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# Impaired expression of an organic cation transporter, *IMPT1*, in a knockout mouse model for kidney stone disease

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**Abstract** The imprinted multimembrane-spanning polyspecific transporter-like gene 1 (*IMPT1*) encodes a predicted protein with organic cation transport capabilities. As a first step in understanding the function of *IMPT1*, we identified the renal structures expressing this gene in knockout mice with adenine phosphoribosyltransferase (APRT) deficiency and 2,8-dihydroxyadenine (DHA) nephrolithiasis. *IMPT1* mRNA was not detected using a standard in situ hybridization (ISH) protocol, but we observed intense staining in cortico-medullary tubules and glomeruli in wild-type mice using an improved reverse transcription-polymerase chain reaction (RT-PCR) ISH procedure. *IMPT1* mRNA expression was significantly decreased in the cortical region in kidney sections from APRT-deficient male mice. APRT-deficient female mice are less severely affected by DHA-induced kidney stone disease, and we observed only a modest reduction in *IMPT1* expression in kidneys from these mice. *IMPT1* expression in *APRT* heterozygous mice was comparable to that in wild-type mice, suggesting imprinting of one of the parental alleles. These findings suggest that decreased *IMPT1* mRNA expression may contribute to the impaired renal function in APRT-deficient male mice, and that RT-PCR ISH is a valuable tool for localizing the site of expression of transcripts that are not detectable using standard ISH procedures.

**Keywords** Adenine phosphoribosyltransferase deficiency · 2,8-Dihydroxadenine nephrolithiasis · Impaired renal transport · Imprinted multimembrane-spanning polyspecific transporter-like gene 1 · In situ hybridization · Reverse transcription-polymerase chain reaction

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## Introduction

In human adenine phosphoribosyltransferase (APRT) deficiency, adenine is metabolized via xanthine dehydrogenase to 2,8-dihydroxyadenine (DHA). This compound is extremely insoluble and its deposition in the kidney can lead to nephrolithiasis and, in some cases, acute or chronic renal failure [19]. Ion transport defects have been described in patients with kidney stone disease and these defects may play an important role in disease pathogenesis [8, 25, 28]. Several renal organic anion and cation transporters have been cloned and analyzed in recent years [4, 30], and the molecular bases of a number of inherited tubular transport defects, including X-linked nephrolithiasis, are beginning to be understood [15, 27, 28]. The availability of animal models for kidney stone disease and the identification of specific cell types showing changes in gene expression in the disease state, provide important experimental approaches for dissecting the role of transport-related genes in the pathophysiology of stone disease.

*APRT* knockout mice mimic the corresponding human disease [5, 17, 26]. They deposit DHA crystals and stones within the kidney, and the most severely affected ones develop renal failure and die by about 6 months of age. The disease phenotype in APRT-deficient male mice is more severe than in female mice [6, 26]. Using mRNA differential display, cDNA microarrays, and reverse transcription-polymerase chain reaction (RT-PCR), we previously identified several genes

showing major changes in expression in kidneys from *APRT* knockout mice compared with control mice and in cultured kidney epithelial cells exposed to DHA crystals [31, 32]. These genes encode proteins such as membrane transporters, metabolic enzymes, hormone-regulated proteins, and extracellular matrix proteins involved in various physiological and pathological processes. The expression of a putative membrane transport protein, imprinted multimembrane-spanning polyspecific transporter-like gene 1 (*IMPT1*), was decreased in *APRT* knockout mice [31].

As a first step in understanding the role of *IMPT1* and other genes showing marked expression changes in kidney stone disease, we developed in situ hybridization (ISH) and RT-PCR ISH procedures to identify the cell types expressing these genes in paraffin-embedded mouse kidney sections [29]. Here we show that *IMPT1* mRNA expression in cortical tubules in *APRT* knockout male mice is impaired to a greater extent than in female mice. This may adversely affect renal transport functions and contribute to the increased disease severity in *APRT* knockout male mice. Our data also support earlier findings that one of the parental *IMPT1* alleles is imprinted [1, 2, 3], and this may have important implications in the dosage regulation of renal transport function under both normal and pathological conditions.

## Materials and methods

All animal studies followed the recommendations of the Public Health Service "Guide for the care and use of laboratory animals". The ISH, RT-PCR ISH, and signal quantification procedures have been described [29]. Briefly, mice were anesthetized and the left kidney removed for solution RT-PCR studies [31]. The animal was then perfused, and the right kidney fixed and embedded in paraffin [6]. Sections 6–8 $\mu$m thick were used for the ISH and RT-PCR ISH studies using digoxigenin-labeled 40-mer oligonucleotide probes. The RT-PCR ISH procedure involved the incorporation of unlabeled nucleotides during PCR, followed by ISH using the same probes as above [10, 13, 16, 33]. The sequences of the forward and reverse *IMPT1* primers were 5′-TGGGAGGCTGAGCACCCATTT-3′ and 5′-GGTCCCTGTGTCTGAAGCGGA-3′, and the sequences of the sense and antisense internal probes were 5′-CCGGGCTGGTATTCAGTCTCTGTACTCTCAACGTAGTCAC-3′ and 5′-GTGACTACGTTGAGAGTACAGAGACTGAATACCAGCCCGG-3′. For solution RT-PCR, total RNA was isolated from frozen kidneys and first strand cDNA synthesis carried out using random primers. An aliquot was then amplified using the same *IMPT1* primers as above and optimized concentrations of primers and competimers for the 18S ribosomal RNA endogenous internal control gene [31].

## Results

### In situ hybridization

We did not detect *IMPT1* mRNA in kidney sections from 1-, 3-, or 6-month old wild-type male mice using our standard ISH protocol (15 ng/$\mu$l probe concentration), or even when the probe concentration was increased to 45 ng/$\mu$l. Messages of high abundance (such as that for kidney androgen protein) could be readily detected with a probe concentration of 5 ng/$\mu$l, and messages of extremely low abundance (such as *APRT* and neomycin) could be detected with a probe concentration of 45 ng/$\mu$l [29]. Furthermore, the expression of *APRT* and neomycin (which was used as a marker to create the knockout mice) in *APRT* heterozygous mice was approximately 50% of that in wild-type and knockout mice, respectively (data not shown). These findings indicated that the level of expression of *IMPT1* mRNA in mouse kidney was below the detection limit of our ISH procedure.

### RT-PCR in situ hybridization

Using RT-PCR ISH, we demonstrated intense staining for *IMPT1* mRNA in cortico-medullary tubular cells and in glomeruli in 1–6-month old *APRT* wild-type male and female mice (Fig. 1). A probe concentration of 1–2 ng/$\mu$l was found to be sufficient for signal detection. All RT-PCR controls were negative, demonstrating the specificity of the hybridization reaction. The signal was detectable with both the sense and antisense *IMPT1* probes, again demonstrating that cDNA synthesis had occurred (data not shown). The *IMPT1* mRNA signal intensities in the above regions in 1-month old *APRT* heterozygous male mice were comparable to those in wild-type mice, suggesting that one of the parental *IMPT1* alleles was imprinted (Fig. 2). There was a significant reduction in signal intensity in the cortical region, but not in the glomeruli, in *APRT* knockout male mice aged 1–6 months (Fig. 2). In APRT-deficient female mice, which are less severely affected by DHA nephrolithiasis, we observed only a modest reduction in *IMPT1* mRNA expression (data not shown).

### Solution RT-PCR

The primer:competimer ratios for the 18S ribosomal control RNA were optimized to give approximately equal amounts of the 18S and *IMPT1* cDNA products, as previously described [31]. These ratios were in the range 2.2:7.8–3.9:6.1, depending on the *APRT* genotype, age, and gender of the animals. RT-PCR was then repeated using these ratios and a comparison made of the *IMPT1* mRNA expression changes in *APRT* knockout compared with wild-type mice of the same age and gender. In line with our RT-PCR ISH data (see above), *IMPT1* mRNA expression was decreased 30% in both 1- and 3-month old APRT-deficient male mice, whereas there was only a modest decrease (approximately 10%) in APRT-deficient female mice.

## Discussion

The official symbol and name for human *IMPT1* is *SLC22A1L* [solute carrier family 22 (organic cation

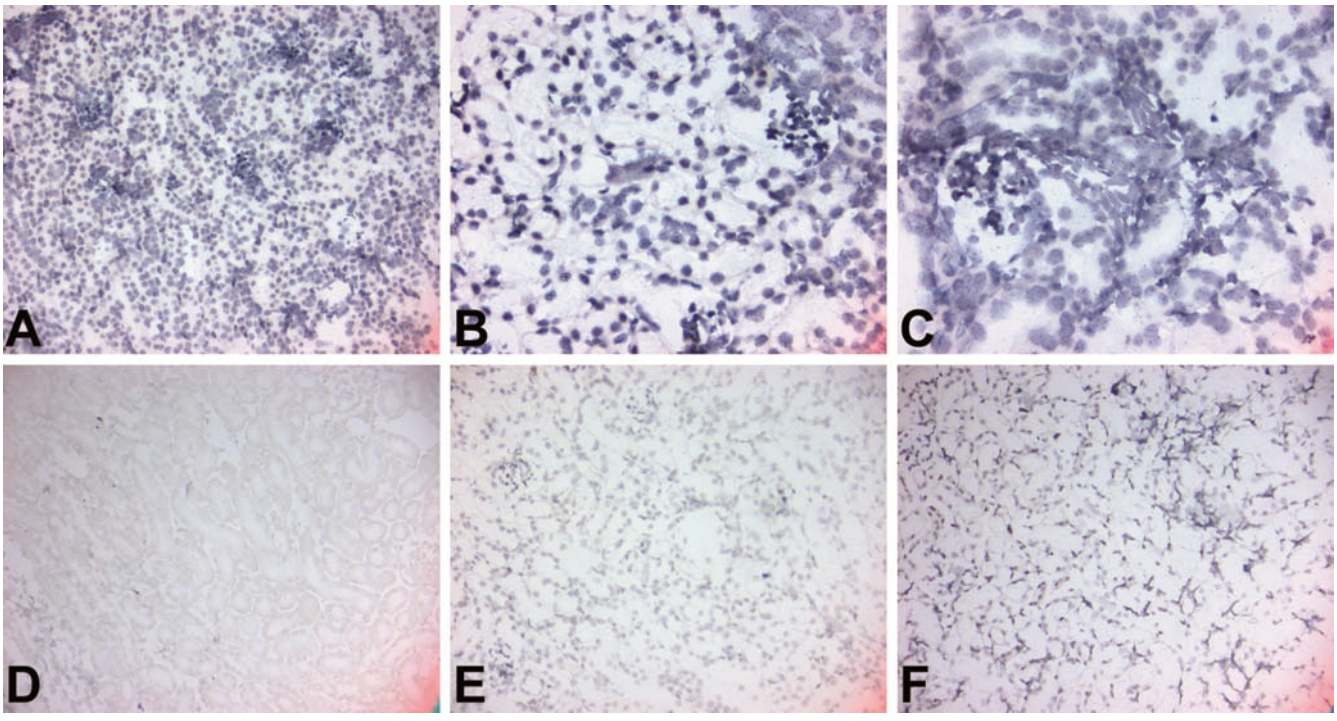


**Fig. 1** Localization of *IMPT1* mRNA in paraffin-embedded kidney sections from one-month-old *APRT* wild-type male mice by RT-PCR ISH. **A** Complete amplification system showing *IMPT1* expression in the nuclei of cortico-medullary tubular cells and in glomeruli (×20). **B** Same section as in **A** focusing on tubular sections (×40). **C** Same section as in **A** focusing on glomeruli (×40). **D** No reverse transcriptase control. **E** No PCR primers control. **F** No DNA polymerase control. All three controls are ×20 and, taken together, they demonstrate the specificity of the RT-PCR ISH reaction. The sections shown here were not counterstained; the bluish-purple signal is due to the deposition of the nitroblue-tetrazolium/5-bromo-4-chloro-3-indolyl phosphate chromogen following the hybridization reaction. To verify the identity of positively-staining structures, selected sections were counterstained with methyl green and adjacent sections were routinely stained with hematoxylin and eosin [6]

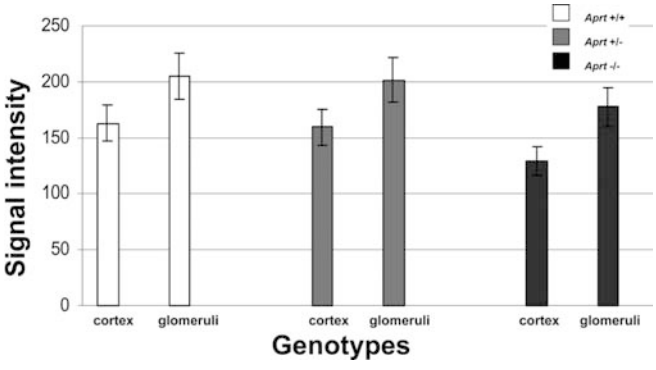


**Fig. 2** Quantification of signal intensity for *IMPT1* mRNA in cortical tubules and glomeruli in 1-month-old male mice of *APRT* genotypes +/+, +/−, and −/−. The error bars represent standard deviations. Similar findings were observed in 3- and 6-months-old male mice. The quantification procedure is described elsewhere [29]. The results are based on expression analyses in 3–5 mice for each group. Using the unpaired *t*-test, there was a significant decrease in *IMPT1* expression in the cortical region ($P = 0.02$), but not in the glomeruli ($P = 0.13$), in *APRT* knockout mice compared with wild-type controls

transporter, member 1-like)], but this gene also has been referred to as *BWR1A/ BWSCR1A* [22], *ITM* [14], *ORCTL2* [18], and *TSSC5* [9]. These names reflect the different experimental systems that have been used to study *IMPT1*, and they suggest that this gene may have multiple biological functions. The predicted product of *IMPT1* is a membrane-associated protein that is homologous to bacterial multi-drug resistance pumps and eukaryotic polyspecific organic cation transporters (OCTs) [2, 18]. At least five OCTs have been characterized, and *OCT1*, the first member of this family to be cloned, encodes a 554 amino acid protein with 12 predicted transmembrane domains [4]. Human *IMPT1* (*ORCTL2*) functions as an OCT in renal proximal tubules [18]. Human *IMPT1* (*TSSC5*) encodes a 424 amino acid protein with predicted ten transmembrane segments [9], clearly indicating that the product of this gene is distinct from the known OCTs.

*IMPT1* is one of at least eight genes on human chromosome 11p15.5 and the corresponding region on mouse chromosome 7 that are subject to parental imprinting [7, 23]. There is preferential expression of the maternal allele in various mouse tissues at fetal stages and a similar allelic expression bias has been observed in human fetal and postnatal tissues [2, 14, 20, 24]. Dosage regulation of *IMPT1* and other genes in the imprinted region may be important for placental function and fetal growth, and for metabolite transport in adult tissues. However, the stringency of allele-specific expression is not absolute and biallelic expression of *IMPT1* and insulin growth factor 2 (*IGF2*, another gene within the imprinted region) has been observed in human

populations [20]. This epigenetic heterogeneity may contribute to variability in metabolite and/or drug transport among individuals.

Abnormalities in the imprinting pattern in the 11p15.5 region have been identified in a number of human tumors, including hepatocarcinomas, Wilm's tumor, Beckwith-Wiedermann syndrome, and in tumor cell lines [7, 21, 23, 24]. The most frequent abnormality in hepatocarcinomas, for example, was gain of imprinting, which led to the loss of expression of *IMPT1* (*SLC22A1L*) and other genes from the maternal chromosome [23, 24]. These findings suggest that, in addition to its potential roles in fetal growth and metabolite transport, *IMPT1* may also function as a tumor suppressor gene [9]. The sequence of *IMPT1* (*ORCTL2*) in the 5′ region partially overlaps with another gene (*ORCTL2S*) that is transcribed in the opposite direction, suggesting that one or both of these genes may be subject to regulation by antisense RNA [1].

In our differential display studies, we observed reduced expression of *IMPT1* in whole kidney mRNA from APRT-deficient mice compared with controls [31], suggesting that the reduced expression may be associated with impairment of metabolite transport in kidney stone disease. At least at the histopathological level, there are significant common features between nephrolithiasis due to DHA and to calcium oxalate monohydrate (COM), the most common cause of stone disease [26]. Our recent cDNA microarray studies have shown that both crystal types trigger the same type of gene expression changes in cultured kidney epithelial cells [32], suggesting that DHA may cause cellular injury via mechanisms similar to those for COM crystals [11, 12].

To better understand the pathological basis of impaired metabolite transport in DHA nephrolithiasis, we used in situ techniques to identify the renal structures expressing *IMPT1* mRNA. We did not detect this mRNA in kidney sections from wild-type or APRT-deficient mice using our standard ISH protocol, but expression was detectable using an improved RT-PCR ISH procedure [10, 13, 16, 33]. This procedure is inherently more specific than direct in situ RT-PCR, in which labeled nucleotides are included in the PCR step. Our studies confirm that RT-PCR ISH can be used to localize the sites of expression of transcripts that may not be detectable using standard ISH procedures.

*IMPT1* mRNA is expressed in organs with metabolite transport functions, including the kidney, liver, intestine, extra-embryonic membranes, and placenta [2]. Immunohistochemical analysis of *IMPT1* (*ORCTL2*) expression in human renal sections demonstrated staining in proximal tubules, in some glomeruli, and at the transition between the glomeruli and the tubules, but there was no significant expression in distal tubules [18]. In the proximal tubules, expression was localized to the apical membrane surface of epithelial cells, which is consistent with its function as a renal transporter. Our findings of impaired *IMPT1* mRNA expression in cortical tubules in mice with kidney stone disease provide strong support for such a function.

Reduced *IMPT1* mRNA expression in APRT-deficient mice may be due to loss of proximal tubules, gain of imprinting, or hormonal changes. Histochemical studies suggest that decreased gene expression, at least in one-month-old APRT-deficient mice, is unlikely to be the result of tubular loss [29]. Whether reduced *IMPT1* expression in APRT-deficient male mice is due to gain of imprinting or hormonal changes is under investigation.

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