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Impact of *ABCB1* Polymorphism on Levetiracetam Serum Concentrations in Epileptic Uygur Children in China

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Background: Interindividual variations in the efficacy of antiseizure medications make epilepsy treatment challenging. This is due to genetic factors such as gene polymorphisms in Adenosine-triphosphate (ATP)-binding cassette sub-family B member 1 (*ABCB1*). In this article, the impact of polymorphisms in the P-glycoprotein-encoding gene, *ABCB1* (C1236T, G2677T/A, and C3435T), on levetiracetam disposition was evaluated in Uygur Chinese children with epilepsy.

Methods: MDR1 C3435T polymorphism was analyzed by polymerase chain reaction–fluorescence staining in situ hybridization. The χ^2 test and Fisher exact test were used to analyze the allelic and genotypic distribution of *ABCB1*, C1236T, G2677T, and *C3435T* between the drug-resistant and drug-responsive groups. Differences in steady-state and dose-corrected levetiracetam serum concentrations between the different genotypes were analyzed using 1-way analysis of variance and Mann–Whitney test.

Results: Total 245 Uygur children with epilepsy were analyzed [drug-resistant, n = 117 (males:females = 53:64) and drug-responsive, n = 128 (males:females = 76:52)]. The frequency of *ABCB1* C1236T, G2677T/A, and *ABCB1* C3435T genotypes, alleles, haplotypes, or diplotypes did not differ significantly between the 2 groups ($P > 0.05$). Significantly higher levetiracetam concentrations and serum concentration/body mass dose were seen in *ABCB1* 2677-GT, TT, GA, and AT genotypes and 3435-TT carriers compared with GG and CC carriers ($P = 0.021$ and $P = 0.002$ versus $P = 0.001$ and $P = 0.000$, respectively).

Conclusions: *ABCB1* G2677T/A and C3435T may affect levetiracetam disposition and therapeutic efficacy in Uygur children with epilepsy. Genetic analysis could be a valuable tool for predicting the response to antiseizure medications before the start of treatment and could contribute to personalized medicine for Uygur children with epilepsy.

Key Words: *ABCB1*, epilepsy, levetiracetam, serum concentration, Uygur children

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BACKGROUND

Epilepsy is one of the most common neurological conditions.[1] To date, the best clinical treatment for this disease relies on the rational use of antiseizure medications (ASMs).[2,3] However, at least one-third of epileptic patients are or will become resistant to treatment and experience recurrent seizures.[4,5]

Transporters, especially P-glycoprotein (P-gp), may affect the absorption of several drugs, because they are actively transported from the blood to the gastrointestinal tract. P-gp belongs to a superfamily of different transporters or efflux pumps, known as Adenosine-triphosphate (ATP)-binding cassette (ABC), involved in multidrug resistance (MDR), and are an expanding area in pharmacogenetics.[6,7] Overexpression of these transporters has been demonstrated in the brains of patients with resistant epilepsy. The overexpression of P-gp in excretory organs suggests that it has a central role in drug elimination and may be coupled to subtherapeutic serum concentrations of ASMs.[8]

The most studied single nucleotide polymorphisms (SNPs) in *ABCB1* are C1236T (rs1128503) in exon 12, G2677T (rs2032582) in exon 21, and C3435T (rs1045642) in exon 26.[9] Multiple studies have demonstrated that there is an association between *ABCB1* polymorphism and ASM resistance.[10–16] However, some studies have shown no association among *ABCB1* polymorphism and ASM resistance.[17–19] These conflicting results reinforce the need to check the functional significance of *ABCB1* polymorphisms in different ethnic groups. In previous studies, it was found that *ABCB1* genetic polymorphisms may affect the efflux activity of transporters in the endothelial cells of the blood–brain barrier, influence the concentration of ASMs, and subsequently contribute to the failure of ASM treatment.[20] These *ABCB1* polymorphisms may be associated with the concentrations and responsiveness of ASMs such as carbamazepine, lamotrigine, oxcarbazepine, and gabapentin.[21–26]

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At present, no report is available on the association of *ABCB1* polymorphisms with levetiracetam serum levels and treatment efficacy. This study was performed to evaluate the association of C1236T, G2677T/A, and C3435T genotypes of *ABCB1* and their haplotypic and diplotypic combinations with levetiracetam serum levels and treatment efficacy in Uygur children with epilepsy in Xinjiang, China.

MATERIALS AND METHODS

Study Participants

Between 2016 and 2019, 245 cases that met the diagnostic criteria for epilepsy were identified at the Department of Neurology and Pediatrics in People's Hospital of Xinjiang Uygur Autonomous Region (Xinjiang, China). Patients were regularly treated with levetiracetam tablets or oral solution. The initial dose was 10 $mg \cdot kg^{-1}$ daily and was increased once a week. The target dose was 20–60 $mg \cdot kg^{-1}$ daily for 3–4 weeks, which was followed by blood sampling after a maintenance dose was reached. This study was approved by the Ethics Committee of People's Hospital of Xinjiang Uygur Autonomous Region (Xinjiang, China). All study participants provided signed informed consent.

Patients were classified as drug-resistant or drug-responsive, according to the definition set by the International League Against Epilepsy.[27] Patients were presumed to be drug-resistant if the treatment with levetiracetam as a monotherapy or in combination with other ASMs correctly prescribed for at least 12 months, at maximal tolerated doses, failed and epileptic seizures persisted.[28] Patients were considered drug-responsive if they were totally free from seizures for at least 1 year during treatment with levetiracetam as a monotherapy or in combination with other ASMs correctly prescribed at optimal tolerated therapeutic doses.[27,29]

Serum Concentration Detection

Chromatography was performed using a Waters ACQUITY UPLC BEH C_{18}, 2.1 × 100 mm, 1.7 μm particle size column, protected by a guard precolumn with a graphite filter. The mobile phase was a mixture of ammonium acetate solution (10 mmol/L) with acetonitrile (88:12, vol/vol). The pH of the mobile phase was set at 4.0 (with acetic acid solution). The flow rate of the mobile phases was 0.1 mL min^{-1}, and the injection volume was 1 μL. The detection wavelength was 210 nm.

DNA Extraction and Genetic Analysis

Genomic DNA extraction was performed using a standard Qiagen kit, following manufacturer's instructions (http://www.qiagen.com/). *ABCB1* C1236T, G2677T, and C3435T were genotyped by a polymerase chain reaction (PCR) assay, using Big Dye™ (BigDye Terminator v1.1, Thermo Fisher Scientific, Waltham, MA), followed by restriction fragment length polymorphism analysis. The following forward and reverse primer sequences were designed for PCR analysis: *ABCB1* rs1128503: 5′-GTTCACTTCA GTTACCCATCTCG-3′ and 5′-TCATCTC ACCATCCCCTCTGT-3′; *ABCB1* rs2032582: 5′-ATTATATC CTTCATCTATGGTTGGC-3′ and 5′-TTAGAGCATAGTA A GCAGTAGGGAG-3′; *ABCB1* rs1045642: 5′-GTTCCTCAAG GCATACAATTATGAC-3′ and 5′-ACCCAGACTCTGTACT TGGACTTAA-3′′. The results of gel electrophoresis and DNA sequencing were stored as images of each genotype (Fig. 1).

Statistics

Statistical analysis was performed using SPSS version 19.0 software (version 4.0.100.1124, Chicago, IL). In addition, linkage disequilibrium (LD) analysis and haplotype construction were performed by using SHEsis online software.[30] The χ^2 test was performed to compare the allelic and genotypic distribution of *ABCB1* between the drug-resistant group (patient group) and the drug-responsive group (control group). Differences in steady-state and dose-corrected levetiracetam serum concentrations between different genotypes were analyzed using 1-way analysis of variance and Mann–Whitney test.

RESULTS

Characteristics of the Study Population

In this study, a total of 245 Uygur children with epilepsy (aged 1–18 years) were included, of which 129 were men and 116 were women. There were 117 drug-resistant patients, constituting the "drug-resistant group" and 128 drug-responsive patients, constituting the "drug-responsive group." There was a statistical difference noted between the serum drug concentrations of levetiracetam and the serum concentration/body mass dose ratios (CDR) between the 2 groups. The clinical characteristics of the patients are presented in Table 1.

Genotype and Allele Frequencies of *ABCB1* Single Nucleotide Polymorphisms

Hardy–Weinberg Genetic Equilibrium Test

All *ABCB1* polymorphisms studied followed the Hardy–Weinberg equilibrium in drug-responsive patients ($P > 0.05$), which indicated that the included patients were representative of the entire group. However, in drug-resistant patients, the G2677T/A polymorphism exhibited a deviation from the Hardy–Weinberg equilibrium.

ABCB1 Genotype and Allele Frequencies

The genotype frequencies of *ABCB1* C1236T did not significantly differ between the drug-resistant and drug-responsive patients with respect to CT [$P = 0.087$, odd ratio (OR) = 0.513, 95% confidence interval (CI) = 0.237–1.109] and TT ($P = 0.166$, OR = 0.576, 95% CI = 0.263–1.262) genotypes (Table 2). No significant differences were observed at the allele level ($P = 0.318$, OR = 0.830, 95% CI = 0.575–1.197).

The genotype frequencies of *ABCB1* G2677T/A did not significantly differ between drug-resistant and drug-responsive patients with respect to TT ($P = 0.127$, OR = 0.517, 95% CI = 0.221–1.210), GA ($P = 0.255$, OR = 1.011, 95% CI = 0.206–1.526), and AT ($P = 0.347$, OR = 0.517, 95% CI = 0.129–2.071) genotypes (Table 2). However, the GT genotype frequency of *ABCB1* G2677T/A was significantly different between the drug-resistant group and the drug-responsive group ($P = 0.046$, OR = 0.484, 95% CI = 0.236–0.993).

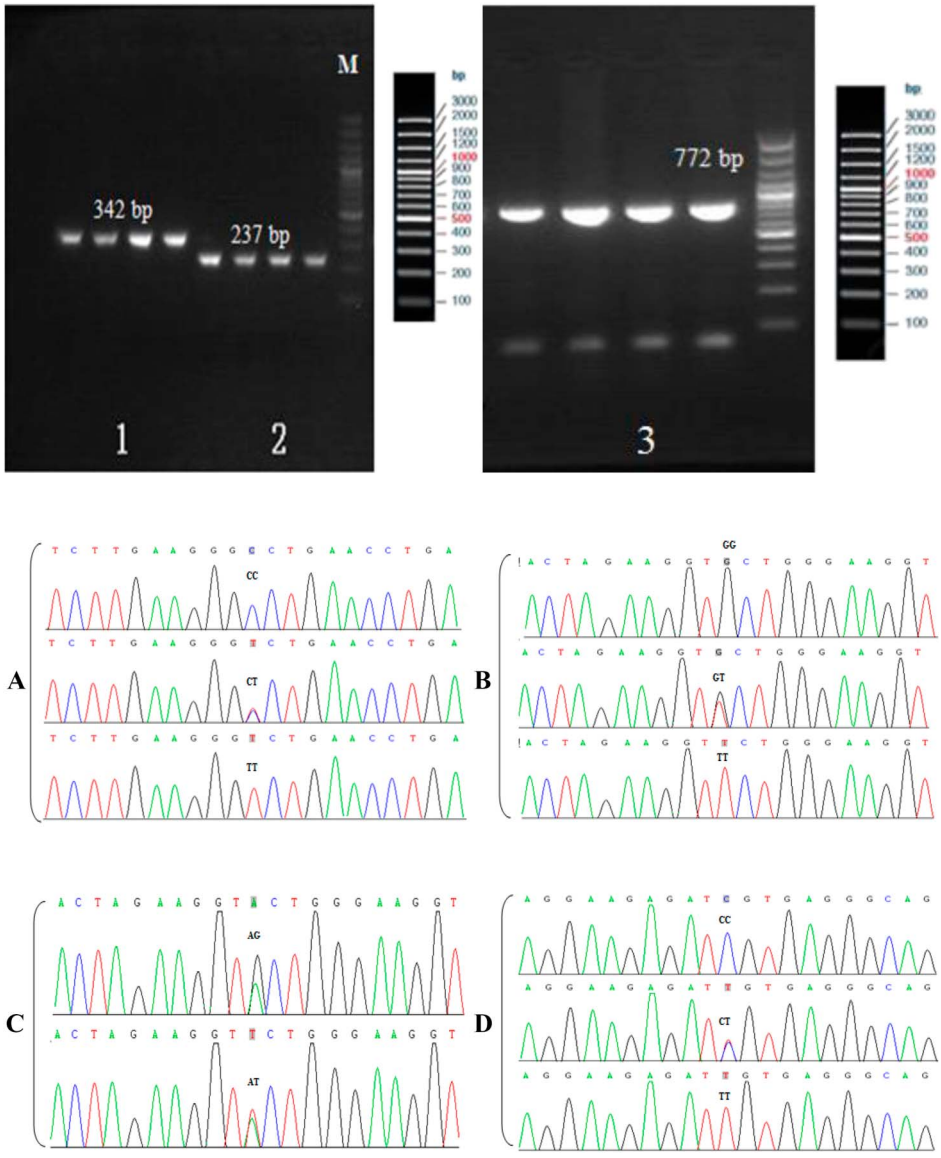

FIGURE 1. Determination of C1236T, G2677T/A, and C3435T genotypes of *ABCB1* by gel electrophoresis after polymerase chain reaction–restriction fragment length polymorphism analysis and verification by DNA sequencing test. (1) PCR amplifications of locus C1236T digested by EcoO109I. M: marker. (2) PCR amplifications of locus G2677T digested by BanI. M: marker. (3) PCR amplifications of locus C3435T digested by MboI. M: marker. The results of DNA sequencing pictures of C1236T, G2677T/A, and C3435T genotypes are shown, and the SNP positions are indicated by letters (A), (B), (C), and (D), respectively.

The genotype frequencies of *ABCB1* C3435T did not significantly differ between drug-resistant and drug-responsive patients with respect to CT (P = 0.128, OR = 0.636, 95% CI = 0.354–1.141) and TT (P = 0.408, OR = 0.747, 95% CI = 0.374–1.492) genotypes (Table 2). No significant differences were observed at the allele level (P = 0.317, OR = 0.834, 95% CI = 0.584–1.191).

Haplotype and Diplotype Association of *ABCB1* Polymorphisms

C1236T was observed to be in strong Linkage disequilibrium (LD) with G2677T/A in the drug-resistant and drug-responsive groups (D' = 0.70 and D' = 0.81, respectively). The P-values were set at <0.05, which suggested that these polymorphisms were in strong LD. The G2677T/A (synonymous) genotype was observed to be in strong LD with C3435T (nonsynonymous) in both the drug-resistant and drug-responsive groups (D' = 0.63 and D' = 0.61, respectively). This tight genetic linkage indicates the importance of haplotypes or multiple genetic variants from *ABCB1* for conducting phenotype–genotype correlation studies and avoiding spurious single SNP associations.

In both the drug-resistant and drug-responsive groups, all haplotypes existed, and the frequencies of each combination of *ABCB1* diplotypes were not significantly different. Table 2 shows 6 diplotype configuration frequencies above 5% in either group. Despite the minor over representation of the 5 diplotypes (CT-GT-CT, TT-GT-CT, TT-TT-TT, CT-GG-CC, CT-GT-CC, and CT-AG-CC), carriers in the drug-resistant group, and the frequencies of each combination of *ABCB1* diplotypes were not significantly different when compared with the drug-responsive group.

TABLE 1. Clinical Characteristics of Patients With Epilepsy (Mean ± SD)

Characteristic	Drug-Resistant Group (n = 117)	Drug-Responsive Group (n = 128)	t/χ^2	*P*
Mean age ± SD, yrs	6.00 ± 4.57	6.46 ± 6.50	0.482	0.630
Gender (M/F)				
Male	53 (45)	76 (59)	3.926	0.066
Female	64 (55)	52 (41)		
Body mass index, $kg \cdot m^{-2}$	24.43 ± 15.26	24.32 ± 15.66	−1.712	0.088
Dose, $mg \cdot kg^{-1} \cdot d^{-1}$	36.42 ± 13.55	37.85 ± 12.07	8.840	0.609
Steady-state plasma concentrations, $\mu g \cdot mL^{-1}$	12.60 ± 4.36	14.09 ± 4.67	6.890	0.037*
CDR, $\mu g \cdot mL^{-1} \cdot kg \cdot mg^{-1}$	0.37 ± 0.13	0.40 ± 0.13	−21.267	<0.001*
Type of seizure, n (%)				
Generalized seizure	101 (86)	90 (70)	7.459	0.006*
Focal seizure	16 (14)	38 (30)		
Drugs of the last visit				
Monotherapy	44 (38)	75 (59)	8.828	0.003*
2 drugs	39 (33)	25 (19)	4.338	0.037
3 drugs	32 (27)	24 (19)	1.807	0.179
4 drugs	2 (2)	4 (3)	0.205	0.651

*P-value < 0.05.

Association Between *ABCB1* Polymorphism and Serum Concentration of Levetiracetam

No significant difference was observed in levetiracetam concentration and CDR with respect to the *ABCB1* C1236T genotype among all the patients (Table 3). However, the *ABCB1* G2677T/A polymorphism significantly influenced levetiracetam concentration and CDR values. Significantly higher levetiracetam concentrations and CDR values were found in *ABCB1* G2677T/A GT, TT, GA, and AT genotype carriers compared with GG carriers ($P = 0.021$ and $P = 0.001$) (Table 3). In addition, the *ABCB1* C3435T polymorphism significantly influenced levetiracetam concentrations and CDR values. Significantly higher levetiracetam concentrations and CDR values were found in *ABCB1* C3435T TT genotype carriers compared with CC and CT carriers ($P = 0.002$ and $P = 0.000$) (Table 3).

DISCUSSION

Epilepsy can occur at any age and its incidence peaks in the first few years of life and in the elderly. ASMs display extensive pharmacological variability between and within patients and a major determinant of differences in response to treatment is pharmacokinetic variability. These differences are attributed to genetic factors, including sex and ethnicity. However, the pharmacokinetics of ASMs can also be affected by age, specific physiological states in life, or pathological conditions.[6] Levetiracetam elimination occurs primarily by renal excretion. In addition, newborns, infants older than 2–3 months, and children show higher drug clearance (normalized for body weight) than adults.[31] Glauser et al [32] found that the apparent clearance rate/bioavailability (CL/F) values in children and infants were comparable with those reported in children aged 6–12 years[33] and higher than those reported in adults.[34] Because all these factors contribute to the overall pharmacological variability in children, pharmacogenetics is only one among the many factors. The impact of pharmacogenetics testing alone is, therefore, insufficient. Serum concentrations should be monitored carefully to examine factors that result in the optimal exposure in each patient.

Several studies have shown that the correlation between MDR1 C3435T polymorphism and drug resistance in epilepsy is not completely consistent in different geographical regions and countries. In many studies, it was confirmed that the high expression of P-gp was closely related to drug resistance in epilepsy.[13–19] Yu et al[13] indicated that *ABCB1* G2677T/A polymorphism may increase the risk of drug-resistant epilepsy in Asians. In addition, Malek et al[18] reported that C1236T, G2677T, and C3435T polymorphisms were involved in ASM resistance in Tunisian patients. By contrast, some studies showed that there is no association between *ABCB1* polymorphism and ASM resistance in epileptic patients.[19–21] Lin et al demonstrated that there were no significant differences in the frequencies of genotypes, alleles, haplotypes, or diplotypes of *ABCB1* polymorphisms between patients with drug-resistant and drug-responsive epilepsy.[19] Furthermore, Lv et al[21] reported that there was no significant association between the MDR1 C3435T polymorphism and overall risk of drug resistance.

Our results demonstrated no significant association between the genotypes, haplotypes, or diplotypes of C1236T, G2677T/A, and C3435T and levetiracetam resistance, which was consistent with the previous reports of Lin et al, Armond et al, and Lv et al . Discrepancies in the results of different studies may be attributed to ethnic differences in the frequencies of *ABCB1* genotypes and haplotypes.

TABLE 2. Genotype and Haplotype and Diplotype Frequencies of *ABCB1* C1236T, G2677T/A, and C3435T Polymorphisms in Drug-Resistant (n = 117) and Drug-Responsive (n = 128) Epilepsy Patients

		Genotype Frequencies		
SNP	**Genotype**	**Drug-Resistance Group, n (%)**	**Drug-Responsive Group, n (%)**	**ORs (95% CI)**
C1236T	CC	13 (11)	24 (19)	0.542 (0.262–1.121)
	CT	57 (49)	54 (42)	1.302 (0.786–2.156)
	TT	47 (40)	50 (39)	1.047 (0.627–1.749)
	C	83 (36)	102 (40)	0.830 (0.575–1.197)
	T	151 (64)	154 (60)	
G2677T/A	GG	15 (13)	29 (23)	0.502 (0.254–0.993)*
	GT	62 (53)	58 (45)	1.361 (0.823–2.250)
	TT	23 (20)	23 (18)	1.117 (0.588–2.122)
	GA	12 (10)	13 (10)	1.011 (0.442–2.314)
	AT	5 (4)	5 (4)	1.098 (0.310–3.894)
	G	104 (45)	129 (50)	0.788 (0.552–1.124)
	T	113 (48)	109 (43)	1.259 (0.882–1.799)
	A	17 (7)	18 (7)	1.036 (0.521–2.061)
C3435T	CC	32 (27)	46 (36)	0.671 (0.390–1.156)
	CT	58 (50)	53 (41)	1.391 (0.840–2.305)
	TT	27 (23)	29 (23)	1.024 (0.564–1.860)
	C	122 (52)	145 (57)	0.834 (0.584–1.191)
	T	112 (48)	111 (43)	
Haplotype	CGC	57 (13)	64 (15)	0.861 (0.586–1.264)
	CGT	36 (8)	38 (9)	0.929 (0.577–1.495)
	CTC	36 (8)	38 (9)	0.929 (0.577–1.495)
	CTT	34 (8)	35 (8)	0.955 (0.584–1.561)
	TGC	64 (14)	70 (16)	0.885 (0.613–1.279)
	TGT	50 (11)	43 (10)	1.166 (0.758–1.794)
	TTC	58 (13)	50 (11)	1.166 (0.779–1.746)
	TTT	74 (17)	63 (14)	1.191 (0.826–1.716)
	Others†	34 (8)	36 (10)	—
Diplotype	CT-GT-CT	24 (21)	19 (15)	1.480 (0.763–2.871)
	TT-GT-CT	18 (15)	14 (11)	1.481 (0.700–3.130)
	TT-TT-TT	15 (13)	16 (12)	1.029 (0.484–2.187)
	CT-GG-CC	8 (7)	5 (4)	1.806 (0.574–5.683)
	CT-GT-CC	7 (6)	10 (8)	0.751 (0.276–2.042)
	Others†	45 (39)	64 (50)	—

*P-value < 0.05.
†Haplotypes and diplotypes with total frequencies below 5% over the 2 groups.

Studies have shown that *ABCB1* genetic polymorphisms are associated with a high incidence of drug-resistant epilepsy, probably because polymorphisms may affect the efflux activity of transporters in endothelial cells of the blood–brain barrier, which in turn influences the concentrations of ASMs, and contributes to the failure of ASMs. In addition, several studies have indicated that *ABCB1* polymorphisms may be associated with the concentrations and responsiveness of drugs such as carbamazepine, lamotrigine, oxcarbazepine, and gabapentin.[24–29] Mila et al[24] demonstrated that *ABCB1* polymorphisms influence lamotrigine concentrations and should be considered for dose adjustment. Shen et al[26] reported that the genetic polymorphism of *ABCB1* rs1045642 is associated with the normalized oxcarbazepine concentration and therapeutic efficacy in patients with epilepsy ($P < 0.05$).

However, no report is available on the association of *ABCB1* polymorphism with levetiracetam serum concentration and treatment efficacy in children with epilepsy. In the current study, we did not find any association between *ABCB1* C1236T genetic polymorphism and levetiracetam serum concentrations and CDR values. However, *ABCB1* G2677T/A and C3435T polymorphisms significantly influenced levetiracetam serum concentrations. The levetiracetam concentration and CDRs were significantly higher in *ABCB1* G2677T/A GT, TT, GA, and AT genotype carriers than in GG carriers, and in *ABCB1* C3435T TT, and CT genotype carriers than in CC carriers. Our findings suggest that 2677-GT, TT, GA, AT, and 3435-TT reduce P-gp activity, promote gastrointestinal absorption of levetiracetam, and ultimately increase its serum concentration.

TABLE 3. Effects of the *ABCB1* Genotypes on Adjusted Levetiracetam Serum Concentrations

SNPs	Genotype	Number (%)	Serum Concentration, $\mu g \cdot mL^{-1}$	F/Z	*P*	CDR, $\mu g \cdot mL^{-1} \cdot kg \cdot mg^{-1}$	F/t	*P*
C1236T	CC	37 (15)	13.04 ± 4.24	F = 0.205	0.814	0.37 ± 0.14	F = 0.302	0.740
	CT	111 (45)	13.56 ± 4.69			0.38 ± 0.14		
	TT	97 (40)	13.29 ± 4.60			0.39 ± 0.12		
	CT + TT versus CC	208 (85)	13.44 ± 4.64	Z = −0.306	0.760	0.39 ± 0.13	Z = −0.875	0.381
G2677T/A	GG	44 (18)	11.76 ± 3.27	F = 2.211	0.068	0.32 ± 0.11	F = 4.906	0.001*
	GT	120 (49)	13.57 ± 4.40			0.38 ± 0.14		
	TT	46 (19)	14.28 ± 5.03			0.43 ± 0.10		
	GA	25 (10)	13.06 ± 4.66			0.38 ± 0.13		
	AT	10 (4)	14.84 ± 7.11			0.45 ± 0.13		
	GT + TT + GA + AT versus GG	201 (82)	13.76 ± 4.73	Z = −2.310	0.021*	0.40 ± 0.13	Z = −3.325	0.001*
C3435T	CC	78 (32)	12.63 ± 3.84	F = 6.392	0.002*	0.34 ± 0.12	F = 17.800	<0.001*
	CT	111 (45)	12.96 ± 4.70			0.38 ± 0.14		
	TT	56 (23)	15.24 ± 4.72			0.47 ± 0.09		
	CT + TT versus CC	167 (68)	13.72 ± 4.81	Z = −1.564	0.118	0.41 ± 0.14	Z = −3.752	<0.001*

*P-value < 0.05 (2-sided) was considered statistically significant.

The lack of a significant association between *ABCB1* C1236T, G2677T/A, and C3435T genotypes and levetiracetam resistance in our study may be attributed to several factors. First, the sample size of our study was not large enough to render sufficient power to detect a significant association. Second, there are multiple genes that could theoretically affect drug resistance in epilepsy, including the sodium channels gene (SCN1A and SCN2A). Third, it is possible that P-gp does not transport levetiracetam. Therefore, a complex induction process can be considered to be involved in the clearance of levetiracetam and drug resistance.

CONCLUSION

The findings of this study suggest that *ABCB1* G2677T/A and C3435T polymorphisms may affect levetiracetam disposition and therapeutic efficacy in Uygur children with epilepsy. Thus, this study may facilitate personalized levetiracetam therapy in patients with epilepsy. Future studies, with larger cohorts, will be used for validation and to explore the underlying regulatory mechanism of action of *ABCB1* genetic variations.

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