SCN1A IVS5N+5 polymorphism and response to sodium valproate: a multicenter study

**Aim:** Approximately 30% of epilepsy patients do not respond to antiepileptic drugs (AEDs). The functional SCN1A IVS5N+5 polymorphism may play a role in response to some AEDs. The purpose of this study was to examine this hypothesis in a cohort study of Malaysian and Hong Kong Chinese epilepsy patients on sodium valproate (VPA) monotherapy and in a meta-analysis. **Patients & methods:** The SCN1A IVS5N+5 polymorphism was genotyped in 583 Malaysian (84%) and Hong Kong Chinese (16%) epilepsy patients receiving VPA monotherapy. The related association studies, including the current study, were meta-analyzed by using fixed- and random-effects models under various genetic models. **Results:** A total of 277 (47.5%) and 306 (52.5%) patients were VPA nonresponsive and responsive, respectively. Unlike Chinese and Indian patients, Malay nonresponsive patients with idiopathic generalized epilepsy showed significant association, probably caused by the small sample size. **Conclusion:** The cohort study and meta-analysis did not demonstrate an association between AED responsiveness and this polymorphism. Future studies with a larger sample size of Malays with idiopathic generalized epilepsy are suggested.

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**KEYWORDS:** alternative splicing, epilepsy, meta-analysis, polymorphism, SCN1A, sodium valproate

Despite the many antiepileptic drugs (AEDs) that act on one or more target molecules in the brain, approximately 30% of epilepsy patients receiving AEDs continue to experience seizures [1]. Voltage-dependent sodium channels are major targets for AEDs [2, 3]. Sodium valproate (VPA) is widely used as a first-line therapy in both generalized and partial epilepsies. This drug suppresses the high frequency firing of neurons through a broad array of mechanisms. A main responsible mechanism for VPA anticonvulsant activity may be blockade of sodium channels via effect on GABAergic system. VPA increases the level of the inhibitory neurotransmitter GABA in the synaptic cleft and thereby enhances GABAergic function to block sustained repetitive firing in neurons [3–5]. The voltage-dependent sodium channel is composed of α- and β-subunits, of which the α-subunit is sufficient for expression of sodium channel function [6]. The α-subunits are encoded by ten genes, which are expressed in different excitable tissues. The sodium channel Na$_1.1$ initiates action potentials in neurons in different parts of the mammalian brain, such as the hippocampus, thalamus and cerebellum, and plays a significant role in epilepsy [7]. This channel forms a pore in the membrane that selectively conducts sodium ions. It comprises a four domain by six segment (4 × 6) architecture: four homologous domains (D1–D4), each containing six α-helical segments (S1–S6). The positively charged residues in the S4 segment or voltage-sensing helix of each domain are involved in action potentials and graded membrane potential changes [8]. Sodium channel-blocking AEDs such as carbamazepine (CBZ), phenytoin (PHT) and lamotrigine bind to this pore and exert their therapeutic effect through selective binding to activated or inactivated channels [6, 9–11].

Any small change in the function of sodium channels can cause epileptic seizures and might influence pharmacological action and response to treatment. Genetic variation is a major determinant of individual phenotypic differences in susceptibility to diseases and response to therapeutic compounds [12]. An SCN1A functional intronic variant (rs3812718, IVS5N+5 G>A) is located at the 5’ splice donor site of a highly conserved region of SCN1A exon 5N. Exon 5 encodes the S4 transmembrane segment or voltage sensor in the first domain of the Na$_1.1$ protein [13]. Conformational changes to a part of this sensor induce rearrangements of the activation gate and opening/closing of the channels during electrical signaling in neurons [5]. The G allele allows both the neonatal (5N) and adult (5A) forms of this exon to be alternatively expressed, while the A allele reduces expression of the 5N form relative to the 5A form [14–16]. The three amino acid changes due to the G>A transition are R516Q, K518Q and R556Q, which are all positively charged residues in the S4 segment of the voltage-sensing loop. The IVS5N+5 G>A variant is linked to the SCN1A A5N allele as reported by a previous study [10].
acid differences between 5N- (Phe, Asn and Phe) and 5A- (Tyr, Asp and Val) containing sodium channels would alter the electrophysiological characteristics of the S4 sensor and its sensitivity to AEDs [13–15]. Evidence has demonstrated a strong relationship between the AA genotype and the maximum doses of CBZ, PHT and lamotrigine in cohort studies of patients with various types of epilepsy [16–19], while another study did not confirm this finding for CBZ [20]. These results brought attention to the probable role of this polymorphism in response to drugs acting on the Na,1.1 channel in epilepsy [21–24]. Since VPA inhibits neural firing through blockade of the Na,1.1 channel, a small difference between 5A- and 5N-containing transcripts may have an effect on sodium channel sensitivity. It is plausible that asparagine (in 5N) is more efficient than aspartate (in 5A) in modifying the sodium channel and thereby its sensitivity and responsiveness to some AEDs, such as CBZ and VPA [16,25]. However, no data are available for VPA, which acts on this channel. In the current study, we examined the association between the SCN1A IVS5N+5 G>A polymorphism and response to treatment in Malaysian and Hong Kong Chinese epilepsy patients receiving VPA monotherapy. A benefit of focusing on VPA monotherapy was the elimination of possible confounding effects of drug interactions on the results. Finally, a meta-analysis was performed from the related data.

Patients & methods

Cohort study

This study was a multicenter collaboration between the University of Malaya Medical Centre (Kuala Lumpur, Malaysia), Universiti Kebangsaan Malaysia Medical Centre (Kuala Lumpur, Malaysia) and the Chinese University of Hong Kong (Shatin, Hong Kong). The study protocol was approved by the ethics committees of the centers involved. Unrelated epilepsy patients of Malay, Indian, Malaysian Chinese and Hong Kong Chinese ethnicities were recruited from epilepsy clinics and diagnosed by neurologists who were blinded to the genotype data. Seizures and epilepsy syndromes were classified according to International League Against Epilepsy guidelines [26,27].

On the basis of definitions of treatment outcomes [28], drug responsiveness was defined as being completely seizure free for at least 1 year during VPA monotherapy. Epilepsy patients were eligible for inclusion if they had been receiving VPA treatment for at least 1 year. Drug resistance was defined as the occurrence of seizures over a period of 1 year during treatment with VPA monotherapy at maximally tolerated therapeutic dosages; however, some of these VPA-resistant patients might be responsive to the second or third AED.

In the present study we excluded patients with generalized epilepsy with febrile seizures plus, severe myoclonic epilepsy of infancy, unreliable record of seizure frequency, significant psychiatric comorbidity, history of pseudoseizures, alcohol or drug abuse, or presence of progressive or degenerative neurological or systemic disorders. Written informed consent was given by all patients or by their guardians in the case of a child. A standardized extraction template was administered to collect demographic details, information on seizure types and frequency and relevant family history from the records.

Genomic DNA was extracted from either whole blood or buccal swabs by standard methods. The SCN1A IVS5N+5 polymorphism was genotyped by using MALDI TOF mass spectrometry (MassARRAY®, Sequenom, CA, USA) at the Hong Kong University Genome Research Centre (Pokfulam, Hong Kong) or by DNA sequencing. All values are presented either as the mean ± standard deviation for continuous data or as frequency for categorical data. The ages of participants at study entry and onset of epilepsy were non-normally distributed, as examined by the Kolmogorov–Smirnov test. Therefore, the frequency of these two variables was compared for VPA nonresponsive and VPA responsive patients by the nonparametric Mann–Whitney U test or the Kruskal–Wallis rank sum test. Differences in categorized data (i.e., gender, seizure type and epilepsy syndrome) between races were calculated by χ2-test. Odds ratios (ORs) with 95% CIs, adjusted for confounders (i.e., ethnicity, gender, age at recruitment, seizure type and epilepsy syndrome), were obtained through a binomial logistic regression analysis. Two-sided tests of statistical significance were used to determine statistically significant p-values (p < 0.05) with the SPSS 15.0 software package (SPSS Inc., IL, USA).

Meta-analysis

Published studies up to May 2012 that determined the distribution of the SCN1A IVS5N+5 genotype in drug nonresponsive and drug-responsive epilepsy patients were included in this meta-analysis. There was no language limitation. Crude ORs with 95% CIs and p-values were calculated from allele and genotype frequencies. Pooled ORs of the SCN1A IVS5N+5 polymorphism were calculated for allele and alternative
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SCN1A IVS5N+5 polymorphism & response to sodium valproate

Results

Cohort study

Demographic characteristics

Table 1. Demographic characteristics of the patients.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Malaysian Malay (n = 166)</th>
<th>Malaysian Indian (n = 125)</th>
<th>Malaysian Chinese (n = 196)</th>
<th>Hong Kong (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NR (n = 82)</td>
<td>R (n = 84)</td>
<td>NR (n = 97)</td>
<td>R (n = 50)</td>
</tr>
<tr>
<td>Age (years), mean ± SD</td>
<td>28 ± 15</td>
<td>25 ± 15</td>
<td>30 ± 18</td>
<td>29 ± 11</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>36 (44)</td>
<td>35 (42)</td>
<td>44 (45)</td>
<td>14 (30)</td>
</tr>
<tr>
<td></td>
<td>36 (44)</td>
<td>35 (42)</td>
<td>44 (45)</td>
<td>14 (30)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>46 (56)</td>
<td>49 (58)</td>
<td>53 (55)</td>
<td>32 (70)</td>
</tr>
<tr>
<td>Seizure type</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Generalized, n (%)</td>
<td>29 (37)</td>
<td>53 (69)</td>
<td>41 (44)</td>
<td>40 (91)</td>
</tr>
<tr>
<td>Partial, n (%)</td>
<td>21 (41)</td>
<td>32 (45)</td>
<td>52 (56)</td>
<td>49 (54)</td>
</tr>
<tr>
<td>Unspecified†</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Etiology</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cryptogenic, n (%)</td>
<td>28 (35)</td>
<td>27 (35)</td>
<td>36 (39)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Idiopathic, n (%)</td>
<td>14 (18)</td>
<td>28 (36)</td>
<td>13 (14)</td>
<td>45 (98)</td>
</tr>
<tr>
<td>Symptomatic, n (%)</td>
<td>37 (47)</td>
<td>22 (29)</td>
<td>43 (47)</td>
<td>36 (40)</td>
</tr>
<tr>
<td>Unspecified†</td>
<td>3</td>
<td>7</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

†Excluded from analysis.
NR: Sodium valproate nonresponsive; R: Sodium valproate responsive; SD: Standard deviation.
subjects (p = 0.03). In Malay and Chinese, symptomatic epilepsy was more common in the VPA-nonresponsive than in the VPA-responsive patients (47 and 47% vs 29 and 40%, respectively); however, their differences were not significant. Malaysian epilepsy patients were much more heterogeneous in seizure type and epilepsy syndrome compared with Hong Kong Chinese subjects. This heterogeneity was mainly caused by switching from other AED monotherapy or polytherapy to VPA monotherapy due to various factors such as drug adverse reactions in Malaysian patients.

Association study

Table 2 lists the allele and genotype frequencies of the SCN1A IVS5N+5 polymorphism in the Malaysian and Hong Kong Chinese epilepsy patients. Distributions of genotypes were consistent with Hardy–Weinberg equilibrium (p > 0.05). In this part, we did analysis for overall epilepsy syndromes as well as for idiopathic generalized epilepsy (IGE) and for symptomatic epilepsy in each ethnicity. Analysis of alleles and genotypes was performed for all epilepsy syndromes in each ethnic group. Symptomatic epilepsy was common in approximately 39% of Malaysian patients; therefore, we performed a stratified analysis for both idiopathic epilepsy and symptomatic epilepsy in the Malaysian Chinese, Indian and Malay subgroups, as causal factors of symptomatic epilepsy differ from idiopathic epilepsy and hence may affect the results.

Alleles

There was an allelic association with response to VPA in the Hong Kong Chinese subgroup (OR: 0.5; 95% CI: 0.3–1.0; p = 0.04); however, adjusted analysis by gender, age at study, seizure type and etiology of epilepsy did not confirm this finding. Seizure type confounded the results. Sub-analysis by epilepsy syndrome and seizure type demonstrated a significant allelic association with response to VPA in Malay IGE patients (OR: 3.6; 95% CI: 1.3–10.2; p = 0.02), as confirmed by adjusted analysis (OR: 3.4; 95% CI: 1.1–10; p = 0.03). However, there was no allelic association with response to VPA in Malay, Indian and Chinese patients with symptomatic epilepsy (p = 1.0; p = 1.0; and p = 0.62, respectively).

Genotypes

A genotype association with response to VPA was observed in the Hong Kong Chinese subgroup (OR: 2.0; 95% CI: 1.1–3.8; p = 0.03); however, adjusted analysis by gender, age at study, seizure type and etiology of epilepsy did not confirm this finding. Seizure type confounded the results. Sub-analysis by epilepsy syndrome and seizure type demonstrated significant genotype association with response to VPA in Malay IGE patients (OR: 0.3; 95% CI: 0.1–0.9; p = 0.02), as confirmed by adjusted analysis (OR: 0.3; 95% CI: 0.1–0.9; p = 0.03). However, no genotype association with response to VPA in Indian and Chinese subgroups with symptomatic epilepsy (p = 0.10 and p = 0.78, respectively) was found; however,
it was significant in Malays ($p = 0.04$), although this finding was not confirmed by the adjusted analysis for confounders.

**Meta-analysis**

We meta-analyzed the pooled allele and genotype data of 1960 subjects (1042 of whom were drug nonresponsive) from six studies, including our data of Malay, Indian, Malaysian Chinese and Hong Kong Chinese epilepsy patients (Table 3). Association with response to AEDs was observed neither before nor after stratified analysis by epilepsy syndrome and seizure type (Figure 1).

**Discussion**

A highly conserved alternative splicing SCN1A IVS5N+5 G>A polymorphism plays an important role in modification of sodium channel function and consequently in epilepsy. Substitution of G for A in the splice donor consensus sequence of this intron modifies the proportion of 5A and 5N transcripts. Both 5N and 5A forms encode a portion of S3 and S3/S4 linker in the first domain of the Na\(_{\text{V}}\)1.1 channel [8,13]. The A allele reduces expression of the 5N form relative to the 5A form, while the G allele allows expression of both 5N and 5A forms [16]. Given the reported association between the IVS5N+5 polymorphism and dosage of Na\(_{\text{V}}\)1.1 channel blockers such as PHT and CBZ [16–18], as well as with response to CBZ [21], the lack of published studies on VPA prompted us to investigate the association between this locus and VPA monotherapy in a cohort study of 583 Malaysian Chinese and Hong Kong Chinese patients with epilepsy. Our cohort data demonstrated that the G allele might be a risk factor for resistance to VPA in Malay IGE patients.

The G allele of the IVS5N+5 polymorphism increases expression of exon 5N [17]. Evidence suggests that Na\(_{\text{V}}\)1.1 channels containing exon 5N are physiologically more sensitive to PHT and lamotrigine and need lower doses of these drugs than channels containing exon 5A. This difference between two types of channels may be caused by the effect of an asparagine residue in the 5N form compared with an aspartic residue in the 5A form (Figure 2) [15,25]. In our cohort study, unlike previous reports, the G allele in VPA-nonresponsive Malays with IGE was significantly more common compared with VPA-responsive patients. However, pooling Malaysian Chinese and Hong Kong Chinese patients with IGE did not verify this finding. Furthermore, we observed no associations between IVS5N+5 and other ethnic subgroups for VPA monotherapy in our cohort study. This discrepancy among subgroups

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**Table 3. Allele and genotype distributions of SCN1A IVS5N+5 polymorphism in the drug-nonresponsive and -responsive epilepsy patients of the eight studies included in the meta-analysis.**

<table>
<thead>
<tr>
<th>Study</th>
<th>Sample</th>
<th>AEDs</th>
<th>Epilepsy</th>
<th>Allele</th>
<th>Ref.</th>
<th>genotype</th>
<th>Allele</th>
<th>Ref.</th>
<th>genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alee et al.</td>
<td>Japan</td>
<td>2008</td>
<td>All types</td>
<td>CIBZ</td>
<td>198 (57)</td>
<td>MR</td>
<td>17 (11)</td>
<td>GA</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Kwan et al.</td>
<td>Hong Kong</td>
<td>2008</td>
<td>All types</td>
<td>Sodium channel blocker</td>
<td>372 (76)</td>
<td>MR</td>
<td>21 (12)</td>
<td>GA</td>
<td>14 (8)</td>
</tr>
<tr>
<td>Sanchez et al.</td>
<td>Spain</td>
<td>2010</td>
<td>All types</td>
<td>AEDs</td>
<td>111 (56)</td>
<td>MR</td>
<td>33 (14)</td>
<td>GA</td>
<td>15 (7)</td>
</tr>
<tr>
<td>Manna et al.</td>
<td>India</td>
<td>2010</td>
<td>All types</td>
<td>CIBZ and OXC</td>
<td>518 (263)</td>
<td>MR</td>
<td>41 (18)</td>
<td>GA</td>
<td>27 (12)</td>
</tr>
<tr>
<td>Present study</td>
<td>Malaysia (Malay)</td>
<td>2012</td>
<td>All types</td>
<td>VPA</td>
<td>166 (52)</td>
<td>MR</td>
<td>43 (16)</td>
<td>GA</td>
<td>27 (10)</td>
</tr>
<tr>
<td>Present study</td>
<td>Malaysia (Indian)</td>
<td>2012</td>
<td>All types</td>
<td>VPA</td>
<td>132 (45)</td>
<td>MR</td>
<td>34 (12)</td>
<td>GA</td>
<td>29 (10)</td>
</tr>
<tr>
<td>Present study</td>
<td>Hong Kong (Chinese)</td>
<td>2012</td>
<td>All types</td>
<td>VPA</td>
<td>96 (50)</td>
<td>MR</td>
<td>17 (9)</td>
<td>GA</td>
<td>37 (19)</td>
</tr>
<tr>
<td>Present study</td>
<td>Japan</td>
<td>2012</td>
<td>All types</td>
<td>Carbamazepine</td>
<td>125 (50)</td>
<td>MR</td>
<td>17 (7)</td>
<td>GA</td>
<td>37 (15)</td>
</tr>
</tbody>
</table>

AED: Antiepileptic drug; CBZ: Carbamazepine; CIBZ: Cis-carbamazepine; NI: Drug nonresponsive; OXC: Oxcarbamazapine; R: Drug responsive; VPA: Sodium valproate.
and with some previous data was perhaps caused by the following factors:

- The small sample size of the Malays with IGE receiving VPA monotherapy (n = 39) may have led to false-positive results. To minimize the occurrence of this error, further association studies with larger sample sizes are suggested;

- IVSSN+5 may not be a causal polymorphism for response to AEDs in epilepsy treatment. Three amino acids differ between 5A and 5N in sodium channels, and one of those amino acids (changing from asparagine in 5N to aspartate in 5A) was sufficient to modify sodium channel sensitivity to AEDs and thereby on drug responsiveness [15,25]. However, our negative data suggest that further in vivo studies are needed to support the relationship between this amino acid in the target channel and response to the commonly prescribed AEDs in epilepsy;

- This polymorphism may not play a causal role alone, but may be in linkage disequilibrium with other loci in the SCN1A gene or in the neighboring genes, such as SCN2A and SCN3A, or more distant genes including SCNB, SCNA and SCNH, thereby affecting sensitivity to treatment. In addition, the relationship between ethnic differences in linkage disequilibrium or interaction of this polymorphism with various loci in SCN1A or other genes and response to AEDs among different populations needs to be taken into account;

- Differences in prescribing practices for AEDs between Malaysian Chinese, Hong Kong Chinese and other epilepsy patients;

- Heterogeneous Malaysian Chinese epilepsy population might affect the cohort study results; however, subanalysis for symptomatic epilepsy did not show any significant association among Malaysian Chinese, Malaysian Indian and Malaysian Malay patients. On the other hand, meta-analysis results may be influenced by the inclusion of studies that included patients with various types of epilepsy.

Our meta-analysis had three major limitations. First, the Malaysian and Hong Kong patients...
in the current cohort study were only on VPA monotherapy, whereas patients in other included studies were treated by different AEDs and often polytherapy. Second, the inclusion criteria for the selection of the studies were heterogeneous for some parameters such as seizure type, epilepsy syndrome and monotherapy or polytherapy. The final limitation was the small number of studies in this meta-analysis, precluding subanalysis by ethnicity, epilepsy syndrome, seizure type or AED type. Moreover, among the previous studies, some AEDs were not sodium channel blockers. Only three studies were carried out in Asians (Chinese, Japanese and Malaysian), while two were carried out in Caucasians (Italian and Spanish).

Conclusion & future perspective
Considering the effect of sodium channel blocking drugs in response to treatment in epilepsy, this cohort study was designed to evaluate the possible link between the SCN1A IVS5N+5 gene polymorphism and response to VPA. In addition, to summarize the previous data and this study, a meta-analysis was also performed. Unlike Malays with IGE, cohort study data were negative in Chinese and in Indians under VPA monotherapy. In conclusion, despite some limitations, our cohort study showed that the positive finding in Malays with IGE is probably caused by a small sample size. Future studies with larger sample sizes from this epilepsy group receiving VPA monotherapy are suggested.
Acknowledgements

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Financial & competing interests disclosure

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

Executive summary

Aim

• The product of the SCN1A gene is a target of antiepileptic drugs (AEDs) and is proposed to play a significant role in resistance to these drugs in approximately 30% of newly diagnosed patients with epilepsy.

Patients & methods

• The current cohort study and meta-analysis examined an association between the SCN1A IVS5N+5 gene polymorphism and response to sodium valproate (VPA) monotherapy and to AEDs, respectively in Malaysian and Hong Kong Chinese epilepsy patients.

Results

• A significant allelic association between the SCN1A IVS5N+5 locus and response to VPA in Malays with idiopathic generalized epilepsy was observed.

• No significant allelic and genotypic relationship between the SCN1A IVS5N+5 gene polymorphism and response to VPA or AEDs was observed in the included studies for meta-analysis.

Conclusion

• The positive finding in Malays with idiopathic generalized epilepsy is probably caused by small sample size.

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** of considerable interest

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