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ORIGINAL ARTICLE

Analysis of regulatory polymorphisms in organic ion transporter genes (*SLC22A*) in the kidney

Ken Ogasawara · Tomohiro Terada · Hideyuki Motohashi · Jun-ichi Asaka · Masayo Aoki · Toshiya Katsura · Tomomi Kamba · Osamu Ogawa · Ken-ichi Inui

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Abstract Organic cation transporters (OCTs) and organic anion transporters (OATs) (SLC22A family) play crucial roles in the renal secretion of various drugs. Messengar ribonucleic acid (mRNA) expression of transporters can be a key factor regulating interindividual differences in drug pharmacokinetics. However, the source of variations in mRNA levels of transporters is unclear. In this study, we focused on single nucleotide polymorphisms (SNP) in the promoter region [regulatory SNPs (rSNPs)] as candidates for the factor regulating mRNA levels of SLC22A. We sequenced the promoter regions of *OCT2* and *OAT1–4* in 63 patients and investigated the effects of the identified rSNPs on transcriptional activities and mRNA expression. In the OCT2 promoter region, one deletion polymorphism (−578_−576delAAG) was identified; −578_−576delAAG significantly reduced OCT2 promoter activity ($p < 0.05$), and carriers of −578_−576delAAG tend to have lower OCT2 mRNA levels, but the difference is not significant. There was no rSNP in the *OAT1* and *OAT2* genes. The five rSNPs of *OAT3* and one rSNP of *OAT4* were unlikely to influence mRNA expression and promoter activity. This is the first study to investigate the influences of rSNPs on mRNA expression of SLC22A in the kidney and to identify a regulatory polymorphism affecting OCT2 promoter activity.

Keywords Transporter · SLC22A · OAT · OCT · SNP · Promoter · rSNP

K. Ogasawara · T. Terada · H. Motohashi · J. Asaka · M. Aoki · T. Katsura · K. Inui (✉)
Department of Pharmacy, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan
e-mail: inui@kuhp.kyoto-u.ac.jp

T. Kamba · O. Ogawa
Department of Urology, Kyoto University Hospital, Faculty of Medicine, Kyoto University, Sakyo-ku, Kyoto 606-8507, Japan

Introduction

The SLC22A family, which comprises organic cation transporters (OCTs) and organic anion transporters (OATs), plays pivotal roles in the renal excretion of various organic compounds, including drugs, toxins, and endogenous metabolites (Inui et al. 2000; Koepsell and Endou 2004). OCT2 (SLC22A2), OAT1 (SLC22A6), OAT2 (SLC22A7), OAT3 (SLC22A8), and OAT4 (SLC22A11) are highly expressed in the human kidney cortex (Motohashi et al. 2002; Terada and Inui 2007) and are known to transport clinically important drugs such as biguanides, histamine (H_2) receptor antagonists, anticancer drugs, antivirals, cephalosporin antibiotics, and nonsteroidal anti-inflammatory drugs (Burckhardt and Burckhardt 2003; Rizwan and Burckhardt 2007; Koepsell et al. 2007).

It has been demonstrated that variations in the expression levels of SLC22A are responsible for the individual variations in pharmacokinetics by clinical studies (Sakurai et al. 2004, 2005) and in vivo animal experiments (Ji et al. 2002; Deguchi et al. 2005). For example, we previously reported that the messenger ribonucleic acid (mRNA) level of OAT3 among OAT1–4 significantly correlated with the elimination rates of cefazolin and phenolsulfonphthalein in patients with renal diseases (Sakurai et al. 2004, 2005). However, factors regulating the interindividual differences in mRNA levels of transporters have not been elucidated.

The promoter region regulates the mRNA expression of genes and single nucleotide polymorphisms (SNPs) in the promoter region [regulatory SNPs (rSNPs)] can alter the transcription of genes (Buckland 2006), suggesting that rSNPs are candidates for the source of the variation in mRNA levels. Recently, we characterized the transcriptional regulation of *MATE1 (SLC47A1)* and identified an rSNP at the Sp1 binding site, which reduced MATE1 promoter activity (Kajiwara et al. 2007). In the SLC22A family, Bhatnagar et al. (2006) found one rSNP in the *OAT1* gene and five rSNPs in the *OAT3* gene, but it is unclear whether these rSNPs affect mRNA levels. In addition, the transcriptional regulation of *SLC22A* genes has been characterized, and *cis*-elements in the proximal promoter regions were identified (Ogasawara et al. 2006, 2007; Asaka et al. 2007; Popowski et al. 2005; Kikuchi et al. 2006; Saji et al. 2008). If rSNPs are located in these *cis*-elements, mRNA expression may be altered. Therefore, we tried to identify rSNPs affecting the mRNA expression of OCT2, OAT1, OAT2, OAT3, and OAT4. In this study, we sequenced these proximal promoter regions spanning 1 kb from the transcription start site.

Materials and methods

Patients

Normal parts of human kidney cortex were obtained from 63 Japanese nephrectomized patients with renal cell carcinoma (RCC) or transitional cell carcinoma at Kyoto University Hospital. This study was conducted in accordance with the Declaration of Helsinki and its amendments and was approved by Kyoto University Graduate School and Faculty of Medicine, Ethics Committee. All patients gave their written informed consent.

Quantification of OCT2 and OAT1–4 mRNA expression

The mRNA expression levels of OCT2 and OAT1–4 were quantified as described previously (Motohashi et al. 2002; Sakurai et al. 2004). Briefly, total RNA was isolated from specimens using a MagNA Pure LC RNA isolation Kit II (Roche Diagnostic GmbH, Mannheim, Germany) and was reverse transcribed to yield complementary DNA (cDNA). Real-time polymerase chain reaction (PCR) was performed using the ABI PRISM 7700 sequence detector (Applied Biosystems, Foster, CA, USA). Glyceraldehyde-3-phosphate dehydrogenase mRNA was used as an internal control.

Genotyping the promoter regions of *OCT2* and *OAT1–4*

Genotyping was investigated by direct sequencing in 63 patients who had data on mRNA expression levels. Genomic DNA was isolated from specimens with a DNA isolation Kit I (Roche Diagnostic GmbH). The promoter regions (about 1 kb) of *OCT2* and *OAT1–4* were amplified by PCR using the specific primers listed in Table 1. The PCR products were sequenced using a multicapillary DNA sequencer RISA384 system (Shimadzu, Kyoto, Japan). The *OAT3* haplotypes were analyzed using SNPAlyze ver. 5.0 (Dynacom, Chiba, Japan).

Preparation of reporter constructs

The OCT2 (−91/+23) and OAT3 (−926/+21) constructs have been previously described (Ogasawara et al. 2006; Asaka et al. 2007). Based on the human genomic sequence (accession number NT_007422), the 1,000-bp flanking region upstream of the transcription start site (Asaka et al. 2007) of the *OCT2* gene was amplified by PCR using genomic DNA from specimens corresponding to the wild type and heterozygote for −578_−576delAAG. The PCR products were isolated by electrophoresis and subcloned into the firefly luciferase reporter vector, pGL3-Basic (Promega, Madison, WI), at KpnI and MluI sites. These reporter plasmids are hereafter referred to as the OCT2 (−1,000/+23) *wt* construct and OCT2 (−1,000/+23) *delAAG* construct. Furthermore, the fragments spanning bp −786 to −476, including bp −578 to −576, were digested with StuI from the OCT2 (−1,000/+23) *wt* and *delAAG* constructs and subcloned into the OCT2 (−91/+23) construct, upstream of the proximal promoter region (−91 to +23) of *OCT2* by blunt ligation. These reporter plasmids are hereafter referred to as the OCT2 (−786_−476, −91/+23) *wt* construct and OCT2 (−786_−476, −91/+23) *delAAG* construct.

Based on the human genomic sequence (accession number NT_033903), the 2,238-bp flanking region upstream of the transcription start site of *OAT4* genes was cloned by PCR using human genomic DNA (Promega). The PCR product was isolated by electrophoresis and subcloned into pGL3-Basic (Promega) at NheI and XhoI sites. This reporter plasmid is hereafter referred to as OAT4 (−2,238/+82). In the *OAT3* and *OAT4* genes, site-directed mutations in the identified rSNPs were introduced into the OAT3 (−926/+21) construct and OAT4 (−2,238/+82) construct with a QuikChange II site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). The nucleotide sequences of these reporter constructs were confirmed using a multicapillary DNA sequencer RISA384 system

Table 1 Primers used for direct sequencing

Name	Sequence (5′ to 3′)	Position	Purpose
OCT2 (NT_007422)			
Forward primer	AAAGGGCAGCAGCCCATGTTCGCA	−1,039 to −1,016	PCR/sequencing
Reverse primer	TCCATGCTCCAGGACATCGTCCACGGT	+207 to +180	PCR
Sequence primer 1	GAGAAGGTCAGAGAGAGAGACTT	−715 to −693	Sequencing
Sequence primer 2	GGCTTGCAACCCACTTCGAATCT	−373 to −351	Sequencing
OAT1 (NT_033903)			
Forward primer 1	CAAGGCTGCAGTGTGCCAAGATTGT	−1,196 to −1,172	PCR
Reverse primer 1	CGTCATACACAATGTCGGGTGATTC	−603 to −627	PCR/sequencing
Forward primer 2	AGACACTATGGACAGAAGACAAT	−701 to −679	PCR/sequencing
Reverse primer 2	GGATGAGATCCAAATAGTCTGCTTT	−277 to −301	PCR
Forward primer 3	GGGCACCCTGTAATTTCCCTGGCAA	−335 to −311	PCR/sequencing
Reverse primer 3	TCCCTTGCAGCTTCTCCTCACTTTG	+88 to +64	PCR
OAT2 (NT_007592)			
Forward primer	GTAGGAGCTGGATTAAACCAGTG	−923 to −901	PCR/sequencing
Reverse primer	TGCTGCTCACCCACCAAATGCTT	+96 to +74	PCR
Sequence primer 1	ATGGGACAGGCAGTTGGAACCAT	−609 to −587	Sequencing
Sequence primer 2	CCTGGTTAGGATCCAGCTTTCAG	−424 to −402	Sequencing
OAT3 (NT_033903)			
Forward primer	ACAGGGCTGCCGTGAGGGTTAAAAG	−919 to −895	PCR/sequencing
Reverse primer	CGATGGGGCTGGTGATCAGTGTAG	+91 to +68	PCR
Sequence primer 1	CAGGAGAGAAGCCTGTCCATTGA	−647 to −625	Sequencing
Sequence primer 2	CATCAGTTGAGAGGGACCTTGAA	−349 to −327	Sequencing
OAT4 (NT_033903)			
Forward primer	GTCCAGACACCCACCCACACCTCA	−1,101 to −1,078	PCR/sequencing
Reverse primer	GGGTGGGGCACTGTCCAATAACCAG	+257 to +233	PCR
Sequence primer 1	CCTGGGCTGCCATAAGGAGTGAA	−777 to −757	Sequencing
Sequence primer 2	TGGTAGGAGGTTCACATCAGGAA	−419 to −397	Sequencing

OCT organic cation transporters, *OAT* organic anion transporters, *PCR* polymerase chain reaction

(Shimadzu). The primers used in PCR amplification and the site-directed mutagenesis are listed in Table 2.

Cell culture, transfection, and luciferase assay

The American opossum kidney (OK) epithelial cell line and the porcine kidney (LLC-PK_1) epithelial cell line were cultured, as described previously (Ogasawara et al. 2006; Asaka et al. 2007). Transfection and the luciferase assay were also carried out as described previously (Ogasawara et al. 2006; Asaka et al. 2007).

Statistical analysis

Data from the luciferase assay were statistically analyzed with a one-way analysis of variance (ANOVA) followed by Scheffé's test. The correlation between haplotype and mRNA expression was analyzed using Kruskal–Wallis test. Comparisons between two genotypes were carried out using the Mann–Whitney *U* test.

Results

Identification of SNPs in the promoter regions of *OCT2*, *OAT3*, and *OAT4*

The promoter regions of *OCT2* and *OAT1–4* were sequenced for about 1 kb from the transcription start site. The results are summarized in Table 3. In the promoter region of *OCT2*, one novel polymorphism was identified, namely, the deletion of AAG at position −578 to −576 (−578_−576delAAG). There were no SNPs in the promoter regions of *OAT1* and *OAT2*. In the *OAT3* gene, five rSNPs, at positions −659_−658, −578, −515, −461, and −19, were identified, and −19C > A had not been reported previously; −659_−658insG, which is a G insertion between −659 and −658, and −578C > G occurred simultaneously. Haplotype analysis showed that OAT3 promoter region was divided into five haplotypes (Table 4). In the *OAT4* gene, one rSNP was identified at position −18.

Table 2 Primers used for preparation of various reporter constructs

Name	Sequence (5′ to 3′)	Position
Cloning of 5′-flanking region		
OCT2 (−1,000/+23)		
Forward primer	GGGGTACCATCCTAAGGCTCACGGCCAAC	−1,000 to −980
Reverse primer	CGACGCGTTCACAGCCCAGTAATCTTCCC	+1 to +23
OAT4 (−2,238/+82)		
Forward primer	GGGCTAGCGGCCATGCTGGCCTTTCGTTT	−2,238 to −2,216
Reverse primer	GGCTCGAGTCCGAGCTGCCCACCAAGTGT	+82 to +62
Site-directed mutagenesis		
OAT3/−659_−658insG		
Forward primer	CATTTGGTCCCCA**G**GGGGGGAAGCGGCTGATC	−676 to −647
Reverse primer	GATCAGCCGCTTCCCCCC**C**TGGGGACCAAATG	−647 to −676
OAT3/−578C > G		
Forward primer	GTCAGAGACACAGACA**G**GGAGGTCCTGGC	−594 to −566
Reverse primer	GCCAGGACCTCC**C**TGTCTGTGTCTCTGAC	−566 to −594
OAT3/−515A > C		
Forward primer	GCGTCCTCAGGGATAGATGA**C**AGTGACATTTTCTG	−535 to −501
Reverse primer	CAGAAAATGTCACT**G**TCATCTATCCCTGAGGACGC	−501 to −535
OAT3/−461T > C		
Forward primer	GAGGCAAATCCCCC**C**TCCCCTACTCGGGAG	−474 to −446
Reverse primer	CTCCCGAGTAGGGGA**G**GGGGATTTGCCTC	−446 to −474
OAT3/−19C > A		
Forward primer	CCTTATATAAGCCCCC**A**TGGGGGAGGCACAAAC	−35 to −3
Reverse primer	GTTTGTGCCTCCCCCA**T**GGGGGCTTATATAAGG	−3 to −35
OAT4/−18C > T		
Forward primer	GTAACATCAAAGCAC**T**CTGTCTTTTTAAATAGAATCTGCC	−33 to +7
Reverse primer	GGCAGATTCTATTTAAAAAGACAG**A**GTGCTTTGATGTTAC	+7 to −33

Restriction enzyme sites are underlined. Mutations introduced into the oligonucleotides are shown in bold

OCT organic cation transporters, *OAT* organic anion transporters

Influences of rSNPs on OCT2, OAT3, and OAT4 promoter activity

To determine whether these polymorphisms influence the promoter activity in vitro, mutations were introduced to compare luciferase activity. At first, we examined the influence of −578_−576delAAG on OCT2 promoter activity using the OCT2 (−1,000/+23) constructs (Fig. 1a). This deletion led to a reduction of 14% in OCT2 promoter activity; however, the OCT2 (−1,000/+23) *wt* construct showed only a 1.5-fold increase in luciferase activity compared with pGL3-Basic. We previously reported that OCT2 reporter constructs of more than 300 bp showed weak promoter activity and the OCT2 (−91/+23) construct had the strongest promoter activity (approximately tenfold) among OCT2 reporter constructs (Asaka et al. 2007). Therefore, the fragment including position −578_−576 was digested and subcloned into the OCT2 (−91/+23) construct, upstream of the proximal promoter region (−91 to +23) of *OCT2*, and luciferase activity was measured. As a result, −578_−576delAAG significantly reduced luciferase activity to one half of the wild-type level ($p < 0.05$; Fig. 1b).

For *OAT3* rSNPs, five reporter constructs carrying each haplotype were prepared. Haplotype 3 corresponds to NCBI reference sequence (accession number NT_033903). Haplotypes 1, 2, 4, and 5 showed increases of 6%, 16%, 22%, and 11% in relative luciferase activity compared with haplotype 3 (Fig. 2a). However, the significant differences among haplotypes were not observed. The *OAT4* rSNP −18C > T led to an increase of 40% in luciferase activity compared with the wild type, but significant difference was not observed (Fig. 2b).

Association of rSNPs with mRNA levels of OCT2, OAT3, and OAT4

Next, we tried to examine whether these rSNPs affect mRNA expression. Messenger RNA levels are regulated by various factors, including hormones, pharmaceutics, and the condition of disease, except for rSNPs. To assess only the effect of rSNP on mRNA expression, we needed to

Table 3 Regulatory single nucleotide polymorphisms (SNPs) of the *OCT2*, *OAT3*, and *OAT4* genes in 63 Japanese nephrectomized patients

Gene	dbSNP (NCBI)	Position	Allele	Allele frequency (%)	Genotype	Number
OCT2	ss94002365	−578_−576	AAG	91.3	AAG/AAG	54
			–	8.7	AAG/−	7
					−/−	2
OAT3	rs3840764	−659_−658	–	18.3	−/−	5
			G	81.7	−/G	13
					G/G	45
	rs948980	−578	C	18.3	C/C	5
			G	81.7	C/G	13
					G/G	45
	rs948979	−515	A	15.9	A/A	4
			C	84.1	A/C	12
					C/C	47
	rs3809069	−461	T	73.8	T/T	36
			C	26.2	T/C	21
					C/C	6
	ss94002361	−19	C	98.4	C/C	61
			A	1.6	C/A	2
					A/A	0
OAT4	rs3759053	−18	C	96.8	C/C	59
			T	3.2	C/T	4
					T/T	0

OCT organic cation transporters, *OAT* organic anion transporters, *dbSNP* Single Nucleotide Polymorphism Database, *NCBI* National Center for Biotechnology Information

Table 4 Haplotypes at the promoter region of organic anion transporter (*OAT*)*3*

Haplotype	−659_−658	−578	−515	−461	−19	Frequency (%)
1	G	G	C	T	C	53.9
2	G	G	C	C	C	26.2
3	–	C	A	T	C	15.9
4	–	C	C	T	C	2.4
5	G	G	C	T	A	1.6

exclude the factors that have an influence on the expression. Therefore, among 63 patients, we focused on 23 males with RCC who had neither complications of renal failure nor diabetes, because these diseases and gender differences were reported to affect the mRNA levels of SLC22A (Urakami et al. 1999; Buist et al. 2002; Ji et al. 2002; Thomas et al. 2003; Sakurai et al. 2004; Monica Torres et al. 2005).

The OCT2 mRNA level of the hetero- or homozygote for −578_−576delAAG was slightly decreased compared with that of the wild type, but this difference was not significant ($p = 0.1462$; Fig. 3a). Next, we analyzed *OAT3* haplotypes for any association with the OAT3 mRNA level and the rSNP at position −18 for any association with the OAT4 mRNA level (Fig. 3b, c). Haplotypes of *OAT3* and rSNP (−18C > T) of *OAT4* did not affect each mRNA expression level (OAT3, $p = 0.5923$; OAT4, $p = 0.6168$).

Discussion

It has been demonstrated that interindividual variation in mRNA expression of transporters regulates drug pharmacokinetics by experiments using human tissue (Sakurai et al. 2004, 2005) and laboratory animals (Ji et al. 2002; Deguchi et al. 2005). Recent studies demonstrated that rSNPs can alter gene transcription (Wang et al. 2005; Buckland 2006), suggesting rSNP to be the cause of the variation in mRNA levels of drug transporters. In the uridine diphosphate-glucuronosyltransferase (*UGT*) *1A1* gene involved in the metabolism of irinotecan, *UGT1A1*28*, which has a seventh dinucleotide (TA) insertion in the $(TA)_6$TAA-box of the UGT1A1 promoter, results in a considerably reduced enzyme expression of about 30–80% (de Jong et al. 2006). Studies have shown that the homozygous *UGT1A1*28* genotype was associated with an increased risk of developing leucopenia and severe delayed-type diarrhea after treatment with irinotecan (de Jong et al. 2006). On the other hand, in transporter genes, it has been reported that an SNP in the intron (intronic SNP; iSNP) of *OCTN1* (*SLC22A4*) influences mRNA expression, a risk factor for rheumatoid arthritis (Tokuhiro et al. 2003), and that an rSNP of *OCTN2* (*SLC22A5*) affects transcription, contributing to the pathogenesis of Crohn's disease (Peltekova et al. 2004). These reports indicated that rSNPs (or iSNPs) have

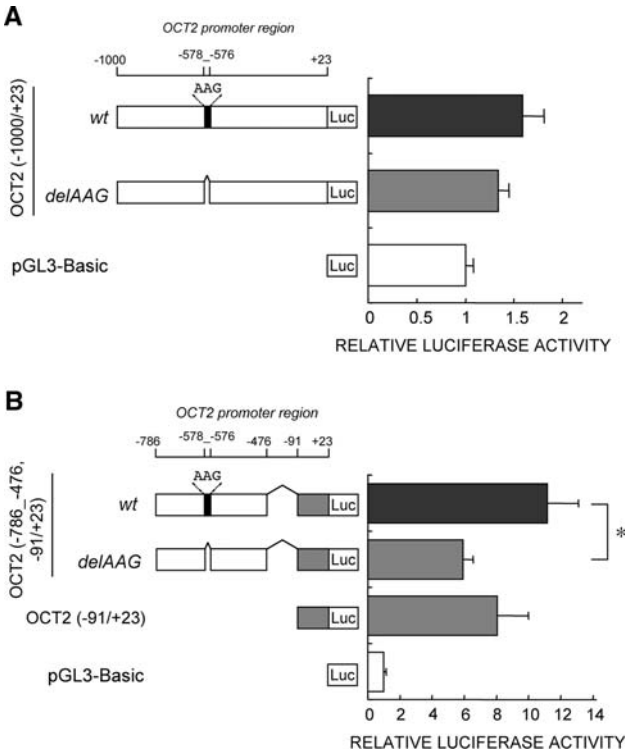

Fig. 1 Effect of −578_−576delAAG on organic cation transporter (OCT)2 promoter activity. Weight of 500 ng of the OCT2 (−1,000/+23) constructs (**a**) and OCT2 (−786_−476, −91/+23) constructs and OCT2 (−91/+23) construct (**b**) were transiently transfected into LLC-PK_1 cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold increase compared with the pGL3-Basic vector and represent the mean ± standard deviation for three replicates. *Asterisks* significantly different from *wt*, $p < 0.05$

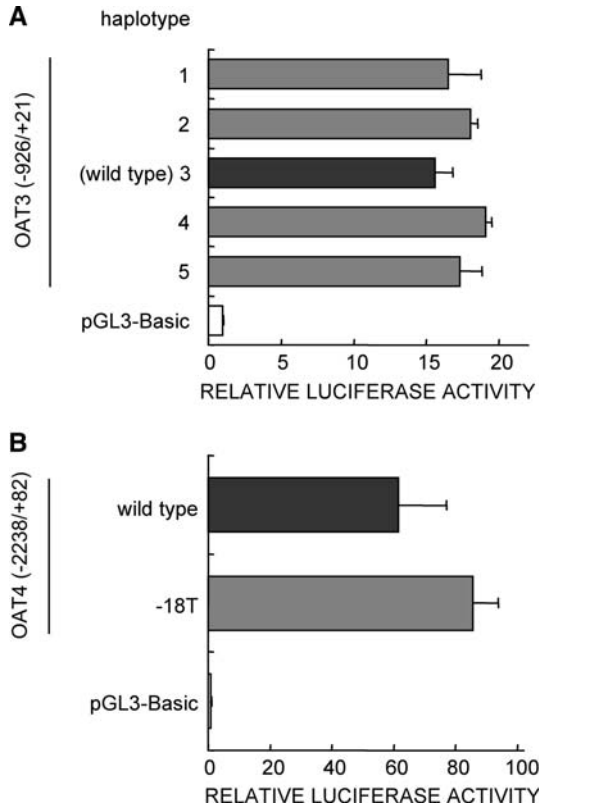

Fig. 2 Effect of haplotypes and regulatory single nucleotide polymorphisms (rSNP) on organic anion transporters (OAT)3 and OAT4 promoter activity. The mutated OAT3 (−926/+21) constructs (**a**) and OAT4 (−2,238/+82) constructs (**b**) (500 ng) were transiently transfected into opossum kidney (OK) cells for luciferase assays. Firefly luciferase activity was normalized to *Renilla* luciferase activity. Data are reported as the relative fold increase compared with pGL3-Basic and represent the mean ± standard deviation for three replicates

an impact on the mRNA expression of drug transporters and pharmacokinetics among individuals.

Expression levels of mRNA are regulated by various factors, including hormones, pharmaceutics, and the extent of disease, except for rSNP. We previously reported that OAT1 mRNA levels are significantly lower in the kidney of patients with renal diseases than in the normal kidney cortex, whereas OAT3 mRNA levels are slightly decreased (Sakurai et al. 2004). It was reported that mRNA and protein expression of OCT2 was reduced in experimental diabetes (Thomas et al. 2003). To assess only the effect of rSNPs on mRNA expression levels, we focused on 23 males with RCC, who had neither complications of diabetes nor renal failure, and we sequenced the promoter regions (about 1 kb) of *OCT2* and *OAT1–4*.

In the *OCT2* gene, we found one polymorphism, a 3-bp deletion (−578_−576delAAG). In vitro promoter analysis suggested that −578_−576delAAG lowers OCT2 mRNA expression. However, in the OCT2 mRNA level, significant differences were not observed between the wild type and hetero- or homozygote for −578_−576delAAG. The poor effect of −578_−576delAAG on luciferase activity in OCT2 (−1,000/+23) constructs may reflect the lack of the difference in OCT2 mRNA level. The change in mRNA level caused by −578_−576delAAG may be masked due to the existence of other factors affecting the expression. Further studies using much larger sample size are needed to clarify whether −578_−576delAAG has an impact on OCT2 mRNA expression in vivo. The OCT2 plays a crucial role in the renal secretion of organic cations (Koepsell et al. 2007), so it is important to investigate the impact of −578_−576delAAG on the renal excretion of cationic drugs.

For *OAT* genes, five rSNPs of *OAT3* and one rSNP of *OAT4* were identified, but these rSNPs were unlikely to influence the mRNA levels and promoter activity of OAT3 and OAT4. In this study, no rSNPs had a functional effect on the mRNA expression of OAT1 and OAT3, which have been proposed to be responsible for the tubular uptake of organic anions into the circulation. The rSNPs of *OAT1* and *OAT3* genes reported by Bhatnagar et al. (2006) were

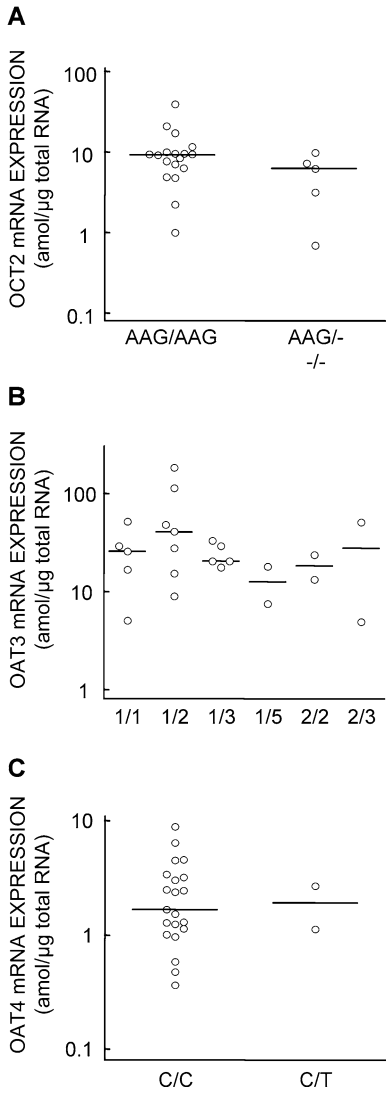

Fig. 3 Association between regulatory polymorphisms and messenger ribonucleic acid (mRNA) levels of the organic cation transporters (*OCT*)*2*, *OAT3*, and *OAT4* genes in 23 nephrectomized patients. The mRNA levels were determined by real-time polymerase chain reaction. Samples were divided according to genotype: OCT2 −578_−576delAAG (**a**) , OAT3 haplotypes (**b**), OAT4 −18C > T (**c**) . The *bars* show the median mRNA expression levels in each genotype

located more upstream of the promoter regions than those analyzed here, but their effects on mRNA expression were not examined in our study. In the *UGT1A1* gene, −3,263T > G (*UGT1A1*60*), an rSNP located 3-kb upstream of the transcription start site is known to lower transcriptional activity of UGT1A1 (Sugatani et al. 2002). To find functional rSNPs, analyses of the more upper promoter region of the *OAT1* and *OAT3* genes may be needed.

We found seven polymorphisms in the OCT2, OAT3, and OAT4 promoter regions, but these polymorphisms were not located at the *cis*-elements for the regulation of *OCT2* and *OAT1-3*. Computational sequence analyses of these promoter regions around the polymorphisms were carried out using TRANSFAC 6.0 at http://www.gene-regulation.com/. It was demonstrated that there are no binding sites for known transcription factors around the polymorphisms and that these polymorphisms have no effect on the binding of transcription factors. The OCT2 promoter activity was reduced by the induction of polymorophism of −578_−576delAAG, but transcription factors may not be associated around this sequence. Further studies are needed to elucidate the molecular mechanisms underlying the reduction of the OCT2 promoter activity by −578_−576delAAG.

In conclusion, we identified a regulatory polymorphism affecting the promoter activity of OCT2. This is the first study to investigate the influences of polymorphisms on mRNA expression in the SLC22A family in the kidney.

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