

Effects of SCN1A and GABA Receptor Genetic Polymorphisms on Carbamazepine Tolerability and Efficacy in Chinese Patients with Partial Seizures: 2-Year Longitudinal Clinical Follow-Up

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Keywords

Carbamazepine; Efficacy; Epilepsy; GABA receptor; Polymorphism; *SCN1A*; Tolerability.

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Received 6 December 2011; revision 30 January 2012; accepted 31 January 2012

doi: 10.1111/j.1755-5949.2012.00321.x

This work was supported by the National Natural Science Foundation of China (81173129), Program for Changjiang Scholars and Innovative Research Team in University (IRT0946), The Science and Technology Plan Key Grant of Hunan Province of China (2009TP4068-2), and The Fundamental Research Funds for the Central Universities (201023100001).

Introduction

Epilepsy is one of the most common chronic neurological conditions and its pathogenesis is unclear. The epileptic symptom is usually one of unprovoked seizures [1]. In general, an imbalance between excitatory and inhibitory neurotransmission has been regarded to be the main cause of seizures [2]. The development of epilepsy is associated with genetic factors. Only approx-

SUMMARY

Aims: To investigate the tolerability and efficacy of carbamazepine treatment in patients with partial-onset seizures and the association with polymorphisms in the sodium channel α -subunit type 1 (*SCN1A*), and *gamma*-aminobutyric acid (GABA) receptor genes among the Chinese Han population. **Methods:** 448 patients were genotyped for single nucleotide polymorphisms selected of the *SCN1A* and GABA-receptor genes. Monotherapy with carbamazepine (CBZ) was administered to the patients. The effectiveness of CBZ treatment was evaluated with regard to efficacy by the decrease in seizures and tolerability by retention rates. **Results:** *SCN1A* rs3812718 A/G with CBZ tolerability ($P = 0.038$) throughout 24 months of clinical follow-up and the *GABRA1* rs2290732 A/G were significantly associated with CBZ tolerability ($P = 0.001$). The maintenance dose and serum level of CBZ in AA genotype carriers of rs3812718 A/G were significantly higher than those of GG genotype carriers between 3 and 12 months of follow-up. The proportion of AA genotype carriers of rs2298771 A/G with seizure free was significantly higher than that of AG+GG genotype carriers from 3 months to 15 months of follow-up ($P < 0.05$). **Conclusion:** rs3812718 A/G and rs2290732 A/G polymorphisms affected the tolerability of CBZ. rs2298771 A/G was associated with efficacy of CBZ treatment.

imately 2% of cases of idiopathic epilepsy have been associated with monogenetic inheritance; most have been correlated with multiple genes [3, 4]. Epilepsy affects approximately 50 million people worldwide [5, 6]. In China, about seven to nine million individuals suffer from various types of epileptic seizures [7].

At present, treatment with antiepileptic drugs (AEDs) is the main approach to the control of epileptic seizures. Such therapy is used in an attempt to restore the balance between excitatory

and inhibitory transmission. Drugs, such as carbamazepine (CBZ), phenytoin, and lamotrigine inhibit hyperexcitable neuron transmission, whereas others, such as valproate, gabapentin, and vigabatrin, induce hypoexcitability. CBZ targets the sodium channel α -subunit type 1 (SCN1A), and is the most important first-line AED for medication of patients with partial seizures in China. In addition, studies have reported that CBZ may also potentiate *gamma*-aminobutyric acid (GABA) receptors [8, 9].

In the treatment of epilepsy, continuation of therapy ("drug tolerability") and seizure remission are the two main characteristics used to assess the effectiveness of AEDs [10, 11]. Usually, the tolerability of AEDs is measured by the retention rates (the proportions of patients who have continued to take the drug over a defined period) and seizure remission by a decrease in the rates of seizures. Investigations have shown that seizure remission could be maintained in 70% of epileptic patients, but that the remaining 30% developed drug resistance in spite of treatment with various AEDs [5, 12–14]. Meanwhile, drug resistance is one of the main factors in nonretention of AEDs.

In previous unpublished work with 448 initial participants, we found that almost 40% of patients with new-onset partial seizures could not continue taking CBZ monotherapy because of lack of efficacy, adverse effects, and other factors, and we also found that seizure remission was maintained after 1 year of treatment (data not published). We still detected heterogeneity in the effects of CBZ treatment that was related to polymorphisms in the *SCN1A* gene. The maintenance doses and serum levels, as well as the retention rates of CBZ in patients undergoing treatment were associated with the polymorphism rs3812718 A/G in *SCN1A*. In addition, a decrease in seizure rates was associated with the rs2298771 A/G polymorphism (data not published).

In the study reported herein, we investigated trends in the efficacy and tolerability of long-term treatment with CBZ using follow-up assessments of the participants at every 3-month intervals. We also investigated the association between genetic polymorphisms of *SCN1A* and of GABA receptors, which comprise α_1 , β_2 , and γ_2 subunits (encoded by the *GABRA1*, *GABRB2*, and *GABRG2* genes, respectively), and the efficacy and tolerability of CBZ treatment in Chinese patients of Han ethnicity with a new onset of partial seizures during 2 years of clinical follow-up.

Methods and Materials

Ethical Approval

The clinical trial of this study was registered in Chinese Clinical Trial Registry and the clinical trial registration number was ChiCTR-TCH-10000813. Before the beginning of the study, informed consent was obtained from each participant. The study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University.

Participants

In total, 448 patients who were more than 12 years old, suffered from idiopathic epilepsy, and had reported a new onset of partial seizures were recruited for the study. The study samples

were composed of 229 males and 219 females, who were administered CBZ monotherapy for the first time. All the patients were recruited from leading hospitals in Changsha City of China. The treatment period lasted from April 2008 to April 2011. The following were the inclusion criteria for the study: 1) patients had received a confirmed diagnosis of partial seizures; and 2) patients had never received AED treatment. The following patients were excluded: 1) patients who were pregnant or lactating; 2) patients with mental disorders, such as schizophrenia; 3) patients who suffered nonepileptic seizures; and 4) patients with any other medical conditions that might influence their liver and renal function. Informed consent was obtained, and the study protocol was approved by the Ethics Committee of Xiangya School of Medicine, Central South University before the study commenced.

Design

Definition and Determination of the Primary Characteristics of CBZ Tolerability and Efficacy

The tolerability and efficacy of CBZ were defined and evaluated as in previous investigations [10, 11]. In the current study, we focused on three primary outcomes: the serum levels of CBZ, tolerability to CBZ, and decrease in the frequency of seizures. The serum levels of CBZ were determined using high performance liquid chromatography (HPLC). The retention rates for CBZ were used to assess tolerability; they were calculated as the proportion of patients who had continued to take CBZ as medication for seizures over the preceding 3 months [15]. The cumulative survival proportion was used to estimate the tolerability of CBZ as long-term therapy. To estimate the efficacy of CBZ, four semi-quantitative levels of decrease in seizure frequency: seizure free (SF), the efficacy of 75%–SF, the efficacy of 50–75%, and the efficacy of <50%, were introduced in this study. We defined the efficacy of seizure free as a "good" response to medication, and the combination of 75%–SF, 50–75% and <50% efficacy as a "bad" response to medication. Our previous unpublished study revealed that the G allele of rs2298771 A/G was a risk factor for a bad response to medication under the dominant model (data not published). In the present study, we compared the efficacy of CBZ therapy in carriers of the AA genotype with that in AG+GG carriers in terms of a decrease in seizure rates.

Treatment Protocol

All recruited participants were treated with CBZ monotherapy. At the first and subsequent clinical visits, which occurred every 3 months during 2 years of treatment, clinical data were collected using questionnaires, reviews of medical records, including medical records, family members, and data from primary treating physicians. In the first visit, the data collected included sex, age, age at the first onset of epilepsy, risk factors for epilepsy, family history of epilepsy, medical and neurological history, classification of epilepsy and epileptic syndrome, number of seizures in the past 3 months, and electroencephalographic (EEG, tested by scalp electrodes), and imaging data obtained. For adolescents, the initial dose of CBZ was 200 mg/d with a weekly increase of 100 mg until

an appropriate value was reached. For adults and elderly patients, the initial dose was 200 mg/d with a daily increase of 100 mg until an appropriate value was reached. At every visit, the doses of CBZ taken, the serum levels of CBZ, and compliance were also recorded. In participants who experienced a recurrence of seizure, the doses of CBZ were increased. In the case of resistance to CBZ or a serious adverse reaction, CBZ was substituted with another AED. Reports of adverse reactions to the drug, including disorders of liver function, serum lipid levels, and changes in blood cells count, and the cause of discontinuation of medication were also recorded.

Selections of Candidate Genes and Single Nucleotide Polymorphisms (SNPs) and Genotyping of SNPs

We selected four candidate genes that are targeted by CBZ: *SCN1A*, *GABRA1*, *GABRB2*, and *GABRG2*. The *SCN1A* gene encodes an excitatory voltage-gated channel and the *GABRA1*, *GABRB2*, and *GABRG2* genes encode the α_1 , β_2 , and γ_2 subunits of the receptor for the inhibitory neurotransmitter GABA, respectively.

We selected SNPs according to a comprehensive consideration of the following criteria: 1) the minor allele frequency (MAF) was more than 10% in the Hapmap CHB (Chinese Han in Beijing) population; 2) tag SNPs was selected by SNPbrowser (version 4.0.1) in the Hapmap database (release 20, January 24, 2006), the selected SNPs had the parameters: pair-wise r^2 ($r^2 \geq 0.8$) and haplotype R^2 ($R^2 \geq 0.8$); and 3) the SNPs had validated functions in the promoter, exons, splice sites of introns or 3'-untranslated region (3'-UTR). Coefficients of linkage disequilibrium (D') and pairwise r values were used to assess the degree of linkage disequilibrium (LD) between pairs of loci using SNPbrowser [16]. All the SNPs selected were confirmed in the HapMap (<http://www.hapmap.org>) and National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/SNP/>) databases. The basic characteristics of the SNPs and the candidate genes are listed in Table 1. DNA was extracted from the peripheral blood of the participants using phenol–chloroform. All DNA samples were shipped to GenScript(Nanjing) Co., Ltd. and all SNPs were genotyped by Sequencing according to the manufacturer's instructions.

Statistical Analysis

All analyses were performed using the Statistical Package for Social Sciences, version 18.0 (SPSS Inc, Chicago, Illinois). The Pearson χ^2 test was used to assess whether the genotypic frequencies among the participants conformed to Hardy–Weinberg equilibrium (HWE), and also to evaluate differences in the proportions of participants who showed particular levels of seizures decrease among the carriers of the various genotypes within the cohort. Analysis of covariance (ANCOVA) was used to assess differences in the maintenance doses and serum levels of CBZ in carriers of various genotypes for all the SNPs, adjusted by age, sex, and body mass index (BMI; defined as the individual's body weight divided by the square of his or her height). Kaplan–Meier survival analysis (using time to event data) was used to assess the CBZ tolerability of

Table 1 Demographics and clinical characteristics of the recruited patients (n = 448)

Characteristics	Patients with epilepsy
Gender (M/F)	229/219
Adolescents (n, %)	141 (31.5)
Adults and elderly patients (n, %)	307 (68.5)
Age (years) (SD)	30.4 (16.7)
Adolescents	15.4 (5.2)
Adults and elderly patients	35.1 (19.5)
Height (m) (SD)	153 (8.7)
Adolescents	146 (12.4)
Adults and elderly patients	164 (7.9)
Weight (kg) (SD)	49.3 (9.5)
Adolescents	44.7 (10.8)
Adults and elderly patients	53.9 (9.8)
BMI (kg/m ²) (SD)	20.4 (3.0)
Adolescents	18.7 (3.6)
Adults and elderly patients	21.0 (3.2)
Seizure type (%)*	–
Simple partial	145 (32.4)
Complex partial	196 (43.8)
Secondary generalization	153 (34.2)
EEG (%)	–
Normal	185 (41.3)
Epileptiform	263 (58.7)

BMI, Body mass index.

EEG, Electroencephalography.

*Some patients might have suffered from more than one type of seizure.

the carriers of different genotypes of the *SCN1A* and GABA receptor genes. Logistic regression analysis was used to adjust the effect among SNPs and other potential factors. The dependent strength between SNPs was evaluated by the haploview software using the r and D' statistics. The test of significance was two-tailed, and alpha was set at 0.05.

Results

Characteristics of the Participants and SNPs Selected

Table 1 shows basic information at baseline for the 448 patients recruited. The 448 patients comprised 229 males and 219 females, of whom 141 were adolescents and 307 adults. The average age of all participants was 30.4 years; their mean height was 153 cm, weight 49.3 kg, and BMI 20.4 kg/m². Of the 448 participants, 145 (32.3%) suffered simple partial seizures, 196 (43.8%) complex partial seizures, 153 (34.2%) secondarily generalized seizures. Some patients might have suffered from more than one type of seizure. On electroencephalogram (EEG) examination, 185 participants (41.3%) showed normal traces and 263 (58.7%) epileptiform abnormalities.

Table 2 lists the basic characteristics of the SNPs selected for the *SCN1A* and *GABA* receptor genes. All SNPs conformed to HWE. The MAF of the all SNPs ranged from 12.0% to 46.2%.

Table 2 Characteristics of the SNPs selected for the *SCN1A* gene and GABA receptor genes in the recruited participants (n = 448)

SNP	Gene	Locus	Location (bp)	Allele	Function	MAF (%)	HWE <i>P</i>	Reference
rs2298771	SCN1A	exon	166601034	A/G	missense	13.0	0.408	[25]
rs3812718	SCN1A	intron	166617790	C/T	splice site	46.2	0.853	[32]
rs4667869	SCN1A	intron	166631605	C/G	NR	34.1	0.399	NR
rs11692675	SCN1A	intron	166634674	C/T	NR	18.9	0.517	NR
rs1020853	SCN1A	intron	166588028	G/T	NR	44.4	0.979	NR
rs10497275	SCN1A	3'-UTR	166554976	A/G	NR	33.1	0.989	NR
rs7577411	SCN1A	3'-UTR	166554040	A/G	NR	12.0	0.200	NR
rs1813502	SCN1A	3'-UTR	166554262	A/G	NR	21.1	0.161	NR
rs12658835	GABRA1	5'-UTR	161275302	A/G	promoter	29.3	0.986	[33]
rs2290732	GABRA1	3'-UTR	161324898	A/G	NR	38.8	0.055	[34]
rs35166395	GABRA1	exon	161281245	C/T	synonymous	42.4	0.602	[33]
rs592403	GABRB2	3'-UTR	160719984	G/T	NR	46.1	0.079	[29]
rs211037	GABRG2	exon	161528280	T/C	synonymous	43.8	1.00	[25]
rs424740	GABRG2	3'-UTR	161581035	A/T	NR	36.3	0.643	[35]

NR, not reported.

Allele, major/minor allele.

MAF, minor allele frequency.

HWE *P*, *P* value for Hardy–Weinberg equilibrium analysis.

Location (bp) from <http://hapmap.ncbi.nlm.nih.gov>.

The rs3812718 A/G and rs2290732 A/G SNPs were positively correlated with retention rates over 24 months of clinical follow-up

Significant differences existed in the retention rates of CBZ with respect to the rs3812718 A/G polymorphism of the *SCN1A* gene over the 24 months of clinical follow-up by Kaplan–Meier survival analysis ($P = 0.038$, Figure 1A). For 24 months of CBZ treatment, the overall retention rate was 50.7%. For the GG genotype, the retention rate was 57.3%, for the AG genotype 52.3%, and for the AA genotype 43.8%. Significant differences also existed in the retention rates of CBZ over the 24 months of clinical follow-up with respect to the rs2290732 A/G polymorphism of the *GABRA1* gene by Kaplan–Meier analysis ($P = 0.001$, Figure 1B). For 24 months of CBZ treatment, the overall retention rate was 50.7%. For the AA genotype it was 62.9%, for the AG genotype 52.5%, and for the GG genotype only 28.2%.

To assess whether rs3812718 and rs2290732 were independent from each other, binary logistic regression analysis was conducted with the tolerability of CBZ as a dependent variable. The results showed that, from the 3-month to the 6-month visit, neither rs3812718 nor rs2290732 was associated with the tolerability of CBZ (data not shown). From the 9-month to the 15-month visit, rs3812718 and rs2290732 were associated independently with the tolerability of CBZ. The association between rs3812718 and tolerability disappeared from the 18-month to the 24-month visit, but this was not the case for rs2290732. These findings indicated that rs3812718 and rs2290732 were associated independently with the tolerability of CBZ (Table 3).

We did not find significant differences in the retention rates for the other SNPs.

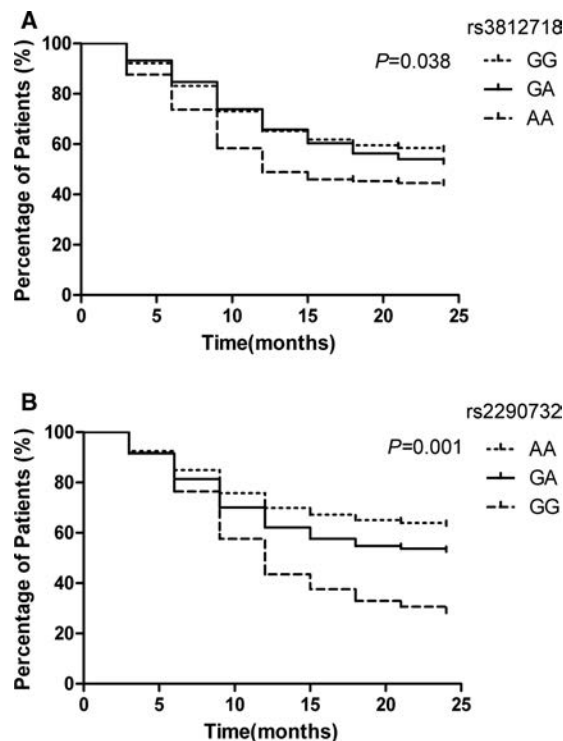


Figure 1 Retention rates for CBZ treatment of carriers of the various genotypes. (A) Retention rates of the carriers of the various rs3812718 A/G genotypes during 24 months of follow-up. (B) Retention rates of the carriers of the various rs2290732 A/G genotypes during 24 months of follow-up. * $P < 0.05$

Table 3 Association between rs3812718 G/A and rs2290732 A/G with the tolerability of CBZ

	9-month visit		12-month visit		15-month visit		18-month visit		21-month visit		24-month visit	
	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
rs3812718	0.520(0.275-0.981)	0.041	0.424(0.210-0.859)	0.017	0.534(0.322-0.887)	0.006	-	-	-	-	-	-
rs2290732	1.55(1.07-2.98)	0.023	2.03(1.23-3.36)	0.010	2.17(1.24-3.55)	0.001	2.31(1.34-3.87)	0.001	2.21(1.31-3.01)	0.001	2.39(1.27-3.99)	0.001

OR, odds ratio; CI, confidence interval.

OR (95% CI) was used to indicate the risk allele using a stepwise multivariate analysis by binary logistic regression; P-value adjusted for age, gender, BMI, seizure type, central adverse effect, allergic reaction, and other SNPs.

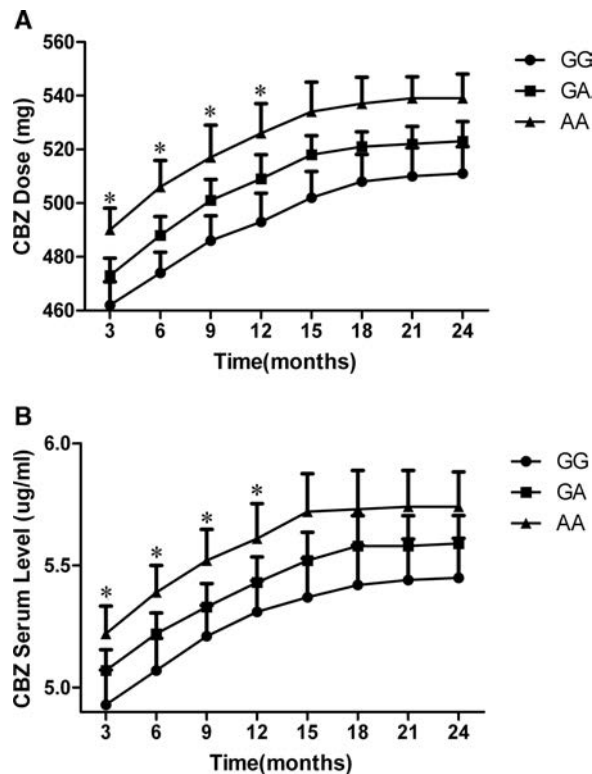


Figure 2 Trends in the maintenance dose and serum levels of CBZ. (A) Trends in the maintenance dose for CBZ (mean ± SE) among rs3812718 A/G genotypes. (B) Trends in the serum levels of CBZ (mean ± SE) among rs3812718 A/G genotypes. P values were analyzed using analysis of covariance (ANCOVA) during each visit to the clinic, after adjustments for age, sex, and BMI as potential compounding covariates. AA genotype carriers vs GG genotype ones. *P<0.05

The rs3812718 A/G SNP was positively correlated with the maintenance doses and serum levels of CBZ over 12 months of clinical follow-up

Figure 2 reveals the trends in the maintenance doses and serum levels of CBZ in the epileptic patients over the 24 months of clinical follow-up. There were relatively sharp increases in the maintenance doses and serum levels of CBZ for carriers of the AA, AG, or GG genotype from 3 to 15 months of treatment. However, the increases were less dramatic from 15 to 24 months of treatment. ANCOVA showed that the maintenance doses and serum levels of CBZ in carriers of the AA genotype of rs3812718 A/G were significantly higher than those of carriers of the GG genotype from 3 to 12 months of follow-up, when adjusted for potential confounding factors, such as age, gender, and BMI (Figure 2, P < 0.05). No significant difference existed between carriers of the AG and GG genotypes within 24 months. Furthermore, the significance difference between AA carriers and GG carriers disappeared from 15 to 24 months of follow-up.

We did not find significant differences in the maintenance doses and serum levels of CBZ in relation to the other SNPs.

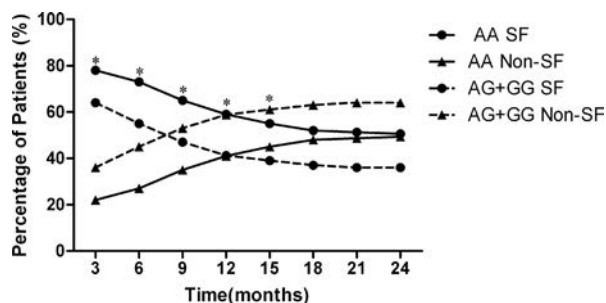


Figure 3 Trends in CBZ efficacy in carriers of various genotypes. The Pearson χ^2 was used to analyze the differences between carriers of the AA genotypes and carriers of the AG+GG genotypes with respect to the level of efficacy. The proportion of patients with seizure free (SF) among AA genotype carriers was significantly higher than that among AA+GG genotype carriers. Non-SF indicated the combination of 75%–SF efficacy + 50–75% efficacy + < 50% efficacy. * $P < 0.05$

The rs2298771 A/G SNP was positively correlated with decrease in seizure rates within 15 months of clinical follow-up

Our data revealed that, from 3 months to 24 months of follow-up, the proportion of patients with seizure free decreased persistently for carriers of either the AA or AG+GG genotypes of the rs2298771 A/G, and the proportion that showed 75%–SF, 50–75%, and <50% efficacy increased gradually (data not shown). This finding indicated that the efficacy of CBZ decreased during an extended treatment period. The Pearson χ^2 analysis showed the significance of the differences in the levels of efficacy between the carriers of the AA and AG+GG genotypes. The proportion of patients with a “good” response to medication among carriers of the AG+GG genotype was significantly lower than that among carriers of the AA genotype from 3 months to 15 months of follow-up (Figure 3, $P < 0.05$). However, no significance differences were found at the other time points.

Meanwhile, we found no significant differences in the levels of efficacy for the other SNPs.

Discussion

We investigated the trends in the retention rates, maintenance doses, and serum levels of CBZ among patients undergoing CBZ treatment during 24 months of clinical follow-up. We also evaluated the efficacy of CBZ for the treatment of epilepsy with respect to polymorphisms in the *SCN1A* and GABA receptor genes. We included both adolescents and adults in our study cohort because adolescents’ physiological characteristics are similar to those of adults. With regard to the demographics and clinical characteristics of the participants, our findings differed in some aspects from those of Hu et al. [17], including the proportion of the distribution of the types of seizure. These differences might originate from differences in the age distribution of the two different cohorts, and in the researchers’ choice for subjects, as well as other environmental factors.

In the treatment of epilepsy, seizure recurrence and resistance to AEDs are two formidable challenges. CBZ can induce upregu-

lation of *SCN1A* and drug transporters [7, 18–20], which might reduce the response of voltage-gated channels and even cause the development of tolerance or resistance to the drug. In our previous unpublished work, logistic regression analysis identified many factors that might affect CBZ retention rates for CBZ treatment, including allergic skin reactions, poor efficacy, central adverse effects, and genetic factors. This is consistent with the findings of Hu et al. [17]. Many previous studies have reported retention rates among patients undergoing AED treatment for epilepsy [17, 21–24]. Using Kaplan–Meier survival curves, we revealed a relationship between the retention rates during long-term CBZ treatment and different genotypes of the *SCN1A* and GABA receptor genes.

Among the genes that encoded different subunits of the GABA receptor, the results of our study indicated that only the rs2290732 A/G SNP of the *GABRA1* gene was correlated positively with the CBZ retention rates over 2 years of clinical follow-up. A recent study [25] has shown that *GABRA1* IVS11+15A (rs2279020) is significantly correlated with drug resistance. The fact that resistance to CBZ leads to poor efficacy is one of the main contributors to a decrease in retention rates. Target mutations could lead to varying sensitivity to CBZ treatment in different patients [26, 27]. Interestingly, our LD analysis found a high-LD value between rs2279020 and rs2290732 in the Caucasian population ($D' = 1$, $r^2 = 1$, HapMap Data Rel 28 Phase, August 10, on NCBI B36 assembly, dbSNP b126).

In a previous study, which involved administration of CBZ to patients with epilepsy for 12 months, we found that the maintenance doses and serum levels of CBZ in carriers of the AA genotype of rs3812718 A/G were significantly higher than those in carriers of the GG genotype, which is consistent with the findings of Tate and colleagues [28]. However, up to now, this finding has been controversial. In the previous study, we elucidated the inconsistencies in other studies [23, 29–31]. The differences among studies may be associated with many factors, including genetic differences, sample size, the age distribution of the cohort, environmental factors, and physicians’ bias. In the current study, the differences appeared from 15 to 24 months of follow-up (Figure 2). This may have originated from changes in the size of the sample. The sample sizes for the visits from 15 to 24 months were smaller than the ones from 3 to 12 months because some patients had ceased to take CBZ. Intriguingly, when Figure 1A was compared with Figure 2, we found that the maintenance doses and serum levels of CBZ in the carriers of different genotypes of rs3812718 A/G showed an inverse association with CBZ tolerability for the respective genotype carriers. This indicated that larger doses of CBZ could provoke more severe adverse reactions, which would affect the tolerability of CBZ.

Polymorphism at rs2298771 A/G could affect the structure and function of *SCN1A*, which is targeted by CBZ. This might have caused the variation in the efficacy of epilepsy treatment among carriers of different genotypes. However, the effect requires further elucidation by electrophysiological and pharmacological investigation during the course of long-term therapy. Moreover, further study is clinically needed to confirm the results.

In summary, in the study reported herein, we revealed that variations in the maintenance dose and serum levels of CBZ in

relation to the rs3812718 A/G polymorphism of the *SCN1A* gene could be partially responsible for the differences in CBZ tolerability that were associated with different genotypes. The rs2290732 A/G polymorphism of the *GABRA1* gene might also affect CBZ tolerability. Polymorphism at rs2298771 A/G could cause variations in drug efficacy because of alterations in the structure and function of the SCN1A receptor. These data might aid clinical management by allowing personalized CBZ treatment to be applied to patients with epilepsy.

Acknowledgments

We thank leading hospitals of Changsha City of China for help and collaboration, as well as our patients and volunteers for their

support for the investigation. We also thank the National Natural Science Foundation of China (81173129), Program for Changjiang Scholars and Innovative Research Team in University (IRT0946), The Science and Technology Plan Key Grant of Hunan Province of China (2009TP4068–2), and The Fundamental Research Funds for the Central Universities (201023100001).

Conflict of Interest

The authors declare no conflict of interest.

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