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Effects of *CYP3A5* and *UGT2B7* variants on steady-state carbamazepine concentrations in Chinese epileptic patients

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Abstract

Carbamazepine (CBZ) is a widely used antiepileptic drug with large interindividual variability in serum concentrations. Previous studies found that CYP3A5*3 (rs776746), UGT2B7*2 (802C>T), and UGT2B7*3 (211G>T) variants could change the enzymes' activity, which may influence drug concentrations. Our study aims to investigate whether these variants affect steady-state CBZ concentrations in Chinese epileptic patients. In our study, 62 epileptic patients who received CBZ as monotherapy were monitored for steady-state CBZ concentrations. We used polymerase chain reaction (PCR)-based Sanger sequencing to assess the variants CYP3A5*3, UGT2B7*2, and UGT2B7*3. The results showed a positive correlation between dose and CBZ serum concentration in all patients and in patients with 3 different variants (all $P < .05$). After CBZ concentrations were normalized by the dose administered, negative correlations between dose-normalized CBZ concentrations and CBZ doses were observed in all patients, and in CYP3A5*3 and UGT2B7*3 patients (all $P < .05$), but not in UGT2B7*2 patients ($P = .1080$). UGT2B7*2 patients exhibited lower dose-normalized CBZ concentrations and larger CBZ dose requirements than UGT2B7*1/*1 patients ($P = .0139$, $P = .032$, respectively). There were no differences between UGT2B7*3, UGT2B7*1/*1 and CYP3A5*3, and CYP3A5*1/*1 patients with regard to steady-state CBZ concentration, dose-normalized concentration, required CBZ dose, and body weight-normalized dose (all $P > .05$). Moreover, a significant difference in body weight-normalized CBZ dose between UGT2B7 GC and TT haplotype patients was observed ($P = .0154$). In conclusion, our study found that the UGT2B7*2 variant, but not the CYP3A5*3 or UGT2B7*3 variant, could affect steady-state CBZ concentrations in epileptic patients.

Abbreviations: BMI = body mass index, CBZ = carbamazepine, PCR = polymerase chain reaction, SNPs = single-nucleotide polymorphisms.

Keywords: cytochrome P450, drug metabolism, epilepsy, genetic polymorphism

1. Introduction

Epilepsy is a neurological disorder characterized by recurrent unprovoked seizures.[1,2] Its clinical manifestation is uncontrolled electrical activity produced by a group of abnormal neurons.[1] Carbamazepine (CBZ) is an older drug, but is still widely used as an antiepileptic drug for the treatment of seizures.[3]

Carbamazepine is almost completely metabolized in the liver, and only approximately 5% of the compound is excreted from the body in its original form.[4] The metabolic processes for CBZ are complex, and over 30 metabolites have been identified in human and animal models.[5–7] CBZ is mainly metabolized by CYP3A4, CYP3A5, and CYP2C8, with CYP3A4 and CYP3A5 predominating.[8] CBZ is also partly metabolized to 2-hydroxy-CBZ and 3-hydroxy-CBZ by CYP3A4 and CYP2B6,[9] and the pharmacologically active metabolite is CBZ-10, 11-epoxide.[8] Because the CYP3A5 contribution to the total CYP3A content is relatively small, the role of CYP3A5 is often ignored[10]; however, recent studies have focused on its functionally and quantitatively vital role in the metabolism of CYP3A substrates.[11,12]

Carbamazepine N-glucuronide and glucuronides of hydroxylated metabolites are produced via the urinary metabolic pathway; therefore, glucuronidation is also important in CBZ metabolism.[13] One study found that glucuronide metabolites are generated from 13 hydroxylated metabolites of CBZ, suggesting an important role for glucuronide not only with regard to CBZ itself but also to its metabolites.[6] Moreover, a sensitive liquid chromatography/mass spectrometry assay study found that CBZ is specifically glucuronidated by the human UGT2B7 enzyme.[13]

Genetic variants can influence the substrate specificity and specific activity of enzymes, binding efficiencies of transcription factors and membrane proteins, and other features and functions. Relevant *CYP3A5* gene polymorphisms have been found, and the

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most common of which is *CYP3A5*3* (rs776746), leading to reduced activity of the CYP3A5 enzyme.[11] Nonetheless, large amounts of CYP3A5 enzyme are expressed in individuals with at least 1 *CYP3A5*1* allele.[11,14] Two nonsynonymous variants, *UGT2B7*2* (802C>T, rs7439366) and *UGT2B7*3* (211G>T, rs12233719), have been identified thus far.[15,16] It has been reported that *UGT2B7*2* decreases,[15,17] increases,[18,19] or does not affect[20,21] activity of the UGT2B7 enzyme. Similarly, *UGT2B7*3* was found to reduce carvedilol glucuronidation activity[22] or was conversely associated with higher enzyme activity.[23] Although more than 30 single-nucleotide polymorphisms (SNPs) of *CYP3A4* have been identified, due to the very low frequencies of different *CYP3A4* alleles,[24] polymorphisms in this gene were not examined in our study. Instead, *CYP3A5*3, UGT2B7*2*, and *UGT2B7*3*—significant variants that can influence enzyme activity—were selected for investigation in our study and were confirmed by in vivo and in vitro analyses.[5–7]

Here, we investigate whether *CYP3A5*3* and *UGT2B7*2, UGT2B7*3* can influence steady-state CBZ concentrations in Chinese epileptic patients. This study is the first to examine the effects of *CYP3A5*3* and *UGT2B7*2, UGT2B7*3* alone, and *UGT2B7*2, UGT2B7*3* haplotypes on steady-state CBZ concentrations in these patients.

2. Methods

2.1. Patients and study design

Patients were enrolled from Xiangya Hospital and The Second Xiangya Hospital. The ethics committee of Xiangya Medical School of Central South University approved the study. Patients over 18 years old and the parents of patients under 18 provided written informed consent, in compliance with the code of ethics of the World Medical Association (Declaration of Helsinki). The Chinese Clinical Trail Register approved the study (registration number: ChiCTR-RO-12002853). Patients were eligible for enrollment if they were treated with CBZ monotherapy and the course of treatment was more than 3 months; CBZ concentration data from therapeutic drug monitoring were available; and clinical data such as seizure types, medication history, CBZ doses, and time were available. Exclusion criteria were as follows: using drugs that could influence metabolic P450 enzymes and the UGT2B7 enzyme; having a history of alcohol and drug abuse and suffering serious side effects of drugs, resulting in poor compliance; and having progressive and degenerative neurological diseases or systemic diseases, including liver and kidney dysfunction. Standardized questionnaires were used to collect clinical and demographic data.

2.2. Blood sampling and drug assays

To ensure the collection of steady-state CBZ plasma concentrations, no dose adjustments were made within 1 month before the collection, and plasma samples were drawn early in the morning before the administration of the morning dose. Blood samples for genotyping were collected by venipuncture. High-performance liquid chromatography–UV (SHIMADZU Inc., Japan) in the therapeutic drug monitoring department of Xiangya Hospital was used to detect CBZ concentrations according to the methods of a previous publication.[25]

2.3. Genotypic analysis

DNA was extracted using the phenol-chloroform method. PCR-based direct sequencing was used to assess *CYP3A5*3*, *UGT2B7*2*, and *UGT2B7*3* variants. *CYP3A5*1* and *CYP3A5*3* were defined as c.219–237 A allele and c.219–237 G allele, respectively. *UGT2B7*1* (268H) and *UGT2B7*2* were defined as c.802 C allele and c.802 T allele, respectively. *UGT2B7*1* (71A) and *UGT2B7*3* were defined as c.211 G allele and c.211 T allele, respectively. *UGT2B7*1/*1* (268H) and *UGT2B7*1/*1* (71A) were defined as c.802 CC genotype and c.211 GG genotype, respectively. The primers designed and annealing temperatures for PCR are provided in Table 1. The PCR reaction contained 2 μL 10× PCR Buffer (Takara, Dalian, China), 1 μL 2.5 mM dNTPs (Takara, Dalian, China), 0.4 μL each 10 μM forward and reverse primers (Boshang Company, Beijing, China), 0.2 μL 5 u/μL rTaq enzyme (Takara, Dalian, China), 1 μL gDNA, and ddH_2O to 20 μL. The amplification conditions were as follows: initial denaturation for 5 minutes at 98°C, followed by 36 cycles of 98°C for 30 seconds, the annealing temperature (specific temperature shown in Table 1) for 30 seconds, and 72°C for 30 seconds, and a 5-minute extension at 72°C. The PCR products were detected using an ABI 3730xl (Applied Biosystems, California, USA).

2.4. Statistical analysis

All statistical analyses were carried out using SPSS software (version 11.0 for Windows; SPSS, Chicago, IL). Measurement data following a normal distribution were expressed as the mean ± standard deviation ($x \pm s$). The unpaired t test or Wilcoxon rank-sum test was used for comparing clinical and demographic data. Hardy–Weinberg equilibrium was analyzed with the chi-square test. The dose-normalized CBZ concentration was calculated using the CBZ concentration (μg/mL) against the CBZ dose (mg). Body weight-normalized doses were calculated using the CBZ dose (mg) against body weight (kg). Stepwise multiple linear regression analysis was applied to evaluate relationships between multiple factors (CBZ doses, *CYP3A5*3*, *UGT2B7*2*, and *UGT2B7*3* variants, body weight, and body mass index [BMI])) and dose-normalized CBZ concentrations. Haplotypes and their frequencies were estimated using haploview software (http://www.broad.mit.edu/personal/jcbarret/haplo/). A P value $<.05$ was considered statistically significant.

3. Results

3.1. Demographic characteristics of patients and genotyping results

In all, 78 patients were eligible for the study; 16 patients were excluded for not meeting the inclusion criteria (n = 5), declining to participate (n = 3), or for other reasons related to the

Table 1

PCR-based Sanger sequencing for *CYP3A5* and *UGT2B7* polymorphisms.

Gene	SNPs	Primer sequence	Annealing temperature
*CYP3A5*3*	rs 776746 (c.219–237A>G)	5'-CTTTAAAGAGCTCTTTTGTCTCTCA-3' 5'- CCAGGAAGCCAGACTTTGAT -3'	57°C
*UGT2B7*2*	rs7439366 (c.802C>T)	5'-TGCCTACATTATTCTAACC-3' 5'-TCTCTGAAAATTCTGCACT-3'	59°C
*UGT2B7*3*	rs12233719 (c.211G>T)	5'-TGCTTTAGCTCTGGGAATTGT-3' 5'-TGCATGAAATTCTCCAAC-3'	58°C

PCR = polymerase chain reaction, SNP = single-nucleotide polymorphism.

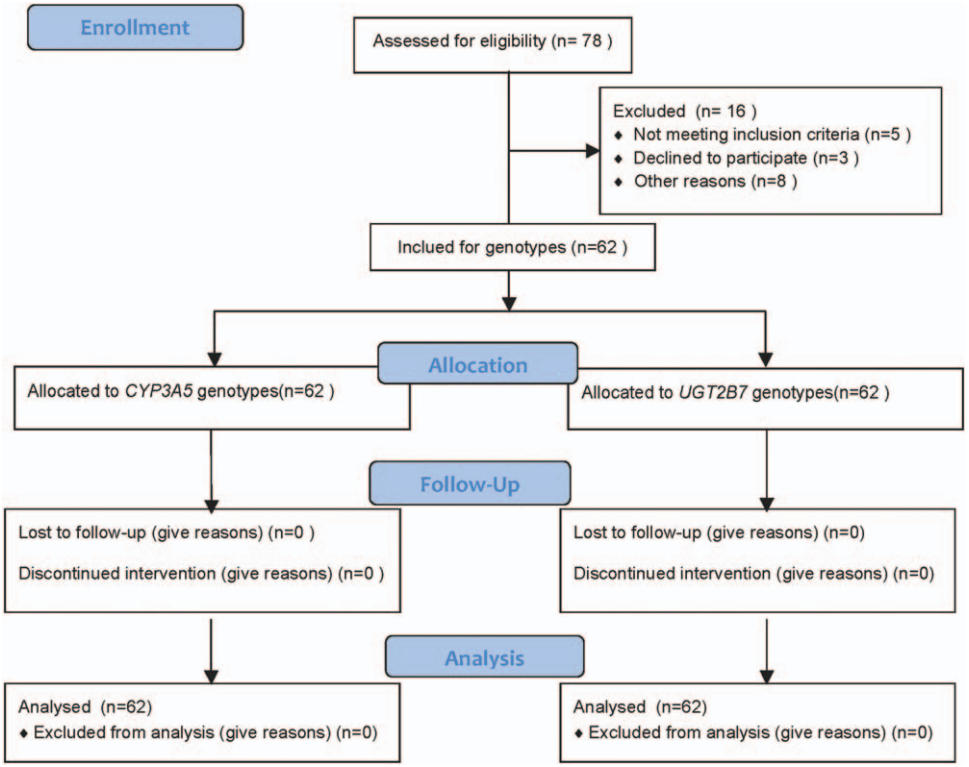

Figure 1. Flow diagram of enrolled patients.

Table 2

Demographic characteristics of all patients and *CYP3A5* genotypic groups.

Parameters	Total patients (n=62)	CYP3A5 expressors (*1/*1+*1/*3) (n=29)	CYP3A5 nonexpressors (*3/*3) (n=33)	*P*
No. sex	Male (32) Female (30)	Male (16) Female (13)	Male (16) Female (17)	.673
Age, y	24.0±16.2	22.9±14.8	25.0±17.6	.498
Body weight, kg	51.3±17.6	51.3±17.6	52.8±12.0	.462
BMI, cm^2/kg	24.9±11.7	21.9±4.00	21.0±2.30	.428

P values for comparisons between CYP3A5 expressors and nonexpressors.
BMI=body mass index.

Table 3

Demographic characteristics of *UGT2B7* genotypic groups.

Parameters	UGT2B7 *1/*1(268H) (n=30)	UGT2B7 *1/*2+*2/*2 (n=32)	*P*	UGT2B7 *1/*1(71A) (n=40)	UGT2B7 *1/*3+*3/*3 (n=22)	*P*
No. sex	Male (14) Female (16)	Male (18) Female (14)	.876	Male (22) Female (18)	Male (10) Female (12)	.314
Age, y	23.2±17.7	24.70±14.9	.598	23.6±15.2	24.4±16.2	.796
Body weight, kg	51.9±15.2	52.6±13.9	.804	51.5±15.9	50.0±13.6	.467
BMI, cm^2/kg	21.5±3.00	21.4±2.90	.394	21.2±2.82	21.8±3.05	.567

P values for comparisons between *UGT2B7* different genotypic groups.
BMI=body mass index.

exclusion criteria (n=8) (Fig. 1). The *CYP3A5* and *UGT2B7* genotypes of all patients are shown in Table 2 and Table 3. Of the 62 patients enrolled, 32 were males and 30 were females. All enrolled patients in our study were local Han Chinese. The average age was 24.0±16.2 years. The average daily carbamazepine dose was 499±278 mg. The average concentration of CBZ was 4.17±2.33 μg/mL. There were no differences between CYP3A5 expressors and nonexpressors regarding age, sex, body weight, or BMI (Table 2). Moreover, there were no differences among *UGT2B7* genotypic groups regarding age, sex, body weight, or BMI (Table 3). The distributions of *CYP3A5**3, *UGT2B7**2, and *UGT2B7**3 are shown in Table 4.

Table 4

Distributions of *CYP3A5* and *UGT2B7* variants among all patients.

Gene	SNPs	Subject numbers			Hardy–Weinberg equilibrium
		wt/wt	wt/mt	mt/mt	*P*
CYP3A5	rs776746(A>G)	6	23	33	.51
UGT2B7	rs7439366(802C>T)	30	26	6	.91
	rs12233719(211G>T)	40	18	4	.33

mt = mutant-type, wt = wild-type.

All allele frequencies were consistent with Hardy–Weinberg equilibrium.

3.2. Correlations between dose and serum concentration of CBZ

Positive correlations between CBZ dose and serum concentration were observed in all patients ($r=0.5819$, $P<.0001$), and CYP3A5 expressors ($r=0.4159$, $P=.0249$) and nonexpressors ($r=0.6252$, $P=.0001$), and *UGT2B7* genotypic groups (*1/*1 (268H): $r=0.415$, $P=.0226$; *1/*2+*2/*2: $r=0.6245$, $P=.0001$; *1/*1(71A): $r=0.5223$, $P=.0005$; *1/*3+*3/*3: $r=0.6993$, $P=.0003$, separately). After CBZ concentrations were normalized according to dose administered, negative correlations between doses and dose-normalized CBZ concentrations were observed in all patients ($r=-0.3239$, $P=.0102$), including CYP3A5 expressors ($r=-0.395$, $P=.034$) and nonexpressors ($r=-0.625$, $P=.023$), and *UGT2B7* genotypic groups (*1/*1 (268H): $r=-0.6321$, $P=.0002$; *1/*1(71A); *1/*3+*3/*3: $r=-0.4740$, $P=.002$, separately). The value of the dose increased and the dose-normalized CBZ concentration decreased in all patients and in CYP3A5 expressors, and also *UGT2B7*1, *UGT2B7*2, *UGT2B7*3 patients (Fig. 2). However, there was no correlation between dose and dose-normalized CBZ concentration in *UGT2B7*1/*2+*2/*2 patients ($r=-0.2895$, $P=.1080$).

3.3. Multiple linear regression analysis of serum CBZ concentration

Multiple linear regression analysis was applied to evaluate relationships among CBZ dose, *CYP3A5*3*, *UGT2B7*2*, and *UGT2B7*3* variants, body weight, BMI, and serum concentration of CBZ, and to compare the significance of each variable. With the equation, R2, β, ΔF, variance inflation factor, the *F* value, and the *P* value were obtained. According to the results, the CBZ dose was significantly correlated with the dose-normalized CBZ concentration ($\beta=0.682$, $P<.001$) (Table 5). When *UGT2B7* *2 was added in step 2, a significant increase was observed in R^2 ($\Delta F=18.515$, $P<.001$). Therefore, the CBZ dose and *UGT2B7* *2 are correlated with the dose-normalized CBZ concentration.

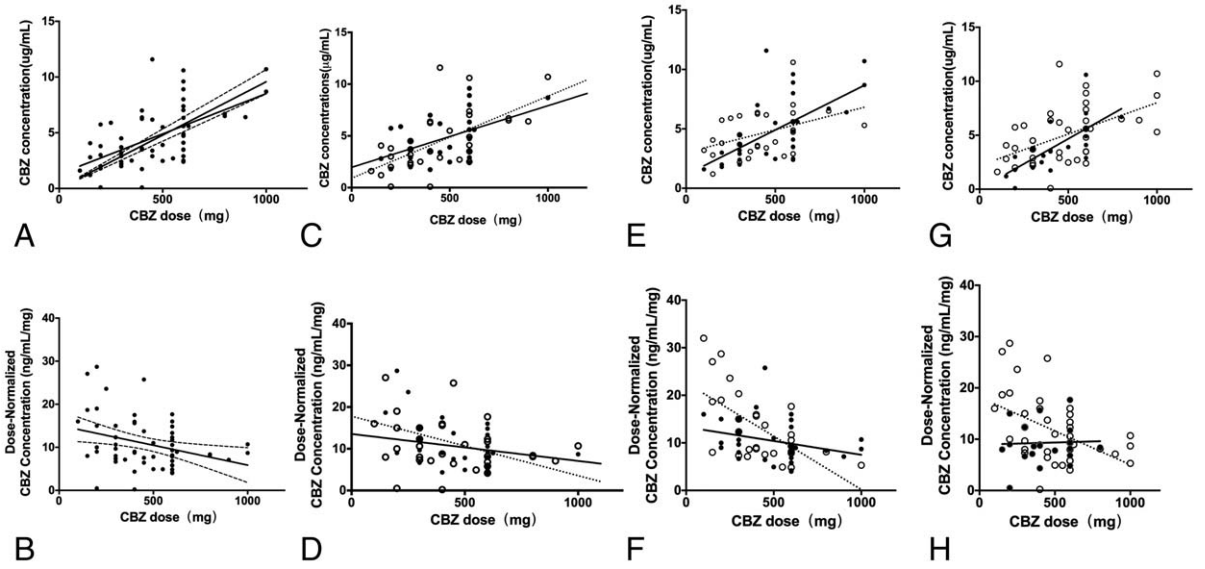

Figure 2. Correlations between doses and serum concentration of CBZ. The figures above present correlations between the dose and serum concentration of CBZ in (Aa) all patients, (C) CYP3A5 expressors and nonexpressors, (E) *UGT2B7*1/*1(268H) and *1/*2+*2/*2 patients, (G) *UGT2B7*1/*1(71A) and *1/*3+*3/*3 patients. The following figures present correlations between the dose and dose-normalized CBZ concentration in (B) all patients, (D) CYP3A5 expressors and nonexpressors, (F) *UGT2B7*1/*1(268H) and *1/*2+*2/*2 patients, (H) *UGT2B7*1/*1(71A) and *1/*3+*3/*3 patients. Open circle, CYP3A5 nonexpressors and *UGT2B7* mutants; closed circle, CYP3A5 expressors and wild-type *UGT2B7*. Solid line, regression line for CYP3A5 expressors and wild-type *UGT2B7*; dotted line, regression line for CYP3A5 non-expressors and mutant *UGT2B7*. CBZ = carbamazepine.

Table 5

Relationship between CBZ concentration and each independent variable using stepwise multiple linear regression.

Step	Equation	Independent variables	Constant	β	R^2	ΔF (P)	VIF	F (P)
1	Y=b0+b1X1	X1, CBZ dose (mg)	b1=−0.014, b0=18.03	−0.682	0.486	—	1.026	185.05 (<.001)
2	Y=b0+b1X1+b2X2	X1, CBZ dose (mg)	b1=−0.012, b0=21.31	−0.498	0.544	18.515 (<.001)	1.049	123.82 (<.001)
		*UGT2B7*2*	b2=−0.245	−0.285			0.998	

β = the standardized coefficients or beta coefficients, R^2 = the coefficient of determination, ΔF = F change, VIF = variance inflation factor.

3.4. Comparison of CBZ serum levels and the required doss relative to the CYP3A5 genotype

After comparing the steady-state CBZ concentration, required dose, dose-normalized concentration, and body weight-normalized dose, we observed no significant difference between CYP3A5 expressors and nonexpressors (Fig. 3). Thus, the *CYP3A5*3* genotype does not appear to affect the CBZ serum concentration or dose.

3.5. Comparison of CBZ serum levels and required doses in relation to UGT2B7*2 and UGT2B7*3 genotypes

Based on a comparison of *UGT2B7*1/*1* and *UGT2B7*1/*2+*2/*2* patients, no differences in steady-state CBZ concentrations and body weight-normalized doses were observed (P=.564, P=.0596, respectively). However, the required CBZ dose and dose-normalized concentration differed between *UGT2B7*1/*1* and *UGT2B7*1/*2+*2/*2* patients (P=.0139, P=.032, respectively) (Fig. 4). In addition, *UGT2B7*2* patients showed a lower dose-normalized concentration and required more doses to maintain a steady-state concentration to control seizures.

In a comparison of *UGT2B7*1/*1* and *UGT2B7*1/*3+*3/*3* patients, we found no differences in steady-state CBZ concentrations, dose-normalized concentrations, required CBZ doses, or body weight-normalized doses (P=.0575, P=.184, P=.676, P=.643, respectively) (Fig. 5). Moreover, we observed that *UGT2B7*3* did not affect the CBZ dose requirement or steady-state CBZ concentration.

3.6. Comparison of CBZ serum levels and required doses in relation to UGT2B7*2 and UGT2B7*3 haplotypes

Significant differences in body weight-normalized CBZ dose between *UGT2B7* GC and TT haplotypes were revealed by analysis of the 4 haplotypes of *UGT2B7*2* and *UGT2B7*3*

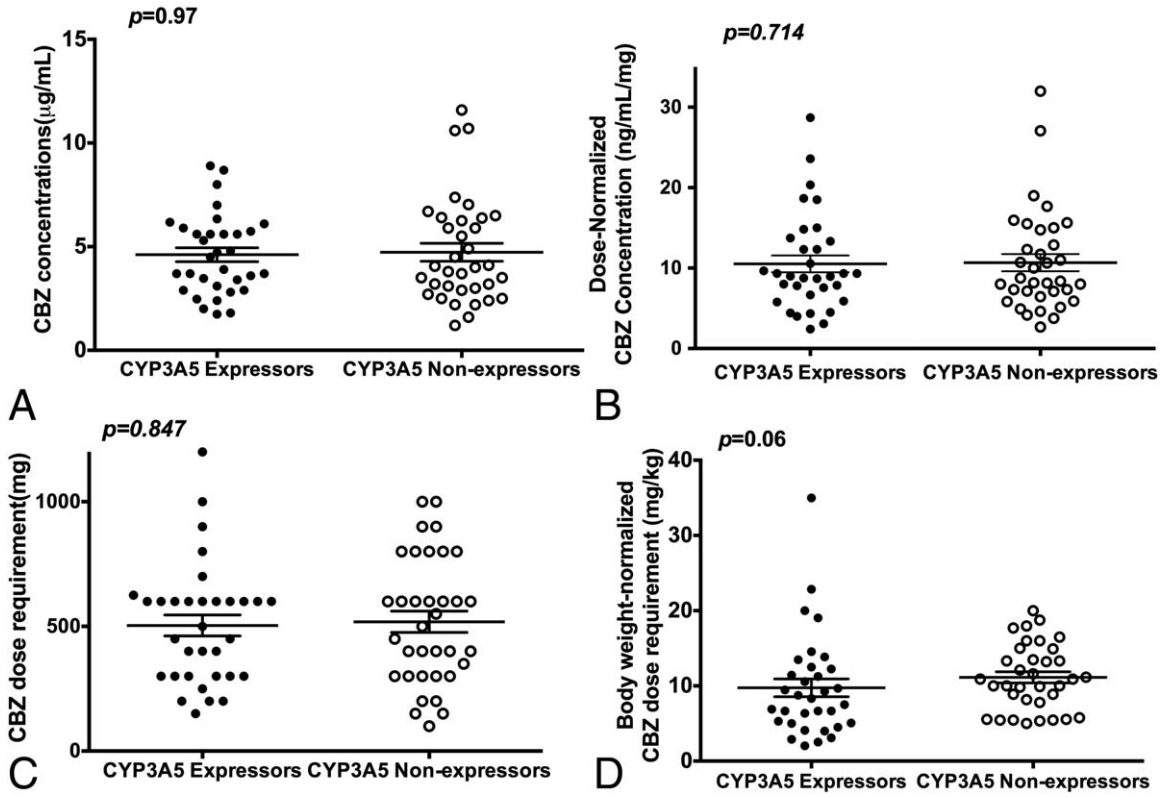

Figure 3. Comparison of CBZ serum levels and required doses in relation to *CYP3A5* genotype. (A) Steady-state CBZ concentration; (B) dose-normalized CBZ concentration; (C) CBZ dose requirement; (D) body weight-normalized CBZ dose requirement. Open circle, CYP3A5 nonexpressors; closed circle, CYP3A5 expressors. CBZ = carbamazepine.

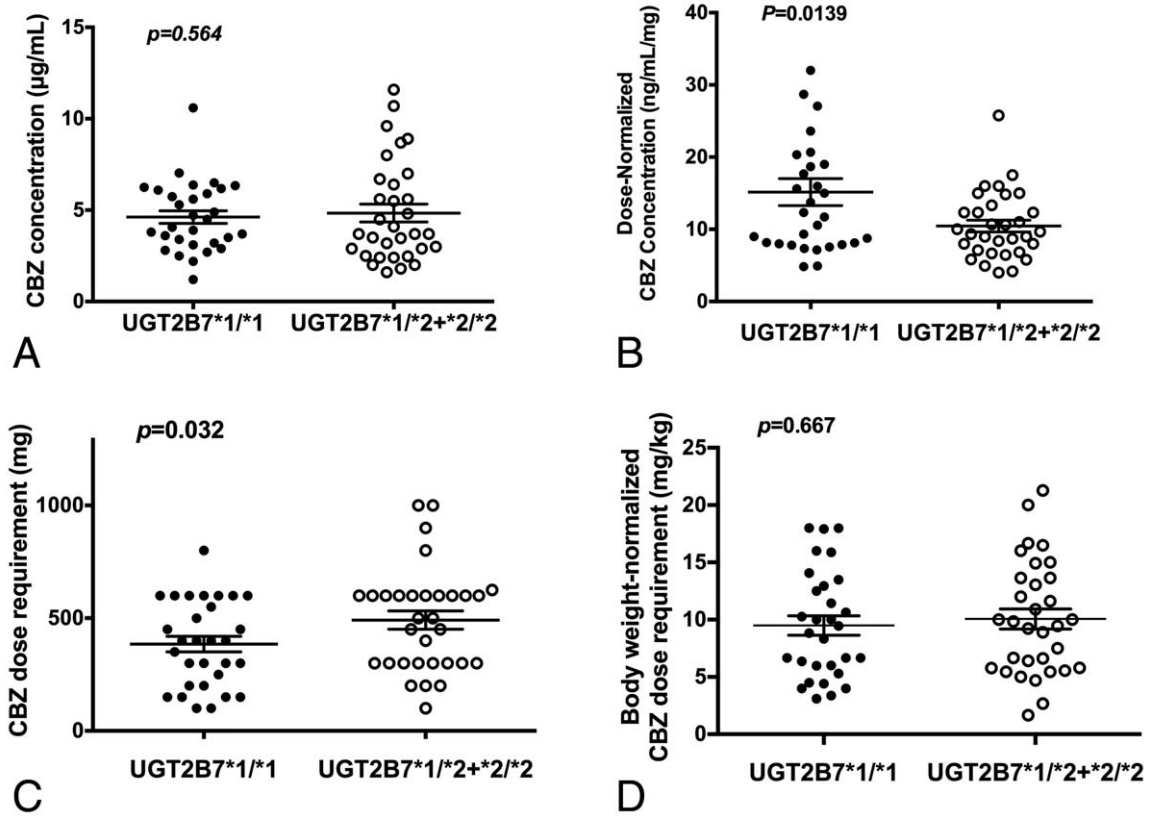

Figure 4. Comparison of CBZ serum levels and required doses in relation to *UGT2B7*2* genotype. (S) Steady-state CBZ concentration; (B) dose-normalized CBZ concentration; (C) CBZ dose requirement; (D) body weight-normalized CBZ dose requirement. Open circle, *UGT2B7**1/*2+*2/*2 patients; closed circle, *UGT2B7**1/*1(268H) patients. CBZ = carbamazepine.

($P = .0154$). For *UGT2B7* GC haplotype patients, a higher body weight-normalized CBZ dose was required for the patient to maintain the steady-state concentration necessary to control seizures. In contrast, we observed no differences in steady-state CBZ concentrations, dose-normalized concentrations, or required CBZ doses among the 4 *UGT2B7* haplotypes (Fig. 6).

4. Discussion

To investigate interindividual variability of CBZ, we carried out a pharmacogenetic study on the relationship between metabolic gene variants of *CYP3A5* and *UGT2B7* and CBZ steady-state concentrations, dose-normalized concentrations, required doses, and body weight-normalized doses. Our results showed the *UGT2B7*2* variant to be significantly associated with dose-normalized CBZ plasma concentrations and required doses in Chinese patients with epilepsy. Conversely, the variants *CYP3A5*3* and *UGT2B7*2* were not found to be associated with CBZ serum concentrations or required doses.

A series of enzymes are involved in the metabolism of CBZ, including CYP3A4, CYP3A5, CYP2C8, UGT2B7, and EPHX1.[8,13,26] Previous studies found that the *CYP3A5*3* variant could influence amlodipine, tacrolimus, and cyclosporine metabolism,[27–29] but that it had no influence on the pharmacokinetics of midazolam or clopidogrel.[30,31] CYP3A5 metabolizes CBZ to carbamazepine 10,11-epoxide, which is the main active metabolite of CBZ.[8] Therefore, we hypothesized that *CYP3A5*3* may influence the steady-state concentration of CBZ in epileptic patients. In our study, the frequency of the *CYP3A5*3* allele was 71.8%, consistent with the frequency in Asians according to the National Center for Biotechnology Information (NCBI) database. In our study, we observed that *CYP3A5*3* did not influence the steady-state CBZ concentration, dose-normalized concentration, required dose, or body weight-normalized dose. Although our results were consistent with the research by Puranik et al,[32] they were inconsistent with other previous studies.[28,33] For example, Park et al[34] found that *CYP3A5*3* affected CBZ concentrations in Korean epileptic patients, and Wang et al[33] found that rs776746 and rs15524 in the *CYP3A5* gene tended to affect CBZ metabolism. However, the latter study enrolled patients who received CBZ plus phenytoin or phenobarbital, which could affect the expression of the P450 enzyme system and could complicate the CBZ metabolism. Moreover, as an inducer of CYP3A4/5 enzymes, CBZ might affect their expression levels.[35] Whether the effect of *CYP3A5* variants on CBZ concentrations has a significant impact on outcomes remains unclear. Further research involving large sample sizes and multiple centers is needed to confirm our negative results for *CYP3A5* variants.

The glucuronidation metabolic pathway of CBZ is an important factor in its pharmacokinetics. CBZ and its main active metabolite CBZ 10,11-epoxide are specifically glucuronidated by UGT2B7.[13] Previous studies reported that the *UGT2B7*2* variant, a missense mutation, can alter metabolic activity of modulating morphine,[19] epirubicin,[36] and efavirenz,[37] but not valproic acid.[38] In our study, the frequencies of the *UGT2B7**2 and *UGT2B7**3 alleles were 30.65% and 8.14%, respectively, which are similar to the frequencies in

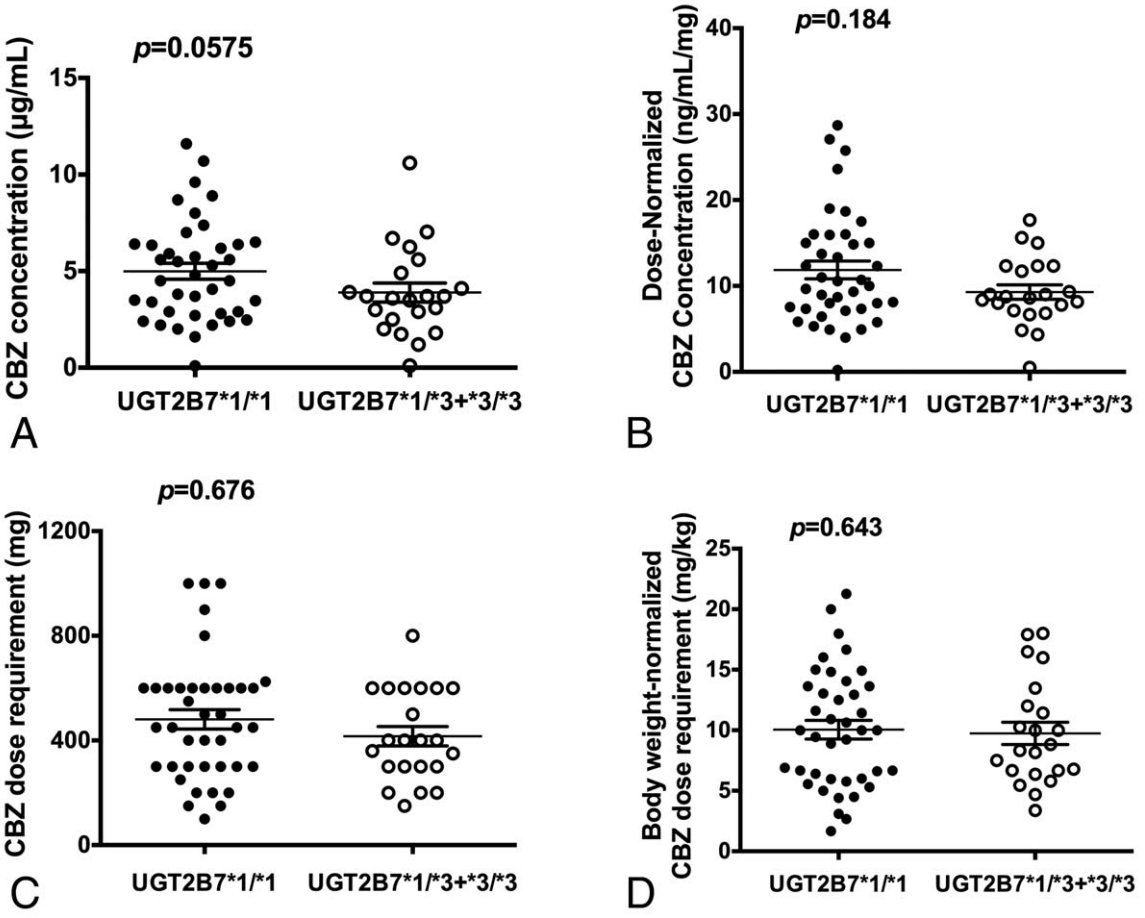

Figure 5. Comparison of CBZ serum levels and required doses in relation to *UGT2B7*3* genotype. (A) Steady-state CBZ concentration; (B) dose-normalized CBZ concentration; (C) CBZ dose requirement; (D) body weight-normalized CBZ dose requirement. Open circle, *UGT2B7**1/*3+*3/*3 patients; closed circle, *UGT2B7**1/*1(71A) patients. CBZ = carbamazepine.

Asians according to the NCBI database. We found no differences in steady-state CBZ concentrations and body weight-normalized doses between *UGT2B7*1/*1* and *UGT2B7*1/*2+*2/*2* patients, though required CBZ doses and dose-normalized concentrations were different between these groups ($P = .0139$, $P = .032$, respectively) (Fig. 4). UG*T2B7*1/*2+*2/*2* patients exhibited a lower dose-normalized steady-state CBZ concentration as compared with *UGT2B7*1/*1* patients, but the former patients require a larger dose to achieve the steady-state CBZ concentration necessary to control seizures. Because different doses are used by epileptic patients to control seizures, we failed to show a correlation between the *UGT2B7*2* genotype and steady-state CBZ concentration, which were not normalized by the CBZ dose. Multiple linear regression analysis also revealed that the CBZ dose and *UGT2B7**2 genotype are correlated with the dose-normalized CBZ concentration. Moreover, we observed significant relationships between the required CBZ dose and *UGT2B7*2* genotype and a positive trend in the body weight-normalized CBZ required dose and *UGT2B7*2* genotype ($P = .0596$). The *UGT2B7*2* mutation replaced the histidine (His) at position 268 to a tyrosine (Tyr), resulting in an increase in enzyme activity. Thus, glucuronidation metabolism during CBZ excretion is increased, resulting in a lower dose-normalized steady-state CBZ concentration than in wild-type *UGT2B7* patients. Our results were consistent with a previous study reporting that *UGT2B7*2* interactively affects the concentration–dose ratio of CBZ.[39] Nonetheless, the relationship between *UGT2B7*2* and CBZ concentration was negative in the study of Ma et al.[40]

We also investigated whether the *UGT2B7*3* (Ala71Ser) variant affects steady-state CBZ concentrations. Although our results showed no significant impact for the *UGT2B7*3* genotype on the CBZ concentration and required dose (Fig. 4), there was a trend toward a lower CBZ concentration and a lower dose-normalized concentration in *UGT2B7*1/*3 +UGT2B7*3/*3* patients ($P = .0575$ and $P = .184$). Research on this variant is limited to date, though it has been demonstrated that *UGT2B7*3* affects the pharmacokinetics of flurbiprofen,[41] zidovudine,[42] and carvedilol,[43] but not R and S-carvedilol. We did not observe any significant effects of *UGT2B7*3* on CBZ concentrations and dose requirements.

In our study, we carried out correlation analysis between dose and serum concentration of CBZ, and found positive correlations between all patients ($r = 0.5819$, $P < .0001$) and *CYP3A5* or *UGT2B7* genotypic groups (all $P < .05$). Although the correlations were significant, the r values were relative small. Therefore, we further carried out multiple linear regression analysis of serum CBZ concentration. According to the results, the CBZ dose was significantly correlated with the dose-normalized CBZ concen-

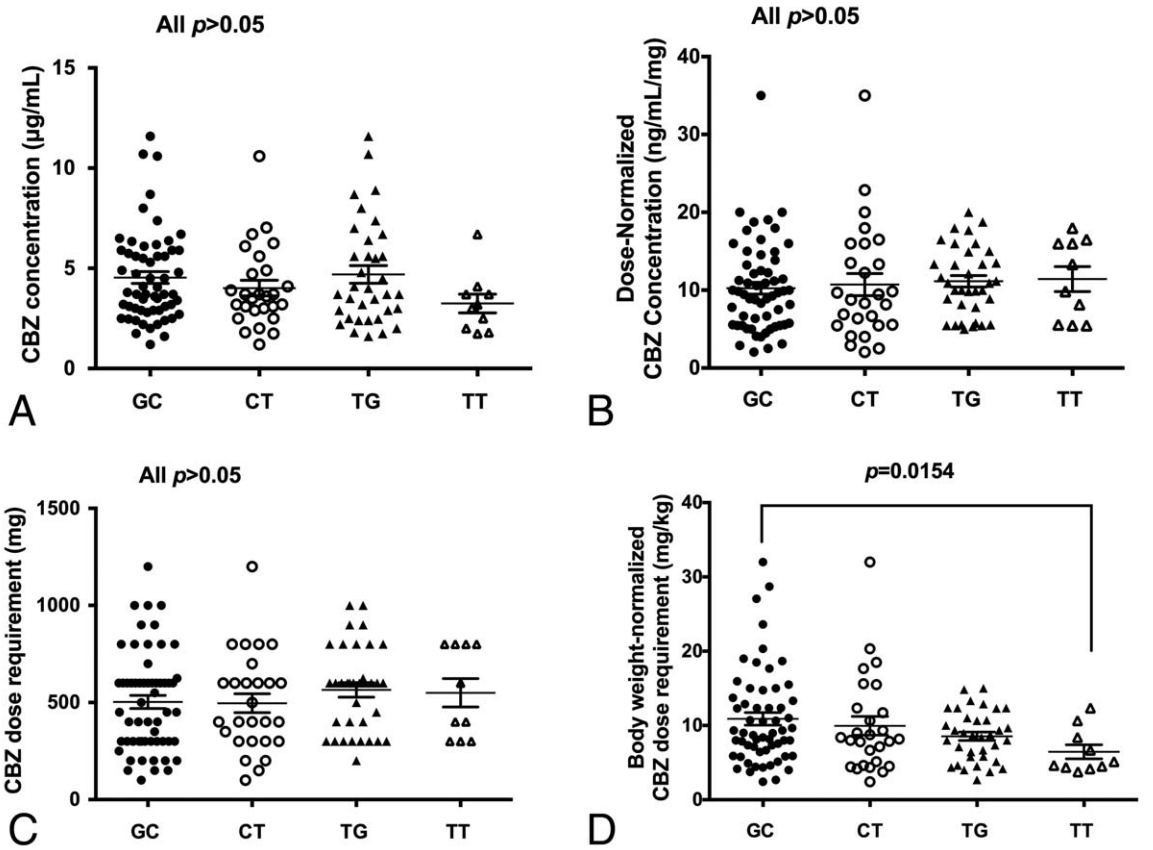

Figure 6. Comparison of CBZ serum levels and required doses in relation to *UGT2B7*2* and **3* haplotypes. (A) Steady-state CBZ concentration; (B) dose-normalized CBZ concentration; (C) CBZ dose requirement; (D) body weight-normalized CBZ dose requirement. CBZ = carbamazepine.

tration ($\beta = 0.682$, $P < .001$) (Table 5). When *UGT2B7* *2 was added in step 2, a significant increase was observed in R^2 ($\Delta F = 18.515$, $P < .001$). Therefore, the CBZ dose and *UGT2B7* *2 are correlated with the dose-normalized CBZ concentration.

There are some limitations to our study. First, despite efforts to enroll more patients, the enrolled sample size was small. Second, we explored the influence of *CYP3A5*3*, *UGT2B7*2*, and *UGT2B7*3* variants on CBZ, but other gene variants may also affect CBZ concentrations. Because the samples size is small, we did not analyze the combination of 3 variants, which is also a limitation. Third, to correlate genetic polymorphisms with metabolism phenotypes, it is better to use the ratio of CBZ's major metabolites to CBZ. All of these aspects limited the results of our research. Larger samples and more genetic polymorphisms should be investigated in future work.

In summary, we explored the effects of *CYP3A5*3*, *UGT2B7*2*, and *UGT2B7*3* variants on steady-state CBZ concentrations in 62 epileptic patients. Our study found that the *UGT2B7*2* variant, but not the *CYP3A5*3* and *UGT2B7*3* variants, can affect steady-state CBZ concentrations in these patients.

Author Contributions

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Writing – review & editing: All authors.

References

[1] Lopez M, Dorado P, Monroy N, et al. Pharmacogenetics of the antiepileptic drugs phenytoin and lamotrigine. Drug Metab Drug Interact 2011;26:5–12.
[2] Qu J, Lu SH, Lu ZL, et al. Pharmacogenetic and case-control study on potassium channel related gene variants and genetic generalized epilepsy. Medicine (Baltimore) 2017;96:e7321.
[3] Beghi E, Perucca E. The management of epilepsy in the 1990s. Acquisitions, uncertainties and priorities for future research. Drugs 1995;49:680–94.
[4] Eichelbaum M, Tomson T, Tybring G, et al. Carbamazepine metabolism in man. Induction and pharmacogenetic aspects. Clin Pharmacokinet 1985;10:80–90.
[5] Madden S, Maggs JL, Park BK. Bioactivation of carbamazepine in the rat in vivo. Evidence for the formation of reactive arene oxide(s). Drug Metab Dispos 1996;24:469–79.
[6] Maggs JL, Pirmohamed M, Kitteringham NR, et al. Characterization of the metabolites of carbamazepine in patient urine by liquid chromatography/mass spectrometry. Drug Metab Dispos 1997;25:275–80.
[7] Lertratanangkoon K, Horning MG. Metabolism of carbamazepine. Drug Metab Dispos 1982;10:1–0.
[8] Kerr BM, Thummel KE, Wurden CJ, et al. Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem Pharmacol 1994;47:1969–79.
[9] Pearce RE, Vakkalagadda GR, Leeder JS. Pathways of carbamazepine bioactivation in vitro I. Characterization of human cytochromes P450 responsible for the formation of 2- and 3-hydroxylated metabolites. Drug Metab Dispos 2002;30:1170–9.

[10] Wrighton SA, Ring BJ, Watkins PB, et al. Identification of a polymorphically expressed member of the human cytochrome P-450III family. Mol Pharmacol 1989;36:97–105.
[11] Kuehl P, Zhang J, Lin Y, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001;27:383–91.
[12] Huang W, Lin YS, McConn DJ2nd, et al. Evidence of significant contribution from CYP3A5 to hepatic drug metabolism. Drug Metab Dispos 2004;32:1434–45.
[13] Staines AG, Coughtrie MW, Burchell B. N-glucuronidation of carbamazepine in human tissues is mediated by UGT2B7. J Pharmacol Exp Ther 2004;311:1131–7.
[14] Hustert E, Haberl M, Burk O, et al. The genetic determinants of the CYP3A5 polymorphism. Pharmacogenetics 2001;11:773–9.
[15] Bhasker CR, McKinnon W, Stone A, et al. Genetic polymorphism of UDP-glucuronosyltransferase 2B7 (UGT2B7) at amino acid 268: ethnic diversity of alleles and potential clinical significance. Pharmacogenetics 2000;10:679–85.
[16] Hirota T, Ieiri I, Takane H, et al. Sequence variability and candidate gene analysis in two cancer patients with complex clinical outcomes during morphine therapy. Drug Metab Dispos 2003;31:677–80.
[17] Blevins-Primeau AS, Sun D, Chen G, et al. Functional significance of UDP-glucuronosyltransferase variants in the metabolism of active tamoxifen metabolites. Cancer Res 2009;69:1892–900.
[18] Coffman BL, King CD, Rios GR, et al. The glucuronidation of opioids, other xenobiotics, and androgens by human UGT2B7Y(268) and UGT2B7H(268). Drug Metab Dispos 1998;26:73–7.
[19] Sawyer MB, Innocenti F, Das S, et al. A pharmacogenetic study of uridine diphosphate-glucuronosyltransferase 2B7 in patients receiving morphine. Clin Pharmacol Ther 2003;73:566–74.
[20] Peterkin VC, Bauman JN, Goosen TC, et al. Limited influence of UGT1A1*28 and no effect of UGT2B7*2 polymorphisms on UGT1A1 or UGT2B7 activities and protein expression in human liver microsomes. Br J Clin Pharmacol 2007;64:458–68.
[21] Bernard O, Tojcic J, Journault K, et al. Influence of nonsynonymous polymorphisms of UGT1A8 and UGT2B7 metabolizing enzymes on the formation of phenolic and acyl glucuronides of mycophenolic acid. Drug Metab Dispos 2006;34:1539–45.
[22] Takekuma Y, Takenaka T, Kiyokawa M, et al. Contribution of polymorphisms in UDP-glucuronosyltransferase and CYP2D6 to the individual variation in disposition of carvedilol. J Pharm Pharmaceut Sci 2006;9:101–12.
[23] Guo D, Pang LF, Han Y, et al. Polymorphisms of UGT1A9 and UGT2B7 influence the pharmacokinetics of mycophenolic acid after a single oral dose in healthy Chinese volunteers. Eur J Clin Pharmacol 2013; 69:843–9.
[24] Human Cytochrome P450 (CYP) Allele Nomenclature Committee. CYP3A4 allele nomenclature; 2017. Available at: http://www.cypalleles.ki.se/cyp3a4.htm. Accessed February 8, 2017.
[25] Daci A, Beretta G, Vllasaliu D, et al. Polymorphic variants of SCN1A and EPHX1 influence plasma carbamazepine concentration, metabolism and pharmacoresistance in a population of Kosovar Albanian epileptic patients. PLoS One 2015;10:e0142408.
[26] Bu HZ, Kang P, Deese AJ, et al. Human in vitro glutathionyl and protein adducts of carbamazepine-10,11-epoxide, a stable and pharmacologically active metabolite of carbamazepine. Drug Metab Dispos 2005;33:1920–4.
[27] Macphee IA, Fredericks S, Mohamed M, et al. Tacrolimus pharmacogenetics: the CYP3A5*1 allele predicts low dose-normalized tacrolimus blood concentrations in whites and South Asians. Transplantation 2005;79:499–502.
[28] Park JY, Kim KA, Park PW, et al. Effect of CYP3A5*3 genotype on the pharmacokinetics and pharmacodynamics of alprazolam in healthy subjects. Clin Pharmacol Ther 2006;79:590–9.
[29] Min DI, Ellingrod VL, Marsh S, et al. CYP3A5 polymorphism and the ethnic differences in cyclosporine pharmacokinetics in healthy subjects. Ther Drug Monit 2004;26:524–8.
[30] Zhang HZ, Kim MH, Guo LZ, et al. CYP2C19 but not CYP2B6, CYP3A4, CYP3A5, ABCB1, PON1 or P2Y12 genetic polymorphism impacts antiplatelet response after clopidogrel in Koreans. Blood Coagul Fibrinolysis 2017;28:56–61.
[31] Chan SW, Xiao Y, Hu M, et al. Associations of the CYP3A5*3 and CYP3A4*1G polymorphisms with the pharmacokinetics of oral midazolam and the urinary 6beta-hydroxycortisol/cortisol ratio as markers of CYP3A activity in healthy male Chinese. J Clin Pharm Ther 2016;41:552–8.
[32] Puranik YG, Birnbaum AK, Marino SE, et al. Association of carbamazepine major metabolism and transport pathway gene polymorphisms and pharmacokinetics in patients with epilepsy. Pharmacogenomics 2013;14:35–45.
[33] Wang P, Yin T, Ma HY, et al. Effects of CYP3A4/5 and ABCB1 genetic polymorphisms on carbamazepine metabolism and transport in Chinese patients with epilepsy treated with carbamazepine in monotherapy and bitherapy. Epilepsy Res 2015;117:52–7.
[34] Park PW, Seo YH, Ahn JY, et al. Effect of CYP3A5*3 genotype on serum carbamazepine concentrations at steady-state in Korean epileptic patients. J Clin Pharm Ther 2009;34:569–74.
[35] Luo G, Cunningham M, Kim S, et al. CYP3A4 induction by drugs: correlation between a pregnane X receptor reporter gene assay and CYP3A4 expression in human hepatocytes. Drug Metab Dispos 2002;30:795–804.
[36] Parmar S, Stingl JC, Huber-Wechselberger A, et al. Impact of UGT2B7 His268Tyr polymorphism on the outcome of adjuvant epirubicin treatment in breast cancer. Breast Cancer Res 2011;13:R57.
[37] Kwara A, Lartey M, Sagoe KW, et al. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. AIDS 2009;23:2101–6.
[38] Liu L, Zhao L, Wang Q, et al. Influence of valproic acid concentration and polymorphism of UGT1A4*3, UGT2B7-161C > T and UGT2B7*2 on serum concentration of lamotrigine in Chinese epileptic children. Eur J Clin Pharmacol 2015;71:1341–7.
[39] Hung CC, Chang WL, Ho JL, et al. Association of polymorphisms in EPHX1, UGT2B7, ABCB1, ABCC2, SCN1A and SCN2A genes with carbamazepine therapy optimization. Pharmacogenomics 2012;13:159–69.
[40] Ma CL, Jiao Z, Wu XY, et al. Association between PK/PD-involved gene polymorphisms and carbamazepine-individualized therapy. Pharmacogenomics 2015;16:1499–512.
[41] Wang H, Yuan L, Zeng S. Characterizing the effect of UDP-glucuronosyltransferase (UGT) 2B7 and UGT1A9 genetic polymorphisms on enantioselective glucuronidation of flurbiprofen. Biochem Pharmacol 2011;82:1757–63.
[42] Yuan L, Qian S, Xiao Y, et al. Homo- and hetero-dimerization of human UDP-glucuronosyltransferase 2B7 (UGT2B7) wild type and its allelic variants affect zidovudine glucuronidation activity. Biochem Pharmacol 2015;95:58–70.
[43] Takekuma Y, Takenaka T, Kiyokawa M, et al. Evaluation of effects of polymorphism for metabolic enzymes on pharmacokinetics of carvedilol by population pharmacokinetic analysis. Biol Pharmaceut Bull 2007; 30:537–42.