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Identification and functional characterization of novel *MATE1* genetic variations in Koreans

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ABSTRACT

Multidrug and toxin extrusion 1 (*MATE1*, SLC47A1), an organic cation transporter, plays an important role in the renal and biliary elimination of various clinical drugs, including the anti-diabetic drug metformin. The goal of this study was to identify and characterize novel genetic variants of *MATE1*. Five variants in the promoter region and two nonsynonymous variants, p.D64G and p.L125F, were identified in 48 DNA samples from healthy Koreans. *MATE1* promoter haplotype 3 containing g.−1975C>A showed a significant increase in reporter activity. Three transcription factors, Nkx-2.5, SREBP-1, and USF-1 were predicted to bind to the promoter in the region of g.−1975C>A. Results from electrophoretic mobility shift assays showed that the g.−1975A allele exhibits greater binding affinity to all of these transcription factors than the g.−1975C allele. In particular, we found that Nkx-2.5 and USF-1 induce *MATE1* transcription. Our study suggests that the common promoter haplotype of *MATE1* changes *MATE1* transcriptional activity regulated by Nkx-2.5, SREBP-1, and USF-1.

1. Introduction

The liver and kidney play important roles in the elimination of various cationic drugs from the body [1]. The human multidrug and toxin extrusion 1 (*MATE1*, SLC47A1) is highly expressed in the liver and kidney and mediates the biliary and renal excretion of many cationic drugs and their metabolites [2]. Various drugs including metformin, verapamil, cimetidine, and procainamide as well as endogenous substances such as guanidine are substrates of *MATE1* [3]. Recent screening samples from large ethnically diverse populations have identified and functionally characterized genetic variants in the *MATE1* coding and basal promoter region [4–6]. A polymorphism in the promoter region, g.−66T>C, has shown a significant decrease in reporter activity and an association with enhanced metformin response [5,6]. Several nonsynonymous variants in the coding region have been known as functional variants having a complete loss or a decrease in transport activity [4]. In addition, two nonsynonymous variants, p.K64N and p.G211V have been identified in Japanese subjects and showed a decrease in transporter activity [7]. In particular, the p.G211V showed a complete loss of transport activity owing to an alteration of protein expression in cell surface membranes. Functional changes in the activity or expression level of *MATE1* caused by genetic variants in coding or non-coding regions may result in changes in the levels of drugs that are substrates of the *MATE1* transporter. For example, reduction in the expression level of *MATE1* in mice has been associated with a higher hepatic concentration of metformin and lactic acidosis [8]. Many clinical studies have been conducted to investigate the association between genetic variants in transporters including *MATE1*, and the pharmacokinetics or pharmacodynamics of clinical drugs, especially metformin [9–12]. Functional variants of organic cation transporter (*OCT*) 1 and *OCT2* could explain the inter-individual variability of metformin pharmacokinetics [13–17] and the common 5′-UTR variant of *MATE2-K*, g.−130G>A was associated with the pharmacokinetics or pharmacodynamics of metformin in previous studies [6,18]. To date, in the case of *MATE1* transporter, one intronic single nucleotide polymorphism (SNP), rs2289669, and one promoter SNP, rs2252281 (g.−66T>C) showed significant associations with metformin response [6,9–11].

The goal of this study was to identify and functionally characterize novel genetic variations in *MATE1* in Koreans. We screened DNA samples from 48 healthy Koreans for variants in the promoter and coding regions of *MATE1* and investigated the function of common haplotypes in the promoter region through *in vitro* luciferase assays and electrophoretic mobility shift assays (EMSAs). Our study provides information about the mechanisms responsible for the transcriptional regulation of *MATE1* and identifies a

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functionally important haplotype that may contribute to variation in the expression level of this transporter.

2. Materials and methods

2.1. Genetic analysis of MATE1 variants

This study was approved by the Institutional Review Board of Ewha Medical Center, Seoul, Korea (ECT 12-15-02). Forty-eight genomic DNA samples were collected from unrelated healthy individuals from the DNA bank of the Korea Pharmacogenomics Research Network at Seoul National University (Seoul, Korea). All subjects provided written informed consent, and the health status of each subject was evaluated through routine physical examinations and laboratory tests. All of the subjects enrolled in this study had an East Asian ethnic background. To identify genetic variants of *MATE1*, we directly sequenced the entire *MATE1* coding region and flanking intronic sequences with an automated genetic analyzer (Life Technologies Corporation, Carlsbad, CA, USA). To identify genetic variants in the promoter region of *MATE1*, a polymerase chain reaction fragment (−2000 to +17 bp from the translational start site) was amplified and directly sequenced using an automated genetic analyzer. Haplotype assembly was performed using the program Haploview (version 4.2, developed by the Broad Institute, Cambridge, MA, USA). Nucleotide location numbers were assigned from the translational start site according to the *MATE1* mRNA sequence (GenBank accession number NM_018242).

2.2. Construction of MATE1 plasmids and variants

To construct reporter plasmids containing the *MATE1* reference sequence in the promoter region, we amplified 2017 bp of *MATE1* using primers containing recognition sites for restriction endonucleases HindIII and NheI (Table 1) from an individual genomic DNA sample with the reference sequence according to NM_018242. The amplified product was inserted into the

Table 1
Oligonucleotide primers used in the construction of *MATE1* reporter plasmids and EMSA.

Primes for *MATE1* promoter cloning[a]	
Reference (2017 bp)	
Sense (HindIII site)	5′-CTA **GCT AGC** GGC TGG TCT CAA AAC TCC TGG GTT C-3′
Antisense (NheI site)	5′-CCC **AAG CTT** TCC TCA GGA GCT TCC ATG TGA CTC G-3′
Primers for *MATE1* mutagenesis PCR[b]	
g.−1975C>A	5′-CAA AAC TCC TGG GTT C**A**A GTG ATC CTC CCA CCT-3′
g.−1374C>T	5′-CAG AGG AGG AAG AGA AAC TA**T** GGA AAG AAT CCC AAG G-3′
g.−1083C>T	5′-GGC CAG GAT GGT CTC GAT **T**TC TTG ACC TTG TGA T-3′
g.−151C>A	5′-GCG CCA GGC G**A**G GGG CGG GCT-3′
g.−66T>C	5′-CTG CGC GGT AC**C** CAC TGC CGG CC-3′
Primers for EMSA	
Reference (g.−1975C)[b]	5′-CCT GGG TTC **C**AG TGA TCC TC-3′
Variant (g.−1975A)[b]	5′-CCT GGG TTC **A**AG TGA TCC TC-3′
Consensus Nkx-2.5[c]	5′-TAA CGC AGT **TCA AGT G**AT TCT GAC TTC TA -3′
Mutant consensus Nkx-2.5[d]	5′-TAA CGC AGT TC**C CTC** GAT TCT GAC TTC TA-3′
Consensus SREBP-1[c]	5′-GAT CCT GAT **CAC GTG** ATC GAG GAG-3′
Mutant consensus SREBP-1[d]	5′-GAT CCT GAT C**CA CA**G ATC GAG GAG-3′
Consensus USF-1[c]	5′-GTG TAG GC**C ACG TG**A CCG GGT GTA AGC TTC-3′
Mutant consensus USF-1[d]	5′-GTG TAG GC**A CTC** TGA CCG GGT GTA AGC TTC-3′

[a] The restriction endonuclease sites were marked by bold-faced letters with underlines.
[b] The SNP sites were marked by bold-faced letters with underlines.
[c] The consensus sequences of transcription factors were marked by bold-faced letters with underlines [19,21,23].
[d] The changes in consensus sequences were marked by bold-faced letters with underlines.

Table 2
Frequency of *MATE1* genetic variations in Koreans.

rs number	Variant	Amino acid substitution	Minor allele frequency	rs number	Variant	Amino acid substitution	Minor allele frequency
Promoter variants							
rs2453579	g.−1975C>A		0.365	–	g.−151C>A		0.010
–	g.−1374C>T		0.010	rs2252281	g.−66T>C		0.281
–	g.−1083C>T		0.010				
Coding variants							
–	c.33C>T	p.R11R	0.010	rs77474263	c.373C>T	p.L125F	0.010
rs77630697	c.191G>A	p.D64G	0.021	rs16960203	c.708C>T	p.L236L	0.083
Noncoding variants							
rs79823921	IVS2 + 90A>T		0.021	–	IVS5−134A>C		0.073
rs12451696	IVS2 + 116A>G		0.458	rs2289668	IVS5−97C>T		0.229
rs77534210	IVS2 + 117G>A		0.073	rs2247437	IVS5−12G>C		0.250
rs17683662	IVS5 + 113T>G		0.010	rs2247436	IVS5−4G>A		0.323
rs2247518	IVS5−162A>C		0.323	rs2440149	IVS7−129C>G		0.083

Data were obtained from DNA samples from 48 unrelated Korean individuals.
The position of the variant is based upon the translational start site.

Table 3
Frequency of *MATE1* haplotypes in Koreans.

	g.−1975C>A	g.−1374C>T	g.−1083C>T	g.−151C>A	g.−66T>C	p.R11R (c.33C>T)	p.D64G (c.191G>A)	IVS2+90A>T	IVS2+116A>G	IVS2+117G>A	p.L125F (c.373C>T)	IVS5+113T>G	IVS5−162A>C	IVS5−134A>C	IVS5−97C>T	IVS5−12G>C	IVS5−4G>A	IVS7−129C>G	p.L236L (c.708C>T)	Frequency
HA									**G**											0.383
HB	**A**				**C**										**T**					0.187
HC													**C**			**C**	**A**			0.147
HD	**A**									**A**			**C**	**C**			**A**	**G**	**T**	0.030
HE										**A**			**C**	**C**			**A**	**G**	**T**	0.022
HF	**A**												**C**			**C**	**A**			0.022
HG													**C**		**T**	**C**	**A**			0.021
HH	**A**				**C**		**A**						**C**			**C**	**A**			0.021

Data were obtained from DNA samples from 48 unrelated Korean individuals.
The minor alleles were marked in bold-faced letter with underlines.

pGL4.11b[*luc*2] vector (Promega Corporation, Fitchburg, WI, USA), and the DNA sequences were confirmed via direct sequencing. Reporter plasmids containing variants of *MATE1* were produced using a QuikChange® II Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA, USA) with the primers listed in Table 1 from the pGL4.11b-*MATE1* plasmid. DNA sequences were confirmed via direct sequencing.

2.3. Measurement of MATE1 promoter activity in vitro

Reporter plasmids containing the reference or variants of *MATE1* were transfected into HCT-116 (human colon carcinoma) cells using Lipofectamine LTX and Plus reagents (Life Technologies). Thirty hours after transfection, reporter activities were measured using a Dual-Luciferase® Reporter Assay System (Promega) according to the manufacturer protocol and quantified using a Glomax 96-well plate luminometer (Promega). The amount of transfected plasmid was equalized by adding pGL4.74 renilla vector. The firefly luciferase to renilla luciferase ratios were determined and defined as the relative luciferase activity. To examine the effect of natural killer homeobox-2.5 (Nkx-2.5) or upstream stimulating factor-1 (USF-1) on the promoter activity of *MATE1*, we co-transfected reference or variant reporter plasmids with increasing amounts of Nkx-2.5-pcDNA3.1 or USF-1-pcDNA3.1 vectors. The *Nkx-2.5* (BC_025711) and *USF-1* (BC_035505) gene were purchased (Thermo Fisher Scientific Inc., Waltham, MA, USA) and inserted into pcDNA3.1(+) vector. The amount of transfected plasmid was equalized by adding pcDNA3.1(+) vector.

2.4. Electrophoretic mobility shift assay (EMSA) of MATE1

Nuclear protein extracts were obtained from HCT-116 cells, and 25 g was incubated with ^{32}P-labeled oligonucleotide (2×10^5 - counts/min) in a buffer containing 7% glycerol, 10 mM HEPES (pH 7.9), 1 mM EDTA (pH 8.0), 1 mM DTT, and 0.2 μg poly(deoxyinosinate–deoxycytidylate) for 30 min at room temperature. The reaction mixtures were loaded on 6% nondenaturing polyacrylamide gel and electrophoresed for 1 h at 80 V. The dried gel was exposed to CP–BU film (Agfa, Mortsel, Belgium) on an intensifying screen for 15 h at −80 °C. The intensity of each band on the film was measured using ImageJ (National Institutes of Health, Bethesda, MD, USA).

2.5. Statistical analysis

P values for the luciferase assay were calculated using one-way analysis of variance followed by Dunnett's two-tailed test. The data were analyzed using SPSS v.20.0 (IBM Corporation, Armonk, NY, USA). Data were expressed as mean values ± standard deviation. *P* values of less than 0.05 were considered statistically significant.

3. Results

3.1. Genetic variations of MATE1 in Koreans

We sequenced the coding and promoter regions of *MATE1* in 48 DNA samples from Koreans andidentified genetic variants in these regions (Table 2). Two nonsynonymous and two synonymous variants were identified. Two of them were polymorphic (minor allele frequency > 1%). The nonsynonymous variants had been previously reported and showed a complete loss or a decrease in transport activity [4]. In promoter region, there were five variants; two of them were polymorphic and three variants, g.−1374C>T, g.−1083C>T, and g.−151C>A were reported at the first time in this study (Table 2). We used Haploview to calculate linkage

Table 4
Frequency of *MATE1* promoter haplotypes in Koreans.

ID	g.−1975C>A	g.−1374C>T	g.−1083C>T	g.−151C>A	g.−66T>C	Frequency
H1	C	C	C	C	T	0.635
H2	**A**	C	C	C	**C**	0.260
H3	**A**	C	C	C	T	0.073
H4	**A**	C	C	**A**	T	0.010
H5	**A**	**T**	C	C	**C**	0.010
H6	**A**	C	**T**	C	**C**	0.010

Data were obtained from DNA samples from 48 unrelated Korean individuals.
The minor alleles were marked in bold-faced letter with underlines.

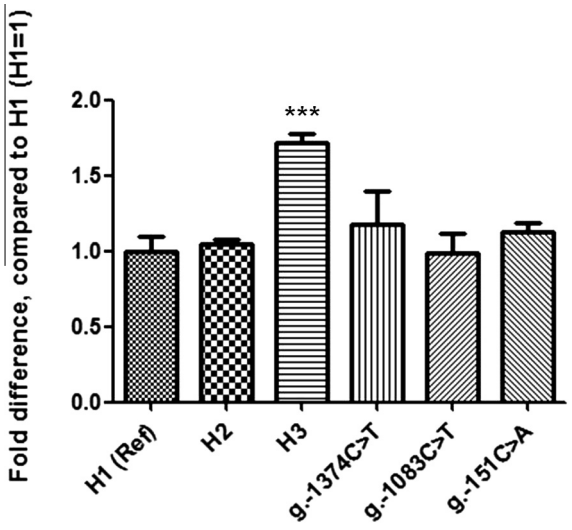

Fig. 1. Luciferase activities of *MATE1* promoter haplotypes. Luciferase activities were measured 30 h after the transfection of reporter plasmids into HCT-116 cells. The reporter activity of each construct was compared with that of the reference (H1). The data shown represent the mean values ± SD from triplicate wells in a representative experiment. ***$P < 0.001$ vs. reference promoter activity.

disequilibrium across the *MATE1* coding and promoter regions. Table 3 shows the frequency distributions of the haplotypes of *MATE1* in Koreans (frequency > 2%). Haplotype A (HA) containing only one intron variant, IVS2 + 116A>G (rs12451696), had the highest frequency, followed by haplotype B (HB), which contains three variants, g.−1975C>A (rs2453579), g.−66T>C (rs2252281), and IVS5−97C>A (rs2289668).

3.2. Promoter activity of MATE1 variants in vitro

To characterize the functional effects of the *MATE1* promoter variants, we constructed *MATE1*-pGL4.11b[*luc*2] reporter plasmids containing 2017 bp of *MATE1* promoter reference sequences and performed the reporter assay 30 h after the transfection of the reporter plasmids into HCT-116 cells. Previously four cell lines, HCT-116, ACHN, HepG2, and HeLa, were tested for measuring *MATE1* promoter activity, and among them, the *MATE1* reference-HCT-116 cells showed the highest promoter activity [5]. Therefore, HCT-116 cells were used to perform reporter assays in this study. To examine the promoter activities of the variants, we constructed the *MATE1* promoter haplotypes using genotype data of the *MATE1* promoter region (Table 4). For the functional analysis, the *MATE1* reference vector (identical to haplotype 1 in Table 4) was used as a reference. The promoter activity of haplotypes 3 (H3), which contains only g.−1975C>A, showed a significant increased reporter gene expression by 72%, compared to that of the reference. While the promoter activity of haplotype 2 (H2), which contains two variants, g.−1975C>A and g.−66T>C, was comparable with that of the reference (Fig. 1). This result may be related to the opposite effect of g.−66T>C on *MATE1* promoter activity; in a previous study, all haplotypes that contain g.−66T>C showed a significant decrease in reporter activity compared to that of the reference [5]. The promoter activities of three novel variants, g.−1374C>T, g.−1083C>T, and g.−151C>A were comparable with that of the reference (Fig. 1).

3.3. Effect of variant g.−1975C>A on the binding affinity of transcriptional factors within the MATE1 promoter region

Owing to the high frequency and increased function of g.−1975C>A, we investigated the mechanisms through which this variant may increase transcriptional activity. First, we identified the transcription factors bound to the promoter region of *MATE1* in the vicinity of g.−1975C>A. Three transcription factors, namely natural killer homeobox-2.5 (Nkx-2.5), sterol regulatory element binding protein-1 (SREBP-1), and upstream stimulating factor-1 (USF-1) were identified using TFSearch (version 1.3, developed by the RWCP, Tokyo, Japan). TFSearch predicted that these transcriptional factors would have a higher binding affinity to the g.−1975A allele than to the g.−1975C allele.

To functionally validate this prediction, we determined whether Nkx-2.5, SREBP-1, and USF-1 could bind to the promoter region containing the g.−1975C>A site through electrophoretic mobility shift assays (EMSAs). Labeled oligonucleotides (2×10^5 counts/min, consensus, Nkx-2.5 (A), SREBP-1 (B), USF-1 (C): lanes 1–3; reference g.−1975C: lanes 4–6; variant g.−1975A: lanes 7–9; Fig. 2) were incubated with nuclear protein extracts (25 μg) obtained from HCT-116 cells. Both reference and variant probes formed DNA–protein complexes, and the intensities of the complexes with the variant probes in particular were increased by 61–70% compared with those with the reference probes (lanes 4 and 7, Fig. 2). To support these results, we performed competition assays using the Nkx-2.5 (A), SREBP-1 (B), and USF-1 (C) unlabeled consensus (lanes 2, 5, and 8, Fig. 2) or mutant (lanes 3, 6, and 9) oligonucleotides. In the competition assay, a 100-fold concentration of unlabeled consensus oligonucleotides competed with labeled consensus (lane 2, Fig. 2), *MATE1* reference (lane 5), or variant (lane 8) probes. However, these binding complexes could not compete with a 100-fold concentration of unlabeled oligonucleotides containing mutated core sequences of each transcriptional factor (lanes 3, 6, 9, Fig. 2). All oligonucleotides for EMSAs are presented in Table 1.

3.4. Effect of Nkx-2.5 and USF-1 on MATE1 promoter activity

Considering transcriptional factors that bind to the *MATE1* promoter region containing the g.−1975C>A site, we investigated the effect of Nkx-2.5 and USF-1 on the promoter activity of *MATE1*. Reference (H1) or variant (H3) reporter constructs with increasing amounts of plasmids containing Nkx-2.5 or USF-1 were co-trans-

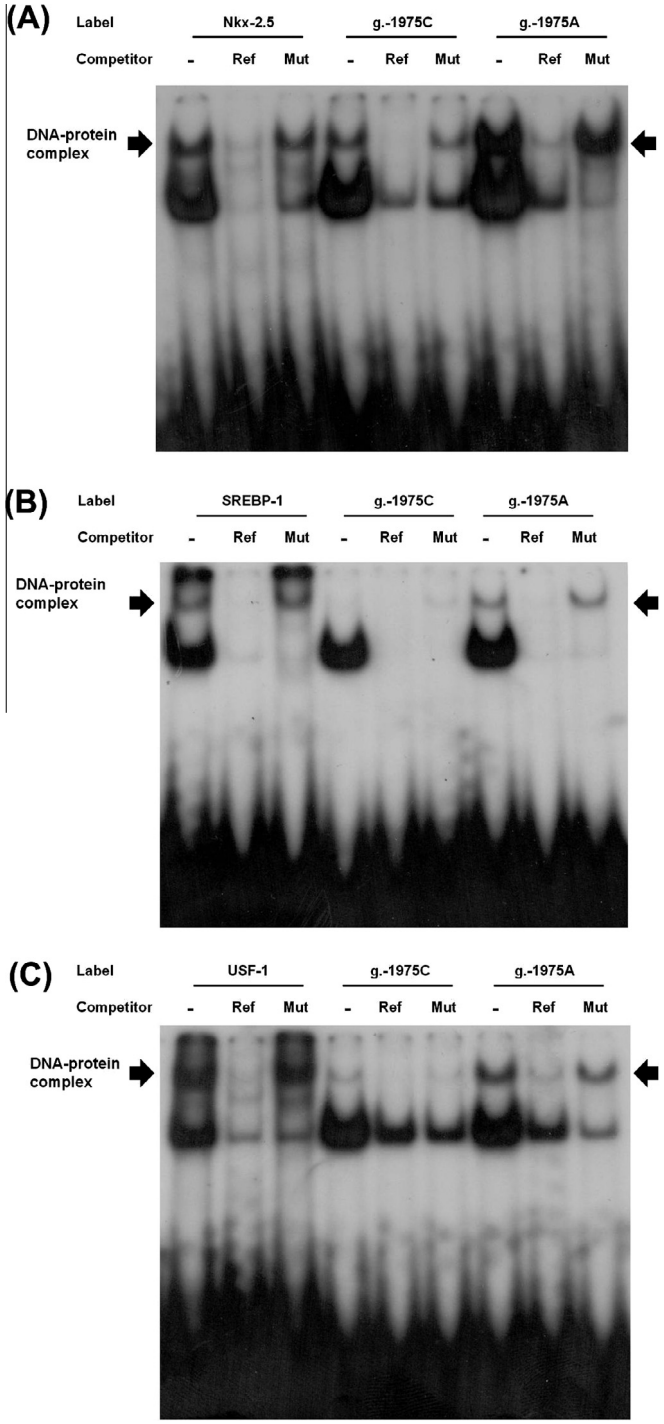

Fig. 2. Electrophoretic mobility shift analysis of *MATE1* reference and variant g.−1975C>A oligonucleotides. (A) Labeled oligonucleotides (Nkx-2.5 consensus: lanes 1–3; reference g.−1975C: lanes 4–6; variant g.−1975A: lanes 7–9) were incubated with nuclear protein extracts (25 μg) obtained from HCT-116 cells. Competition reactions were performed using a 100-fold molar excess of unlabeled consensus (lanes 2, 5, and 8) or mutant (lanes 3, 6, and 9) Nkx-2.5 oligonucleotides. The arrow indicates the position of the DNA–protein complex. (B) Labeled oligonucleotides (SREBP-1 consensus: lanes 1–3; reference g.−1975C: lanes 4–6; variant g.−1975A: lanes 7–9) were incubated with nuclear protein extracts. Competition reactions were performed using a 100-fold molar excess of unlabeled consensus (lanes 2, 5, and 8) or mutant (lanes 3, 6, and 9) SREBP-1 oligonucleotides. (C) Labeled oligonucleotides (USF-1 consensus: lanes 1–3; reference g.−1975C: lanes 4–6; variant g.−1975A: lanes 7–9) were incubated with nuclear protein extracts. Competition reactions were performed using a 100-fold molar excess of unlabeled consensus (lanes 2, 5, and 8) or mutant (lanes 3, 6, and 9) USF-1 oligonucleotides.

fected into HCT-116 cells. We observed that both transcriptional factors produced a dose-dependent induction of the *MATE1* promoter activity (Fig. 3).

4. Discussion

This study was conducted to identify and functionally characterize genetic variants in the coding and promoter regions of *MATE1* in Koreans. Several previous studies have identified and functionally investigated genetic variations of *MATE1* [4,5,7]. Choi et al. [5] have focused on the basal promoter region of *MATE1* in ethnically diverse US populations, whereas other studies have reported several nonsynonymous variants of *MATE1* that showed significant changes in transport activities [4,7]. In this study, we screened the coding and wide ranges (up to −2000 bp from the translational start site) of the promoter region of *MATE1* in 48 samples from healthy Koreans and found two previously reported nonsynonymous variants, p.D64G and p.L125F [4,7], and five promoter variants including three that are novel. Using luciferase assays, we found one common *MATE1* promoter haplotype containing one variant, g.−1975C>A, that showed a significant increase in reporter activity. Transcriptional factor biding site analyses predicted three transcriptional factors, Nkx-2.5, SREBP-1, and USF-1, as possible regulators of *MATE1* transcription. In particular, each would have a higher biding affinity for g.−1975A than for g.−1975C allele.

Nkx-2.5 is a transcription factor belonging to the natural killer homeobox gene family and a critical regulator during cardiac development [19]. Nkx-2.5 is also a potent inducer of sodium/iodide symporter in thyroid [20]. *MATE1* contains the sequence TCCAGTG, which is similar to the consensus sequence of Nkx-2.5, TYAAGTG. The variant g.−1975C>A results in TCAAGTG, an exact match to the consensus sequence of Nkx-2.5. To date, three members of the SREBP family, SREBP-1a, SREBP-1c, and SREBP-2 have been reported [21]. Among them, SREBP-1a and SREBP-1c are encoded by a single gene with differences in exon 1 owing to alternative splicing [21]. A previous study has reported that SREBP-1c upregulates the expression of glucose transporter 4 in adipocytes [22]. USF-1 is expressed ubiquitously and is a key regulator of various genes involved in stress and immune responses, cell cycle and proliferation, glucid–lipid metabolism, and other processes [23]. Despite the various roles of this transcriptional factor, few studies have investigated its role in the transcription of transporters. Previous studies have reported that USF-1 induces *OCT2* transcription [24,25]. SREBP-1 and USF-1 are thought to bind to the E-box regulatory elements CANNTG [21,23]. We observed that *MATE1* contains the sequence CCAGTG, which is similar to the E-box regulatory elements. Like Nkx-2.5, the variant g.−1975C>A results in CAAGTG, an exact match to the consensus sequence of SREBP-1 and USF-1. Therefore, we predicted that all 3 transcription factors would have a binding preference to the variant DNA sequence rather than to the reference sequence. The EMSA results supported our hypothesis. The intensity of each DNA–protein complex was increased in the presence of g.−1975C>A (Fig. 2). In addition, we observed that Nkx-2.5 and USF-1 act as inducers of *MATE1* promoter activity (Fig. 3).

A recent study has investigated the association between g.−66T>C, a variant in the *MATE1* promoter region, and metformin pharmacokinetics and pharmacodynamics [6]. In this study, diabetic patients carrying this variant showed enhanced metformin response. However, the results found that g.−66T>C has no effect on the pharmacokinetics of metformin in healthy volunteers. In our study, the promoter activity of *MATE1* promoter haplotype 2 (H2), containing the polymorphisms g.−1975C>A and g.−66T>C, was similar to that of the reference (Fig. 1), where as the promoter

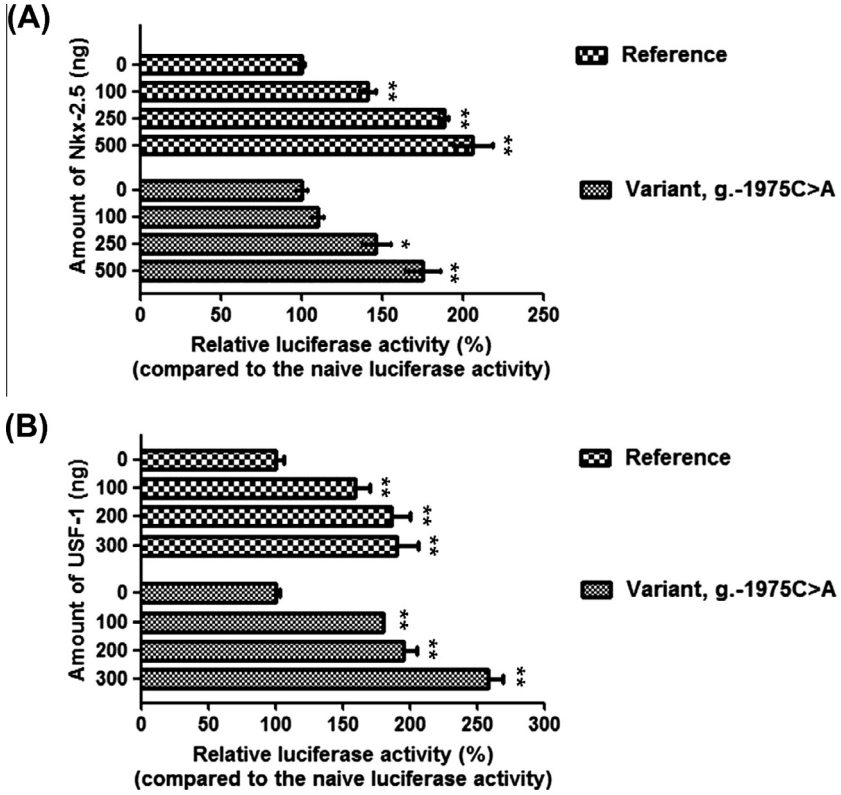

Fig. 3. Effects of Nkx-2.5 and USF-1 on the promoter activity of *MATE1*. Promoter activities were measured 30 h after the co-transfection of the reference or variant reporters and various amounts of Nkx-2.5 (A) or USF-1 (B) plasmids into HCT-116 cells. The reporter activity of each construct was compared with that of the naïve promoter. Data shown represent mean ± SD from triplicate wells in a representative experiment. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. the naïve promoter activity.

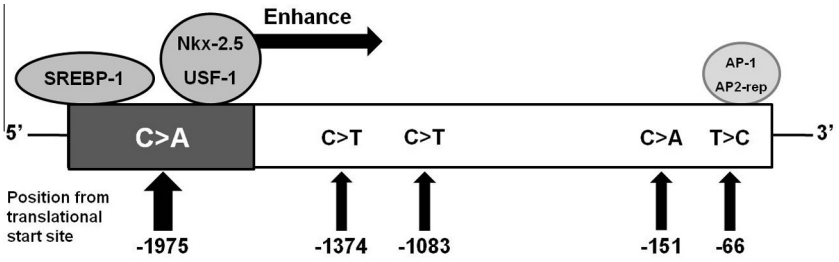

Fig. 4. Schematic of the interaction of the *MATE1* promoter with transcription factors proposed to be involved in *MATE1* regulation. The transcription factors Nkx-2.5, SREBP-1, and USF-1 bind to the site containing the variant g.−1975C>A; in particular, each has a higher binding affinity for the g.−1975A allele. Nkx-2.5 and USF-1 induce *MATE1* transcription. Other variants found in Koreans in this study are included in the schematic. Variant g.−66T>C showed significantly reduced promoter activity owing to a reduction in the binding potency of the transcriptional activator, AP-1, and enhanced binding potency of the repressor, AP-2rep, to the g.−66C allele in a previous study [5].

activity of H3, containing only g.−1975C>A, was significantly increased compared to that of the reference (Fig. 1). Our result is consistent with that of a previous study reporting decreased promoter activities of haplotypes containing g.−66T>C [5]. To investigate the effect of g.−1975C>A on metformin pharmacokinetics, we performed retrospective analysis of 45 genomic DNA samples from healthy Koreans participated in metformin pharmacokinetics study. After removing the data for 8 subjects with the variant g.−66T>C, we found that 2 subjects carrying H3 showed increased renal clearance or secretion of metformin compared with that in the reference group, although we were unable to conduct statistical analysis owing to the small numbers of subjects (data not shown). The frequencies of genetic variants or haplotypes may differ according to ethnicity. Therefore, confirmation of the effect of *MATE1* haplotypes on metformin pharmacokinetics requires clinical study in diverse populations with the novel *MATE1* variant, g.−1975C>A.

In conclusion, we characterized genetic variants in the promoter region of *MATE1* and found that one common promoter haplotype in Koreans, H3, showed significantly increased promoter activity. The mechanism appeared to be related to the increased binding of the transcription factors Nkx-2.5 and USF-1, which induce transcription, to the region containing variant g.−1975C>A, the polymorphism present in H3. In addition, we found that SREBP-1 may be involved in the regulation of *MATE1* transcription (Fig. 4). Our studies revealed that a common haplotype in the *MATE1* promoter changes the transcriptional activity of *MATE1*. The presence of this haplotype may affect the pharmacokinetics or drug response of various drugs that are *MATE1* substrates. Further functional evaluations of this haplotype including *in vivo* assay or clinical study are necessary to confirm our suggestion.

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References

[1] Y. Shitara, T. Horie, Y. Sugiyama, Transporters as a determinant of drug clearance and tissue distribution, Eur. J. Pharm. Sci. 27 (2006) 425–446.
[2] M. Otsuka, T. Matsumoto, R. Morimoto, S. Arioka, H. Omote, Y. Moriyama, A human transporter protein that mediates the final excretion step for toxic organic cations, Proc. Natl. Acad. Sci. USA 102 (2005) 17923–17928.
[3] Y. Tanihara, S. Masuda, T. Sato, T. Katsura, O. Ogawa, K. Inui, Substrate specificity of MATE1 and MATE2-K, human multidrug and toxin extrusions/H(+)-organic cation antiporters, Biochem. Pharmacol. 74 (2007) 359–371.
[4] Y. Chen, K. Teranishi, S. Li, S.W. Yee, S. Hesselson, D. Stryke, S.J. Johns, T.E. Ferrin, P.Y. Kwok, K.M. Giacomini, Genetic variants in multidrug and toxic compound extrusion-1, hMATE1, alter transport function, Pharmacogenom. J. 9 (2009) 127–136.
[5] J.H. Choi, S.W. Yee, M.J. Kim, L. Nguyen, J.H. Lee, J. Kang, S. Hesselson, R.A. Castro, D. Stryke, S.J. Johns, P.Y. Kwok, T.E. Ferrin, M.G. Lee, B.L. Black, N. Ahituv, K.M. Giacomini, Identification and characterization of novel polymorphisms in the basal promoter of the human transporter, MATE1, Pharmacogenet. Genomics 19 (2009) 770–780.
[6] S.L. Stocker, K.M. Morrissey, S.W. Yee, R.A. Castro, L. Xu, A. Dahlin, A.H. Ramirez, D.M. Roden, R.A. Wilke, C.A. McCarty, R.L. Davis, C.M. Brett, K.M. Giacomini, The effect of novel promoter variants in MATE1 and MATE2 on the pharmacokinetics and pharmacodynamics of metformin, Clin. Pharmacol. Ther. 90 (2013) 186–194.
[7] M. Kajiwara, T. Terada, K. Ogasawara, J. Iwano, T. Katsura, A. Fukatsu, T. Doi, K. Inui, Identification of multidrug and toxin extrusion (MATE1 and MATE2-K) variants with complete loss of transport activity, J. Hum. Genet. 54 (2009) 40–46.
[8] K. Toyama, A. Yonezawa, S. Masuda, R. Osawa, M. Hosokawa, S. Fujimoto, N. Inagaki, K. Inui, T. Katsura, Loss of multidrug and toxin extrusion 1 (MATE1) is associated with metformin-induced lactic acidosis, Br. J. Pharmacol. 166 (2012) 1183–1191.
[9] M.L. Becker, L.E. Visser, R.H. van Schaik, A. Hofman, A.G. Uitterlinden, B.H. Stricker, Genetic variation in the multidrug and toxin extrusion 1 transporter protein influences the glucose-lowering effect of metformin in patients with diabetes: a preliminary study, Diabetes 58 (2009) 745–749.
[10] M.L. Becker, L.E. Visser, R.H. van Schaik, A. Hofman, A.G. Uitterlinden, B.H. Stricker, Interaction between polymorphisms in the OCT1 and MATE1 transporter and metformin response, Pharmacogenet. Genomics 20 (2010) 38–44.
[11] K.A. Jablonski, J.B. McAteer, P.I. de Bakker, P.W. Franks, T.I. Pollin, R.L. Hanson, R. Saxena, S. Fowler, A.R. Shuldiner, W.C. Knowler, D. Altshuler, J.C. Florez, Common variants in 40 genes assessed for diabetes incidence and response to metformin and lifestyle intervention in the diabetes prevention program, Diabetes 59 (2010) 2672–2681.
[12] K. Toyama, A. Yonezawa, M. Tsuda, S. Masuda, I. Yano, T. Terada, R. Osawa, T. Katsura, M. Hosokawa, S. Fujimoto, N. Inagaki, K. Inui, Heterozygous variants of multidrug and toxin extrusions (MATE1 and MATE2-K) have little influence on the disposition of metformin in diabetic patients, Pharmacogenet. Genomics 20 (2010) 135–138.
[13] Y. Shu, C. Brown, R.A. Castro, R.J. Shi, E.T. Lin, R.P. Owen, S.A. Sheardown, L. Yue, E.G. Burchard, C.M. Brett, K.M. Giacomini, Effect of genetic variation in the organic cation transporter 1, OCT1, on metformin pharmacokinetics, Clin. Pharmacol. Ther. 83 (2008) 273–280.
[14] I.S. Song, H.J. Shin, E.J. Shim, I.S. Jung, W.Y. Kim, J.H. Shon, J.G. Shin, Genetic variants of the organic cation transporter 2 influence the disposition of metformin, Clin. Pharmacol. Ther. 84 (2008) 559–562.
[15] Z.J. Wang, O.Q. Yin, B. Tomlinson, M.S. Chow, OCT2 polymorphisms and in-vivo renal functional consequence: studies with metformin and cimetidine, Pharmacogenet. Genomics 18 (2008) 637–645.
[16] Y. Chen, S. Li, C. Brown, S. Cheatham, R.A. Castro, M.K. Leabman, T.J. Urban, L. Chen, S.W. Yee, J.H. Choi, Y. Huang, C.M. Brett, E.G. Burchard, K.M. Giacomini, Effect of genetic variation in the organic cation transporter 2 on the renal elimination of metformin, Pharmacogenet. Genomics 19 (2009) 497–504.
[17] M.V. Tzvetkov, S.V. Vormfelde, D. Balen, I. Meineke, T. Schmidt, D. Sehrt, I. Sabolić, H. Koepsell, J. Brockmöller, The effects of genetic polymorphisms in the organic cation transporters OCT1, OCT2, and OCT3 on the renal clearance of metformin, Clin. Pharmacol. Ther. 86 (2009) 299–306.
[18] J.H. Choi, S.W. Yee, A.H. Ramirez, K.M. Morrissey, G.H. Jang, P.J. Joski, J.A. Mefford, S.E. Hesselson, A. Schlessinger, G. Jenkins, R.A. Castro, S.J. Johns, D. Stryke, A. Sali, T.E. Ferrin, J.S. Witte, P.Y. Kwok, D.M. Roden, R.A. Wilke, C.A. McCarty, R.L. Davis, K.M. Giacomini, A common 5′-UTR variant in MATE2-K is associated with poor response to metformin, Clin. Pharmacol. Ther. 90 (2011) 674–684.
[19] B. Hu, Y.M. Wu, Z. Wu, S.H. Phan, Nkx2.5/Csx represses myofibroblast differentiation, Am. J. Respir. Cell Mol. Biol. 42 (2010) 218–226.
[20] M. Dentice, C. Luongo, A. Elefante, R. Romino, R. Ambrosio, M. Vitale, G. Rossi, G. Fenzi, D. Salvatore, Transcription factor Nkx-2.5 induces sodium/iodide symporter gene expression and participates in retinoic acid- and lactation-induced transcription in mammary cells, Mol. Cell Biol. 24 (2004) 7863–7877.
[21] M. Foretz, C. Pacot, I. Dugail, P. Lemarchand, C. Guichard, X. Le Lièpvre, C. Berthelier-Lubrano, B. Spiegelman, J.B. Kim, P. Ferré, F. Foufelle, ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose, Mol. Cell Biol. 19 (1999) 3760–3768.
[22] S.S. Im, S.K. Kwon, S.Y. Kang, T.H. Kim, H.I. Kim, M.W. Hur, K.S. Kim, Y.H. Ahn, Regulation of GLUT4 gene expression by SREBP-1c in adipocytes, Biochem. J. 399 (2006) 131–139.
[23] S. Corre, M.D. Galibert, Upstream stimulation factors: highly versatile stress-responsive transcription factors, Pigment. Cell Res. 18 (2005) 337–348.
[24] J. Asaka, T. Terada, K. Ogasawara, T. Katsura, K. Inui, Characterization of the basal promoter element of human organic cation transporter 2 gene, J. Pharmacol. Exp. Ther. 321 (2007) 684–689.
[25] M. Aoki, T. Terada, M. Kajiwara, K. Ogasawara, I. Ikai, O. Ogawa, T. Katsura, K. Inui, Kidney-specific expression of human organic cation transporter 2 (OCT2/SLC22A2) is regulated by DNA methylation, Am. J. Physiol. Renal Physiol. 295 (2008) F160–F170.