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Comprehensive Variant Screening of the UGT Gene Family

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Purpose: *UGT1A1*, *UGT2B7*, and *UGT2B15* are well-known pharmacogenes that belong to the uridine diphosphate glucuronyltransferase gene family. For personalized drug treatment, it is important to study differences in the frequency of core markers across various ethnic groups. Accordingly, we screened single nucleotide polymorphisms (SNPs) of these three genes and analyzed differences in their frequency among five ethnic groups, as well as attempted to predict the function of novel SNPs. **Materials and Methods:** We directly sequenced 288 subjects consisting of 96 Korean, 48 Japanese, 48 Han Chinese, 48 African American, and 48 European American subjects. Subsequently, we analyzed genetic variability, linkage disequilibrium (LD) structures and ethnic differences for each gene. We also conducted *in silico* analysis to predict the function of novel SNPs. **Results:** A total of 87 SNPs were detected, with seven pharmacogenetic core SNPs and 31 novel SNPs. We observed that the frequencies of *UGT1A1* *6 (*rs4148323*), *UGT1A1* *60 (*rs4124874*), *UGT1A1* *93 (*rs10929302*), *UGT2B7* *2 (*rs7439366*), a part of *UGT2B7* *3 (*rs12233719*), and *UGT2B15* *2 (*rs1902023*) were different between Asian and other ethnic groups. Additional *in silico* analysis results showed that two novel promoter SNPs of *UGT1A1 -690G>A* and *-689A>C* were found to potentially change transcription factor binding sites. Moreover, *673G>A* (*UGT2B7*), *2552T>C*, and *23269C>T* (both SNPs from *UGT2B15*) changed amino acid properties, which could cause structural deformation. **Conclusion:** Findings from the present study would be valuable for further studies on pharmacogenetic studies of personalized medicine and drug response.

Key Words: SNP, uridine diphosphate glucuronyltransferase, *UGT1A1*, *UGT2B7*, *UGT2B15*, personalized medicine

INTRODUCTION

Uridine diphosphate glucuronyl transferase (UGT) is an enzyme that catalyzes the addition of glucuronic acid to a substrate, known as a glucuronidation reaction, which plays a major role in phase II drug metabolism.[1,2] The enzyme is expressed

in most parts of the body, including liver, kidney, intestine, lung, skin, prostate, and brain. In this study, we have selected three genes of the *UGT* gene family, *uridine diphosphate glucuronosyl transferase 1 family, polypeptide A1* (*UGT1A1*); *uridine diphosphate glucuronosyl transferase 2 family, polypeptide B7* (*UGT2B7*); and *uridine diphosphate glucuronosyl transferase 2 family, polypeptide B15* (*UGT2B15*), and then sequenced for single nucleotide polymorphisms (SNPs) in the three *UGT* genes. We also calculated and compared allele frequencies, linkage disequilibrium (LD), and haplotype structures in five ethnic groups consisting of Korean, Han Chinese, Japanese, African American, and European American subjects.

UGT1A1 is located on chromosome 2q37, and encodes for a part of the UGT enzyme. The gene is also known to have several isoforms due to the splicing of exons at various locations. A preferred substrate for the enzyme is bilirubin, which is hypothesized to be a cellular antioxidant in the human body.[3] Previous studies have found that polymorphisms of *UGT1A1* are associated with diseases such as head and neck cancers, colorectal cancer, Gilbert syndrome, and coronary artery disease.[4-7] Located on chromosome 4q13, *UGT2B7* is known to glucosidate hydesoxycholic acid in the liver, as well as glucuronidate steroid hormones and fatty acids.[8,9] It also conjugates various classes of major drugs including analgesics, carboxylic non-steroidal anti-inflammatory drugs, and anti-carcinogens.[9] In addition, *UGT2B7* polymorphisms were found to have associations with an increased risk of bladder cancer, colorectal cancer, and a treatment method for breast cancer.[10-12] *UGT2B15* is also located on chromosome 4q13, and encodes for an enzyme that has unique conjugation with xenobiotic and endogenous compounds, such as 7-hydroxylated coumarins, flavonoids, anthraquinones, and other drugs.[13] Furthermore, UGT2B15 is also known to glucuronidate anti-anxiety drugs such as lorazepam and oxazepam.[14,15]

In this study, we directly sequenced 288 genomic DNA samples from diverse ethnicities, which yielded 87 SNPs in *UGT1A1*, *UGT2B7*, and *UGT2B15*. Our results might aid research on pharmacogenomics of these important genes.

MATERIALS AND METHODS

Study subjects

DNA from a total of 288 subjects was used in the present study and was obtained from 96 Koreans, 48 African Americans, 48 European Americans, 48 Japanese, and 48 Han Chinese subjects. The 96 Korean DNA samples were unrelated population controls. Other ethnic groups were obtained as anonymous, unrelated DNA samples from the Human Variation Panels prepared by the Coriell Institute (Camden, NJ, USA).

Sequencing analysis of UGT family genes

Promoters, all exons, and exon-intron boundaries (+/- 50 bp) were polymerase chain reaction-amplified and directly sequenced using a ABI PRISM 3730 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Primers for the amplification and sequencing analysis were designed by using Primer3 software (http://frodo.wi.mit.edu) based on the sequence of respective genes (Ref. genome seq.: NG_002601.2, AC_111000.3, and NT_167250.1 for *UGT1A1*, *UGT2B7*, and *UGT2B15*, respectively) (Supplementary Table 1, only online).[16] Sequence variants were verified by chromatograms using SeqMan software.

Statistical analysis

The χ^2 tests were used to determine whether individual variants were in Hardy-Weinberg equilibrium at each locus in each ethnic group. Pairwise LD was estimated for the SNPs in each gene using standardized summary statistics D' and r^2,[17] which were calculated using HaploView software.[18] Haplotype blocks were assigned using the D' confidence interval algorithm also using HaploView. For *in silico* analysis, we used the FastSNP program to predict the potential binding site changes caused by promoter SNPs.[19] Fisher's exact test was calculated using the Statistical Analysis System 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Sequencing results of *UGT1A1*, *UGT2B7*, and *UGT2B15*

We included a total of 288 subjects (96 Koreans and 48 Han Chinese, 48 Japanese, 48 African American and 48 European American) for direct sequencing of *UGT1A1*, *UGT2B7* and *UGT2B15*. As a result, we detected 33 SNPs from *UGT1A1*, 18 SNPs from *UGT2B7*, and 36 SNPs from *UGT2B15* (Table 1). All SNPs were in Hardy-Weinberg equilibrium for five ethnic groups (data not shown). Among the observed SNPs, There were eight novel SNPs from *UGT1A1*, seven from *UGT2B7*, and 16 from *UGT2B15*, amounting to a total

Table 1. MAF of 87 SNPs from *UGT1A1*, *UGT2B7*, and *UGT2B15*

Gene	SNP ID	Position	Amino acid change	Allele change	MAF				
					KR	HC	JP	AA	EA
UGT1A1	*rs4124874*	Promoter	.	G>T	0.297	0.271	0.292	0.106	0.426
	rs10929302	Promoter	.	G>A	0.125	0.083	0.083	0.354	0.240
	rs35815287	Promoter	.	C>T	-	-	0.010	-	-
	rs34118072	Promoter	.	T>G	-	-	-	0.031	-
	rs3755319	Promoter	.	A>C	0.286	0.219	0.298	0.333	0.479
	rs28900395	Promoter	.	G>A	0.005	-	0.011	-	-
	*-1224G>A**	Promoter	.	G>A	-	-	-	-	0.010
	rs28899472	Promoter	.	C>T	-	-	-	-	0.031
	*-997G>A**	Promoter	.	G>A	0.161	0.135	0.191	0.177	0.125
	rs6723506	Promoter	.	A>G	0.010	0.031	0.032	0.031	0.021
	*-741A>G**	Promoter	.	A>G	0.005	0.010	-	-	-
	*-690G>A**	Promoter	.	G>A	0.005	-	-	-	-
	*-689A>C**	Promoter	.	A>C	0.286	0.181	0.234	0.438	0.375
	rs34916116	Promoter	.	C>A	-	-	-	0.010	0.021
	rs35665780	Promoter	.	C>G	-	0.010	-	-	-
	rs4148323	Exon1	R71G	G>A	0.172	0.223	0.106	-	-
	rs35003977	Exon1	V225G	T>G	-	-	-	0.014	-
	rs35350960	Exon1	P229Q	C>A	0.016	-	-	-	-
	rs6708136	Intron1	.	C>T	-	-	-	0.031	0.031
	rs4148327	Intron2	.	T>C	0.062	0.021	0.031	-	-
	rs34650714	Intron2	.	C>T	-	-	-	0.188	-
	rs34082659	Intron2	.	C>T	-	-	-	-	0.031
	rs28900402	Intron2	.	C>T	-	-	-	0.073	-
	rs2302538	Intron2	.	T>C	0.052	0.021	0.042	0.354	0.115
	*7501G>C**	Intron2	.	G>C	0.005	0.010	-	-	-
	rs12471326	Intron2	.	T>C	-	-	-	0.010	0.052
	rs35129844	Intron2	.	C>A	-	-	-	0.010	-
	*7724A>C**	Intron3	.	A>C	0.005	-	-	-	-
	rs35523971	Intron3	.	T>G	-	-	-	0.010	-
	rs10445705	Intron3	.	G>A	-	-	-	0.062	-
	*12460T>G**	3'UTR	.	T>G	-	0.010	-	-	-
	rs10929303	3'UTR	.	C>T	0.150	0.146	0.167	0.438	0.271
	rs1042640	3'UTR	.	C>G	0.150	0.146	0.167	0.198	0.250
UGT2B7	*rs6600880*	Promoter	.	A>T	-	-	-	0.010	-
	*-1711A>T**	Promoter	.	A>T	-	-	-	0.010	-
	*-1692A>G**	Promoter	.	A>G	-	-	-	0.010	-
	rs73823857	Promoter	.	T>C	-	-	-	0.073	0.073
	*-534G>A**	Promoter	.	G>A	-	-	-	0.021	-
	rs116572954	Promoter	.	G>A	-	-	-	0.031	-
	rs73823859	Promoter	.	G>A	-	-	-	0.073	0.073
	rs7668282	Promoter	.	T>C	0.084	0.073	0.062	0.052	0.010
	*-3A>T**	Promoter	.	A>T	-	-	-	0.010	0.010
	rs12233719	Exon1	A71S	G>T	0.137	0.052	0.043	-	-
	rs28365063	Exon1	R124R	A>G	0.147	0.135	0.146	0.093	0.185
	*673G>A**	Exon1	E225K	G>A	-	-	-	-	0.033
	rs7439366	Exon2	H268Y	C>T	0.323	0.255	0.271	-	0.468
	*2247G>A**	Intron2	.	G>A	-	0.010	-	-	-
	*6164T>A**	Intron2	.	T>A	-	0.011	0.011	-	0.011
	rs4348159	Exon4	Y354Y	C>T	0.037	0.021	0.125	0.177	0.073
	rs28365064	Intron4	.	T>A	-	-	-	0.042	-
	rs11302069	Intron4	.	A>-	0.319	0.292	0.312	0.281	0.417

Table 1. Continued

Gene	SNP ID	Position	Amino acid change	Allele change	MAF				
					KR	HC	JP	AA	EA
	rs7696472	Promoter	.	T>C	0.474	0.417	0.427	0.260	0.438
	rs62299491	Promoter	.	T>C	-	0.010	-	0.104	0.260
	rs75261806	Promoter	.	G>A	-	-	-	0.011	-
	*-1774C>T**	Promoter	.	C>T	0.010	-	-	-	-
	*-1720G>T**	Promoter	.	G>T	0.005	-	-	-	-
	rs77529204	Promoter	.	T>C	0.026	0.021	-	-	-
	rs7686914	Promoter	.	A>G	0.479	0.438	0.448	0.333	0.490
	*-1400C>T**	Promoter	.	C>T	0.005	-	-	-	-
	*-1357A>C**	Promoter	.	A>C	-	0.010	-	-	-
	*-1240C>T**	Promoter	.	C>T	0.005	-	-	-	-
	*-1163T>C**	Promoter	.	T>C	-	0.010	-	-	-
	rs62317005	Promoter	.	T>C	-	0.010	-	0.106	0.250
	rs76571221	Promoter	.	C>T	0.174	0.292	0.255	0.074	0.073
	rs78497667	Promoter	.	A>G	0.140	0.188	0.160	0.021	0.052
	rs1902023	Exon1	D85Y	G>T	0.484	0.289	0.436	0.309	0.298
	*412A>G**	Exon1	M138V	A>G	-	-	-	0.010	-
	rs76161856	Intron1	.	A>G	-	-	-	0.031	-
UGT2B15	*2552T>C**	Exon2	C283R	T>C	0.010	0.031	-	-	-
	rs1993282	Intron2	.	A>G	-	-	-	0.021	-
	*2594T>A**	Intron2	.	T>A	-	-	-	0.031	-
	rs2045100	Intron2	.	A>T	0.312	0.250	0.292	0.240	0.135
	rs62298394	Intron2	.	T>A	-	0.010	-	0.104	0.208
	rs115196363	Intron3	.	T>C	0.161	0.277	0.250	0.052	0.062
	rs116368924	Intron3	.	A>C	-	0.021	0.021	0.135	0.271
	*15608G>A**	Intron4	.	G>A	-	-	-	0.021	-
	*16461C>T**	Exon5	A398V	C>T	-	-	-	-	0.021
	*16462G>T**	Exon5	A398A	G>T	0.005	-	0.021	-	-
	rs4148268	Intron5	.	T>C	0.026	0.031	0.031	-	0.010
	*23269C>T**	Exon6	S483L	C>T	-	-	-	0.031	-
	*23403G>A**	Exon6	V494V	G>A	-	0.010	0.010	-	-
	*23609T>A**	3'UTR	.	T>A	0.005	-	-	-	-
	rs3100	3'UTR	.	C>T	0.161	0.104	0.146	0.281	0.354
	*23684G>-**	3'UTR	.	G>-	-	-	-	0.156	0.042
	rs4148271	3'UTR	.	A>T	0.208	0.281	0.250	0.01	-
	rs35791822	3'UTR	.	G>T	-	0.010	-	0.115	0.228
	rs34930215	3'UTR	.	C>T	-	0.011	-	0.130	0.239

MAF, minor allele frequency; KR, Korean; HC, Han Chinese; JP, Japanese; AA, African American; EA, European American; UTR, untranslated region; SNP, single nucleotide polymorphism.
"-" indicates the SNP was monomorphic in the particular ethnic group.
*SNPs indicate the novel SNPs discovered in our study.

of 31 novel SNP discoveries (Supplementary Fig. 1, only online). One novel *UGT2B7* SNP (673G>A, E225K) and five novel *UGT2B15* SNPs (*412A>G*, M138V; *16461C>T*, A398V; *16263G>T*, A398A; *23269C>T*, S483L; *23403G>A*, V494V) were located in coding regions.

LD structures of the three genes

The LD structures of the three genes in the study were inferred by only using SNPs with detectable minor allele frequencies (MAFs) in at least one ethnic group, and the results were shown in Supplementary Fig. 2 (only online). Overall, MAFs of most SNPs were not high enough to form distinct LD structures or haplotype blocks. However, Asian ethnic groups (Korean, Han Chinese, and Japanese) displayed similar LD structures when compared with African American or European American subjects.

Analysis of SNP frequencies across five ethnic groups

Generally, MAFs of Asian ethnic groups were similar, while MAFs of African American and European American groups deviated from Asians in several SNPs (Table 1, Supplementary Fig. 3, only online). Among the numerous SNPs of *UGT1A1*, which showed MAF differences among ethnic groups, *6 (*rs4148323*), *60 (*rs4124874*), and *93 (*rs10929302*) are known as core markers of *UGT1A1*. In all three SNPs, MAFs of Asians were similar, while those in European American and African American subjects were either significantly higher or lower than those in Asians. In *UGT2B7*, the MAF of core marker *2 (*rs7439366*) was higher in European Americans than in Asians, while it was monomorphic in African Americans. On the other hand, frequency differences in a part of the *3 (*rs12233719*) allele were not as drastic, but MAFs in Koreans were higher than those in other ethnic groups. In *UGT2B15*, MAFs of the core marker *2 (*rs1902023*) in Koreans and Japanese were larger than those in Han Chinese, African Americans, and European Americans. Detailed information of each gene's core markers are summarized in Table 2, with their position, allele change, amino acid change, star allele nomenclature, MAF, and any known roles in pharmacogenetics. Three core markers of *UGT1A1*,*7 (*rs34993780*), *28 (*rs8175347*), and *29 (*rs55750087*), were monomorphic in all studied ethnic groups, and thus not shown in any results. We conducted Fisher's exact test to find statistically significant differences in allele frequencies of core markers between Koreans and other populations (Supplementary Table 2, only online).

***In silico* analysis of novel SNPs**

In order to predict the function of the novel SNPs detected in our study, we conducted *in silico* analysis using the FastSNP program. The program analyzes for a potential transcription factor binding site change caused by a promoter SNP. As a result, in *UGT1A1*, *-690G>A* was estimated to induce Ras responsive element binding protein 1 (RREB1) and specificity protein 1 (Sp1) binding sites onto the promoter, while *-689A>C* could lead to an additional binding site for Sp1 only (data not shown).

DISCUSSION

In the field of pharmacogenetics, studying the frequency

Table 2. Detailed Information on Core Markers of *UGT1A1*, *UGT2B7*, and *UGT2B15*

Gene	SNP	Position, allele change, and amino acid change	Star allele nomenclature	Notes	MAF				
					KR	HC	JP	AA	EA
UGT1A1	*rs8175347*†	TATA box	*28	Enzyme activity reduced[36] Reduced dose of irinotecan recommended[37]	-	-	-	-	-
	rs4124874	-3279T>G	*60	Related to Gilbert's syndrome[20]	0.297	0.271	0.292	0.106	0.426
	rs10929302	-3152G>A	*93	Affects bilirubin level[38]	0.125	0.083	0.083	0.354	0.240
	rs4148323	211G>A (G71R)	*6	Enzyme activity reduced[39]	0.172	0.223	0.106	-	-
	rs35350960	686C>A (P229Q)	*27	Enzyme activity reduced[40]	0.016	-	-	-	-
	rs55750087†	1099C>G (R367G)	*29	Enzyme activity reduced[40]	-	-	-	-	-
	rs34993780†	1456T>G (Y486D)	*7	Enzyme activity reduced[39]	-	-	-	-	-
UGT2B7	*rs12233719*	211G>T (A71S)	A part of *3	Studied in Japanese ethnic group[41]	0.137	0.052	0.043	-	-
	rs7439366	2100C>T (H268Y)	*2	Luciferase activity decreased[42]	0.323	0.255	0.271	-	0.468
UGT2B15	*rs1902023*	253G>T (D85Y)	*2	Decreased clearance of lorazepam and oxazepam (*in vivo*) Increased activity on androgens and decreased glucuronidation of oxazepam (*in vitro*)[14,15,43]	0.484	0.289	0.436	0.309	0.298

MAF, minor allele frequency; KR, Korean; HC, Han Chinese; JP, Japanese; AA, African American; EA, European American; SNP, single nucleotide polymorphism.

Monomorphic frequencies are denoted as "-".

†These SNPs do not appear on the present study's results because they are monomorphic.

differences of core markers across various ethnic groups is important for personalized drug treatment. Therefore, the present study analyzed the SNPs of UGT family genes *UGT1A1*, *UGT2B7*, and *UGT2B15* across five ethnic groups. Although the LD structures of the three genes did not exhibit much difference between Asians and other ethnic groups, the MAFs of several SNPs differed depending on ethnicity. Interestingly, most of the SNPs with large MAF discrepancies among ethnic groups were pharmacogenetic core markers of each gene.

From our result, three *UGT1A1* core markers, *6 (*rs4148323*), *60 (*rs4124874*), and *93 (*rs10929302*), showed large MAF differences among ethnic groups. Previously, researchers found *6 (*rs4148323*), *28 (*rs8175347*), and *60 (*rs4124874*) to influence the expression levels of *UGT1A1*, and discrepancies in frequency across ethnic groups led to varying effects of irinotecan application depending on the ethnicity.[20-22] In addition, a study of a Japanese population reported that *27 (*rs35350960*), a genetic variant unique to Japanese subjects, could increase susceptibility to irinotecan side effects.[23] Although we failed to detect *27 (*rs35350960*) or *28 (*rs8175347*) in the present study, higher MAFs of *6 (*rs4148323*) and *60 (*rs4124874*) in Asian ethnic groups suggest that the two alleles could be important pharmacogenetic factors for irinotecan application in Korean and other Asian cancer patients.

As a core marker for *UGT2B7*, *2 (*rs7439366*) is known to be related with Vadimezan (5,6-dimethylxanthenone-4-acetic acid, previously called DMXAA), an anti-cancer drug.[24] A previous study reported that there was a five-fold inter-individual variation in *UGT2B7* expression level in the liver.[25] Subsequently, such variation led to differing degrees of reaction to Vadimezan individually, as reduced enzyme expression caused by inhibition of Vadimezan metabolism. In our study's results, the MAF of *2 (*rs7439366*) was considerably lower in Asians compared to Caucasians, while a part of *UGT2B7* *3 (*rs12233719*) was polymorphic in Asian only. Therefore, we suspect that higher MAF of *2 (*rs7439366*) in Europeans may lead to increased resistance to Vadimezan clearance in Caucasians, while *3 (*rs12233719*) may cause increased resistance in Asians.

UGT2B15 is known to glucuronidate drugs such as oxazepam, lorazepam, and rofecoxib.[14,26,27] Its core marker *2 (*rs1902023*) has been linked with varying degrees of reaction toward the drugs. The minor allele of *2 (*rs1902023*) was found to lower the glucuronidation level of oxazepam in the liver, while also lowering the systemic clearance of lorazepam.[14,15] Such studies have shown that *2 (*rs1902023*) could be used as a pharmacogenetic marker to personalize treatments with oxazepam or lorazepam. In the present study, the MAFs of *2 (*rs1902023*) varied across the five studied ethnic groups. Interestingly, whereas other investigated SNPs often showed clear MAF differences between Asian and other ethnic groups, *2 (*rs1902023*) showed differences among Asian ethnic groups as well. While Koreans and Japanese showed higher MAFs than European Americans (MAF=0.484, 0.436, and 0.298, respectively), Han Chinese MAFs were similar to those for European Americans (MAF=0.289). The result suggests that Korean and Japanese ethnic groups tend to possess increased resistance toward lorazepam or oxazepam clearance in comparison to other ethnic groups.

In the present study the direct sequencing of *UGT1A1*, *UGT2B7*, and *UGT2B15* yielded a number of novel SNPs for each gene. We performed additional *in silico* analyses to predict the function of these thirty two novel SNPs. As a result, a couple of SNPs located in the promoter region of *UGT1A1* were simulated to change transcription factor (TF) binding sites. As stated above, *-690G>A* induced RREB1 and Sp1 binding sites, and *-689A>C* only introduced Sp1 binding site. RREB1 binds to Ras-responsive elements (RREs) of gene promoters has been shown to increase the expression of the bound gene.[28] In a previous study, *calcitonin* (*CT*) with RREB1 bound to its promoter showed an increased expression of *CT* promoter-reporter construct during Ras- or Raf-induced differentiation, which subsequently suggested that RREB1 might play a role in Ras/Raf signal transduction in medullary thyroid cancer.[28] Although no study has reported a binding of RREB1 to *UGT1A1*, our *in silico* analysis results suggest that the binding of RREB1 might cause expression change of *UGT1A1*, which could affect the overall function of the gene.

Another predicted TF, Sp1 is known to bind at GC-rich promoter sites and enhance gene transcription. It has been associated with various forms of cancer and drug treatments.[29,30] Furthermore, other UGT family members such as *UGT1A4*, *8*, *9*, and *10* are known to have a binding site for Sp1 and are regulated by it.[31,32] Therefore, the discovery of a potential binding site for Sp1 in *UGT1A1* could suggest a new pathway of how Sp1 might increase cancer risk or drug delivery.

Besides the discovery of promoter SNPs, we also found exonic SNPs that induced amino acid change. In *UGT2B7*, *673G>A* caused an amino acid change of Glu225Lys. A pre-

vious study showed that such amino acid change, which reverses the amino acid property from acidic to basic, caused a conformational change of the protein structure.[33] Among the six novel exonic SNPs of *UGT2B15*, *2552T>C* (Cys283 Arg) and *24269C>T* (Ser483Leu) were found to change the amino acid properties. Cys283Arg showed an amino acid property change from polar to basic, while the amino acid property of Ser483Leu was modified from polar to nonpolar. Such amino acid substitutions have been shown to result in significant changes in the protein structures in previous studies.[34,35] Therefore, the three exonic SNPs listed above could cause structural modification of proteins, although functional studies would be required to confirm our results.

Although our study sequenced three UGT family genes to examine frequency differences across different ethnic groups and discover novel SNPs, several limitations are present in the study. First, we did not detect core SNPs with low frequencies, such as *UGT1A1* *28 (*rs8175347*), because of the small number of samples. The small number of samples also may skew the results in this study. Also, although samples originated from five different ethnic groups, a majority of samples were of Asian descent, which might have limited the effectiveness of the population study. Lastly, no functional study was conducted to further confirm the roles of the SNPs, although *in silico* analyses were performed to compensate for the lack of functional analysis to an extent.

In summary, the present study analyzed *UGT1A1*, *UGT2B7*, and *UGT2B15* by directly sequencing 288 subjects from five ethnic groups. As a result, core markers for each gene showed significant MAF differences from Asians in comparison to other population. Furthermore, we discovered 31 novel SNPs and predicted the function of the newly discovered SNPs by *in silico* analyses. Our results offer new insight into the pharmacogenetic role of *UGT1A1*, *UGT2B7*, and *UGT2B15* in various ethnicities as well as potential novel therapeutic targets with the discovery of novel SNPs.

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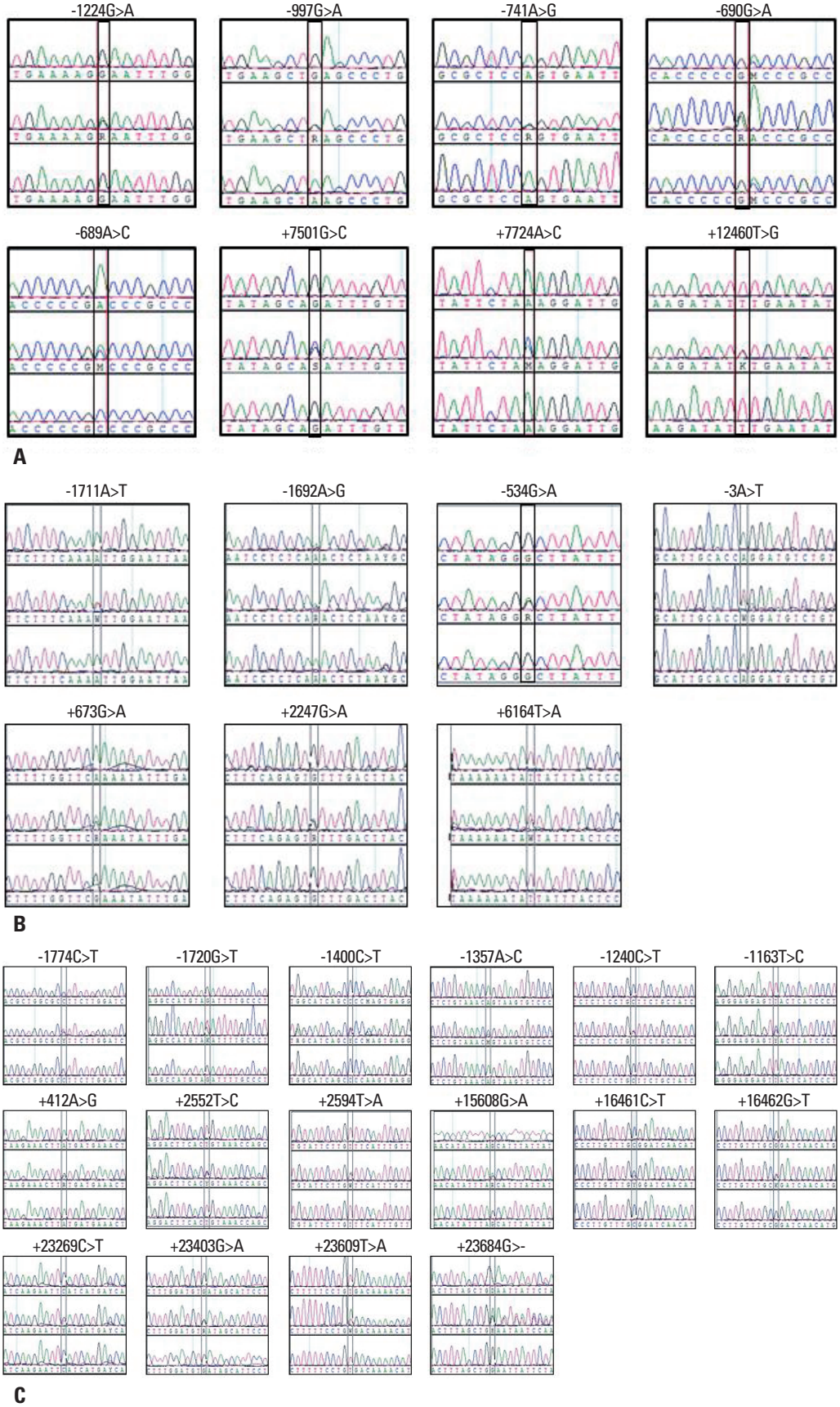
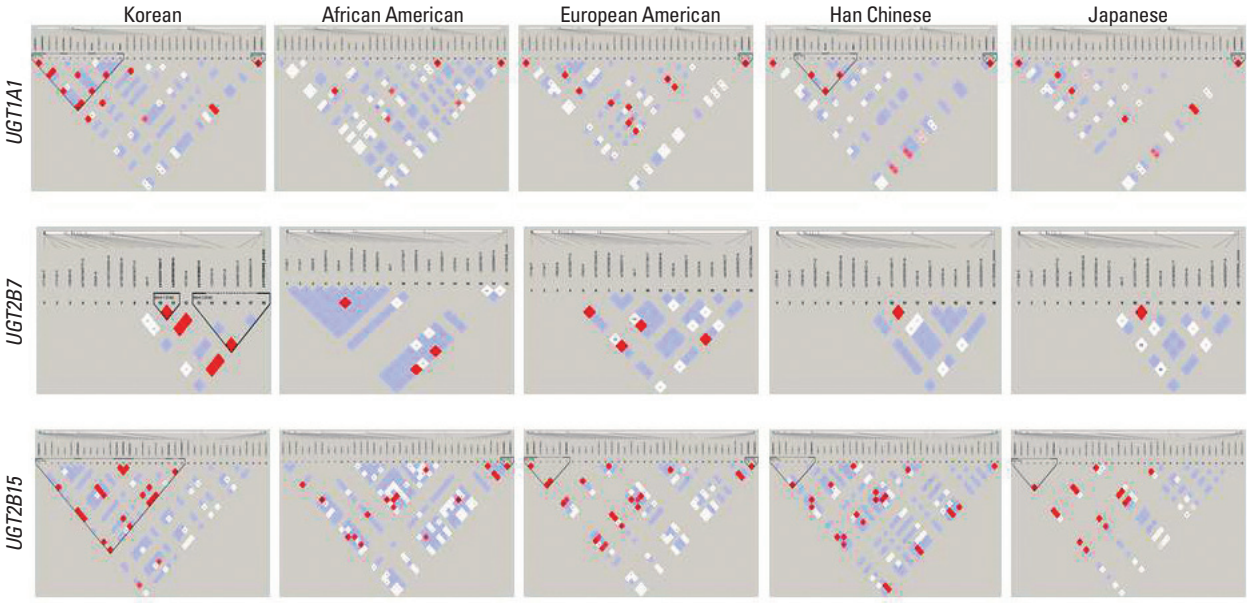
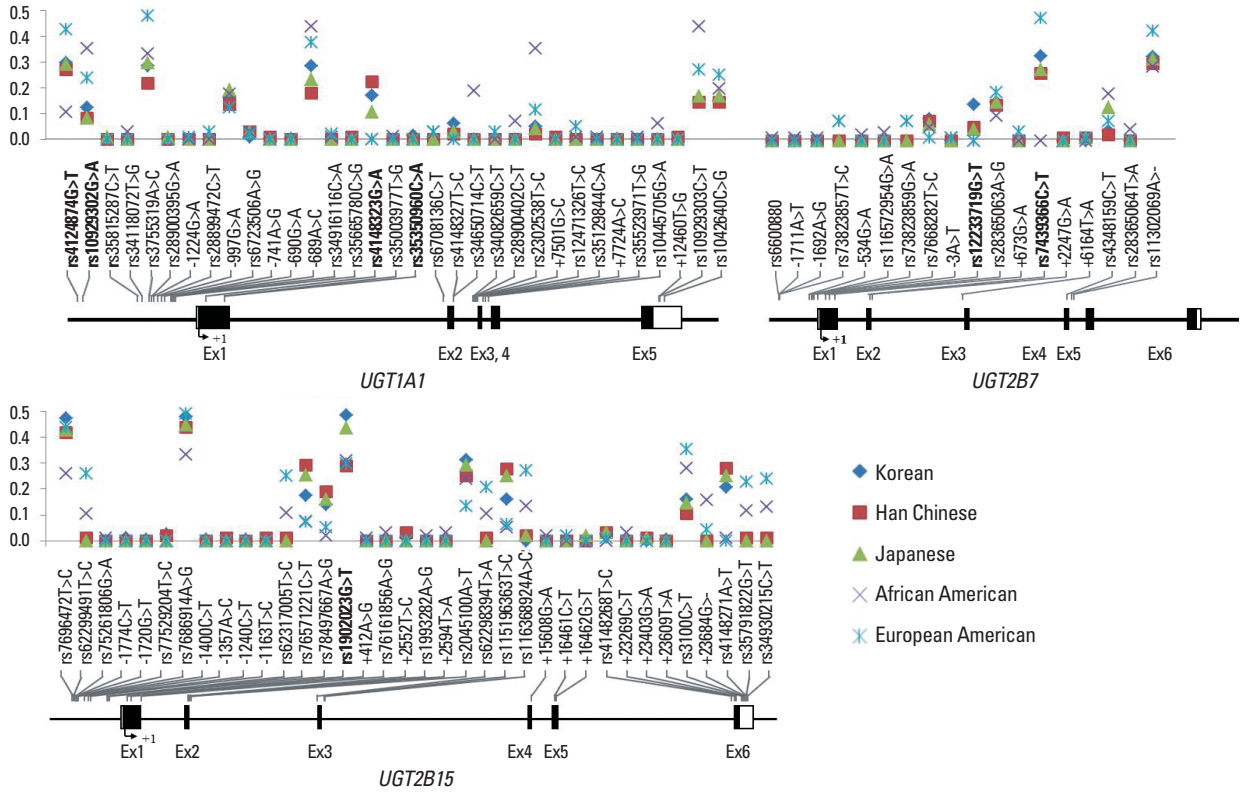


Supplementary Fig. 1. Chromatograms of novel SNPs for (A) *UGT1A1*, (B) *UGT2B7*, and (C) *UGT2B15*. SNP, single nucleotide polymorphism.



Supplementary Fig. 2. LD blocks of *UGT1A1*, *UGT2B7*, and *UGT2B15* in Korean, African American, European American, Han Chinese, and Japanese subjects. Haplotype blocks are shown with separate black lines. LD, linkage disequilibrium.



Supplementary Fig. 3. A physical map and MAF of *UGT1A1*, *UGT2B7*, and *UGT2B15* SNPs among Korean, African American, European American, Han Chinese, and Japanese subjects. Bold SNPs indicate pharmacogenetic core SNPs. Novel SNPs are labeled with their location and allele change. MAF, minor allele frequency; SNP, single nucleotide polymorphism; Ex, Exon.

Supplementary Table 1. Primer Information of *UGT1A1*, *UGT2B7*, and *UGT2B15*

Gene	Name	Forward seq. (5'-3')	Reverse seq. (5'-3')
UGT1A1	UGT1A1_01P	CCAGGTACACAGCAGAAGCA	GCAAGTATTGTGCAGCCAGA
	UGT1A1_02P	CTGGCCAGTGATGTGTATGG	GCTCACCTGAACCAACCAAT
	UGT1A1_03P	GCAAACCAGGGAGTTACAGC	AGGAAATGAACAGGTGGGTG
	UGT1A1_03-1P	AGGGCCAGTTGGCTCTATTT	AGAACACAACTGTACCCCCG
	UGT1A1_04P	TCACGATTTCTAAGTTCCTGCTC	GGCAAAAACCAATCGATACA
	UGT1A1_05P	CTCCCTGCTACCTTTGTGGA	CCGTCAGCATGACATCAAAG
	UGT1A1_06P	AGACGTACCCTGTGCCATTC	TAAACCACGTCGCACAGAAA
	UGT1A1_06-1P	CCAACCCATTCTCCTACGTG	TCATCCAGAAGATGATGCCA
	UGT1A1_07P	TGGATTTTGCATCTCAAGGA	AATAGTTGGGAAGTGGCAGG
	UGT1A1_08P	CTGATCCTCCCACTCTGTTAAA	TAAACACCATGGGAACCAGC
	UGT1A1_09P	AAATTTCTGCAAGGGCATGT	TCCAGCCTAGGTGACAGAGC
	UGT1A1_10P	TTGTGCCACTACACTCCAGC	TTGGAAATGACTAGGGAATGG
	UGT1A1_11P	CAGTGGCCTTCATCACCTTT	ACCATCTGCAGAAGCCAAAA
	UGT1A1_12P	GAGTGCGGGATTCAAAGGT	GCGTGTGTGTGTGAACCTCTA
UGT2B7	UGT2B7_1P	CACTTAATTGCTAAAAAGTGCTAATG	GCTCAGTGCCCTGCAATTAT
	UGT2B7_2P	TCTTTGCCCCTCCAAAAGTA	TGGTATTTTGTGACTGGCTTCTT
	UGT2B7_3P	AAGGAAAGTTGATCATTTCATAATGT	TTGTTTCTGCAGTCCATTTG
	UGT2B7_4P	AAGGGCTCTCCAACTGATTG	TGGAAGCTGAAGATGCCAGT
	UGT2B7_5P	GCACCAGGATGTCTGTGAAA	AGCTCTGCTTCAAAGACACAAA
	UGT2B7_6P	TTTCTTATAAATACACATGGGCAAAA	CCAACCCTATTTTCAAAGTCTCC
	UGT2B7_7P	CCCTTACACACATGCACACA	AAAAATGCAACCACAATTTTCA
	UGT2B7_8P	GTTGGCCACACGTAGGTTTT	ACAGAGAGACAGCCCAGGAA
	UGT2B7_9P	TCCTCAATCCTAGCACCACC	AAAAAGGATGAAACTCACACTCA
	UGT2B7_10P	AGAGAGGAGTCTTGCCGATG	CAGTGGACTTCTTAATGATCTTGTG
UGT2B15	UGT2B15_1P	GTTTGCAGATTTTTAATGAGGCA	CTCAGCCCACCTGCAACC
	UGT2B15_2P	CTCCTAGGATTTGGCACCAG	CCCCCTCTCCAGAATACACA
	UGT2B15_3P	TTCTCTAATTTGACTCAGCTTCACA	TATCGTGGTGCAAGTAATGTCTTC
	UGT2B15_4P	TACTAGGAAACATTGAACTGTACACAC	TTATCCAATGGCTGTATTCTGTG
	UGT2B15_5P	ATGGACGTCAGTCTTTCTGCT	TCACTTAATTCTGACATAACAACAGG
	UGT2B15_6P	GCTCTGTAAAGATGCAGTTTTGAAT	TGACTCTCTGGGTGTCCTGT
	UGT2B15_7P	TCTCTGAATACATTGCCTTCCTT	ACCTCCCATATTCCCCTCAC
	UGT2B15_8P	TCTCCTTTCAAAGAAACTGAATGA	TGCCAACGTTATTATCAACCA
	UGT2B15_9P	TGGGTATTCAACTTACCTCAAACTT	CTGAACAAAGAGATTTTTATTCTGACC
	UGT2B15_10P	CCTAGGGAACAGTTTTGCTTT	GTGGGGTTGAAATGTAACTTTG
	UGT2B15_11P	ACTTTCCCACCGAAAATTCC	CAGGAAAAAGGAAATCCTCCA
	UGT2B15_12P	TGCGTGGCAACTGTGATATT	CTTTCGTGTGTAACTTTTGGATT

Supplementary Table 2. The Comparison of Core Marker Minor Allele Frequencies between Koreans and Other Ethnic Groups

Gene	SNP	KR vs. CH		KR vs. JP		KR vs. AA		KR vs. EA	
		P	$P^{cor.}$	P	$P^{cor.}$	P	$P^{cor.}$	P	$P^{cor.}$
UGT1A1	*rs4124874G>T*	0.074	NS	0.808	NS	1.05E-10*	2.52E-09*	0.050*	NS
	rs10929302G>A	0.294	NS	0.294	NS	1.13E-05*	2.71E-04*	0.0151*	NS
	rs4148323G>A (R71G)	0.318	NS	0.309	NS	3.01E-06*	7.22E-05*	3.01E-06*	7.22E-05*
UGT2B7	*rs12233719G>T* (A71S)	0.056	NS	0.030	NS	2.39E-05*	5.74E-04*	3.42E-05*	8.21E-04*
	rs7439366C>T (H268Y)	0.076	NS	0.188	NS	4.36E-11*	1.05E-09*	0.002*	0.048*
UGT2B15	*rs1902023G>T* (D85Y)	0.002*	0.048*	0.377	NS	0.01*	NS	0.006*	NS

KR, Korean; CH, Chinese; JP, Japanese; AA, African American; EA, European American.

Values indicate the *p* value of difference between the two ethnic groups calculated by Fisher's exact test.

*Values indicate numbers below 0.05. *p* values were adjusted for the multiple testing by applying Bonferroni correction (n=24, which is a number of total tests in the table).