

Impact of *ABCB1* Polymorphisms on Lacosamide Serum Concentrations in Uygur Pediatric Patients With Epilepsy in China

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Background: P-glycoprotein, encoded by *ABCB1* (or *MDR1*), may contribute to drug resistance in epilepsy by limiting gastrointestinal absorption and brain access to antiseizure medications. The study aimed to evaluate the impact of *ABCB1* polymorphisms on lacosamide (LCM) serum concentrations in Uygur pediatric patients with epilepsy.

Methods: The serum concentrations of LCM were determined by ultrahigh performance liquid chromatography, and the *ABCB1* polymorphism was analyzed through polymerase chain reaction–fluorescence staining in situ hybridization. The χ^2 test and the Fisher exact test were used to analyze the allelic and genotypic distributions of *ABCB1* polymorphisms between the drug-resistant and drug-responsive patient groups. Differences in steady-state and dose-corrected LCM serum concentrations between different genotypes were analyzed using the one-way analysis of variance and the Mann–Whitney test.

Results: A total of 131 Uygur children with epilepsy were analyzed, and of them, 41 demonstrated drug resistance. The frequency of the GT genotype of *ABCB1* G2677T/A was significantly higher in the drug-resistant group than that in the drug-responsive group ($P < 0.05$, OR = 1.966, 95% CI, 1.060–3.647). Patients with the G2677T/A-AT genotype had a statistically significantly lower concentration-to-dose (CD)

value than patients with the G2677T/A-GG genotype (mean: 0.6 ± 0.2 versus 0.8 ± 0.5 mcg/mL per mg/kg, $P < 0.001$). Significantly lower LCM serum concentrations were observed in *ABCB1* C3435T CT and TT genotype carriers than those in the CC carriers ($P = 0.008$ and $P = 0.002$), and a significantly lower LCM CD value was observed in *ABCB1* C3435T CT genotype carriers than that in the CC carriers ($P = 0.042$).

Conclusions: *ABCB1* G2677T/A and C3435T polymorphisms may affect LCM serum concentrations and treatment efficacy in Uygur pediatric patients with epilepsy, leading to drug resistance in pediatric patients.

Key Words: *ABCB1*, epilepsy, lacosamide, Uygur

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BACKGROUND

Epilepsy is a neurological brain disorder, and according to an epidemiological survey, the incidence of epilepsy among children in China is approximately 7 cases observed per 1000 individuals.¹ The current primary clinical treatment of epilepsy comprises antiseizure medications (ASMs).² However, approximately 25%–30% of patients with epilepsy fail to become seizure free or become resistant to treatment and, as a result, experience recurrent seizures.³

Lacosamide (LCM) is a novel n-methyl-D-aspartic acid receptor glycine site antagonist, which plays a unique anti-convulsive role by selectively enhancing the slow inactivation of voltage-gated sodium channels.⁴ In August and October 2008, LCM was approved in Europe and the United States, respectively, for the treatment of partial-onset seizures with or without secondary generalization in adults, adolescents, and children as of age 4 years with epilepsy.^{5,6} Multiple studies have demonstrated that LCM exhibits favorable short-term and long-term efficacy, tolerability, and safety in the treatment of patients with epilepsy.^{7–9} LCM was approved in China in 2018. In our previous study, we demonstrated that the LCM showed 69% effectiveness in treating patients with epilepsy.¹⁰ However, in our routine treatment drug monitoring studies, we demonstrated that an increased number of children with epilepsy developed drug resistance.

Genetic factors play an important role in the underlying mechanism of antiepileptic drug resistance.¹¹ P-glycoprotein (P-gp), encoded by the *ABCB1* (or *MDR1*) gene, is an extensively studied drug efflux transporter involved in epilepsy, which may limit gastrointestinal absorption and limitation

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of brain access to ASMs.¹² Previous studies have shown that *ABCB1* genetic polymorphisms may affect the activity of the transporter efflux in the endothelial cells of the blood–brain barrier, thereby influencing the concentrations of ASMs and causing treatment failure.¹¹ The single-nucleotide polymorphisms (SNPs) G2677T (rs2032582) in exon 21 and C3435T (rs1045642) in exon 26 are the most studied SNPs of the *ABCB1* gene.^{13,14} Several studies have indicated that *ABCB1* polymorphisms may influence the concentration and responsiveness of ASMs (eg, carbamazepine, lamotrigine, oxcarbazepine, and gabapentin).^{15–20} Zhang et al²¹ demonstrated that LCM is a substrate of P-gp.

At present, no reports have been published showing the association between *ABCB1* polymorphism and LCM serum concentrations and treatment efficacy. This study was performed to evaluate the association of G2677T/A and C3435T genotypes of *ABCB1* and their haplotypic and diplotypic combinations with LCM serum concentrations and efficacy in Uyur pediatric patients with epilepsy. The purpose of this study was to provide a valuable tool for predicting the clinical efficacy of LCM before treatment and hence contribute to personalized treatment of Uyur pediatric patients with epilepsy.

MATERIALS AND METHODS

Collection of Demographic Details of Pediatric Patients

A total of 165 pediatric patients with epilepsy who were administered LCM from 2018 to 2021 at the People's Hospital of Xinjiang Uyur Autonomous Region, China, were initially included in this study. A total of 34 pediatric patients with epilepsy were excluded because of incomplete data. Finally, 131 pediatric patients with epilepsy who received LCM treatment were included.

All pediatric patients met the criteria for the diagnosis of epilepsy issued by the International League against Epilepsy in 2017.²² This study was approved by the Ethics Committee of People's Hospital of Xinjiang Uyur Autonomous Region (Xinjiang, China; ethical approval number: KY2019120614). Parents of all patients signed an informed consent form.

All subjects were regularly administered LCM tablets in accordance with the study protocol. Participants were presumed to be drug resistant if treatment with LCM using monotherapy or combined with other ASMs administered for at least 12 months at maximally tolerated doses failed and epileptic seizures persisted.²³ In addition, drug responsiveness was considered when the patient was completely free from seizures for at least 1 year during treatment with LCM using monotherapy or combined with other ASMs administered at optimal tolerated therapeutic doses.

Materials and Reagents

LCM was purchased from UCB Pharma, Brussels, Belgium (purity >99%). Methanol and acetonitrile (chromatogram chemicals) were acquired from Fisher Ltd, Shanghai, China. Ammonium acetate was purchased from

Sangon Biotech, Shanghai, China. A Qiagen DNA extraction kit was purchased from Gentra Ltd, Chicago, IL. A sequencing reaction kit was purchased from Sangon Biotech.

Therapeutic Drug Monitoring of LCM

The initial dose of LCM was 2 mg·kg⁻¹ daily, which was increased once a week. The target dose was 5–20 mg·kg⁻¹ daily for 3–4 weeks, which was followed by blood sampling after a maintenance dose was administered. From each pediatric patient, 4–6 mL of venous blood was obtained for drug assays just before the morning LCM dose was administered (approximately 12 hours after the evening dose, trough concentration).

Blood was divided into 2 tubes: 2–3 mL was transferred into an EDTA anticoagulant tube (used for DNA extraction) and 2–3 mL was transferred into a biochemical tube. Biochemical tubes were immediately centrifuged at 4000g (–4°C) for 5 minutes, and serum was transferred into a clean tube and stored at –80°C.

The LCM serum concentrations were measured using validated ultrahigh performance liquid chromatography; Waters Ltd, Shanghai, China. Chromatography was performed using Waters ACQUITY UPLC BEH (C18, 2.1 × 100 mm, 1.7 μm). Ammonium dihydrogen phosphate solution (10 mmol·l⁻¹)–methanol (55:45, v/v, adjust pH 4.0 with phosphoric acid) was used as the mobile phase. The flow rate was 0.2 mL·minute⁻¹. The injection volume was 2 μL. The detection wavelength was 210 nm.

The method was linear within 0.5–40 mcg·mL⁻¹ for LCM ($r = 0.9997$). The intraday and interday precision as measured by the relative SD values were between 1.36% and 4.50%. Recovery ranged from 96.58% to 106.22%. All serum samples were stable for up to 3 hours at ambient temperature, 24 hours at 4°C, 30 days at –80°C, and after 6 successive freeze–thaw cycles (24 hours per cycle) without any significant degradation.

DNA Extraction and Genetic Analysis

Genomic DNA was extracted from whole blood using a standard method (<http://www.qiagen.com/>). Two SNPs in the *ABCB1* gene (G2677T and C3435T) were genotyped through a polymerase chain reaction (PCR) assay using BigDye (Applied Sanger sequencing Technologies) followed by restriction fragment length polymorphism (RFLP) analysis.

PCR and RFLP products were analyzed by gel electrophoresis using 2% agarose gels. Amplification of the polymorphisms was performed by PCR using the following forward and reverse primers: *ABCB1* G2677T/A (rs2032582): 5'-GCTTTAGTAATGT TGCCGTGAT-3' and 5'-GAAGAACAGTGTGAAGACAATGG-3', and *ABCB1* C3435T (rs1045642): 5'-ATCACACAACTTTTCCTTAATCTC-3' and 5'-ACCCAGACTCTGTACTTGGGA CTTAA-3'.

The fragments were amplified with 10.0 mM dNTPs, 10 mM MgCl₂, nuclease-free water, reaction buffer, 20 μmol/L of primers, and 5.0 U Taq polymerase in a final volume of 25 μL using a PCR Master Mix. The following conditions were used for PCR of the SNPs (rs2032582 and rs1045642): 95°C for 5 minutes, 30 cycles (94°C for 30 seconds, 63°C for 30 seconds, and 72°C for 60 seconds), and 72°C for 10 minutes.

TABLE 1. Sequences of Primers Used in the Study and the Sizes of the Amplicons for Each SNP

SNP	Exon	Primer Forward	Primer Reverse	Amplicon Size (bp)
<i>ABCB1</i> G2677T/A	21	GCTTTAGTAATGTTGCCGTGAT	GAAGAACAGTGT GAAGACAATGG	748
<i>ABCB1</i> C3435T	26	ATCACACAAACTTTT CCTTAATCTC	ACCCAGACTCTGT ACTTGGACTTAA	714

bp, base pair.

The details of the primers and fragment sizes are provided in Table 1. The amplicon size was 748 bp and 714 bp in the *ABCB1* G2677T/A (rs2032582) and *ABCB1* C3435T (rs1045642), respectively. The results of PCR–RFLP were confirmed through DNA sequencing of a random selection of samples (5%) using the ABI Prism 3730XL Genetic Analyzer (Applied Biosystems, Carlsbad, CA) and ABI Prism BigDye Terminator v3.1 Cycle Sequencing Kit. The results of gel electrophoresis and DNA sequencing of each genotype are presented in Figure 1.

Statistical Analysis

Analyses were performed using SPSS version 19.0 software (version 4.0.100.1124, Chicago, IL), and a *P* value of <0.05 was considered statistically significant. The χ^2 test (2 × 2 contingency tables) was performed to compare the allelic and genotypic distributions of *ABCB1* G2677T, C3435T, and *ABCB1* polymorphisms between the drug-resistance group and the drug-responsive group. The one-way analysis of variance and the Mann–Whitney test were used to analyze the differences in LCM serum concentrations in pediatric patients with epilepsy with different genotypes.

RESULTS

Characteristics of Pediatric Patients

In this study, a total of 131 Uyghur pediatric patients with epilepsy (aged 4–14 years) were included. The mean age at the initiation of LCM therapy was 7.8 years, and 63% of pediatric patients with epilepsy were male (n = 82). Most of the children with epilepsy (99%) received LCM twice a day, and only 1 child (1%) was administered LCM once daily. At the last follow-up, 27 pediatric patients were administered LCM monotherapy, 67 pediatric patients received valproic acid combined therapy, 45 pediatric patients received oxcarbazepine combined therapy, 37 pediatric patients received levetiracetam combined therapy, 17 pediatric patients received lamotrigine combined therapy, 3 pediatric patients received topiramate combined therapy, and 1 pediatric patient received clonazepam combined therapy. The clinical characteristics of the patients are presented in Table 2.

Pediatric patients were divided into 2 groups: the drug-responsive group (n = 90, 69%) and the drug-resistant group (n = 41, 31%). Multivariate analysis of the factors affecting LCM response showed that there were no significant differences between the drug-sensitive group and the drug-resistant group regarding age, sex, weight, body mass index, medication time, LCM dose, LCM serum concentration, and the ratio of

concentration-to-dose (CD) (*P* > 0.05). However, significant differences were observed in the types of seizures, abnormal electroencephalogram, and concomitant ASMs between the drug-responsive group and the drug-resistant group who were administered LCM (*P* < 0.05) (Table 2).

To further analyze the influence of each factor affecting the response to LCM, comparisons between groups and response outcomes were performed using the χ^2 test or Fisher exact test for qualitative variables, and the Student *t* test or Mann–Whitney *U* test was used for quantitative variables. Significant differences were observed in the type of seizure (focal onset and combined generalized and focal onset), abnormal electroencephalogram, monotherapy, concomitant cytochrome P450 enzyme inducers (phenytoin, carbamazepine, oxcarbazepine, and lamotrigine), and concomitant sodium channel blockers (valproic acid, levetiracetam, and topiramate) between the drug-responsive group and the drug-resistant group (*P* < 0.05) (Table 2).

Genotype and Allele Frequencies of ABCB1 SNPS

Hardy–Weinberg Genetic Equilibrium Test

All *ABCB1* polymorphisms studied followed Hardy–Weinberg equilibrium in drug-resistant and drug-responsive patients (*P* > 0.05), which indicated that the research subjects were representative of the Uyghur pediatric patients with epilepsy.

ABCB1 Genotype and Allele Frequencies

The genotype frequencies of *ABCB1* G2677T/A did not differ significantly between drug-resistant and drug-responsive pediatric patients with respect to TT (*P* = 0.149, OR = 1.161, 95% CI, 0.544–2.475), AG (*P* = 0.384, OR = 1.465, 95% CI, 0.618–3.475), and AT (*P* = 0.809, OR = 1.123, 95% CI, 0.436–2.895) genotypes (Table 3). However, the frequency of the GG genotype of *ABCB1* G2677T/A was significantly lower in the drug-resistant group compared with that of the drug-responsive group (*P* < 0.05, OR = 0.374, 95% CI, 0.202–0.693), and the frequency of the GT genotype of *ABCB1* G2677T/A was significantly higher in the drug-resistant group compared with that of the drug-responsive group (*P* < 0.05, OR = 1.966, 95% CI, 1.060–3.647) (Table 3).

The frequency of *ABCB1* C3435T genotypes did not differ significantly between drug-resistant and drug-responsive patients for the CC genotype (*P* = 0.317, OR = 0.751, 95% CI, 0.428–1.317), CT genotype (*P* = 0.391, OR = 1.278, 95% CI, 0.729–2.239), and TT genotype (*P* = 0.841, OR = 1.084, 95% CI, 0.493–2.383) (Table 3). No significant differences in frequency distribution of alleles were observed

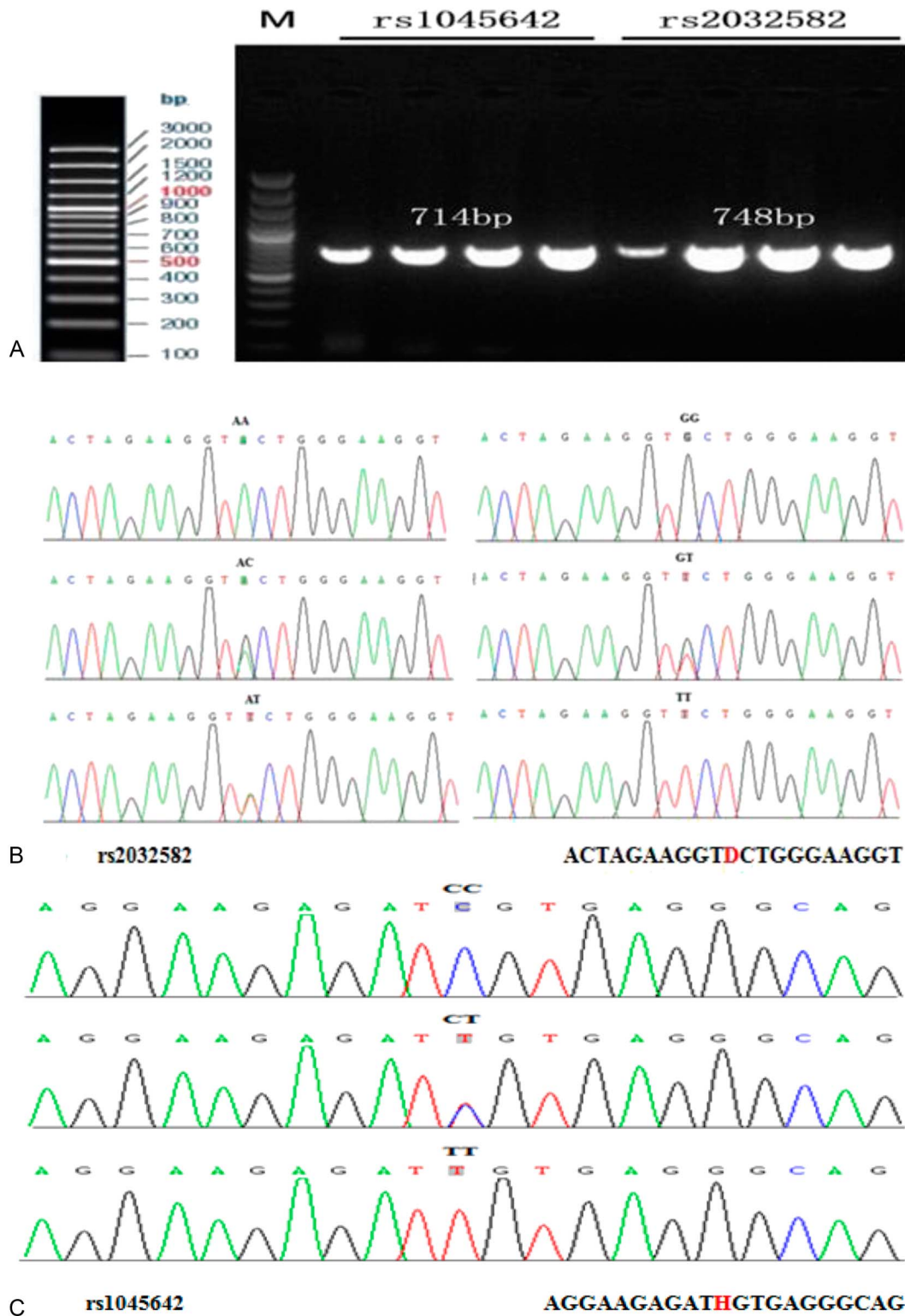


FIGURE 1. Determination of G2677T/A and C3435T genotypes of *ABCB1* through gel electrophoresis after PCR-RFLP analysis and verification by DNA sequencing. M: marker. A, PCR amplification of the loci G2677T and C3435T digested by *Ban*I. M: marker. B, The results of DNA sequencing of the G2677T/A genotype. C, The results of DNA sequencing of the C3435T genotype.

TABLE 2. Statistical Analysis of Qualitative and Quantitative Variables Between the Effective Group and Ineffective Group (the Mean ± SDs)

Characteristic	Resistance Group, (n = 41)	Responsive Group (n = 90)	Multivariate Analysis		Univariate Analysis		
			F/ χ^2	P	95% Confidence Interval	t/ χ^2	P
Mean age ± SD (yrs)	8.8 ± 5.0	7.4 ± 3.9	2.686	0.104	−2.914 to 0.274	−1.639	0.104
Gender (M/F)							
Male	23 (56)	59 (66)	2.102	0.147	0.861 to 2.702	2.102	0.147
Female	18 (44)	31 (34)					
Weight (kg)	35.5 ± 23.8	29.4 ± 14.9	3.127	0.079	−12.811 to 0.718	−1.768	0.079
Body mass index (kg·m ^{−2})	19.0 ± 5.5	18.2 ± 3.7	1.073	0.302	−2.458 to 0.769	−1.036	0.302
Medication time (yrs)	1.6 ± 1.3	1.6 ± 1.4	0.014	0.907	−0.149 to 0.133	−0.114	0.909
Type of seizure, n (%)							
Generalized onset	9 (22)	24 (27)	11.598	0.009*	0.687 to 2.505	0.676	0.411
Focal onset	12 (29)	39 (43)			1.028 to 3.318	4.253	0.039*
Combined generalized and focal onset	14 (34)	13 (14)			0.157 to 0.636	10.965	<0.001**
Unknown onset	6 (15)	14 (16)			0.502 to 2.322	0.038	0.845
Abnormal EEG, n (%)	38 (93)	56 (62)	27.556	<0.001**	0.052 to 0.292	27.556	<0.001**
LCM dose (mg/(kg·d))	7.2 ± 3.4	7.0 ± 2.4	0.142	0.707	−1.371 to 0.980	−0.333	0.740
LCM serum concentration (mcg/mL ^{−1})	5.6 ± 3.0	5.5 ± 2.5	0.053	0.818	−1.184 to 0.952	−0.214	0.829
CD (mcg/mL per mg/kg)	0.9 ± 0.6	0.9 ± 0.5	0.315	0.576	−0.277 to 0.161	−0.530	0.598
Concomitant ASMs, n (%)							
Monotherapy	4 (5)	23 (19)	11.558	0.073	0.080 to 0.628	9.280	0.002*
Valproic acid	27 (35)	40 (33)			0.609 to 1.963	0.089	0.765
Oxcarbazepine	21 (27)	24 (20)			0.765 to 2.861	1.363	0.243
Levetiracetam	16 (21)	21 (18)			0.601 to 2.442	0.287	0.592
Lamotrigine	6 (8)	11 (9)			0.325 to 2.379	0.064	0.800
Topiramate	2 (3)	1 (1)			0.313 to 2.715	1.020	0.312
Clonazepam	1 (1)	0 (0)			1.748 to 2.311	1.005	0.316
Concomitant ASMs, n (%)							
Monotherapy	4 (10)	23 (25)	18.824	<0.001**	1.355 to 6.642	7.792	0.005*
Polytherapy (with inducers)	10 (24)	35 (39)			1.100 to 3.726	5.214	0.022*
Polytherapy (with sodium channel blockers)	27 (66)	32 (36)			0.162 to 0.518	18.007	<0.001**

Bold indicates statistical significance.

P* < 0.05; *P* < 0.001.

CD, concentration-to-dose ratio; EEG, electroencephalogram.

between the 2 groups (*P* = 0.556, OR = 0.841, 95% CI, 0.471–1.498).

ABCB1 Polymorphisms and Drug Responsiveness: Haplotype and Diplotype Frequencies

In the patient groups, the polymorphisms of the 2 loci, G2677T/A and C3435T, in the ABCB1 gene were observed with strong linkage disequilibrium (LD) (*D'* = 0.70). The frequencies of 3-marker haplotypes are presented in Table 3. There were 6 possible haplotypes, which were estimated and compared between the drug-resistant group and the drug-responsive group. In the drug-resistant and drug-responsive groups, all haplotypes were present, and all haplotypes except A-T were overrepresented at a percentage higher than 5%.

We also performed a diplotype analysis of G2677T/A and C3435T polymorphisms. Table 3 shows that in either group, the 6 diplotype configuration frequencies were above 5%. The diplotype (GT-CT) carrier frequency was significantly higher in the drug-resistant group compared with that in the drug-responsive group (*P* < 0.05, OR = 1.994, 95% CI, 1.013–3.926). However, the diplotype (GG-CT) carrier frequency was significantly lower in the drug-resistant group compared with that in the drug-responsive group (*P* < 0.05, OR = 0.206, 95% CI, 0.043–0.981).

Associations Between ABCB1 Polymorphisms and LCM Serum Concentrations

The mean LCM dosages during the maintenance phase were 7.2 ± 3.4 mg/(kg·d) and 7.0 ± 2.4 mg/(kg·d) in the

TABLE 3. Genotype, Haplotype, and Diplotype Frequencies of *ABCB1* Polymorphisms in Drug-Resistant (n = 41) and Drug-Responsive (n = 90) Pediatric Patients With Epilepsy

SNP	Genotype	Genotype Frequencies		Odds Ratio (95% Confidence Interval)	χ^2	P
		Drug-Resistance Group, n (%)	Drug-Responsive Group, n (%)			
<i>ABCB1</i> G2677T/A	GG	9 (22)	39 (43)	0.374 (0.202–0.693)	10.051	0.002*
	GT	15 (37)	21 (23)	1.966 (1.060–3.647)	4.667	0.031*
	TT	7 (17)	13 (15)	1.161 (0.544–2.475)	0.149	0.700
	AG	6 (14)	9 (10)	1.465 (0.618–3.475)	0.758	0.384
	AT	4 (10)	8 (9)	1.123 (0.436–2.895)	0.058	0.809
	G [#]	39 (48)	108 (60)	0.615 (0.351–1.078)	2.899	0.089
	T [#]	33 (40)	55 (31)	1.484 (0.828–2.658)	1.769	0.184
	A [#]	10 (12)	17 (9)	1.480 (0.593–3.692)	0.712	0.399
<i>ABCB1</i> C3435T	CC	16 (39)	41 (46)	0.751 (0.428–1.317)	1.003	0.317
	CT	19 (46)	36 (40)	1.278 (0.729–2.239)	0.734	0.391
	TT	6 (15)	13 (14)	1.084 (0.493–2.383)	0.040	0.841
	C [#]	51 (62)	118 (66)	0.841 (0.471–1.498)	0.347	0.556
	T [#]	31 (38)	62 (34)			
Haplotype	G–C	28 (29)	63 (34)	0.793 (0.436–1.442)	0.579	0.447
	G–T	14 (14)	29 (16)	0.855 (0.393–1.860)	0.157	0.692
	T–C	18 (19)	31 (17)	1.145 (0.556–2.358)	0.136	0.713
	T–T	24 (25)	38 (21)	1.254 (0.648–2.428)	0.452	0.502
	A–C	9 (9)	16 (8)	1.137 (0.420–3.078)	0.064	0.800
	A–T	4 (4)	7 (4)	—	—	—
Diplotype	GT–CT	12 (29)	15 (17)	1.994 (1.013–3.926)	4.065	0.044*
	GG–CC	8 (20)	28 (31)	0.556 (0.291–1.064)	3.185	0.074
	AG–CC	6 (15)	7 (8)	2.029 (0.819–5.028)	2.407	0.121
	TT–TT	5 (12)	8 (9)	1.379 (0.554–3.434)	0.479	0.489
	AT–CT	4 (10)	7 (8)	1.278 (0.482–3.384)	0.244	0.621
	TT–CT	1 (2)	5 (5)	0.388 (0.073–2.047)	1.332	0.248
	GG–CT	1 (2)	8 (9)	0.206 (0.043–0.981)	4.714	0.030*
	Others†	4 (10)	12 (13)	0.744 (0.310–1.785)	0.442	0.506

Bold indicates statistical significance.

* $P < 0.05$; ** $P < 0.001$.

†Haplotypes and diplotypes with total frequencies below 5% over the 2 groups.

drug-resistant and drug-responsive groups, respectively. The mean LCM serum concentrations were 5.6 ± 3.0 mcg/mL and 5.5 ± 2.5 mcg/mL in the drug-resistant and drug-responsive groups, respectively.

Table 4 shows that the *ABCB1* G2677T/A polymorphism had a significant influence on the LCM CD value. Patients with the G2677T/A-AT genotype had a statistically significantly lower CD value compared with patients with the G2677T/A-GG genotype (mean: 0.6 ± 0.2 versus 0.8 ± 0.5 mcg/mL per mg/kg, $P < 0.001$) (Table 4 and Fig. 3).

Moreover, the *ABCB1* C3435T polymorphism had a significant influence on LCM serum concentrations and CD values. Significantly lower LCM serum concentrations were observed in *ABCB1* C3435T CT and TT genotype carriers compared with those in CC carriers ($P = 0.008$ and $P = 0.002$), and a significantly lower LCM CD value was observed in *ABCB1* C3435T CT genotype carriers compared with that in CC carriers ($P = 0.042$) (Table 4 and Figs. 2, 3).

DISCUSSION

Several studies have demonstrated that the correlation between *ABCB1* polymorphism and drug resistance of epilepsy is not consistent in different geographical regions and countries.^{3,24–33} Our research group previously studied the effect of the polymorphisms in the P-glycoprotein-encoding gene, *ABCB1* (G2677T/A and C3435T), on the clinical efficacy of levetiracetam in Uyur children with epilepsy.³⁴ We found that *ABCB1* G2677T/A and C3435T may affect levetiracetam disposition and the therapeutic efficacy in Uyur children with epilepsy.³⁴ Moreover, multiple studies have demonstrated an association between *ABCB1* polymorphism and ASM resistance.^{3,24–30} Ponnala et al²⁴ revealed that CT and TT genotype carriers of the *MDR1* gene demonstrated more recurrent seizures compared with other carriers. The *MDR1* C3435T gene polymorphism affects serum phenytoin levels ($P < 0.015$). Taur et al²⁵ found that the P-gp activity was higher in nonresponders (n = 68) compared with that in the responders (n = 47) ($P < 0.001$). Moreover, Stasiotek et al²⁸ found that the C3435T poly-

TABLE 4. Effects of the *ABCB1* Genotypes on Adjusted LCM Serum Concentrations

SNP	Genotype	Number (%)	Serum Concentration (ug/mL)	95% Confidence Interval	F/t	P	CD (mcg/mL per mg/kg)	95% Confidence Interval	F/t	P
<i>ABCB1</i> G2677T/A	GG	48 (37)	5.3 ± 2.7	—	<i>F</i> = 2.258	0.067	0.8 ± 0.5	—	<i>F</i> = 5.674	<0.001**
	GT	36 (28)	6.1 ± 2.1	−1.907 to 0.347			1.0 ± 0.4	−0.432 to 0.194		
	TT	20 (15)	6.2 ± 3.5	−2.284 to 0.434			1.2 ± 0.8	−0.649 to 0.105		
	AG	15 (11)	4.0 ± 1.8	−0.209 to 2.809			0.5 ± 0.3	−0.046 to 0.609		
	AT	12 (9)	5.2 ± 2.3	−1.498 to 1.794			0.6 ± 0.2	−0.094 to 0.565		
<i>ABCB1</i> G2677T/A	GG	48 (37)	5.3 ± 2.7	−1.270 to 0.660	<i>t</i> = −0.628	0.532	0.8 ± 0.5	−0.287 to 0.105	<i>t</i> = −0.919	0.360
	GT + TT + GA + AT	83 (63)	5.6 ± 2.6				0.9 ± 0.6			
<i>ABCB1</i> C3435T	CC	57 (44)	6.4 ± 2.8	—	<i>F</i> = 5.026	0.008*	1.0 ± 0.6	—	<i>F</i> = 3.249	0.042*
	CT	55 (42)	4.9 ± 2.5	0.308 to 2.597			0.8 ± 0.5	0.0006 to 0.505		
	TT	19 (14)	5.0 ± 2.2	−0.259 to 2.949			1.0 ± 0.6	−0.358 to 0.349		
<i>ABCB1</i> C3435T	CC	57 (44)	6.4 ± 2.8	0.520 to 2.330	<i>t</i> = 3.120	0.002*	1.0 ± 0.6	−0.014 to 0.388	<i>t</i> = 1.841	0.068
	CT + TT	74 (56)	5.0 ± 2.4				0.8 ± 0.5			

Bold indicates statistical significance.
P* < 0.05; *P* < 0.001.

morphism of the *MDR1* gene may be associated with the incidence of drug-resistant epilepsy among Polish children. Furthermore, Yu et al²⁶ demonstrated that the *ABCB1* G2677T/A polymorphism may increase the risk of drug-resistant epilepsy in Asians.

Some studies demonstrated an absence of association between *ABCB1* polymorphism and resistance to ASMs in patients with epilepsy.^{31–33} Haerian et al³¹ suggested that *ABCB1* C1236T, G2677T/A, and C3435T haplotypes do not contribute to the response to ASM treatment in epilepsy. Vahab et al³² indicated that there are no statistically significant differences between the *ABCB1* allele and genotype frequencies of refractory and drug-responsive epilepsy among patients. Moreover, Dong et al³³ showed a lack of association between the *ABCB1* (C1236T, G2677T/A, and C3435T) gene polymorphisms and pharmacoresistant epilepsy in a western Chinese pediatric population.

Our results suggest that GT genotypes of *ABCB1* G2677T/A, that is, the mutant heterozygous type, were more frequent in patients who demonstrated resistance to LCM and that the GG genotypes of *ABCB1* G2677T/A, that is, the wild type, were more frequent in patients who were responsive to LCM. Hence, the mutant heterozygous type was associated with an increased risk of LCM resistance. Our results are consistent with the findings presented in the previous report by Ponnala et al,³⁴ Stasiołek et al,²⁶ and Yu et al.²⁵ However, the observations are

inconsistent with the results published by Haerian et al,³¹ Vahab et al,³² and Dong et al.³³ These conflicting results reinforce the need to examine the functional significance of *ABCB1* polymorphisms in different ethnic groups. Discrepancies in the results of different studies may be attributed to ethnic differences in the frequencies of *ABCB1* genotypes and haplotypes.

At present, haplotype data are obtained through statistical calculation and are based on the distribution frequency of the genotypes in the population of interest.³⁵ The group led by Chouchi and Kwan et al showed that a haplotype of the *ABCB1* gene was associated with drug resistance.^{3,36} However, results presented by other groups, such as Zimprich et al and Vahab et al, did not indicate that a haplotype of the *ABCB1* gene was associated with drug resistance.^{32,35} The results of our study showed that there were no significant differences in the distribution frequencies of the *ABCB1* G2677T/A and C3435T haplotype combination between the drug-resistant and drug-responsive groups. G-C, G-T, T-C, and T-T haplotypes were the predominant types in our drug-resistant and drug-responsive groups (>10%). In our study, we also performed diplotype analysis of G2677T/A and C3435T polymorphisms. The GT-CT, GG-CC, and TT-TT diplotypes were the predominant types in our drug-resistant and drug-responsive groups (>10%). Except for GT-CT and GG-CT diplotypes, no statistically significant differences in the frequency of all other diplotype combina-

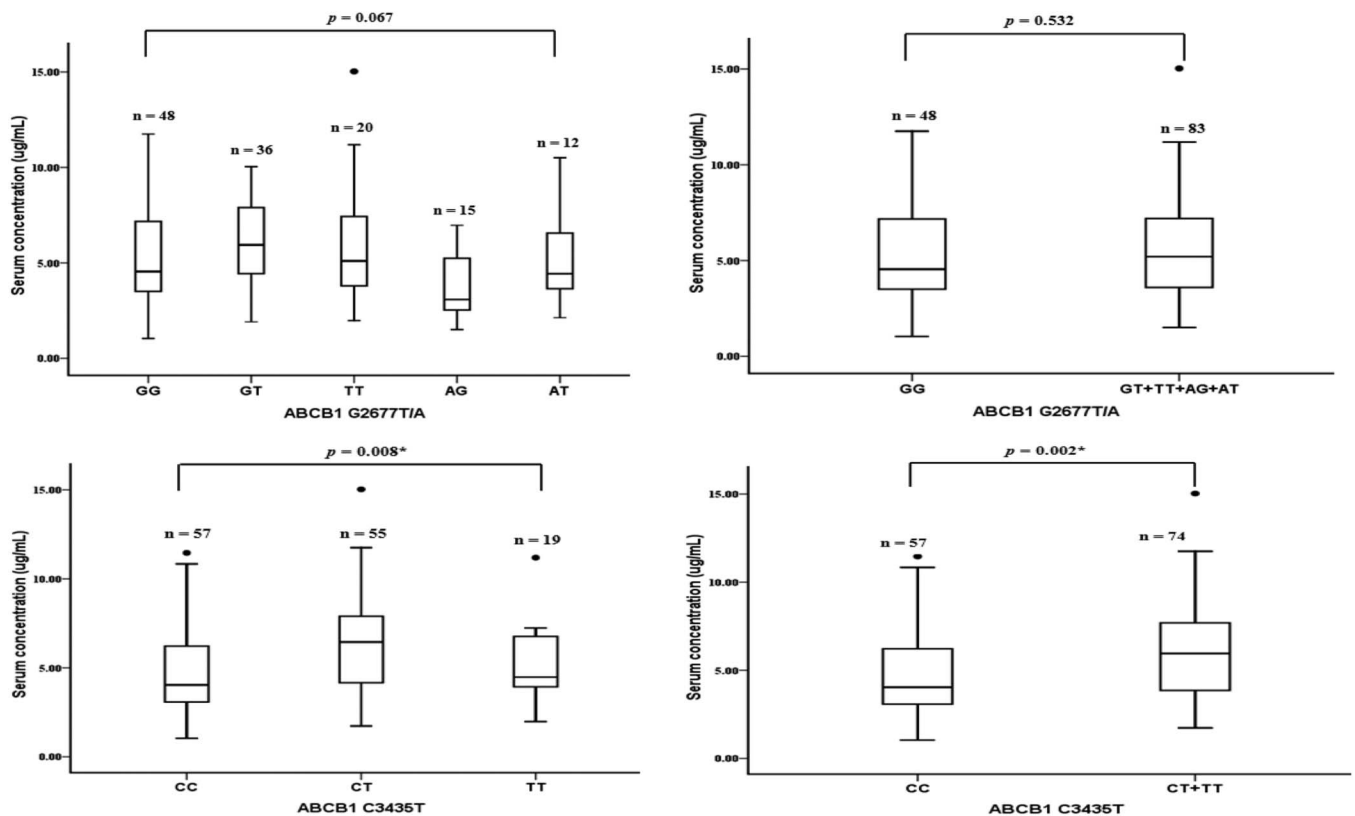


FIGURE 3 Box plots illustrating the associations between *ABCB1* G2677T/A and *ABCB1* C3435T allele with LCM serum concentrations.

tions of *ABCB1* G2677T/A and C3435T were observed between the 2 groups.

Several studies have indicated that *ABCB1* polymorphisms may be associated with ASM (eg, carbamazepine, lamotrigine, oxcarbazepine, and gabapentin) concentration and responsiveness.^{15–19} Lovric et al¹⁶ demonstrated that *ABCB1* polymorphisms influenced lamotrigine trough concentrations and should be taken into account for dose adjustment. Tran et al¹⁹ indicated that *ABCB1* 2677G>T/A genotypes significantly influenced the absorption rate constant ($P < 0.05$) of gabapentin in healthy Korean individuals. Wang et al¹⁷ indicated that the *ABCB1* gene may contribute to responsiveness to carbamazepine and carbamazepine-10, 11-epoxide transport among patients with epilepsy who were treated with carbamazepine in combination with phenytoin or phenobarbital. Shen et al¹⁸ reported that the genetic polymorphism of *ABCB1* rs1045642 was associated with a normalized oxcarbazepine concentration and the therapeutic efficacy in patients with epilepsy ($P < 0.05$). Meng et al¹⁵ demonstrated that Chinese patients with the *ABCB1*3435-TT genotype had significantly lower adjusted carbamazepine concentrations than patients with the 3435-CC genotype.

No reports are available demonstrating the associations of *ABCB1* polymorphism with LCM serum concentrations and efficacy in pediatric patients with epilepsy. This study was the first to evaluate the impact of polymorphisms in the P-glycoprotein-encoding gene *ABCB1* on LCM disposition in

Chinese Uyghur pediatric patients with epilepsy. The results of this study show that *ABCB1* G2677T/A and *ABCB1* C3435T polymorphisms had a significant effect on the LCM serum concentration. Significantly lower CD values were found in *ABCB1* G2677T/A AG and AT genotype carriers compared with those in GG genotype carriers ($P < 0.001$). In addition, the *ABCB1* C3435T polymorphism had a significant effect on LCM serum concentrations and CD values. Moreover, significantly lower LCM serum concentrations were found in *ABCB1* C3435T CT and TT genotype carriers compared with those in CC carriers ($P < 0.05$), and significantly higher LCM CD values were found in *ABCB1* C3435T CT genotype carriers compared with those in CC carriers ($P < 0.05$). Our study results suggest that 2677-AG, AT and 3435-CT, TT may reduce P-gp activity, inhibit gastrointestinal absorption of LCM, and ultimately reduce serum concentration of LCM.

At present, identifying the underlying mechanism of ASM drug resistance is a significant challenge in the treatment of epilepsy. Current studies on the mechanism(s) underlying ASM resistance mainly include the target hypothesis and transporter hypothesis.^{37,38} In this study, we explored the correlation between drug transporter P-gp and LCM resistance and demonstrated that *ABCB1* G2677T/A and C3435T gene polymorphisms correlated with the development of drug resistance in pediatric patients who were treated with LCM. Changes in sodium ion channels and GABA_A receptors can cause seizures, which were found to be related to epileptic drug resistance. LCM plays an antiepileptic

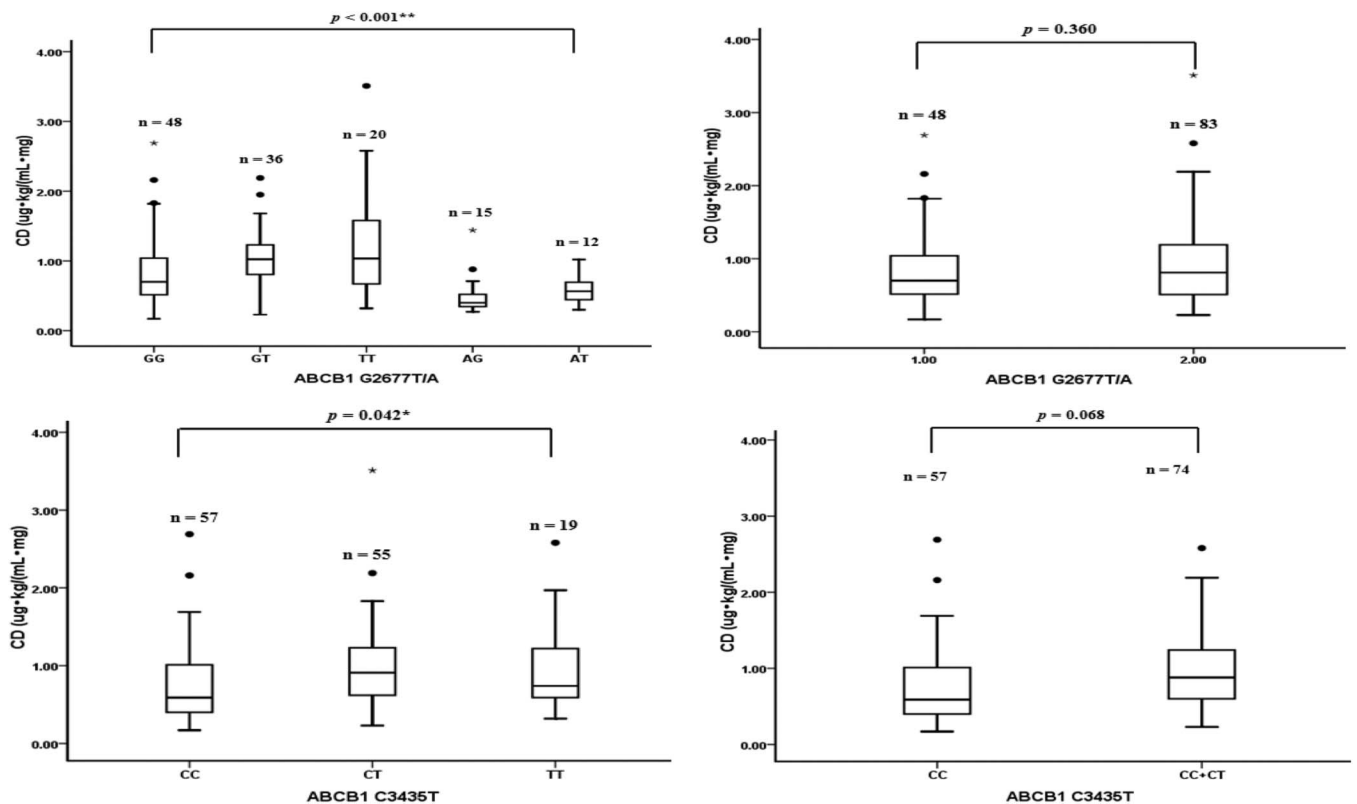


FIGURE Box plots illustrating the associations between *ABCB1* G2677T/A and *ABCB1* C3435T allele with the LCM concentration-to-dose (CD) ratio.

role mainly by binding to the sodium channel subunit to inactivate it. However, if the expression of the sodium channel subunit is reduced or its structure is changed, LCM cannot play an antiepileptic role, leading to drug resistance in patients with epilepsy. We will test the point hypothesis in a follow-up study and will explore the relationship between sodium ion channels and GABA_A receptors, and drug resistance in epilepsy.

This study has some limitations. First, multiple factors may affect LCM pharmacokinetics and treatment outcomes; therefore, the possibility of confounders remains, such as other SNPs. Second, some objective factors, including sex and regional differences, and subjective factors, including clinical efficacy evaluation, epileptic drug resistance judgment, and the patient’s expression of the condition, limited this study. Finally, the sample size was small. Therefore, the association between *ABCB1* polymorphisms and LCM serum concentrations in patients with epilepsy should be verified using large ambidirectional methods.

CONCLUSION

ABCB1 G2677T/A and C3435T polymorphisms may affect LCM serum concentrations and treatment efficacy in Uyghur pediatric patients with epilepsy, leading to drug resistance in pediatric patients. Future studies should be performed with a larger cohort and explore the regulatory mechanism of *ABCB1* genetic variations.

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