# A variant 2677A allele of the *MDR1* gene affects fexofenadine disposition

*Background and Objectives:* There have been considerable disagreements regarding the influence of *MDR1* (*ABCB1*) polymorphisms on the disposition of P-glycoprotein (P-gp) substrates. We speculated that the unknown function of the A allele of exon 21 G2677T/A (Ala893Ser/Thr) provides one of the reasons for the contradictory results. This study was performed to clarify the effects of major *MDR1* gene polymorphisms, including a variant A allele in exon 21, on fexofenadine pharmacokinetics.

*Methods:* We investigated the occurrence of 3 high-frequency single-nucleotide polymorphisms (SNPs) in exons 12 (C1236T), 21 (G2677T/A), and 26 (C3435T) of the *MDR1* gene in 232 healthy Koreans, using a polymerase chain reaction–restriction fragment length polymorphism method, and performed haplotype analysis on these 3 SNPs. A single oral dose of 180 mg fexofenadine hydrochloride was administered to 33 healthy Korean male volunteers, who were divided into 6 groups based on the *MDR1* genotype for the G2677T/A polymorphism in exon 21 and the C3435T polymorphism in exon 26.

*Results:* A strong linkage disequilibrium was observed among the 3 SNPs. The frequencies of the 4 major haplotypes, 1236C-2677A-3435C, C-G-C, T-G-C, and T-T-T, were 16.4%, 18.6%, 21.6%, and 32.2%, respectively. Fexofenadine disposition varied considerably among the groups. In the 2677AA/3435CC genotype group (n = 3), the values of area under the concentration-time curve from time 0 to 24 hours [AUC(0-24)] were significantly lower ($P = .014$) than those of the other 5 genotype groups (GG/CC, GT/CT, TT/TT, GA/CC, and TA/CT). As compared with the 2677GG/3435CC subjects, the AUC(0-24) values were 17% lower in the 2677AA/3435CC subjects and 47% higher in the 2677TT/3435TT subjects (GG/CC versus AA/CC versus TT/TT, 4017 ± 1137 ng · h/mL versus 3315 ± 958 ng · h/mL versus 5934 ± 2,064 ng · h/mL; $P = .018$). By stratification for genotypes at position 3435, homozygous 3435TT subjects were found to have significantly higher AUC(0-24) ($P = .024$) and maximum plasma concentration ($P = .040$) values than CC subjects [AUC(0-24), 5934 ± 2064 ng · h/mL versus 3998 ± 1241 ng · h/mL; maximum plasma concentration, 958 ± 408 ng/mL versus 673 ± 242 ng/mL].

*Conclusions:* The plasma concentrations of fexofenadine after a single oral administration were lower in 2677AA/3435CC subjects than in subjects with the other 5 genotype combinations of the SNPs of G2677T/A and C3435T. These findings confirm the importance of analyzing *MDR1* haplotypes and provide a plausible explanation for the conflicting results regarding the effect of *MDR1* polymorphisms on the disposition of P-gp substrates. (Clin Pharmacol Ther 2004;76:418-27.)

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P-glycoprotein (P-gp), the product of the multidrug resistance gene (*MDR1*), is a member of the superfamily of adenosine triphosphate–binding cassette transporters.[1] It is an integral membrane protein of 170 kd and is composed of 2 homologous halves; each half contains 6 transmembrane domains and an intracellular binding site for adenosine triphosphate. P-gp was first identified in cancer cells as a protein responsible for resistance to a variety of structurally unrelated anticancer agents. P-gp is expressed not only in tumor cells but also in the cells of several normal tissues, where it functions protectively against xenobiotics.[2-4] P-gp limits its absorption from the gastrointestinal tract and promotes the efflux of these compounds into bile and urine. The expressed amounts are highly variable between subjects; up to 8-fold variations have been found by small intestine biopsy,[5] and 55-fold variations have been found in normal and perineoplastic liver biopsy specimens.[6] P-gp transports many structurally diverse and clinically important drugs including the anticancer drugs, cardiac glycoside digoxin, human immunodeficiency virus protease inhibitors, immunosuppressants, antibiotics, and glucocorticoids.[5,7-10] Considering the effect of P-gp on drug disposition, in particular with respect to its synergism in the intestine with the cytochrome P450 3A4 enzyme,[11-14] it would appear that polymorphisms in the *MDR1* gene may affect the disposition of P-gp substrates.

At present, 28 single nucleotide polymorphisms (SNPs) have been found at 27 positions, and 11 SNPs alter the amino acid sequences of P-gp.[15] Several groups have reported that a synonymous polymorphism, C3435T in exon 26, is associated with lower *MDR1* expression[16,17] and higher plasma levels of *MDR1* substrates,[16,18-20] but other studies have contradicted these results.[21-24] On the other hand, there are also studies that have not found an association between reported SNP and *MDR1* expression in the placenta[25] and duodenum[26] or disposition of P-gp substrates.[27,28] Another frequent SNP with an amino acid exchange, G2677T/A (Ala893Ser/Thr) in exon 21,[29,30] is located in the second transmembrane spanning domain, and there is also disagreement regarding the effects of this polymorphism (G2677T/A) on *MDR1* transport activities.[19,23,25,26] The reasons for these discrepancies concerning *MDR1* polymorphisms and P-gp expression or function are unclear. However, we speculated that the unknown function of the 2677A allele in exon 21 is a possible reason for these contradictory results. The G2677T/A polymorphism is unique, with 3 allelic variants at the same gene locus. Moreover, ethnic differences in the allelic frequencies of G2677T/A have also been observed. Of the 3 allelic variants, the allelic frequency of the variant A allele ranged from 3.3% to 36% in Asians[18,31-35] compared with 1.9% to 10% in white subjects[27,30,34] and 0.5% in black subjects.[34] This G2677T/A polymorphism was found to be in significant linkage disequilibrium with the C3435T polymorphism and with another synonymous polymorphism, C1236T, in exon 12.[31,32] In particular, it has been reported that the variant 2677A allele, as well as the wild-type 2677G allele, is strongly linked to the wild-type 3435C allele. The majority of clinical studies on the effects of *MDR1* polymorphisms on expression and function have been conducted on the silent mutation C3435T and on the nonsynonymous frequent mutation G2677T. Until recently, no report was available on the function of the 2677A allele variant. Accordingly, subjects with the 2677A allele have been evaluated mainly as subjects with the 2677G or 2677T allele or the linked 3435C allele. Thus the activity of the variant 2677A allele needs to be evaluated by haplotype analysis to avoid misinterpretations.

To clarify the effects of the major *MDR1* gene polymorphisms on fexofenadine pharmacokinetics, we designed this study to identify the activity of the variant 2677A allele in healthy male subjects, who were divided into 6 groups based on genotype combinations of SNPs G2677T/A and C3435T.

## METHODS

***Subjects.*** A total of 232 healthy unrelated Korean volunteers living in Seoul and neighboring areas (155 men and 77 women, aged 21-39 years) were genotyped for the *MDR1* exon 12 C1236T, exon 21 G2677T/A (Ala893Ser/Thr), and exon 26 C3435T polymorphism. Of these 232 Korean volunteers who had been genotyped for the *MDR1* polymorphism, 33 also participated in the pharmacokinetic study of fexofenadine. They ranged in age from 21 to 39 years and in weight from 57 to 82 kg (Table I). The subjects were classified into the 6 groups based on the *MDR1* genotype for the G2677T/A and C3435T polymorphisms. The demographic data of the genotype groups did not differ. The genotypes at positions 2677 and 3435 of the subjects were as follows: 2677GG/3435CC (n = 5), 2677GT/3435CT (n = 6), 2677TT/3435TT (n = 6), 2677GA/3435CC (n = 7), 2677TA/3435CT (n = 6), and 2677AA/3435CC (n = 3). All subjects were considered to be eligible on the basis of medical history, physical examination, vital signs, electrocardiography, and routine laboratory tests, namely, hematology, biochemistry, urinalysis, hepatitis B and C, human immunodeficiency virus, and urine drug screening with the

**Table I.** Characteristics of healthy male volunteers grouped by genotypes in exon 21 (G2677T/A) and exon 26 (C3435T)

| *MDR1 genotype* | | *Subjects* | | | |
|---|---|---|---|---|---|
| *G2677T/A* | *C3435T* | *(No.)* | *Age (y)* | *Weight (kg)* | *Height (cm)* |
| GG | CC | 5 | 27 (21-39) | 70 (64-79) | 178 (170-183) |
| GT | CT | 6 | 21 (21-21) | 68 (59-75) | 173 (170-176) |
| TT | TT | 6 | 24 (21-26) | 67 (57-74) | 174 (170-178) |
| GA | CC | 7 | 22 (21-25) | 72 (61-82) | 174 (170-179) |
| TA | CT | 6 | 22 (21-22) | 71 (62-78) | 176 (169-183) |
| AA | CC | 3 | 24 (21-28) | 71 (69-80) | 176 (170-180) |

Values are given as mean and range.

REMEDi HS (Bio-Rad Laboratories, Hercules, Calif). Drugs, alcohol, grapefruit products, caffeine, and smoking were not permitted 1 week before the study or during the study. All subjects gave written informed consent before entry into the study. The study protocol was approved by the Institutional Review Board of Seoul National University Hospital (SNUH) (Seoul, Korea), and the study was conducted at the SNUH Clinical Trial Center. All procedures were performed in accord with the recommendations of the Declaration of Helsinki on biomedical research involving human subjects and with the International Conference on the Harmonization of the Technical Requirements for the Registration of Pharmaceuticals for Human Use–Good Clinical Practice (ICH-GCP) guidelines.

***Study protocol.*** On the day of the study, each subject received a 180-mg oral dose of fexofenadine (Allegra tablet; HanDok/Aventis Pharma, Seoul, Korea), after an overnight fast. Two hours after administration, the subjects were given 200 mL of water and were maintained in a fasting state for 4 hours after drug administration; they remained in a semirecumbent position after drug administration until lunch. An indwelling venous catheter was inserted into a forearm vein to collect 10 mL of blood immediately before and at 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 12, and 24 hours after dosing. Blood samples were collected in heparinized tubes and centrifuged for 10 minutes at 4°C and 1500*g* within 30 minutes of collection. Plasma was separated and stored at −70°C until required for analysis.

***Identification of MDR1 polymorphisms.*** The genotypes of *MDR1,* C1236T,[31] G2677T,[30] G2677A,[25] and C3435T,[16] were identified by polymerase chain reaction (PCR)–restriction fragment length polymorphism (RFLP) analysis as described previously. Whole blood (8 mL) was obtained from each subject, and genomic deoxyribonucleic acid (DNA) was extracted from peripheral lymphocytes by use of a QIAamp DNA Blood Mini Kit (QIAGEN, Hilden, Germany). The oligonucleotides used for PCR were commercially synthesized at Bioneer Co Ltd (Daejeon, Korea). For allele-specific PCR, in a total volume of 20 μL, approximately 100 ng of genomic DNA was added to a PCR mixture consisting of 0.25 to 0.5 μmol/L of each specific primer pair, 10× PCR buffer with 1.5-mmol/L magnesium chloride, 0.2-mmol/L each deoxyribonucleotide triphosphate, and 0.5 U of recombinant *Taq*DNA polymerase (Takara, Shiga, Japan). PCR was carried out in a GeneAmp PCR System 2400 (Perkin Elmer, Boston, Mass). After amplification, the DNA was digested with restriction enzyme at 37°C. Digested PCR products were analyzed by electrophoretic separation on an agarose gel stained with ethidium bromide.

***Analysis of fexofenadine.*** Fexofenadine concentrations in plasma were determined by HPLC by use of fluorescence detection with the modification of the previously used method.[35,36] In a glass tube, 1.0 mL of plasma, 1 μg of flurbiprofen (internal standard), and 150 μL of 5% perchloric acid were added and then extracted with 5-mL mixtures of hexane/1-butanol (8:1 [vol/vol]) by mixing in a vortex blender for 1 minute. After centrifugation (1500*g* at 4°C for 15 minutes), the organic layer was transferred into another tube and evaporated to dryness. The dried residue was then reconstituted in 100 μL of mobile phase, and 60 μL was injected onto the HPLC system. The mobile phase used for the fexofenadine assay consisted of a mixture of 30-mmol/L ammonium acetate/acetonitrile (7.2:2.8 [vol/vol]) and was adjusted to pH 6.5 with phosphoric acid. The HPLC system consisted of a model 303 solvent delivery system (Gilson, Villiers-le-Bil, France), a model 234 autosampling device (Gilson), and an FP-2020 Intelligent fluorescent detector (Jasco International, Tokyo, Japan). Chromatographic separation was performed by use of Capcell Pak C8 UG120 5-μm, 250 × 4.6–mm column (Shiseido, Tokyo, Japan) and a New-Guard RP-8 7-μm, 15 × 3.2–mm Brownlee column

**Table II.** Allelic and genotypic frequencies of *MDR1* gene in 232 Korean subjects

| *SNP* | *Allele* | *Effect* | *Frequency* and 95% CI (%)* | *Genotype* | *Frequency and 95% CI (%)* |
|---|---|---|---|---|---|
| Exon 12 C1236T | C | Synonymous | 38.1 (33.7-42.6) | C/C | 11.6 (7.5-15.8) |
| | T | | 61.9 (57.4-66.3) | C/T | 53.0 (46.6-59.4) |
| | | | | T/T | 35.3 (29.2-41.5) |
| Exon 21 G2677T/A | G | 893Ala | 43.8 (39.2-48.3) | G/G | 19.8 (14.7-25.0) |
| | T | 893Ser | 37.5 (33.1-41.9) | G/T | 34.5 (28.4-40.6) |
| | A | 893Thr | 18.8 (15.2-22.3) | T/T | 11.2 (7.1-15.3) |
| | | | | G/A | 13.4 (9.0-17.7) |
| | | | | T/A | 18.1 (13.1-23.1) |
| | | | | A/A | 3.0 (0.8-5.2) |
| Exon 26 C3435T | C | Synonymous | 63.1 (58.8-67.5) | C/C | 38.4 (32.1-44.6) |
| | T | | 36.9 (32.5-41.2) | C/T | 49.6 (43.1-56.0) |
| | | | | T/T | 12.1 (7.9-16.3) |

CI, Confidence interval.
*Allelic frequency was calculated by the law of Hardy-Weinberg.

(Perkin-Elmer, Norwalk, Conn). The mobile phase was delivered at a flow rate of 0.9 mL/min, and the column eluant was monitored fluorometrically at an excitation wavelength of 225 nm and an emission wavelength of 275 nm. Under these conditions, the fexofenadine and internal standard eluted at 20 minutes and 22 minutes, respectively. Data were analyzed with the Unipoint data analysis system (Gilson). The lower quantification limit was 10 ng/mL, and the coefficients of determination ($r^2$) of the calibration curves (range, 10-1,500 ng/mL) were all greater than 0.9999. The mean accuracy values of quality control samples (n = 6 for each) at 10- to 500-ng/mL concentrations ranged from 97.7% to 105.3%, and the mean within-day and between-day precisions (coefficients of variation) were less than 6.3% and 7.7%, respectively.

***Pharmacokinetic analysis.*** Pharmacokinetic parameters were calculated by noncompartmental analysis with WinNonlin Professional 4.0.1 (Pharsight Corp, Mountain View, Calif). Actual blood sampling times were used, and the maximum plasma concentration ($C_{max}$) and time to reach $C_{max}$ ($t_{max}$) presented are observed values. The area under the plasma concentration–time curve (AUC) of the orally administered fexofenadine was calculated by use of the linear trapezoidal rule. The terminal half-life ($t_{1/2}$) was derived by least-squares regression analysis as applied to the postpeak linear portion of the log plasma drug concentration versus time curve.

***Statistical analysis.*** Allelic and genotypic frequencies of the various SNPs were assessed for deviation from Hardy-Weinberg equilibrium by use of the Fisher exact test. The estimation of haplotypic frequencies for *MDR1* was performed by maximum likelihood estimation, based on the expectation-maximization algorithm, by use of Arlequin software (version 2.0; Arlequin, Geneva, Switzerland).

Data from 3 or more different genotype groups were compared by use of the Kruskal-Wallis test or ANOVA with the Tukey-Kramer multiple comparison test. Differences between the pharmacokinetic parameters of the 2 genotypic groups were determined by use of the Wilcoxon rank sum test. A $P$ value of $< .05$ was considered statistically significant. All statistical analyses were performed with SAS statistical software (version 8.0; SAS Institute Inc, Cary, NC).

## RESULTS

***MDR1 genotype distribution and haplotypes.*** The genotypic and allelic frequencies of exons 12 (C1236T), 21 (G2677T/A), and 26 (C3435T) in 232 healthy Korean subjects are shown in Table II. The observed genotypic frequency distribution was consistent with Hardy-Weinberg equilibrium (each $P > .05$). The allelic frequencies of the 1236T, 2677T, 2677A, and 3435T variants were 61.9%, 37.5%, 18.8%, and 36.9%, respectively. In exon 21, G2677T/A genotyping revealed GG in 19.8%, GT in 34.5%, TT in 11.2%, GA in 13.4%, TA in 18.1%, and AA in 3.0%. In exon 26, the frequencies of the CC, CT, and TT genotypes were 38.4%, 49.6%, and 12.1%, respectively.

Linkage disequilibrium for all pairs of exons 12, 21, and 26 were observed by use of the expectation-maximization algorithm ($P < .01$). Of 12 possible haplotypes, 10 were observed in the Korean subjects. Four major haplotypes, 1236C-2677A-3435C, C-G-C, T-G-C, and T-T-T, constituted 16.4%, 18.6%, 21.6%, and 32.2% of all haplotypes, respectively, adding up to a total of 88.8%.

***Fexofenadine disposition.*** Subjects were classified into 12 groups based on C1236T-G2677T/A-C3435T genotypes, and multiple comparisons of pharmacoki-

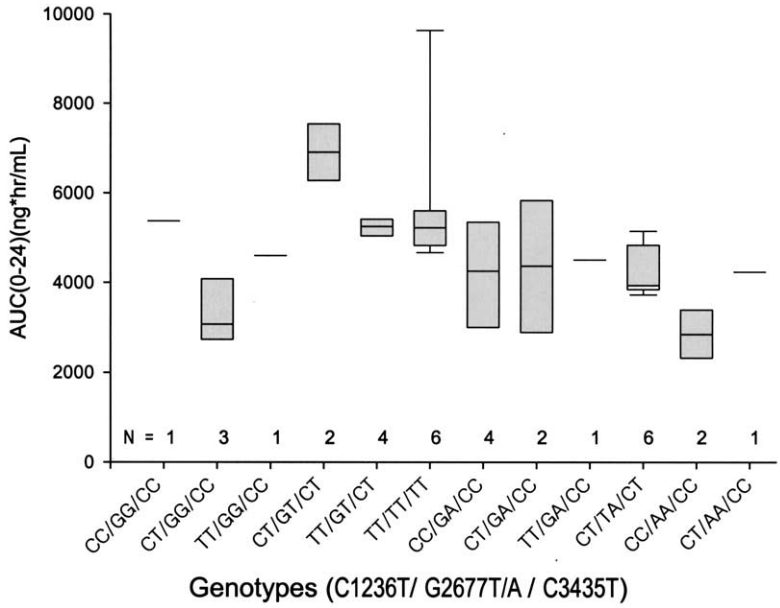


**Fig 1.** Values for area under concentration-time curve from time 0 to 24 hours [AUC(0-24)] for fexofenadine after 180-mg oral dose in relation to *MDR1* polymorphisms with combinations of C1236T in exon 12, G2677T/A in exon 21, and C3435T in exon 26. *Plots* depict the median and 10th, 25th, 75th, and 90th percentiles as *vertical boxes* with *error bars*. $P > .05$ by Kruskal-Wallis test among genotypes.

netic parameters of fexofenadine in these groups did not identify significant differences (Fig 1). With regard to the effect of the G2677T/A and C3435T genotypes on fexofenadine disposition, the following results were analyzed without considering the C1236T variation, because of the limited number of subjects.

Thirty-three male subjects revealed considerable fexofenadine pharmacokinetic variability. AUC from time 0 to 24 hours [AUC(0-24)] and $C_{max}$ ranged between 2109 and 10,075 ng·h/mL (4.8-fold) and between 359 and 1622 ng/mL (4.5-fold), respectively. The pharmacokinetic parameters of subjects grouped by the genotypes G2677T/A and C3435T are given in Table III. In the 2677AA/3435CC subject group (n = 3), AUC(0-24) values were found to be significantly lower than those of the other 5 groups (GG/CC, GT/CT, TT/TT, GA/CC, and TA/CT) ($P = .014$) (Table III).

With regard to subjects homozygous for the wild-type allele GG and homozygous for the variant allele TT and AA at position 2677, a significant difference was observed in AUC(0-24) values (GG versus TT versus AA, 4017 ± 1137 ng · h/mL versus 5934 ± 2064 ng · h/mL versus 3315 ± 958 ng · h/mL; $P = .018$). Homozygous variant subjects (AA) had low fexofenadine plasma concentrations versus homozygous variant subjects (TT), whereas homozygous wild-type subjects (GG) presented intermediate levels (Fig 2, *A*). Stratification of the data according to the number of variant alleles (wild type versus one variant allele versus 2 variant alleles) in exon 21 showed no significant pharmacokinetic parameter difference between the groups (Fig 2, *B*).

When stratified according to genotype at position 3435 in exon 26, homozygous 3435TT subjects had significantly higher AUC(0-24) (TT versus CC, 5934 ± 2064 ng · h/mL versus 3998 ± 1241 ng · h/mL; $P = .024$) and $C_{max}$ (TT versus CC, 958 ± 408 ng/mL versus 673 ± 242 ng/mL; $P = .040$) values than CC subjects (Table III, Fig 2, *C*). AUC(0-24) and $C_{max}$ values in 3435CT subjects were intermediate between those in the 2 homozygous carriers.

## DISCUSSION

Knowledge of the genotype-phenotype correlation and frequency distribution of functional SNPs may be a valuable tool for individualizing drug therapy. This information can also be useful for explaining interindividual and interethnic drug response or side effect differences. However, there is no consensus on the effects of the *MDR1* genotype in terms of its disposition

**Table III.** Pharmacokinetic characteristics after oral administration of 180 mg fexofenadine

| *Genotype* | *AUC(0-24) (ng · h/mL)* | $C_{max}$ *(ng/mL)* | $t_{max}$ *(h)* | $t_{1/2}$ *(h)* |
|---|---|---|---|---|
| G2677T/A in exon 21/C3435T in exon 26 | | | | |
| GG/CC (n = 5) | 4017 ± 1137 | 628 ± 189 | 2.4 ± 1.2 | 5.0 ± 0.9 |
| GT/CT (n = 6) | 5786 ± 976 | 927 ± 128 | 2.0 ± 1.1 | 4.2 ± 1.2 |
| TT/TT (n = 6) | 5934 ± 2064 | 958 ± 408 | 2.4 ± 2.1 | 4.5 ± 0.5 |
| GA/CC (n = 7) | 4277 ± 1450 | 782 ± 280 | 1.4 ± 0.6 | 4.7 ± 0.7 |
| TA/CT (n = 6) | 4239 ± 611 | 829 ± 255 | 2.3 ± 1.2 | 5.6 ± 1.7 |
| AA/CC (n = 3) | 3315 ± 958* | 494 ± 81 | 1.7 ± 0.3 | 4.8 ± 0.6 |
| *P* value (Kruskal-Wallis test among genotypes) | .014 | NS | NS | NS |
| C3435T in exon 26 | | | | |
| CC (n = 15) | 3998 ± 1241 | 673 ± 242 | 1.8 ± 0.9 | 4.8 ± 0.7 |
| CT (n = 12) | 5012 ± 1120 | 878 ± 199 | 2.2 ± 1.1 | 4.9 ± 1.6 |
| TT (n = 6) | 5934 ± 2064† | 958 ± 408† | 2.4 ± 2.1 | 4.5 ± 0.5 |
| *P* value (Kruskal-Wallis test among genotypes) | .024 | .040 | NS | NS |

Values are given as mean ± SD.

AUC(0-24), Area under concentration-time curve from time 0 to 24 hours; $C_{max}$, peak plasma concentration; $t_{max}$, time to reach peak plasma concentration; $t_{1/2}$, half-life; NS, not significant.

*$P < .05$ by ANOVA with Tukey-Kramer post hoc test in contrast to other groups.

†$P < .05$ by ANOVA with Tukey-Kramer post hoc test for CC versus TT.

to P-gp substrates, although many investigations have been performed.

After a single oral dose of fexofenadine, AUC(0-24) values were 17% lower in 2677AA/3435CC subjects and 47% higher in 2677TT/3435TT subjects versus 2677GG/3435CC subjects. Considering the limitations of the small number of subjects and multiple comparison tests, we paid attention to the tendency of lower plasma concentrations in the 2677A allele by use of descriptive statistics as well. This finding is supported by the significantly lower AUC(0-24) in the heterozygous 2677TA/3435CT group compared with that in the 2677GT/3435CT group ($P = .013$). In accord with our findings, Verstuyft et al[20] recently reported reduced digoxin plasma concentrations in 2 subjects with the 2677AA and 2677GA genotypes versus subjects with the 2677GG, 2677GT, and 2677TT genotypes. In addition, Siegmund et al[26] detected a trend of higher duodenal P-gp content and lower talinolol bioavailability in white subjects with the 2677GA genotype versus those with the 2677GT and 2677TT genotypes. However, in an in vitro study of 6 haplotypes containing the 2677A allele, Morita et al[37] did not find an association between haplotype combinations of G2677T/A and C3435T and the transport activities of *MDR1* proteins expressed in LLC-PK1 cells. Differences regarding the variant A allele in vitro and in vivo might result from an association with the regulation of *MDR1* expression. Accordingly, further studies are needed to determine whether individuals with the A allele variant show higher P-gp expression in the small intestine versus individuals with other genotypes.

Because the 2677A allele is a rare allelic variant in white subjects and black subjects, on many occasions, studies have not genotyped the 2677A variant in subjects[19,23] or have counted it together with 2677T even if genotyped.[18,25,26] In the latter case, the transport activities of the 2677A and 2677T alleles were noticeably different in the present study; therefore a detailed analysis of carriers of the 2 variant alleles at position 2677 (TT versus TA versus AA) showed a statistically significantly lower AUC(0-24) in subjects with the AA genotype versus those with the TT or TA genotype ($P = .020$) and a significantly lower AUC(0-24) in subjects with the TA genotype than in those with the TT genotype ($P = .045$). A similar lower AUC(0-24) trend was observed in carriers harboring one variant allele with the 2677GA genotype than in those with the GT genotype, without being statistically significant ($P = .074$). As a result, when such subjects were grouped according to the number of variant alleles at position 2677, 2 different variants were grouped as 1 group and the differences between the groups were set off as shown in Fig 2, *B*. In addition, in the former case, if studies have not genotyped the A allele mutation in subjects by PCR-RFLP, misinterpretation occurs, because GA and AA genotypes are included in the GG genotype and the TA genotype is included in the GT genotype. The frequencies of the GA, TA, and AA genotypes were not low, at 13.4%, 18.1%, and 3.0% in

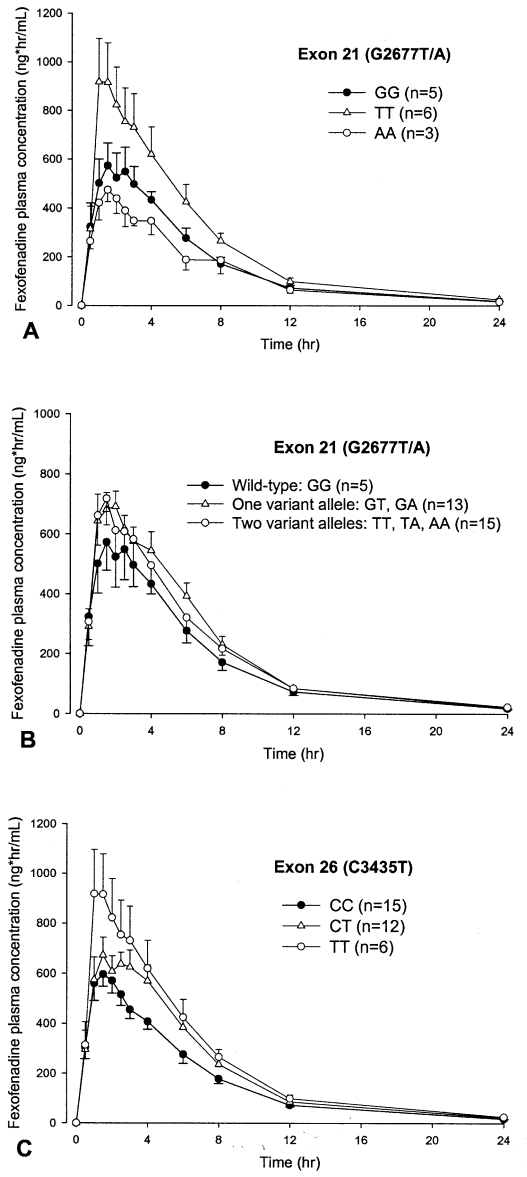


**Fig 2.** Plasma concentration–time curves after administration of 180 mg fexofenadine orally according to genotype. **A**, G2677T/A in exon 21. *Solid circles*, GG (n = 5); *open triangles*, TT (n = 6); *open circles*, AA (n = 3). $P = .018$ between all groups by Kruskal-Wallis test. **B**, G2677T/A in exon 21. *Solid circles*, wild type (GG) (n = 5); *open triangles*, 1 variant allele (GT, GA) (n = 13); *open circles,* 2 variant alleles (TT, TA, AA) (n = 15). $P = .290$ between all groups by Kruskal-Wallis test. **C**, C3435T in exon 26. *Solid circles*, CC (n = 15); *open triangles*, CT (n = 12); *open circles*, TT (n = 6). $P = .024$ between all groups by Kruskal-Wallis test. Values are given as mean ± SE.

Koreans, respectively, whereas they were 2.0%, 1.8%, and 0%, respectively, in white German subjects.[30] Therefore, despite interethnic differences with regard to the functional 2677A allele, it is thought that the study analysis methods that did not consider the 2677A variant may have influenced the inconsistent results of relationship studies between *MDR1* genotype and phenotype.

When the subjects were stratified by C3435T polymorphism, the TT group was found to have a significantly higher AUC and $C_{max}$ than the CC group. This might be related to the fact that all subjects with the 2677A allele at exon 21 also had the 3435C allele at exon 26 and all subjects with the 2677T allele at exon 21 had the 3435T allele at exon 26. Therefore, when identification of the *MDR1* polymorphism in subjects is solely based on the C3435T polymorphism, subjects who have different haplotypes are included in the study, which can cause discrepancies. A recent study by Kroetz et al[34] reported that there was great interethnic variability in the total number and frequency of *MDR1* haplotypes. A total of 25 haplotypes were found in white subjects compared with 55 haplotypes in black subjects, with only 20 haplotypes being present in both populations. Ethnic differences in the distribution of genotypes and allelic frequencies also suggest that haplotype analysis should be performed instead of detection of 1 or several SNPs. In a study in which subjects were enrolled on the basis of the *MDR1* genotype for G2677T/A and C3435T polymorphisms,[19,32] in agreement with our results, 2677GG/3435CC carriers showed a lower concentration of P-gp substrate than the 2677TT/3435TT carriers, which supports the above-mentioned facts. Similarly, Johne et al[18] reported a significantly lower AUC from time 0 to 4 hours for digoxin in haplotype 11 (2677G/3435C) carriers than in noncarriers. However, a nonsignificant trend of higher plasma fexofenadine concentrations in *MDR*1/*1* haplotype (2677GG/3435CC) compared with *MDR*2/*2* haplotype (2677TT/3435TT) was reported in another study.[23] Discrepancies in the results might be speculated to result from the potential gender difference and interethnic variability in *MDR1* haplotypes. Gender differences have been noted in the hepatic expression of *MDR1*, with women displaying only one third to one half of the hepatic P-gp level of men.[38] Although one study has provided limited information regarding the gender difference in intestinal expression of *MDR1,* it was performed without classification according to gender-matched genotype and its findings are not consistent with our results.[23] In addition, because the patterns of linkage disequilibrium in a different population group are different, some subjects

would be misclassified if the same tagging SNPs were used and interpreted in the same way.[39]

Fexofenadine, an active metabolite of terfenadine, is a histamine $H_1$-receptor antagonist with no sedative or other central nervous system effect. Human mass balance studies reported a recovery of approximately 80% and 11% of the carbon 14–labeled fexofenadine hydrochloride dose in feces and urine, respectively.[40] Fexofenadine was found to be a substrate for P-gp–mediated efflux transporter, as well as organic anion transporting polypeptides (OATPs),[41] which are involved in the uptake of various endogenous and xenobiotic compounds. Thus far, 9 members of the OATP family have been reported in humans.[42] Among these, OATP-A (SLCO1A2) is predominantly expressed in the brain and OATP-C (SLCO1B1) and OATP8 (SLCO1B3) exhibit specific liver expression, whereas OATP-B (SLCO2B1) has a broad tissue distribution in several tissues, including the small intestine, brain, liver, kidney, and placenta.[43] In the intestine both OATP(s) and P-gp transporters are located on the luminal surface of epithelial cells, which counteract intestinal absorption effects. However, fexofenadine has been shown to be a substrate of human OATP-A,[41,44] which is expressed selectively in brain. The intestinal transporters responsible for fexofenadine uptake have not yet been identified, although several reports suggest that P-gp–independent transporters are involved in the translocation of fexofenadine in the small intestine.[44,45] In addition, the oral exposure of fexofenadine was increased by coadministering P-gp inhibitors, such as erythromycin and verapamil, a finding that appeared despite the fact that these inhibitors also inhibited OATP.[46] Taken together, these facts suggest that the disposition of fexofenadine appears to be largely dependent on P-gp transporter activities, but further studies are required to determine the multiple transport mechanisms involved in the disposition of fexofenadine and relevant variations that might have interfered with *MDR1* function.

In conclusion, we have shown for the first time that the 2677A allele variant in exon 21 shows lower plasma concentrations of fexofenadine than the wild-type or T allele variant. Subjects with the 3435TT genotype showed higher plasma concentrations than subjects with the 3435CC genotype, which confirms the importance of analyzing *MDR1* haplotypes instead of analyzing 1 or several SNPs. The findings described here suggest a plausible explanation for the conflicting results of studies regarding the influence of *MDR1* polymorphisms on the disposition of P-gp substrates.

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