmann, MacDonald, Eustace, et al., 2005; Singh, Akhras, & Shiozawa, 2015). Lack of adherence to allopurinol therapy only is a highly unlikely explanation for the differences in DHA excretion observed between the two study drugs.

It is uncertain whether these differences in pharmacologic properties of allopurinol and febuxostat contribute to the marked difference in efficacy of the two drugs in reducing urinary DHA excretion. As demonstrated in Paper III and other reported studies, allopurinol in doses less than 400 mg/day may not effectively reduce new stone formation or stabilize kidney function in APRT deficiency patients (Bollee et al., 2010; Edvardsson et al., 2001). Thus, a daily dose of at least 400 mg is recommended. However, urinary DHA excretion may remain detectable despite treatment with allopurinol 400 mg daily, unlike with febuxostat 80 mg, suggesting that higher doses of allopurinol may be required to adequately control dihydroxyadeninuria. As the level of reduction in urinary DHA excretion required for optimal prevention of adverse renal outcomes is unknown (Harambat et al., 2012), it remains unclear whether the difference in urinary DHA excretion observed between the two drugs in Paper VII is clinically significant. Therefore, the level of reduction in urinary DHA required for prevention of adverse outcomes warrants further study. Finally, febuxostat may also have some safety benefit as its association with hypersensitivity is less common than has been observed for allopurinol (Jordan & Gresser, 2018). Rarely, allopurinol-associated hypersensitivity may be manifested as the Stevens-Johnson syndrome.

5.4.3 Dietary purine intake and urinary 2,8-dihydroxyadenine excretion

No effect of variable dietary purine intake urinary DHA excretion was detected although both intra-individual and inter-day variability were noted. While current treatment recommendations include low-purine intake, very limited number of human studies exist to support this practice. One single-patient study observed less urinary DHA excretion on a low-purine diet compared to a high-purine intake (Simmonds et al., 1977). Nevertheless, it remains unclear whether the effect of a restricted purine intake would be clinically significant in patients already receiving XOR inhibitor treatment. Interestingly, murine DHA nephropathy is easily induced by feeding mice a 0.25% adenine diet, as the excessive adenine intake causes the conversion of adenine by XOR to DHA (Correa-Costa et al., 2011). In this model, DHA crystal deposition and precipitation leads to crystal-associated chronic tubulointerstitial nephropathy identical the human disease via inflammasome-mediated mechanisms (Ludwig-Portugall et al., 2016). Based on the above data, a human dietary study will need to be repeated in a larger population, preferably in a clinical research unit, to ascertain whether dietary restrictions may be beneficial for patients, especially those unable to tolerate XOR inhibitor treatment.

5.4.4 Kidney transplantation

The optimal treament for ESKD in patients with APRT deficiency is kidney transplantation, although historically the outcomes have been rather poor compared with other causes of CKD. Most previously reported cases of kidney transplantation in patients with APRT deficiency have demonstrated premature allograft loss or chronic allograft dysfunction in patients who were not on XOR inhibitor treatment at the time of transplantation (Benedetto et al., 2001; Brown, 1998; de Jong et al., 1996; Eller et al., 2004; Gagne et al., 1994; Kaartinen et al., 2014; Samih H. Nasr et al., 2010), usually due to missed diagnosis. Similar observations were also evident in the study described in Paper V, as all 10 allografts biopsies from untreated patients showed disease recurrence, which lead to the premature loss of 4 grafts in 2 patients. By contrast, patients who were receiving treatment with an XOR inhibitor at the time of kidney transplantation demonstrated better long-term allograft function and survival. Although the small study sample in Paper V precludes meaningful statistical analysis of allograft outcomes, the patients who initiated XOR inhibitor therapy in adequate doses pre-transplant and remained compliant with the treatment appeared to experience graft survival

similar to what has been reported for kidney transplantation in general.

Delayed graft function requiring dialysis in the first post-transplant week was reported in roughly a quarter of the cases described in Paper V, both in patients who initiated XOR inhibitor therapy before transplantation and those who did not, all of whom received deceased donor transplants. Interestingly, DHA crystal-induced injury seems to be much more aggressive in allografts than in native kidneys. As plasma DHA measurements are currently unavailable, it has not been determined if and how effectively dialysis clears DHA from plasma. Hence, it is conceivable that DHA may accumulate before transplantation in patients with kidney failure in the absence of XOR inhibitor therapy, flooding the allograft immediately following the transplant surgery, resulting in early graft dysfunction (de Jong et al., 1996; Eller et al., 2004; Kaartinen et al., 2014; Samih H. Nasr et al., 2010). Furthermore, ischemia-reperfusion injury at the time of transplantation may render the graft more susceptible to crystal deposition and injury.

5.5 Strengths and limitations

The studies described in this thesis have several strengths, all of which provide further insight into the understanding of this rare disorder. Paper I presents the most comprehensive information on known pathogenic mutations in patients with APRT deficiency to date, as well as important, novel insights into the prevalence of the disorder.

The largest cohort of patients with this rare disorder is described in Paper III, utilizing the complete data and long observation time available through the RKSC APRT Deficiency Registry to accurately assess the clinical course and long-term outcomes of the disease. Thus, the study is an important addition to the literature. Similarly, the population of patients presenting with APRT deficiency in childhood is one of the two largest reported, and as in Paper III, the multiple longitudinal SCr values available for most patients facilitated the accurate ascertainment of AKI and trends in kidney function over time.

The series on kidney transplant recipients with APRT deficiency is the first paper ever published (Paper V) in which the clinical course is examined in patients receiving XOR inhibitor therapy prior to transplantation, establishing the importance of timely and adequate XOR inhibitor treatment. In Paper VI, the great number of samples from patients with APRT deficiency and the novel UPLC-MS/MS assay allowed for unique and detailed analysis of urinary DHA excretion, as well as evaluation of the method for diagnostic purposes. The first clinical trial reported in patients with APRT deficiency (Paper VII)

provided important information on the absolute effect of allopurinol and febuxostat on urinary DHA excretion.

One of the major strengths of the studies presented in this thesis is the abundance of high-quality longitudinal data, including the wealth of SCr values available in the RKSC APRT Deficiency Registry. These studies report data that include more than 1000 patient-years of clinical information from 63 patients from the Registry and 4 referrals to The APRT Deficiency Research Program, making this the largest patient cohort with complete clinical data and the longest observation time reported. The APRT Biobank included a large number of samples available from this population, namely urine specimens which enabled the assessment of DHA excretion in individuals both on and off XOR inhibitor treatment.

The studies also have some limitations. The data collection in Paper I, although detailed, cannot completely capture all cases of APRT deficiency worldwide. A relatively small sample size as is expected for any rare disease, with even smaller subgroups for studies of some of the outcomes, is an additional shortcoming. The data, which had a significant retrospective and observational component, are also hampered by heterogeneity in terms of treatment and variable level of documentation, laboratory testing and duration of follow-up (Papers III-V). The large proportion of Icelandic patients, where the awareness of APRT deficiency appears high, may also limit the conclusions that can be drawn regarding variability in clinical presentation and outcomes between countries (Paper III). The small number of patients with advanced CKD limited the ability to assess the effect of reduced kidney function on urinary DHA excretion in Paper VI. Moreover, information on dietary intake was neither available for patients nor controls, except in the small study of the effect of dietary purine intake on urinary DHA excretion. Several factors were limiting in the execution of the clinical trial in Paper VII. The trial was not randomized and all patients first received allopurinol, then febuxostat. Notably, urine DHA was not quantified at the end of the second washout period. Finally, the study was not blinded to the participants, although the laboratory personnel did not have information on the drug treatment assignments.

6 Conclusions

APRT deficiency is a rare disorder with a variable clinical course that can lead to kidney failure in the absence of early institution of XOR inhibitor therapy. The prevalence in populations other than Iceland and Japan seems to be very low, although the disorder may still be underdiagnosed in some areas and ethnic groups. Missed diagnosis is still common, mostly due to misidentification of DHA crystals in urine and renal tissue, often in the setting of progressive CKD or renal failure. The diagnosis of the disorder should be confirmed by demonstrating absence of APRT enzyme activity or a pathogenic variant in both alleles of the *APRT* gene.

The clinical presentation of APRT deficiency may be more variable than previously suggested. Both AKI and progressive CKD are highlighted as major features of APRT deficiency, while nephrolithiasis still remains the most common presenting manifestation. Timely treatment with an XOR inhibitor appears to slow the progression of CKD, even in severely affected individuals. Indeed, allograft outcomes were markedly improved in patients who received XOR inhibitor treatment prior to or at the time of kidney transplantation. Large doses of allopurinol may be needed to adequately prevent recurrence of DHA nephropathy. As delay in diagnosis and initiation of appropriate pharmacotherapy is a major cause of premature graft loss in patients with APRT deficiency, it is important to increase the awareness of the disorder among physicians caring for patients with CKD. Indeed, the relatively frequent occurrence of advanced CKD and even kidney failure at diagnosis is concerning and suggests a lack of familiarity with this treatable condition. This underscores the importance of a kidney biopsy in younger patients with unexplained CKD.

High urinary DHA excretion was observed in all patients with APRT deficiency and the excretion was much greater in men than women. A strong correlation between 24-h urinary DHA excretion and DHA-to-Cr ratio in first morning void urine samples was demonstrated and suggests that timed collections may be replaced with first morning void samples to monitor the effect of pharmacotherapy and treatment adherence. No DHA was detected in urine samples from heterozygotes and healthy individuals, while high urinary excretion was observed in patients with APRT deficiency, suggesting that the novel UPLC-MS/MS urinary DHA assay, which uses an isotope-

labeled internal standard for absolute DHA quantification, can be added to the list of diagnostic tests for APRT deficiency. The clinical trial, comparing conventional doses of both allopurinol and febuxostat, revealed a marked decrease in urinary DHA excretion in patients with APRT deficiency for both drugs. Interestingly, febuxostat was significantly more efficacious than allopurinol in reducing DHA excretion in the prescribed doses. The clinical significance of the difference in urinary DHA excretion observed between these drugs warrants further study, as the effect on long-term renal outcomes may very well be improved with enhanced suppression of DHA excretion.

Future studies should focus on the potential of newborn screening in high-risk populations such as the Icelandic population and on incorporating the diagnostic possibilities of the UPLC-MS/MS urinary DHA assay. Not all patients have tolerated XOR inhibitor therapy, in most cases due to debilitating eye symptoms of unknown etiology. The nature of these symptoms must be determined. The effect of dietary restrictions should be studied in a controlled setting to determine whether such interventions may be beneficial for patients in the absence of pharmacotherapy. Although there is ample evidence for DHA crystal-induced inflammation in animal models of APRT deficiency, no human studies have yet demonstrated elevated plasma or urine inflammatory biomarker levels in individuals affected with the disorder. Such studies need to be carried out to search for potential treatment targets.

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Paper I

Allele Frequency of Variants Reported to Cause Adenine Phosphoribosyltransferase Deficiency

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Running title: Allele frequency of causative variants in APRT deficiency

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Abstract

Adenine phosphoribosyltransferase deficiency is a rare, autosomal recessive disorder of purine metabolism that causes nephrolithiasis and progressive chronic kidney disease. The small number of reported cases indicates an extremely low prevalence, although it has been suggested that missed diagnoses may play a role. We assessed the prevalence of APRT deficiency based on the frequency of causally-related *APRT* sequence variants in a diverse set of large genomic databases.

A thorough search was carried out for all *APRT* variants that have been confirmed as pathogenic under recessive mode of inheritance, and the frequency of the identified variants examined in six population-based genomic databases: the deCODE genetics database, the UK Biobank, the 100,000 Genomes Project, the Genome Aggregation Database, the Human Genetic Variation Database and the Korean Variant Archive. The estimated frequency of homozygous genotypes was calculated using the Hardy-Weinberg equilibrium.

A total of 62 identified pathogenic *APRT* variants were identified, including five novel variants. Most common where the missense variants, c.194A>T (p.(Asp65Val)) in Iceland and c.407T>C (p.(Met136Thr)) in Japan, as well as the splice-site variant c.400+2dupT in the European population. Twenty-nine variants were detected in at least one of the six genomic databases. The by far highest cumulative minor allele frequency (cMAF) of pathogenic variants outside of Iceland and Japan was observed in the Irish population (0.2%), though no APRT deficiency cases have been reported in Ireland.

The large number of cases in Japan and Iceland is consistent with a founder effect in these populations. There is no evidence of widespread underdiagnosis based on the current analysis.

Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600; 614723) is a rare disorder of purine metabolism that is inherited in an autosomal recessive manner. In the absence of functional APRT activity, adenine is oxidized to 2,8-dihydroxyadenine (DHA) by xanthine oxidoreducatase (XOR; xanthine dehydrogenase/oxidase). Poor solubility of DHA in the urine leads to stone formation and crystal nephropathy (1, 2). Radiolucent kidney stones are the most common manifestation of the disorder, followed by chronic kidney disease (CKD). Approximately 15-20% of patients have end-stage kidney failure (ESKD) at the time of diagnosis and in a number of cases the disorder is first recognized following kidney transplantation in the setting of severe allograft dysfunction due to disease recurrence. Treatment with the XOR inhibitors allopurinol and febuxostat effectively reduces DHA synthesis and excretion, alleviating stone burden and kidney injury (3).

The human *APRT* gene, located on chromosome 16q24, is 2466 base-pairs long and contains five exons encoding a 180 amino acid protein (NP_000476.1) (4). The Human Gene Mutation Database (HGMD) lists 51 reported disease-causing variants to date. More than 300 patients have been reported worldwide, the majority of cases coming from Japan, France and Iceland (3). The most commonly reported variants are a missense variant in exon 3, c.407T>C (p.(Met136Thr)), in patients from Japan (5), a T-insertion at the splice donor site of intron 4, c.400+2dupT, among Europeans (6, 7) and a missense variant in exon 3, c.194A>T (p.(Asp65Val)), which accounts for all known cases of APRT deficiency in Iceland (6, 8). While individuals homozygous for disease-causing variants have been shown to have completely abolished enzyme activity, heterozygous carriers have partial function but do not appear to have any biochemical or clinical abnormalities (9).

Although the small number of reported cases worldwide indicates that APRT deficiency is an extremely rare condition, the high number of patients identified in Japan, France and Iceland

has raised concerns that the prevalence may be underestimated in many populations. Furthermore, the commonly reported occurrence of delayed or missed diagnosis suggests that lack of awareness among clinicians might contribute to the low number of reported cases (3, 9, 10). However, a founder effect is a likely explanation for the high frequency of a single pathogenic variant and disease prevalence in the Icelandic (6, 11) and the Japanese populations (5, 12).

As whole-genome or whole-exome sequencing has become more widely available, studies on the molecular basis of rare diseases using open-access population-based data have provided further insight into their pathogenicity and allele frequency (13-15). The aim of this study was to search for all *APRT* variants confirmed as disease-causing under recessive mode of inheritance that have been reported in the literature and registered in online disease databases to date, and assess the frequency of these variants in a set of large population based genomic databases containing information on individuals of diverse geographic locations and ethnicity.

Methods

Ethical considerations

The study was approved by the National Bioethics Committee of Iceland (NBC 09-072) and the Icelandic Data Protection Authority.

Search for known pathogenic APRT variants

In order to identify all sequence variants documented to cause APRT deficiency, we performed a search strategy using three major sources:

1) Medical literature and databases. We performed a web-based search and assessement of variants in reported cases of APRT deficiency using PubMed, the Human Gene Mutation Database (HGMD) (16), OMIM (17) and ClinVar (18) through November 2019.
2) Expanded web sources. To identify *APRT* variants, we also assessed the full text of published articles, conference abstracts and book chapters identified using PubMed® (https://pubmed.ncbi.nlm.nih.gov/) and Google® (http://www.google.com), with the terms „APRT deficiency", "adenine phosphoribosyltransferase deficiency", "2,8-dihydroxyadenine", "2,8-dihydroxyadeninuria" and "2,8-DHA".
3) APRT Deficiency Registry of the Rare Kidney Stone Consortium (RKSC, http://www.rarekidneystones.org). Information on pathogenic variants was obtained from the APRT Deficiency Registry and through personal communication to the research group. The RKSC was established in 2009 to study hereditary kidney stone disorders and has since maintained a private database with clinical information on participating individuals. As of September 2019, 63 patients from eight countries were enrolled in the Registry, 56 of whom had undergone genetic testing with the identification of biallelic disease-causing *APRT* variants.

The variants are reported using nomenclature recommended by the Human Genome Variation Society, including the reference sequence numbers (NM_000485.2 and NG_008013.1) and transcript number (ENST00000426324.6).

As consequence at the RNA level has not been reported for the vast majority of the variants, a prediction analysis of the variant effect at the splice junction on the splicing was carried out using consensus sequence frequencies, Maximum Entropy score and the varSEAK online splice prediction tool (https://varseak.bio/).

Search for allelic frequency of known pathogenic APRT variants in genomic databases

We looked for the identified pathogenic variants, as described above, in multiple public whole-genome and exome databases. The individual and cumulative frequency of these disease-causing variants was specifically reviewed, as such information can be used to estimate the expected prevalence of the disorder.

We used six databases in all, containing information on sequence variation for over 300,000 individuals from various geographic locations and ethnic groups:

1) The database at deCODE genetics (19) comprises whole-genome sequencing data from 53,964 Icelandic, 3153 Swedish, 8831 Danish, 2920 Norwegian and 1365 Irish subjects enrolled in various studies.
2) The UK Biobank Project (20), a large prospective cohort study with exome sequencing of approximately 50,000 individuals (by 2019).
3) The 100,000 Genomes Project (21) contains data on whole genome sequencing of 63,737 patients with rare diseases from across the UK.
4) The Genome Aggregation Database (gnomAD) (22) browser (version 2.1.1) includes whole-genome and exome sequencing data from 141,456 unrelated individuals sequenced as a part of various disease-specific and population genetic studies of diverse

countries and ethnicities. The database includes whole-exome sequencing from 8128 African/African-American subjects, 17,296 Latinos, 5040 Ashkenazi Jews, 9197 East Asian subjects, 10,824 Finnish and 56,885 Non-Finnish Europeans, 15,308 South Asian subjects and 3070 individuals classified as „Other".

5) The Human Genetic Variation Database of Japan (23, 24) comprises exome sequencing of 1208 individuals and genotyping information for common variations from 3248.
6) The Korean Variant Archive (KOVA) (25) includes exome sequencing on 1055 healthy Korean individuals. The minor allele frequency (MAF) of each causally-related sequence variant among different ancestries was exctracted.

Determination of cumulative allelic frequency of known pathogenic APRT variants and estimation of homozygous genotype frequency

To determine the cumulative minor allele frequency (cMAF) of any of the reported *APRT* variants, we used the sum of the allele frequency of individual variants in databases of genome and exome sequences available for a given ethnic or geographic group. We note that each of the individual variants are rare and none are reported to be present on the same haplotype. As APRT deficiency is an autosomal recessive disease, each affected individual is expected to carry two copies of a disease-causing variant, the same (homozygous) or different variants (compound heterozygous). The Hardy-Weinberg equilibrium principle was used to calculate the expected genotype frequencies; for heterozygous (2pq) and homozygous (p^2) genotypes using the minor and total allele counts, where p is equal to the cMAF of causally-related variants and q is (1-p).

Results

Pathogenic APRT variants

Using a comprehensive search strategy, 62 pathogenic variants in the *APRT* gene were identified in homozygous or compound heterozygous patients with APRT deficiency (Supplemental Table 1). All 62 variants were detected in patients with clinical findings characteristic of APRT deficiency and/or abolished APRT enzyme function (94% of variants). In total, 438 cases of APRT deficiency from 33 countries have been identified worldwide, of which 359 (81.9%) have received a molecular diagnosis, to our knowledge. Thirty-nine variants were detected in only one affected individual each. The remaining 23 variants were observed in two or more cases, including 9 which were observed in at least five individuals. There were 28 missense variants and the remaining 35 correspond to nonsense, insertion or deletion (indel), frameshift and start loss and splice variants. Of 9 presumed splice variants, 6 were predicted to have a splicing effect and 1 a likely splicing effect (Supplemental Table 2).

We also noted two sequence variants that we do not classify as pathogenic, each of which were reported in a single heterozygous individual with partial APRT enzyme deficiency; a missense variant, c.266G>A (p.(Arg89Gln)), from Australia and the c.346G>A (p.(Ala116Thr)) missense variant from China. These variants were quite common in the databases searched; in gnomAD, the p.(Ala116Thr) variant had an allele frequency of 0.23% (46/19,942) in the East Asian population and p.(Arg89Gln) an allele frequency of 0.41% (125/30,584) in the South Asian population. However, neither of the two variants have been found in confirmed cases of APRT deficiency and, thus, were not included in our set of 62 causally-related variants.

Of the 62 pathogenic *APRT* variants, 57 had already been reported in the literature. The missense variants p.(Met136Thr) and p.(Asp65Val) and the splice-site variant c.400+2dupT

were most commonly reported. Additionally, five novel pathogenic variants were discovered through our APRT Deficiency Research Program. A C-to-G substitution in intron 1 (c.81-3C>G) was identified in homozygous state in two affected siblings in the US and one patient in Italy, and a C-to-T substitution in exon 1 (c.58C>T; p.(Pro20Ser)) was found in a homozygous patient in the UK. Two compound heterozygous patients from the US had a sequence variant that had not been previously described in addition to one already reported variant; one of these two patients had a frameshift variant, c.23dupT (p.(Val9Glyfs*2)), while the other had a missense variant in exon 3, c.264G>T (p.(Lys88Asn)). The fifth novel variant was identified in a patient from India who was referred to our program but had already been found to be a compound heterozygote during diagnostic testing at his home institution, carrying a missense variant in exon 3, c.227C>T (p.(Ala76Val)) in addition to one reported variant.

The three most common pathogenic APRT variants

All identified cases harboring the three most common variants causing APRT deficiency in a homozygous and compound heterozygous state are presented in Table 1. For these three variants, we summarized the counts of reported cases and the frequencies of these variants in different ethnic groups and geographic locations represented in the large genomic population databases. These three variants were observed in 510 of the 718 causally-related alleles (71.0%) among APRT deficiency cases.

The missense variant, c.407T>C (p.(Met136Thr))

The T-to-C missense variant at codon 136 in exon 5, p.(Met136Thr) has been described in 171 patients of Japanese descent, all but one were previously published in the literature. The additional case was identified in the APRT Deficiency Registry of the RKSC in a Japanese patient living in the US. This pathogenic variant was also found in the Human Genetic

Variation Database in Japanese cohorts from Tohoku University (MAF 1.3%, Alt/Ref 1/76) and the University of Tokyo (MAF 0.3%, Alt/Ref 2/666). This geographic distribution is consistent with previous reports (12). In the Korean Variant Archive (KOVA) the MAF of this variant was 0.05% (1/1898). The variant was not detected in other genomic databases. We note that the number of Japanese in the gnomAD database is very low, or only 150.

The missense variant, c.194A>T (p.(Asp65Val))

Fourty-one cases of APRT deficiency carrying the p.(Asp65Val) variant have been reported. Of these, 37 are from Iceland, all of whom are homozygous. One patient from Spain was homozygous for the same variant and three other patients were compound heterozygotes; one from France, one from the UK and one from Australia. On whole-genome sequencing of 53,964 Icelanders in the deCODE database, we observed this *APRT* variant in 1299 alleles. The minor allele frequency (MAF) was 1.2% (1299/107,928). Icelanders have ancestries originating from Scandinavia and the British Islands. Out of 14,904 whole-genome sequenced Scandinavian individuals at deCODE, only two heterozygotes were identified carrying the p.(Asp65Val) variant, and one single heterozygote was identified in the UK (Supplemental Table 3).

The splice-site variant, c.400+2dupT

A T insertion at the splice donor site in intron 4 (c.400+2dupT) was found in a total of 41 cases, all of European descent. This variant has been identified in 13 homozygotes and 23 compound heterozygotes from Europe, including France (n=29), Germany, Austria, Belgium, Italy and Poland. The variant has also been found in patients in the US (n=6) and Australia (n=1). The variant was consistently present in European populations in the genomic databases (Supplemental Tables 3-5). The highest frequency were observed among Irish (MAF 0.18%; 5/2730) and Southern European (MAF 0.043%; 5/11,592) individuals.

Allelic frequency of the 62 pathogenic APRT variants in population databases

We searched for the presence and assessed the frequency of the 62 pathogenic *APRT* variants within the publicly available databases, by ethnicity and geographic location. Out of the 62 variants, we observed 29 in the population databases.

The three most common variants in terms of case numbers are described above. Two of these variants, p.(Asp65Val) and p.(Met136Thr), are clearly indicative of a founder effect in Iceland and Japan, respectively, with very low frequency in the public databases. The third variant, c.400+2dupT, shows a wide distribution among cases from multiple European countries and the US, and is the disease-causing variant with the largest occurence in public databases. Besides the three aforementioned variants, the most commonly reported were ~~the~~ c.294G>A (p.(Trp98*)) in 24 Japanese cases and the c.521_523del (p.(Phe174del)) was observed in 4 cases from European countries and 3 in the US.

Iceland is the country where the highest fraction of the population has been sequenced (around 1 in 6 Icelanders). In addition to the p.(Asp65Val) variant discussed above, we discovered five individuals heterozygous for one of the two other known pathogenic *APRT* variants in the deCODE database out of 53,964 Icelanders (Table 2). Of these, the c.1A>C (p.(Met1?)) nonsense variant, previously reported in patients from Hungary and France, was observed in four Icelanders with a MAF of 0.004% (4/107,928).

Estimation of homozygous APRT genotype frequency

Based on the Hardy-Weinberg principle, the cMAF of p.(Asp65Val) in the Icelandic population was 1.2 %, with a predicted frequency of homozygous individuals of 1 in 6840 (Table 3). In the Scandinavian countries (Denmark, Sweden and Norway), the cumulative allele frequency of any of the pathogenic variants in 14,904 individuals is similar among the three nations, or around 0.05%. This would correspond to an expected number of

homozygotes of about 1 in 4 million individuals, or five cases for a total population of 20 million people. We note that no cases of APRT deficiency have been reported in these three countries. In Ireland, the cMAF of the pathogenic variants observed was 0.26% (7/2730 alleles), represented by five copies of c.400+2dupT and by a single copy of two other disease-causing variants. The expected frequency of Irish individuals homozygous for a pathogenic variant is 1 in 152,100, corresponding to roughly 30 homozygous subjects given the size of the Irish nation.

The overall frequency of the reported pathogenic variants in gnomAD was similar among the European (Non-Finnish) population, with a calculated allelic frequency of 0.05% (63/128,838). Frequencies of causally-related variants in Latinos and East Asians was similar to that observed in gnomAD for Europeans, whereas other groups had lower frequencies. Thus, in the East Asian population (1.7 billion), there could be as many as 300 cases and up to 170 cases in the Latin American population (642 million).

Discussion

A comprehensive search for APRT deficiency cases, using multiple web-based resources and the APRT Deficiency Registry of the RKSC, identified 62 pathogenic *APRT* variants in a total of 359 patients undergoing molecular diagnosis. Five novel variants were discovered through the APRT Deficiency Registry. Three variants are by far most common among the APRT deficiency cases, namely the missense variants p.(Asp65Val), p.(Met136Thr) and the splice variant c.400+2dupT, accounting for 71% of the disease-associated alleles.
Twenty-nine of the 62 pathogenic variants were detected in large genomic population databases and were assessed for the individual and cumulative frequencies. In particular, when assessing the frequency of the Icelandic founder variant (1 out of 42 Icelanders heterozygous) in large datasets from their ancestral populations in Scandinavia and UK, we found that the variant is approximately 100 times less common in these geographic locations. Thus, the high carrier rate of the missense variants, p.(Asp65Val), in the Icelandic population of 1.2% clearly represents a founder effect. Not surprisingly, analysis of the large Icelandic genomic data disclosed a number of additional undiagnosed homozygous individuals.
The c.400+2dupT variant was observed in cases from many countries in Europe, the US and Australia, as well as in most of the public databases used in the current study. In a report of a French APRT deficiency cohort, this variant was detected in 54% of the patient population, all of whom originated from metropolitan France except for one Italian family (26). The allele frequency of the c.400+2dupT variant was 40% in the case series. The same group of investigators also detected an allele frequency of this variant of 0.98% (2/204) by newborn screening (26).

A previous study assessing the frequency of the p.(Meth136Thr) variant observed an allele frequency of 0.37% (7/1910) among Japanese, 0.26% (2/756) among Koreans while it was

absent in samples from Taiwanese individuals. In the same study, the geographical distribution in Japan was determined to be rather uniform (12).

When calculating the cMAF of reported causally-related variants to assess the expected genotypic frequency, we found that there may be as many as 50 individuals in Iceland homozygous for p.(Asp65Val) and 200 in Japan homozygous for p.(Met136Thr). As expected, the two described founder variants in Iceland and Japan are very rare outside of their resepective countries. In the European (non-Finnish) population, the cMAF of any pathogenic variants was 0.05%. The cMAF in the Irish population was higher and is consistent with approximately 30 homozygous individuals. Interestingly, no cases have been diagnosed in Ireland to the best of our knowledge, indicating that the disease may be underreported or underdiagnosed in that country.

Two *APRT* missense variants, c.266G>A (p.(Arg89Gln)) (9) and c. 346G>A (p.(Ala116Thr)) (27), have been reported in individuals with decreased enzyme function that is comparable to the heterozygous carrier state of other pathogenic *APRT* variants. It is unclear if and to what extent individuals homozygous for these variants will develop clinical manifestations of APRT deficiency. Multiple copies of both variants were present in the genomic databases that were analyzed and the allele frequencies were quite high in both the East Asian and South Asian populations. If proven to be disease-causing, there could be roughly 20,000 homozygotes in South Asia and 5000 in East Asia, assuming the Hardy-Weinberg equilibrium. However, as no cases of APRT deficiency caused by these two variants have been reported, a classical phenotype appears unlikely as the disease would then be seriously underdiagnosed. Hence, homozygozity for these variants would either be expected to cause a very mild phenotype or no clinical disease expression at all.

Of the 62 pathogenic variants, 33 were not observed in any of the public genomic databases. Most were found in one or two cases except for c.188G>A (p.(Gly63Asp)) which was found

in five patients, most of whom were of Lebanese decent. The reason for this may be the high proportion of consanguineous marriages in Lebanon, previously reported to be approximately 35%, and/or that Middle East populations might be underrepresented in the genomic databases used in this study (28).

We also examined sequence variants that have not been detected in patients with APRT deficiency in the largest population database with the highest allele count, gnomAD (Supplemental Table 6). Notably, the c.362A>G (p.(Gln121Arg)) variant was quite common among the African population in gnomAD, with a MAF of 0.5% and two homozygous individuals. The confirmation of this variant or any other uncharacterized novel variants as disease-causing in patients with APRT deficiency would require testing with a functional assay in order to assess pathogenicity.

Recent introduction of high-throughput sequencing technologies and advancements in bioinformatics for variant assessment have facilitated studies of the epidemiology of monogenic diseases. The creation of large public databases of human genomic information have generated possibilities to estimate genotypic frequencies based on population-based data. Furthermore, exome sequencing has led to the discovery of many novel causal genes in rare diseases (29-32). Moreover, previously unknown pathogenic variants have been unraveled and undiagnosed cases identified. Finally, the allele frequency and rare causally-related variants can be assessed in multiple populations like in the current study.

The prevalence of a monogenic disease depends on the frequency of all the pathogenic variants in a population. The allele frequency of each variant can vary in different populations and minor alleles with much higher frequencies than would be expected for a rare disorder should be carefully interpreted. Correct annotation of genetic variants is important in clinical practice as misinterpretation may lead to incorrect diagnoses or harmful and ineffective treatments. A limited number of variants will generally be encountered within

a given population with close to random mating. By contrast, the occurrence of the disease in inbred populations depends on the mating choice and each causally-related variant can be extremely rare, sometimes explaining only a single family.

The present study has several limitations that are noteworthy. The data collected relies largely on published reports and is therefore probably incomplete. Although the reports were carefully examined, there is always a possibility that the cases were miscounted or missed. Homozygous status in consanguinous unions, that are frequent in certain countries, is likely to result in underestimation of the disease prevalence in a population under study. It should be noted that publicly available genomic datasets are still scarce and the inclusion of certain ethnic groups is very limited or nonexistent. Additional work is required in order to characterize the prevalence of APRT deficiency around the globe. Although the gnomAD database contains data from various genomic projects in the US, the American population is not identifiable in the database so that allele frequencies cannot be assessed. Studies of certain ethnic groups would also be of particular interest, for example the South Asian and African populations. In the future, genomic information from high-risk groups, namely patients with nephrolithiasis and/or unexplained CKD presenting at a relatively young age, might become publicly available. Finally, the interpretation of previously unknown sequence variants remains challenging and APRT enzyme activity testing among individuals homozygous for such variants would be of great interest.

In conclusion, we identified 62 confirmed pathogenic *APRT* variants, five of which have not been reported previously. Three of the variants account for the majority of cases, two of which are mostly confined to a single country, Japan and Iceland. The high carrier rate of the missense variant, p.(Asp65Val), in the Icelandic population of 1.2% clearly represents a founder effect. While there is no clear indication of extensive underdiagnosis, this may be the case in certain areas. Furthermore, data are lacking for many countries. Hence, future work

should assess the situation in countries and populations where the number of expected cases is higher than currently reported. An updated list of all variants causally related to APRT deficiency will be made publicly available and maintained on the Rare Kidney Stone Consortium website.

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Conflict of Interest Statement

None of the authors declare financial or other conflicting interests.

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Tables

Table 1. The frequency of the three most common pathogenic *APRT* variants among all identified cases worldwide and in open-access genomic databases.

	Identified cases		Genomic databases		
	Homozygotes	**Compound heterozygotes**	**Allele count**	**Allele number**	**Allele frequency (%)**
c.407T>C (p.(Met136Thr))					
Japan	126	44			
Japan (HGVD)			3	2326	0.13
USA	1				
KOVA (Korea)			1	1898	0.05
gnomAD					
East Asian			1	18360	0.005447
African			0	16760	0.000
Ashkenazi Jewish			0	9956	0.000
European (Finnish)			0	20398	0.000
European (Non-Finnish)			0	112090	0.000
Latino			0	34518	0.000
Other			0	6066	0.000
South Asian			0	30590	0.000
c.194A>T (p.(Asp65Val))					
Iceland	37				
Iceland (deCODE)			1299	107928	1.2
Sweden (deCODE)			1	6306	0.016
Denmark (deCODE)			1	17662	0.006
UK		1			
UK (100,000 Genomes Project)			1	127474	0.0008
Spain	1				
France		1			
Australia		1			
gnomAD					
European (Non-Finnish)			1	124598	0.0008026
Estonian			1	4768	0.02097
African			0	24072	0.000
Ashkenazi Jewish			0	10156	0.000
East Asian			0	19736	0.000
European (Finnish)			0	24188	0.000
Latino			0	35102	0.000
Other			0	7032	0.000
South Asian			0	30262	0.000
c.400+2dupT					
Europe	13	23			
UK (Biobank)			22	1000000	0.022
UK (100,000 Genomes Project)			4	127474	0.003
Ireland (deCODE)			5	2730	0.18315018
Denmark (deCODE)			4	17662	0.02264749
USA	6				
Australia		1			
Unknown	1				
gnomAD					
European (Non-Finnish)			27	128838	0.02096
Southern European			5	11592	0.04313
Other non-Finnish European			10	32948	0.03035
North-Western European			11	50708	0.02169

Swedish	1	26100	0.003831
African	1	24940	0.004010
South Asian	1	30614	0.003266
Latino	1	35430	0.002822
Ashkenazi Jewish	0	10350	0.000
East Asian	0	19942	0.000
European (Finnish)	0	24890	0.000
Other	0	7280	0.000

Abbreviations: gnomAD, Genome Aggregation Database; HGVD, Human Genome Variation Database; KOVA, Korean Variation Archive.

Table 2. Pathogenic *APRT* variants detected in the deCODE genetics database

No.	Reference sequence	Position (Build 38)	Location	Base change	Amino acid change	Mutation	Alt/Ref	MAF (%)	Country of origin
1	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1299/107928	1.2	Iceland
2	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	4/107928	0.003706	Iceland
3	NM_000485.2	88810141	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	1/107928	0.000927	Iceland
4	NM_000485.2	88810141	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	5/17662	0.028309	Denmark
5	NG_008013.1	88810067	Intron 4	c.400+2dupT	-	Indel	4/17662	0.02264749	Denmark
6	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	2/17662	0.01132374	Denmark
7	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1/17662	0.00566187	Denmark
8	NM_000485.2	88811899	Exon 1	c.1A>C	p.(Met1?)	Nonsense	1/6306	0.015857913	Sweden
9	NM_000485.2	88810550	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1/6306	0.015857913	Sweden
10	NM_000485.2	88809717	Exon 5	c.521_523del	p.(Phe174del)	Deletion	1/6306	0.015857913	Sweden
11	NG_008013.1	88810067	Intron 4	c.400+2dupT	-	Indel	5/2730	0.18315018	Ireland
12	NM_000485.2	88809700	Exon 5	c.541T>C	p.(*181Argext*?)	Stop lost	2/2730	0.07326007	Ireland
13	NM_000485.2	88810494	Exon 3	c.250G>A	p.(Val84Met)	Missense	2/5840	0.0342465	Norway
14	NM_000485.2	88809717	Exon 5	c.521_523del	p.(Phe174del)	Deletion	1/5840	0.0171232876	Norway

Abbreviations: Alt/ref, alternate/reference alleles; MAF, minor allele frequency.

Table 3. *APRT* cumulative allele count, allele frequency and expected homozgous frequency of pathogenic observed in population databases

	Allele count (Alt/Ref)	Allele frequency (%)	Expected frequency of homozygotes (1 in)	Expected carrier frequency (1 in)
gnomAD				
African	9/24940	0.0360	7,679,057	1386
Latino	11/35430	0.0508	3,712,044	964
Ashkenazi Jewish	1/10350	0.0097		
East Asian	9/19946	0.0451	4,911,641	1109
Finnish	1/24920	0.0080		
European (Non-Finnish)	63/128828	0.0489	4,181,571	1023
South Asian	5/30614	0.0163	37,488,680	3062
UK Biobank				
All	77/1000000	0.071	1,686,625	650
100,000 Genomes Project				
All	11/127474	0.0086	134,294,386	5795
deCODE				
Iceland	1304/107928	1.2082	6840	42
Denmark	12/17662	0.0678	2,166,293	736
Sweden	3/6306	0.0476	4,418,404	1052
Norway	3/5840	0.0514	3,789,511	974
Ireland	7/2730	0.18315	298,116	274
HGVD	3/2326	0.1289	601,142	388
KOVA	1/1898	0.052687	3,602,404	950

Abbreviations: gnomAD, Genome Aggregation Database; HGVD, Human Genome Variation Database; KOVA, Korean Variation Archive.

Supplemental Tables

Supplemental Table 1. Pathogenic *APRT* variants reported in patients with adenine phosphoribosyltransferase deficiency

	Reference sequence	Build 37	Build 38	rsID	Location	Base change	Amino acid change	Variant type	Compound heterozygotes (n)	Homozygotes (n)	Country
1	NM_000485.2	88876105_88878308?	88809697_88811900?			c.(-1_1*)?	-	Gross alteration	2†	0	Japan(1)
2	NM_000485.2	88878307	88811899	rs930107496	Exon 1	c.1A>G	p.(Met1?)	Start lost?	4†	0	Hungary(2), France(3)
3	NM_000485.2	88878306	88811898	rs1180937573	Exon 1	c.2T>C	p.(Met1?)	Start lost?	0	1†	India*
4	NM_000485.2	88878305	88811897	rs918734933	Exon 1	c.3G>A	p.(Met1?)	Start lost?	3†	2†	Turkey(4), UK*, India*, Japan(5)
5	NM_000485.2	88878285	88811877	rs1261219212	Exon 1	c.23dupT	p.(Val9Glyfs*2)	Frameshift	1†	0	USA*
6	NM_000485.2	88878250	88811842		Exon 1	c.58C>T	p.(Pro20Ser)	Missense	0	1†	UK±
7	NG_008013.1	88878067	88811659	rs761838152	Intron 1	c.81-3C>G	-	Splice	1†	2†	USA±, Italy*
8	NG_008013.1	88878066	88811658	rs751779314	Intron 1	c.81-2A>G	-	Splice	0	1†	French Cohort(3)
9	NM_000485.2	88878063	88811655		Exon 2	c.82G>C	p.(Asp28His)	Missense	0	1†	Spain(6)
10	NM_000485.2	88878061	88811653		Exon 2	c.84C>A	p.(Asp28Glu)	Missense	1†	0	Italy(7)
11	NM_000485.2	88878047	88811639		Exon 2	c.98T>C	p.(Leu33Pro)	Missense	1†	0	Japan(8)
12	NM_000485.2	88878026	88811618		Exon 2	c.119G>C	p.(Arg40Pro)	Missense	0	1†	Morocco(4)
13	NM_000485.2	88877985	88811577	rs752977102	Exon 2	c.160C>G	p.(His54Asp)	Missense	2†	0	USA(9), UK*
14	NM_000485.2	88877964_88877965insA	88811556_88811557insA		Exon 2	c.180_181insT	p.(Ile61fs*49)	Frameshift	1†	1†	Greece(2), USA(2)
15	NG_008013.1	88877936_88877961del	88811528_88811553del		Exon 2 /Intron 2	c.184_187+22del	-	Deletion	1†	0	Italy(7)
16	NG_008013.1	88876967	88810559		Intron 2	c.188-3C>G	-	Splice	0	1†	Italy(10)
17	NG_008013.1	88876856_88877109del	88810448_88810701del		Intron 2/ Exon 3	c.188-145_296del	-	Indel	1†	2†	Austria(2), Italy(11, 12)
18	NM_000485.2	88876964	88810556		Exon 3	c.188G>A	p.(Gly63Asp)	Missense	0	5†	Lebanon(4), Finland (Middle East origin)(13), Australia (Lebanese)(14), USA*
19	NM_000485.2	88876958	88810550	rs104894506	Exon 3	c.194A>T	p.(Asp65Val)	Missense	3†	38†	Iceland(2), Britain(2), France(3), Spain(4), Australia(15)
20	NM_000485.2	88876953	88810545	rs369681854	Exon 3	c.199C>T	p.(Arg67*)	Nonsense	1†	1†	France(3), India*
21	NM_000485.2	88876952	88810544	rs762509151	Exon 3	c.200G>A	p.(Arg67Gln)	Missense	3†	1	USA(2), Japan(16) and China(17)
22	NM_000485.2	88876925	88810517	-	Exon 3	c.227C>T	p.(Ala76Val)	Missense	1†	0	India±
23	NM_000485.2	88876902	88810494	rs200392753	Exon 3	c.250G>A	p.(Val84Met)	Missense	1	0	Japan(16)
24	NM_000485.2	88876893	88810485	rs3169258	Exon 3	c.259C>T	p.(Arg87*)	Nonsense	1†	2†	Pakistan(2), USA*, UK±
25	NM_000485.2	88876891_88876894dup	88810483_88810486dup	rs281860265	Exon 3	c.258_261dup	p.(Lys88Profs*23)	Frameshift	1†	6†	Japan(1, 18)
26	NM_000485.2	88876888	88810480	-	Exon 3	c.264G>T	p.(Lys88Asn)	Missense	1†	0	USA*
27	NM_000485.2	88810458_88810464del	88810458_88810464del	rs776240467	Exon 3	c.280_286del	p.(Gly94Leufs*41)	Frameshift	1†	1†	Turkey(2), Hungary(2)
28	NM_000485.2	88876865_88876866del	88810457_88810458del	rs563575862	Exon 3	c.286_287del	p.(Thr96Serfs*13)	Frameshift	0	1†	Portugal(4),
29	NM_000485.2	88876864_88876865del	88810454_88810455del	rs1437920638	Exon 3	c.289_290del	p.(Leu97Valfs*12)	Frameshift	2†	5†	French cohort(3)

30	NM_000485.2	88876858	88810450	rs104894507	Exon 3	c.294G>A	p.(Trp98*)	Nonsense	8†	16†	Japan(16, 19)
31	NM_000485.2	88876841	88810433	-	Exon 3	c.311A>G	p.(Glu104Gly)	Missense	0	1†	Senegal(4)
32	NG_008013.1	88876829dup	88810421dup	rs281860263	Intron 3	c.321+2dup	-	Splice	0	2†	Germany(20)
33	NM_000485.2	88876549	88810141	rs104894508	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	1†	1	Canada(2), French cohort(3)
34	NM_000485.2	88876544	88810136	rs767177754	Exon 4	c.334A>T	p.(Ile112Phe)	Missense	1†	0	Bermuda(2)
35	NM_000485.2	88876526	88810118	rs370665100	Exon 4	c.352G>C	p.(Glu118Gln)	Missense	2†	0	French cohort(3)
36	NM_000485.2	88876519	88810111	rs776948275	Exon 4	c.359G>T	p.(Gly120Val)	Missense	0	1	Spain(21)
37	NM_000485.2	88876507	88810099	-	Exon 4	c.371T>G	p.(Val124Gly)	Missense	0	1†	France(3)
38	NM_000485.2	88876498	88810090		Exon 4	c.380A>G	p.(Asp127Gly)	Missense	0	1†	UK(22)
39	NM_000485.2	88876480	88810072		Exon 4	c.398G>A	p.(Gly133Asp)	Missense	0	1	Japan(16)
40	NG_008013.1	88876477	88810069		Intron 4	c.400+1G>T	-	Splice	0	1†	Iraq(23)
41	NG_008013.1	88876475	88810067		Intron 4	c.400+3A>T	-		1†	0	French cohort(24)
42	NG_008013.1	88876476dup	88810068dup	rs745594160	Intron 4	c.400+2dupT	-	Indel	24†	20†	Italy(12), France(3), Poland(25), Germany(2), Austria(26), USA*, Belgium(2), Australia(15)
43	NM_000485.2	88876489	88810081		Exon 5	c.389T>C	p.(Leu130Pro)	Missense	1†	0	French cohort(24)
44	NM_000485.2	88876242	88809834	rs28999113	Exon 5	c.407T>C	p.(Met136Thr)	Missense	44†	127†	Japan(1), USA*
45	NM_000485.2	88876221	88809813		Exon 5	c.428T>C	p.(Leu143Pro)	Missense	1†	0	France(4)
46	NM_000485.2	88876210	88809802	rs745872435	Exon 5	c.439C>T	p.(Gln147*)	Nonsense	0	1†	Italy(27)
47	NM_000485.2	88876201	88809793	rs281860266	Exon 5	c.448G>T	p.(Val150Phe)	Missense	1†	0	Germany(25)
48	NM_000485.2	88876192	88809784		Exon 5	c.457T>C	p.(Cys153Arg)	Missense	1†	0	Bermuda(2)
49	NM_000485.2	88876187_88876188del	88809779_88809780del		Exon 5	c.461_462del	p.(Val154Glufs*9)	Frameshift	2	0	China(17)
50	NM_000485.2	88876175_88876177del	88809767_88809769del		Exon 5	c.472_474del	p.(Glu158del)	Deletion	1±	0	France(4)
51	NM_000485.2	88876158	88809750	rs768425517	Exon 5	c.491G>A	p.(Gly164Asp)	Missense	1†	0	France(28)
52	NM_000485.2	88876139del	88809731del		Exon 5	c.510del	p.(Val171Tyrfs*82)	Deletion	1†	0	French cohort(3)
53	NM_000485.2	88876129del;88876122_88876123del	88809721del;88809714_88809715del		Exon 5	c.520del; 526_527del	-	Deletion	1†	0	French cohort(24)
54	NM_000485.2	88876126_88876128del	88809718_88809720del	rs121912681	Exon 5	c.521_523del	p.(Phe174del)	Deletion	4†	3†	Belgium(2), USA*, French cohort(3), UK±
55	NM_000485.2	88876125	88809717	-	Exon 5	c.524C>T	p.(Ser175Phe)	Missense	1†	0	France
56	NM_000485.2	88876125_88876127del	88809717_88809719del	-	Exon 5	c.522_524del	p.(Ser175del)	Deletion	1†	0	USA*
57	NM_000485.2	88876123	88809715	-	Exon 5	c.526C>T	p.(Leu176Phe)	Missense	2†	0	France(4)
58	NM_000485.2	88876119_88876123del	88809711_88809715del	rs755380873	Exon 5	c.526_530del	p.(Leu176Alafs*3)	Frameshift	0	1†	India(29)
59	NM_000485.2	88876117	88809709	rs1165408563	Exon 5	c.532C>T	p.(Gln178*)	Nonsense	1†	0	France(3)
60	NM_000485.2	88876108	88809700	rs758634272	Exon 5	c.541T>C	p.(*181Argext*?)	Nonsense	1†	0	France(4)
61	NM_000485.2	88876107	88809699	rs387906584	Exon 5	c.542G>C	p.(*181Serext*?)	Nonsense	0	1†	Japan(30)
62	NM_000485.2	88876106	88809698	-	Exon 5	c.543A>T	p.(*181Cysext*?)	Nonsense	0	2†	UK(22)

*From the APRT deficiency Registry of the Rare Kidney Stone Consortium. ±Personal communication. †Enzyme function completely abolished *in vivo*.

Supplemental Table 2. Analysis of splice site strength distributions for known pathogenic *APRT* variants using consensus sequence frequencies, the Maximum Entropy score and the varSEAK online splice prediction tool

Reference sequence	Chromosome	Build 38	rsID	Location	Base change	Variant type	Frequencies of donor and acceptor nudeobase changes	Score alt (%)*	Score ref (%)*	Delta score (%)	Maxentscan alt±	Delta maxentscan	Class‡	Splice site prediction	varSEAK results
NG_008013.1	16	88811659	rs761838152	Intron 1	c.81-3C>G	Splice	0.8%	-49.68	-10.66	-39.02	-5.76	-11.42	4 (likely splicing effect)	3' acceptor splice site prediction: Exon skipping. Likely loss of function for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.81-3C%3EG
NG_008013.1	16	88811658	rs751779314	Intron 1	c.81-2A>G	Splice	0.1%	< no AG >	-10.66	NA	-2.29	-7.96	5 (splicing effect)	3' acceptor splice site prediction: Exon skipping. No AG. Loss of function for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.81-2A%3EG
NG_008013.1	16	88810559		Intron 2	c.188-3C>G	Splice	0.8%	-40,56	20.75	-61.31	-0.51	-9.75	5 (splicing effect)	3' acceptor splice site prediction: Use of cryptic site 44 nt downstream of 3' ss. Loss of function for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.188-3C%3EG
NG_008013.1	16	88810421dup	rs281860263	Intron 2	c.321+2dup	Splice		-18.01	86.39	-104.4	3.39	-7.18	5 (splicing effect)	5' donor splice site prediction: Loss of function for authentic splice site. Exon skipping. Strong decrease of score for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.321%2b2dup
NG_008013.1	16	88810069		Intron 4	c.400+1G>T	Splice	0.1%	< no GT >	46.89	NA	-0.14	-8.50	5 (splicing effect)	5' donor splice site prediction: Loss of function for authentic splice site. Exon skipping. No GT. Loss of function for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.400%2B1G%3ET
NG_008013.1	16	88810067		Intron 4	c.400+3A>T	Splice	3.4%	-66.14	46.89	-113.03	0.95	-7.42	5 (splicing effect)	5' donor splice site prediction: Loss of function for authentic splice site. Exon skipping. Strong decrease of score for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&hgvs=c.400%2B3A%3ET
NG_008013.1	16	88810068dup	rs745594160	Intron 4	c.400+2dupt	Indel		-44.33	46.89	-91.22	-4.78	-13.15	5 (splicing effect)	5' donor splice site prediction: Loss of function for authentic splice site. Exon skipping. Strong decrease of Score for authentic splice site.	Https://varseak.bio/ssp.php?Gene=APRT&transcript=NM_000485.3&variant=&hgvs=c.400%2b2dupt
NG_008013.1	16	88811528_88811553del		Exon 2/ Intron 2	c.184_187+22del	Deletion		NA	NA	NA	-8.61	-11.53	NA†	NA	Na
NG_008013.1	16	88810448_88810701del		Intron 2/ Exon 3	c.188-145_296del	Indel		NA	NA	NA	-7.93	-16.56	NA†	NA	Na

*varSEAK Score: Likelihood that this variant is predicted to be a functional splice site (positive values) or not a functional splice site (negative values), reaching from -100% to +100%. For splice sites that are as likely to work as they are not likely to work, the score is 0 %.

±MaxEntScan: This splice site model assigns a log-odd ratio (MaxENT score) to a given sequence. The higher the score, the higher the probability that the sequence is a true splice site. From Yeo and Burge, 2004 [http://web.mit.edu/~fyfer/tmp-public/yeo-2004-jcompiol-maxent.pdf].

‡Classes given by the online varSEAK tool. Possible classes are: Class 1: No splicing effect. Class 2: Likely no splicing effect. Class 3: Unknown splicing effect. Class 4: Likely splicing effect. Class 5: Splicing effect.

†This variant was not suitable for the varSEAK online splice prediction tool. The delta_MaxEntScan score was therefore manually curated. These larger deletions are expected to result in abnormal splicing as suggested by the low delta_MaxEntScan scores.

Supplemental Table 3. Known pathogenic *APRT* variants found in the 100.000 Genomes Project database

No.	Reference sequence	Position (Build 37)	Position (Build 38)	rs ID	Location	Base change	Amino acid change	Variant type	Allele count	Allele number	*MAF (%)*
19	NM_000485.2	88876958	88810550	rs104894506	Exon 3	c.194A>T	p.(Asp65Val)	Missense	1	127,474	0.001
42	NG_008013.1	88876476dup	88810068dup	rs745594160	Intron 4	c.400+2dup	-	Indel	4	127,474	0.003
35	NM_000485.2	88876526	88810118	rs370665100	Exon 4	c.352G>C	p.(Glu118Gln)	Missense	1	127,474	0.001
13	NM_000485.2	88877985	88811577	rs752977102	Exon 2	c.160C>G	p.(His54Asp)	Missense	2	127,474	0.002
4	NM_000485.2	88878305	88811897	rs918734933	Exon 1	c.3C>T	p.(Met1?)	Nonsense	2	127,474	0.002
54	NM_000485.2	88876126_88876128del	88809718_88809720del	rs121912681	Exon 5	c.521_523del	p.(Phe174del)	Deletion	1	127,474	0.001

Abbreviations: MAF, minor allele frequency.

Supplemental Table 4. Known pathogenic *APRT* variants found in the Genome Aggregation Database (gnomAD) (v2.1.1)

No.	Reference sequence	Position (Build 37)	Position (Build 38)	rs ID	Location	Base change	Amino acid change	Variant type	Allele count	MAF (%)
42	NG_008013.1	88876476dup	88810068dup	rs745594160	Intron 4	c.400+2dupT	-	Indel	30	0.010630
54	NM_000485.2	88876126_88876128del	88809718_88809720del	rs121912681	Exon 5	c.521_523del3	p.(Phe174del)	Indel	10	0.006977
23	NM_000485.2	88876902	88810494	rs200392753	Exon 4	c.250G>A	p.(Val84Met)	Missense	9	0.003224
13	NM_000485.2	88877985	88811577	rs752977102	Exon 3	c.160C>G	p.(His54Asp)	Missense	7	0.002806
30	NM_000485.2	88876858	88810450	rs104894507	Exon 3	c.294G>A	p.(Trp98*)	Nonsense	6	0.002423
51	NM_000485.2	88876158	88809750	rs768425517	Exon 3	c.491G>A	p.(Gly164Asp)	Missense	4	0.001596
47	NM_000485.2	88876201	88809793	rs281860266	Exon 3	c.448G>T	p.(Val150Phe)	Missense	4	0.001600
35	NM_000485.2	88876526	88810118	rs370665100	Exon 3	c.352G>C	p.(Glu118Gln)	Missense	4	0.001418
34	NM_000485.2	88876544	88810136	rs767177754	Exon 5	c.334A>T	p.(Ile112Phe)	Missense	3	0.001064
29	NM_000485.2	88876862_88876865del	88810454_88810457del	rs1437920638	Exon 5	c.289_290del	p.(Leu97Valfs*12)	Frameshift	3	0.001075
24	NM_000485.2	88876893	88810485	rs3169258	Exon 3	c.259C>T	p.(Arg87*)	Nonsense	3	0.001210
7	NG_008013.1	88878067	88811659	rs761838152	Intron 1	c.81-3C>G	-	Splice	3	0.001521
4	NM_000485.2	88878305	88811897	rs918734933	Exon 1	c.3G>A	p.(Met1?)	Nonsense	3	0.002043
32	NM_000485.2	88876549	88810141	rs104894508	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	2	0.000799
20	NM_000485.2	88876953	88810545	rs369681854	Exon 3	c.199C>T	p.(Arg67*)	Nonsense	2	0.000818
16	NG_008013.1	88876953	88810545	rs766646831	Intron 2	c.188-3C>G	-	Splice	2	0.000827
5	NM_000485.2	88878285	88811877	rs1261219212	Exon 1	c.23dupT	p.(Val9Glyfs*2)	Frameshift	2	0.001017
2	NM_000485.2	88878307	88811899	rs930107496	Exon 1	c.1A>G	p.(Met1?)	Nonsense	2	0.001378
61	NM_000485.2	88876107	88809699	rs387906584	Exon 5	c.542G>C	p.(*181Serext*?)	Nonsense	1	0.003186
60	NM_000485.2	88876108	88809700	rs758634272	Exon 5	c.541T>C	p.(*181Argext*?)	Nonsense	1	0.000399
59	NM_000485.2	88876117	88809709	rs1165408563	Exon 5	c.532C>T	p.(Gln178*)	Nonsense	1	0.000399
58	NM_000485.2	88876119_88876123del	88809711_88809715del	rs755380873	Exon 5	c.526_530del	p.(Leu176Alafs*3)	Frameshift	1	0.000399
46	NM_000485.2	88876210	88809802	rs745872435	Exon 5	c.439C>T	p.(Gln147*)	Nonsense	1	0.000400
44	NM_000485.2	88876242	88809834	rs28999113	Exon 5	c.407T>C	p.(Met136Thr)	Missense	1	0.000403
28	NM_000485.2	88876865_88876866del	88810457_88810458del	rs563575862	Exon 3	c.286_287del	p.(Thr96Serfs*13)	Frameshift	1	0.000404
26	NM_000485.2	88876888	88810480	rs138781159	Exon 3	c.264G>T	p.(Lys88Asn)	Missense	1	0.000403

21	NM_000485.2	88876952	88810544	rs762509151	Exon 3	c.200G>A	p.(Arg67Gln)	Missense	1	0.000408
8	NG_008013.1	88878066	88811658	rs751779314	Intron 1	c.81-2A>G	-	Splice	1	0.000497

Abbreviations: MAF, minor allele frequency.

Supplemental Table 5. Known pathogenic *APRT* variants found in the UK Biobank

No.	Reference sequence	Position (Build 37)	Position (Build 38)	rs ID	Location	Base change	Amino acid change	Variant type	Allele count	Allele number	*MAF (%)*
13	NM_000485.2	88877985	88811577	rs752977102	Exon 2	c.160C>G	p.(His54Asp)	Missense	24	100,000	0.024
42	NG_008013.1	88876476dup	88810068dup	rs745594160	Intron 4	c.400+2dup	-	Indel	22	100,000	0.022
35	NM_000485.2	88876526	88810118	rs370665100	Exon 4	c.352G>C	p.(Glu118Gln)	Missense	15	100,000	0.015
54	NM_000485.2	88876126_88876128del	88809718_88809720del	rs121912681	Exon 5	c.521_523del	p.(Phe174del)	Deletion	3	100,000	0.003
32	NM_000485.2	88876549	88810141	rs104894508	Exon 4	c.329T>C	p.(Leu110Pro)	Missense	3	100,000	0.003
20	NM_000485.2	88876953	88810545	rs369681854	Exon 3	c.199C>T	p.(Arg67*)	Nonsense	2	100,000	0.002
60	NM_000485.2	88876108	88809700	rs758634272	Exon 5	c.541T>C	p.(*181Argext*?)	Nonsense	1	100,000	0.001
46	NM_000485.2	88876210	88809802	rs745872435	Exon 5	c.439C>T	p.(Gln147*)	Nonsense	1	100,000	0.001

Abbreviations: MAF, minor allele frequency.

Supplemental Table 6. *APRT* variants of uncertain significance found in the Genome Aggregation Database (gnomAD) (v2.1.1)

No.	Reference sequence	Position (Build 37)	Position (Build 38)	rs ID	Location	Base change	Amino acid change	Variant type	Allele count	Allele number	MAF (%)
1	NM_000485.2	88876886	88810478	rs150156607	Exon 3	c.266G>A	p.(Arg89Gln)	Missense	237	279,314	0.06
2	NM_000485.2	88877960	88811552	rs201579274	Exon 3	c.185C>T	p.(Ala62Val)	Missense	140	236,708	0.06
3	NM_000485.2	88876516	88810108	rs8191494	Exon 4	c.362A>G	p.(Gln121Arg)	Missense	130	282,280	0.05
4	NM_000485.2	88876502	88810094	rs75205792	Exon 4	c.376G>C	p.(Val126Met)	Missense	58	282,292	0.02
5	NM_000485.2	88876532	88810124	rs201944035	Exon 4	c.346G>A	p.(Ala116Thr)	Missense	58	282,044	0.02
6	NM_000485.2	88876537	88810129	rs151240811	Exon 4	c.341A>C	p.(Lys114Thr)	Missense	45	282,040	0.02
7	NM_000485.2	88876505	88810097	rs376629164	Exon 4	c.373G>A	p.(Val125Ile)	Missense	34	282,286	0.01
8	NM_000485.2	88876851	88810443	rs551418842	Exon 3	c.301T>C	p.(Tyr101His)	Missense	34	247,540	0.01
9	NM_000485.2	88876836	88810428	rs780098835	Exon 3	c.316G>A	p.(Gly106Arg)	Missense	23	278,010	0.008
10	NM_000485.2	88877970	88811562	rs768268700	Exon 3	c.175G>A	p.(Asp59Asn)	Missense	21	242,620	0.009

Abbreviations: MAF, minor allele frequency.

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Paper II

Urolithiasis
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ORIGINAL PAPER

Are conventional stone analysis techniques reliable for the identification of 2,8-dihydroxyadenine kidney stones? A case series

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Abstract
We have recently encountered patients incorrectly diagnosed with adenine phosphoribosyltransferase (APRT) deficiency due to misidentification of kidney stones as 2,8-dihydroxyadenine (DHA) stones. The objective of this study was to examine the accuracy of stone analysis for identification of DHA. Medical records of patients referred to the APRT Deficiency Research Program of the Rare Kidney Stone Consortium in 2010–2018 with a diagnosis of APRT deficiency based on kidney stone analysis were reviewed. The diagnosis was verified by measurement of APRT enzyme activity or genetic testing. Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra of pure crystalline DHA and a kidney stone obtained from one of the confirmed APRT deficiency cases were generated. The ATR-FTIR spectrum of the kidney stone matched the crystalline DHA spectrum and was used for comparison with available infrared spectra of stone samples from the patients. Of 17 patients referred, 14 had sufficient data available to be included in the study. In all 14 cases, the stone analysis had been performed by FTIR spectroscopy. The diagnosis of APRT deficiency was confirmed in seven cases and rejected in the remaining seven cases. Comparison of the ATR-FTIR spectrum of the DHA stone with the FTIR spectra from three patients who did not have APRT deficiency showed no indication of DHA as a stone component. Misidentification of DHA as a kidney stone component by clinical laboratories appears common among patients referred to our program. Since current clinical protocols used to interpret infrared spectra for stone analysis cannot be considered reliable for the identification of DHA stones, the diagnosis of APRT deficiency must be confirmed by other methods.

Keywords APRT deficiency · Infrared spectroscopy · Kidney stone composition · Misdiagnosis · Nephrolithiasis

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Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600) is a rare inherited disorder of purine metabolism that leads to excessive renal excretion of 2,8-dihydroxyadenine (DHA), resulting in kidney stones and crystal nephropathy. End-stage kidney disease occurs in 15–20% of patients [1, 2]. In some cases, APRT deficiency is not diagnosed until after kidney transplantation, often in the setting of allograft failure due to disease recurrence [3, 4].

Radiolucent DHA kidney stones are the most common feature of APRT deficiency, reported in up to 60% of those affected [1, 5], and as many as one-third of patients experience recurrent stones. Treatment with the oxidoreductase (XOR; xanthine dehydrogenase/oxidase) inhibitors, allopurinol and febuxostat, has been shown to prevent stone formation and halt the progression of chronic kidney disease (CKD) in patients with APRT deficiency [2, 5]. Early diagnosis of the disorder is a prerequisite for timely institution of pharmacotherapy.

The diagnosis of APRT deficiency is confirmed by identification of biallelic pathogenic variants in the *APRT* gene or abolished enzyme function in red blood cell lysates [6, 7]. Analysis of urine crystal or kidney stone material has also been considered diagnostic of the disorder, using the recommended techniques of X-ray diffraction crystallography or infrared spectroscopy [6, 8, 9]. Fourier transform infrared (FTIR) spectroscopy is currently the most commonly used method in clinical stone analysis laboratories [10, 11].

We have recently encountered cases where kidney stones were misidentified as DHA stones by infrared spectroscopy. The objective of this study was to examine the accuracy of stone analysis for identification of DHA as a kidney stone component.

Methods

Ethical approval

The study was approved by the National Bioethics Committee of Iceland (NBC 09-072) and the Icelandic Data Protection Authority. The clinical and research activities reported are consistent with the Principles of the Declaration of Helsinki.

Study population

This was a retrospective study of all patients referred to the APRT Deficiency Research Program of the Rare Kidney Stone Consortium (RKSC, https://www.rarekidneystones.org/) at Landspitali–The National University Hospital in Reykjavik, Iceland, from 2010 to 2018, with a presumptive diagnosis of the disorder based on kidney stone analysis.

Clinical data and diagnostic testing

Clinical information was obtained from the APRT Deficiency Registry that was established in 2010 to collect observational data from patients with the disease worldwide. Registry variables include age at presentation, first kidney stone event and number of clinical kidney stone episodes; results of laboratory studies, including serum creatinine (SCr) measurements, urine microscopy, renal imaging studies and kidney stone analysis; and XOR inhibitor treatment. Urinary DHA excretion was measured using an ultra-high performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) assay as previously described [12]. APRT enzyme activity measurement or mutation analysis of the *APRT* gene was performed to confirm or rule out the diagnosis of APRT deficiency.

Evaluation of kidney stone spectra

To facilitate the study of individual patient stone analysis results, attenuated total reflection-Fourier transform infrared (ATR-FTIR) reference spectra were generated in the Glynn Laboratory of Bioenergetics at University College London by one of the authors (PRR). First, a reference spectrum of pure crystalline DHA, supplied by Santa Cruz Biotechnology (cat. no. sc-498575), was produced. To ensure its crystalline state and purity, the DHA was solubilised in alkali and recrystallised after neutralisation. ATR-FTIR reference spectra were also generated for solid urea, sodium hydrogen urate, uric acid, hydroxyapatite (Sigma H-0252; calcium hydroxide phosphate, dried at pH 6.8 with 1 mM phosphate) and ammonium acid urate. All these chemicals were purchased from Sigma Aldrich Chemical Company except ammonium acid urate which was synthesised by addition of 0.6 mL of 0.2 M ammonium sulphate to 1 mL of 11 mM sodium hydrogen urate at pH 6.5–7.0 and 80 °C. Ammonium acid urate precipitated on cooling to 4 °C and was washed with water and dried. The ATR-FTIR spectra were recorded with a Bruker IFS 66/S spectrometer, fitted with a liquid nitrogen-cooled MCT-A detector and a silicon ATR microprism (3 mm diameter; 3 reflections; DuraSampl*IR* II, SensIR/Smith Detection). The frequencies quoted have an accuracy to ± 1 cm^{-1}. Sample spectra were recorded *versus* a background spectrum of the clean prism surface. Reference materials were either loaded onto the prism surface as a suspension in several µL of distilled water, followed by thorough drying with a gentle stream of dry argon gas, or placed on the prism as solids and pressed to maximise surface contact. In addition, an ATR-FTIR spectrum of a

stone sample obtained from one of the patients with confirmed APRT deficiency (case 13; Table 1) was recorded. This spectrum closely matched the ATR-FTIR spectrum of pure crystalline DHA and was used as a reference to assess the original infrared spectra of kidney stone specimens from three patients (cases 4, 5 and 8; Table 1) that were available for examination. These spectra had been recorded in clinical laboratories and were supplied as transmittance spectra with little useful information outside the fingerprint region (from ~ 2000 to 750 cm^{-1}). The spectra were compared to the reference DHA stone spectrum in the same transmittance form and wavenumber range.

Statistical analysis

Descriptive statistics were carried out using SPSS (IBM SPSS Statistics, version 21.0; 2012). Data are presented as number, percentage, and median (range).

Results

Seventeen patients were referred to the APRT Deficiency Research Program with the presumptive diagnosis of APRT deficiency based on analysis of kidney stone composition demonstrating DHA. Information on the stone analysis method used in two patients who subsequently had the diagnosis of APRT deficiency confirmed was not available. In one case, stone analysis carried out by X-ray diffraction indicated DHA as a stone component, but the diagnosis of APRT deficiency was excluded by genetic testing and measurement of APRT activity. The details of stone analysis results were not available. These three cases were excluded from further analysis because of incomplete data. In the remaining 14 cases, kidney stone analysis had been carried out using FTIR spectroscopy. The characteristics of the 14 patients are shown in Table 1. Their median age was 29 years (range, 2–58 years) and 7 were females. The diagnosis of APRT deficiency was confirmed by analysis of APRT enzyme activity and/or genetic testing in 7 out of 14 patients, all of whom had kidney stones composed of 100% DHA. Urine samples were available for five of the seven patients, three of whom had a high urine DHA-to-Cr ratio, ranging from 14.9 to 37.2 mg/mmol, while two individuals on allopurinol therapy had levels that were low (2.5 mg/mmol) or below the limit of quantitation. The stone analysis spectra could not be acquired for any of these seven cases. However, analysis of a kidney stone sample obtained from case 13 (Table 1) revealed an FTIR spectrum which corresponded closely to that of the pure crystalline DHA, indicating that it was indeed an essentially pure (100%) DHA stone (Fig. 1). This stone spectrum was used as a reference in the evaluation of available infrared spectra.

In seven cases, the diagnosis of APRT deficiency made by stone analysis was rejected by confirmatory testing. The first case was a 37-year-old female from the United States who experienced her second kidney stone episode with infrared spectroscopy-based stone analysis revealing 100% DHA. Treatment with allopurinol was initiated. No DHA crystals were observed on urine microscopy and analysis of a 24-h urine sample showed a DHA level below the limit of quantitation. The diagnosis of APRT deficiency was excluded by demonstration of normal APRT enzyme function. Subsequently, allopurinol was discontinued.

The second case was referred by the same physician as the first case for evaluation of suspected APRT deficiency based on stone composition by FTIR analysis reported as 100% DHA. This 58-year-old male with a family history of gout had passed three kidney stones during a 16-month period, the last of which was sent for analysis. Metabolic evaluation showed a low urine pH and high urinary uric acid excretion, which is strongly inconsistent with APRT deficiency. Sequencing of the *APRT* gene did not disclose pathogenic variants. Repeat stone analysis revealed calcium oxalate as the principal stone constituent.

Two patients were referred from the United Kingdom. The first patient (case 3) was a 45-year-old female with a history of recurrent kidney stones and high serum uric acid levels who passed an 8 mm stone that was reported to be composed of 64% DHA, 26% calcium oxalate and 10% uric acid, determined using infrared spectroscopy. However, replotting of the fingerprint region of the supplied FTIR transmittance spectrum with the equivalent spectrum of the confirmed DHA stone did not disclose characteristics consistent with DHA (Fig. 2). The majority of dominant features of the spectrum appeared to most closely resemble urea, suggesting that significant soluble contaminants were present in the analysed sample. The second patient (case 4) was a 28-year-old male with a first kidney stone reported to contain 12% DHA, 69% calcium phosphate and 18% calcium oxalate by FTIR analysis. Again, replotting of the fingerprint region of the supplied FTIR transmittance spectrum with the equivalent spectrum of the validated DHA stone failed to reveal any features consistent with DHA, and the majority of the stone composition could instead be assigned to a hydroxyapatite (calcium phosphate hydroxide)-like material plus residual water (Fig. 3). Urine DHA was not detected in either of these patients, while the female patient reportedly had elevated 24-h urinary uric acid excretion. Both patients had normal APRT activity and genetic testing did not disclose a pathogenic variant in the *APRT* gene. The results of the stone analysis for the female patient were re-evaluated by the original laboratory, again using FTIR spectroscopy, revealing a purine component that was not DHA.

Two brothers from South Africa (cases 5 and 6), 22 and 26 years of age, were referred to our program after infrared

Table 1 Characteristics of patients with kidney stones reported to contain 2,8-dihydroxyadenine

Case	Sex	Country	Age at stone analysis (years)	Stone events (number)	SCr at last follow-up (μmol/L)	Stone analysis method	Proportion of DHA in stone material	Urine DHA-to-Cr ratio (mg/mmol)	Genetic testing	APRT enzyme analysis
1	Female	USA	34	1	NA	FTIR	100%	BLQ	NA	Normal
2	Male	USA	58	4	88	FTIR	100%	NA	Normal	NA
3	Female	UK	45	1	95	FTIR	64%	BLQ	Benign variant	NA
4	Male	UK	28	1	70	FTIR	12%	BLQ	NA	Normal
5	Male	S-Africa	22	1	88	FTIR	60%	BLQ	Normal	Normal
6	Male	S-Africa	26	1	80	FTIR	30%	BLQ	Normal	Normal
7	Female	Australia	11	NA	NA	FTIR	Trace	BLQ	Normal	Normal
8	Male	USA	37	5	153	FTIR	100%	NA	Pathogenic biallelic mutations	No enzyme function
9	Female	USA	22	7	106	FTIR	100%	14.9	Pathogenic biallelic mutations	NA
10	Female	USA	47	6	72	FTIR	100%	NA	Pathogenic biallelic mutations	No enzyme function
11	Male	USA	30	2	71	FTIR	100%	BLQ*	NA	No enzyme function
12	Female	India	2	2	35	FTIR	100%	26.3	Pathogenic biallelic mutations	No enzyme function
13	Male	Italy	2	2	49	FTIR	100%	2.5*	Pathogenic biallelic mutations	No enzyme function
14	Female	UK	55	4	69	FTIR	100%	37.2	Pathogenic biallelic mutations	NA

BLQ below limit of quantitation, *DHA* 2,8-dihydroxyadenine, *FTIR* Fourier transform infrared spectroscopy, *SCr* serum creatinine, *NA* not available

*On treatment with an XOR inhibitor

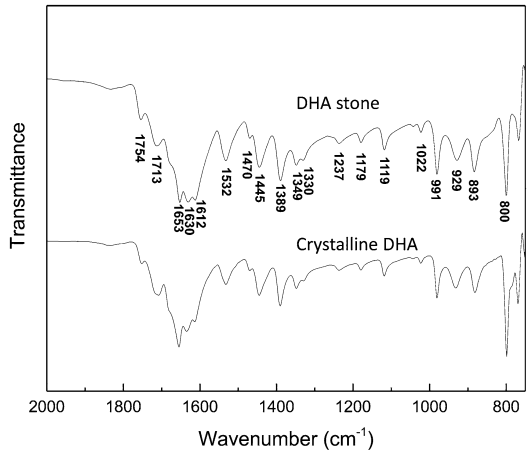

Fig. 1 Comparison of the fingerprint region of attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectra of a 2,8-dihydroxyadenine (DHA) stone (obtained from case 13) and pure crystalline DHA. Spectra were recorded in ATR mode and are plotted as transmittance spectra. All have been scaled to roughly the same signal intensities for ease of comparison. Wavenumbers of the stone spectrum that closely match those of crystalline DHA are labelled

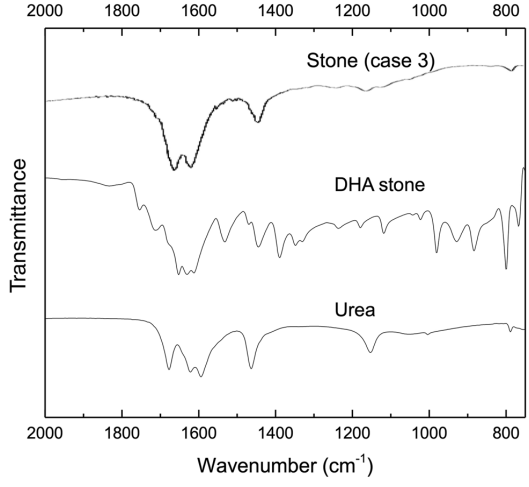

Fig. 2 Comparison of the fingerprint region of the Fourier transform infrared spectrum from case 3 with reference spectra. The 2,8-dihydroxyadenine (DHA) stone and crystalline urea reference spectra were recorded in attenuated total reflection mode and are plotted as transmittance spectra. All have been scaled to roughly the same signal intensities for ease of comparison. The spectrum from case 3 shows no features consistent with DHA

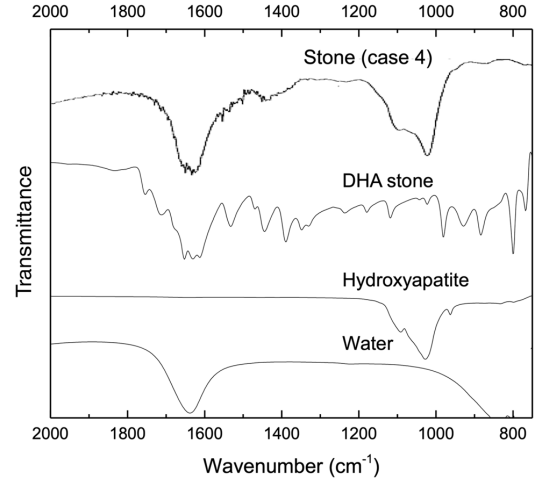

Fig. 3 Comparison of the fingerprint region of the Fourier transform infrared (FTIR) spectrum from case 4 with reference spectra. The 2,8-dihydroxyadenine (DHA) stone, hydroxyapatite (calcium phosphate hydroxide) and liquid water reference spectra were recorded in attenuated total reflection mode and are plotted as transmittance spectra. All have been scaled to roughly the same signal intensities for ease of comparison. The supplied FTIR spectrum from case 4 does not correspond to DHA. The majority of the stone composition is compatible with hydroxyapatite (calcium phosphate hydroxide)-like material plus residual water

analysis of stone material was reported as 60% and 30% DHA, respectively. Both had urine DHA below the limit of quantitation and the APRT enzyme function was normal. Genetic analysis did not reveal pathogenic variants in the *APRT* gene.

The final patient (case 7) was a female from Australia who presented with hematuria and multiple bilateral kidney stones at age 11 years. Kidney stone composition, analysed using infrared spectroscopy, was reported as calcium phosphate with trace amounts of DHA. The patient was placed on treatment with allopurinol and increased fluid intake was recommended. However, the fingerprint region of the supplied FTIR transmission spectrum again showed no correspondence to the spectrum of the DHA stone (Fig. 4). Instead, a close match was found to the infrared spectrum of ammonium acid urate [13, 14]. APRT enzyme activity proved to be normal on two separate occasions. Urine testing was negative for DHA, but metabolic screening performed at the referring institution showed slightly elevated urinary cystine levels, possibly consistent with heterozygous mutation in *SLC7A9*. Treatment with allopurinol was subsequently discontinued.

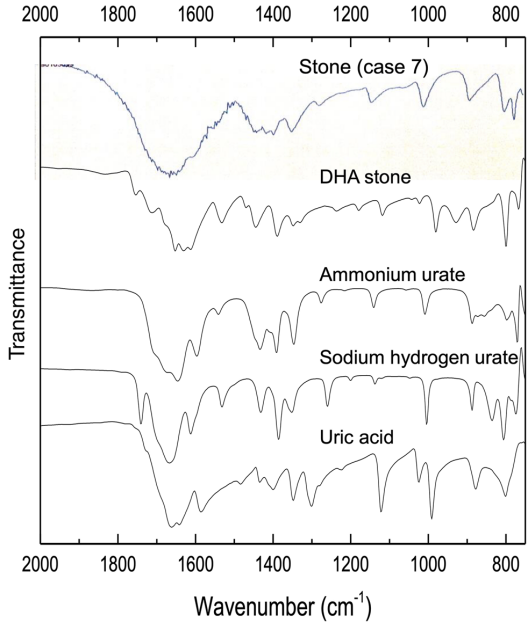

Fig. 4 Comparison of the fingerprint region of the Fourier transform infrared spectrum from case 7 with reference spectra. The 2,8-dihydroxyadenine (DHA) stone, ammonium acid urate, sodium hydrogen urate and uric acid reference spectra were recorded in attenuated total reflection mode and are plotted as transmittance spectra. All have been scaled to roughly the same signal intensities for ease of comparison. The supplied FTIR transmission spectrum shows no correspondence to the spectrum of the DHA stone

Discussion

This study shows that 7 out of 14 patients who had analysis of kidney stone composition performed, using infrared spectroscopy, were erroneously diagnosed with APRT deficiency. This finding highlights shortcomings of the clinical infrared protocols used for stone analysis in reliably identifying DHA as a stone constituent and emphasizes the need for confirming the diagnosis of APRT deficiency, either by demonstrating absence of APRT enzyme activity or a pathogenic variant in both alleles of the *APRT* gene. In addition, measurement of urinary DHA excretion appears to be a reliable method for diagnosing APRT deficiency.

Analysis of stone composition is an essential component of the clinical evaluation of nephrolithiasis, providing clues regarding the pathophysiology and facilitating the selection of targeted treatment for prevention of recurrent stone episodes [15, 16]. Wet chemical methods, which were commonly used for analysis of stone composition in the past, are inaccurate and often lead to a wrong diagnosis [10]. Furthermore, these methods fail to identify rare purine stones resulting from genetic disorders, including DHA stones which are confused with uric acid stones [17, 18]. Consequently, only physical methods such as X-ray diffraction crystallography and FTIR spectroscopy are currently considered acceptable for the analysis of kidney stone composition [16].

First introduced as a stone analysis procedure in 1955 [19], FTIR spectroscopy is currently preferred in most laboratories worldwide as the analysis time is short and the cost of the equipment is significantly less than for X-ray crystallography [17, 20]. Morphologic examination coupled to FTIR analysis is considered important in the diagnosis of rare types of kidney stones, including DHA calculi [9]. The same technique is useful for analysis of crystal composition when no stones are available, including in kidney biopsies when crystal nephropathy is present.

In FTIR spectroscopy, the sample is irradiated with a broadband infrared beam. Absorption occurs at frequencies associated with specific infrared-active molecular vibrational normal modes, which increase in parallel with the number of atoms. Hence, most molecules composed of more than a few atoms have characteristic absorption spectra with multiple bands. Infrared absorption spectra of the stone samples can be analysed by comparison to reference spectra of pure substances to determine the chemical composition of the stone [10, 11]. However, infrared spectra in the 'fingerprint' region, below 1800 cm^{-1}, tend to be complex and mixtures of components may have overlapping bands from the different constituents. In general, published reports on stone analysis employing infrared spectroscopy have not included details of the reference infrared spectra that were used, so the robustness of the deconvolution methods cannot be independently verified. A number of additional factors can complicate the analysis. Pure compounds may exist in different ionic, crystalline or hydration states which can result in significant differences in their infrared signatures. Moreover, the relative intensities of bands of a pure substance are different when the spectra are recorded in transmission *versus* ATR mode and this must be considered when comparing spectra measured in these modes. In the case of ATR-FTIR spectra, caution is needed to ensure that material representative of the whole sample is present in the very thin (several microns) infrared-active volume. In many instances, spectra are presented in transmittance form and although useful for highlighting minor bands, transmittance (as opposed to absorbance) band intensities of a pure compound will not remain at a constant ratio to each other when bands are strongly absorbing. When fitting reference spectra to stone spectra, care must also be taken to account for possible artefacts caused by broad baseline drifts by beamline water vapour interference. All these factors will complicate the accurate deconvolution of component mixtures in dried stone samples, making automated analysis of spectra

difficult without additional expert scrutiny of data. Indeed, several reports have highlighted the inaccuracies of current automated methods of infrared stone analysis [10, 17, 20], as was clearly the case in the three examples reported here. However, the quality of FTIR spectra recorded in ATR mode can be extremely high when optimally performed (Fig. 1), which is particularly simple in terms of sample handling. Furthermore, the wavenumber range is extendable both above and below the typical fingerprint region, providing additional bands that can be diagnostic for DHA [21]. Accurate recording of ATR-FTIR spectra in combination with a more global analysis of the full spectral range in comparison to reference compounds could provide a far more robust method for DHA detection and quantitation.

The misidentification of kidney stone components as DHA in our study is striking. Although FTIR spectroscopy is currently the most widely used stone analysis method, it is limited by the quality of the reference libraries available and the choice of computer algorithm chosen for matching sample spectral data with the reference library [10, 11, 20]. Indeed, incorrect results are known to occur and are more common when stones contain a mixture of constituents [10, 22]. It is noteworthy that the majority of misidentified stone specimens from the patients included in the current study reportedly contained less than 70% DHA. Stones from patients with APRT deficiency are typically composed of pure DHA [23–25], though occasional mixed stones containing calcium salts have been reported [26, 27]. Thus, stone analysis reports of mixed stones containing DHA should raise a suspicion of erroneous interpretation.

The misdiagnosis of the cases reported herein resulted from incorrect assignment of the infrared spectra in most cases. Although the infrared spectrum of DHA is very specific and the identification of DHA can be definitive when performed by trained laboratory personnel [9], untrained operators can incorrectly attribute spectra of other materials such as uric acid and its salts to DHA. Stringent quality control measures in clinical laboratories are considered essential for improving the accuracy of kidney stone analysis [11].

Clinicians caring for patients with rare kidney stone disorders must be familiar with the potential misidentification of DHA stones from infrared spectra and should invariably confirm the diagnosis of APRT deficiency using APRT enzyme function analysis or sequencing of the *APRT* gene to search for pathogenic mutations affecting both alleles. Indeed, due to increasing availability and markedly reduced cost, genetic testing is becoming a favoured diagnostic method for APRT deficiency. The *APRT* gene should be included in high-throughput next-generation sequencing panels for rare types of CKD and kidney stone disease [28]. Finally, our recently described UPLC-MS/MS assay for measurement of urine DHA is a promising alternative method for the diagnosis of APRT deficiency [12].

Misdiagnosis of the patients in this case series as having APRT deficiency could have led to lifelong XOR inhibitor therapy. These medications, particularly allopurinol, are associated with adverse effects. Erroneous stone analysis results could also lead to missed cases of APRT deficiency, thereby precluding the institution of appropriate therapy. Incorrect analysis or failure to identify a stone constituent may also result in inadequate therapy of other stone types.

In conclusion, misidentification of DHA as a kidney stone component by clinical laboratories appears common among patients referred to our program. The determination of kidney stone composition is often based on automated analysis of FTIR spectra of varying quality and thus is subject to error. The diagnosis of APRT deficiency should always be confirmed by enzyme activity measurement, genetic testing or detection of DHA in urine samples.

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Compliance with ethical standards

Conflict of interest None of the authors declare financial or other conflicting interests.

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Paper III

AJKD
Original Investigation

Kidney Disease in Adenine Phosphoribosyltransferase Deficiency

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Background: Adenine phosphoribosyltransferase (APRT) deficiency is a purine metabolism disorder causing kidney stones and chronic kidney disease (CKD). The course of nephrolithiasis and CKD has not been well characterized. The objective of this study was to examine long-term kidney outcomes in patients with APRT deficiency.

Study Design: An observational cohort study.

Setting & Participants: All patients enrolled in the APRT Deficiency Registry of the Rare Kidney Stone Consortium.

Outcomes: Kidney stones, acute kidney injury (AKI), stage of CKD, end-stage renal disease, estimated glomerular filtration rate (eGFR), and changes in eGFR.

Measurements: Serum creatinine and eGFR calculated using creatinine-based equations.

Results: Of 53 patients, 30 (57%) were females and median age at diagnosis was 37.0 (range, 0.6-67.9) years. Median duration of follow-up was 10.3 (range, 0.0-31.5) years. At diagnosis, kidney stones had developed in 29 (55%) patients and 20 (38%) had CKD stages 3 to 5, including 11 (21%) patients with stage 5. At latest follow-up, 33 (62%) patients had experienced kidney stones; 18 (34%), AKI; and 22 (42%), CKD stages 3 to 5. Of 14 (26%) patients with stage 5 CKD, 12 had initiated renal replacement therapy. Kidney stones recurred in 18 of 33 (55%) patients. The median eGFR slope was −0.38 (range, −21.99 to 1.42) mL/min/1.73 m^2 per year in patients receiving treatment with an xanthine dehydrogenase inhibitor and −5.74 (range, −75.8 to −0.10) mL/min/1.73 m^2 per year in those not treated prior to the development of stage 5 CKD ($P = 0.001$).

Limitations: Use of observational registry data.

Conclusions: Progressive CKD and AKI episodes are major features of APRT deficiency, whereas nephrolithiasis is the most common presentation. Advanced CKD without a history of kidney stones is more prevalent than previously reported. Our data suggest that timely therapy may retard CKD progression.
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INDEX WORDS: End-stage renal disease; chronic kidney disease (CKD); adenine phosphoribosyltransferase (APRT) deficiency; purine metabolism disorder; nephrolithiasis; kidney stone; crystal nephropathy; estimated glomerular filtration rate (eGFR); renal function; acute kidney injury (AKI); disease progression; kidney failure; renal replacement therapy (RRT).

Adenine phosphoribosyltransferase (APRT) deficiency is an uncommon autosomal recessive disorder of purine metabolism that leads to kidney stones and chronic kidney disease (CKD).[1,2] The absence of APRT activity prevents the recycling of adenine, which instead is catabolized by xanthine dehydrogenase (XDH) to 2,8-dihydroxyadenine (2,8-DHA), a poorly soluble substance excreted by the kidney resulting in heavy crystalluria (Fig 1). More than 40 pathogenic mutations in the coding region of *APRT* have been identified in more than 400 affected people from more than 25 countries,[3,4] most of whom are from France, Iceland, and Japan, whereas fewer than 15 patients originate in the United States.[2] All known pathogenic mutations abolish enzyme function.[1,5]

The phenotype is characterized by radiolucent kidney stones, the most commonly reported clinical manifestation of APRT deficiency, followed by progressive CKD secondary to crystal nephropathy. Kidney failure requiring renal replacement therapy (RRT) is the presenting feature in ∼15% of adult

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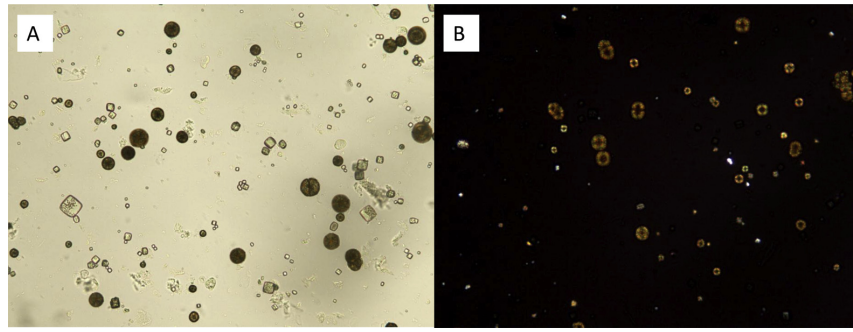

Figure 1. Urinary 2,8-dihydroxyadenine crystals. (A) The characeristic medium-sized crystals are brown with a dark outline and central spicules. (B) The same field viewed with polarized light microscopy shows that the small- and medium-sized crystals appear yellow and produce a central Maltese cross pattern (A, B: original magnification, ×400).

cases.[1,5] In a number of instances, APRT deficiency has first been recognized after kidney transplantation, when transplant dysfunction occurs.[6,7] Other reported clinical manifestations include hematuria and lower urinary tract symptoms. A significant number of patients are asymptomatic at diagnosis.[1,2,8] Treatment with the XDH inhibitor allopurinol has been shown to effectively prevent the progression of kidney disease, and the recently introduced nonpurine XDH inhibitor, febuxostat, has provided an alternative therapeutic option.

Limited data exist on kidney stone recurrence, and the course of kidney function over time has not been well characterized in patients with APRT deficiency. In order to closely examine long-term kidney outcomes, we analyzed data from all persons currently enrolled in the APRT Deficiency Registry of the Rare Kidney Stone Consortium.

METHODS

Study Design

This was an observational cohort study using data from the APRT Deficiency Registry of the Rare Kidney Stone Consortium (www.rarekidneystones.org/). The study was approved by the National Bioethics Committee of Iceland (NBC 09-072) and the Icelandic Data Protection Authority, and informed consent was obtained from all living participants. The clinical and research activities reported are consistent with the Principles of the Declaration of Helsinki. Data from all 53 patients (from Iceland, 33; United States, 13; Austria, 2; Italy, 2; United Kingdom, 1; India, 1; and from Norway of Turkish descent, 1) who enrolled in the registry before November 11, 2014, were included. Limited data for 23 of the 33 Icelandic patients have previously been reported by our group[1] and 5 of the non-Icelandic cases were included in earlier publications.[7,9,10]

Clinical Data

Registry data included age at diagnosis; kidney manifestations, including kidney stones, acute kidney injury (AKI), and stage of CKD; lower urinary tract symptoms; results of urologic imaging studies, kidney stone analysis, and kidney biopsies; surgical treatment of kidney stones; XDH inhibitor treatment; RRT; and cause of death. For calculation of estimated glomerular filtration rate (eGFR) in children, height measurements were obtained from medical records or extrapolated from data points on the growth chart when recent measurements were not available. Laboratory studies included serum creatinine (Scr) measurements; results of urine microscopy, including assessment of 2,8-DHA crystals; *APRT* genotype; and APRT activity.

Definitions

Symptomatic kidney stone events were defined as either patient-reported stone passage or abdominal pain associated with hematuria and/or a stone confirmed by an imaging study. Urinary tract stones identified by imaging only were considered asymptomatic. Stone recurrence was defined as detection of a stone in patients previously shown to be stone free by imaging study. eGFR was calculated from Scr, using the CKD-EPI (CKD Epidemiology Collaboration) creatinine equation in adults[11] and the modified Schwartz equation[12] in children. Nonstandardized Scr values were reduced by 5% before eGFR was calculated, as previously described.[13] All patients were considered to have CKD based on presumed structural damage associated with renal 2,8-DHA crystal deposition. The KDIGO (Kidney Disease: Improving Global Outcomes) classification system was used to stage CKD.[14] Available Scr values were used to identify episodes of AKI, defined according to KDIGO criteria as an increase in Scr level $\geq$ 26.5 μmol/L ($\geq$ 0.3 mg/dL) within 48 hours or 1.5 or more times baseline within 7 days.[15]

Statistical Analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics, version 21.0; 2012). Data are presented as number, percentage, and median and range. Chi-square analysis was used to compare the prevalence of CKD in Icelandic patients and those from other countries. eGFR slopes and CKD staging were based on annual eGFR values derived from the lowest available Scr measurement in each calendar year, excluding all Scr values obtained during episodes of AKI; patients receiving RRT were assigned an eGFR of 10 mL/min/1.73 m^2. Comparison of eGFR slopes of patients receiving XDH inhibitor treatment and those who were untreated prior to the development of end-stage kidney failure were compared using Mann-Whitney U test. Wilcoxon signed rank test was used to compare eGFR slopes of patients before and after the initiation of pharmacotherapy. $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics

The 53 patients belonged to 42 families. Fifty patients were white; 1, African American; 1, Indian; and 1, of Japanese descent. Thirty (57%) patients were female. Median age at diagnosis was 37.0 (range, 0.6-67.9) years, and the age of symptomatic and asymptomatic patients was 40.6 (range, 0.6-67.9) and 25.7 (range, 3.6-39.2) years, respectively. Median duration of follow-up after diagnosis was 10.3 (range, 0.0-31.5) years, and median age at last follow-up was 43.2 (range, 2.7-75.0) years. Median number of Scr values per patient was 10 (range, 2-130). Clinical characteristics of the 53 patients at the time of diagnosis are presented in Table 1. The most frequently noted clinical manifestation was nephrolithiasis (n = 29); 20 patients had CKD stages 3 to 5, which occurred in 9 patients without a history of kidney stones, and 16 patients had AKI or a history of the condition. Five asymptomatic individuals, identified by the detection of 2,8-DHA crystals on urine microscopy, were diagnosed in childhood or early adulthood. Kidney stones were a more frequent presentation than CKD stages 3 to 5 in childhood, while advanced CKD was more commonly observed in adults (Fig 2).

Table 2 outlines the clinical course and management of all 33 patients who eventually developed kidney stones. Twenty-nine had experienced 80 symptomatic stone events at the time of diagnosis. In 20 of those patients, stones were first detected a median of 10.5 (range, 0.8-47.9) years before the diagnosis of APRT deficiency, but in 9 patients, the disorder was diagnosed during the initial stone event.

Two of the 16 patients who had AKI prior to diagnosis required transient hemodialysis therapy, whereas 2 additional patients who developed AKI at a later stage responded well to conservative treatment. Causes of AKI included biopsy-proven crystal nephropathy in 7 patients, urinary tract obstruction from stones in 9, and volume depletion in 2. Ten of the 18 patients with AKI developed CKD stages 3 to 5 at a median of 1.6 (range, 0.3-29.5) years after the AKI event.

Stages of CKD and the requirement for RRT are presented in Table 3. Median age of the 20 patients who had developed CKD stages 3 to 5 at diagnosis was 44.5 (range, 11.9-67.9) years. Two additional patients had CKD stages 3 to 5 during follow-up. Six patients had initiated RRT a median of 3.8 (range, 1.1-7.4) years prior to the diagnosis of APRT deficiency, including 4 with recurrent 2,8-DHA transplant nephropathy. In addition, 1 Icelandic patient died of complications of kidney failure in 1967, before APRT deficiency was first described. Of 14 patients with CKD stage 5 at latest follow-up, 12 had initiated RRT, of whom 11 had undergone kidney transplantation and received a total of 15 transplants. Three patients died with a functioning transplant at a median age of 43.2 (range, 36.7-51.9) years.

Thirteen of the 20 (65%) patients from countries other than Iceland had developed CKD stages 3 to 5 at diagnosis compared with only 7 of the 33 (21%) Icelandic patients ($P = 0.001$; Fig 3). Eight non-Icelandic patients had already initiated RRT at diagnosis, compared to no Icelandic patient.

Table 1. Clinical Characteristics at Diagnosis of APRT Deficiency

Characteristic	Value
Female sex	30 (57)
Age	
Median (range), y	37.0 (0.6-67.9)
<18 y	14 (26)
≥18 y	39 (74)
Kidney stones	29 (55)
Chronic kidney disease stages 3-5	20 (38)
Renal replacement therapy	8 (15)
Acute kidney injury	16 (30)
Lower urinary tract symptoms	15 (28)
Reddish-brown diaper stains in infancy	11 (21)
2,8-DHA crystalluria	34 (64)
Asymptomatic	5 (9)
Family screening	3
Incidental finding	2

Note: N = 53. Unless otherwise indicated, values are given as number or number (percentage).

Abbreviations: APRT, adenine phosphoribosyltransferase; DHA, dihydroxyadenine.

Diagnosis of APRT Deficiency

The diagnosis of APRT deficiency was initially suggested by detection of urinary 2,8-DHA crystals in 34 cases, histologic findings of crystal nephropathy in 9, and kidney stone analysis in 8 cases. Two cases were diagnosed postmortem by review of autopsy findings that were consistent with 2,8-DHA crystal nephropathy. In 52 of the 53 cases, the diagnosis of APRT deficiency was confirmed by genetic testing (n = 45) and/or the absence of APRT activity in red blood cell lysates (n = 11). A total of 10 different pathogenic variants in the *APRT* gene were identified, including 4 novel mutations. All 33 Icelandic patients were homozygous for the missense variant c.194A>T (p.Asp65Val; ie, an adenine to thymine change at nucleotide 194 of the complementary DNA, leading to an aspartate to valine substitution at amino acid 65) and 4 American patients were found to be homozygous for c.400+2dup (IVS4+2insT; ie, a duplication of the thymine found at nucleotide 2 of the fourth

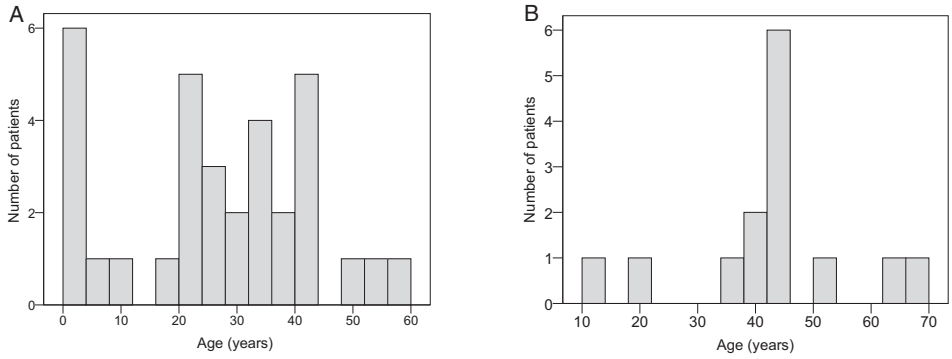

Figure 2. Age distribution (A) at the occurrence of the first kidney stone event and (B) at the detection of chronic kidney disease stage 5 in patients with adenine phosphoribosyltransferase deficiency.

intervening sequence of *APRT*), which results in aberrant splicing and deletion of exon 4. Genetic testing or measurement of APRT activity were not carried out in one case because blood or other tissue samples could not be obtained. The diagnosis was confirmed by detection of large quantities of urinary 2,8-DHA using ultra high-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) in our research laboratory.

A median delay in diagnosis of 7.5 (range, 0.4-47.9) years occurred in 37 patients following their first symptomatic stone event or detection of elevated Scr level. Misidentification of radiolucent kidney stones as uric acid calculi in 4 patients and confusion of renal histopathologic findings with other forms of crystal nephropathy in 6 patients contributed to the diagnostic delay, while urinary 2,8-DHA crystals were not correctly identified in 17 cases.

Table 2. Kidney Stones in Patients With APRT Deficiency

Variable	Value
Total no. of patients with kidney stones	33 (62)
At time of diagnosis	29 (55)
During follow-up	4 (8)
Age	
At first kidney stone event, y	26.4 (0.3-56.4)
≥18 y at first episode	26 (49)
Kidney stones before diagnosis of APRT deficiency	20 (38)
Delay from first clinical stone event to diagnosis, y	10.5 (0.8-47.9)
Kidney stone recurrence	18 (34)
Off XDH inhibitor treatment	2 (4)
On XDH inhibitor treatment	16 (30)
Allopurinol dosage, mg	200 (100-600)
Kidney stone events	
1	14 at diagnosis; 7 during f/u
2-3	10 at diagnosis; 8 during f/u
4-5	2 at diagnosis; 2 during f/u
>5	3 at diagnosis; 0 during f/u
Asymptomatic stones	5 at diagnosis; 2 during f/u
Urologic procedures	
Extracorporeal shockwave lithotripsy	4 at diagnosis; 6 during f/u
Endoscopic surgery	6 at diagnosis; 9 during f/u
Open or percutaneous surgery	4 at diagnosis; 3 during f/u

Note: Values are given as number (percentage), median (range), or number of patients meeting the listed criterion at the time of diagnosis or during f/u.

Abbreviations: APRT, adenine phosphoribosyltransferase; f/u, follow-up; XDH, xanthine dehydrogenase.

Pharmacologic Treatment

Although 52 of the 53 patients were prescribed therapy with allopurinol at some point, none of the 11 patients who had developed CKD stage 5 by the time of diagnosis had received XDH inhibitor therapy. Clinical features of patients who received allopurinol

Table 3. Stages of CKD and RRT in Patients With APRT Deficiency

	At Diagnosis	At Last Follow-up
CKD stage		
1	19	19
2	14	12
3a	2	2
3b	2	3
4	5	3
5	11	14
RRT		
Functioning transplant	3	7
Dialysis	5	2

Note: Values are given as number of patients.

Abbreviations: APRT, adenine phosphoribosyltransferase; CKD, chronic kidney disease; RRT, renal replacement therapy.

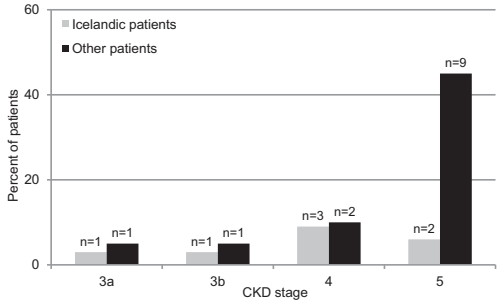

Figure 3. Chronic kidney disease (CKD) stages 3 to 5 in patients with adenine phosphoribosyltransferase deficiency from Iceland and other countries at the time of diagnosis.

treatment prior to initiation of RRT and those who did not are presented in Fig 4. Thirty-eight adult patients initiated allopurinol treatment at a median age of 42.2 (range, 20.5-62.8) years with a daily dose of 100 (n = 7), 150 (n = 3), 200 (n = 21), or 300 mg (n = 7). In 14 children, allopurinol therapy was begun at a median age of 3.3 (range, 0.6-16.6) years in a median daily dose of 8 (range, 3-11) mg/kg or total dose of 100 (range, 25-200) mg. The prescribed dose of allopurinol increased over time, and at latest follow-up, 13 adult patients were receiving doses as high as 400 to 600 mg/d regardless of kidney function. Allopurinol therapy was discontinued in 7 patients after a median of 7.1 (range, 0.4-16.4) years of treatment due to presumed adverse reactions that included itching, hair loss, and severe ocular symptoms, such as pain, photosensitivity, and blurred vision. These patients were taking a median of 300 (range, 100-600) mg of allopurinol daily. All 7 of these patients were subsequently prescribed febuxostat, 80 mg/d, when the drug became available. Febuxostat use was discontinued in

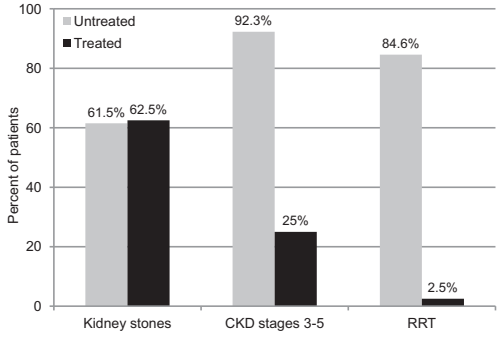

Figure 4. Kidney stones, chronic kidney disease (CKD) stages 3 to 5, and initiation of renal replacement therapy (RRT) in patients with adenine phosphoribosyltransferase deficiency who did and did not receive xanthine dehydrogenase inhibitor treatment.

2 patients due to severely blurred vision and punctate keratitis in 1 patient and ocular dryness in the other. Of 12 minimally symptomatic patients who began XDH inhibitor therapy at a median age of 7.5 (range, 1.0-39.2) years, none subsequently developed kidney stones, AKI, or CKD stages 3 to 5. Absence of urinary 2,8-DHA crystals was generally considered indicative of adequate drug dosing. However, quantitative assessment of crystalluria was not systematically performed.

Eighteen (34%) patients had 35 clinical stone events while receiving allopurinol treatment (Table 2), most frequently at a daily dose of 300 mg (n = 8). One patient with CKD stage 3b, 1 with stage 4, and 1 with stage 5 had temporary improvement in kidney function after initiation of XDH inhibitor treatment. Nevertheless, 2 additional patients developed CKD stages 3 to 5 (Table 3).

Evolution of Kidney Function in Patients with APRT Deficiency

Median eGFR in the 53 patients was 68 (range, 3-165) mL/min/1.73 m^2 at diagnosis, which included several patients with AKI, and 73 (range, 10-163) mL/min/1.73 m^2 at latest follow-up. A box plot of eGFRs in different age groups demonstrated a relatively low median eGFR, particularly after age 40 years (Fig 5). Median eGFR slopes were −0.38 (range, −21.99 to 1.42) mL/min/1.73 m^2 per year in patients receiving pharmacotherapy and −5.74 (range, −75.8 to −0.10) mL/min/1.73 m^2 per year in those not treated prior to the development of CKD stage 5 ($P = 0.001$). In 7 patients who had serial Scr measurements available before and after the initiation of XDH inhibitor therapy, median eGFR slopes were −3.01 (range, −14.43 to 0.92) and 1.76 (range, −0.7 to 13.50) mL/min/1.73 m^2 per year, respectively ($P = 0.04$). Finally, the median eGFR slope was 1.88 (range, −4.16 to 5.12) mL/min/1.73 m^2 per year in 9 patients with CKD stage 3 or 4 when pharmacotherapy was initiated.

DISCUSSION

Our findings demonstrate a highly variable clinical presentation and course of kidney disease in patients with APRT deficiency. The most commonly observed clinical manifestations were nephrolithiasis, episodes of AKI, and progressive CKD, eventually requiring dialysis or kidney transplantation in a significant proportion of patients. Interestingly, a considerable number of patients with advanced CKD had no history of kidney stones. Patients who progressed to kidney failure almost invariably had not received XDH inhibitor treatment, whereas timely pharmacotherapy retarded or prevented CKD progression. Lack of awareness of APRT deficiency appears to have

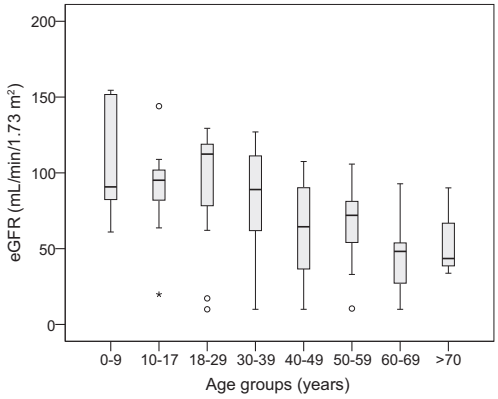

Figure 5. Box plot of estimated glomerular filtration rates (eGFRs) in different age groups of patients with adenine phosphoribosyltransferase deficiency. Patients contributed data to every age group for which they had a serum creatinine value available, and for each individual the mean of all eGFRs in any given age group was used. Patients receiving renal replacement therapy were assigned an eGFR of 10 mL/min/1.73 m^2.

resulted in marked treatment delay, particularly in non-Icelandic patients.

Approximately half the patients in our study had developed kidney stones by the time of diagnosis. This is substantially less than in the largest previously reported series from France,[5] in which 90% of the 40 patients with available clinical data had had kidney stones at diagnosis. Intriguingly, recurrent stone events were observed more frequently in our patients despite the use of similar allopurinol doses.[5,8] Reasons for this observed difference in stone recurrence may include the longer median observation period and relative completeness and quality of data obtained at scheduled annual follow-up visits for a large proportion of our cohort, reducing the likelihood that stone events were missed. Differences in adherence to allopurinol therapy may also have played a role.

One-third of our patients had episodes of AKI, which previously has only rarely been reported for APRT deficiency. In the aforementioned French cohort,[5] only a single case of AKI was noted. A likely explanation for the relatively high frequency of AKI in our study is the availability of numerous Scr values over many years for most patients, allowing more sensitive detection of AKI. Although recovery of kidney function occurred in all these patients, our findings suggest that AKI may contribute to disease progression.

The proportion of patients with CKD stages 3 to 5 in our cohort was similar to that observed in the French study.[5] However, the absence of nephrolithiasis in many of our patients with CKD stages 3 to 5 is striking because stone disease has generally been considered the characteristic feature of APRT deficiency. Another noteworthy finding of our study is the much lower prevalence of advanced CKD or kidney failure in Icelandic patients compared with those from other countries. This observation together with the relatively low number of reported cases in these countries suggests lack of awareness of APRT deficiency as a cause of CKD and crystal nephropathy among nephrologists and renal pathologists. Misinterpretation of kidney biopsy findings contributed to diagnostic delay in at least 6 patients in our study. When histologic examination of a renal tissue reveals a crystal-associated tubulointerstitial lesion, 2,8-DHA nephropathy should always be considered.[2,7,16] Polarized light microscopy facilitates the identification of renal parenchymal crystal deposits, but caution must be exercised to avoid confusion of 2,8-DHA with oxalate.[2,7] Whereas crystal nephropathies are associated with inflammation and fibrosis leading to progressive kidney damage, the pathogenesis of crystal-induced injury in humans remains elusive. The best characterized mediator of crystal-induced inflammation is the intracellular NLRP3 inflammasome, which has been shown to cause direct injury to tubular cells, tubulointerstitial inflammation, and kidney failure in oxalate nephropathy.[17,18]

Earlier studies[5,8] have described improvement in eGFR following the onset of allopurinol therapy. When we included acutely elevated Scr values obtained at the time of diagnosis, we observed a similar improvement in eGFR, which may in part reflect the effect of pharmacologic therapy. In contrast, we carefully excluded episodes of AKI when characterizing the course of kidney function. As previously reported,[1,5] treatment with an XDH inhibitor, primarily allopurinol, clearly stabilized or improved kidney function in our cohort, while eGFR invariably declined in untreated cases. Allopurinol treatment preserved kidney function and prevented stone formation for decades in a subgroup of patients with minimal or no symptoms at diagnosis. The frequent development of advanced CKD and end-stage kidney failure in patients not receiving XDH inhibitor treatment underscores the importance of timely diagnosis and pharmacotherapy.

Presumed adverse reactions precluded the use of allopurinol or febuxostat in several patients in our study.[19] However, the eye symptoms that led to discontinuation of XDH inhibitor therapy have not been previously reported for these drugs except as a part of allopurinol hypersensitivity syndrome.[20,21] Although APRT deficiency has not been clearly demonstrated to affect other organ systems than the kidneys and urinary tract, 2 cases of corneal dystrophy were reported in Belgium in 1986 and the authors concluded that corneal crystal deposition was a probable cause, although this was not histologically

confirmed.[22] No other reports of ocular manifestations in patients with APRT deficiency exist. Nevertheless, the frequently reported eye symptoms by the patients in our study warrant further investigation to determine whether corneal 2,8-DHA crystal deposits occur.

The XDH inhibitor dose required to adequately reduce urinary 2,8-DHA excretion has not been defined. The adverse outcomes observed in our cohort, particularly progressive transplant dysfunction, suggest a need for higher allopurinol doses than have generally been used, perhaps in the range of 600 to 800 mg/d. In the past, it has been recommended to avoid using allopurinol doses higher than 200 to 300 mg/d in patients with CKD based on a report of increased risk for allopurinol hypersensitivity syndrome.[23] Later studies have contradicted this notion.[24] However, it is recommended to begin with a low dose because recent work has shown that higher starting doses of allopurinol may increase the risk for allopurinol hypersensitivity syndrome.[25] The response to therapy has generally been monitored by assessment of 2,8-DHA crystals in urine sediment, which may not be accurate enough to guide drug treatment. Therefore, reliable methods for urinary 2,8-DHA measurement are needed. An assay using UPLC-MS/MS for urinary 2,8-DHA measurement is currently being developed by our group.

The reason for the relatively high number of APRT deficiency cases reported in Japan, France, and Iceland compared to the United States is not clear. Based on reported heterozygote frequency rates of 0.4% to 1.2%,[4,26] one might expect 3,000 to 6,000 cases of APRT deficiency in the United States. Strikingly, a recent report of kidney stone composition from 43,545 US patients did not reveal a single 2,8-DHA stone, which would suggest a lower prevalence.[27] Because APRT deficiency is a preventable cause of CKD, strategies to increase awareness among clinicians and pathologists are important. The disorder should be considered in the differential diagnosis of radiolucent kidney stones and unexplained CKD and in cases of kidney transplant dysfunction of unclear cause.

Demonstrating absence of APRT activity in red blood cell lysates and the identification of pathogenic mutations in both copies of the *APRT* gene are the only definitive methods for the diagnosis of APRT deficiency.[2] Based on reports in the literature and our own experience, urine microscopy has not proved to be reliable enough as the sole method for diagnosis of APRT deficiency. The 2,8-DHA crystals have frequently either been overlooked or not recognized or misidentified. Infrared spectroscopy, which is considered the gold-standard technique for stone analysis, has, in experienced hands, been shown to be a reliable test for detecting 2,8-DHA in stone materials. However, the 2,8-DHA spectrum can be confused with that of other stone-forming components, as illustrated by 3 cases recently referred to us. Hence, identification of 2,8-DHA in stones with the infrared technique cannot be considered diagnostic of APRT deficiency. We believe that measurement of urinary 2,8-DHA using UPLC-MS/MS may emerge as a valuable diagnostic test for APRT deficiency in coming years.

Strengths of the current study include the abundance of data entered into the APRT Deficiency Registry of the Rare Kidney Stone Consortium, which contains more than 950 patient-years of clinical information, making this the largest patient cohort with complete clinical data and the longest observation time reported to date. Furthermore, the availability of multiple Scr values for most patients facilitated accurate ascertainment of AKI, CKD staging, and characterization of the course of kidney function.

Limitations of the study include a small sample size, as is expected for any rare disease, and the use of observational registry data. As a result, the duration of observation varied, as did the scope of laboratory evaluation. Moreover, the large proportion of patients from Iceland, where the awareness of APRT deficiency appears high, may limit the conclusions that can be drawn regarding variability in clinical presentation and outcomes between countries. However, factors such as the rate of 2,8-DHA production may influence clinical expression and warrant further investigation.

In conclusion, our study indicates that the clinical presentation of APRT deficiency may be more variable than previously suggested. Both AKI and progressive CKD have emerged as major features of APRT deficiency, whereas nephrolithiasis remains the most common presenting manifestation. Timely pharmacologic therapy appears to slow the progression of CKD, even in severely affected individuals. The relatively frequent occurrence of advanced CKD and even kidney failure at diagnosis is concerning and suggests a lack of familiarity with this treatable condition. This underscores the importance of kidney biopsy in younger patients with unexplained CKD.

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Contributions: Conception and study design: RP, VOE; statistical analysis: HLR, OSI; critical review of results: HLR, IMA, OSI, RP, VOE; supervision and mentorship: OSI, RP, VOE. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. VOE takes responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

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Paper IV

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ORIGINAL ARTICLE

Long-term renal outcomes of APRT deficiency presenting in childhood

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Abstract

Background Adenine phosphoribosyltransferase (APRT) deficiency is a hereditary purine metabolism disorder that causes kidney stones and chronic kidney disease (CKD). The purpose of this study was to examine the course of APRT deficiency in patients who presented in childhood.

Methods The disease course of 21 (35%) patients in the APRT Deficiency Registry of the Rare Kidney Stone Consortium, who presented with manifestations of APRT deficiency and/or were diagnosed with the disorder before the age of 18 years, was studied. The effect of pharmacotherapy on renal manifestations and outcomes was thoroughly assessed.

Results Fourteen children were placed on allopurinol, 100 (25–200) mg/day, at the age of 2.6 (0.6–16.5) years. Six of these patients had experienced kidney stone events and three had developed acute kidney injury (AKI) prior to allopurinol treatment. During 18.9 (1.7–31.5) years of pharmacotherapy, stones occurred in two patients and AKI in three. Six adult patients started allopurinol treatment, 200 (100–300) mg/day, at age 29.8 (20.5–42.4) years. Five of these patients had experienced 28 stone episodes and AKI had occurred in two. Stone recurrence occurred in four patients and AKI in two during 11.2 (4.2–19.6) years of allopurinol therapy. Lack of adherence and insufficient dosing contributed to stone recurrence and AKI during pharmacotherapy. At latest follow-up, estimated glomerular filtration rate (eGFR) was 114 (70–163) and 62 (10–103) mL/min/1.73 m^2 in those who initiated treatment as children and adults, respectively. All three patients with CKD stages 3–5 at the last follow-up were adults when pharmacotherapy was initiated.

Conclusion Timely diagnosis and treatment of APRT deficiency decreases renal complications and preserves kidney function.

Keywords Kidney stones · Nephrolithiasis · Chronic kidney disease · Kidney failure · Crystal nephropathy · Kidney transplantation · Allopurinol · Children

Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600) is a rare autosomal recessive disorder of adenine metabolism that leads to kidney stones and progressive chronic kidney disease (CKD) [1–3]. In the absence of APRT activity, adenine is converted by xanthine oxidoreductase (XOR; xanthine dehydrogenase/oxidase) to the poorly soluble 2,8-dihydroxyadenine (DHA) which is excreted in the urine in excessive amounts. Affected individuals develop kidney stones and/or progressive CKD due to DHA crystal nephropathy [2, 3]. At least 20% of reported adult patients developed end-stage renal disease (ESRD), most commonly in the fifth decade of life [3].

Radiolucent kidney stones are by far the most commonly reported childhood manifestation of APRT deficiency [1, 4]. Other well-known clinical features in children include reddish-brown diaper stains in young children, acute kidney injury (AKI) due to bilateral obstructive DHA calculi, recurrent urinary tract infections, and hematuria [1, 2, 5–7]. Many individuals remain asymptomatic for years or even decades and, not uncommonly, the disease is recognized by family screening or incidentally when DHA crystals (Fig. 1) are

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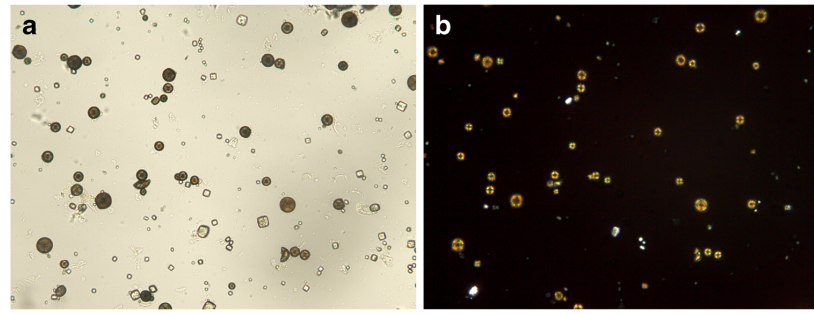

Fig. 1 Urinary 2,8-dihydroxyadenine crystals. **a** The characteristic medium-sized crystals are brown with a dark outline and central spicules (original magnification × 400). **b** The same field viewed with polarized light microscopy shows that the small- and medium-sized crystals appear yellow in color and produce a central Maltese cross pattern (original magnification × 400)

detected on routine urine microscopy [1, 4]. The diagnosis is confirmed by absent APRT enzyme activity in red cell lysates and/or the identification of biallelic pathogenic variants in the *APRT* gene [8].

The outcomes of kidney disease in APRT deficient patients who from early childhood have received treatment with an XOR inhibitor, mostly allopurinol and more recently febuxostat, appear to be favorable [2, 3]. To date, only one published study has focused on the disease presentation in children [4] and limited data exist on the long-term evolution of kidney function in treated and untreated individuals [3]. The aim of the present study was to compare the disease course in patients with APRT deficiency who initiated XOR inhibitor treatment prior to the age of 18 years with those who did not receive pharmacologic therapy until adulthood.

Methods

Ethical approval

The study was approved by the National Bioethics Committee of Iceland (NBC 09-072) and the Icelandic Data Protection Authority. All living patients or their legal guardians gave a written informed consent for participation in the study. The clinical and research activities reported herein are consistent with the principles of the Declaration of Helsinki.

Study design

Description of registry data and definitions, which have previously been described by our group [3], is briefly outlined below. The present study is based on extensive observational data from patients in the APRT Deficiency Registry of the Rare Kidney Stone Consortium (RKSC, http://www.rarekidneystones.org/). Included in the current study were 21 (35%) of the 60 enrolled patients, who all presented with clinical manifestations of APRT deficiency or were diagnosed before the age of 18 years. The latest follow-up was on December 31, 2017. Limited data on 20 of the 21 patients have previously been reported [3].

Clinical data

The registry data included age at initial disease presentation and at diagnosis; clinical manifestations; laboratory tests, including serum creatinine (SCr) values, results of urine microscopy, including assessment of DHA crystalluria, APRT enzyme activity, *APRT* genotype, imaging studies, kidney biopsies and kidney stone analyses; XOR inhibitor treatment, surgical management of kidney stones and renal replacement therapy (RRT); and causes of death.

Definitions

Symptomatic kidney stones were defined as either a patient-reported stone passage or abdominal pain associated with hematuria and/or a urinary tract stone confirmed with an imaging study. Kidney stones identified by imaging only were considered asymptomatic. Stone recurrence was defined as the detection of a stone in a patient with a history of kidney stones who has previously been shown to be stone-free by medical imaging. Glomerular filtration rate estimates (eGFR) were derived from SCr using the modified Schwartz (CKiD) equation in children [9] and the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation in adults [10]. For calculation of eGFR in children, height measurements were either obtained from the registry or extrapolated from data points on individual patient's growth charts when recent measurements were not available. Non-standardized SCr values were reduced by 5% before eGFR was calculated, as previously described [11]. All patients were considered to have CKD based on presumed kidney damage associated with DHA crystal deposition. The Kidney Disease Improving Global Outcomes (KDIGO) classification system was used to stage CKD [12]. Serial SCr values were used to identify episodes of AKI, defined according to the KDIGO criteria as an increase in SCr of ≥ 26.5 μmol/L (≥ 0.3 mg/dL)

within 48 h or ≥ 1.5 times baseline, presumed to have occurred over seven days [13].

Statistical analysis

Statistical analyses were performed using SPSS (IBM SPSS Statistics version 21.0, Armonk, NY, USA). Data are presented as number, percentage, and median (range). For the purpose of eGFR analyses, individuals receiving RRT were assigned an eGFR of 10 mL/min/1.73 m^2. Slopes of eGFR and staging of CKD were based on all eGFR calculations available for each patient, excluding values obtained during episodes of AKI. Slopes and eGFR trajectory lines were calculated by fitting a linear regression line through available eGFR values for the 18 patients who had more than one SCr value available in our registry. The long-term renal outcomes (kidney stones, AKI, CKD, ESRD, and eGFR slope) of patients who were placed on XOR inhibitor treatment prior to 18 years of age was compared with those not receiving pharmacotherapy until they had reached adulthood (≥ 18 years of age).

Results

Patient characteristics and presenting features

Of the 21 patients in the registry who either presented with clinical manifestations of APRT deficiency or were diagnosed with the disorder at < 18 years of age, 16 were from Iceland, one from Austria, one from Italy, one Norwegian child of Turkish descent, one from the USA, and one from India. Twelve (57%) patients were females and the age at first presentation was 1.6 (0.2–16.5) years. Clinical characteristics of the patients at presentation are described in Table 1. Reddish-brown diaper spots and kidney stones were the most common presenting features, occurring in 13 (62%) and 11 (52%) patients, respectively. The median (range) age at the first symptomatic stone event among the 11 patients was 3.4 (0.3–6.9) years. Three patients presented with AKI due to obstructive stone disease, one of whom required transient hemodialysis. No patient had reached CKD stages 3–5 at the time of presentation, while at diagnosis four had progressed to CKD stage 3 or above, including one who had initiated RRT for ESRD.

Diagnosis of APRT deficiency

The diagnosis of APRT deficiency was initially suggested by detection of urinary DHA crystals in 18 patients and by stone analysis in two. The diagnosis was confirmed by genetic testing ($n = 19$) and/or absent APRT activity ($n = 4$) in all cases. All of the 16 Icelandic patients shared the same biallelic variant, c.194A>T (p.Asp65Val), in the *APRT* gene, whereas the Austrian and the US patients were found to be homozygous for c.400 + 2dup (IVS4 + 2insT), resulting in aberrant splicing and deletion of exon 4. The patient from India was homozygous for the c.2T>C (p.Met1?) variant in exon one, affecting the translation initiation codon.

The age at diagnosis was 4.8 (0.6–42.4) years, while a diagnostic delay of 10.4 (0.6–39.2) years occurred in 13 patients following their first stone event ($n = 10$) and/or detection of diaper stains ($n = 3$). The diagnostic delay was caused by misidentification of urinary DHA crystals in eight patients, erroneous kidney stone analysis (presumed uric acid calculi) in two, absence of stone analysis in two cases, and failure to recognize diaper stains in one case.

Treatment and outcomes

The timeline of clinical events, diagnosis and XOR inhibitor treatment for each patient with APRT deficiency is shown in Fig. 2. In 14 patients, the diagnosis was made and allopurinol treatment commenced in childhood, in the daily dose of 100 (25–200) mg or 6.0 (3.0–20.8) mg/kg. Six of these 14 patients had already experienced eight kidney stone events when XOR inhibitor treatment was initiated at the age of 2.6 (0.6–16.5) years. After 18.9 (1.7–31.5) years of drug therapy, one additional patient, who was known to be poorly adherent to the treatment, had experienced an incident kidney stone, and one receiving insufficient allopurinol dose, 200 mg per day, had suffered stone recurrence. Both these patients had DHA crystalluria confirmed by urine microscopy. Three patients required stone removal procedures, one at the time of presentation and two patients underwent two urological interventions each shortly following diagnosis. One patient underwent unilateral nephrectomy due to irreversible kidney damage caused by an obstructing stone. Four of the 14 patients had experienced AKI episodes, one prior to diagnosis and treatment initiation and three following onset of pharmacologic therapy. Two of these patients had been prescribed insufficient doses (75–200 mg/day) of allopurinol and one had been placed on allopurinol 400 mg/day but was non-adherent to the treatment as evidenced by DHA crystalluria. None of the 14 patients who were started on pharmacotherapy in childhood had developed CKD stages 3–5 at the time of the last follow-up. Five of these patients had not developed any clinical events at the last follow-up.

Six patients first received allopurinol therapy as adults, in a daily dose of 200 (100–300) mg. Five had experienced a total of 28 kidney stone events at the age of 29.8 (20.5–42.4) years when they were diagnosed with the disorder and allopurinol treatment was started (Fig. 2). After 11.2 (4.2–19.6) years of pharmacotherapy, seven stone recurrences had occurred in four of these patients— three were non-adherent with drug treatment and one was receiving an insufficient allopurinol

Table 1 Clinical characteristics of children ($n = 21$) with adenine phosphoribosyltransferase deficiency at the time of first presentation, classified according to whether the diagnosis of the disorder was made before or after the age of 18 years

	Diagnosis at age < 18 years (n = 15)	Diagnosis at age ≥ 18 years (n = 6)
Females	8 (53)	4 (67)
Age at first presentation, years	1.5 (0.2-16.5)	4.4 (0.5-7.1)
Age at diagnosis, years	2.5 (0.6-16.5)	35.5 (20.5-42.4)
Diagnostic delay, years	1.2 (0.6-10.4)	29.2 (20.1-39.2)
Reddish-brown diaper stain in infancy	12 (80)	1 (17)
Kidney stones	7 (47)	4 (67)
Acute kidney injury	2 (13)	1 (17)
Chronic kidney disease stages 3-5	0	0
Asymptomatic crystalluria	2 (13)	1 (17)

Data are presented as number (percentage) and median (range)

dose of 200 mg per day. All these patients had DHA crystals detected on urine microscopy. One patient who initiated treatment at the age of 20 years remained free of clinical events. Stone removal procedures were carried out in two patients, one of whom underwent 18 procedures before diagnosis, while the other had three surgical interventions performed following diagnosis of the disorder. One patient underwent partial nephrectomy in adulthood due to severe kidney damage caused by ureteral obstruction. Three of the six patients suffered six episodes of AKI, five before pharmacologic therapy was initiated and one following prescription of allopurinol treatment. The patient who developed AKI during pharmacotherapy was poorly adherent to the treatment and the episode occurred during a symptomatic kidney stone event. Three of these six patients had progressed to CKD stages 3–5 at latest follow-up, one had stage 3A, one stage 3B, and one patient had reached stage 5 CKD requiring RRT. One patient had improved from stage 3A to stage 2. At the last follow-up, five of the six patients who initiated treatment with an XOR inhibitor as adults were receiving allopurinol in a daily dose of 300 (200–400) mg.

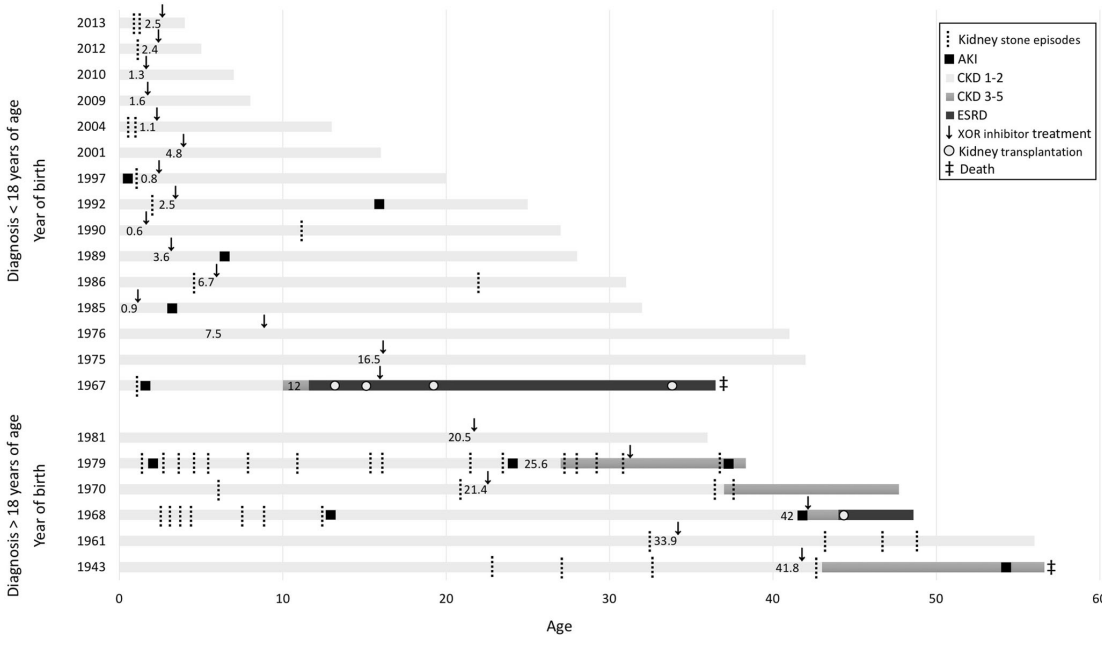

Fig. 2 Timeline of major renal manifestations and treatment in patients with adenine phosphoribosyltransferase deficiency according to age below or above 18 years at diagnosis. Year of birth is shown on the y-axis. Age at initiation of xanthine oxidoreductase inhibitor (allopurinol or febuxostat) treatment is indicated within each patient's timeline. The patient born in the year 1967 did not receive drug treatment prior to progressing to kidney failure. *CKD* chronic kidney disease, *ESRD* end-stage renal disease

Febuxostat 80 mg/day had been prescribed in one patient, in whom allopurinol therapy was discontinued due to an adverse reaction (pruritus). One patient in this group died at the age of 56 years from breast cancer.

At disease presentation, the eGFR in the 21 patients was 83 (35–165) mL/min/1.73 m^2, despite the inclusion of several patients with AKI. Eighteen patients who had two or more SCr values available were included in the assessment of the evolution of kidney function, excluding two patients who only had a single SCr value and the patient who developed ESRD at the age of 11 years. At the last follow-up, the eGFR was 114 (70–163) mL/min/1.73 m^2 and the median eGFR slope (n = 12) was 0.04 (− 5.28 to 2.79) mL/min/1.73 m^2 per year in the group of patients who started allopurinol treatment in childhood. As these 14 patients only had SCr values available after they had started pharmacotherapy, the potential effect of XOR inhibitor treatment on kidney function could not be studied. For the six patients who initiated drug treatment as adults, the eGFR at the last follow-up was 62 (10–103) mL/min/1.73 m^2 and the median eGFR slope (n = 6) was − 0.47 (− 1.32 to 0.38) mL/min/1.73 m^2 per year. Four of these six patients had SCr values available prior to and after initiation of pharmacotherapy, all of whom displayed improvement in kidney function following onset of treatment. The median eGFR slope was − 0.47 (− 1.23 to − 0.26) before starting therapy and 0.85 (0.29 to 5.13) mL/min/1.73 m^2 per year after treatment initiation. The results for both patient groups are depicted in Fig. 3.

The case of the patient who was diagnosed with APRT deficiency at the age of 11 years but did not receive pharmacotherapy until after his second kidney transplant warrants more in-depth description. The boy first presented with AKI at the age of 18 months due to bilateral obstructive stone disease requiring three percutaneous stone removal procedures and transient hemodialysis. By the time of diagnosis at 11 years of age, the boy had already progressed to ESRD. Due to unknown reasons, allopurinol treatment was not initiated at that time. He later received four kidney transplants, at age 14, 16, 19, and 34 years. Treatment with allopurinol, 300 mg/day, was started at 16 years of age when a biopsy disclosed a recurrence of DHA nephropathy in his second renal allograft, resulting in graft loss three years later. The third kidney transplant functioned for eight years with allopurinol therapy. Following a fourth deceased donor kidney transplant, the patient died from sepsis and liver failure associated with intestinal perforation at the age of 36 years.

Discussion

This study of patients with APRT deficiency presenting in childhood demonstrates improved long-term renal outcome in those initiating treatment with an XOR inhibitor before 18 years of age, even when started late in childhood. A substantial proportion of those who experienced significant diagnostic delay and did not receive pharmacotherapy until after the age of 18 years had progressive CKD, which was not seen in the much larger group of patients who started treatment early. Furthermore, almost all patients who initiated pharmacologic treatment after 18 years of age experienced multiple recurrent kidney stone episodes prior to onset of therapy, which were much less frequent in those commencing treatment prior to the age of 18 years. Interestingly, poor treatment adherence and/or insufficient drug doses contributed to all recurrent kidney stone and AKI episodes that occurred in both patient groups following initiation of pharmacotherapy. The

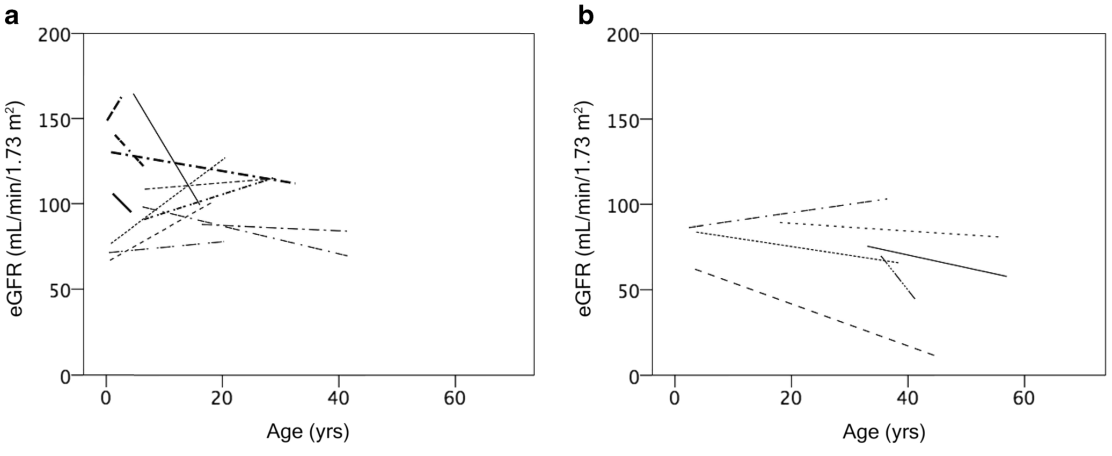

Fig. 3 Changes in estimated glomerular filtration rate (eGFR) over time in patients (**a**, n = 12) who received xanthine oxidoreductase inhibitor treatment before the age of 18 years and in those (**b**, n = 6) who initiated drug therapy in adult life. eGFR trajectory lines were created by fitting a linear regression line through all available eGFR values for each patient, excluding episodes of acute kidney injury (AKI)

most common presenting features of APRT deficiency were reddish-brown diaper spots in infancy and kidney stones, while AKI and asymptomatic DHA crystalluria were less commonly observed.

Nephrolithiasis was the presenting feature in half of our patient population and almost 60% had developed kidney stones at the time of diagnosis. This is lower than previously described in a study from France [4], where 80% of patients presented with a symptomatic kidney stone event, subsequently leading to a diagnosis of APRT deficiency. The higher proportion of patients with kidney stones as the presenting feature in the French cohort may have resulted from failure to identify other less apparent manifestations of the disorder in that study, such as diaper stains, lower urinary tract symptoms, and asymptomatic crystalluria. As expected, a much higher stone burden was seen in the group of patients in our study who first initiated drug treatment in adult life, and most of these stones were formed before the diagnosis of APRT deficiency was made and pharmacotherapy initiated. Approximately one third of the patients experienced stone recurrence after the diagnosis of APRT deficiency, despite treatment with allopurinol or febuxostat, which is similar to what was observed in the French study [4]. Lack of adherence to treatment or insufficient allopurinol dosing appears to have contributed to new stone formation in all the patients in our study.

Acute kidney injury episodes caused by bilateral obstructive stone disease was the presenting feature in three patients, which is similar to the findings in the previously mentioned French study [4]. However, episodes of AKI were more commonly observed in our patient cohort during the follow-up period than previously described, likely due to the advantage of the comprehensive SCr dataset in our registry which allowed for a more detailed analysis of changes in kidney function than has been possible in other published studies. The proportion of patients with AKI at diagnosis in the French study may have been higher than recognized in view of the large number of patients who experienced marked improvement in kidney function during the first few months of allopurinol therapy [4]. Diagnostic delay or inadequate pharmacologic therapy due to either poor adherence or insufficient XOR inhibitor dosing appears to have contributed to all the AKI episodes observed in the current study. Furthermore, episodes of AKI may have contributed to progression of CKD among patients who experienced significant delay in diagnosis and treatment.

Chronic kidney disease stage 3 or above did not develop in any of the patients in our study who initiated treatment at an early age, in contrast to two thirds of those who did not start pharmacotherapy until in adult life. These findings are similar to those in the French cohort, in which none of the patients diagnosed in childhood developed advanced CKD [4]. Although their study period and follow-up time was considerably shorter than in the present study, this finding emphasizes the importance of early institution of pharmacotherapy.

Our extensive set of SCr values allowed a detailed analysis of the evolution of kidney function over time. In the group that initiated allopurinol treatment in childhood, an improvement in eGFR was observed followed by preservation of kidney function, which was within normal limits at the end of the study period. This finding is in concert with the results of the previously mentioned French study, in which all patients started pharmacotherapy at a relatively young age [4]. Interestingly, the patients who experienced a prolonged diagnostic delay experienced improvement in eGFR after initiation of treatment with an XOR inhibitor, but the eGFR level remained much lower than in the group receiving pharmacotherapy at an early age. The initial eGFR rise on treatment can possibly be explained by clearance of intratubular DHA crystals, while the subsequent decline in kidney function is likely caused by chronic DHA crystal nephropathy which is characterized by chronic tubulointerstitial inflammation, fibrosis, and progressive nephron loss.

The transplanted kidney appears to be particularly susceptible to DHA crystal nephropathy which tends to recur early in the post-transplant period and is frequently severe, leading to shortened allograft survival, even despite XOR inhibitor therapy [14]. This phenomenon is well illustrated by the case of the unfortunate patient who had already progressed to ESRD at the time of diagnosis of APRT deficiency when he was 11 years of age, and who later underwent a total of four kidney transplantation procedures. This case also underscores the phenotypic variability of APRT deficiency as the disease was unusually aggressive, resulting in ESRD in childhood.

Diagnostic delay is an important determinant of adverse renal outcomes and, in contrast to many other rare causes of CKD, effective treatment of APRT deficiency is available. The most frequent reasons for missed diagnosis are lack of awareness of the disease resulting in failure to recognize urinary DHA crystals, misidentification of radiolucent kidney stones as uric acid calculi, and confusion of renal histopathological findings with other forms of crystal nephropathy [3]. The observed variability in disease expression between individuals, as illustrated by only one third of our overall study population having symptomatic disease during the first two decades of life, makes the diagnosis even more difficult. Reddish-brown diaper stains in infancy, reported in approximately 60% of patients in the current study, is an important presenting feature that should be taken seriously [3]. Indeed, APRT deficiency must be considered in all such patients and in the differential diagnosis of kidney stones and CKD in children and young adults. The lack of awareness of rare diseases frequently results in unacceptable delay in diagnosis and treatment, often with grave consequences.

Timely institution of treatment with an XOR inhibitor is extremely important as both allopurinol and febuxostat have

been shown to effectively reduce urinary DHA excretion [15]. The currently recommended daily dose of allopurinol in children is 10 mg/kg and at least 400–600 mg in adults and, if well tolerated, this dose should be maintained, even in individuals with advanced CKD [3, 15]. The currently recommended starting dose of febuxostat is 80 mg/day in adults as a single daily dose [15], but no dosing recommendations are currently available for children. Both drugs are usually well tolerated. Monitoring of pharmacotherapy, which is important to ensure adequate dosing and adherence, has traditionally been carried out using urine microscopy where the disappearance of urine DHA crystals has been considered indicative of adequate treatment. However, detection of crystalluria may not be reliable enough for monitoring of drug treatment. Our group has recently developed a urinary DHA assay using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS), which has the potential to greatly facilitate both clinical diagnosis and therapeutic monitoring of pharmacotherapy in patients with APRT deficiency [16]. As the current study is based on observational registry data only, we did not have the opportunity to include the novel urinary DHA assay in the current work.

Most of the cases in our study come from Iceland where all patients are homozygous for the same missense mutation (p.Asp65Val), presumably due to a founder effect. It is noteworthy that no genotype-phenotype correlation has been identified in APRT deficiency, a finding that would be expected as all known pathogenic *APRT* variants completely obliterate the enzyme activity. Thus, the phenotypic differences observed must be due to other factors.

Our study has several strengths, including the comprehensive dataset. At the time of analysis, the registry contained more than 600 patient-years of clinical information on individuals included in the present study, making this the largest pediatric clinical dataset available and the longest observation time reported to date. The abundance of available SCr values allowed for the identification of AKI episodes and detailed characterization of the long-term evolution of kidney function. The main limitation is the retrospective nature of part of the dataset, thus lacking standardization.

In conclusion, our data clearly demonstrate a much more favorable renal outcome in patients with APRT deficiency who are diagnosed and treated early. The diagnostic evaluation of young patients with stone disease or CKD of unknown etiology at any age should include screening for rare disorders such as APRT deficiency. Timely pharmacologic therapy appears to reduce stone burden and slow or possibly prevent the progression of CKD, even in severely affected individuals. Our results further highlight the significant variability in the clinical characteristics of APRT deficiency which may include a long asymptomatic period, making the diagnosis extremely difficult. The relatively frequent occurrence of advanced CKD and even kidney failure at the time of diagnosis is concerning and suggests a lack of familiarity with this treatable condition.

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Compliance with ethical standards

The study was approved by the National Bioethics Committee of Iceland (NBC 09-072) and the Icelandic Data Protection Authority. All living patients or their legal guardians gave a written informed consent for participation in the study. The work described has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Conflict of interest The authors declare that they have no conflict of interest.

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Paper V

Kidney Transplant Outcomes in Patients With Adenine Phosphoribosyltransferase Deficiency

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Background. Adenine phosphoribosyltransferase (APRT) deficiency is a rare, hereditary cause of kidney stones and chronic kidney disease (CKD) which is characterized by 2,8-dihydroxyadenine renal parenchymal crystal deposition. The aim of this study was to examine outcomes of kidney transplantation in APRT deficiency patients. **Methods.** Included were 13 patients in the APRT Deficiency Registry of the Rare Kidney Stone Consortium, 2 from Westmead Hospital in Sydney, Australia, and 2 from Necker Hospital in Paris, France. The CKD-EPI and CKiD equations were used to calculate glomerular filtration rate estimates. Allograft survival was analyzed employing the Kaplan-Meier method. The Wilcoxon-Mann-Whitney test was used to compare alllograft outcomes according to xanthine oxidoreductase (XOR) inhibitor treatment status at transplantation. **Results.** Seventeen patients (9 females) received 22 kidney transplants. Age at first transplantation was 47.2 (14.9–67.0) years. Ten patients received XOR inhibitor therapy pretransplant (11 allografts), while 8 patients did not receive such treatment before transplantation (11 allografts). Two-year allograft survival was 91% and 55% in the 2 groups, respectively (P = 0.16). The median (range) estimated glomerular filtration rate at 2 years posttransplant was 61.3 (24.0–90.0) mL/min/1.73 m^2 when XOR inhibitor therapy was initiated before transplantation, and 16.2 (10.0–39.0) mL/min/1.73 m^2 (P = 0.009) when such treatment was not administered pretransplant. **Conclusions.** Kidney allograft outcomes are good in APRT deficiency patients beginning XOR inhibitor therapy pretransplant. Delay in such treatment is a major cause of premature graft loss in these patients. Increased awareness among clinicians is imperative, promoting early diagnosis of APRT deficiency and pharmacotherapy initiation before kidney transplantation.

(*Transplantation* 2020;00: 00–00)

INTRODUCTION

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600) is a rare autosomal recessive disorder of purine metabolism. Absence of a functional APRT enzyme results in the conversion of adenine to 2,8-dihydroxyadenine (DHA), catalyzed by xanthine oxidoreductase (XOR;

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The authors declare no conflicts of interest.

R.P., V.O.E., D.S.M., and O.S.I. participated in conception and study design. H.L.R., I.M.S.A., J.L., and M.D. were involved in data acquisition. H.L.R. and O.S.I. were involved in statistical analysis. H.L.R., I.M.S.A., O.S.I., R.P., V.O.E., J.L., M.D., B.K., and D.S.M. participated in critical review of the results. H.L.R. drafted the paper. O.S.I., R.P., D.S.M., and V.O.E. participated in supervision and mentorship. Each author contributed to the important intellectual content during manuscript writing and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved. V.O.E. and R.P. take responsibility that this study has been reported honestly, accurately, and transparently; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned have been explained.

Previous Presentation: Part of this work was presented in an abstract form at the American Society of Nephrology Kidney Week, November 11–16, 2014; Philadelphia, PA.

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also known as xanthine oxidase/dehydrogenase). DHA is poorly soluble in the urine at physiological pH, leading to precipitation and formation of recurrent radiolucent kidney stones and crystal nephropathy. DHA nephropathy is characterized by widespread DHA crystal deposits, predominantly in the form of large aggregates in the tubular lumen, and smaller crystals in tubular epithelial cells and the interstitium, accompanied by tubulointerstitial inflammation and fibrosis.[1,2]

Chronic kidney disease (CKD), sometimes without a history of nephrolithiasis, is a common presenting feature of APRT deficiency in adults, and 15%–20% of patients have already reached end-stage kidney disease (ESKD) at the time of diagnosis.[3-5] Furthermore, the disorder is often first recognized in the setting of disease recurrence following kidney transplantation. Three case series[2,6,7] and several single-patient reports[8-13] of kidney transplantation in patients with APRT deficiency have previously been published, commonly describing disease recurrence and unfavorable outcomes.

Treatment with the XOR inhibitors, allopurinol or febuxostat, reduces DHA production, and in turn, its renal excretion and can prevent or halt the progression of crystal nephropathy,[14] preserving and even improving kidney function.[3] Allopurinol is frequently prescribed in the daily dose of 200–300 mg/day although higher doses (400–600 mg) are likely needed to minimize or prevent recurrent kidney stone formation and renal parenchymal DHA crystal deposition in the native kidney.[2,3] Early disease recurrence in transplanted kidneys and accelerated allograft loss has been noted in both untreated and treated patients, suggesting that higher doses of allopurinol may be needed to preserve graft function.[6,8]

Limited data exist on the outcome of kidney transplantation in patients with APRT deficiency and the role of XOR inhibitor treatment in preserving kidney allograft function. The aim of this study was to examine the outcome of kidney transplantation in patients with APRT deficiency, in particular the effect of XOR inhibitor treatment status at the time of transplantation.

MATERIALS AND METHODS

Ethics

The study was approved by the National Bioethics Committee of Iceland (NBC 09–072) and the Icelandic Data Protection Authority. All living patients consented to participation in the study. The clinical and research activities reported herein are consistent with the principles of the Declarations of Helsinki and Istanbul.

Study Population

Thirteen (21%) of the 61 patients enrolled in the APRT Deficiency Registry of the Rare Kidney Stone Consortium (http://www.rarekidneystones.org/) who had undergone kidney transplantation were included in the study. These patients were from Austria (n = 1), Iceland (n = 2), India (n = 1), Italy (n = 2), and the United States (n = 7). In addition, 2 patients from Australia and 2 patients from France who had received a kidney transplant were included. Transplant outcome data on 8 of these 17 patients have previously been reported.[2,6,7,11,15] The diagnosis of APRT deficiency was confirmed by the identification of biallelic pathogenic *APRT* mutations or completely abolished APRT enzyme function.

Clinical Data

Sources of data included the APRT Deficiency Registry of the Rare Kidney Stone Consortium and individual patient records at participating hospitals through June 2019. In addition to basic demographic information, the following data were collected: age and clinical characteristics at presentation and diagnosis of APRT deficiency; age at onset of renal replacement therapy; dialysis and modality before kidney transplantation; age at transplantation, number of kidney allografts and donor type; date and cause of graft loss; date and cause of death; immunosuppressive therapy; treatment with allopurinol or febuxostat, including dose before and after transplantation; laboratory studies including serum creatinine (SCr) measurements, results of urine microscopy, including assessment of DHA crystals, *APRT* genotype, APRT enzyme activity measurement results, results of urological imaging; and kidney allograft biopsy findings.

Glomerular filtration rate estimates (eGFR) were calculated from SCr using the modified Schwartz (CKiD) equation in children[16] and the CKD Epidemiology Collaboration equation in adults.[17] As previously described, nonstandardized SCr values were reduced by 5% before eGFR was calculated.[18] Staging of CKD was according to the Kidney Disease: Improving Global Outcomes classification system.[19] Graft loss was defined as initiation of dialysis, retransplantation, or death with a functioning graft. Delayed graft function was defined as the need for dialysis in the first posttransplant week. Posttransplant acute kidney injury (AKI) was defined according to the Kidney Disease: Improving Global Outcomes AKI criteria.[20]

Analytical Considerations

Data are presented as number, percentage and median (range). In the analysis, allografts were grouped according to whether or not the patients were receiving XOR inhibitor treatment before kidney transplantation, and the Wilcoxon-Mann-Whitney test and Fisher's exact test were used to compare the group of patients who initiated XOR inhibitor treatment pretransplant to those who first started such treatment following transplant surgery. Patients receiving dialysis were assigned an eGFR of 10 mL/min/1.73 m^2. Kaplan-Meier analysis was used to estimate death-censored allograft survival and groups were compared using the log-rank test. Statistical analyses were performed using SPSS (IBM SPSS Statistics version 21.0, 2012, Armonk, NY).

RESULTS

Clinical Characteristics of Patients at Diagnosis of APRT Deficiency

Of 17 patients who had undergone kidney transplantation, 9 (53%) were females.

The clinical characteristics of the patients at the time of diagnosis of APRT deficiency are presented in Table 1. The median age at diagnosis was 44.5 (11.9–67.9) years, by which time 13 of the 17 patients (76%) had initiated renal replacement therapy for ESKD. Of the 17 patients, 15 had a diagnostic delay of 7.8 (1.1–47.9) years following presentation of APRT deficiency. Eleven patients (65%) were diagnosed with APRT deficiency before the first kidney transplantation; 1 of the 11 patients had CKD stage 3a, 3 had reached CKD stages 4–5, and 7 were already on hemodialysis. The diagnosis of APRT deficency was suggested by detection of

TABLE 1.

Clinical characteristics at the time of diagnosis of APRT deficiency

Patient	Sex	History of kidney stones	Age at diagnosis (y)	Diagnostic delay (y)	Kidney function (eGFR, mL/min/1.73 m^2)	Age at kidney biopsy (y)	Original native kidney biopsy findings
1	M	No	62	1.1	ESKD	62	DHA crystal nephropathy; global glomerulo sclerosis (21 of 45 glomeruli), severe, interstitial fibrosis and arteriosclerosis
2	F	No	43	5.1	ESKD	38	Crystals thought to be consistent with primary hyperoxaluria
3	M	Yes	43	11.1	ESKD		NA
4	F	Yes	68	47.9	ESKD		NA
5	F	No	52	7.5	ESKD		NA
6	F	No	52	6.0	ESKD	45	Interstitial inflammation with inflammatory infiltrate; refractory golden-brown crystalline material seen
7	F	Yes	59	24.0	ESKD		NA
8	M	Yes	12	10.4	ESKD		NA
9	F	Yes	49	7.8	9	42	Tubulointerstitial fibrosis; presumed calcium oxalate crystal deposits
10	M	Yes	42	39.2	17	42	DHA crystals; interstitial inflammation
11	F	Yes	45	1.4	ESKD		NA
12	F	No	21	0	ESKD	21	Tubulointerstitial nephritis with extensive calcium oxalate deposits
13	M	No	40	4.7	ESKD	35	Chronic interstitial nephritis; crystals seen but not identified
14	F	Yes	24	4.0	54		NA
15	M	Yes	50	20.0	ESKD	50	Small number of scattered tubules contain intraluminal polarizable crystals believed to be calcium oxalate
16	M	No	43	0.2	ESKD	42	DHA crystals with advanced glomerulosclerosis, tubular atrophy and interstitial fibrosis
17	M	Yes	52	20.0	6		NA

APRT, adenine phophoribosyltransferase; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; NA, not available.

DHA crystals on urine microscopy in 2 of these 11 cases, kidney stone analysis in 1 case and renal histological findings of crystal nephropathy in 5 individuals. Three patients were diagnosed through family screening of index cases, 2 of whom had a personal history of kidney stone disease.

The diagnosis of APRT deficiency was not made until after kidney transplantation in 6 patients. In 5 of the patients, the diagnosis was made following their first transplantation and in 1 individual after the failure of 2 kidney allografts. The presumed cause of ESKD in these 6 patients was primary hyperoxaluria (n = 2), chronic interstitial nephritis (n = 2), kidney stone disease (n = 1), and CKD of unknown causes (n=1).

Pharmacotherapy of APRT Deficiency and Kidney Allograft Outcomes

The 17 patients received 22 kidney allografts as outlined in Table 2. The first kidney transplantation was carried out at the median age of 47.2 (14.9–67.0) years, 1.8 (0.7–13.5) years after reaching ESKD in 14 cases, while 3 patients underwent preemptive transplantation. The maintenance immunosuppression regimen consisted of a calcineurin inhibitor (18 allografts) or sirolimus (1 allograft) in conjunction with mycophenolate mofetil and steroids; 2 patients received cyclosporine and azathioprine without steroids and azathioprine was the sole immunosuppressive agent in 1 case.

Ten patients received treatment with allopurinol 200 (100–300) mg/day for 2.6 (0.1–15.6) years before the transplantation of 11 allografts (Table 2). Delayed graft function was noted in the case of 3 kidney allografts, all from deceased donors, and AKI in 4 allografts, occurring within the first posttransplant week in 2 cases. A transplant biopsy was obtained from 8 allografts in 7 patients (Table 3). Recurrence of DHA crystal nephropathy (Figure 1) was found in 3 of these allografts, at 10 days, 8 weeks, and 4 months after transplantation. At the time of biopsy, 1 of the patients (No. 16) was taking allopurinol in the daily dose of 150 mg, while the remaining 2 allografts were from the same patient (No. 12) who was prescribed allopurinol 300–600 mg/day before and following both transplants, though significant medication nonadherence following the first transplant was acknowledged. Renal histopathological findings suggestive of acute rejection were found in 2 cases, immediately posttransplant in 1 case and 1 month following transplant surgery in the other: no crystals were observed. One patient (No. 13) died with a functioning graft 4 months after transplantation from bacterial sepsis associated with peritonitis and 1 graft was lost nearly 8 years posttransplant with an allograft biopsy consistent with chronic allograft nephropathy (patient No. 8). No DHA crystals were detected.

Eight patients did not receive XOR inhibitor therapy before transplantation of 11 kidney allografts (Table 2). In the case of 9 allografts, XOR inhibitor treatment was initiated at a median of 0.1 (0.1–2.9) years posttransplant. Delayed graft function was noted in 2 allografts, both coming from deceased donors, and AKI was observed in 7 transplants. Recurrence of DHA nephropathy was observed in all 10 allograft biopsies that were performed (Table 3), which was significantly more common than in those receiving treatment pretransplant (P = 0.004; Table 4). Four allografts belonging to 2 patients were lost due to disease recurrence, 1.3 (0.1–3.4) years posttransplant. Both patients were untreated before and after their first transplant, but initiated treatment with allopurinol 200 and 300 mg/day at 1 and 7

TABLE 2.

Pharmacotherapy of APRT deficiency and kidney allograft outcomes

Patient	Treatment before Tx	Age at RRT (y)	Graft number	Age at Tx (y)	Type of donor	Treatment with XOR inhibitor	Outcome	Last follow-up (y after Tx)	Latest eGFR (mL/min/1.73 m^2)
1	No	62	1	65	DD	Febuxostat 40 mg/d from 3 wk post-Tx	Recurrence of DHA nephropathy 3 wk post-Tx; functioning graft	1.6	25
2	No	38	1	39	DD	None	Graft lost due to recurrence of DHA nephropathy 1 mo post-Tx	0.1	ESKD
	No	39	2	42	DD	Allopurinol 200 mg/d from 6 mo post-Tx	Recurrence of DHA nephropathy 3 d post-Tx and allograft failure at 13 mo post-Tx; died while on dialysis	1.1	ESKD
3	No	42	1	43	DD	Allopurinol 300 mg/d from 4 mo post-Tx	Died with a functioning graft	0.5	41
4	No	65	1	67	DD	Allopurinol 150 mg/d from 3 wk post-Tx	Functioning graft	6.5	28
5	No	45	1	46	DD	Allopurinol 150 mg/d from 3 y post-Tx	Died with a functioning graft	5.4	29
6	No	50	1	51	DD	Allopurinol 300 mg/d from 5 wk post-Tx, later febuxostat 80 mg/d	Recurrence of DHA nephropathy 24 d post-Tx; graft lost after 19 mo	1.7	AKI/dialysis
7	No	44	1	58	LRD	Allopurinol 300 mg/d from 2 mo post-Tx	Functioning graft	9.7	37
8	No	12	1	15	DD	None	Graft lost 18 mo post-Tx	1.5	ESKD
	No	16	2	17	DD	Allopurinol 300 mg/d from d 28 post-Tx	Recurrence of DHA nephropathy 1 mo post-Tx; graft lost after 3.4 y	3.4	ESKD
	Yes	20	3	21	DD	Allopurinol 300 mg/d	Chronic allograft failure	7.8	ESKD
	No	27	4	35	DD	Allopurinol 150 mg twice-a-wk, from d 36 post-Tx	Recurrence of DHA nephropathy 1 mo post-Tx; died with a functioning graft	1.4	15
9	Yes	54	1	56	DD	Allopurinol 300 mg/d for 7 y pre-Tx, subsequently 600 mg/d	Functioning graft	13.3	50
10	Yes	46	1	46	LUD	Allopurinol 400 mg/d for 3 y pre-Tx	Functioning graft	4.4	71
11	Yes	43	1	47	DD	Allopurinol 300 mg/d for 2 y pre-Tx	Functioning graft	4.4	45
12	Yes	21	1	22	LRD	Allopurinol 300 mg/d for 1 y pre-Tx, later also febuxostat 120 mg/d	Graft lost due to recurrence of DHA nephropathy 5 y post-Tx	5.2	ESKD
	Yes	28	2	29	LUD	Allopurinol 600 mg/d and febuxostat 120 mg/d	Functioning graft	1.8	69
13	Yes	36	1	41	LRD	Allopurinol 200 mg/d for 1 mo pre-Tx, subsequently 400 mg/d	Died with a functioning graft	0.3	39
14	Yes	41	1	41	DD	Allopurinol 300-400 mg/d for 15 y pre-Tx, subsequently 600 mg/d	Functioning graft	3.9	80
15	Yes	50	1	53	LUD	Allopurinol for 2 y pre-Tx, subsequently 600 mg/d	Functioning graft	2.8	65
16	Yes	42	1	47	DD	Allopurinol 150 mg/d for 4 y pre-Tx, subsequently 300 mg/d and febuxostat 40 mg/d	Recurrence of DHA nephropathy 10 d post-Tx; functioning graft	1.0	60
17	Yes	66	1	66	DD	Allopurinol 200 mg/d for 12 y pre-Tx, subsequently 300 mg/d	Functioning graft	2.1	82

AKI, acute kidney injury; APRT, adenine phophoribosyltransferase; DD, deceased donor; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; LRD, living-related donor; LUD, living-unrelated donor; RRT, renal replacement therapy; Tx, kidney transplantation.

TABLE 3.

Clinical features at the time of kidney allograft biopsy and renal histopathological findings

Patient	Allograft	Delayed graft function	Time from Tx (mo)	Treatment with XOR inhibitor	Renal histolopathogical findings	eGFR (mL/min/1.73 m^2)
1	1	No	0.75	Febuxostat 40 mg/d	Numerous intratubular DHA crystal deposits	23
2	1	No	0.75	None	Extensive crystal deposits	Dialysis
	2	No	0.03	None	Acute tubular necrosis; no crystals	6
			0.1	None	Intratubular crystal deposits	25
			4	None	Extensive crystal deposits within the interstitium	29
			5	None	Mild focal interstitial fibrosis and tubular atrophy associated with mild inflammation; polarizable intraluminal crystals in several tubules	20
			12	Allopurinol 200 mg/d	Minimal interstitial fibrosis and focal tubular atrophy; occasional intratubular crystals	17
3	1	No	3.5	None	Intratubular crystal deposits	40
			4	None	Subjectively more crystals within the tubules	–
4	1	No	0.75	None	Diffuse intratubular crystals identified as DHA	8
5	1	No	36	None	Intratubular crystals assumed to be uric acid	20
			37	None	Diffuse crystal nephropathy with tubulointerstitial inflammatory infiltrates; crystals thought to be uric acid	10
			39	Allopurinol 150 mg/d	Persistence of intratubular brown crystals	23
6	1	Yes	0.1	None	No crystals detected	Dialysis
			0.75	None	Crystal deposits presumed to be oxalate	24
			1.2	Allopurinol 300 mg/d	Crystal deposits suspected to be DHA	26
			2.1	Allopurinol 300 mg/d	DHA crystals identified	43
			12	Febuxostat 80 mg/d	Crystals present (15%); severe tubular atrophy and interstitial fibrosis (>50%)	48
7	1	No	2	Allopurinol 300 mg/d	DHA crystals present	52
			12	Allopurinol 300 mg/d	No crystals detected	47
8	2	Yes	1	None	Multiple strongly birefringent DHA crystals in tubules	–
			12	Allopurinol 300 mg/d	Crystal deposits within tubules and interstitium	–
			19	Allopurinol 300 mg/d	No crystals detected	–
	3	Yes	1	Allopurinol 300 mg/d	No crystal detected	–
	4	No	0.25	None	No crystals detected	–
			1.2	None	Intratubular crystal deposits	–
			2	Allopurinol 150 mg/d × 2/wk	Multiple deposits of highly birefringent crystals	11
9	1	Yes	0.25	Allopurinol 300 mg/d	Acute tubular necrosis; no crystals	–
12	1	No	0.75	Allopurinol 600 mg/d[a]	–	51
			4	Allopurinol 300 mg/d[a]	Extensive DHA crystal deposits	20
			5	Allopurinol 450 mg/d[a]	20%–30% reduction in DHA crystals	20
			12	Allopurinol 300 mg/d, febuxostat 80 mg/d[a]	Some reduction in DHA crystals	28
			24	Allopurinol 300 mg/d, febuxostat 80 mg/d[a]	Rare crystals within the interstitium	24
			36	Allopurinol 300 mg/d, febuxostat 80 mg/d[a]	>100 crystals within the parenchyma	23
	2	No	2	Allopurinol 300 mg/d, febuxostat 80 mg/d	Rare intratubular DHA crystal deposits	69
13	1	No	3	Allopurinol 400 mg/d	No crystals detected	39
15	1	No	4	Allopurinol 600 mg/d	–	50
			12	Allopurinol 600 mg/d	–	64
16	1	Yes	0.3	Allopurinol 150 mg/d	DHA crystals in ~30% of tubules	Dialysis
			0.6	Allopurinol 300 mg/d	DHA crystals in ~40% of tubules	Dialysis
			2	Allopurinol 600 mg/d	Subjectively fewer DHA crystals	33
			12	Allopurinol 300 mg/d, febuxostat 80 mg/d	Scant DHA crystals	45
17	1	No	3	Allopurinol 300 mg/d	No crystals detected	99

The shaded area denotes allografts where xanthine oxidoreductase inhibitor treatment was not initiated before kidney transplantation.
[a]Reported nonadherence to XOR inhibitor treatment.
DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; Tx, kidney transplantation; XOR, xanthine oxidoreductase.

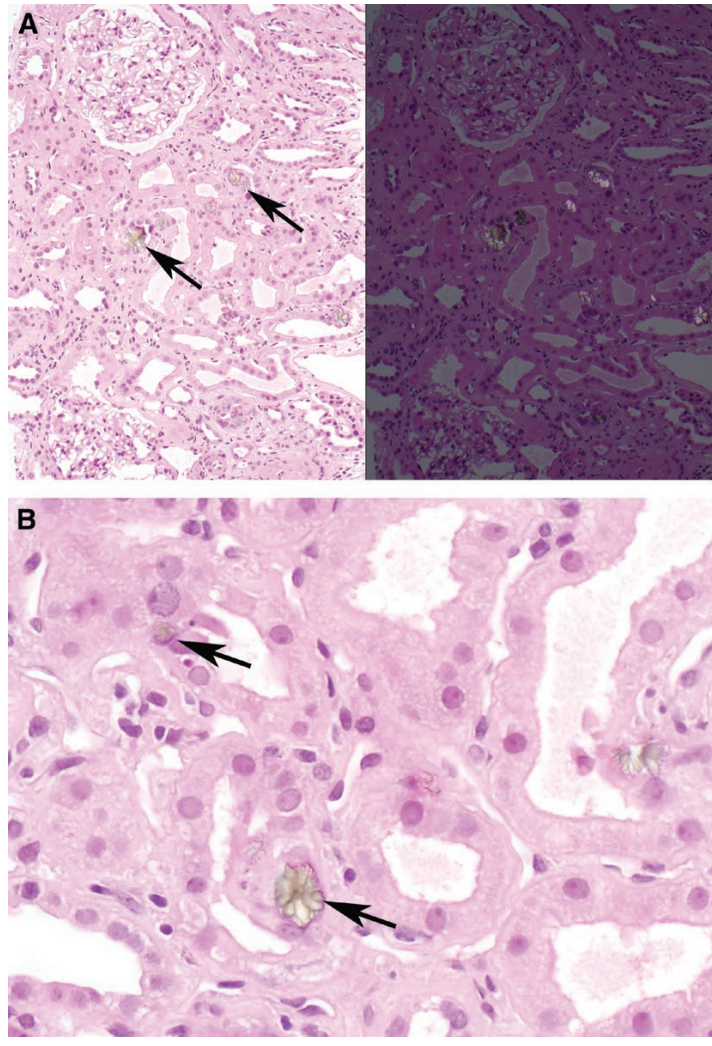

FIGURE 1. Recurrent 2,8-dihydroxyadenine nephropathy in an allograft from 1 of the patients (No 12; Table 2). A, On a hematoxylin and eosin stained section, numerous brown crystals (arrows) are seen within tubular lumens and within the tubular epithelial cytoplasm (left panel). Upon examination under polarized light, these crystals are strongly birefringent (right panel). B, On high magnification (×600) of the hematoxylin and eosin stained section, small brown crystals (arrows) are often seen within the tubular epithelial cytoplasm.

months following the second transplant, respectively. Five patients who initiated pharmacotherapy posttransplant had persistent biopsy-proven DHA allograft nephropathy, 4 of whom were treated with allopurinol in doses ranging from 150 mg twice a week to 300 mg daily. Three patients died with a functioning graft (Table 2); 1 patient (No. 3) from miliary tuberculosis at 5 months posttransplant, 1 patient (No. 5) from breast cancer 5.8 years posttransplant, and the third one (No. 8) from sepsis 1.4 years following a fourth kidney transplant. One patient (No. 2), who had lost 2 allografts due to disease recurrence, expired while on hemodialysis 8 years after the second transplant. Another patient (No. 6) developed acute liver failure 1.5 years posttransplant, suspected to be drug-induced, though the offending agent was not identified. The patient died while on hemodialysis for AKI 2 months later, in the setting of multiorgan failure.

The median eGFR at 6 months posttransplant was 61.5 (22.5–93.0) mL/min/1.73 m^2 in patients who received XOR inhibitor therapy pretransplant, while it was 24.9 (9.6–53.3) mL/min/1.73 m^2 in those who did not receive such treatment before transplantation (P = 0.003; Table 4). Similarly, the graft function was superior in the XOR inhibitor-treated group at 2 years posttransplant, with a median eGFR of 61.3 (24.0–90.0) mL/min/1.73 m^2 compared with 16.2 (10.0–39.0) mL/min/1.73 m^2 in the untreated group (P = 0.009; Table 4). At 2 years posttransplant, the allograft survival was 91% in the group receiving XOR inhibitor treatment pretransplant versus 55% in the untreated group, but the difference did not reach statistical significance (P = 0.16; Figure 2). In general, patients receiving higher allopurinol doses appeared to be less likely to experience disease recurrence (Tables 2 and 3).

DISCUSSION

In this study of kidney transplant outcomes in patients with APRT deficiency, allograft function was superior in patients who initiated XOR inhibitor therapy pretransplant

TABLE 4.

Allograft outcomes in patients who initiated xanthine oxidoreductase inhibitor treatment before kidney transplantation compared with those who did not receive such treatment until posttransplant, or not at all

	No XORi pretransplant	XORi pretransplant	*P*
Number of patients	8	10	
Number of grafts	11	11	
Age at transplant, y	42.8 (14.9–67.0)	45.5 (20.7–66.2)	0.974
Delayed graft function	2	3	1.0
Posttransplant acute kidney injury	7	4	0.395
eGFR, mL/min/1.73 m^2			
At 6 mo	24.9 (9.6–53.3) [9 grafts]	61.5 (22.5–93) [10 grafts]	0.003
At 12 mo	27.5 (10.0–67.5) [9 grafts]	64.8 (28–93.8) [10 grafts]	0.035
At 2 y	16.2 (10.0–39.0) [6 grafts]	61.3 (24.0–90.0) [8 grafts]	0.009
Biopsy-proven recurrence of DHA nephropathy	10	3	0.004
Graft loss due to recurrence of DHA nephropathy	4	1	0.31
Death	5	1	0.043
Death with a functioning graft	3	1	0.275

Data are presented as median (range).
eGFR, estimated glomerular filtration rate; XOR, xanthine oxidoreductase; XORi, xanthine oxidoreductase inhibitor.

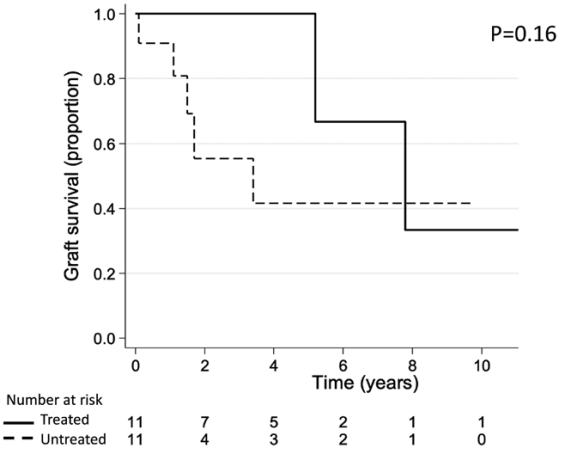

FIGURE 2. Kaplan-Meier curve of death-censored kidney allograft survival from the time of kidney transplantation in patients who received treatment with an xanthine oxidoreductase inhibitor pretransplant (solid line) and those who did not (broken line).

compared with those who either first started this therapy posttransplant or did not receive such treatment at all. Timely initiation of pharmacotherapy in adequate doses appears to be a major determinant of kidney allograft function in patients with APRT deficiency.

Most previously reported cases of kidney transplantation in patients with APRT deficiency have demonstrated premature allograft loss or chronic allograft dysfunction in patients who were not on XOR inhibitor treatment at the time of transplantation,[6,8,10-12,21,22] usually due to missed diagnosis of this rare disorder. Similar observations were also evident in our study, as all 10 allografts biopsies from untreated patients showed disease recurrence, which lead to the premature loss of 4 grafts in 2 patients. These 2 patients were first placed on treatment with allopurinol at 4 weeks and 6 months following their second transplant in the setting of decreased graft function, and subsequently progressed to allograft failure. By contrast, patients who were receiving treatment with an XOR inhibitor at the time of kidney transplantation demonstrated better long-term allograft function and survival. One patient treated with low dose of allopurinol experienced early biopsy-proven disease recurrence which prompted a dose increase. Poor adherence to pharmacotherapy was reported for 1 patient in the present study due to severe episodic eye symptoms, including blurry vision, burning pain, and photophobia, resulting in subsequent allograft loss. Although our small study sample precludes meaningful statistical analysis of allograft outcomes, the patients who initiated XOR inhibitor therapy in adequate doses pretransplant and remained compliant with the treatment appeared to experience graft survival similar to what has been reported for kidney transplantation in general.

Delayed graft function requiring dialysis in the first posttransplant week was reported in roughly a quarter of the cases described herein, both in patients who initiated XOR inhibitor therapy before transplantation and those who did not, all of whom received deceased donor transplants. In patients receiving treatment pretransplant, delayed graft function prompted a large increase in allopurinol dosage in 2 of the 3 cases, with subsequent stabilization of allograft function. One of these 2 patients underwent a kidney biopsy revealing extensive tubular crystal deposition that diminished with higher doses of allopurinol as observed on a repeat biopsy, with improved kidney function. A kidney biopsy performed a month later in the third patient on a stable allopurinol dose of 300 mg/day showed signs of acute cellular rejection but no crystal deposits. Two patients who experienced delayed graft function and did not receive treatment with an XOR inhibitor pretransplant had early biopsy-proven disease recurrence. Treatment with allopurinol was subsequently started in both patients. It is noteworthy that the diagnosis of APRT deficiency had been made some years before kidney transplantation in 1 of these 2 patients but treatment with an XOR inhibitor was not initiated for unknown reasons. In addition to ischemic kidney injury, DHA crystal nephropathy likely contributed to the delay in allograft function in the cases where XOR inhibitor treatment was lacking or inadequate.[6,21,23]

Our data clearly demonstrate superior allograft outcomes among patients on XOR inhibitor therapy at the time of kidney transplantation and through the posttransplant period compared with those who did not initiate treatment until several weeks posttransplant, or not at all. Initiation of XOR inhibitor treatment before transplantation may be important as the risk of disease recurrence appears to be particularly high in the early posttransplant period. Most patients in our study who received XOR inhibitor therapy pretransplant had initiated the treatment >12 months before the transplant surgery. Interestingly, DHA crystal-induced injury seems to be much more aggressive in allografts than in native kidneys.[3] As plasma DHA measurements are currently unavailable, it has not been determined if and how effectively dialysis clears DHA from plasma. Hence, it is conceivable that DHA may accumulate before transplantation in patients with kidney failure in the absence of XOR inhibitor therapy and flood the kidney allograft immediately following the transplant surgery, resulting in early graft dysfunction.[6,10,11,22] Furthermore, ischemia-reperfusion injury at the time of transplantation may render the graft more susceptible to crystal deposition.

We noted disease recurrence in all allograft biopsies from patients who either did not receive XOR inhibitor treatment pretransplant or received inadequate doses. By contrast, no signs of DHA crystal nephropathy were noted in the majority of transplants treated with pretransplant allopurinol in the daily dose of 300 mg or greater. One patient who began treatment with a low dose of allopurinol (150 mg/d) before transplantation had early disease recurrence and required hemodialysis for 3 weeks posttransplant. Recurrence of DHA nephropathy in patients treated with similarly low allopurinol doses has been reported previously,[6,22] indicating that higher doses are needed. Even in cases of delayed diagnosis, prompt initiation of XOR inhibitor therapy can have beneficial impact on graft outcomes, as seen in the present study where graft function improved in several cases following institution of allopurinol therapy.

The XOR inhibitor dose and duration of treatment pretransplant required to successfully prevent progressive DHA allograft nephropathy is currently not known and requires further study. However, based on our experience we recommend treatment with allopurinol in the dose of 400 mg/day for a minimum of 3 months before the transplantation. Recent data do not suggest increased risk of adverse effects in individuals with advanced CKD,[24] and therefore we do not routinely lower allopurinol doses in APRT deficiency patients with reduced kidney function. In fact, patients with progressive allograft dysfunction may need higher allopurinol doses. In patients who do not tolerate allopurinol, febuxostat should be prescribed in the daily dose of 80 mg. Microscopic assessment of crystalluria is widely used to monitor XOR inhibitor treatment but lacks precision and is associated with significant interobserver variations. Our group recently developed a UPLC-MS/MS assay for quantification of urine DHA that holds great promise for the future.[25] However, additional studies must be performed to determine the level of urine DHA that must be achieved to prevent crystal deposition and kidney allograft injury.

Traditionally, DHA crystal deposition has been believed to cause diffuse tubular obstruction, resulting in progressive CKD. More recent studies suggest that inflammatory mechanisms do play an important role in crystal-induced kidney injury, including NLPR3 inflammasome activation provoked by crystal uptake into intracellular lysosomes, leading to nephron destruction.[1] Future studies will provide better understanding of the pathobiological mechanisms in crystal nephropathies, hopefully leading to the discovery of novel therapeutic options.

Misinterpretation of kidney biopsy findings was common in the present study, similar to previously reported cases.[6,21,26] Observation of crystal deposits in the renal parenchyma should always prompt further evaluation, in which case it is particularly important to rule out DHA nephropathy as effective treatment is available. When kidney sections are viewed under light microscopy, the crystals are brown with hematoxylin and eosin stain, have a needle, rod, or rhomboid shape, and are strongly birefringent. Importantly, caution must be taken not to confuse DHA crystals with oxalate deposits.[6] Indeed, primary hyperoxaluria was the original histologic diagnosis that was erroneously made in 4 patients (5 allografts) in the current study, significantly delaying the initiation of pharmacotherapy.

Our study has limitations, including the small sample size and retrospective observational design which is hampered by variable level of documentation, testing and duration of follow-up. Nevertheless, the series of APRT deficiency patients in this report represents the largest transplant dataset described for this rare disease to date.

In conclusion, this study demonstrates improved allograft outcomes among patients with APRT deficiency who receive treatment with an XOR inhibitor before or at the time of kidney transplantation. Thus, increased awareness among clinicians is imperative for promoting early diagnosis of APRT deficiency and initiation of XOR inhibitor treatment pretransplant. Notably, large doses of allopurinol may be needed to adequately prevent recurrence of DHA nephropathy, apparently 400 mg/day or greater. Moreover, it may be necessary to initiate the treatment several weeks or even months before transplantation to minimize the risk of disease recurrence due to enhanced susceptibility of the kidney allograft. As delay in diagnosis and appropriate pharmacotherapy is a major cause of premature graft loss in patients with APRT deficiency, it is important to increase the awareness of the disorder among physicians caring for patients with kidney failure.

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Urinary 2,8-dihydroxyadenine excretion in patients with adenine phosphoribosyltransferase deficiency, carriers and healthy control subjects

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ABSTRACT

Background: Adenine phosphoribosyltransferase (APRT) deficiency is a rare autosomal recessive disorder of adenine metabolism that results in excessive urinary excretion of the poorly soluble 2,8-dihydroxyadenine (DHA), leading to kidney stones and chronic kidney disease. The purpose of this study was to assess urinary DHA excretion in patients with APRT deficiency, heterozygotes and healthy controls, using a recently developed ultra-performance liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) assay.

Methods: Patients enrolled in the APRT Deficiency Registry and Biobank of the Rare Kidney Stone Consortium (http://www.rarekidneystones.org/) who had provided 24-h and first-morning void urine samples for DHA measurement were eligible for the study. Heterozygotes and healthy individuals served as controls. Wilcoxon-Mann-Whitney test was used to compare 24-h urinary DHA excretion between groups. Associations were examined using Spearman's correlation coefficient (r_s).

Results: The median (range) 24-h urinary DHA excretion was 138 (64–292) mg/24 h and the DHA-to-creatinine (DHA/Cr) ratio in the first-morning void samples was 13 (4–37) mg/mmol in APRT deficiency patients who were not receiving xanthine oxidoreductase inhibitor therapy. The 24-h DHA excretion was highly correlated with the DHA/Cr ratio in first-morning void urine samples ($r_s = 0.84$, $p < .001$). DHA was detected in all urine samples from untreated patients but not in any specimens from heterozygotes and healthy controls.

Conclusions: High urinary DHA excretion was observed in patients with APRT deficiency, while urine DHA was undetectable in heterozygotes and healthy controls. Our results suggest that the UPLC-MS/MS assay can be used for diagnosis of APRT deficiency.

1. Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600) is a rare autosomal recessive disorder of purine metabolism that results in excessive renal excretion of the poorly soluble 2,8-dihydroxyadenine (DHA), leading to kidney stones, acute kidney injury (AKI) and chronic kidney disease (CKD) [1–3]. Radiolucent kidney stones are the most common clinical manifestation of APRT deficiency [1–3], whereas 15–20% of adult cases have end-stage kidney disease secondary to DHA crystal-induced nephropathy at diagnosis [2,3]. The xanthine oxidoreductase (XOR; xanthine dehydrogenase/oxidase) inhibitors allopurinol and febuxostat effectively reduce urinary DHA ex-

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cretion [4] and have been found to ameliorate disease manifestations, including kidney stone formation and progressive CKD [1–3]. While the typical round and brown urinary DHA crystals are highly suggestive of APRT deficiency, abolished APRT enzyme activity in red cell lysates and/or identification of biallelic pathogenic mutations in the *APRT* gene have been required for definitive diagnosis of the disorder [5].

Therapeutic drug monitoring is traditionally performed by urine microscopy where the absence or at least reduction of DHA crystals is thought to indicate adequate XOR inhibitor treatment [5]. Several methods for quantifying DHA have been described, including techniques based on high-performance liquid chromatography coupled to a multichannel ultraviolet detector or tandem mass spectrometry [6,7] and capillary electrophoresis [8–10]. Our group recently developed an ultra-performance liquid chromatography - electrospray tandem mass spectrometry (UPLC-MS/MS) assay with isotopically labeled internal standard for absolute urinary quantification of DHA [4,11].

The aims of the present work were to use the UPLC-MS/MS assay to measure urinary DHA excretion in APRT deficiency patients, heterozygotes and healthy control subjects, to correlate the 24-h urinary DHA excretion with the DHA-to-creatinine (DHA/Cr) ratio in single-void urine specimens and to test the sensitivity and specificity of the UPLC-MS/MS urinary DHA assay for the diagnosis of APRT deficiency.

2. Methods

2.1. Ethical approval

The study was approved by the Icelandic National Bioethics Committee (NBC 09–072 and 13–115-S1) and the Icelandic Data Protection Authority. All study participants gave written informed consent for their participation. The clinical and research activities reported are consistent with the ethical principles of the Declaration of Helsinki.

2.2. Study subjects and clinical data

Patients enrolled in the APRT Deficiency Registry and Biobank of the Rare Kidney Stone Consortium (http://www.rarekidneystones.org/) who had donated urine samples for DHA measurement before April 30, 2018, were eligible for participation in the study. Individuals heterozygous for pathogenic *APRT* mutations and healthy subjects who were not taking medications affecting the metabolism or excretion of purines served as controls. Registry data included clinical manifestations, medication use (including the XOR inhibitors allopurinol and febuxostat), height, weight, serum Cr, urine Cr and results of APRT activity measurements and genetic testing. The Cr measurements were traceable to a reference method based on isotope dilution mass spectrometry (IDMS). The CKD-EPI equation was used to estimate glomerular filtration rate (eGFR) in adults [12] and the CKiD Schwartz equation in patients < 18 years of age [13].

2.3. Determination of urine 2,8-dihydroxyadenine

Biobanked 24-h urine specimens and single-void urine samples were used for the study. All participants were on their habitual diet when the urine samples were collected. 24-H urinary DHA excretion was measured in patients with APRT deficiency, both on and off XOR inhibitor treatment, and in control subjects. Correlation between the DHA/Cr ratio in first-morning void urine specimens and 24-h urinary DHA excretion, both obtained in the same 24-h period, was assessed in all available sample pairs, regardless of XOR inhibitor treatment status.

Urine DHA was measured using the UPLC-MS/MS assay developed by our group and expressed as mg/24 h in timed samples and as DHA/Cr ratio in mg/mmol in single-void specimens as previously described [11]. The lower limit of DHA detection and quantification were 20 and 100 ng/mL, respectively. Urinary DHA excretion values based on timed urine samples were adjusted for collection time (median collection time 1375 (range, 870–1670) min) and standardized to 1440 min (24 h).

DHA crystalluria detected by urine microscopy in our laboratory was semiquantitatively graded as 0, (+), 1+, 2+, 3+ and 4+ by an experienced medical laboratory scientist before the urine specimens were frozen for storage.

2.4. Statistical analyses

Data are presented as numbers or median (range). Wilcoxon-Mann-Whitney test was used to compare 24-h urinary DHA excretion between groups. When participants had more than one 24-h urine sample available, the mean urinary DHA excretion for each individual was used in the analysis. Spearman's correlation coefficient (r_s) was used to assess the correlation between 24-h urinary DHA excretion and the DHA/Cr ratio and DHA crystalluria in first-morning void urine samples, as well as weight, body surface area (BSA) and eGFR. Outliers were identified by visual examination of the scatter plot. The sensitivity and specificity of the UPLC-MS/MS urinary DHA assay for diagnosis of APRT deficiency was examined by comparing the urinary DHA concentration in samples from patients, carriers and healthy controls.

3. Results

3.1. Characteristics of participants

Thirty-three of the 60 patients in the APRT Deficiency Registry, 22 of whom were women, provided urine samples for DHA measurement. Most patients had multiple samples available; there were a total of 91 24-h urine collections, 165 first-morning void and 24 random urine specimens (Table 1). A single 24-h and a first-morning void urine sample pair, obtained in the same 24-h period, was available for 11 patients who were not receiving XOR inhibitor therapy. Eight of these 11 patients also donated paired 24-h and first-morning void urine samples after they had been placed on XOR inhibitor treatment. In addition, paired urine samples were obtained from 4 heterozygotes and 10 healthy control subjects for DHA measurement.

Twenty-one of the 33 patients had a past history of kidney stones, 11 had experienced AKI episodes and 8 patients had developed CKD stage 5, one of whom was on dialysis and 6 had undergone kidney transplantation. Six patients had an asymptomatic course. At the time of last urine sampling, one patient had eGFR of 14 mL/min/1.73 m^2 when a first-morning void urine sample was obtained, while another had eGFR of 40 mL/min/1.73 m^2 at the time of a 24-h urine collection. All other patients had eGFR > 60 mL/min/1.73 m^2.

Table 1
Urine samples from patients with adenine phosphoribosyltransferase deficiency available for analysis.

	Homozygotes			
	Paired		Not paired	
No XOR inhibitor therapy	Samples (n)	Patients (n)	Samples (n)	Patients (n)
24-h samples	19	11	12	7
First-morning void samples	19	11	25	13
Random samples			24	4
XOR inhibitor therapy				
24-h samples	17	8	43	21
First-morning void samples	17	8	104	33

Abbreviations: XOR, xanthine oxidoreductase.

3.2. Urinary 2,8-dihydroxyadenine excretion

Fourteen patients (10 women) with APRT deficiency who were not receiving XOR inhibitor therapy had 31 24-h urine collections available for the determination of DHA excretion. The median 24-h urinary DHA excretion was 138 (64–292) mg, but DHA was not detected in any 24-h urine specimens from the 4 heterozygotes and 10 healthy controls (Table 2). The urinary DHA excretion was significantly greater in men, 233 (95–289) mg/24 h compared with 129 (64–291) mg/24 h in women ($p = .03$; Fig. 1A), and did not change when corrected for weight or BSA. No association was observed between the urinary DHA excretion and age (rs = − 0.332, p = .068), weight ($r_s = 0.36$, $p = .203$; Fig. 1B), BSA ($r_s = 0.36$, $p = .208$; Fig. 1C) or eGFR ($r_s = 0.35$, $p = .227$; Fig. 1D). A marked intra-individual variability in urinary DHA excretion was seen in 5 untreated patients who had multiple urine collections available at different time points (Fig. 2). The DHA/Cr ratio was determined in 44 first-morning void urine samples available from 19 untreated patients and 24 random urine samples from 4 untreated patients, yielding a median of 12.7 (3.8–37.2) mg/mmol and 15.5 (10.4–19.3) mg/mmol, respectively. A total of 121 first-morning void urine samples were available from 33 patients while they were receiving XOR-inhibitor treatment. No DHA was detected in 44 of these samples (from 21 patients), whereas the DHA/Cr ratio was 4.5 (0.4–24.8) mg/mmol in 77 samples (from 25 patients).

3.3. Correlation of 2,8-dihydroxyadenine excretion assessed in timed and single-void urine specimens

Thirty-six pairs of 24-h and first-morning void urine samples collected by 11 patients in the same 24-h period (Table 1), were available for analysis of the correlation between the DHA/Cr ratio in the first-morning void specimens and 24-h DHA excretion. The correlation was highly significant, both before ($r_s = 0.78$, $p < .001$) and after removing 3 outliers ($r_s = 0.84$, p < .001; Fig. 3). Seventeen of these 36 sample pairs were obtained during treatment with an XOR inhibitor and 19 while off pharmacotherapy.

3.4. Correlation between 24-h 2,8-dihydroxyadenine excretion and crystalluria

A highly significant correlation was observed between 24-h urinary DHA excretion and DHA crystalluria ($r_s = 0.810$, $p < .001$; Fig. 4) in 91 24-h urine samples (60 obtained during XOR inhibitor treatment and 31 off pharmacotherapy) available for the analysis (Table 1). Interestingly, urinary DHA excretion in the range of 19–88 mg/24 h was noted in 16 of the 40 urine samples that were deemed negative for DHA crystals. All 16 samples were from patients treated with allopurinol in a daily dose of 300–400 mg, while the remaining 24 timed urine samples were collected during treatment with allopurinol 300–600 mg/day (n = 12) or febuxostat 80 mg/day (n = 12), all of which had undetectable levels of DHA.

To test the accuracy of the UPLC-MS/MS urinary DHA assay for the diagnosis of APRT deficiency, we included a total of 99 urine samples (24-h collections, $n = 31$; first-morning void specimens, $n = 44$; random specimens, $n = 24$) from 21 untreated patients and 28 samples (24-h collections, $n = 14$; first-morning void specimens, n = 14) from 14 unaffected controls. Urine DHA was detected in all patient specimens, but not in any of the samples from the 4 heterozygotes and the 10 healthy control subjects. Thus, the detection of urine DHA using the novel UPLC-MS/MS assay confers 100% sensitivity and specificity for the diagnosis of APRT deficiency in patients who are not receiving XOR inhibitor treatment.

4. Discussion

In the current study, all patients with APRT deficiency had high, albeit variable, urinary DHA excretion that was significantly greater in men than women. There was a strong correlation between DHA excretion in timed urine samples and the DHA/Cr ratio in first-morning void urine specimens. Importantly, DHA was not detected in urine samples from heterozygotes, healthy individuals and many patients on XOR inhibitor therapy.

Our results demonstrate a wide range of urinary DHA excretion in patients with APRT deficiency. While intra- and inter-day variability in

Table 2
Urinary DHA excretion in patients with adenine phosphoribosyltransferase deficiency (not receiving xanthine oxidoreductase inhibitor therapy), heterozygotes and healthy control subjects.

	Homozygotes	Heterozygotes	Healthy control subjects
Number of participants	19	4	10
Age, years	35.4 (16.1–67.0)	47.4 (36.6–56.6)	25.0 (23.5–30.1)
eGFR, mL/min/1.73 m^2	101 (14–131)	94 (55–100)	106 (78–125)
Weight, kg	79.4 (52–112)	77.2 (62–116)	72.4 (58–93)
BSA, m^2	1.9 (1.5–2.4)	1.9 (1.7–2.5)	1.8 (1.7–2.2)
24-h urine collections			
Number of participants	14	4	10
Number of samples	31	4	10
DHA/24 h, mg	138.2 (63.5–291.5)	BLQ*	BLQ*
Cr, mmol/kg/24 h	0.15 (0.10–0.20)	0.18 (0.15–0.24)	0.15 (0.10–0.21)
First-morning void urine samples			
Number of participants	19	4	10
Number of samples	44	4	10
DHA/Cr ratio, mg/mmol	12.7 (3.8–37.2)	BLQ*	BLQ*
Random urine samples			
Number of participants	4	NA	NA
Number of samples	24		
DHA/Cr ratio, mg/mmol	15.5 (10.4–19.3)		

Abbreviations: BLQ, below limit of quantification (< 100 ng/mL); BSA, body surface area; Cr, creatinine; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate; NA, not available; *$p < .005$.

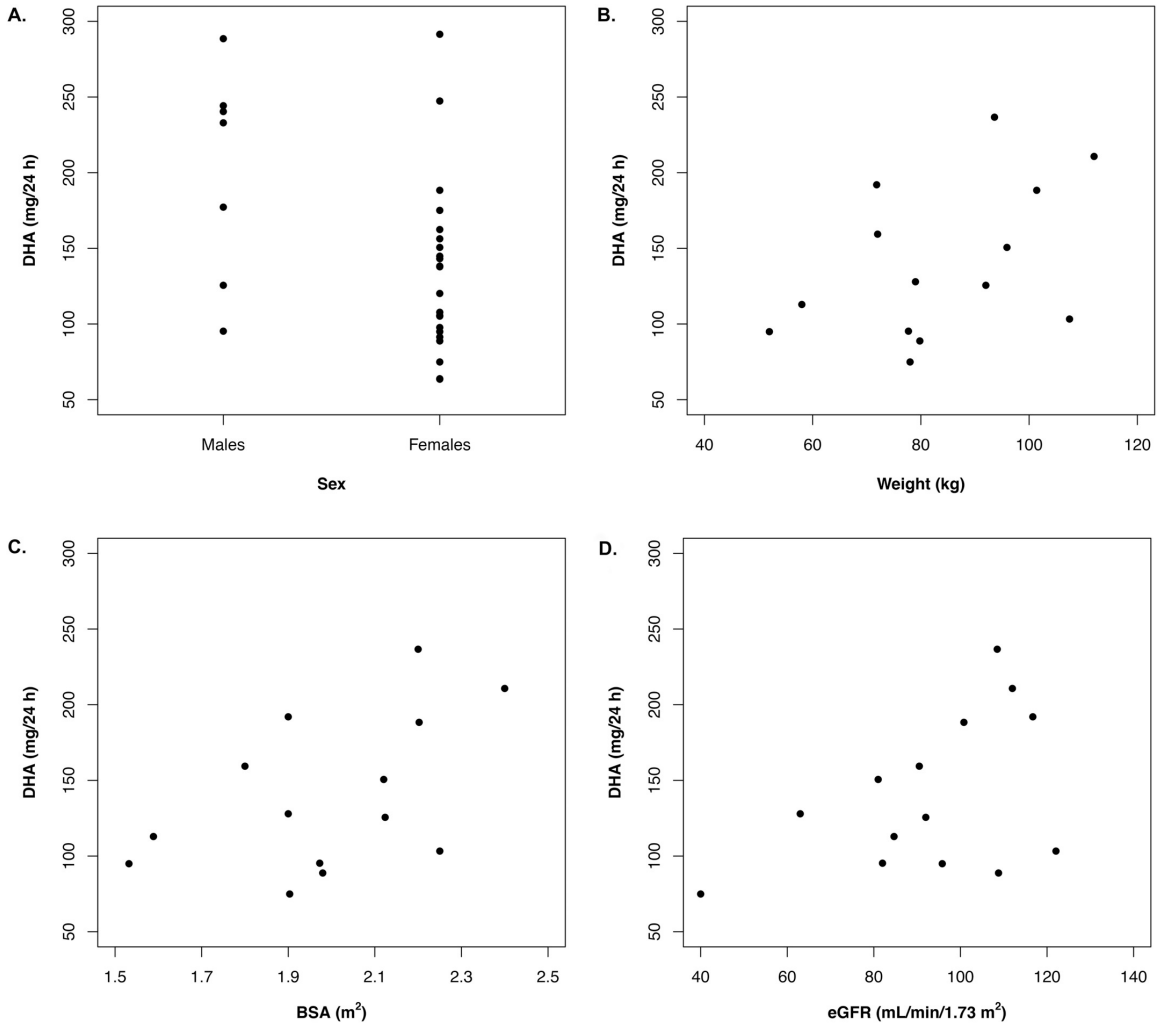

Fig. 1. Scatter plot of 24-h urine DHA with (A) sex (p = .03), (B) weight (r_s = 0.36, p = .203), (C) BSA (r_s = 0.36, p = .208), and (D) eGFR (r_s = 0.35, p = .227) in patients with adenine phosphoribosyltransferase deficiency who were not receiving xanthine oxidoreductase inhibitor treatment.
Abbreviations: BSA, body surface area; DHA, 2,8-dihydroxyadenine; eGFR, estimated glomerular filtration rate.

the accuracy of quantification obtained with our UPLC-MS/MS DHA assay is within ± 15% [11], this does not explain the wide variation in urinary DHA excretion seen in our patient cohort. A more likely explanation is variability in dietary or systemic adenine load and/or the rate at which adenine is converted to DHA by XOR. Furthermore, multiple types of human intestinal bacterial taxa use APRT to metabolize adenine to adenosine monophosphate through adenine and adenosine salvage pathways [14,15]. Therefore, modulation of adenine metabolism by the gut microbiome, affecting the amount of adenine available for intestinal absorption, may have contributed to the variable urinary DHA excretion in the APRT deficiency patients. High-purine diet may also increase the systemic adenine load. One single-patient report showed a decrease in urinary DHA excretion on a purine-restricted diet when compared with a purine-rich diet, suggesting a significant contribution of dietary adenine intake to the systemic adenine supply [16]. Rodents fed adenine-enriched diet are well known to develop adenine or DHA nephropathy within weeks, a form of kidney damage closely mimicking human DHA nephropathy [17]. Finally, recently reported inter-individual differences in plasma XOR activity in humans [18] might be a plausible mechanism for the variability in renal DHA excretion observed in our patient cohort.

No obvious explanation exists for the significantly higher urinary DHA excretion in men compared with women, a finding that has not been reported previously. The larger muscle mass in men may have

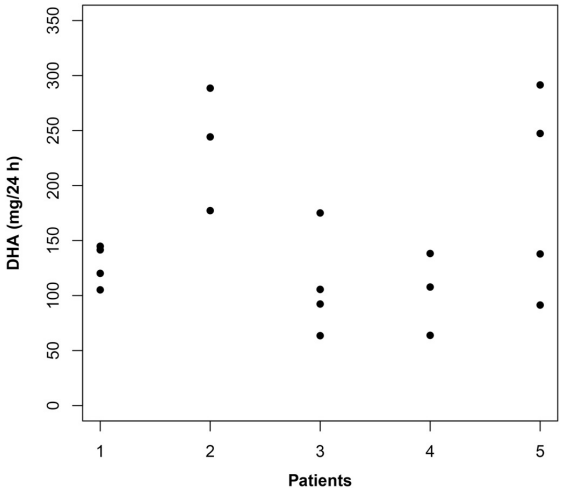

Fig. 2. 24-h urinary DHA excretion in five patients with adenine phosphoribosyltransferase deficiency who were not receiving xanthine oxidoreductase inhibitor treatment. For each patient, measurements were carried out in urine samples obtained at different time points. Abbreviations: DHA, 2,8-dihydroxyadenine.

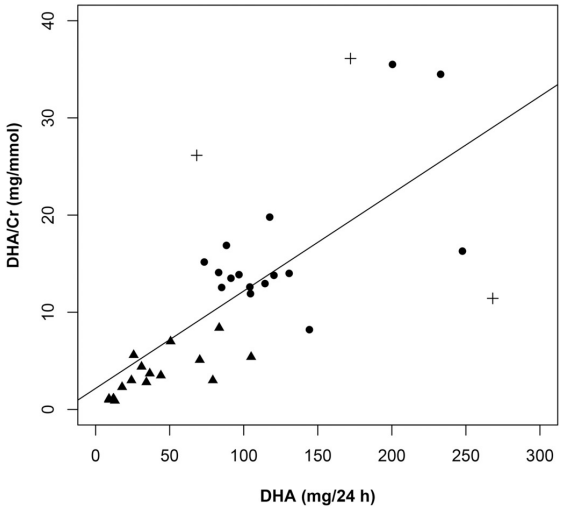

Fig. 3. Scatter plot of 24-h urinary DHA excretion versus DHA/Cr ratio in first-morning void urine samples from patients with adenine phosphoribosyltransferase deficiency who were treated (▲) or not treated (●) with xanthine oxidoreductase inhibitor (r_s = 0.84, p < 0.001). *Outliers (+) were excluded from the correlation analysis. Abbreviations: Cr, creatinine; DHA, 2,8-dihydroxyadenine.

contributed to some of the differences observed, although we did not find any significant variability in DHA excretion when correlated to BSA. The generally larger food portions consumed by men may also have contributed to the sex discrepancies, despite the lack of data to support this notion. Interestingly, there are reports showing differences

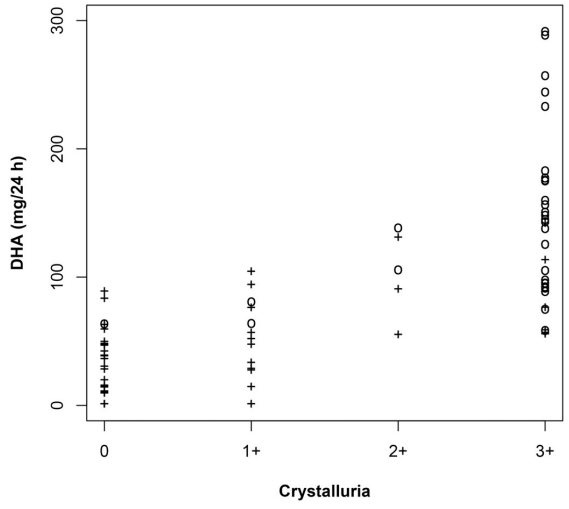

Fig. 4. 24-h urinary DHA excretion versus microscopic DHA crystalluria in patients with adenine phosphoribosyltransferase deficiency who were treated (+) or not treated (○) with xanthine oxidoreductase inhibitor (r_s = 0.810, $p < .001$). No patient had 4+ crystalluria.
Abbreviations: DHA, 2,8-dihydroxyadenine.

in the intestinal microbiome between the sexes [15], a fact that may have implications for the variation in DHA excretion between men and women. The small number of men in our study may have amplified any errors associated with urine sampling, such as inaccuracy in the documentation of collection time which may have falsely increased the DHA excretion. Additionally, also due to the small sample size, the differences in urinary DHA excretion between the sexes may simply have occurred by chance.

The intra-individual variability in DHA excretion noted in the current study could also be affected by sampling or measurement errors, but temporal changes in systemic adenine load, and less likely XOR activity, cannot be excluded. Although we did not find any correlation between urinary DHA excretion and eGFR, it is important to note that only one untreated patient had severe renal failure at the time of urine sampling.

Urinary DHA excretion rates have previously only been reported for a few patients with APRT deficiency and the values in both randomly collected [6] and timed [10,16] urine specimens have been at the very low end of or markedly below the range seen in our patients. While this finding may be explained by dietary factors or other influences mentioned earlier, methodological differences in the measurement methods used may also play a role. Our UPLC-MS/MS urinary assay uses an isotope-labeled internal standard for absolute DHA quantification which has been lacking in previously reported techniques. The isotope-labeled internal standard corrects for errors which might be present during sample preparation and analysis.

The close correlation between 24-h urinary DHA excretion and the DHA/Cr ratio in first-morning void urine samples in the present study is an important discovery. Based on this finding, the determination of 24-h urinary DHA excretion can be replaced with DHA/Cr ratio in first-morning void urine samples for monitoring the effectiveness of pharmacotherapy and treatment adherence, both in the clinic and clinical research studies. Assessment of the renal excretion of various biomarkers

has been challenging through the years, due in part to the tedious nature of 24-h urine sampling which is associated with poor compliance and errors. Hence, the measurement of solute/Cr ratio in random urine samples has been increasingly used for quantification of renal solute excretion. The best examples are protein/Cr and albumin/Cr ratio in random urine samples which have been shown to correlate well with 24-h urinary protein or albumin excretion, respectively, the correlation coefficient being over 0.8 in most reports [19–21], as in the present study.

A strong correlation was also observed between 24-h urinary DHA excretion and DHA crystalluria, currently widely used for therapeutic monitoring. For example, absence of crystalluria mostly correlated with unquantifiable amounts of DHA. However, it is noteworthy that significant urinary DHA excretion, up to 88 mg/24 h, was detected in several timed urine collections despite the absence of microscopic crystalluria, whereas DHA levels in this range were generally found in samples with 1–3 + DHA crystalluria. This finding suggests that DHA crystalluria is not reliable enough for assessment of the effectiveness of XOR inhibitor therapy and should be used with a degree of caution. Currently, it is not known if persistent low-level DHA excretion enhances the risk of kidney disease progression.

The gold standard methods for the diagnosis of APRT deficiency, enzyme activity measurements and genetic testing, are cumbersome and not widely available. Hence, a more rapid method for screening and diagnosis of this rare and frequently underdiagnosed disease would be of great value. Since urine DHA was undetectable in heterozygotes and healthy control subjects and abundant in patients with APRT deficiency, our UPLC-MS/MS method for absolute quantification of urinary DHA excretion appears to be highly accurate in identifying patients with the disorder. Indeed, our results suggest that detection of any urine DHA using our assay can be considered diagnostic of APRT deficiency in individuals who have not recently received large parenteral adenine load as occurs with massive blood transfusions [22].

Strengths of our study include the relatively large number of urine samples available from this rare disease population, including timed urine collections. Another important advantage is the use of our highly sensitive and specific UPLC-MS/MS assay that includes an isotope-labeled internal standard, allowing for accurate quantification of the urinary DHA concentration. Nevertheless, the study is limited by a small number of participants, although this would be expected for a rare disease. The small number of patients with advanced CKD limited our ability to assess the effect of reduced kidney function on DHA excretion. Moreover, information on dietary intake was not available for the participants.

In conclusion, high urinary DHA excretion was observed in patients with APRT deficiency and the excretion was greater in men than women. The strong correlation between 24-h urinary DHA excretion and DHA/Cr ratio in first-morning void samples suggests that timed collections may be replaced by first-morning void samples for monitoring the effectiveness of XOR inhibitor therapy and treatment adherence. DHA was not detected in urine samples from heterozygotes and healthy individuals suggesting that our robust and reliable UPLC-MS/MS urinary DHA assay can be added to the list of diagnostic tests for APRT deficiency. Future studies should focus on factors that might affect production and urinary excretion of DHA in patients with APRT deficiency, as well as the impact of different levels of DHA excretion on the progression of CKD.

Details of the contributions of individual authors

Conception and study design: VE, RP, HLR, OSI; laboratory work and DHA measurements: UAT, MTh, GSO; statistical analysis: HLR, OSI; critical review of results: HLR, IMA, OSI, RP, VE, UAT, MTh; supervision and mentorship: VE, RP, OSI. HLR and VE wrote the first manuscript draft. Each author contributed important intellectual content during manuscript drafting and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy or integrity of any portion of the work are appropriately investigated and resolved.

Competing interest statement

HLR, IMA, OSI, RP, SGO, VE and UAT declare no financial or other competing interests. MT owns stock in ArcticMass.

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Data availability and material statement

Data used for this project are kept in the APRT Deficiency Registry of the Rare Kidney Stone Consortium. Biological samples used were stored in the Rare Kidney Stone Consortium Biobank, housed at Landspitali–The National University Hospital of Iceland in Reykjavik.

Take home message (synopsis)

High urinary 2,8-dihydroxyadenine (DHA) excretion was observed in all patients with APRT deficiency, while DHA was not detected in urine samples from *APRT* heterozygotes and healthy individuals, suggesting that our robust and reliable UPLC-MS/MS urinary DHA assay can be added to the list of diagnostic tests for APRT deficiency.

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Original Article

Comparison of the effect of allopurinol and febuxostat on urinary 2,8-dihydroxyadenine excretion in patients with APRT deficiency: A clinical trial

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ABSTRACT

Introduction: Adenine phosphoribosyltransferase (APRT) deficiency is a rare, but significant, cause of kidney stones and progressive chronic kidney disease. The optimal treatment has not been established. The purpose of this pilot study was to compare the effect of the xanthine oxidoreductase inhibitors allopurinol and febuxostat on urinary 2,8-dihydroxyadenine (DHA) excretion in APRT deficiency patients.

Materials and methods: Patients listed in the APRT Deficiency Registry of the Rare Kidney Stone Consortium, currently receiving allopurinol therapy, were invited to participate. The trial endpoint was the 24-h urinary DHA excretion following treatment with allopurinol (400 mg/day) and febuxostat (80 mg/day). Urinary DHA was measured using a novel ultra-performance liquid chromatography - electrospray tandem mass spectrometry assay.

Results: Eight of the 10 patients invited completed the study. The median (range) 24-h urinary DHA excretion was 116 (75–289) mg at baseline, and 45 (13–112) mg after 14 days of allopurinol therapy ($P = 0.036$). At the end of the febuxostat treatment period, 4 patients had urinary DHA below detectable limits (< 20 ng/mL) compared with none of the participants following allopurinol treatment ($P = 0.036$). The other 4 participants had a median 24-h urinary DHA excretion of 13.2 (10.0–13.4) mg at the completion of febuxostat therapy ($P = 0.036$).

Conclusion: Urinary DHA excretion in APRT deficiency patients decreased with conventional doses of both allopurinol and febuxostat. Febuxostat was, however, significantly more efficacious than allopurinol in reducing DHA excretion in the prescribed doses. This finding, which may translate into improved outcomes of patients with APRT deficiency, should be confirmed in a larger sample.

1. Introduction

Adenine phosphoribosyltransferase (APRT) deficiency (OMIM 102600) is a rare autosomal recessive disorder of adenine metabolism resulting in the generation and renal excretion of large amounts of the poorly soluble and nephrotoxic metabolite 2,8-dihydroxyadenine (DHA) [1,2]. Adenine accumulates in affected patients due to the abolished APRT enzyme activity and is converted by xanthine oxidoreductase (XOR; xanthine dehydrogenase/oxidase) to DHA in excessive quantities [3].

APRT deficiency patients frequently develop serious renal complications, including recurrent radiolucent kidney stones and progressive chronic kidney disease (CKD) caused by DHA crystal nephropathy. At least 15% of patients reported to date have already developed end-stage

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Table 1
Assessment and treatment schedule of study participants.

Protocol activity	First visit	Wash-out period 1	Allopurinol (400 mg/day)	Wash-out period 2	Febuxostat (80 mg/day)
	Day 1	Days 1–7	Days 8–21	Days 22–28	Days 29–42
Physical examination	X				
Serum Cr measurement	X				
24-H urine samples collected[a]		Day 7	Day 21	NA	Day 42
First morning void urine sample[a]		Day 7	Day 21	NA	Day 42
Adverse events			Monitored continuously		

Abbreviations: Cr, creatinine. NA, not assessed.
[a] 2,8-Dihydroxyadenine and creatinine were measured in these urine samples.

kidney failure at diagnosis [4], which frequently is not confirmed until after kidney transplantation when disease recurrence in the allograft has occurred [5].

The XOR inhibitor allopurinol is an effective therapy for preventing new kidney stone formation, renal DHA deposition and progressive crystal nephropathy in individuals with APRT deficiency. The drug decreases DHA synthesis and thereby reduces crystalluria [1,2,4,6]. Febuxostat, a selective non-purine XOR inhibitor [7], has also been reported to decrease DHA crystalluria in APRT deficiency patients [8], providing an attractive alternative treatment option for those who are intolerant of allopurinol. A reliable method to guide the titration of either XOR inhibitor has been lacking due to inability to measure urinary DHA. The monitoring of drug treatment is currently performed by urine microscopy where the absence of urinary DHA crystals is considered indicative of adequate therapy. However, this indirect method has several limitations that render it unsatisfactory as the only approach for therapeutic drug monitoring. Based on our own personal experience some patients with minimal or no crystalluria continue to form stones, while others with persistent crystalluria do not develop new stones or evidence of CKD progression. Thus, the dosing of XOR inhibitor therapy has simply been empiric and has been modified by the degree of DHA crystalluria or by clinical events such as recurrent kidneys stones or progressive CKD. In adults, allopurinol has commonly been prescribed in doses ranging from 200 to 300 mg/day [5]. Several reports of recurrent allograft DHA nephropathy despite treatment with allopurinol in this dosage range [5], has prompted us to use doses higher than 300 mg/day. Recently, our group developed a high-throughput ultra-performance liquid chromatography - electrospray tandem mass spectrometry (UPLC-MS/MS) assay for measurement of DHA in urine samples, which has the potential to greatly improve monitoring of pharmacotherapy in patients with APRT deficiency [9].

The aim of this exploratory pilot study was to compare the efficacy of allopurinol and the non-purine XOR inhibitor febuxostat in reducing urinary DHA excretion in patients with APRT deficiency.

2. Materials and methods

2.1. Ethics committee approval

The study was approved by the Icelandic National Bioethics Committee (NBC 13-115-S1), the Icelandic Medicines Agency (EudraCT No. 2013-00975-33) and the Icelandic Data Protection Authority. This clinical trial is registered at www.clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT02752633). All study participants gave a written informed consent for their participation.

2.2. Study design and setting

This exploratory pilot study was an open-label, crossover, single-center, non-randomized clinical trial designed to compare the effect of allopurinol (400 mg/day) and febuxostat (80 mg/day) on urinary DHA excretion in individuals with APRT deficiency. These doses were chosen as they are currently recommended in the management of APRT deficiency. The study was conducted between May 2013 and May 2015 as the participants were enrolled at different times. The only study site was Landspitali - The National University Hospital of Iceland in Reykjavik, Iceland. The Data Safety Monitoring Board (DSMB) constituted by the National Institutes of Health had oversight responsibility of the Data Safety Monitoring Plan for this clinical trial. The monitoring board reviewed accrual, patterns and frequencies of all adverse events, and protocol compliance every 6–12 months.

2.3. Participants

Study participants were recruited from a group of patients with confirmed APRT deficiency, who were enrolled in the National Institutes of Health-supported APRT Deficiency Registry of the Rare Kidney Stone Consortium (RKSC, http://www.rarekidneystones.org/). Confirmation of APRT deficiency was based upon the determination of known biallelic pathogenic APRT mutations or absent APRT activity in red blood cell lysates. Participants were eligible for inclusion if they: a) were currently receiving allopurinol therapy (the recommended treatment for patients with APRT deficiency); b) were willing to interrupt their allopurinol therapy for a total of 3 weeks as outlined below; and c) were at least 18 years of age. There were no exclusion criteria if the above inclusion criteria were met. Patients with reduced kidney function were not excluded.

2.4. Study interventions

An overview of the treatment and assessment schedule is presented in Table 1. At baseline, after a 7-day washout period, all participants were prescribed 400 mg of allopurinol in a single daily dose for 14 days. Following a second 7-day washout period, all participants were prescribed 80 mg of febuxostat in a single daily dose for another 14 days. The order of the interventions was not varied as it was not considered important due to the relatively short half-life of both study drugs (febuxostat, 5–8 h; allopurinol 1–2 h and oxypurinol, the active allopurinol metabolite, 15–16 h) [10,11]. 24–H and first morning void urine samples were collected at baseline and at the end of the allopurinol and febuxostat treatment periods (on days 7, 21 and 42), respectively. To minimize a potential adverse effect of variations in dietary purine intake on the results, the participants were asked to keep a food record while they collected the first 24-h urine sample and adhere to the same diet when they collected the other two 24-h urine samples. No further measures were taken to control dietary purine intake during the study period. At the completion of the study, all patients were advised to return to their regular allopurinol dosing regimens.

2.5. Laboratory testing

During the study, all participants were asked to donate three pairs of 24-h and first morning void urine specimens as described in Table 1. All urine samples were collected without additives or preservatives and

stored at room temperature and returned to the laboratory on days 8, 22 and 43, the same days the 24-h collections were completed. Immediately before aliquoting, the 24-h collection bottles were inverted 3 times and the urine samples were then frozen and stored at − 80 °C.

Urinary DHA and adenine were measured using the UPLC-MS/MS assay developed by our group, as previously described [9]. Prior to the UPLC-MS/MS analysis, the urine samples were diluted 1:15 (v/v) with 10 mM NH_4OH, followed by the addition of internal standard. The samples were subsequently mixed for 3 minutes and centrifuged at 3100 rpm for 10 minutes at 4 °C before injection into the UPLC-MS/MS system. The lower limit of detection for DHA is 20 ng/mL, the lower limit of quantification 100 ng/mL and the upper limit of quantification 5000 ng/mL. The intra- and inter-day accuracy and precision coefficients of variation have been shown to be well within ± 15% for quality control samples. The 24-h urinary DHA and adenine excretion was measured and the urinary DHA/creatinine (DHA/Cr) and adenine/Cr ratios in first morning void urine samples were calculated. Urine and serum creatinine concentrations were measured with an isotope dilution mass spectrometry (IDMS)-standardized laboratory method. Estimates of glomerular filtration rate (eGFR) were derived from serum creatinine values using the Chronic Kidney Disease Epidemiology Collaboration equation [12]. Plasma uric acid was measured at baseline and at the conclusion of both allopurinol and febuxostat therapy, applying standard laboratory methods.

2.6. Outcome measures

The primary trial endpoint was the 24-h urinary DHA excretion and the urinary DHA/Cr ratio after two weeks of treatment with the two study drugs, allopurinol (400 mg/day) and febuxostat (80 mg/day).

2.7. Statistical analysis

The urinary DHA excretion is expressed as mg/24 h and as DHA/Cr ratio in mg/mmol. Data are presented as a median (range). Differences in the median urinary DHA excretion and the urinary DHA/Cr ratio between periods off pharmacotherapy and on treatment with the two study drugs, allopurinol and febuxostat, were assessed using the Wilcoxon signed-rank test.

3. Results

Eight of the 10 patients who were invited to participate in the clinical trial completed the study. One participant discontinued participation due to pregnancy and one did not accept the invitation. One individual inadvertently reversed the order of the allopurinol and febuxostat treatment periods, but as he had otherwise adhered to the protocol, his data were included in the analysis.

The clinical characteristics of the participating patients are presented in Table 2. Only one individual had stage 3 CKD; the others had CKD stage 1 or 2. The median (range) 24-h urinary DHA excretion was 116 (75–289) mg at baseline. Following 14 days of allopurinol therapy, the DHA excretion was 45 (13–112) mg and was below the lower limit of quantification (100 ng/mL) in all 8 cases after 14 days of febuxostat treatment (P = 0.036). At the end of febuxostat therapy, 4 participants had urinary DHA below detectable limits (< 20 ng/mL) and the other 4 had a median urinary DHA excretion of 13 (10 − 13) mg/24 h (P = 0.036) (Table 3). The median urinary DHA/Cr ratio in first morning void urine samples was 16.1 (8.2–34.5) mg/mmol at baseline, 5.3 (1.1–8.4) mg/mmol on allopurinol (P = 0.036) and below lower limit of quantification at the completion of febuxostat treatment in all participants (P = 0.036) (Table 4). Four of these individuals with a quantifiable value had a median urinary DHA/Cr ratio of 1.0 (0.8–1.1) mg/mmol. The urinary adenine excretion, which was 32 (18–46) mg/24 h at baseline, increased to 96 (26–139) mg/24 h on allopurinol and to 112 (54–158) mg/24 h at the end of febuxostat treatment. The plasma uric acid concentration was 257 (180–454) μmol/L at baseline, and decreased to 179 (131–295) μmol/L following two weeks of

Table 3
24-H urinary 2,8-dihydroxyadenine (DHA) excretion at baseline and at the completion of allopurinol and febuxostat treatment periods.

Patient	Baseline	Allopurinol	Febuxostat
	DHA/24 h (mg)	DHA/24 h (mg)	DHA/24 h (mg)
1	89	32	13[a]
2	126	54	13[a]
3	233	112	10[a]
4	151	35	ND
5	289	90	13[a]
6	106	27	ND
7	75	13[a]	ND
8	95	75	ND

Abbreviations: ND, not detectable (limit of detection < 20 ng/mL).
[a] Below limit of quantification (< 100 ng/mL).

Table 4
2,8-Dihydroxyadenine-to-creatinine ratio in first morning void urine samples at baseline and at the completion of allopurinol and febuxostat treatment periods.

Patient	Baseline	Allopurinol	Febuxostat
	DHA/Cr (mg/mmol)	DHA/Cr (mg/mmol)	DHA/Cr (mg/mmol)
1	12.5	4.4	0.9[a]
2	22.8	7.0	ND
3	34.5	5.4	1.1[a]
4	8.2	2.8	0.8[a]
5	17.4	8.4	1.1[a]
6	13.9	5.6	ND
7	15.2	1.1[a]	ND
8	16.9	5.1	ND

Abbreviations: Cr, creatinine; DHA, 2,8-dihydroxyadenine.
ND, not detectable (limit of detection < 20 ng/mL).
[a] Below limit of quantification (< 100 ng/mL).

Table 2
Clinical characteristics of study participants.

Patient	Age (years)	Sex	eGFR (mL/min/1.73 m^2)	Age at diagnosis (years)	Major clinical feature
1	33	Female	103	0.5	Crystalluria
2	61	Male	90	23	Asymptomatic
3	28	Male	103	0.8	Kidney stones
4	38	Female	87	3.3	LUTS
5	52	Male	99	24.2	Kidney stones
6	62	Female	83	33.1	Kidney stones
7	67	Female	37	52.2	CKD
8	56	Male	80	32.9	Kidney stones

Abbreviations: eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; LUTS, lower urinary tract symptoms.

Table 5
24-H 2,8-dihydroxyadenine excretion and 2,8-dihydroxyadenine-to-creatinine ratio in first morning void urine samples on allopurinol therapy (400 mg/day) during and following the study.

Patient	Allopurinol[a]		Allopurinol[b]	
	DHA/24 h (mg)	DHA/Cr (mg/mmol)	DHA/24 h (mg)	DHA/Cr (mg/mmol)
2	54	7.0	47	3.5
4	35	2.8	27	3.0
5	90	8.4	88	3.0
6	27	5.6	18[c]	2.3
7	13[c]	1.1[c]	9[c]	1.0[c]
8	75	5.1	39[c]	3.7

Abbreviations: Cr, creatinine; DHA, 2,8-dihydroxyadenine; ND, not detectable (limit of detection < 20 ng/mL).
[a] Allopurinol treatment period.
[b] During allopurinol treatment following study completion.
[c] Below limit of quantification (< 100 ng/mL).

allopurinol and to 137 (88–221) μmol/L at the end of the febuxostat treatment period. No adverse events were observed.

Six of the 8 participants had their urinary DHA excretion re-evaluated when they had resumed allopurinol therapy in the daily dose of 400 mg, at a median of 6 (2 – 12) months following the study completion. The median 24-h urinary DHA excretion of these 6 individuals was 33 (9–88) mg, compared with 45 (13–112) mg at the completion of allopurinol therapy during the study period. The median urinary DHA/Cr ratio in first morning void urine samples from these 6 patients was 3.0 (1.0–3.7) mg/mmol 6 months following the study completion, while it was 5.3 (1.1–8.4) mg/mmol during the study. Individual patient data are displayed in Table 5.

4. Discussion

In this pilot study, a marked reduction of urinary DHA excretion was observed with both allopurinol and febuxostat therapy in patients with APRT deficiency. However, febuxostat decreased the urinary DHA excretion much more effectively than allopurinol. Importantly, the urinary DHA excretion remained substantial despite conventional doses of allopurinol, suggesting that higher doses may be required to adequately control dihydroxyadeninuria.

As this is the first published study designed to compare the effect of allopurinol and febuxostat on the urinary DHA excretion in patients with APRT deficiency, no prior data are available for comparison. The more powerful reduction of urinary DHA excretion observed during febuxostat treatment may simply reflect more effective XOR inhibition compared with allopurinol in the doses prescribed. This notion is supported by both a larger increase in urinary adenine excretion and a greater reduction of plasma uric acid concentration when the participants changed from allopurinol to febuxostat. Whereas a carryover effect of allopurinol cannot be excluded, this would very unlikely be due the short half-life of allopurinol and its active metabolite, oxypurinol. Thus, it can be assumed that urinary DHA had returned to baseline before the febuxostat treatment commenced. Lack of compliance during allopurinol therapy only is a highly unlikely explanation of the differences in urinary DHA excretion observed between the two study drugs. Since dose-equivalence studies comparing allopurinol and febuxostat in lowering DHA are not available, underdosing of allopurinol rather than a true difference in the efficacy of the two drugs in mediating XOR inhibition may have contributed to the superior effect on urinary DHA excretion observed with febuxostat treatment. Moreover, higher doses of allopurinol might have led to a more complete inhibition of DHA generation and should be studied in this patient population. Doses of allopurinol up to 800 mg/day are approved by US Food and Drug Administration and the European Medicines Agency but have not been tested in a systematic way. Studies in patients with gout have shown that febuxostat, in a dose of 80–120 mg daily, resulted in a greater proportion of individuals achieving serum uric acid levels < 6 mg/dL (357 μmol/L), compared with allopurinol 300 mg daily, or approximately 80% vs. 40% [13,14].

There are several differences in the actions of the two study drugs. Whether these differences, beyond a greater inhibitory effect of febuxostat on XOR, contribute to the greater efficacy of the drug in patients with APRT deficiency is not known. Allopurinol is a purine analogue that inhibits enzymes involved both in purine and pyrimidine synthesis and metabolism. In addition to inhibiting XOR, allopurinol and its metabolites also impede purine nucleoside phosphorylase, a mediator of purine metabolism, and orotidine-5′-monophosphate decarboxylase, which is required in the synthesis of pyrimidines [15]. Febuxostat is a non-purine inhibitor of XOR only [16]. Allopurinol, a relatively weak inhibitor of XOR and a purine analogue, also undergoes metabolism by XOR which results in the production of its most active metabolite, oxypurinol. It is oxypurinol that binds very tightly to the reduced form of XOR, resulting in a robust competitive inhibition of the enzyme function. Febuxostat, on the other hand, is a potent inhibitor of XOR, blocking both the reduced and oxidized forms of the enzyme. We cannot comment on whether these differences in pharmacologic properties contribute to the marked discrepancy in the effectiveness of the two drugs in decreasing urinary DHA excretion.

Recent work has shown that allopurinol in doses < 400 mg/day may not effectively diminish new stone formation or stabilize kidney function in APRT deficiency patients [1,4,6]. Therefore, we have recommended a daily dose of at least 400 mg which is the dose we elected to use in the current study. However, as the level of reduction in urinary DHA excretion required for optimal prevention of adverse renal outcomes is unknown [4,17], it is not clear if the difference in urinary DHA excretion observed between the two study drugs is clinically significant. Nevertheless, the urinary DHA excretion remains substantial despite allopurinol 400 mg daily, unlike with febuxostat 80 mg, suggesting that higher doses of allopurinol may be required to adequately control dihydroxyadeninuria. Finally, febuxostat may also have some safety benefit as its association with hypersensitivity is extremely rare compared with allopurinol. Rarely, allopurinol-associated hypersensitivity may be manifested as the Stevens-Johnson syndrome.

The novel, rapid and robust UPLC-MS/MS-based urinary DHA assay was instrumental in this study. This assay has the potential to greatly improve pharmacotherapy monitoring in patients with APRT deficiency and will form the basis for future studies of the optimal reduction of urinary DHA excretion required for prevention of recurrent stone formation and progressive kidney disease. Although the assay is not yet commercially available, we do offer urinary DHA measurements for all patients participating in our studies and for other individuals on a case-by-case basis.

Limitations inherent in this study include a small number of participants which is to be expected for a rare disease. Measuring hard endpoints, including incidence of stones or changes in eGFR would take years or even decades to complete due to the small number of participants and low event rate. Other notable limitations are lack of both a crossover design and a measurement of urinary DHA at the end of the second washout period. Nevertheless, a long-lasting pharmacodynamic effect of allopurinol would be unlikely due to the short half-life of the active metabolite, oxypurinol. While the study was not blinded to the participants, laboratory personnel were blinded to the drug treatment assignments. As 7 of 8 participants had well-preserved eGFR, we were unable to examine whether febuxostat is more effective in patients with advanced CKD. Before we discovered the robust effect of febuxostat presented here, we were reluctant to discontinue allopurinol therapy in patients with more advanced CKD. Since febuxostat is largely metabolized by the liver and the active allopurinol metabolites are cleared by the kidneys, the former may be easier to dose adequately and perhaps is safer in individuals with CKD [18].

The study has several important strengths, including the use of a new mass spectrometry-based method for accurate quantification of urinary DHA excretion in the participants. The participation of a well characterized population of APRT deficiency patients and the abundant clinical data from the APRT Deficiency Registry of the RKSC also add strength to this work.

In conclusion, this clinical trial revealed a marked decrease in urinary DHA excretion in patients with APRT deficiency using conventional doses of both allopurinol and febuxostat. Interestingly, febuxostat was significantly more efficacious than allopurinol in reducing DHA excretion in the prescribed doses. These results need to be confirmed in a larger patient sample. Furthermore, it will be important to include higher doses of allopurinol, in the range of 600–800 mg daily, in future studies. The clinical significance of the difference in urinary DHA excretion observed between these drugs warrants further study, as the effect on long-term renal outcomes may be improved with enhanced XOR inhibition and greater reduction of DHA excretion.

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Conflict of interests

The results presented in this article have not been published previously in whole or part, except in abstract form. None of the authors declared financial or other conflicting interests.

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