Citation: Clin Transl Sci (2017) 10, 93–101; doi:10.1111/cts.12451

ARTICLE

A Study on *CYP2C19* and *CYP2D6* Polymorphic Effects on Pharmacokinetics and Pharmacodynamics of Amitriptyline in Healthy Koreans

S Ryu[1], S Park[1], JH Lee[1], YR Kim[1], HS Na[1], HS Lim[2], HY Choi[2], IY Hwang[1], JG Lee[1], ZW Park[1], WY Oh[1], JM Kim[1] and SE Choi[1,*]

We performed a double-blinded, genotype-based stratification study to explore the pharmacokinetics and pharmacodynamics of amitriptyline according to *CYP2C19* and *CYP2D6* genotype in Korean subjects. Twenty-four healthy adults were grouped by genotype of *CYP2C19* and *CYP2D6*. After a single dose of 25 mg of amitriptyline, blood samples were collected and anticholinergic effects were measured. The extent of *N*-demethylation of amitriptyline significantly decreased in subjects carrying two nonfunctional alleles of *CYP2C19*. The extent of hydroxylation of amitriptyline or nortriptyline was significantly reduced in subjects carrying two *CYP2D6* decreased functional alleles compared with those with no or one decreased functional allele. The overall metabolic pathway of amitriptyline was more likely to be dominated by *CYP2C19* than *CYP2D6*. The gene variations of *CYP2C19* and *CYP2D6* did not change the pharmacodynamic effect. The findings of this study will provide useful information on individualized drug treatment with amitriptyline considering both *CYP2D6* and *CYP2C19* gene variations.
Clin Transl Sci (2017) **10**, 93–101; doi:10.1111/cts.12451; published online on 15 March 2017.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
✔ Two major genes involved in the metabolism of amitriptyline, *CYP2C19* and *CYP2D6*, are highly polymorphic and show interethnicity variability leading to different drug responses.

WHAT QUESTION DID THIS STUDY ADDRESS?
✔ What are the consequences of the combinations of *CYP2C19* and *CYP2D6* polymorphism on the metabolism of amitriptyline? How do these genotypes affect the pharmacokinetics and pharmacodynamics of amitriptyline?

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE?
✔ The extent of *CYP2D6*-mediated amitriptyline hydroxylation decreased significantly in the *CYP2D6*10/*10* group. The highest systemic exposure to amitriptyline was shown in the *CYP2C19* poor metabolizer group (**2/*2, *2/*3, or *3/*3*). The dominant metabolic pathway of amitriptyline seemed to be *CYP2C19*-mediated *N*-demethylation rather than *CYP2D6*-mediated hydroxylation.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE
✔ The findings of this study reveal that genetic polymorphism of *CYP2C19* and *CYP2D6* should be considered for individualized drug treatment with amitriptyline. Furthermore, *CYP2C19* poor metabolizer should be given careful consideration due to a potentially high exposure to amitriptyline, which may cause an increased risk of anticholinergic side effects.

Amitriptyline is a tricyclic antidepressant (TCA) with sedative effects and has been used widely worldwide since it was developed in the 1960s.[1] Its mechanism of action is not fully understood. However, it is believed to enhance the activity of neurotransmitters such as norepinephrine, serotonin, or dopamine by blocking their reuptake, but with weak influences on dopamine compared with serotonin.[2] Amitriptyline is mainly metabolized by *CYP2C19 via N*-demethylation into nortriptyline[3] and amitriptyline and nortriptyline are further transformed to *(E)*-10-OH-amitriptyline and *(E)*-10-OH-nortriptyline, respectively, *via* hydroxylation by *CYP2D6*[4] (**Figure 1**). Through hydroxylation, both the *(Z)* and *(E)* hydroxylated isomers and their enantiomers can be produced but the *CYP2D6*-mediated hydroxylation leads to the (–)-*(E)*-hydroxyl form as the major product.[5] Both amitriptyline and nortriptyline are active compounds and used in the treatment of depression, but amitriptyline, a tertiary amine, tends to have higher serotonergic effect than the secondary amine, nortriptyline, which gives greater noradrenergic activity.[6] Another minor pathway transforms amitriptyline into the less active metabolite, *(E)*-10-OH-amitriptyline by *CYP2D6*.[4] The hydroxylated metabolites are less active than their respective parent drugs with approximately half the potency[7] and are further glucuronidated forming water-soluble substrates that are excreted in the urine.[8] The pharmacogenetic effects on pharmacokinetics or pharmacodynamics of amitriptyline have not been widely studied compared with those of nortriptyline due to its complex

[1]Clinical Research Division, National Institute of Food and Drug Safety, Ministry of Food and Drug Safety, Republic of Korea; [2]Department of Clinical Pharmacology and Therapeutics, College of Medicine, University of Ulsan, Asan Medical Center, Republic of Korea. *Correspondence: SE Choi (choi77@korea.kr)
Received 11 October 2016; accepted 13 January 2017; published online on 15 March 2017. doi:10.1111/cts.12451

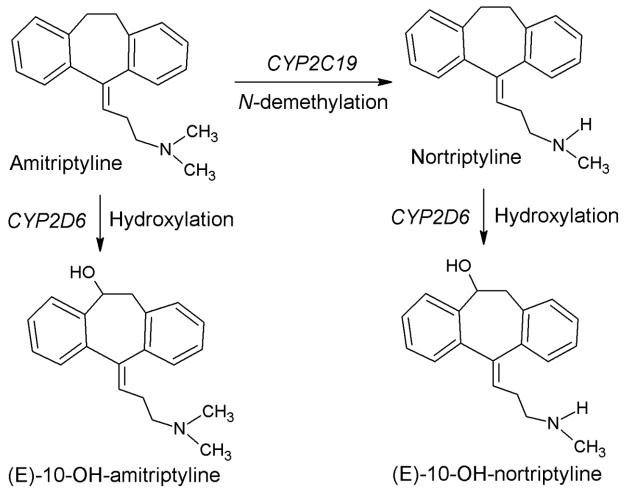

Figure 1 Metabolic pathway of amitriptyline.

pharmacology (e.g., amitriptyline is metabolized by various isozymes such as *CYP2C19* and *CYP2D6*, while nortriptyline is mainly metabolized by CYP2D6[9]).

Amitriptyline has several side effects, such as blurred vision, dry mouth, and drowsiness, and cardiac side effect. High plasma concentrations of amitriptyline are associated with high risk of anticholinergic side effects due to the binding of TCAs to cholinergic receptors,[10] and a higher risk of orthostatic hypotension and drowsiness than other TCAs due to its affinity for the α-adrenergic receptors.[11] The hydroxymetabolites of amitriptyline and nortriptyline have negligible anticholinergic side effects, as they have much less affinity for the muscarinic acetylcholine receptors than the parent compounds (e.g., 10-hydroxy nortriptyline has only one-eighteenth the affinity of nortriptyline[7]). Although amitriptyline has been replaced by serotonin-selective reuptake inhibitors due to its higher toxicity and lower tolerability, it is still used commonly used in migraine prevention, postherpetic pain, fibromyalgia, or diabetic peripheral neuropathies as well as the second line of treatment for depression.[8,12]

CYP2D6 and *CYP2C19* are cytochrome P450 subfamily enzymes and reported as highly polymorphic.[13–15] The frequencies of polymorphic variants of *CYP2D6* and *CYP2C19* occur to various extents among ethnic groups. For *CYP2D6*, the nonfunctional alleles represent 26% of the variability, primarily the **4* allele and less commonly the **5* allele in the European Caucasian population, while the median frequency of nonfunctional alleles in Asian is ~6.4%, primarily *CYP2D6*5*, while the **4* is extremely rare among this population.[16–19] Meanwhile, the decreased functional allele, *CYP2D6*10,* is more frequently found in Asians with a comparatively higher frequency of about 42% (mean value) vs. 2.8% (mean value) reported in Caucasians.[6] For *CYP2C19* in East Asians, the most distinct ethnicity-oriented difference was found in the frequencies of nonfunctional alleles **2* and **3* and increased functional allele **17*. The frequency of **2* in Asians was reported to be more than twice that occurring in Caucasians or Africans (29% of East Asians, 12% of Americans, 15% of Europeans and Africans) and the **3* allele was comparatively commonly found in Asians with a frequency of 8.9% but found at less than 0.1% in Europeans or Americans. Meanwhile, the frequency of **17* in Asians is only 2.7%, which is much lower than other ethnicities (18% of Americans, 21% of Europeans, and 16% of Africans).[6] Ethnic differences for *CYP2D6* and *CYP2C19* allele frequencies are highlighted in **Table 1**.

Table 1 Highlights of ethnic differences on allele frequencies of major variants of *CYP2D6* and *CYP2C19*

	Frequency (%)[a]	
Genotype	**Asian**	**Caucasian**
CYP2D6		
**4*	0.45	18[b]
**5*	5.8	2.8[b]
**10*	42	2.8[b]
CYP2C19		
**2*	29	15[c]
**3*	8.9	0.4[c]
**17*	2.7	21[c]

[a]Data are extracted from the study by Hicks *et al.* (ref. 6).
[b]Caucasian represents European and North American.
[c]Caucasian represents European only.

This substantial difference in the allele frequencies of the drug-metabolizing enzymes can lead to interindividual or interethnicity variability in drug response. Reduced efficacy or higher toxicity due to interindividual variability contributes to treatment failure or noncompliance. Approximately 62% of the depressive patients were reported to show no effective response to their treatment.[20–22]

The Clinical Pharmacogenetics Implementation Consortium (CPIC) developed a gene–drug guideline regarding *CYP2D6* and *CYP2C19* genotypes and dosing of TCAs. The guideline suggests a 25% dose reduction for an intermediate metabolizer who carries one reduced functional and one nonfunctional allele (such as *CYP2D6*4/*41, *5/*9, *4/*10*) and a 50% reduction or consideration of another treatment for a poor metabolizer who carries two nonfunctional alleles (such as **4/*4, *3/*4, *5/*5, *5/*6*). However, this guideline does not specifically provide a dosage for the *CYP2D6*10/*10* diplotype, which occurs at high frequency in East Asian populations. This study aimed to evaluate the effects of *CYP2D6* and *CYP2C19* polymorphism on the pharmacokinetics and pharmacodynamics of amitriptyline in Koreans, with a particular focus on *CYP2D6*10/*10* and *CYP2C19*2/*2, *2/*3, or *3/*3* alleles, which are relatively frequent in East Asian populations.

METHODS

Subjects

We recruited consecutive adult volunteers and performed genotyping and laboratory screening until at least six for each genotype group (as shown in **Supplementary Table 1**) were fulfilled. This study design was approved by the Institutional Review Boards (Asan Medical Center and National Institute of Food and Drug Safety) and was conducted in agreement with the principles of the Helsinki Declaration. The subjects were informed of the aims and design of the study and gave written consent. All subjects were healthy according to the

laboratory screening and medical examination. The subjects were screened for their genotypes before enrollment into this study and allocated into four groups according to their *CYP2D6* and *CYP2C19* diplotypes. The intention for Groups I, II, and III was to compare the pharmacokinetic and pharmacodynamic variability of the drug caused mainly by *CYP2D6* variants under similar circumstances to the *CYP2C19* genotypes. Meanwhile, the intention for Group IV was to compare the differences between *CYP2C19* variants and other groups.

The subjects were randomized for double-blinding in order to avoid bias either by subjects or investigators.

The inclusion criteria were 19–45 years old, healthy males with a body weight of not less than 60 kg. The exclusion criteria were current gastrointestinal disease state or having such history, drug or alcohol abuse, smoking or clinically relevant laboratory abnormalities, severe illness (e.g., cardiovascular disease, epilepsy, glaucoma), other relevant psychiatric disease (e.g., schizophrenia, bipolar disorder), or genetic disorder (e.g., galactose intolerance, Lapp lactase deficiency, glucose-galactose absorption disorder).

Study design

The subjects and investigators were blinded to the subjects' genotypes until the end of this study. The subjects were hospitalized 2 days prior to administration of the drug and a baseline checkup for blood pressure and pulse rate was obtained over a period of 24 h 1 day prior so that these effects could be compared before and after drug administration. After the baseline checkup was taken, the subjects took a single oral dose of 25 mg of amitriptyline (Etravil, Dongwha Pharmaceuticals, Republic of Korea) with 200 mL of water the following morning. The subjects fasted for 10 h and drinking water was restricted 1 h prior to administration. Standardized meals and water were served after 4 h and 10 h for lunch and dinner, respectively. Water intake other than at meal times was strictly controlled up to 24 h after drug administration because anticholinergic assessment of a dry mouth could be affected by water intake. The subjects remained in the hospital for 2 days after administration and returned to the hospital for up to 5 days after, for remaining blood sampling and measurements.

Blood samples were collected at 0 (predose), 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h after amitriptyline administration from all subjects. Blood samples (9 mL) were collected in a heparin-treated tube in an icebox and centrifuged (at 4°C, 1,800 g for 8 min) within 45 min of the sampling. Each 1.2 mL of plasma was separated into an Eppendorf tube and stored frozen at –20°C until analyzed. All subjects were given a 100-mm piece of white paper to record their self-rating intensity of dry mouth and drowsiness. Also, blood pressure in a supine and vertical position and pulse rate were measured at the designated timepoint for pharmacodynamic evaluation.

Genotyping

For the genotyping of both *CYP2D6* and *CYP2C19*, DNA was extracted from the blood samples of each subject using a QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The *CYP2D6* gene was amplified by polymerase chain reaction (PCR) and processed further by the ABI PRISM SNaPshot Multiplex Kit (Applied Biosystems, Foster City, CA) to detect the *CYP2D6*10* allele. Separately, gene deletion *(*5*) and the presence of duplication of the *CYP2D6 genes* were analyzed by PCR as described by previous studies using a specific primer set.[43,44] The assessment of *CYP2C19*2, *3* and **17* variants was performed using the same procedure as described for *CYP2D6*10* but with a different PCR primer and SNaPshot probe. The PCR primer and SNaPshot probe used in our study for the analysis of *CYP2D6* and *CYP2C19* is summarized in **Supplementary Table 2**.

Pharmacokinetic measurements

The plasma concentrations of amitriptyline, nortriptyline, *(E)*-10-OH-amitriptyline, *(E)*-10-OH-nortriptyline were measured using liquid chromatography coupled to a tandem mass spectrometry (LC-MS/MS) with a few modifications.[45–47] Doxepin was used as an internal standard. The chromatographic separation was performed on a BEH C18 column (100 $\times$ 2.1 mm, 1.7 μm, Waters, Milford, MA) in an ultraperformance LC system (UPLC; Waters) with a mobile phase consisting of 10 mM ammonium acetate (adjusted to pH 3.5 with formic acid) and acetonitrile. The retention times were 2.83, 2.78, 1.55, 1.99, and 2.50 min for amitriptyline, nortriptyline, *(E)*-10-OH-amitriptyline, *(E)*-10-OH-nortriptyline, and doxepine, respectively. The mass transitions of amitriptyline, nortriptyline, *(E)*-10-OH-amitriptyline, *(E)*-10-OH-nortriptyline, and doxepin were detected by multiple reaction monitoring (MRM) at m/z 278.4→91.0, m/z 264.2→91.0, m/z 294.3→276.3, m/z 280.2→231.1, and m/z 280.3→107.1, respectively. The lower limit of quantification (LLOQ) was 0.5 ng/mL for amitriptyline, nortriptyline, and *(E)*-10-OH-amitriptyline and 0.1 ng/mL for *(E)*-10-OH-nortriptyline.

Pharmacodynamic measurements

Anticholinergic effects (dry mouth and drowsiness ratings) were evaluated at 0 (predose), 1, 2, 3, 4, 6, 8, 12, 24, 48, 72, and 96 h and heart effects (supine and vertical blood pressure and pulse rate) were measured at 0 (predose), 1, 2, 4, 6, 8, 12, and 24 h after amitriptyline administration. A visual analog scale (VAS) rating assessment was performed as described previously.[40,48] The subjects rated their dry mouth sensation on a 100 mm VAS (0 = normally moist; 100 = extremely dry) at the designated timepoints. The subjects rated their subjective assessments of drowsiness using a 100 mm VAS (0 = extremely alert; 100 = extremely drowsy). Subjects who fell asleep during the designated timepoint were given the maximal score of 100 for that period.

Supine blood pressure and pulse rate were measured after the subjects had laid on the bed for more than 1 min and vertical blood pressure and pulse rate were obtained when the subject changed their position to standing. The subjects were instructed not to change their position abruptly to avoid any other side effects, such as dizziness. Both anticholinergic effects and blood pressure and pulse rate were measured 2 days prior to drug administration on a matched timepoint basis (0, 1, 2, 4, 6, 8, 12, and 24 h for –2 days) to obtain a baseline profile.

Table 2 Demographics and genotype characteristics of the subjects included in this study

Characteristics	Group I	Group II	Group III	Group IV
n	6	6	6	6
Age (yrs)	25.83 ± 3.43	24.17±4.96	25.33 ± 2.50	24.67 ± 4.84
Height (cm)	175.35 ± 4.71	175.55 ± 6.05	177.65 ± 6.76	172.33 ± 4.73
Body weight(kg)	72.16 ± 6.22	69.03 ± 7.65	76.98 ± 8.23	70.07 ± 5.62
BMI (kg/m^2)	23.47 ± 1.78	22.36 ± 1.60	24.38 ± 2.13	23.62 ± 2.06
Genotype				
CYP2D6 (n)	*1/*1 (6)	*1/*10 (4)	*10/*10 (6)	*1/*1 (1)
		*1/*10 (1)†		*1/*10 (2)
		*1/*5 (1)†		*1/*5 (1)
				*10/*10 (1)
				*5/*10 (1)
CYP2C19 (*n*)	*1/*1 (1)	*1/*1 (3)	*1/*1 (4)	*2/*2 (4)
	*1/*2 (4)	*1/*2 (3)	*1/*2 (1)	*2/*3 (1)
	*1/*3 (1)		*1/*3 (1)	*3/*3 (1)

Age, height, body weight, and BMI are expressed as mean ± standard deviation.
†Indicates gene duplication.

Safety evaluation
Safety assessments were performed for all the subjects who were given the drug during the study. The assessments included self-reporting or adverse event monitoring, medical checkups, vital signs, 12-lead EKG, and laboratory parameters. The serious side effects and the reported adverse events were evaluated based on the associations with the drug treatment.

Statistics
Pharmacokinetic and pharmacodynamic parameters were analyzed using descriptive statistical analysis and the results are presented as the mean ± SD. Analysis of variance (ANOVA) and Kruskal–Wallis tests were performed to compare the *P*-value of each group. For the statistical test, a *P*-value $\leq$ 0.05 was considered significant.

Pharmacokinetic evaluation
Pharmacokinetics was assessed for all of 24 subjects whose samples had been collected up to 72 h. Major pharmacokinetic parameters were obtained by noncompartmental analysis using Phoenix WinNonlin (v. 6.1, Pharsight, Mountain View, CA). AUC, C_{max}, T_{max}, $T_{1/2}$, CL, and Vz were determined for amitriptyline, nortriptyline, *(E)*-10-OH-amitriptyline, and *(E)*-10-OH-nortriptyline. The ratios of the AUC of the metabolites to the parent drug (nortriptyline to amitriptyline and hydroxyl metabolites to their respective parent drugs) were also calculated for each genotype group.

Pharmacodynamic evaluation
A pharmacodynamic evaluation was performed for all of 24 subjects whose data were obtained up to at least 8 h after drug treatment. VAS scores for dry mouth and sedation were reported as mean scores at each timepoint for each genotype group from day –1 to 96 h. Supine/vertical blood pressure and pulse rate were assessed from day –1 to 24 h. Pulse rate was reported as the difference at each timepoint from the baseline value on the day –1 so that the interday variability could be considered. The area under the pulse rate difference vs. time curve was calculated using Phoenix WinNonlin software to compare the data between groups.

RESULTS
Demographics
A total of 24 out of 53 healthy volunteers who underwent laboratory screening for genotyping were enrolled in this clinical trial. The demographics and genotype characteristics of the subjects are shown in **Table 2**. The mean age was 25 years and the mean height was 175.2 cm with a mean body weight of 72.2 kg.

Genotypes
The genotyping of 53 volunteers who provided informed consent was performed and the results are summarized in **Supplementary Table 1**. The *CYP2D6*5, *10* and *CYP2C19*2* and **3* alleles were assessed in our study, because they are frequently found in the Korean population. The *CYP2C19*17* was also assessed to allow us to exclude subjects carrying this increased function allele.

There were 12 subjects with two functional *CYP2D6* alleles *(*1/*1)*, 17 subjects with only one functional allele *(*1/*5, *1/*10)*, and 24 subjects who did not carry a normal function allele *(*5/*10, *10/*10)*. The gene duplication was found in two subjects in Group II. The plasma concentrations of analytes in those subjects were not different from subjects without duplication in this group. Also, there were 24 subjects with two functional *CYP2C19* alleles *(*1/*1)*, 23 subjects with only one functional allele *(*1/*2, *1/*3)*, and six subjects who did not carry a normal function allele *(*2/*2, *2/*3, *3/*3)*. There was no subject with *CYP2C19*17* allele identified.

Pharmacokinetics
Pharmacokinetics for amitriptyline, nortriptyline, *(E)*-10-OH-amitriptyline, and *(E)*-10-OH-nortriptyline were evaluated on the plasma samples from 24 subjects by noncompartmental analysis. Four compounds showed monoexponential decay and the half-lives of amitriptyline and nortriptyline ranged between 24.5–32.3 h and 31.5–67.8 h, respectively. The terminal volume of distribution of amitriptyline ranged between

Table 3 Pharmacokinetics of amitriptyline, nortriptyline, (*E*)-10-OH-amitriptyline, (*E*)-10-OH-nortriptyline after a single oral administration of amitriptyline, 25 mg

	Genotype group								
	I (*n* = 6) (*CYP2C19* W/W, W/V)		II (*n* = 6) (*CYP2C19* W/W, W/V)		III (*n* = 6) (*CYP2C19* W/W, W/V)		IV (*n* = 6) (*CYP2C19* V/V)		
Parameters	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	***P*-values***
Amitriptyline									
AUC_{last} (ng•h/mL)	265.60	27.4	250.21	19.0	288.96	24.9	477.54	34.9	0.015
C_{max} (ng/mL)	15.57	51.0	14.28	54.2	15.02	54.3	22.06	27.2	0.125
T_{max} (h)†	3.5	1.5∼6.0	4.0	1.5∼6.0	3.5	2.1∼6.0	3.0	1.5∼6.0	0.872
CL (L/h)	93.08	26.5	97.53	19.8	84.39	24.0	51.50	32.9	0.011
V_z (L)	3,958.44	45.1	3,356.18	23.3	3,488.93	23.4	2,381.43	39.3	0.132
$t_{1/2\beta}$ (h)	29.83	42.3	24.52	27.8	29.05	16.1	32.27	18.5	0.193
Nortriptyline									
AUC_{last} (ng•h/mL)	171.16	28.7	207.56	26.3	323.36	40.8	127.51	31.6	0.007
C_{max} (ng/mL)	3.92	32.0	4.74	43.4	5.43	40.5	2.13	14.9	0.005
T_{max} (h)†	8.98	3.0∼12.1	6.0	6.0∼12.0	12.0	2.1∼24.0	9.0	5.0∼24.0	0.527
$t_{1/2\beta}$ (h)	31.85	31.7	31.46	32.0	63.01	67.8	58.46	39.6	0.046
(E)-10-OH-amitriptyline									
AUC_{last} (ng•h/mL)	85.83	27.3	63.66	11.3	34.06	46.2	112.20	46.7	0.004
C_{max} (ng/mL)	6.17[a]	50.6	4.74[ab]	39.8	2.56[b]	57.1	5.4	67.5	0.082
T_{max} (h)†	3.5	1.5∼6.0	3.0	1.5∼4.0	3.0	2.1∼4.0	1.5	1.5∼8.0	0.705
$t_{1/2\beta}$ (h)	24.35	49.7	25.30	45.3	15.34	54.7	31.68	22.6	0.061
(E)-10-OH-nortriptyline									
AUC_{last} (ng•h/mL)	273.64	30.61	276.16	37.43	232.41	55.34	157.07	60.86	0.123
C_{max} (ng/mL)	6.91	32.93	6.17	40.88	3.83	54.07	2.76	71.02	0.021
T_{max} (h)†	6.98	5.0∼1.0	7.93	6.0∼12.0	11.01	7.9∼48.0	11.00	6.0∼23.9	0.062
$t_{1/2\beta}$ (h)	25.39	17.99	33.21	45.95	53.88	47.78	38.98	33.56	0.071

AUC_{last}, area under the curve to the last measurable concentration; C_{max}, maximum concentration; T_{max}, time to maximum concentration; CL, apparent clearance, calculated using dose and $AUC_{0\infty\infty}$; V_z, volume of distribution based on terminal elimination phase; $t_{1/2\beta}$, terminal elimination half-life.
Mean, arithmetic mean; CV, coefficient of variation = (standard deviation / mean) × 100 (%).
**P*-values were calculated by the Kruskal–Wallis test.
†Median and range.

2,381.4–3,958.4 L and clearance ranged between 51.5–97.5 L/h (**Table 3**).

In Group IV, which consisted of *CYP2C19* with two non-functional variants *(*2/*2, *2/*3 or *3/*3)*, the AUC_{last} of amitriptyline was higher than the other groups (477.54 ng*h/mL in Group IV vs. 250.21-288.96 ng*h/mL in other groups, $P = 0.015$) (**Table 3**). The C_{max} of amitriptyline was higher in Group IV but the difference was not significant (22.06 ng/mL in Group IV vs. 14.28-15.57 ng/mL in the other groups, $P = 0.125$). Also, the AUC_{last} of nortriptyline was significantly lower in Group IV ($P = 0.007$). The mean plasma concentration of amitriptyline was highest in Group IV and nortriptyline was lowest in the same group (**Figure 2**). In the absorption phase of both amitriptyline and nortriptyline, double peaks were observed in the plasma concentration vs. time curve and this was likely caused by enterohepatic recirculation as reported in a previous study.[23]

Distinct correlations between biotransformation from amitriptyline to nortriptyline and *CYP2C19* genotypes were demonstrated in the subanalysis of the *CYP2C19* allele variants. The ratio of AUC (nortriptyline/amitriptyline) was 1.17 in the two functional alleles group, while it was 0.74 in the one functional allele group, and 0.29 in the two nonfunctional alleles group. The correlation between hydroxylation of amitriptyline or nortriptyline into their hydroxyl metabolites and *CYP2D6* genotypes were assessed by subanalysis of the *CYP2D6* genotypes. The ratio of AUC ((*E*)-10-OH-amitriptyline/amitriptyline) was 0.27 in the two functional alleles group, while it was 0.20 in the one functional allele group, and 0.09 in the two decreased functional alleles group. The ratio of AUC ((*E*)-10-OH-nortriptyline/nortriptyline) was 1.90 in the two functional alleles group, while it was 1.38 in the one functional allele group, and 0.69 in the two nonfunctional alleles group (**Table 4**).

Pharmacodynamics

The VAS ratings for dry mouth were noticeably increased up to 6 h after drug treatment and rapidly recovered within 12 h to return to the baseline level (**Supplementary Figure 1**). There was no difference between groups. Drowsiness appeared to increase after drug treatment but it was not significant compared with baseline. Blood pressure in the supine or vertical position did not change regardless of drug treatment. However, there was a tendency toward increased pulse rate after drug treatment and this increase was more remarkable when subjects were standing. The area under the pulse rate difference vs. time curve did not show a significant difference between groups. The time to maximum effect was slightly different in anticholinergic effects (6 h) and orthostatic effects (7–10 h). In all, there were no significant differences between the genotype groups for dry mouth, drowsiness, blood pressure, or pulse rate.

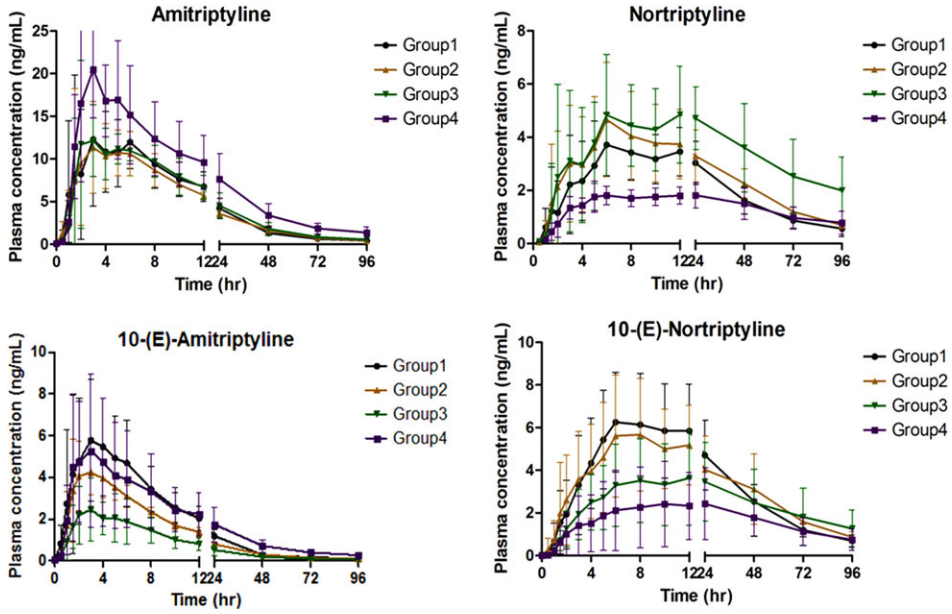

Figure 2 Plasma concentration–time curves for each genotype groups after a single oral administration of amitriptyline, 25 mg. Data are expressed as mean ± SD (each group, $n = 6$).

Table 4 AUC and C_{max} ratio stratified by *CYP2C19* and *CYP2D6* genotypes after a single oral administration of amitriptyline, 25 mg

***CYP2C19* genotypes**							
	***CYP2C19* genotypes**						
	W/W (*n* = 8)		**W/V (*n* = 10)**		**V/V (*n* = 6)**		
Parameters	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	***P*-values***
AUC_{last} ratio (nortriptyline /amitriptyline)	1.17[a]	34.35	0.74[a]	26.02	0.29[b]	17.56	0.0003
C_{max} ratio (nortriptyline /amitriptyline)	0.40[a]	32.26	0.31[a]	21.48	0.11[b]	17.66	0.001
***CYP2D6* genotypes**							
	***CYP2D6* genotypes**						
	W/W (*n* = 7)		**W/V (*n* = 9)**		**V/V (*n* = 8)**		
Parameters	**Mean**	**CV%**	**Mean**	**CV%**	**Mean**	**CV%**	***P*-values***
AUC_{last} ratio (10-OH-amitriptyline/amitriptyline)	0.27[a]	41.49	0.20[a]	20.35	0.09[b]	63.12	0.002
C_{max} ratio (10-OH-amitriptyline/amitriptyline)	0.34[a]	44.91	0.23[ab]	23.29	0.12[b]	75.93	0.007
AUC_{last} ratio (10-OH-nortriptyline/nortriptyline)	1.90[a]	43.22	1.42[ab]	23.95	0.70[b]	67.30	0.007
C_{max} ratio (10-OH-nortriptyline/nortriptyline)	2.05[a]	40.24	1.38[ab]	28.72	0.69[b]	62.48	0.004

[a,b]Values designated by the same letter are not significantly different by *post-hoc* analysis using Dunn's multiple comparison test.
V, *CYP2D6*10, CYP2D6*5*; W, the *CYP2D6* genotypes other than *CYP2D6*10* and *CYP2D6*5.*
V, *CYP2C19*2, CYP2C19*3;* W, the *CYP2C19* genotypes other *than CYP2C19*2* and *CYP2C19*3.*
AUC_{last}, area under the curve to the last measurable concentration.
AUC and C_{max} ratios after molar conversion of the concentration unit.
CV, coefficient of variation = (standard deviation / mean) × 100 (%).
**P*-values were calculated using Kruskal–Wallis test.

Safety

Among the enrolled 24 subjects who were given the drug, eight adverse events were reported from individuals. Only four adverse events (one for dry eyes, two for headaches, and one for head heaviness) were regarded as relevant to the test drug. All the reported events were mild and fully recovered. No serious adverse event was observed.

DISCUSSION

The genotyping results in our study showed that 18 out of 53 were *CYP2D6*10* heterozygotes and 20 were *CYP2D6*10* homozygotes. This gave an allele frequency of 56.3% *CYP2D6*10*, which was in a similar or a slightly higher range than previous reports of Korean populations.[24–29] Meanwhile, seven subjects had *CYP2D6* with the nonfunctional allele **5* heterozygote (three with **1/*5*, four with **10/*5*) and no **5* homozygote was found, which gave an allele frequency of 6.6%. This result is similar to previous studies.[17,27,29] The high frequency of *CYP2D6*10* in East Asians compared with Caucasians causes a lower metabolic clearance of *CYP2D6* substrate drugs.[17,30] The functional alleles of *CYP2D6* are dominant in European Caucasians, with a frequency of 71%, but only up to 50% of the functional alleles were reported

in Asians.[16] In our study, other decreased and nonfunctional *CYP2D6* variants other than *5 and *10 were not tested, which might remain as a limitation in this study with regard to genotype accuracy. *CYP2C19*2* and **3* were found at a frequency of 23.6% and 9.4%, respectively, which conforms with other studies.[6,31] There was no *CYP2C19*17* allele found in our study.

The results of this study showed that the hydroxylation of amitriptyline or nortriptyline into their 10-hydroxy metabolites was substantially affected by *CYP2D6* genotypes. The metabolic ratio of amitriptyline to *(E)*-10-OH-amitriptyline was significantly different between *CYP2D6* genotype subgroups (0.27, 0.20, and 0.09 in the groups with two, one, and no functional allele, respectively; $P = 0.002$). The same tendency in the metabolic ratio of nortriptyline was shown in the *CYP2D6* genotype subgroups (1.90 vs. 1.42 vs. 0.70, for groups with two, one, and no functional alleles, respectively; $P = 0.007$). Some other studies reported a similar association between *CYP2D6* allele variety and hydroxylation of nortriptyline.[32–34]

Some researchers suggest that *CYP2D6* enzyme activity can be graded as an activity score by summing the allele activity value for two haplotype alleles. Using this approach, the activity scores of *CYP2D6* diplotypes with **1/*1, *1/*10,* and **10/*10* have activity scores of 2.0, 1.5, and 1.0, respectively, and all of them are classified as extensive metabolizers.[6] In our study, the metabolic ratio of amitriptyline into (*E*)-10-OH-amitriptyline in *CYP2D6*10/*10* was significantly lower compared with those in *CYP2D6*1/*1 or *1/*10* (~0.09 vs. 0.27 or 0.20, respectively), while the difference between *CYP2D6*1/*1* and **1/*10* was not significant. This result demonstrated that the enzyme activity of *CYP2D6*10/*10* was different from those of *CYP2D6*1/*1* or **1/*10*. Therefore, we suggest that *CYP2D6*10/*10* subjects, who comprise about a quarter of the population (based on a **10* allele frequency of 50%), not be grouped with *CYP2D6*1/*1* and **1/*10* subjects. Some studies reported similar results on other drugs, showing the reduced activities of *CYP2D6**10/**10* compared with *CYP2D6*1/*10* or **1/*1*.[17,34–38]

Our result also clearly demonstrated that *N*-demethylation of amitriptyline into nortriptyline was affected by *CYP2C19* genotypes. The metabolic ratio of amitriptyline to nortriptyline was significantly different between *CYP2C19* allele groups (1.17, 0.74, and 0.29 in the groups with two, one, and no functional allele, respectively; $P = 0.0003$). But due to the small number of the subjects in each group, we could not draw definitive conclusions of pharmacokinetic differences between various allele combinations of *CYP2D6* and *CYP2C19*.

By considering the polymorphic effect of both *CYP2D6* and *CYP2C19* on the pharmacokinetics of amitriptyline, the extent of the systemic exposure of amitriptyline was shown to be highest in the *CYP2C19* PMs, regardless of *CYP2D6* allele variants, and there was no difference between *CYP2D6* allele groups. This result indicated that the *CYP2C19*-mediated demethylation pathway was more dominant than the hydroxylation pathway mediated by *CYP2D6* for overall metabolism of amitriptyline in the studied genotypes. Mellstrom *et al.* reported that the amitriptyline demethylation rate was 1,750–9,230 pmol per mg of protein, while the corresponding rate for the hydroxylation was 305–871 pmol per mg of protein at a high concentration of substrate (100 μM) from adult human livers.[4] In another *in vitro* study comparing cDNA expressed *CYP1A2, 3A4, 2C19, 2D6,* and *2E1*, *CYP2C19* showed the highest reaction capacity with regard to the metabolism of amitriptyline.[39]

Earlier studies reported that there were associations between dry mouth anticholinergic effects and plasma concentration of amitriptyline or nortriptyline.[40–42] In the study by Steimer *et al.*, the mean concentration of plasma amitriptyline was 92.65 ng/mL, where 150 mg of amitriptyline was administered in 50 depressive disorder patients and a lower risk of side effects was reported in patients with two functional *CYP2D6* alleles combined with only one functional *CYP2C19* allele.[42] Gupta *et al.* reported an association between plasma amitriptyline concentration and anticholinergic effects but not with plasma nortriptyline concentration, in a comparison of a single 75-mg osmotic controlled release tablet (OROS) or three 25-mg immediate-release (IR) tablets every 8 h or three doses of 25 mg IR tablet at night.[40] Kin *et al.* reported that patients administered 75 mg of nortriptyline for up to 7 weeks showed anticholinergic side effects associated with higher plasma nortriptyline levels, which ranged between 82–133 ng/Ml.[41] However, we found no pharmacodynamic differences between groups, although there were differences in plasma amitriptyline or nortriptyline levels between groups. This might be explained partially by the low drug dose we used in this study. The maximum concentration of plasma amitriptyline and nortriptyline ranged between 14.28–22.06 ng/mL and 1.13–3.92 ng/mL, respectively, which were very low compared with the above-mentioned studies that reported correlations between plasma concentrations and pharmacodynamic effects. It seemed that an oral single dose of 25 mg of amitriptyline might not have been sufficient to evaluate the anticholinergic side effects in our genotype stratified study. Nevertheless, it still remains a limitation of our study. VAS scores for dry mouth and drowsiness were maxima up to 6 h after the drug uptake and recovered to baseline within 12 h. Considering that the time to maximum concentration (T_{max}) of amitriptyline and nortriptyline was ~3.5 h and 8 h (as mean of all groups), respectively, it was plausible that the anticholinergic effect was caused more likely by amitriptyline than by nortriptyline. This implication was comparable with a previous study that reported the correlations of nortriptyline concentrations (not amitriptyline) with side effects (DOTES scale) in depressed patients.[42]

We found that the plasma concentration of *(E)*-10-OH-nortriptyline was higher than those of its parent drug in all groups except in the *CYP2D6* with no functional allele. This is in agreement with the results of other studies that reported similar or higher plasma concentrations of the hydroxyl metabolite of nortriptyline than the parent drug itself.[7,32,41] We surmise that this observation was caused by a higher fraction of hydroxyl metabolites into the central fluid rather than into the tissue compared with their parent drugs due to its increased hydrophilicity afforded by the hydroxyl moiety.

CONCLUSION

The effect of polymorphisms of two major metabolizing enzymes, *CYP2D6* and *CYP2C19*, on the pharmacokinetics and pharmacodynamics of amitriptyline was studied with a focus on the major variations in the Korean population. The pharmacokinetic parameters were dependent on the genotypes of both *CYP2C19* and *CYP2D6*. The extent of demethylation of amitriptyline into nortriptyline, which was mediated by *CYP2C19*, decreased significantly in *CYP2C19* PMs. The extent of hydroxylation of amitriptyline or nortriptyline into its hydroxyl metabolites, which were mediated by *CYP2D6*, was significantly decreased in subjects with a *CYP2D6*10/*10* genotype. The dominant metabolic pathway of amitriptyline seemed to be *CYP2C19*-mediated *N*-demethylation rather than *CYP2D6*-mediated hydroxylation, and the highest systemic exposure of amitriptyline was shown in the *CYP2C19* nonfunctional allele group regardless of *CYP2D6* variants. However, we could not relate the gene variations of *CYP2C19* and *CYP2D6* to the pharmacodynamic effect. Nevertheless, the findings of this study will provide useful information on individualized drug treatment with amitriptyline, considering both *CYP2D6* and *CYP2C19* gene variations.

Acknowledgments. We thank Mi Jo Kim for advice and suggestions on the research design. The views expressed in this article are the personal views of the authors and do not reflect the position of Korean Ministry of Food and Drug Safety.

Author Contributions. S.R. wrote the article; S.C., S.R., H.N., and J.K. deigned the research; Y.K., J.L., I.H., J.L., Z.P., H.L., and H.C. performed the research; S.R., S.P., W.Y.,H.L., and H.C. analyzed the data.

Conflict of Interest. This research was supported by a grant (13181MFDS704) from the Ministry of Food and Drug Safety, Republic of Korea.

1. Sneader, W. Hormone Analogues (Drug Discovery: John Wiley & Sons, 2006), p. 188–225.
2. Tatsumi, M., Groshan, K., Blakely, R.D. & Richelson, E. Pharmacological profile of antidepressants and related compounds at human monoamine transporters. *Eur. J. Pharmacol.* **340**, 249–258 (1997).
3. Venkatakrishnan, K., Greenblatt, D.J., von Moltke, L.L., Schmider, J., Harmatz, J.S. & Shader, R.I. Five distinct human cytochromes mediate amitriptyline N-demethylation in vitro: dominance of CYP 2C19 and 3A4. *J. Clin. Pharmacol.* **38**, 112–121 (1998).
4. Mellstrom, B. & von Bahr, C. Demethylation and hydroxylation of amitriptyline, nortriptyline, and 10-hydroxyamitriptyline in human liver microsomes. *Drug Metab. Dispos. Biol. Fate Chem.* **9**, 565–568 (1981).
5. Breyer-Pfaff, U. The metabolic fate of amitriptyline, nortriptyline and amitriptylinoxide in man. *Drug Metab. Rev.* **36**, 723–746 (2004).
6. Hicks, J.K. *et al.* Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6 and CYP2C19 genotypes and dosing of tricyclic antidepressants. *Clin. Pharmacol. Ther.* **93**, 402–408 (2013).
7. Nordin, C. & Bertilsson, L. Active hydroxymetabolites of antidepressants. Emphasis on E-10-hydroxy-nortriptyline. *Clin. Pharmacokinet.* **28**, 26–40 (1995).
8. Rudorfer, M.V. & Potter, W.Z. Metabolism of tricyclic antidepressants. *Cell. Mol. Neurobiol.* **19**, 373–409 (1999).
9. Bertilsson, L. Metabolism of antidepressant and neuroleptic drugs by cytochrome p450s: clinical and interethnic aspects. *Clin. Pharmacol. Ther.* **82**, 606–609 (2007).
10. Hiemke, C. *et al.* AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry* **44**, 195–235 (2011).
11. Preskorn, S.H., Dorey, R.C. & Jerkovich, G.S. Therapeutic drug monitoring of tricyclic antidepressants. *Clin. Chem.* **34**, 822–828 (1988).
12. Drug Facts and Comparisons, 2010 Edition. 2010 ed. St. Louis MO, Wolters Kluwer Health; 2010.
13. Ingelman-Sundberg, M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends Pharmacol. Sci.* **25**, 193–200 (2004).
14. Hsu, H.L., Woad, K.J., Woodfield, D.G., Helsby, N.A. A high incidence of polymorphic CYP2C19 variants in archival blood samples from Papua New Guinea. *Human genomics.* **3**, 17–23 (2008).
15. Kirchheiner, J. CYP2D6 phenotype prediction from genotype: which system is the best? *Clin. Pharmacol. Ther.* **83**, 225–227 (2008).
16. Bradford, L.D. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics* **3**, 229–243 (2002).
17. Johansson, I., Oscarson, M., Yue, Q.Y., Bertilsson, L., Sjoqvist, F. & Ingelman-Sundberg, M. Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. *Mol. Pharmacol.* **46**, 452–459 (1994).
18. LLerena, A., Naranjo, M.E., Rodrigues-Soares, F., Penas, L.E.M., Farinas, H. & Tarazona-Santos, E. Interethnic variability of CYP2D6 alleles and of predicted and measured metabolic phenotypes across world populations. *Expert Opin. Drug Metab. Toxicol.* **10**, 1569–1583 (2014).
19. Gaedigk, A., Sangkuhl, K., Whirl-Carrillo, M., Klein, T. & Leeder, J.S. Prediction of CYP2D6 phenotype from genotype across world populations. *Genet Med.* **19**, 69–76 (2017).
20. Spear, B.B., Heath-Chiozzi, M. & Huff, J. Clinical application of pharmacogenetics. *Trends Mol. Med.* **7**, 201–204 (2001).
21. Sheehan, D.V., Keene, M.S., Eaddy, M., Krulewicz, S., Kraus, J.E. & Carpenter, D.J. Differences in medication adherence and healthcare resource utilization patterns: older vs. newer antidepressant agents in patients with depression and/or anxiety disorders. *CNS Drugs* **22**, 963–973 (2008).
22. Etchepare, F., Pambrun, E., Verdoux, H. & Tournier, M. Trends in patterns of antidepressant use in older general population between 2006 and 2012 following publication of practice guidelines. *Int. J. Geriatr. Psychiatry* (2016) In press.
23. Gard, H., Knapp, D., Walle, T., Gaffney, T. & Hanenson, I. Qualitative and quantitative studies on the disposition of amitriptyline and other tricyclic antidepressant drugs in man as it relates to the management of the overdosed patient. *Clin. Toxicol.* **6**, 571–584 (1973).
24. Kim, M.K. *et al.* Effect of the CYP2D6 genotype on the pharmacokinetics of tropisetron in healthy Korean subjects. *Eur. J. Clin. Pharmacol.* **59**, 111–116 (2003).
25. Roh, H.K., Kim, C.E., Chung, W.G., Park, C.S., Svensson, J.O. & Bertilsson, L. Risperidone metabolism in relation to CYP2D6*10 allele in Korean schizophrenic patients. *Eur. J. Clin. Pharmacol.* **57**, 671–675 (2001).
26. Yoon, Y.R. *et al.* Relationship of paroxetine disposition to metoprolol metabolic ratio and CYP2D6*10 genotype of Korean subjects. *Clin. Pharmacol. Ther.* **67**, 567–576 (2000).
27. Lee, S.Y., Sohn, K.M., Ryu, J.Y., Yoon, Y.R., Shin, J.G. & Kim, J.W. Sequence-based CYP2D6 genotyping in the Korean population. *Ther. Drug Monitor.* **28**, 382–387 (2006).
28. Jin, S.K. *et al.* Influence of CYP2D6*10 on the pharmacokinetics of metoprolol in healthy Korean volunteers. *J. Clin. Pharm. Ther.* **33**, 567–573 (2008).
29. Lee, S.J. *et al.* Discovery of novel functional variants and extensive evaluation of CYP2D6 genetic polymorphisms in Koreans. *Drug Metab. Dispos. Biol. Fate Chem.* **37**, 1464–1470 (2009).
30. Roh, H.K., Dahl, M.L., Johansson, I., Ingelman-Sundberg, M., Cha, Y.N. & Bertilsson, L. Debrisoquine and S-mephenytoin hydroxylation phenotypes and genotypes in a Korean population. *Pharmacogenetics* **6**, 441–447 (1996).
31. Roh, H.K., Dahl, M.L., Tybring, G., Yamada, H., Cha, Y.N. & Bertilsson, L. CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. *Pharmacogenetics* **6**, 547–551 (1996).
32. Dalen, P., Dahl, M.L., Bernal Ruiz, M.L., Nordin, J. & Bertilsson, L. 10-Hydroxylation of nortriptyline in white persons with 0, 1, 2, 3, and 13 functional CYP2D6 genes. *Clin. Pharmacol. Ther.* **63**, 444–452 (1998).
33. Kirchheiner, J. *et al.* CYP2D6 and CYP2C19 genotype-based dose recommendations for antidepressants: a first step towards subpopulation-specific dosages. *Acta Psychiatr. Scand.* **104**, 173–192 (2001).
34. Yue, Q.Y. *et al.* Pharmacokinetics of nortriptyline and its 10-hydroxy metabolite in Chinese subjects of different CYP2D6 genotypes. *Clin. Pharmacol. Ther.* **64**, 384–390 (1998).
35. Senda, C. *et al.* Influence of the CYP2D6*10 allele on the metabolism of mexiletine by human liver microsomes. *Br. J. Clin. Pharmacol.* **52**, 100–103 (2001).
36. Sakuyama, K. *et al.* Functional characterization of 17 CYP2D6 allelic variants (CYP2D6.2, 10, 14A-B, 18, 27, 36, 39, 47–51, 53-55, and 57). *Drug Metab. Dispos. Biol. Fate Chem.* **36**, 2460–2467 (2008).
37. Huang, J., Chuang, S.K., Cheng, C.L. & Lai, M.L. Pharmacokinetics of metoprolol enantiomers in Chinese subjects of major CYP2D6 genotypes. *Clin. Pharmacol. Ther.* **65**, 402–407 (1999).
38. Fukuda, T. *et al.* The decreased in vivo clearance of CYP2D6 substrates by CYP2D6*10 might be caused not only by the low-expression but also by low affinity of CYP2D6. *Arch. Biochem. Biophys.* **380**, 303–308 (2000).
39. Olesen, O.V. & Linnet, K. Metabolism of the tricyclic antidepressant amitriptyline by cDNA-expressed human cytochrome P450 enzymes. *Pharmacology* **55**, 235–243 (1997).
40. Gupta, S.K., Shah, J.C. & Hwang, S.S. Pharmacokinetic and pharmacodynamic characterization of OROS and immediate-release amitriptyline. *Br. J. Clin. Pharmacol.* **48**, 71–78 (1999).

41. Kin, N.M. *et al.* Clinical relevance of serum nortriptyline and 10-hydroxy-nortriptyline measurements in the depressed elderly: a multicenter pharmacokinetic and pharmacodynamic study. *Neuropsychopharmacology* **15**, 1–6 (1996).
42. Steimer, W. *et al.* Amitriptyline or not, that is the question: pharmacogenetic testing of CYP2D6 and CYP2C19 identifies patients with low or high risk for side effects in amitriptyline therapy. *Clin. Chem.* **51**, 376–385 (2005).
43. Lovlie, R., Daly, A.K., Molven, A., Idle, J.R. & Steen, V.M. Ultrarapid metabolizers of debrisoquine: characterization and PCR-based detection of alleles with duplication of the CYP2D6 gene. *FEBS Lett.* **392**, 30–34 (1996).
44. Ishiguro, A., Kubota, T., Sasaki, H. & Iga, T. A long PCR assay to distinguish CYP2D6*5 and a novel CYP2D6 mutant allele associated with an 11-kb EcoRI haplotype. *Clin. Chim. Acta* **347**, 217–221 (2004).
45. Shen, Y., Zhu, R.H., Li, H.D., Liu, Y.W. & Xu, P. Validated LC-MS (ESI) assay for the simultaneous determination of amitriptyline and its metabolite nortriptyline in rat plasma: application to a pharmacokinetic comparison. *J. Pharm. Biomed. Anal.* **53**, 735–739 (2010).
46. Bhatt, M. & Shah, S. Development and validation of amitriptyline and its metabolite in human plasma by ultra performance liquid chromatography-tandem mass spectrometry and its application to a bioequivalence study. *Biomed. Chromatogr. BMC* **24**, 1247–1254 (2010).
47. Chae, J., In, B., An, J., Kim, E. & Ki, K. Quantitative Determination of Amitriptyline and Its Metabolite in Rat Plasma by Liquid Chromatography-tandem Mass Spectrometry. *Bull. Korean Chem. Soc.* **33**, 2163–2167 (2012).
48. Dalén, P., Dahl, M.L., Roh, H.K., Tybring, G., Eichelbaum, M., Wilkinson, G.R. *et al.* Disposition of debrisoquine and nortriptyline in Korean subjects in relation to CYP2D6 genotypes, and comparison with Caucasians. *Br. J. Clin. Pharmacol.* **55**, 630–634 (2003).

Supplementary information accompanies this paper on the *Clinical and Translational Science* website. (http://onlinelibrary.wiley.com/journal/10.1111/(ISSN)1752-8062)