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Analyses of Single Nucleotide Polymorphisms and Haplotype Linkage of the Human *ABCB1* (*MDR1*) Gene in Korean

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Single nucleotide polymorphisms (SNPs) in the *MDR1* gene that are responsible for drug efflux can cause toxicity. Therefore, this study determined the SNPs of the Korean *MDR1* gene, and analyzed the haplotypes and a linkage disequilibrium (LD) of the SNPs determined. The frequency of 9 SNPs from the *MDR1* gene was determined by PCR-RFLP analyses of 100 to 500 healthy individuals. The frequcies of the SNPs were C3435T (47.7%), G2677T (37.6%), G2677A (4.4%), T1236C (21.7%), T129C (8%), A2956G (2.5%), T307C (1.5%), A41aG (9.2%), C145G (0%), and G4030C (0 %). Analyses of the haplotype structure and an estimation of the LD of the combined polymorphisms demonstrated that the frequency of the 1236T-2677G-3435T haplotype is much higher in Koreans (14.1%) than in Chinese and western black Africans and the C3435T SNP in Koreans appears to have LD with T129C in Koreans for the first time. These results provide insight into the genetic variation of *MDR1* in Koreans, and demonstrated the possibility of a new LD in this gene.

Key words: *ABCB1*, Korean, SNP, Haplotype, Linkage disequilibrium

INTRODUCTION

A 170-kDa phospho-glycoprotein (P-gp) encoded by the human *MDR1* gene acts as an ATP–dependent membrane efflux pump that belongs to the ATP-binding cassette (ABC) superfamily (*ABCB1*) of membrane transporters. P-gp is found in both normal tissues and cancer cells, and is responsible for the efflux of chemotherapeutics or toxic metabolites. Hence, P-gp decreases the absorption of a drug from the intestine, kidney, and liver, and promotes the secretion of drugs into the bile and urine (Schwab *et al.*, 2003; Gottesman and Pastan, 1993). P-gp overexpression in cancer cells is a major obstacle in cancer chemotherapy because it causes multidrug resistance to hydrophobic cytotoxic agents including the vinka alkaloids, epipodophylotoxins, anthracyclines, colchicines or taxol, which comprise structurally and mechanically of unrelated anticancer drugs (Schwab *et al.*, 2003; Gottesman and Pastan, 1993).

A single nucleotide polymorphism (SNP) in the *MDR1* gene has been documented at a noncoding sequence change in exon 26 of the *MDR1* gene (C3435T) at a wobble position, which was found to affect the intestinal expression of *MDR1* and the plasma concentrations of orally administered digoxin (Hoffmeyer *et al.*, 2000). Therefore, nucleotide variations in the *MDR1* gene can affect the pharmacokinetics and pharmacodynamics of various drugs, and cause toxicity and/or reduce the efficacy of chemotherapeutics. The SNP frequency on the *MDR1* gene of various ethnic groups was shown to have a different effect on the drug efflux pump in multidrug resistant human patients and animal models (see review: Marzolini *et al.*, 2004). For example, the highest frequency of the homozygous mutant genotype in exons 12, 21, and 26 at positions 1236, 2677, and 3435, respectively, (TT-TT-TT) was found in Indians (31%) followed by 19% in Chinese, and 15% in Malays. Furthermore, in heart transplant patients, cyclosporine exposure ($AUC_{0\text{-}4\ h}$, $AUC_{0\text{-}12\ h}$ and C_{max}) was high in those patients with the T-T-T haplotypes compared with those with the C-G-C haplotypes. This demonstrates that haplotype analysis of the gene should be included instead of SNP detection (Chowbay *et al.*, 2003).

Currently, a total of 29 SNPs in Chinese, Japanese, German, Caucasians have been found in the human

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MDR1 gene (Casocobi *et al.*, 2001; Hoffmeyer *et al.*, 2000; Marzolini *et al.*, 2004). It has also been reported that a strong LD between multiple SNPs at the *MDR1* locus and the common alleles or haplotypes is associated with an altered P-gp function (Kim *et al.*, 2001). Although the *MDR1* SNP in Asians has been reported mainly for Japanese and Chinese (Schwab *et al.*, 2003), knowledge of the *MDR1* SNPs in different ethnic groups is still limited and the pharmacogenetic analysis of *MDR1* should be analyzed further. Furthermore, knowledge of the extent of the LD and haplotype structure of the *MDR1* gene is quite limited. In Koreans, only the 3 most frequent SNPs located in exons 12, 21, and 26 of the *MDR1* gene in 232 healthy subjects along with the corresponding haplotype analysis have been reported (Yi *et al.*, 2004). However, SNP analyses on the other *MDR1* loci remain unclear, and the LD pattern has not been analyzed. Therefore, this study characterized the frequencies of 9 SNPs, which was made up of the 7 most frequent SNPs including the 3 most frequent SNPs and 2 frequently encountered SNPs (A41aG, and T129C) in Japan, and analyzed the *MDR1* LD pattern and haplotype structure. Unexpectedly, this study found significantly different frequencies of the C1236T SNP and four major haplotypes compared with the previous report (Yi *et al.*, 2004). This suggests that these changes are in LD and could contribute to a variation in the pharmacokinetics of drugs.

MATERIALS AND METHODS

Subjects

Five hundred healthy volunteers (Women: 320, men: 180) were enrolled in the SNP analyses study between May 2004 and November 2004 in a local internal medicine clinic in In-Cheon city, Kyoung-gi Province. Their mean (±SD) age was 55 ± 5 years (range, 50-60 years). All the subjects provided written informed consent. The median age of the participants was approximately 53.2 years. The medical ethics review boards of the participating hospitals approved the study protocol. The serum concentrations of alkaline phosphatase, γ-glutamyltransferase, AST, ALT, albumin, total protein, bilirubin, urea nitrogen, and creatinine were recorded at the time of the visit for diagnosis.

Extraction of genomic DNA

The peripheral blood was sampled from the subjects. The genomic DNA was extracted from the cells via phenol-chloroform extraction after proteinase K extraction and ethanol precipitation using G-SPIN™ (Intron, Korea), as suggested by the supplier.

Genotyping of *MDR1* gene

For 9 *MDR1* SNP analyses, a polymerase chain reaction (PCR)–based restriction fragment length polymorphism (PCR-RFLP) assay was performed using the primers in Table I, as previously reported (Tanabe *et al.*, 2001) or newly designed primers in the case where the PCR products formed multiple DNA bands (C145G, T129C) after the reaction. Most of the PCR products were designed to be within 300 bp. PCR was carried out using the following steps: an initial denaturation of 5 minutes at 94°C, followed by 35 cycles of denaturation at 94°C for 30 seconds, primer annealing for 30 seconds at 50-60°C,

Table I. Genotyping procedures for the *MDR1* polymorphisms using the genomic DNA

	5' Primer	3' Primer	Length (bp)	Restriction Enzyme	Cutting position	W/W	W/V	V/V
A-41aG	5' TAAATGCGAATCCCGAGAAAA 3'	5' TCCCGGCCGGATTGACTGAA 3'	243	BsrI	108,243	108,135,243	108,135,243	108,135
C-145G	5' ATCAGCATTCAGTCAATCCGGGC CGGGAGC 3'	5' GGAAGAAGATACTCCGACTT TAGTGGAAA 3'	219	Bpu1102I	84,,121	84,135	37,84,98,135	84,37,98
T-129C	5' ATCAGCATTCAGTCAATCCGGGC CGGGAGC 3'	5' GGAAGAAGATACTCCGACTT TAGTGGAAA 3'	219	MspA1I	138,170	170,49	32,49,138,170	32,49,138
T-307C	5' AATGGAGACTAAAGAGTCAT AAATG 3'	5' ACCTGGTCATGTCTTCCTCC 3'	104	BanII	72	104	32,72,104	32,72
T-1236C	5' TTTTTCTCACGGTCCTGGTAG 3'	5' CATCCCCTCTGTGGGGTCATA 3'	147	HaeIII	33,68	68,79	33,35,68,79	33,35,79
G-2677A	5' TACCCATCATTGCAATAGCAG 3'	5' TTTAGTTTGACTCACCTTCCC 3'	107	AfaI	83	107	24,83,107	83,24
G-2677T	5' TGCAGGCTATAGGTTCCAGG 3'	5' TTTAGTTTGACTCACCTTCCCG 3'	224	BanI	198	198,26	224,198,26	224
A-2956T	5' TTGTGTTTGTGCTTTCCAGAG 3'	5' TTAGGCCTTCCGTGCTGTAGC 3'	171	NcoI	47	47,124	47,124,171	171
C-3435T	5' TTGATGGCAAAGAAATAAAGC 3'	5' CTTACATTAGGCAGTGACTCG 3'	207	NdeII	145	62,145	62,145,207	207
G-4030C	5' TCCTCAGTCAAGTTCAGAGTC 3'	5' GACACTTTATGCAAACATTTC 3'	242	Alw26I	26,55,71	26,29,187	16,26,29,171,187	16,26,29,171

Primer sequence used for amplifying the PCR fragments that contained the distinct *MDR1* polymorphisms, restriction endonucleases, and RFLP fragment sizes of the wild type (W) or variant allele (V).

and 30 seconds extension at 72°C. The amplified reaction products were purified using the Qiagen PCR purification kit (Qiagen, Valencia, Calif), followed by digestion with the restriction enzyme as shown in Table I. The PCR products produced by the *Streptococcus pneumoniae* chromosomal DNA as a template and the relevant oligonucleotide sequences as primers were used as the positive controls to confirm that the PCR product containing the SNP had been digested by the restriction enzyme of choice. When the mutation was present, digestion of the PCR product produced different fragments. Direct DNA sequencing was performed if there were pseudo-positive or negative results after PCR-RFLP to determine the nucleotide sequence of the PCR product.

LD and haplotype analyses

The Hardy-Weinberg equilibrium test, haplotype analysis, and LD were determined using the web site (http://ngri.re.kr/HapAnalyzer). The allelic frequency of the 9 SNPs was determined using the Hardy-Weinberg equilibrium test. Eleven haplotypes were detected in pairwise of 3 SNPs (T1236C, G2677T/A, C3435T) using Haplotype analyses. The HapAnalyzer software provides two measures, $|D'|$ and r^2 with a confidence interval and a *p*-value, and shows the LD blocks by computing the confidence interval between each set of pairwise SNP loci (Jung *et al.*, 2004). The LD values between each set of pairwise SNP loci were also visualized by its equivalent image format, which was provided by a Microsoft Excel spread sheet and a text file format.

Statistical analysis

The observed allele frequencies were compared using the Hardy-Weinberg test. The Mann-Whitney test was used to examine the differences between the haplotype carriers and noncarriers. A p value < 0.05 was considered significant.

RESULTS

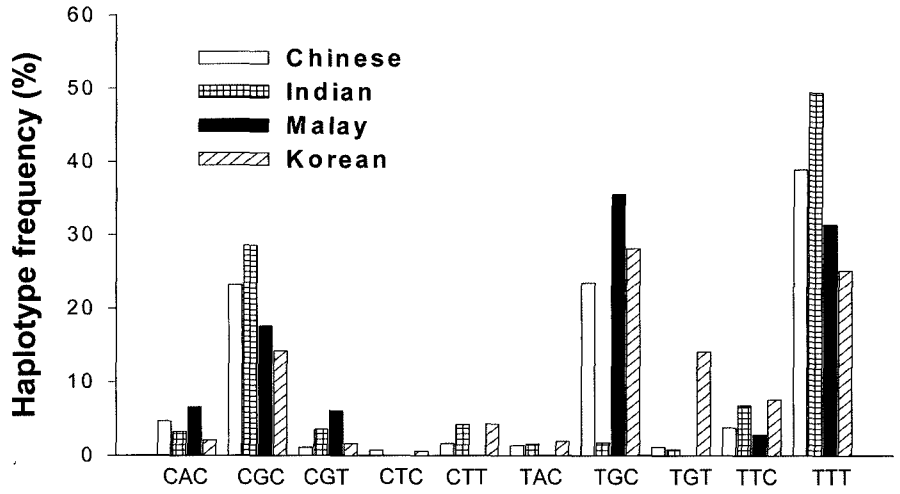
MDR1 polymorphism in genomic DNA by RFLP

Nine *MDR1* SNPs were examined using PCR-RFLP analyses from 100 to 500 healthy subjects. The *MDR1* gene mutation is unaffected by age (Brenner and Klotz, 2004). Therefore, the frequency of the 9 SNPs determined from the blood samples of the 50-60 years old Koreans would be valid for SNP analysis. The allele frequencies of the 9 *MDR1* SNPs and the sample numbers were C3435T 47.7% (n=500), G2677T 37.6% (n=500), G2677A 4.4% (n=500), T1236C 21.7% (n=500), A41aG 9.2% (n=388), T129C 8% (n=100), A2956G 2.5% (n=100), and T307C 1.5% (n=100). However, 2 SNPs, C-145G and G4030C, were not detected in 100 DNA samples (Table II). There were 4 point mutations (T307C, G2677T/A, and A2956G) among the 9 SNPs that caused an amino acid exchange in *MDR1*, but not the others (C3435T, T1236C, A41aG, T129C, C-145G, and G4030C) (Table II). The frequency of the heterozygote and homozygote C3435T SNP in exon 26 were 59.8% and 17.8%, respectively. The heterozygote and homozygote SNP frequency at exon 21 in position 2677 was 49.6% and 17.2%, respectively (Table II). In addition, two non-coding SNPs in the Korean

Table II. Positions, sequences, and frequencies of the *MDR1* variants in the Korean genomic DNA

	MDR1 Exon/Position	Effect	Analyzed individuals, *N*	Genomic DNA				
				Genotype, *N*			Allele frequency (%)	
				W/W	W/V	V/V	W	V
A-41aG	5'-flanking/-41	Noncoding	388	320 (A/A)	66 (A/G)	2 (G/G)	91 (A)	9.2 (G)
C-145G	1a/-145	Noncoding	100	100 (C/C)	0 (C/G)	0 (G/G)	100 (C)	0 (G)
T-129C	1b/-129	Noncoding	100	84 (T/T)	16 (T/C)	0 (C/C)	92 (T)	8 (C)
T-307C	5/307	Phe103Leu	100	97 (T/T)	3 (T/C)	0 (C/C)	98.5 (T)	1.5 (C)
T-1236C	12/1236	Gly412Gly	500	330 (T/T)	123 (T/C)	47 (C/C)	78.3 (T)	21.7 (C)
G-2677T	21/2677	Ala893Ser	500	166 G/G)	208 (G/T)	82 (T/T)	58 (G)	37.6 (T)
G-2677A	21/2677	Ala893Thr	500		40 (G/A) 4 (A/T)	0 (A/A)		4.4 (A)
A-2956G	24/2956	Met986Val	100	95 (A/A)	5 (A/G)	0 (G/G)	97.5 (A)	2.5 (G)
C-3435T	26/3435	Ile1145Ile	500	112 (C/C)	299 (C/T)	89 (T/T)	52.3 (C)	47.7 (T)
G-4030C	28/4030	Noncoding	100	100 (G/G)	0 (G/C)	0 (C/C)	100 (G)	0 (C)

* PCR-RFLP-based genotyping was developed to detect the new and known variations using genomic DNA. The SNPs that are located in introns are presented as (exon+/-n), i.e., n nucleotides upstream (-) or downstream (+) of the exons.
* Allelic frequency was calculated based on the Hardy-Weinberg method.
* Wild type (W) or variant allele (V).



	CAC	CGC	CGT	CTC	CTT	TAC	TGC	TGT	TTC	TTT	Total
Chinese	4.679	23.163	1.1	0.733	1.644	1.365	23.448	1.191	3.756	38.923	100
Indian	3.251	28.54	3.517	0	4.208	1.587	1.745	0.875	6.812	49.465	100
Malay	6.579	17.562	6.122	0	0	0	35.526	0	2.832	31.378	100
Korean	2.1	14.2	1.6	0.6	4.3	1.9	28.1	14.1	7.6	25.1	99.6

Fig. 1. Haplotype frequency of the most frequent three SNP loci in Koreans. *The order of the SNP loci in the haplotype is SNP 1236 in exon 12 - SNP 2677 in exon 21 - SNP 3435 in exon 26 (CC, CT, TT) in *MDR1* gene.

population, A41aG and T129C, were found to be quite frequent, 9.2% and 8%, respectively.

Haplotype and LD analysis

The most frequent 3 SNPs (T1236C, G2677T/A, C3435T) were further analyzed for their haplotype. The frequency of the 11 haplotypes in T1236C-G2677T/A-C3435T loci were T-G-C (28.1%), T-T-T (25.1%), C-G-C (14.2%), T-G-T (14.1%), T-T-C (7.6%), C-T-T (4.3%), C-A-C (2.1%), T-A-C (1.9%), C-G-T (1.6%), C-T-C (0.6%), and T-A-T (0.4%). The frequencies of the 4 haplotypes, T-G-C (28.1%), T-T-T (25.1%), C-G-C (14.2%), T-G-T (14.1%) were also relatively high (Fig. 1).

The LD was measured in a pairwise manner to determine if there was any relationship between a specific SNP and the other SNPs (Fig. 2). Any crosslink between each SNP was denoted as the (D') value. Only the 5 SNPs that had a > 8% frequency were selected because a low SNP frequency does not allow an analysis of the relationship between 2 SNPs. C3435T SNP was shown to have relationship with the T1236C (D' value of 0.64), T129C (D' value of 0.58), A41aG (D' value of 0.32), and G2677T (D' value of 0.37) SNPs. In addition, the G2677T and T129C SNPs showed a D' value of 0.58, which demonstrates a correlation between them. This suggests that C3435T SNP has some relationship with the other 4 SNPs, whereas the G2677T SNP has a relationship with

	A41aG	T129C	T1236C	G2677T	C3435T
A41aG					
T129C	0.19				
T1236C	0.17	0.05			
G2677T	0.14	0.58*	0.06		
C3435T	0.32*	0.58*	0.64*	0.37*	

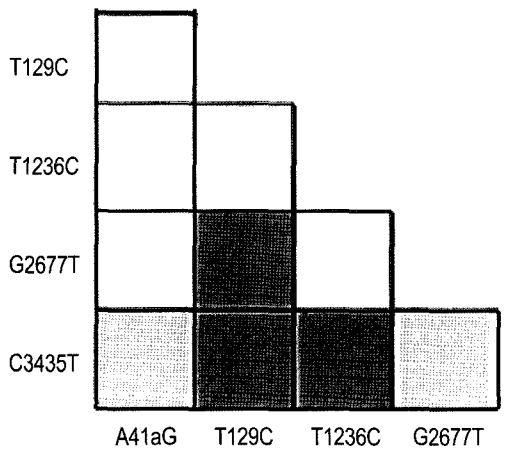


Fig. 2. Linkage Disequilibrium (LD) analysis for a set of 4 SNP in Koreans. LD was measured using the statistic |D' | in a pairwise manner across 4 SNPs (allelic frequency >0.08) in HapAnalyzer. |D' |>0.5: red, 0.5.>= |D' |>=0.2: orange, |D' |<0.2 : white.

the T129C SNP only. Therefore, the 5 SNPs appear to have a direct or indirect relationship with each other. In contrast, there was no correlation between sex gender and SNP/haplotype frequency (data not shown).

DISCUSSION

In this study, the frequencies of 3 SNPs from 500 samples showed that C3435T (47.7%) was the most frequent followed by G2677T (37.6%), and T1236C (21.7%). In contrast, Yi *et al.* (2004) reported that T1236C was the most frequent (38.1%) followed by G2677T (37.5%), and C3435T (36.9%) from 232 samples. In addition, the frequencies of the other 6 SNPs (A-41aG, T-129C, C-145G, T307C, A2956G, and G4030C) in Koreans are reported for the first time. It was previously reported that in exon 21, G2677T/A genotyping revealed GG in 19.8% of samples, GT in 34.5%, TT in 11.2%, GA in 13.4%, TA in 18.1%, and AA in 3.0% (Yi *et al.*, 2004). However, in this study, genotyping showed GG in 33.2% of samples, GT in 41.6%, TT in 16.4%, GA in 8.0%, TA in 0.8%, and AA in 0%. Furthermore, it was reported that in exon 26, C3435T the frequencies of the CC, CT, and TT genotypes were 38.4%, 49.6%, and 12.1%, respectively

Table III. Genetic polymorphism of *MDR1* in various ethnic groups

Location	Position	Variation W/V	Variant allele frequency (%)									
			Caucasians				Africans		Asians			Korean
			Ho[3]	Ca[6]	Si[23]	Ge[24]	Sc[12]	Ki[7]	Ito[25]	Ta[9]	Tan[15]	Ryu
Promoter	5' flanking/-41	A/G							7.3	9.4		9.2
Exon 1a	Exon 1a/-145	C/C							1	1		0
Exon 1b	Exon 1b/-129	T/C	5.9							8.3	1.6	8
Intron 1	Exon 2/-4	C/T										
Intron 1	Exon 2/-1	G/A	5.6	9	3.7	7						
Exon 2	Exon 2/61	A/G	9.3	11.2	8.9	13						
Intron 4	Exon 5/-35	G/C	0.6									
Intron 4	Exon 5/-25	G/T	16.5									
Exon 5	Exon 5/307	T/C	0.6	0								1.5
Intron 6	Exon 6/+139	C/T	40.6	37.2	35.8	39						
Intron 6	Exon 6/+145	C/T	1.2									
Exon 7	Exon 7/548	A/G										
Exon 11	Exon 11/1199	G/A	6.5	5.5	2.9	2						
Exon12	Exon 12/1236	C/T	37.8	41	34.3			15	61.5	64.6	68.6	78.3
Intron 12	Exon 12/+44	C/T	5.9	4.9	7.5							
Exon 13	Exon 13/1474	C/T										
Intron 16	Exon 17/-76	T/A	45.3	46.2	49.3							
Intron 17	Exon 17/+137	A/G	0.6									
Exon 21	Exon 21/2650	C/T										
Exon 21	Exon 21/2677	G/T		41.6	40.3	38		15		41.7	43.7	37.6
		G/A		1.9	3.7	10		0		21.8	5.8	4.4
Exon 24	Exon 24/2956	A/G								0		2.5
Exon 24	Exon 24/2995	G/A										
Exon 26	Exon 26/3320	A/C		0.2								
Exon 26	Exon 26/3396	C/T	0.3									
Exon 26	Exon 26/3421	T/A										
Exon 26	Exon 26/3435	C/T	48.1	53.9	50.7	51	10	26		49	40.4	47.7
Exon 28	Exon 28/4030	G/C								0		0
Exon 28	Exon 28/4036	A/G								25	38	

* Ho, Hoffmeyer *et al.*, 2000; Ca, Cascorbi *et al.*, 2001; Si, Siegmund *et al.*, 2002; Ge, Gerloff *et al.*, 2002; Sc, Schaeffeler *et al.*, 2001; Ki, Kim *et al.*, 2001; Ito, Ito *et al.*, 2001; Ta, Tanabe *et al.*, 2001; Tan, Tang *et al.*, 2002; Ryu, this study.
* Wild type (W) or variant allele (V).

(Yi *et al.*, 2004). In contrast, this study showed that the frequency of the CC, CT, and TT genotypes were 22.4%, 59.8%, and 17.8%, respectively. This indicates a significant difference in both the G2677T/A and C3435T SNP genotype frequencies between this study and Yi *et al.* (2004)'s study.

The most frequent C3435T SNP in exon 26 in the Korean population, which does not accompany any amino acid change, was similar to those reported in East Asians and Caucasians (Schaeffeler *et al.*, 2001; Ameyaw *et al.*, 2001; Hoffmeyer *et al.*, 2000; Cascorbi *et al.*, 2001) (Table III). However, frequency of the C3435T SNP in Africans was approximately 10-26% lower than in Koreans (Schaeffeler *et al.*, 2001, Kim *et al.*, 2001) indicating that the African population has the more wild type allele, C, than the East Asian and Caucasian populations (Table 3). Exon 21 mutant alleles, T/T and A/A, in the G2677T/A SNP give rise to an amino acid change (G-T; Ala-Ser, G-A; Ala-Thr) in such a manner that the lipophilic residue (Ala) is changed into a hydrophilic residue (Ser, Thr), resulting in higher resistance to drugs such as adriamycin and vinblastine (Kioka *et al.*, 1989). Our results show that the frequency of the exon G2677T/A haplotype in Koreans was the A (4.4%), T (37.6%), and wild type G alleles (58%). Although the frequencies of the A mutant allele in Chinese, Malay, and Indian subjects (<10%) were similar to those in Koreans (Tang *et al.*, 2002; Chowbay *et al.*, 2003; Balram *et al.*, 2003), the frequency of the A allele in the Japanese subjects was >20% (Tanabe *et al.*, 2001), suggesting a distinct SNP haplotype frequency within the East Asian population. In addition, the frequencies of the A and T alleles in Africans (0 and 15%, respectively [Kim *et al.*, 2001]) were much lower than those in Koreans (Table III). The 3rd most frequent SNP, T1236C SNP, detected at the exon 12, which affected the expression and function of P-gp (Marzolini *et al.*, 2004; Kim *et al.*, 2001; Goto *et al.*, 2002; Illmer *et al.*, 2002), was similar to those reported in other Asian populations (Japanese, Chinese, Indian, Malay) but much higher than those reported in Caucasians (34-41%) and Africans (15%) (Table III).

When the LD for all pairs of exons 12, 21, and 26 (3 SNPs T1236C, G2677T/A, and C3435T) were examined, of the 12 possible haplotypes, 11 and 12 were observed in the Korean and Chinese subjects, respectively (Zhang *et al.*, 2005), and these haplotype numbers were much larger than those in western black Africans (6 haplotypes; Allabi *et al.*, 2005), Benineses (8 haplotypes), African-Americans (8 haplotypes), Caucasians (10 haplotypes) (Kroetz *et al.*, 2003; Allabi *et al.*, 2005), Malays (6 haplotypes), Chinese (10 haplotypes), and Indians (9 haplotypes; Tang *et al.*, 2002). This study also examined whether or not there was any correlation between the haplotype and blood pressure as well as between the SNP and blood pressure in normal Korean subjects. However, none could be found (data not shown).

Yi *et al.* (2004) reported that the frequencies of the 4 major haplotypes in Koreans, 1236C-2677A-3435C, C-G-C, T-G-C, and T-T-T, were 16.4%, 18.6%, 21.6%, and 32.2%, respectively, making a total of 88.8% (Yi *et al.*, 2004) whereas the present study revealed the frequencies to be 2.1%, 14.2%, 28.1%, and 25.1%, respectively. In addition, the previous study reported that the frequency of the T-G-T haplotype (7.5%; Yi *et al.*, 2004) was almost half the value obtained in this study (14.1%) indicating that there is a discrepancy in the frequencies of the 4 major haplotypes between these results and previous results in Korean.

When the frequency of the T-G-T haplotype in Koreans was compared with those in other Asians, the frequency of the T-G-T haplotype was much higher in Koreans (14.1%) than in Chinese (1.19%) and western black Africans (Beninese; 7.5%) (Allabi *et al.*, 2005; Fig. 1). In addition, when the frequencies of the 3 haplotypes in Koreans, T-G-C (28.1%), T-T-T (25.1%), and C-G-C (14.2%), were compared with those in other Asian groups (Chinese, Malays, Indians), the frequency of the haplotype T-G-C (28.1%) in Koreans was similar to those in Chinese (23.4%), and Malays (35.5%), whereas the frequency of T-G-C haplotype was low (1.7%) in Indians. In contrast, the frequencies of the T-T-T (25.1%), and C-G-C (14.2%) haplotypes in Koreans were lower than those in Chinese, Malays, and Indians (Tang *et al.*, 2002) (Fig. 2). The T-G-C (23.4-35.5%) and T-T-T (25.1-38.9%) haplotypes in Chinese, Malay, and Korean population were relatively higher than those in Benineses (6.16% and 0.45%, respectively), African-Americans (4% and 7.5%, respectively), and Caucasians (1% and 4%, respectively) (Kroetz *et al.*, 2003) even though the C-G-C haplotype was the most frequent haplotype (79.3%) in Beninese, and the second highest (72.6%) in African-Americans (Kroetz *et al.*, 2003; Allabi *et al.*, 2005). This demonstrates that these haplotypes frequencies could be distinctive features in ethnic groups. The C-T-C, C-T-T, T-A-C, and T-G-T haplotypes were not detected in Malays, and the C-T-C haplotype was not found in Indians (Tang *et al.*, 2002). However, frequency of the T-A-T haplotype in Koreans (0.4%) was similar to those reported in other Asian groups (0.9%; Zhang *et al.*, 2005).

Recently Yi *et al.* (2004) and Shon *et al.* (2005) reported that the 2677A allele variant in exon 21 in healthy subjects was correlated with lower plasma concentrations of fexofenadine than either the wild type or T allele variant, and subjects with the 3435TT genotype showed higher plasma concentrations than the subjects with the 3435CC genotype. This indicates that *MDR1* haplotypes analysis

is more important than either single or multiple SNPs analyses. However, a comparison could not be made because pharmacokinetic analysis of these and other haplotypes has not been reported. Therefore, further analyses on the pharmacokinetics of these haplotypes would be needed to draw a general conclusion on the relationship between thehaplotypes and pharmacokinetics.

Two synonymous SNPs (T1236C and C3435T) and a non-synonymous SNP (G2677T, Ala893Ser) were found to be linked in 62% of European Americans and 13% of African Americans (Kim *et al.*, 2001; Goto *et al.*, 2002; Illmer *et al.*, 2002). This study also confirmed this similar linkage in Koreans, and the T1236C was linked to an exon 26 (C3435T) and exon 21 (G2677T) SNPs in 64% and 37% of cases, relatively. Interestingly, a LD was found for the first time between the C3435T polymorphism and the T129C polymorphism even though the G2677T/A SNP is linked with exon1b T129C SNP, and alter the expression and function of P-gp (Marzolini *et al.*, 2004; Kim *et al.*, 2001; Tanabe *et al.*, 2001). Further pharmacokinetic studies on this LD will be needed to determine the significance of this finding.

Overall, these results suggest that Korean *MDR1* gene polymorphisms and haplotypes are different from those in Caucasians and Africans as well as in other Asians. A LD was also found in the C3435T SNP with T129C, and T1236C. In addition, the T129C and G2677T SNPs were linked.

Abbreviation

LD, linkage disequilibrium; PCR-RFLP, polymerase chain reaction (PCR)-based restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

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