



SCN1A and SCN2A polymorphisms are associated with response to valproic acid in Chinese epilepsy patients

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Abstract

Purpose There is a large inter-individual variation in the efficacy of valproic acid (VPA) against epilepsy. The genetic polymorphism influence of sodium channels on VPA response remains a matter of debate. The aim of the study was to explore the effect of *SCN1A* and *SCN2A* gene polymorphisms on VPA response in the treatment of epilepsy among Chinese patients.

Methods A total of 354 epileptic patients with VPA treatment were genotyped for five single nucleotide polymorphisms (SNP), including *SCN1A* rs10188577 T>C, rs2298771 T>C, rs3812718 G>A, and *SCN2A* rs2304016 A>G, rs17183814 G>A. A binary logistic regression analysis was performed to evaluate the association of genotype with VPA antiepileptic effects, adjusting the influence of confounding factors.

Results Genotype distributions of all selected SNPs were consistent with the Hardy–Weinberg equilibrium in epilepsy patients. *SCN1A* rs3812718 and *SCN2A* rs2304016 were found to be significantly associated with VPA response, both in monotherapy and in VPA-based polytherapy. Patients with the rs3812718 A allele were more frequently seen in the VPA-responsive group ($P < 0.05$), and the rs2304016 G allele was related to an increased risk of resistance to VPA therapy ($P < 0.05$).

Conclusions Our study revealed that *SCN1A* rs3812718 and *SCN2A* rs2304016 polymorphisms might be markers of VPA response in Chinese epilepsy patients.

Trial registration ChiCTR-1800016477

Keywords Epilepsy · Valproic acid · *SCN1A* · *SCN2A* · Antiepileptic efficacy

Introduction

Epilepsy is a severe neurological disease that affects 65 million people worldwide. Seizure-related disability, mortality, comorbidity, stigma, and costs are major burdens for epileptic

patients [1, 2]. Antiepileptic drugs (AEDs) are the mainstay treatment for epileptic seizures, and both traditional and new-generation AEDs are used. However, even with the most effective medication, about 30% of patients still experience recurrent seizures [3, 4]. Drug resistance is multifactorial and accumulating evidence indicates that genetic variations can affect an individual response to a drug at pharmacokinetics and pharmacodynamics levels [5].

Valproic acid (VPA) is one of the most frequently prescribed AEDs in epilepsy treatment with unsurpassed efficacy against most generalized seizure types and focal seizures [6]. VPA is also recommended as the preferred treatment for seizures that are difficult to classify at the time of diagnosis [7]. However, large inter-patient variations of VPA antiepileptic efficacy exist, and variations in VPA plasma concentrations can only explain part of the difference in pharmacodynamics.

Epilepsy is an ion channel disease involving multiple ion channels, such as chloride, calcium, potassium, and sodium. About 25% of genes identified in epilepsy encode ion channels [8]. Voltage-gated sodium (Nav) channels are vital for the

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initiation and propagation of neuronal action potentials [9]. Nav channels are the major targets for many first-line AEDs, such as carbamazepine (CBZ), oxcarbazepine (OXC), phenytoin (PHT), lamotrigine (LTG), and VPA. VPA eliminates the high-frequency repetitive electrical activation of central nervous cells and delays the recovery of inactivated sodium ion channels, persistently reducing the electrical conduction of sodium ions [10–12]. In addition, long-term VPA treatment can upregulate the expression of sodium channels on cell surfaces [12].

Nav channels are composed of both α and β subunits, of which the α subunit is the functional subunit. VPA and other sodium channel blockers bind to the α subunit to exert their therapeutic effects [13]. The defects of the sodium channel subunit slow down its inactivation and prolong the time of depolarization of cell membranes, leading to the generation and propagation of seizures [14]. Nav1.1 and Nav1.2 channel subtypes are the primary sodium channels in the central nervous system (CNS), which are encoded by *SCN1A* and *SCN2A*, respectively [9]. Nav1.1 is mainly expressed in GABAergic neurons [14, 15]. *SCN1A*, encoding the α subunit of the Nav1.1 channel, is the gene most frequently associated with epilepsy. Mutations in *SCN1A* are responsible for genetic epilepsy with a wide range of severities [9]. Similarly, the *SCN2A* gene, which codes for the α subunit of the Nav1.2 channel, is closely associated with many types of epilepsy. Genetic variations in *SCN1A* and *SCN2A* may be the major determinants of individual phenotypic differences in response to AEDs, which has aroused a great interest among researchers.

At present, many investigators have studied the association between gene polymorphisms of *SCN1A* and *SCN2A*, and AED response, but few studies focused on VPA response and results were conflicting. A cohort study in Hong Kong Chinese and Malaysian epilepsy patients with VPA monotherapy did not show that *SCN1A* gene polymorphism influenced VPA response [16]. A study carried out by Yip and colleagues in Caucasian patients found that *SCN1A* rs10188577 was the only single nucleotide polymorphism (SNP) associated with an AED response (including VPA) [17]. Moreover, a study in a Chinese epilepsy cohort revealed that *SCN2A* rs2304016 was related to VPA response [18].

In this study, we intended to explore the effect of *SCN1A* and *SCN2A* gene polymorphisms on VPA efficacy in a Chinese Han epileptic population. We aimed to provide genetic evidence for personalized VPA treatment by identifying genetic markers that can predict VPA treatment resistance.

Materials and methods

Patients

The study recruited a total of 419 unrelated Chinese Han epilepsy patients from the neurology outpatient

department at Xiangya Hospital, Central South University. All patients had a history of two or more clinically definite, unprovoked epileptic seizures. Out of the 419 patients, 318 were treated with oral VPA monotherapy, and the other 101 patients received at least 3 AEDs because their initial VPA monotherapy failed. Patients' demographic and clinical data were collected at their first visit, such as gender, age, body weight, height, seizure type, age at onset of seizure, family history of epilepsy, medical and neurological history, frequency and duration of seizures, and epilepsy treatment regimen. Medical records were subsequently collected every 3 months during 1-year VPA treatment, and recordings from each follow-up phone call or visit included doses of AEDs at the time of follow-up, patient compliance, and responsiveness to AEDs. The final evaluated value of VPA concentration represented the most recent trough plasma level at the maximum maintenance dose during the study period. Patients were excluded if treating physician or patient thought that VPA treatment was contraindicated or if there was a history of other progressive neurological disease. Patients with poor medication adherence were also excluded.

Written informed consent was given for all patients or their guardians before recruitment. The study was approved by the hospital Medical Ethics Committee and was registered in the Chinese Clinical Trial Registry (ChiCTR-1800016477).

Definition of drug resistance and responsiveness

In accordance with the consensus proposal of the International League Against Epilepsy (ILAE) Commission [4], VPA monotherapy patients were divided into two groups according to their VPA response: VPA-responsive and VPA-resistant. VPA-responsive patients were defined as those who were completely free of seizures for at least 1 year during VPA monotherapy. VPA-resistant patients were defined as those with an occurrence of at least four seizures during 1 year of VPA monotherapy at the maximum tolerated dose.

Genotyping

Candidate SNPs were selected based on database results and published papers. First, data on *SCN1A* and *SCN2A* SNPs in the southern Chinese population (CHS) were downloaded from the 1000 Genomes Project. Second, tag SNPs were selected using the Haploview software. SNPs with minor allele frequency > 0.1 were selected for tag SNP screening, and an $R^2 > 0.8$ was considered to represent a linkage disequilibrium. Finally, referring to the published literatures and F -scores from F-SNP

Table 1 Information of selected SNPs

Gene	SNP	Chr	Genetic position	Allele	Function	F-score	MAF in CHS (1000 genomes)
SCN1A	rs10188577	2	Intron	T>C	Intron variant	0.242	0.210
	rs2298771	2	Exon	T>C	p.A1067T	0.933	0.105
	rs3812718	2	Intron	G>A	Splicing site	0.176	0.381
SCN2A	rs2304016	2	Intron	A>G	Splicing site	0.217	0.110
	rs17183814	2	Exon	G>A	p.R19K	0.917	0.186

SNP, single nucleotide polymorphism; Chr, chromosome; MAF, minor allele frequency

database (<http://compbio.cs.queensu.ca/F-SNP/>), the following five SNPs were identified: *SCN1A* rs10188577 T>C, rs2298771 T>C, rs3812718 G>A, *SCN2A* rs2304016 A>G, and rs17183814 G>A. Details of the selected SNPs are presented in Table 1.

Peripheral venous blood samples (2 mL) were obtained from each patient. Following the manufacturer's instructions, genomic DNA was extracted from blood samples using E.Z.N.A.SQ Blood DNA Kit II (Omega Bio-Tek, GA, USA). The selected SNPs were genotyped using the SNPscan™ assay technique (cat#: G0104K, Genesky Inc. Shanghai, China) as previously described [19]. To ensure the quality of genotyping, random duplicated sample genotyping was undertaken as an internal control and no genotyping error was observed for all SNPs.

Statistical analysis

Statistical analyses were performed using the SPSS software (IBM SPSS Statistics 24.). Continuous variables were analyzed by Kolmogorov–Smirnov test to confirm normal distribution. Normal distributed data were expressed as mean \pm standard deviation (SD) and mean comparisons were calculated by an independent samples *t* test. For non-normal distribution, data were presented as median (1st–3rd quartile) and were compared by Mann–Whitney *U* test. Categorical variables were reported as frequency and percentage. The differences in the categorized data between the responsive group and the resistant group were analyzed using chi squared test (χ^2 test) or Fisher's exact test when it is appropriate. The χ^2 test was also used to assess deviations from the Hardy–Weinberg equilibrium (HWE). Genotypes containing fewer than five individuals were combined with heterozygotes to create a dominant model. Binary logistic regression was applied to adjust potential covariates. Associations of VPA response and sodium channel gene polymorphisms were expressed as odds ratios (ORs) and risks were estimated with 95% confidence intervals (CIs). A two-tailed *P* value < 0.05 was considered to be statistically significant.

Results

Association study in VPA monotherapy

A total of 318 epilepsy patients undergoing VPA monotherapy were enrolled in this study. However, 19 patients did not complete their 1-year follow-up. The drug responses of 46 patients were undetermined because their seizures occurred less than four times during 1 year of VPA treatment. Hence, 253 VPA monotherapy patients were included in the final analysis. The demographics and clinical characteristics of patients with VPA monotherapy are depicted in Table 2. Among these patients, 125 patients were classified into the VPA-responsive group and 128 were in the VPA-resistant group. No statistically significant differences were found between the two groups regarding their gender, age, seizure type, course of epilepsy, and VPA daily dose. Patients in the VPA-responsive group were much younger when experienced their first seizure than those in the VPA-resistant group ($P = 0.048$). Additionally, VPA trough concentration in the VPA-resistant group was significantly higher than that in the VPA-responsive group (53.38 ± 27.10 vs. 45.54 ± 23.49 $\mu\text{g/mL}$, $P = 0.016$).

Table 3 lists the allelic and genotypic frequencies of *SCN1A* and *SCN2A* SNPs in patients on VPA monotherapy. Genotypic distributions agreed with the HWE ($P > 0.05$, Table S1). Distributions of alleles and genotypes were similar between the VPA-responsive and the VPA-resistant groups. After correcting for confounding factors (age, age at onset of seizures, and course of epilepsy), the results of binary logistic regression analysis revealed that *SCN1A* rs3812718 G>A was associated with a positive response to VPA monotherapy, and *SCN2A* rs2304016 was associated with a weaker response to VPA monotherapy. Regarding the genotypic frequencies of the *SCN1A* rs3812718 G>A polymorphism, carriers with a GA or AA genotype were more common in the VPA-responsive group (GG vs. GA: OR = 0.40, 95% CI = 0.18–0.93, $P = 0.034$; GG vs. AA: OR = 0.41, 95% CI = 0.18–0.90, $P = 0.027$). In the dominant model, both GA and AA genotypes were significantly more frequent in the VPA-

Table 2 Demographics and clinical characteristics of the epilepsy patients with VPA therapy

Characteristics	VPA-responsive group (<i>n</i> = 125)	VPA-resistant group (<i>n</i> = 128)	<i>P</i> value	Resistant group (<i>n</i> = 229)	<i>P</i> value*
Males, <i>n</i> (%)	81 (64.8)	78 (60.9)	0.525	145 (63.3)	0.782
Age (years)	6.9 (3.5–14.4)	10.5 (3.8–32.7)	0.075	7.7 (4.0–17.3)	0.427
Seizure type (primary/secondary)	70/55	66/62	0.479	118/111	0.420
Age at onset of seizure (years)	3.0 (1.0–10.5)	6.3 (1.0–25.8)	0.048	3.3 (0.8–13.0)	0.593
Course of epilepsy (years)	2.1 (1.1–4.2)	1.6 (0.9–4.9)	0.289	2.0 (1.0–5.0)	0.746
Duration of VPA treatment (years)	1.1 (0.5–2.3)	1.0 (0.3–1.8)	0.025	1.2 (0.4–2.9)	0.585
VPA daily dose (mg/kg)	18 ± 16	18 ± 8	0.992	20 ± 9	0.113
VPA trough concentration (µg/mL)	45.54 ± 23.49	53.38 ± 27.10	0.016	56.96 ± 25.72	0.00006

VPA, valproic acid

*VPA-responsive group compared with resistant group

responsive group than the VPA-resistant group (88.0% vs. 78.1%, $P = 0.037$), as confirmed in logistic analysis (OR = 0.41, 95% CI = 0.19–0.87, $P = 0.020$). Compared with the *SCN2A* rs2304016 AA genotype, the AG genotype was associated with an increased risk of VPA resistance (OR = 2.36, 95% CI = 1.08–5.16, $P = 0.031$). However, the difference was only marginally significant under the dominant model (OR = 2.07, 95% CI = 0.99–4.34, $P = 0.054$).

Association study in VPA-based polytherapy

We also recruited 101 VPA-based polytherapy patients who were resistant to initial VPA monotherapy. Consistent with the definition of VPA resistance, those patients, who experienced more than four seizures with VPA-based polytherapy at maximum tolerated doses during 1-year follow-up, were combined with 128 VPA monotherapy-resistant patients as the VPA-resistant group. The VPA-responsive group included 125 VPA monotherapy-responsive patients. As can be seen in Table 2, the two groups were similar in age, age at study entry and onset of seizures, course of epilepsy, and duration of VPA treatment. The distributions of gender and seizure type between groups had no statistical difference. However, at an equivalent VPA daily dose, patients in the VPA-resistant group had a significantly higher VPA trough concentration than the VPA-responsive group (56.96 ± 25.72 vs. 45.54 ± 23.49 µg/mL, $P = 0.00006$).

As shown in Table 4, *SCN1A* rs3812718 G>A was significantly associated with VPA response. Patients with GA or AA genotypes were more prone to have a better response to VPA treatment (GG vs. GA: OR = 0.38, 95% CI = 0.19–0.78, $P = 0.008$; GG vs. AA: OR = 0.40, 95% CI = 0.19–0.85, $P = 0.017$). In the dominant model, the GG genotype was less common in the VPA-responsive group compared with the VPA-resistant group (OR = 0.39, 95% CI = 0.20–0.77, $P = 0.007$). In addition, the A allele was a protective factor for complete freedom from seizures (OR = 0.70, 95% CI = 0.50–0.99, $P = 0.043$). *SCN2A* rs2304016 was also

significantly related to an increased risk of VPA resistance. Compared with the responsive group, AG genotype was more frequent in the resistant group (OR = 2.47, 95% CI = 1.19–5.12, $P = 0.015$). In the dominant model, G-containing genotype carriers were more likely to be resistant to VPA therapy (OR = 2.14, 95% CI = 1.07–4.25, $P = 0.031$).

SCN1A rs10188577, rs2298771, and *SCN2A* rs17183814 had no effect on VPA therapy in our cohorts.

Discussion

VPA is a broad-spectrum AED and it is widely used in the treatment of various seizures. Individual responses to VPA are significantly varied and pharmacogenetics plays an important role in explaining this difference. In the present study, *SCN1A* rs3812718 G>A was significantly associated with a drug response both in VPA monotherapy and VPA-based polytherapy. Compared with wild homozygote carriers, patients with rs3812718 AA and GA genotypes were more sensitive to VPA therapy and had better seizure control. Patients with the *SCN2A* rs2304016 A allele were more likely to achieve seizure freedom after VPA treatment.

Our results are similar to a subgroup analysis in Malay idiopathic generalized epilepsy (IGE) patients, which revealed that the rs3812718 G allele might be a risk factor for resistance to VPA monotherapy [16]. Results of previous studies on the correlation between patients' response to sodium channel blockers and rs3812718 polymorphism were inconclusive. Some suggested that the G allele was a protective factor resulting in a better response [20–22], while others found that rs3812718 had no effect on AED response [23–26]. A meta-analysis published in 2013 revealed that rs3812718 polymorphism and AED response were unrelated [27]; however, the result warrants for an update. Hung and colleagues found that in the Taiwanese population, maintenance doses and the maintenance dose-adjusted concentrations (CDR) of CBZ in GA and AA genotypes were lower than those in the GG genotype

Table 3 Distribution of genotypes and alleles among VPA-responsive group and VPA-resistant group

Gene	SNP	Genotype/ allele	VPA-responsive <i>n</i> (%)	VPA-resistant <i>n</i> (%)	<i>P</i> value	Odds ratios (95% CI)	Adjusted <i>P</i> value	
<i>SCN1A</i>	rs10188577 T>C	TT	85 (68.0)	83 (64.8)	0.580	Reference		
		CT	38 (30.4)	40 (31.3)		1.04 (0.58–1.88)	0.899	
		CC	2 (1.6)	5 (3.9)		–	–	
	Dominant	CT+CC	40 (32.0)	45 (35.2)	0.595	1.13 (0.64–2.00)	0.680	
		T	208 (83.2)	206 (80.5)	0.426	Reference		
	rs2298771 T>C	Dominant	C	42 (16.8)	50 (19.5)		1.20 (0.73–1.96)	0.474
			TT	103 (82.4)	107 (83.6)	0.948	Reference	
			CT	20 (16.0)	19 (14.8)		0.83 (0.40–1.75)	0.630
		Dominant	CT+CC	22 (17.6)	21 (16.4)	0.800	0.83 (0.40–1.68)	0.597
			T	226 (90.4)	233 (91.0)	0.811	Reference	
		rs3812718 G>A	Dominant	C	24 (9.6)	23 (9.0)		0.83 (0.44–1.59)
	GG			15 (12.0)	28 (21.9)	0.112	Reference	
	GA			64 (51.2)	58 (45.3)		0.40 (0.18–0.93)	0.034
	Dominant		AA	46 (36.8)	42 (32.8)		0.41 (0.18–0.90)	0.027
			AA+GA	110 (88.0)	100 (78.1)	0.037	0.41 (0.19–0.87)	0.020
Dominant	G		94 (37.6)	114 (44.5)	0.113	Reference		
	A	156 (62.4)	142 (55.5)		0.70 (0.48–1.03)	0.069		
<i>SCN2A</i>	rs2304016 A>G	Dominant	AA	109 (87.2)	101 (78.9)	0.092	Reference	
			AG	14 (11.2)	26 (20.3)		2.36 (1.08–5.16)	0.031
			GG	2 (1.6)	1 (0.8)		–	–
		Dominant	AG+GG	16 (12.8)	27 (21.1)	0.079	2.07 (0.99–4.34)	0.054
			A	232 (92.8)	228 (89.1)	0.144	Reference	
		rs17183814 G>A	Dominant	G	18 (7.2)	28 (10.9)		1.73 (0.88–3.40)
	GG			100 (80.0)	99 (77.3)	0.863	Reference	
	AG			23 (18.4)	27 (21.1)		1.31 (0.66–2.59)	0.433
	Dominant		AA	2 (1.6)	2 (1.6)		–	–
			AG+AA	25 (20.0)	29 (22.7)	0.606	1.33 (0.68–2.58)	0.400
	Dominant		G	223 (89.2)	225 (87.9)	0.644	Reference	
		A	27 (10.8)	31 (12.1)		1.30 (0.71–2.37)	0.396	

[28]. Conversely, a British study suggested that the maximum doses or serum levels of CBZ or PHT in AA genotype carriers were higher than those in GG genotype carriers [29]. Another study on a Taiwanese cohort reproduced these results, which indicated that PHT maintenance dose for the GG genotype was lower than that for the AA genotype [30]. An adequate drug concentration is required for antiepileptic efficacy. Therapeutic drug monitoring is recommended in epilepsy treatment to ensure that AED plasma concentration lies within the therapeutic window [31]. In our population, patients in the responsive group had a significantly lower VPA trough

concentration than those in the resistant group. Therefore, the response of a patient may be the best evaluation criteria for efficacy.

Located at the 5' splice donor site of a highly conserved region of *SCN1A* exon 5, rs3812718 (namely IVS5N+5, IVS5-91) G>A is a common functional intronic variant. It modulates the proportion of human brain *SCN1A* transcripts incorporating the canonical (5A) or alternative (5N) exon 5 [32]. The α subunit of Nav channel contains voltage sensors and an ion-conducting pore in four internally repeated domains (I–IV), each of which consists of six transmembrane

Table 4 Distribution of genotypes and alleles among VPA-based polytherapy patients

Gene	Genotype/ allele	Responsive group <i>n</i> (%)	Resistant group <i>n</i> (%)	<i>P</i> value	Odds ratio (95% CI)	Adjusted <i>P</i> value	
SCN1A	rs10188577 T>C						
		TT	85 (68.0)	157 (68.6)	0.631	Reference	
		CT	38 (30.4)	64 (27.9)		0.89 (0.53–1.51)	0.671
		CC	2 (1.6)	8 (13.5)		–	–
	Dominant	CT+CC	40 (32.0)	72 (31.4)	0.914	0.95 (0.57–1.58)	0.836
		T	208 (83.2)	378 (82.5)	0.822	Reference	
		C	42 (16.8)	80 (17.5)		1.02 (0.65–1.59)	0.937
	rs2298771 T>C						
		TT	103 (82.4)	189 (82.5)	0.826	Reference	
		CT	20 (16.0)	38 (16.6)		1.00 (0.53–1.88)	0.994
		CC	2 (1.6)	2 (0.9)		–	–
	Dominant	CT+CC	22 (17.6)	40 (17.5)	0.975	0.94 (0.51–1.74)	0.851
		T	226 (90.4)	416 (90.8)	0.851	Reference	
		C	24 (9.6)	42 (9.2)		0.90 (0.51–1.57)	0.898
	rs3812718 G>A						
	GG	15 (12.0)	52 (22.7)	0.048	Reference		
	GA	64 (51.2)	101 (44.1)		0.38 (0.19–0.78)	0.008	
	AA	46 (36.8)	76 (33.2)		0.40 (0.19–0.85)	0.017	
Dominant	AA+GA	110(88.0)	177 (77.3)	0.014	0.39 (0.20–0.77)	0.007	
	G	94 (37.6)	205 (44.8)	0.065	Reference		
	A	156 (62.4)	253 (55.2)		0.70 (0.50–0.99)	0.043	
SCN2A	rs2304016 A>G						
		AA	109 (87.2)	183 (79.9)	0.057	Reference	
		AG	14 (11.2)	45 (19.7)		2.47 (1.19–5.12)	0.015
		GG	2 (1.6)	1 (0.4)		–	–
	Dominant	AG+GG	16 (12.8)	46 (20.1)	0.085	2.14 (1.07–4.25)	0.031
		A	232 (92.8)	411 (89.7)	0.177	Reference	
		G	18 (7.2)	47 (10.3)		1.74 (0.93–3.24)	0.081
	rs17183814 G>A						
		GG	100 (80.0)	168 (73.4)	0.326	Reference	
		AG	23 (18.4)	58 (25.3)		1.48 (0.82–2.66)	0.191
		AA	2 (1.6)	3 (1.3)		–	–
	Dominant	AG+AA	25 (20.0)	61 (26.6)	0.164	1.49 (0.84–2.64)	0.177
		G	223 (89.2)	394 (86.0)	0.228	Reference	
		A	27 (10.8)	64 (14.0)		1.42 (0.84–2.40)	0.192

α -helical segments (S1–S6) and a pore loop connecting S5 and S6 [9]. Exon 5 encodes the S4 transmembrane segment or voltage sensor in the first domain (DI) of Na_v1.1. Investigators have demonstrated that Na_v1.1-5N is primarily expressed during the neonatal periods and Na_v1.1-5A is the adult form [29, 33]. However, strict developmental regulation of this splicing event may be relaxed as evidenced by the detection of Na_v1.1 transcripts (up to 50% of transcripts) consisting of exon 5N in the adult human brain [29, 33]. Exon 5N makes up 50% GG genotype transcripts, while it only makes up 1% of transcripts in the AA genotype. Of note,

the A allele can disrupt the consensus splice donor sequence immediately following exon 5N, therefore completely blocking Nova2-mediated (a specific nervous system splicing factor) incorporation of exon 5N into mature *SCN1A* mRNA transcripts and affecting the translating protein [33]. As a result, a three-amino-acid difference between channels containing 5N-(Phe, Asn, and Phe) and 5A-(Tyr, Asp, and Val) may alter the sensitivity to AEDs [34].

Our study provides evidence that rs3812718 may affect VPA response. However, the results in our cohort and the Malay IGE cohort were contrary to previous studies.

Generally, drugs exert their pharmacological action through binding to their specific targets, and changes in those targets alter drug effects. Severe myoclonic epilepsy of infancy (SMEI) is caused by haplo insufficiency of Nav1.1 channel. Clinical practice suggests that sodium channel blockers should be avoided in these SMEI patients, which could worsen the disease [35]. Studies have shown that OXC and LTG exacerbate symptoms of some patients with Dravet syndrome, while VPA therapy is effective [36]. Dravet syndrome is also caused by *SCN1A* mutation. CBZ, PHT, and OXC mainly block sodium channels to create an antiepileptic effect. The multiplicity of cellular targets of VPA action, ranging from channels and receptors to pathways involved in gene expression regulation, makes the identification of antiseizure mechanisms a particularly difficult task. In addition, VPA is an inhibitor of histone deacetylase and displays antiepileptogenesis and neuroprotection activity in some animal models [37]. In view of the multiple extra- and intra-cellular targets of VPA, it could play an antiepileptic role through other pathway when something is wrong with the sodium channels. To some extent, this may explain why CBZ and PHT perform better in rs3812718 GG carriers while VPA performs better in the A allele carriers. For this reason, we need more studies with large samples to confirm our results.

Evidence has shown that *SCN1A* IVS5-91 G>A polymorphism is associated with a susceptibility to epilepsy but not with drug responsiveness [38]. A meta-analysis reported that the A allele was a risk factor of epilepsy both in North Indian and Caucasian populations [39]. Without a healthy control, we are not sure if this polymorphism is related to the susceptibility to epilepsy in our population.

Given Nav1.2 plays a vital role in epileptogenesis, we also tried to find the association between *SCN2A* gene polymorphisms and VPA resistance in our cohort. The result suggested that *SCN2A* rs2304016 A>G is a possible marker for VPA resistance, and G allele carriers seem more likely to be resistant to VPA therapy. However, this result is contrary to work done by Li and colleagues. They found that the AA genotype was a risk factor for VPA monotherapy resistance in Chinese epilepsy patients [18]. Furthermore, a multicenter study from Hong Kong in epileptics treated with AEDs revealed that rs2304016 did not affect the response of sodium channel blocker AEDs [40]. The SNP rs2304016 is located in the putative branch site for splicing exons 7 and 8, and a functional study demonstrated that this locus polymorphism did not affect splicing, nor did it influence mRNA expression [40]. It is possible that rs2304016 A>G polymorphism has no effect on drug response itself but is in linkage disequilibrium with a functional genetic variant that influences drug efficacy. In future studies, differences among genotypes at the protein level are worthwhile to explore.

Our study has the following limitations: (1) types of epilepsy were only roughly divided into primary and secondary

seizures according to etiologies as it was not easy to select homogeneous individuals in this clinical study. However, a logistic regression analysis indicated that seizure type was not a confounder; (2) the study follow-up was only 1 year, and therapeutic effect of VPA therapy could be delayed; (3) the study sample size was relatively small which was not suitable for the Bonferroni correction to counteract the problem of multiple comparisons; and (4) the study population should be better defined with regard to age. It is necessary in future studies to investigate the molecular mechanism of how an IVS5-91 G>A splice-site polymorphism influencing treatment efficacies of VPA and other AEDs.

In conclusion, we have shown that *SCN1A* IVS5-91 G>A polymorphism was not only associated with VPA monotherapy response but also with VPA polytherapy response. Furthermore, the AA and GA genotypes were protective factors for a better antiepileptic response. *SCN2A* rs2304016 polymorphism also affected VPA efficacy. Pharmacogenomics diagnostics is an important part of precision medicine. If the above associations are confirmed in a prospective trial with a large population, the determination of *SCN1A* rs3812718 genotype and *SCN2A* rs2304016 genotype may help to identify epileptic patients who will benefit the most from VPA therapy.

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Contributions of authors Zanling Zhang designed the study. Lihong Shi performed the data analysis and wrote the manuscript. Miaomiao Zhu, Huilan Li, and Zhipeng Wen recruited patients and conducted patients' follow-up. Jia Luo and Cong Lin extracted the DNA. Xiaoping Chen reviewed the manuscript and critically revised the intellectual content of the manuscript.

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Compliance with ethical standards

All procedures performed in studies involving human participants were in accordance with the ethical standards of Xiangya Hospital Central South University Medical Ethics Committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

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