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# Association study of *CYP3A5* genetic polymorphism with serum concentrations of carbamazepine in Chinese epilepsy patients

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### Abstract

*Objective:* To investigate the association between the *CYP3A5* genetic polymorphism and the serum concentrations of carbamazepine (CBZ), to provide guidance for individualized drug dosing. *Methods:* Eighty-four epilepsy patients taking CBZ were included in this study. Their clinical data were recorded and CBZ serum concentrations were measured. The *CYP3A5* 6986 genetic polymorphism was assessed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Patients were divided according to genotype into *CYP3A5* expressor (*CYP3A5*1/*1* genotype and *CYP3A5*1/*3* genotypes) and non-expressor groups (*CYP3A5*3/*3*). The two groups were compared for the total dose of CBZ, dose of CBZ/kg body weight, serum drug concentration, dose-corrected serum concentration, and standardized serum concentration. *Results:* The total dose of CBZ and the dose of CBZ/kg body weight was higher in the *CYP3A5* expressor group than the non-expressor ($P$ = 0.043 and $P$ = 0.014, respectively). The dose-corrected and standardized serum concentrations were lower in the *CYP3A5* expressor group than the non-expressor ($P$ = 0.001 and $P$ < 0.001, respectively). There was however, no significant difference in serum drug concentration between the two groups ($P$ = 0.487).
*Conclusions:* There was a close relationship between *CYP3A5* genetic polymorphism and the serum concentrations of carbamazepine.

## INTRODUCTION

Seventy to 80% of epileptic patients have partial epilepsy.[1] Carbamazepine (CBZ) is often used as monotherapy in partial epilepsy with or without secondary generalization. There are differences in therapeutic efficacy in patients taking CBZ. These differences in therapeutic response may be partly due to individual differences in serum concentrations of the drug, which may in turn be determined by the genetic polymorphisms of metabolic enzymes. *CYP3A5* is one of the main enzymes that metabolize CBZ. Few studies have been previously performed to determine the relationship between *CYP3A5* genetic polymorphisms and serum level of CBZ. Our objective in this study was to determine the relationship between *CYP3A5* polymorphisms and CBZ serum concentrations, and the therapeutic effects of the drug in a cohort of Chinese epilepsy patients.

## METHODS

*Inclusion criteria*

The inclusion criteria were: (1) Partial epilepsy with or without secondary generalization; (2) CBZ was taken regularly for more than a week with good adherence; (3) Other antiepileptic drugs and drugs which could affect the activities of liver drug metabolizing enzymes had not been administered; (4) There was no serious liver or renal disease.

*Drug serum concentration determination*

CBZ was taken (P.O.) regularly for more than a week to establish a steady state prior to drug serum concentration measurement. Venous blood was collected from patients on empty stomach in the early morning. The blood samples were pre-dose samples.

Serum concentrations of CBZ were determined using the Dimension Clinical Biochemistry System and CRBM Flex kits (particle-enhanced turbidimetric immunoassay) at the Inspection

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Department, First Hospital of Jinlin University, Jilin, China. Its coefficient of variation was 31%, and the sensitivity of the analytical method was 0.5µg/ml or 2.1µmol/L. The therapeutic level of CBZ was taken as 4 to 12 µg/mL. Dose-corrected serum concentration, which takes into account the effect from different doses, was calculated as the ratio of serum concentration to CBZ dose/day. Standardized serum concentration, which takes into account the effects of the dose and body weight, was calculated as the ratio of serum concentration to CBZ dose/kg body weight/day.

*DNA extraction*

Four milliliters of collected peripheral venous blood from epileptic patients was infused into an anticoagulation tube with 2% EDTA, and DNA was extracted using a Blood Genomic DNA Purification Kit. There was a blank control prepared to look for environmental DNA contamination and samples cross-contamination.

*CYP3A5 genetic polymorphism analysis by PCR-RFLP*

*PCR primer:* The forward primer was 5’-GGCAACATGACTTAGACAG-3’, and the reverse primer was 5’-GGTCCAAACAGGGAAGAAATA-3’.

*PCR reaction system:* The bulk volume of 50 µL consisted of 0.7 µL forward primer, 0.7 µL reverse primer, 0.4 µL (5 U/µL) Taq enzyme, 3 µL DNA templates, 5 µL 10×PCR buffer, and 39.2 µL sterilized dd$H_2O$.

*PCR reaction procedure:* The reaction mixtures were kept at 95 °C for 1 min to denature the DNA fully. Denaturing was at 95 °C for 45 seconds, annealing at 57 °C for 45 seconds and extended at 72 °C for 30 seconds, repeated for 33 cycles. After these cycles were completed, the reaction mixtures were extended at 72 °C for a further 10 minutes. After amplification, the 298 bp DNA band was detected by 2% agarose gel electrophoresis for 30 min, and visualized using a gel imaging system.

*Genotype determination by PCR-RFLP:* The PCR product was digested by restriction enzyme *Ssp* I at 37 °C for 3 h. The bulk volume of the PCR-RFLP reaction system was 20 µL, consisting of 0.5 µL *Ssp* I, 10 µL gene fragment after PCR amplification, 2 µL 10×NEB buffer, and 7.5 µL sterilized dd$H_2O$. The product was detected by 3% agarose gel electrophoresis for 45 min, and visualized using a gel imaging system.

*Gene Sequencing:* In order to check the accuracy of PCR-RFLP, gene fragments after PCR amplification of several samples were analyzed with an automatic sequencer at Shanghai Shenggong Company.

*Statistical Analysis*

All data were analyzed by Statistical Package for the Social Sciences (SPSS) 13.0 software (SPSS Inc., IL, USA). Data were expressed as means ± standard deviation (SD). The Kolmogorov-Smirnov normal distribution and homogeneity tests for variance were used for a single sample of data. Student’s *t*-test and the chi-square ($\chi^2$)-test were used for two samples of measurement and enumeration data, respectively. Linear regression analysis was used for dependability analysis between two variables. Probability (*P*)-values $< 0.05$ were considered statistically significant.

## RESULTS

Eighty-four epilepsy patients (53 males, 31 females, age range 16-77 years, body weight range 55-80 kg) treated with CBZ monotherapy were included in this analysis. Of the 84 patients, 19 suffered from generalized seizures, 65 from partial seizures and 11 had mixed seizures. One serum sample in each patient was taken for CBZ quantitation.

*Clinical data of patients in the CYP3A5 expressor and non-expressor groups*

According to the genotypes, patients were divided into a CYP3A5 expressor group and a CYP3A5 non-expressor group. The CYP3A5 expressor group consisted of the genotypes *CYP3A5*1/*1* (AA) and *CYP3A5*1/*3* (AG). The CYP3A5 non-expressor group included the *CYP3A5*3/*3* genotype (GG). There were no statistically significant differences in age, gender, weight, CBZ dose, or CBZ dose/kg body weight between the two groups (Table 1, Fig. 1). The gender was compared by $\chi^2$-test, and others by Student’s *t*-test.

*Efficacy comparison between the CYP3A5 expressor and non-expressor groups*

For pateints treated with CBZ monotherapy, drug efficacy was defined as seizure-free or a 50% or

**Table 1: Comparison of the clinical data of patients between the CYP3A5 expressor and non-expressor groups.**

| | CYP3A5 expressor group | CYP3A5 non-expressor group | *P-value* |
|---|---|---|---|
| Quantity | AA 3, AG 29 | GG 52 | |
| Gender | Male 21, Female 11 | Male 32 ; Female 20 | 0.706 |
| Age, years | 35.8 ± 14.3 | 36.3 ± 17.7 | 0.883 |
| Weight, kg | 64.1 ± 6.1 | 66.3 ± 6.1 | 0.112 |
| CBZ dose, mg | 475.00 ± 141.42 | 441.35 ± 167.66 | 0.347 |
| CBZ dose, mg/kg | 7.52 ± 2.48 | 6.70 ± 2.53 | 0.152 |

CBZ: carbamazepine

greater reduction in seizure frequency for at least one year, up to the date of the last follow-up visit. Drug inefficacy was defined as no change or less than 50% reduction in seizure frequency for at least one year, up to the date of the last follow-up visit, The efficacy comparison between the *CYP3A5* expressor and non-expressor groups is shown in Table 2. There was no statistically significant difference in efficacy between the two groups ($\chi^2 = 0.005$, $P = 0.943$).

*Products of PCR amplification and Ssp I PCR-RFLP*

After PCR amplification of *CYP3A5*, the 298 bp DNA band was detected by 2% agarose gel electrophoresis (Figure 2). After *Ssp* I PCR-RFLP, two products (168bp and 130bp) of *CYP3A5*3/*3* genotype (GG genotype) were obtained, three (148bp, 130bp and 20bp) of *CYP3A5*1/*1* genotype (AA genotype), and four (168bp, 148bp, 130bp and 20bp) of *CYP3A5*1/*3* genotype (AG genotype). The 20 bp DNA band was not detected in the electropherogram. Therefore, two bands of GG and AA genotypes, and three bands of AG genotype, could be seen in the electropherogram (Figure 3). The sequencing results are shown in Figure 4.

*Genotype analysis*

The percentages of AA, AG, and GG genotypes in the 84 epilepsy patients were 3.6%, 34.5%, and 61.9%, respectively. The percentages of

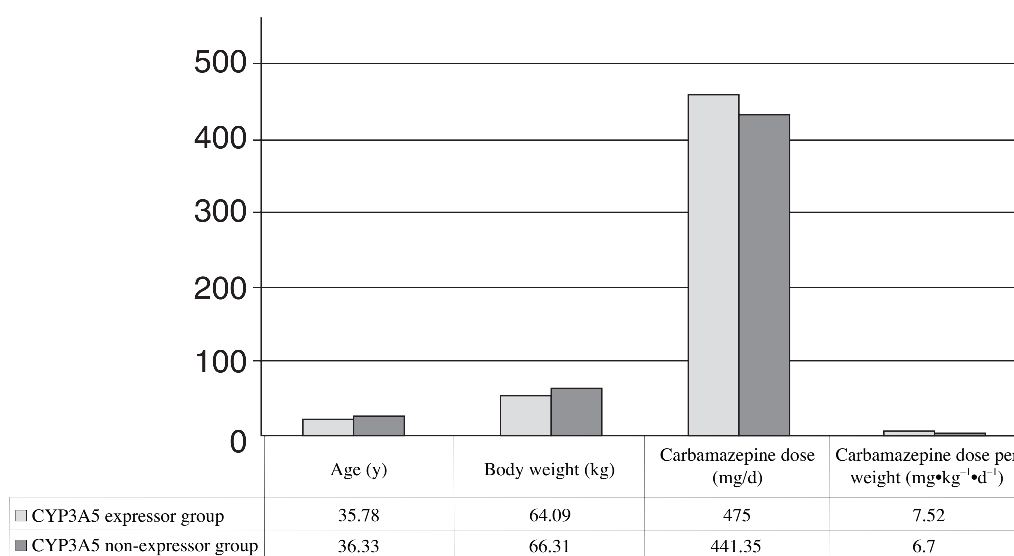


Figure 1. Comparison of the clinical data of patients between the *CYP3A5* expressor and non-expressor groups.

**Table 2: Comparison of clinical efficacy to carbamazepine between the CYP3A5 expressor and non-expressor groups**

| | Effective | Ineffective | Effective rate (%) |
|---|---|---|---|
| *CYP3A5* expressor group | 23 | 9 | 71.9 |
| *CYP3A5* non-expressor group | 37 | 15 | 71.2 |
| Total | 60 | 24 | 71.4 |

Effective: seizure-free or a 50% or greater reduction in seizure frequency for at least one year, up to the date of the last follow-up visit; Ineffective: no change or less than 50% reduction in seizure frequency for at least one year, up to the date of the last follow-up visit, for patients treated with CBZ monotherapy

allelomorphic gene A and G were 20.8% and 79.2%, respectively, which fit the Hardy-Weinberg genetic balance. Patients were divided into the *CYP3A5* expressor group (*CYP3A5*1/*1* and *CYP3A5*1/*3* genotypes) and the *CYP3A5* non-expressor group (*CYP3A5*3/*3*). There were no statistically significant differences in sex, age, or weight between these two groups.

*CYP3A5 genetic polymorphism and CBZ dosage*

The CBZ doses for the *CYP3A5* expressor and non-expressor groups were 512.50 ± 128.89 mg and 441.35 ± 167.66 mg, respectively, and the difference was statistically significant ($t$ = 2.054, $P$ = 0.043). The CBZ dose/kg body weight in the *CYP3A5* expressor group was 8.07 ± 2.21 mg/kg, higher than the 6.70 ± 2.53 mg/kg for the *CYP3A5* non-expressor group, a statistically significant difference ($t$ = 2.523, $P$ = 0.014).

*Association of CYP3A5 genetic polymorphism and serum drug concentration, dose-corrected serum concentration, and standardized serum concentration of CBZ*

The CBZ serum drug concentrations in the *CYP3A5* expressor and non-expressor groups were 7.03 ± 1.78 μg/mL and 7.37 ± 2.29 μg/mL, respectively; the difference was of no statistical significance ($P$ = 0.487). The dose-corrected serum concentration of CBZ in the *CYP3A5* expressor group was 14.08 ± 3.18 ng/(mL·mg), lower than the 18.01 ± 5.62 ng/(mL·mg) of the non-expressor group, the difference was statistically significant ($P$ = 0.001). The standardized serum concentration of CBZ in the *CYP3A5* expressor group was 0.90 ± 0.20 μg·kg/(mL·mg), lower than the 1.19 ± 0.38 μg·kg/(mL·mg) of the non-expressor group, the difference was statistically significant ($P$ < 0.001).

## DISCUSSION

*CYP3A5* is located on human chromosome 9, mapped to q21.1-22.1, coding 502 amino acids. Individual and racial differences are evident in its expression and activities. While *CYP3A5* is expressed in a minority of Caucasians and Asians, it is present in the majority of African Americans, and when present can be 50% of the total *CYP3A* enzymes in the liver.[2] The expression of *CYP3A5* is a very important factor influencing the total amount of *CYP3A* enzymes in the human liver, and the most important factor determining individual differences in *CYP3A* activities, therapeutic effects and the toxicity of drugs metabolized by *CYP3A*.

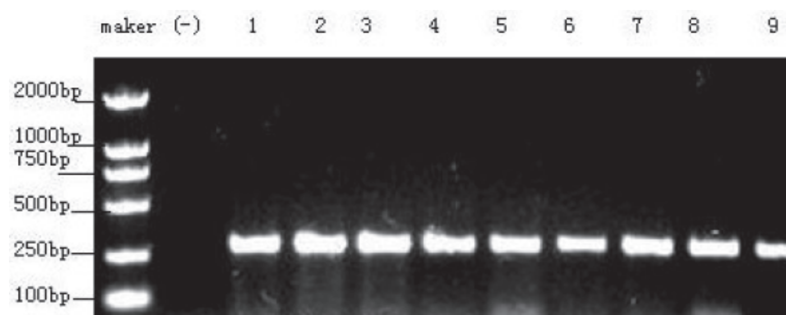


Figure 2. Electropherogram of the PCR products of *CYP3A5* 6986A/G
Notes: Marker is in the left lane; (-), negative control; lanes 1-9 are the PCR products.

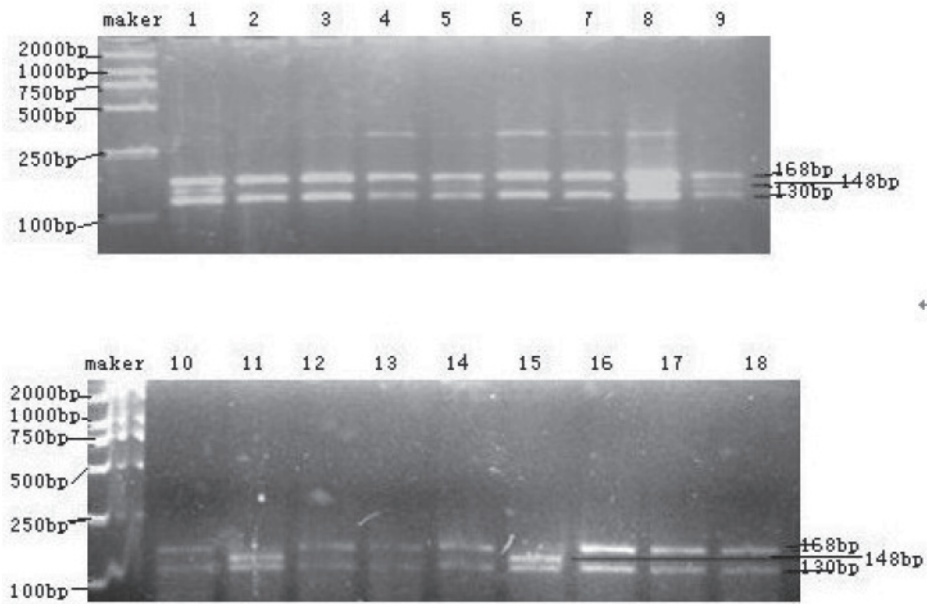


Figure 3. Electropherogram of the PCR-RFLP products of *CYP3A5* 6986A/G
Notes: Marker is in the left lane; lanes 1, 5, 8, 9 of AG genotype; lanes 2-4, 6, 7, 10, 12-14, 16-18 of GG genotype; lanes 11 and 15 of AA genotype.

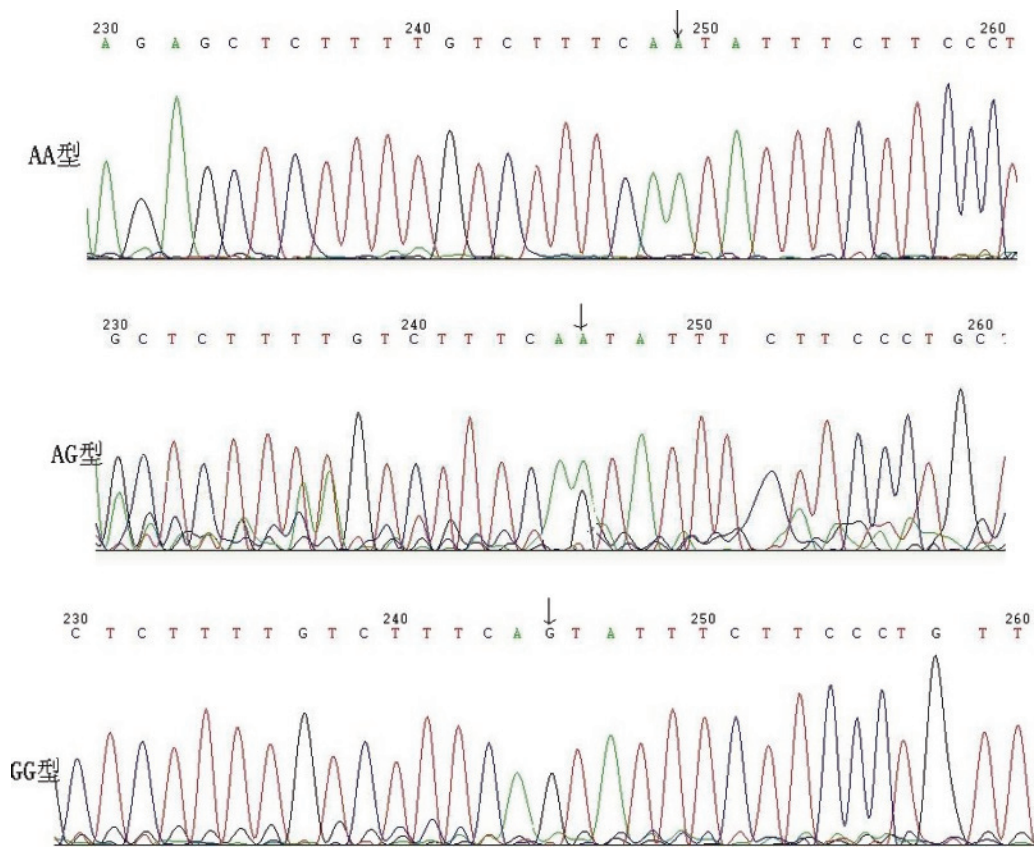


Figure 4. The sequencing results of *CYP3A5* 6986A/G

The difference in *CYP3A5* activities is caused by single-nucleotide polymorphisms (SNPs).[3] The SNP 6986 A→G (*CYP3A5*3*) in intron 3, is very important for *CYP3A5*. Several splice variant mRNAs produced by *CYP3A5*3* result in a premature stop codon and leads to *CYP3A5* deficiency. Therefore, *CYP3A5* can be produced only when there is more than one *CYP3A5*1* in allelomorphic genes. The occurrence frequency of *CYP3A5*3* in Chinese is 72.7%.[4]

A change in *CYP3A5* activities may affect the metabolism of drugs that are substrates of this enzyme.[5] The metabolism of substrates such as sirolimus[6], tacrolimus[2] and amlodipine[7], has been shown to be altered by *CYP3A5* polymorphisms. The metabolism of other substrates such as midazolam[8], nifedipine[9], and diltiazem[10], however, was not affected.

CBZ could be eliminated almost completely by biotransformation in the liver, mainly by the isoenzymes *CYP3A4* and *CYP3A5*, and partially by *CYP2C8* and *CYP1A2*.[11,12] Liver enzyme induction of CBZ could enhance the metabolism itself. In this study, the doses of CBZ and of CBZ/kg body weight for the *CYP3A5* expressor group were significantly higher than those of the *CYP3A5* non-expressor group. The difference in serum drug concentration between the two groups had no statistical significance. However, compared to those of *CYP3A5* non-expressor group, the dose-corrected serum concentration and standardized serum concentration of *CYP3A5* expressor group were significantly lower. In other words, the average serum concentration of the *CYP3A5* non-expressor group was higher than that of the *CYP3A5* expressor group, and not influenced by gender, age, weight, or dose. Park, *et al.*[13] reported similar results in their research. However the results of Seo, *et al.*[14] were contrary, when a pharmacokinetic model of an oral administration to study CBZ elimination and *CYP3A5* genotypes in human was used. It was considered that the combined use of valproate sodium, phenytoin (diphenylhydantoin), phenobarbitone and CBZ in the patients, and the difference of autoinduction caused by different CBZ doses, might cause the change of metabolism velocity.

One of the limitations in this study is that only one sample was taken from each study subject and the administration time of the CBZ dose before sampling in these patients were not the same. This variability may affect the interpretations of the CBZ concentrations between the two study groups.

In summary, the metabolism of CBZ was regulated by *CYP3A5* polymorphic expression. Patients with either *CYP3A5*1/*1* or *CYP3A5*1/*3* genotype require higher doses of CBZ, while those with the *CYP3A5*3/*3* genotype need less, in order to reduce the risk of adverse effects of the drug and to avoid unnecessary dosing. The practical significance of this study is its usefulness for guiding clinical personalized medicine.

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