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Differential role of sodium channels *SCN1A* and *SCN2A* gene polymorphisms with epilepsy and multiple drug resistance in the north Indian population

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The *SCN1A* and *SCN2A* genes encode α subunits of the neuronal voltage-gated sodium channel, which are targets for various antiepileptic drugs such as carbamazepine, phenytoin, valproate and others.
- Recent studies have demonstrated that various genetic variants of these channel genes play important role in the pathogenesis and therapy of epilepsy.

WHAT THIS STUDY ADDS

- This study demonstrates a significant association between the *SCN1A* c.3184 A→G; AG genotype and epilepsy.
- However *SCN2A* c.56 G→A; allele 'A' was significantly associated with multiple drug resistance in epilepsy in north Indian population.

AIMS

To evaluate sodium channel genes as candidates for epilepsy susceptibility and their role in therapeutic efficacy, we screened coding single-nucleotide polymorphism of *SCN1A* p.Thr 1067 Ala or c.3184 A→G (rs2298771) and *SCN2A* p.Arg19Lys or c.56 G→A (rs17183814) in north Indian epilepsy patients.

METHODS

The genotyping was performed in 160 control subjects and 336 patients with epilepsy, of whom 117 were drug resistant and 219 were drug responsive. Therapeutic drug monitoring for phenytoin, carbamazepine, phenobarbital and valproate was also performed in 20% of the patients to confirm compliance.

RESULTS

AG genotype of *SCN1A* 3184 A→G polymorphism was significantly higher and associated in epilepsy patients [$P = 0.005$; odds ratio (OR) 1.76, 95% confidence interval (CI) 1.19, 2.61], whereas A variant of *SCN2A* c.56 G→A was associated with multiple drug resistance in north Indian patients with epilepsy ($P = 0.03$; OR 1.62, 95% CI 1.03, 2.56).

CONCLUSIONS

Overall, results indicate a differential role of genetic polymorphisms of sodium channels *SCN1A* and *SCN2A* in epilepsy susceptibility and drug response.

Introduction

Epilepsy is a common episodic neurological condition that is heterogeneous in clinical presentation. Approximately 70% of all patients with epilepsy lack an obvious extraneous cause, and genetics is presumed to be the predominant factor underlying the disorder. Most epilepsy phenotypes result from interactions between genes and environmental factors. The genetic variation could affect the aetiology, prognosis and consequences of epilepsies to varying degrees in different individuals, including responsiveness to antiepileptic drugs (AEDs). Voltage-gated sodium channels, essential for action potential generation, also have a critical role in membrane excitability. The genes coding for channel components are considered to be a major class of genes associated with various epilepsy phenotypes. The voltage-gated sodium ion channels consist of α and β subunits. Each α subunit is associated with one or more β subunits to form functional voltage-gated ion channels. Defects in subunits of sodium channels render them susceptible to slow inactivation, i.e. membrane remains depolarized for a longer time that can result in epileptogenesis and spread of seizures [1, 2]. In fact, altered sodium channel transcript levels in human epilepsy have been found in brain tissues, suggesting a potential role for sodium channels in the pathophysiology of epilepsy [3]. In the last decade, various coding and noncoding sequence variations of voltage-gated sodium channels *SCN1A*, *SCN2A*, *SCN8A* and *SCN9A* have been identified in patients with seizures, ataxia, and sensitivity to pain [4].

Apart from their role in nerve conduction and the process of epileptogenesis, these voltage-gated sodium channels are also recognized as the major targets with respect to AED efficacy [5, 6]. Several single nucleotide polymorphisms (SNPs) in the sodium channel genes have been described so far, but only a few including *SCN1A* p. Thr1067Ala or c.3184 A→G (rs2298771) and *SCN2A* p.Arg19Lys or c.56 G→A (rs17183814) gene polymorphisms are found to have functional significance in different neurological disorders [7]. In a proband of the Japanese family with missense mutations in *SCN2A* gene, the patient had partial epilepsy after febrile seizures (FS) [8]; along with other genetic variants identified, the *SCN2A* c.56 G→A variant was also observed. Although it was not a disease-causing mutation, it is believed that this variant in *SCN2A* gene along with other genes could have modified the phenotype of the individual affected in that family [8]. This polymorphism causes amino acid substitution (Arg19Lys) in a cytoplasmic portion of the channel and this Arg19 is a moderately conserved residue. Moreover, this *SCN2A* c.56 G→A variant that codes for lysine was significantly more frequent in patients with FS associated with afebrile seizures including generalized epilepsy with febrile seizures (GEFS) 1 than in controls [9]. Along with other genetic variants, c.*SCN2A* 56 G→A allele may be a possible modifying factor for epilepsy susceptibility and therapeutic response. Defects in *SCN1A* are a cause of severe myoclonic epilepsy in infancy, GEFS plus type 2 [10] and intractable childhood epilepsy with generalized tonic-clonic seizures [11]. As the causal relation between *SCN2A* 56 G→A, *SCN1A* c.3184 A→G polymorphisms and FS associated with afebrile seizures or idiopathic generalized epilepsy has not been proven genetically, identification or confirmation of the association in different populations would be important in establishing a role for the *SCN1A* and *SCN2A* gene in the development of seizures.

Ionic currents generated through sodium channels are inhibited by a number of different types of therapeutically important AEDs. There is substantial evidence to suggest that most AEDs such as carbamazepine, phenytoin and oxcarbazepine interact with voltage-gated sodium, calcium and potassium channels [12, 13]. A significant association has been found between intronic *SCN1A* IVS5–91 G→A SNP and maximum doses in regular usage of carbamazepine and phenytoin [14–16]. However, this polymorphism was not found to be associated with carbamazepine dosage in Austrian epilepsy patients [17], suggesting population or ethnic variations. Thus, on the basis of previous observations and current knowledge that variations in voltage-gated sodium channel genes are involved in susceptibility to epilepsy and could influence interindividual variation in response to AEDs, we investigated the role *SCN1A* c.3184 A→G and *SCN2A* c.56 G→A gene polymorphisms in modulating these aspects in north Indian patients with epilepsy.

Material and methods

Patients and controls

The present study comprised 336 epilepsy patients and 160 healthy controls. All epilepsy patients were recruited from the Department of Neurology, Sanjay Gandhi Post Graduate institute of Medical Sciences (SGPGIMS) (Lucknow India). The patients were diagnosed and classified according to guidelines from the International League against Epilepsy. Exclusion criteria included severe adverse drug reactions, poor compliance with AEDs, unreliable record of seizure frequency, history of pseudo seizures, alcohol or drug abuse, or any other malignant diseases such as brain tumour, secondary metastasis, hepatic or renal failure. An informed consent was signed by each participant or responsible adult and they were personally interviewed for information on ethnicity, seizure frequency, and duration of seizure, compliance and other habits. All controls, refractory and responsive patients were of the same ethnic origin. The study was approved by the Ethics Committee of SGPGIMS (Lucknow, India).

Control subjects were recruited from the staff of SGPGIMS as well as from the general population of the region. All control subjects were unrelated to the patients, and belonged to the same ethnicity and geographical

Table 1
Primer sequences used in the study

SNP	Primer sequence	Restriction enzyme	References
***SCN1A* c.3184 A→G(rs2298771)**	5′-TGCACAAAGGAGTAGCTTATG-3′ 5′-AGTCAAGATCTTTCCCAATTTCAG-3′	*PvuII*	[21]
***SCN2A* c.56 G→A(rs17183814)**	5′-AATCACCTTTTATTCTAATGGTC-3′ 5′-CAGTGAAGGCAACTTACTAAGA-3′	*ScrFI*	[22]

area. These subjects were not reported to have a history of epileptic seizures or any other neuropsychiatric disorder. Mean ages were similar in patients and control groups.

Definition of drug resistance and responsiveness

The main criterion for drug resistance was the occurrence of at least four seizures over a period of 1 year with three appropriate AEDs at maximum tolerated doses [18, 19]. Patients who had undergone surgery for seizure control were considered refractory irrespective of their outcome after surgery. The epilepsy patients who had complete freedom from seizures for at least 1 year from the last follow-up visit were considered drug responsive.

Laboratory protocols: sample collection and genotyping of SCN1A *c.3184 A→G and* SCN2A *c.56 G→A*

Blood samples (5 ml) were taken in ethylenediamine tetraaceticacid vials from patients and DNA was isolated using the salting out method with slight modifications [20]. The plasma was separated and stored at −20°C for drug level assay. We genotyped total 992 chromosomes of 336 epilepsy patients and 160 healthy controls. Genotyping was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as reported previously (Table 1). Twenty percent of samples from patients including samples of each genotype were re-genotyped by different laboratory personnel and results were found to be concordant. Fragments containing polymorphic sites were amplified with forward and reverse primers by PCR in a final volume of 20 µl containing 50–100 ng genomic DNA. PCR conditions were as follows: a denaturing step at 95°C for 5 min, then 30 cycles at 94°C for 30 s, 60°C for *SCN2A* and 57°C for *SCN1A* for 30 s, 72°C for 30 s, and a final incubation at 72°C for 7 min. After amplification, PCR products were digested using specific restriction endonucleases. The *SCN2A* c.56 G→A polymorphism was identified by loss of the *ScrFI* restriction site (G allele 178, 130, 64 and 28 bp; and A allele 206, 130 and 64 bp). The *SCN1A* c.3184 A→G polymorphism was identified by *PvuII* restriction site (A allele, 168 bp; G allele, 145 and 23 bp).

Statistical analysis

The relationship between various genotypes and responsiveness was examined using binary logistic regression. Association was expressed as odds ratios (OR) or risk estimates with 95% confidence intervals (CI). The association was considered significant when *P*-value was <0.05. All analyses were performed using SPSS statistical analysis software, version 15.0 (SPSS Inc., Chicago, IL, USA). The sample size was calculated using the QUANTO 1.1 program (http://hydra.usc.edu/gxe). The desired power of the study was set at 80%. Relative risks for power calculation were set at 2.0.

Results

Table 2 shows the demographic profile of all 336 epilepsy patients comprising 24.9% (86) idiopathic, 41.4% (143) symptomatic and 30.0% (107) with cryptogenic aetiology. Among them, 58.3% (201) patients presented generalized and 39.1% (135) partial seizures. Out of all epilepsy patients, 34.8% (117) showed drug resistance and 65.2% (219) had drug-responsive epilepsy. The mean age of the drug-resistant patients was 23.8 ± 12.1 years *vs.* drug-responsive patients, in whom it was 24.4 ± 10.8 years. Mean age of onset for seizures in epilepsy patients was 15.7 ± 10.2 years; it was 14.2 ± 10.74 for nonresponders and 16.5 ± 9.9 for responders. Compliance was confirmed in about 20% patients, measuring antiepileptic drug levels using high-performance liquid chromatography. Mean carbamazepine, phenytoin and valproate levels were 8.26 ± 5.25, 11.27 ± 8.12 and 68.0 ± 36.22 µg ml^{-1}, respectively, in epilepsy patients; these were in therapeutic range.

SCN1A *c. 3184 A→G and* SCN2A *c.56 G→A gene polymorphisms and susceptibility to epilepsy*

We determined the genotypic and allelic frequencies in 336 sporadic epilepsy patients and 160 normal healthy controls by PCR-RFLP assay (Table 3a,b). In our study population the observed genotype distribution of these polymorphisms in control subjects was consistent with Hardy–Weinberg equilibrium (HWE) ($P = 0.97$ for *SCN1A* and $P = 0.52$ for *SCN2A*). In the patient population *SCN2A* genotype distribution was consistent with HWE, whereas this was not the case for *SCN1A* genotypes. The frequency of AG genotype of *SCN1A* c.3184 A→G polymorphism was significantly higher in epilepsy patients *vs.* healthy controls

Table 2

Demographic profiles of epilepsy patients

	Total epilepsy (336)	Responder (219)	Drug resistant (117)	Controls (160)
Male	242 (72.1%)	155 (70.8%)	87 (74.4%)	70 (43.5%)
Female	94 (27.9%)	64 (29.2%)	30 (25.6%)	90 (56.5%)
Age (years)	24.2 ± 11.3	24.37 ± 10.8	23.79 ± 12.1	24.94 ± 11.4
Age of onset		16.55 ± 9.9	14.22 ± 10.7	–
AED therapy at the last clinic visit				
Monotherapy	112 (33.2%)			
Polytherapy	224 (66.8%)			
Aetiology				
Idiopathic	86 (25.6%)	52 (23.8%)	34 (29.1%)	–
Symptomatic	143 (42.6%)	95 (43.4%)	48 (41.0%)	–
Cryptogenic	107 (31.8%)	72 (32.8%)	35 (29.1%)	–
Seizure type				
Generalized	201 (59.8%)	126 (57.5%)	75 (64.1%)	–
Partial	135 (40.2%)	93 (42.5%)	42 (35.9%)	–

Table 3a

Distribution of *SCN1A* rs2298771 polymorphism; genotype and allele frequencies in epilepsy patients *vs.* healthy controls

Genotypes/alleles	All patients with epilepsy (*n* = 336)	Healthy controls (*n* = 160)	Odds ratio (95% CI)	*P*-value
AA	144 (42.9%)	88 (55.0%)	reference	–
AG	179 (53.3%)	62 (38.8%)	1.76 (1.192, 2.61)	0.005
GG	13 (3.9%)	10 (6.3%)	0.79 (0.334, 1.88)	0.60
A*	467 (69.5%)	238 (74.4%)	reference	–
G*	205 (30.5%)	82 (25.6%)	1.27 (0.94, 1.72)	0.11

*Alleles.

Table 3b

Distribution of *SCN2A* rs17183814 polymorphism; genotype and allele frequencies in epilepsy patients *vs.* healthy control

Genotypes/alleles	All patients with epilepsy (*n* = 336)	Healthy controls (*n* = 160)	Odds ratio (95% CI)	*P*-value
RR	257 (76.5%)	112 (70.0%)	reference	–
RK	71 (21.1%)	46 (28.8%)	0.69 (0.43, 1.03)	0.07
KK	8 (2.4%)	2 (1.3%)	1.74 (0.36, 8.34)	0.48
R*	585 (87.1%)	270 (84.4%)	reference	–
K*	87 (12.9%)	50 (15.6%)	0.80 (0.55, 1.17)	0.25

*Alleles.

(*P* = 0.005; OR 1.76, 95% CI 1.19, 2.61) (Table 3a). However, we did not observe any significant differences in genotypic or allelic frequency between the epilepsy patients and healthy controls for the *SCN2A c.*56 G→A gene polymorphism (Table 3b).

SCN1A *c.3184 A→G and* SCN2A *c.56 G→A polymorphism in drug-resistant epilepsy*

Genotype or allelic frequencies of *SCN1A* c.3184 A→G polymorphism did not differ significantly in drug-resistant *vs.* drug-responsive epilepsy patients for AG (*P* = 0.98; OR 0.99, 95% CI 0.62, 1.58) or GG (*P* = 0.78; OR 1.17) (Table 4a). Similarly, the genotype frequencies of *SCN2A* c.56 G→A also did not differ significantly in drug-resistant *vs.* -responsive patients for RK (*P* = 0.97; OR 1.44, 95% CI 0.84, 2.48) or KK (*P* = 0.09; OR 3.49, 95% CI 0.81, 14.97) genotypes (Table 4b). However, variant allele frequency of *SCN2A* c.56 G→A SNP was significantly higher in drug-resistant patients with epilepsy *vs.* drug-responsive patients (*P* = 0.03; OR 1.62, 95% CI 1.03, 2.56). It suggests that *SCN2A* c.56 G→A polymorphism modulates drug response behaviour in north Indian epilepsy patients.

Table 4a

Distribution of *SCN1A* rs2298771; genotype and allele frequencies in drug-resistant *vs.* drug-responsive patients with epileps

Genotypes/alleles	Drug resistant (*n* = 117)	Drug responder (*n* = 219)	Odds ratio (95% CI)	*P*-value
AA	50 (42.7%)	94 (42.9%)	reference	–
AG	62 (53.0%)	117 (53.4%)	0.99 (0.62, 1.58)	0.98
GG	5 (4.3%)	8 (3.7%)	1.17 (0.36, 3.78)	0.78
A*	162 (69.2%)	305 (69.6%)	reference	–
G*	72 (30.8%)	133 (30.4%)	1.01 (0.72, 1.43)	0.91

*Alleles.

Table 4b

Distribution of *SCN2A* rs17183814 gene polymorphism in drug-resistant and -responsive patients with epilepsy

Genotypes/alleles	Drug-resistant epilepsy (*n* = 117)	Drug-responsive epilepsy (*n* = 219)	Odds ratio (95% CI)	*P*-value
RR	83 (70.9%)	174 (75.9%)	reference	–
RK	29 (24.8%)	42 (19.2%)	1.44 (0.84, 2.48)	0.97
KK	5 (4.3%)	3 (1.4%)	3.49 (0.81, 14.97)	0.09
R*	195 (83.3%)	390 (89.0%)	reference	–
K*	39 (16.7%)	48 (11.0%)	1.62 (1.03, 2.56)	0.03

*Alleles.

Discussion

In the present study of the essential role of sodium channels in epilepsy, we analysed two genetic polymorphisms, *SCN1A* c.3184 A→G and *SCN2A* c.56 G→A. Association was found with epilepsy at different genetic levels. Our observations suggest involvement of *SCN1A* c.3184 A→G, AG genotype in increasing risk for developing epilepsy, but it does not modulate drug response. However, *SCN2A* c.56 G→A polymorphism was found to be associated with multidrug resistance phenotype. Thus, our findings support the fact that sodium channels play a role in epilepsy at multiple levels and may also be involved in the differential effects of AEDs in epilepsy patients.

Initiation and propagation of seizures is due to misfiring of neurons in the brain, and >300 mutations in *SCN1A* gene have thus far been identified in epilepsy [7] and other neurological disorders. We found an association of *SCN1A* c.3184 A→G polymorphism with overall susceptibility to epilepsy. However, a study from Taiwan failed to show any association of this polymorphism in epilepsy patients with FS [21]. In our cohort, the number of patients with FS was limited, and the aetiology of other sporadic epilepsies is different from FS. The *SCN1A* c.3184 A→G results in change of amino acid threonine to alanine at highly consensus conserved site in the coding region of the *SCN1A*, and possibly affects functioning of the inactivation gate in the cytosol regulating efflux and influx of sodium ions. Another possibility is that this polymorphism is in linkage disequilibrium with some other genetic variants of the same gene that imparts risk for epilepsy.

The *SCN2A* c.56 G→A genetic variation under study showed no association in overall epilepsy susceptibility. Previously, this polymorphism studied in German idiopathic generalized epilepsy patients also failed to show any association with epilepsy susceptibility [23]. A similar study [24] carried out in Japanese population by Nakayama *et al*. also found no association in epilepsy patients with FS. These studies suggest this particular polymorphism in *SCN2A* gene is not involved in epilepsy susceptibility.

Until now, most pharmacogenetic studies in epilepsy have explored the role of multidrug transporter gene *ABCB1* in drug-resistant epilepsy and have yielded conflicting results indicating population-specific differences. However, very few studies have correlated drug targets like sodium channels in drug resistance and therapeutic dosage in patients with epilepsy. Therefore, we looked for an association of the two polymorphisms with drug-resistance phenotype in our patient groups. Even though *SCN1A* c.3184 A→G polymorphism was associated with generalized epilepsy, it showed no influence on multidrug resistance phenotype in patients with epilepsy. Similarly, Kwan *et al*. [25] also found no involvement of this polymorphism in drug resistance epilepsy. However, *SCN1A* IVS5–91 G→A intronic polymorphism of same gene has been reported to show population-specific association with carbamazepine and multiple drug resistance epilepsy [14–16, 25].

The *SCN2A* c.56 G→A polymorphism (rs17183814) was found to be independently involved in drug resistance in north Indian epilepsy patients. In our cohort, association

was rather weak as it was observed only at the allele level. The association at allele level may be due to the low number of AA genotype in the drug-resistant and responsive groups and the effect of AA genotype not reaching statistical significance. Sills *et al*. [26] also found a weak association of the polymorphism with drug resistance in a cohort of 400 Scottish patients. Alhough the exact mechanism by which it affects therapeutic response is unknown, it is believed that AEDs block action potential propagation by decreasing neurotransmitter release, which reduces focal firing that decreases spread of seizures. Although it is difficult to predict the mechanism of altered drug response, it appears that amino acid change from arginine to lysine somehow interferes with stabilization of biological membranes by AEDs. Limitations of the present study were that only two SNPs in sodium channel genes were screened in the study population; however, the effect and presence of other genetic variations in the same gene or other candidate genes cannot be ignored. Also, an *in vitro* functional study to examine the electrophysiological effects of the SNPs/combinations of SNPs and mutations may provide insight into the molecular mechanism underlying the pathogenesis of epilepsy and the aetiology of variable drug response.

Overall, the results of our study suggest differential behaviour of channel gene polymorphisms in epilepsy and its therapy. However, it may be added that in addition to genetic factors, there are multiple causes of drug resistance in epilepsy that include past treatment history [27] and other clinical factors such as type of epilepsy, duration of seizure, and number of seizures prior to initiation of drug therapy.

In summary, we observed a differential influence of genetic variations in the genes coding for two α units of sodium channels in epilepsy phenotypes. Until now, very few studies have explored the role of these genetic variants in epilepsy and multiple drug resistance; it would be desirable to study them at the functional level and also to replicate them in larger cohorts.

Competing interests

None declared.

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