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Evaluating the Role of Genetic Variants on first-line antiepileptic drug response in North India: Significance of *SCN1A* and *GABRA1* Gene Variants in Phenytoin Monotherapy and its Serum Drug Levels

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SUMMARY

Aim: The present study aimed to evaluate association of genetic variants on drug response and therapy optimization parameters in patients treated with first-line antiepileptic drugs (AEDs). Genetic variants from ion channels, their functionally related genes, and synaptic vesicle cycle (SVC) genes with a potential role in epilepsy pathophysiology were thus prioritized. **Methods:** A total of 12 genes from ion channels and related gene set and seven genes from SVC comprising 155 SNPs were genotyped and evaluated with drug response, dose levels, and drug levels in 408 patients with epilepsy. **Results:** Both *GABRA1* and *SCN1A* variants showed haplotypic and diplotypic associations in response to phenytoin (PHT). Diplotype analysis of *GABRA1* variants revealed association of rs12658835|rs7735530 (AG/AG) (P-value$_{corrected}$ = 0.034, OR = 3.75, 95% CI = 1.36–11.05) and rs12658835|rs7735530|rs7732641|rs2279020 (AGCA/AGCA) (P-value$_{corrected}$ = 0.035, OR = 2.48, 95% CI = 0.96–6.41) with recurrent seizures. *SCN1A* haplotype rs6432860|rs3812718 (AC: P-value$_{corrected}$ = 0.022, OR = 2.72, 95% CI = 1.39–5.35) and diplotype (AC/AC: P-value$_{corrected}$ = 0.034, OR = 6.42, 95% CI = 1.10–65.76) were further observed to be associated with recurrent seizures. With respect to therapy optimization parameters, we observed significantly lower dose-adjusted drug levels at maximum dose of PHT in patients carrying AC/AC diplotype (P-value = 0.021). **Conclusion:** The results further substantiate the role of *GABRA1* in PHT mode of action and contribution of *SCN1A* in response and therapy optimization with PHT monotherapy.

Introduction

Epilepsy is a complex neurological disorder characterized by recurrent and unprovoked seizures. It affects nearly 50 million people worldwide with a prevalence of 5.3/1000 person in India [1]. A variety of antiepileptic drugs (AEDs) are available in the market with newer drugs also developing constantly. However, first-generation AEDs mainly carbamazepine (CBZ), phenytoin (PHT), valproate (VP), and phenobarbitone (PB) still remain as the major prescriptions [2]. Despite availability of appropriate therapy, patient response is highly variable with 30% failing to respond to treatment [3]. Mechanism of action of first-generation AEDs is considered to be well known with their possible metabolizing enzymes, transporters, and targets well characterized and well explored in pharmacogenetic studies conducted for understanding the variable patient response. [4]. The results have been inconclusive and conflicting with no recommendations available to date for prediction of antiepileptic dose, drug level, and drug response on the basis of genetic markers. Therefore, due to unavailability of clinically applicable results, disparities overshadow patient response, with no uniformity among individuals in terms of dosing and response [4–9].

This hints toward the need for further exploration of mechanisms of action of AEDs. Majorly, ion channels and receptors such as sodium channels, calcium channels, GABA and glutamate receptors, GABA transporters, and GABA aldehyde dehydrogenase are known to be the targets of AEDs. Both CBZ and PHT are known to target voltage-dependent sodium channels, whereas VP and PB are known to target GABAergic neurotransmitter system [10–12]. Additionally, potentiation of GABAergic neurotransmission has also been reported with CBZ and PHT [13]. In addition, there may be many other potential molecules in nervous system. For instance *SV2A*, a nonion channel molecule belonging to presynaptic region of neurons is a possible target of levetiracetam [14]. Of the

presynaptic gene assembly, genes such as synapsin, syntaxin, and syndapin have been well explored for their role in epilepsy genetics in humans. On the other hand, genes such as dynamin 1 and *SNAP-25* have only been explored for genetics in mouse models. However, the understanding of presynaptic gene involvement in AEDs mode of action is still in its infancy [14–18]. Therefore, gene prioritization for pharmacogenetic studies may thus be reviewed and may also include evaluation of epilepsy pathophysiology genes other than classic ion channel genes [11,19].

Therefore, to understand the interpatient variability, genetic variants from ion channels and their functionally related genes (mainly sodium channels, GABA and glutamate receptors, GABA transporter, and GABA aldehyde dehydrogenase) along with the presynaptic (synaptic vesicle cycle [SVC]) genes with potential involvement in disease pathophysiology were evaluated for their influence on variable AED response and therapy optimization parameters, that is, dose and dose-adjusted drug levels of patients.

Materials and Methods

Subjects

A total of 478 epilepsy patients of North Indian ethnicity were enrolled from the Outpatient Department of Neurology at the Institute of Human Behaviour and Allied Sciences (Delhi, India). Prior to patient enrollment, the study protocol was approved by institutional ethics committee. All the enrolled patients fulfilled the inclusion and exclusion criteria of the study. Inclusion criteria were as follows: patients above five years of age with at least two unprovoked seizures, on treatment with any of the four first-line AEDs, that is, PHT, CBZ, VP, PB, or their combinations/multitherapy (MT). Exclusion criteria were as follows: patients with gross neurological deficits such as mental retardation, motor deficits, and imaging abnormalities including tumor, multiple neurocysticercosis, tuberculoma, vascular malformation and atrophic lesions, patients who had severe hepatic and renal disorders, and pregnant women [20]. Diagnosis and treatment were performed by an experienced neurologist. For seizure types, seizure diagnosis, and their classification, guidelines of International League Against Epilepsy (ILAE) 1981 and 1989 were followed [21,22]. A detailed questionnaire of the project which included gender, age at seizure onset, type of seizure, baseline seizure frequency, AED prescription, neurological examination, brain imaging was used for data collection. Other baseline evaluations of the patients included all routine investigations such as biochemistry profile, hematology profile, serum drug-level estimation, and DNA extraction. Blood samples were collected from all the patients after obtaining written consent forms in accordance with the institutional ethics committee approval. Patients were followed up at 2nd, 4th, 8th, and 12th months from the date of enrollment and were evaluated for seizure control, compliance to medications, side effects, and therapeutic drug monitoring (TDM). At the end of one-year study duration, all patients were assessed for seizure control based on the number of seizures experienced during the follow-up duration which did not include the initial 2-month period during which all patients were assumed to attain the steady-state drug levels. Patients who remained seizure free in the last 10 months, despite appropriate AED treatment, were kept in "no-seizure" group, whereas those with one or more seizures during the same period were kept in "recurrent seizure" group. Out of the enrolled 478 patients, a total of 408 patients completed the study duration (Table 1). An additional cohort of 170 ethnicity matched unrelated healthy individuals were also enrolled for the study.

Genes and SNP Prioritization

Genomic DNA was extracted from peripheral blood cells by utilizing a modified version of salting out method [23]. A total of 12 ion channels, their functionally related genes, and seven SVC genes comprising 210 SNPs were prioritized (Tables S1 and S2). The genes prioritized for the present study thus comprised ion channels and their functionally related genes: sodium channel genes encoding α subunit type 1 (*SCN1A*), α subunit type 2 (*SCN2A*), and β subunit type 1 (*SCN1B*), GABA and glutamate receptors including *GABRA1*, *GABRA6*, *GABRB3*, *GABRG2*, *GRIK1* and *GRIN1*, GABA transporter *(SLC6A11)*, and GABA aldehyde dehydrogenase *(ALDH5A1)*. Among the presynaptic genes (SVC genes), a comprehensive gene list of *SNAP25, STX1A, STXBP1, SYN2, SYT1, VAMP2, EFHC1* was prioritized. SNPs were prioritized on the basis of literature reports stating their genetic association [14,15] and probable functional significance depending on the gene location and prediction softwares (SNP function prediction, FuncPred which gives integrative results for PolyPhen, SNP3D, miRNA binding, etc.) [24]. With the aim of covering the entire gene, SNPs from noncoding sequences, that is, intron and other 3′ and 5′ UTRs, as well as coding region, that is, synonymous and nonsynonymous SNPs, were prioritized. Additionally, SNPs from 5′ and 3′ upstream region of the gene were also prioritized. A total of 186 SNPs which passed the assay designing and initial optimization of reactions were finally genotyped by iPLEX Gold, Sequenom MassARRAY Genetic Analysis System (Sequenom, Inc., San Diego, CA, USA) using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. To determine precision, a random 5% of the samples were later regenotyped by sequencing using the BigDye Terminator kit (version 3.1; Applied Biosystems, Foster City, CA, USA). Further to rule out the issue of population stratification, nine randomly chosen autosomal microsatellite markers not linked to epilepsy (D10S548, D10S196, D10S1653, D11S937, D11S901, D13S218, D13S175, D20S115, and D20S107) were also genotyped in all the samples. The results were then analyzed on GeneScan module of the Genotyper software, version 3.7 (Applied Biosystems).

Quantitation of AEDs in Serum Samples

Total serum drug concentration of PHT, CBZ, VPA, and PB were measured by Auto-analyzer from Logitech Pvt. Ltd. (Model Echo) using cloned enzyme donor immunoassay (CEDIA®) II Assay kits by Microgenics Corporation, USA. All measurements and interpretations were performed in the neuropsychopharmacology department of IHBAS under the supervision of an experienced clinical pharmacologist. The inter-run assay precision for AEDs studied was <10% for follow-ups and evaluation. Due to the variability in oral doses among patients, dose-adjusted serum concentrations were calculated for each patient by dividing the

Table 1 Demographic characteristics of 408 epilepsy patients

Phenotypic characteristics	Phenytoin (PHT) (n = 94 [23.04%])	Carbamazepine (CBZ) (n = 168 [41.18%])	Valproate (VP) (n = 85 [20.83%])	Phenobarbitone (PB) (n = 32 [7.84%])	Multitherapy (MT) (n = 29 [7.11%])	Total (n = 408 [100%])
Age (years)						
Mean ± SD (n)	22.1 ± 10.6, (94)	21.4 ± 8.60, (166)	20.1 ± 8.10, (85)	26.7 ± 12.7, (32)	22.3 ± 10.3, (29)	22.2 ± 9.54, (406)
Body weight (kg)						
Mean ± SD (n)	47.6 ± 12.6, (91)	47.1 ± 11.9, (166)	49.0 ± 13.8, (82)	51.0 ± 8.97, (32)	51.5 ± 19.9, (28)	48.2 ± 13.0, (399)
Age at seizure onset (years) (n [%])						
<5	11 (12.0)	15 (8.98)	3 (3.61)	2 (6.25)	5 (17.2)	36 (8.93)
6 to 15	34 (37.0)	82 (49.1)	46 (55.4)	17 (53.1)	16 (55.2)	195 (48.4)
16 to 25	32 (34.8)	51 (30.5)	26 (31.3)	7 (21.9)	5 (17.2)	121 (30.0)
Above 25	15 (16.3)	19 (11.4)	8 (9.64)	6 (18.7)	3 (10.3)	51 (12.7)
Gender (n [%])						
Male	77 (81.9)	55 (32.7)	63 (74.1)	19 (59.4)	24 (82.8)	238 (58.3)
Female	17 (18.1)	113 (67.3)	22 (25.9)	13 (40.6)	5 (17.2)	170 (41.7)
Seizure type (n [%])						
Generalized tonic clonic seizures (GTCS)	68 (72.3)	91 (54.2)	48 (56.5)	25 (78.1)	15 (51.7)	247 (60.5)
Simple partial seizures (SPS)	2 (2.13)	3 (1.79)	2 (2.35)	0 (0.00)	2 (6.90)	9 (2.21)
Simple partial seizures with secondary generalization (SPS sec. gen.)	18 (19.1)	41 (24.4)	6 (7.06)	4 (12.5)	7 (24.1)	76 (18.6)
Complex partial seizures (CPS)	0 (0.00)	11 (6.55)	3 (3.53)	0 (0.00)	1 (3.45)	15 (3.68)
Complex partial seizures with secondary generalization (CPS sec.gen.)	5 (5.32)	17 (10.1)	6 (7.06)	1 (3.13)	4 (13.8)	33 (8.09)
Others	1 (1.06)	3 (1.79)	20 (23.5)	2 (6.25)	0 (0.00)	26 (6.37)
Seizure control (n [%])						
No seizure	57 (60.6)	97 (57.7)	50 (58.8)	21 (65.6)	7 (24.1)	232 (56.9)
Recurrent seizure	37 (39.4)	71 (42.3)	35 (41.2)	11 (34.4)	22 (75.9)	176 (43.1)
Dose and drug-level parameters maintenance dose ((mg/day)/kg)						
Median (range), n	4.95 (2.50–12.5), 78	10.9 (2.70–26.7), 146	15 (6.70–42.8), 75	1.87 (1.00–4.70), 29	–	10 (1.00–42.8), 328
Serum drug levels (mg/L)						
Median (range), n	12.02 (3.8–24.5), 64	9.4 (3.1–117.8), 115	84 (6–141), 55	22.51 (2.30–48.8), 22	–	12.05 (2.30–141), 256
Dose-corrected serum drug levels ([mg/L]/[[mg/day]/kg])						
Median (range), n	2.42 (1.00–6.80), 62	0.86 (0.30–9.80), 115	5.72 (0.20–12.8), 55	11.7 (1.40–21.7), 22	–	1.74 (0.20–21.7), 254

SD, standard deviation.

steady-state concentration (mg/L) by the daily dose (mg/day). The "maintenance dose" of a given drug was defined as the dose, which remained unchanged for successive visits in the 12-month period. On the other hand "maximum dose" was defined as the maximum dose prescribed to the patients during the follow-up time period. To obtain the average steady-state serum drug levels at maintenance dose, mean of drug-level measurements over a period when consecutive doses were documented was used. Similarly, average serum levels at maximum dose corresponded to drug levels over the period when consecutive maximum documented dose was used.

Statistical Analysis

Statistical analysis was performed by utilizing the statistical package PLINK (http://pngu.mgh.harvard.edu/purcell/plink/) [25], STATA (StataCorp. 2009, *Stata Statistical Software: Release 11*, College Station, TX: StataCorp LP), SPSS, and R statistical analysis software (version 3.0.2). On the basis of initial statistical quality control (QC) of the genotyped SNPs, SNPs with MAF<0.01 and Hardy–Weinberg equilibrium (HWE) *P*-value <0.001 were dropped from further analysis. The remaining 155 SNPs were carried forward for genotype–phenotype correlation. Normality of the continuous dependent variables was tested using Shapiro–Wilk test. As the data on dose and dose-adjusted drug levels showed non-normal distribution, Mann–Whitney *U*-test and Kruskal–Wallis test were used for comparing their distribution among two (diplotype and dominant model) or three comparable groups (genotypes). Odds ratio (OR) and 95% confidence intervals (CIs) were calculated for each marker and were adjusted for age, gender, weight, and age at onset of patients using binary logistic regression. To reduce correction for multiple comparisons, *P*-values were computed for dominant model of inheritance only based on visual inspection of genotypes. A $P < 0.05$ was considered as statistically significant. Correction for multiple comparisons was applied by means of false discovery rate (FDR) approach. All the significantly associated SNPs were then observed for linkage disequilibrium (LD) based on healthy controls of same ethnicity using tagger algorithm of Haploview 4.2 [26]. SNPs showing high LD (r^2 >0.9) were dropped from further analysis. Remaining significantly associated SNPs were further subjected to haplotype identification by means of sliding window approach of PLINK [25]. Prior to haplotype construction, PHASE (Ver. 2.1) software based on the Bayesian algorithm was used for phasing the entire genotype data with parameter value of 100 iterations a thinning interval of 10, and a burn-in value of 100 in the Markov chain Monte Carlo simulations was used [27]. This was followed by diplotype analysis of the significantly associated haplotypes. Significantly associated diplotypes were than evaluated for association with therapy optimization parameters. To test for population stratification, the genotype frequencies of unlinked markers in cases and controls were compared using Pearson's chi-square test utilizing STRAT software [28].

Results

Of the 408 patients who completed the one-year study duration, 379 (92.65%) were on monotherapy (24.80% [PHT], 44.33% [CBZ], 22.43% [VP], and 8.44% [PB]). Among monotherapy patients, 225 belonged to "no-seizure" group (59.37%) and 154 belonged to "recurrent seizure" (40.63%) group. Maintenance dose of PHT, CBZ, PB, and VPA showed considerable interindividual variation (Median [range]—PHT: 250 [50–500] mg/day, CBZ: 500 [150–1200] mg/day, VP: 750 [400–2000] mg/day, and PB: 90 (60–210) mg/day) (Table 1). The test for population stratification using all of the nine unlinked markers was not significant in our study suggesting ethnically homogeneous population ($\chi^2 = 76.67$, df = 60, *P*-value = 0.072).

Association of Genetic Variants

Association analysis with drug response was first performed in a pooled cohort of all patients on different drug therapies. Later stratified analysis was conducted in each drug therapy group for association with drug response, dose levels, and drug levels.

All Drugs

Pooled analysis of all drug groups revealed significant associations for a total of 15 SNPs spanning five genes, but none of them could remain significant after accounting corrections for multiple comparisons (Table S2). Further, haplotype analysis was performed on SNPs with significant uncorrected *P*-values. However, none of the haplotype could remain significant postcorrection for multiple comparisons.

Phenytoin (PHT)

Upon stratification for PHT treatment, we observed significant association of 13 SNPs from three genes including *SCN1A* (rs2298771, rs6432860, rs3812718), *GABRA6* (rs3811995, rs13184586, rs3219151), and *GABRA1* (rs12658835, rs7735530, rs7732641, rs1157122, rs2279020, rs2290732, rs998754), but none could remain significant postcorrection for multiple comparisons (Table S3). Among all the variants, rs1157122 of *GABRA1* had the most significant uncorrected *P*-value of 8.28×10^{-4}. Further evaluation of LD information on SNPs showing significant but uncorrected association in healthy control population revealed tight LD among following associated SNPs: rs6432860 and rs2298771 of *SCN1A* ($r^2 = 0.92$), rs3219151 with rs3811995 ($r^2 = 0.906$) and rs13184586 ($r^2 = 0.929$) of *GABRA6*, rs7732641 with rs1157122 of *GABRA1* ($r^2 = 0.98$), and rs2290732 with rs998754 ($r^2 = 0.99$) of *GABRA*. Exclusion of tagged SNPs from every gene was followed by haplotype analysis of the remaining five SNPs from *GABRA1* (rs12658835, rs7735530, rs7732641, rs2279020, and rs2290732) and two SNPs (rs6432860 and rs3812718) from *SCN1A*. *GABRA1* showed several significantly associated haplotypes ranging from two- to five-marker combinations and *SCN1A* also showed a significant two-marker haplotype. Of the several significantly associated haplotypes, the longest five-marker haplotype for *GABRA1* was rs12658835|rs7735530|rs7732641|rs2279020|rs2290732 (AATGA: $P_{corrected}$ value = 0.022, OR = 0.30, 95% CI = 0.12–0.77) and (AGCAG: $P_{corrected}$ value = 0.022, OR = 2.34, 95% CI = 1.11–4.94). With respect to *SCN1A*, we observed significant association of a two-marker haplotype

rs6432860|rs3812718 (AC: $P_{corrected}$ value = 0.022, OR = 2.72, 95% CI = 1.39–5.35) only. Diplotype analysis of significant *GABRA1* haplotypes further revealed significant overrepresentation of rs12658835|rs7735530 (AG/AG: $P_{corrected}$ value = 0.0349, OR = 3.75, 95% CI = 1.36–11.05) and rs12658835|rs7735530|rs7732641 (AGC/AGC: $P_{corrected}$ value = 0.0349, OR = 3.22, 95% CI = 1.20–9.10) in patients with "recurrent seizures" (Table 2A). Furthermore, *SCN1A* also showed significant overrepresentation of rs6432860|rs3812718 (AC/AC: $P_{corrected}$ value = 0.0349, OR = 6.42, 95% CI = 1.10–65.76) in patients with "recurrent seizures" (Table 2B).

Analysis of therapy optimization parameters failed to reveal association of *GABRA1* variants with dose and drug levels (Table S4). However, significantly higher levels of "dose-adjusted

Table 2 Association analysis of haplotypes and diplotypes with antiepileptic drug response

(A) Significantly associated haplotypes with antiepileptic drug response

Gene	SNPS	Haplotype	Recurrent seizures	No seizures	OR (95% CI)	*P*-value*
Phenytoin						
GABRA1	rs12658835|rs7735530	AA	9.59	26.1	0.33 (0.13–0.83)	**0.0060**[a]
		AG	90.4	73.8	3.61 (1.57–8.28)	**0.0060**[a]
	rs2279020|rs2290732	AG	74.6	55.0	2.17 (1.16–4.07)	**0.0078**[a]
		GA	25.3	44.9	0.41 (0.21–0.82)	**0.0078**[a]
	rs7732641|rs2279020	CA	72.6	55.2	2.07 (0.68–6.32)	**0.0170**[c]
		TG	9.52	24.4	0.30 (0.12–0.77)	**0.0105**[a]
	rs7735530|rs7732641	AT	9.59	24.3	0.30 (0.12–0.78)	**0.0118**[a]
		GC	90.4	75.7	2.84 (1.21–6.66)	**0.0118**[a]
	rs12658835|rs7735530|rs7732641	AAT	9.72	24.8	0.33 (0.12–0.86)	**0.0118**[a]
		AGC	90.3	75.2	3.22 (1.46–7.08)	**0.0118**[a]
	rs7732641|rs2279020|rs2290732	CAG	74.3	55.0	2.33 (1.08–5.04)	**0.0094**[a]
		TGA	9.93	24.7	0.30 (0.12–0.77)	**0.0140**[b]
	rs7735530|rs7732641|rs2279020	ATG	9.59	24.3	0.30 (0.12–0.77)	**0.0118**[a]
		GCA	72.6	54.9	3.03 (1.28–7.19)	**0.0159**[c]
	rs7735530|rs7732641|rs2279020|rs2290732	ATGA	10.0	25.2	0.30 (0.12–0.77)	**0.0119**[a]
		GCAG	74.3	54.2	1.36 (0.48–3.88)	**0.0071**[a]
	rs12658835|rs7735530|rs7732641|rs2279020	AATG	9.59	24.6	0.30 (0.12–0.77)	**0.0108**[a]
		AGCA	72.6	54.5	2.90 (1.22–6.93)	**0.0138**[b]
	rs12658835|rs7735530|rs7732641|rs2279020|rs2290732	AATGA	10.0	25.5	0.30 (0.12–0.77)	**0.0109**[a]
		AGCAG	74.3	53.7	2.34 (1.11–4.94)	**0.0061**[a]
SCN1A	rs6432860|rs3812718	AC	40.5	19.3	2.72 (1.39–5.35)	**0.0015**[a]
Carbamazepine						
STX1A	rs867500|rs4363087	GT	54.9	39.4	1.97 (1.24–3.13)	**0.0048**[d]
Phenobarbitone						
ALDH5A1	rs2247845|rs1054899	TC	13.6	38.1	0.25 (0.04–1.34)	**0.0419**[e]

(B) Significantly associated diplotypes in phenytoin group of patients

Phenotypic groups	Gene	Diplotype		Recurrent seizures (n%)	No seizures (n%)	OR (95% CI)	*P*-value*
Phenytoin drug response	*GABRA1*	rs12658835|rs7735530	AG	29 (78.4)	28 (49.1)	3.75 (1.36–11.0)	**0.0046**[a]
		rs2279020|rs2290732	AG	19 (51.3)	17 (29.8)	2.48 (0.96–6.41)	**0.0359**[b]
		rs7732641|rs2279020	CA	19 (51.3)	17 (29.8)	2.48 (0.96–6.41)	**0.0359**[b]
		rs7735530|rs7732641	GC	29 (78.4)	32 (56.1)	2.83 (1.02–8.37)	**0.0273**[b]
		rs12658835|rs7735530|rs7732641	AGC	28 (75.7)	28 (49.1)	3.22 (1.20–9.10)	**0.0104**[a]
		rs7735530|rs7732641|rs2279020	GCA	19 (51.3)	17 (29.8)	2.48 (0.96–6.41)	**0.0359**[b]
		rs12658835|rs7735530|rs7732641|rs2279020	AGCA	19 (51.3)	17 (29.8)	2.48 (0.96–6.41)	**0.0359**[b]
	SCN1A	rs6432860|rs3812718	AC	7 (18.9)	2 (3.51)	6.42 (1.10–65.8)	**0.0131**[a]

SNPs with $P < 0.05$ were included for haplotype analysis, haplotype with minimum frequency <0.05 was excluded from the study, *P*-values in bold remained significant after correction, *FDR corrected *P*-values, [a]0.022, [b]0.023, [c]0.025, [d]0.050, [e]0.0189; OR, odds ratio; CI, confidence interval. Haplotypes which withstood FDR correction were included for diplotype analysis. *P*-values in bold remained significant after correction. *FDR corrected *P*-values [a]0.0349, [b]0.0359; OR, odds ratio; CI, confidence interval.

Table 3 Effects of SCN1A diplotypes, genotypes, and model on PHT maintenance doses, maximum doses, and corresponding adjusted serum PHT concentrations

Genotypic	*SCN1A_* rs6432860			*SCN1A_ rs3812718*		
Maintenance	Genotypic	n (mean rank)	*P*-value	Genotypic	n (mean rank)	*P*-value
Dose corrected by weight (mg/day per kg)	GG	46 (41.0)	0.166	TT	23 (41.2)	0.171
	GA	26 (40.8)		CT	39 (42.4)	
	AA	6 (22.7)		CC	16 (30.1)	
Dug levels corrected by dose (mg/L per mg/kg)	GG	34 (33.9)	0.208	TT	15 (34.4)	0.691
	GA	22 (30.9)		CT	33 (31.4)	
	AA	6 (19.8)		CC	14 (28.6)	
Maximum						
Dose corrected by weight (mg/day per kg)	GG	49 (44.9)	0.867	TT	25 (45.5)	0.629
	GA	30 (45.2)		CT	14 (46.1)	
	AA	9 (40.2)		CC	19 (39.5)	
Dug levels corrected by dose (mg/L per mg/kg)	GG	35 (38.8)	**0.042**	TT	16 (38.4)	0.472
	GA	24 (33.5)		CT	35 (34.9)	
	AA	9 (20.3)		CC	17 (30.1)	

Dominant model	*SCN1A_* rs6432860			*SCN1A_* rs3812718		
Maintenance	Dominant model	n (mean rank)	*P*-value	Dominant model	n (mean rank)	*P*-value
Dose corrected by weight (mg/day per kg)	GG	46 (41.0)	0.489	TT	23 (41.2)	0.673
	GA+AA	32 (37.4)		CT+CC	55 (38.8)	
Dug levels corrected by dose (mg/L per mg/kg)	GG	34 (33.9)	0.246	TT	15 (34.4)	0.475
	GA+AA	28 (28.6)		CT+CC	47 (30.6)	
Maximum						
Dose corrected by weight (mg/day per kg)	GG	49 (44.9)	0.883	TT	25 (45.5)	0.810
	GA+AA	39 (44.0)		CT+CC	63 (44.1)	
Dug levels corrected by dose (mg/L per mg/kg)	GG	35 (38.8)	0.065	TT	16 (38.4)	0.362
	GA+AA	33 (29.9)		CT+CC	52 (33.3)	

Diplotype	*SCN1A*_rs6432860|rs3812718		
Maintenance	Diplotype	n (mean rank)	*P*-value
Dose corrected by weight (mg/day per kg)	AC (+)	6 (22.7)	0.06
	AC (–)	72 (40.9)	
Dug levels corrected by dose (mg/L per mg/kg)	AC (+)	6 (19.8)	0.1
	AC (–)	56 (32.7)	
Maximum			
Dose corrected by weight (mg/day per kg)	AC (+)	9 (40.2)	0.6
	AC (–)	79 (45.0)	
Dug levels corrected by dose (mg/L per mg/kg)	AC (+)	9 (20.3)	**0.02**
	AC (–)	59 (36.7)	

Data were represented as n (mean rank) number and mean rank, *P*-values for diplotype and dominant model were calculated by Mann–Whitney *U*-test and for genotype were calculated by Kruskal–Wallis test. No significant association was observed., AC (–): AC diplotype present, AC (+): AC diplotype absent. *P*-values in bold are significantly associated and < 0.05.

serum drug levels" at maximum dose were observed in patients carrying GG genotype of rs6432860 of *SCN1A* (*P*-value = 0.042). This is in contrast to significantly lower "dose-adjusted drug levels" at maximum dose of PHT in patients harboring *SCN1A* AC/AC diplotype (*P*-value = 0.021) (Table 3).

Carbamazepine (CBZ)

With respect to CBZ treatment, a total of nine SNPs from six genes namely *SCN2A* (rs1007722), *GABRA6* (rs13184586, rs3219151), *GABRA1* (rs7732641), *GABRG2* (rs209353), *STX1A* (rs6956879,

rs867500, rs4363087), and *SNAP25* (rs3787283) were significantly associated with drug response, but none could withstand corrections for multiple comparisons (Table S2). Further LD determination in healthy control population revealed strong LD between rs3219151 and rs13184586 ($r^2 = 0.93$) of *GABRA6*. As a result, only three genetic variants from *STX1A* could be carried forward for further haplotype analysis. A 2–3 marker sliding window approach identified a borderline association of two-marker haplotype rs867500|rs4363087 (GT: $P_{corrected}$ value = 0.050, OR = 1.97, 95% CI = 1.24–3.13) with a higher frequency (54.93%) in patients with "recurrent seizures" compared to patients with "no seizure" (39.38%) (Table 2A). However, diplotype combination of haplotypic variants could not reveal significant association which limited any further analysis with CBZ dose and drug levels.

Valproate (VP)

In case of valproate monotherapy, we observed significant association of six SNPs from four genes *CACNA1E* (rs4652678, rs199930), *SCN1A* (rs1813502, rs3812718), *GABRB3* (rs878960), and *GRIK1* (rs466476), but none could withstand correction for multiple comparisons (Table S2). Further, LD information on variants from *CACNA1E* and *SCN1A* in healthy controls did not reveal any significant linkage. However, none of the haplotypes from both genes showed any significant association. The absence of any haplotype association refrained from conducting further association analysis of diplotypes and their influence on dose and drug levels.

Phenobarbitone (PB)

With respect to association analysis in patients on PB treatment, a total of eight SNPs from four genes namely *GABRG2* (rs209353), *ALDH5A1* (rs2247845, rs1054899), *SCN1B* (rs67777826, rs5839 2252, rs2278995, rs2278996), and *GRIK1* (rs2832495) revealed significant *P*-values which could not remain significant postcorrection for multiple comparisons (Table S2). The LD determination in healthy control population did not reveal any significant linkage. Lastly, haplotype analysis did not yield any significant association which limited any further analysis.

Discussion

The identification of a genetic marker as a predictor for treatment response has been a long felt need in epilepsy therapeutics. We hypothesized that genetic variants from ion channels and functionally related genes and genes from synaptic vesicle cycle (SVC) may affect response to first-line AEDs. We conducted a comprehensive association study of 155 SNPs across 12 such genes (Table S5). Although single-marker analysis of all the genetic markers with drug response provided significant association signals across several loci, none could withstand correction for multiple comparisons. Single SNP may not be the actual causal variant rather more than one SNP might be responsible. SNPs do not transmit independently; rather, there is an intrinsic dependency of SNPs due to their combination as linked units into haplotypes and diplotypes. Haplotypes and diplotypes are in fact more informative and have higher statistical power. Therefore, in addition to single SNP association analysis, a multimarker/haplotype approach was adopted to increase the power of the study, thus providing a more holistic view of association [29]. The multimarker approach further led us to identification of significant associations of *SCN1A* and *GABRA1* haplotypes and diplotypes in response to PHT monotherapy in patients with epilepsy.

GABA is well known to be a principal inhibitory neurotransmitter and alteration in expression or activity of genes encoding these receptors (GABAergic) is believed to be one of the main causes behind seizure pathophysiology which could further lead to altered drug response. In the present study, majority of the *GABRA1* variant haplotypes and diplotypes were observed to be overrepresented in patients with recurrent seizures. The *GABRA1* haplotypes comprised of SNPs spanning from 5′ UTR (rs12658835) to 3′ UTR (rs2290732) of the gene and included several intronic SNPs (rs7735530-intron 3, rs7732641-intron 6, rs2279020-intron 8) as well. Although the longest associated four-marker diplotype rs12658835|rs7735530|rs7732641|rs2279020 did not have 3′ UTR SNP, the varied gene locations of SNPs reflected the gene coverage of the associated region. This further highlighted the presence of long regions of high linkage within the gene and led us to identification of causal variants in our population. Based on the functional prediction of the associated SNPs, we observed that 5′ UTR SNP rs12658835 is present on the transcription factor binding site which may ultimately lead to impaired transcription of the *GABRA1*. Further, it was also observed that the 3′ UTR SNP rs2290732 is present on a miRNA binding site which may ultimately influence the *GABRA1* gene regulation. In conclusion, our data highlight the potential influence of *GABRA1* gene as a whole on the development of recurrent seizure phenotype in epilepsy patients despite being on adequate treatment.

A recent report by Zhou et al. highlighted the importance of 3′ UTR region of *GABRA1* on CBZ tolerance [30]. It was observed that carriers of 3′ UTR SNP rs2290732 GG genotype were less tolerant to CBZ therapy. This may be attributed to poor seizure control or development of adverse drug reactions (ADRs). Significant association of another *GABRA1* variant rs2279020 with drug resistance was earlier observed in patients treated with CBZ, PHT, or VPA [31]. In light of evidence supporting the possible binding of both PHT and CBZ on $GABA_A$ receptor, CBZ associations may provide directions for genetic marker search for PHT response. Similar to results reported by Zhou et al., our study also showed higher frequency of G allele of rs2290732 as a part of associated haplotypes (rs2279020_rs2290732:AG = 74.65%, rs7732641_rs2279 020_rs2290732: CAG = 74.27%, rs7735530_rs7732641_rs22790 20_rs2290732: GCAG = 74.29%, rs12658835_rs7735530_rs773 2641_rs2279020_rs2290732: AGCAG = 74.28%) and diplotypes (rs2279020_rs2290732:AG/AG = 51.35%) in recurrent seizure patients (Table 2A) [30]. An earlier report by Kumari et al. showed significant association of another SNP rs2279020 with drug resistance in ethnically similar North Indian epilepsy patients [32]. In the same year, Kim et al. reported the significant association of a gene–gene interaction model of four SNPs from *GABRA1*, *EAAT3,* and *GAT3* including 5′ UTR SNP rs12658835 from *GABRA1* with epilepsy drug resistance [33]. Similarly, in our study, A allele of rs12658835 was observed to have higher frequency in diplotypic combination with other *GABRA1* variants in patients showing "recurrent seizures." In addition, *SCN1A* SNPs were also observed to be associated in response to PHT in our study. A two-

Table 4 A comparative summary of pharmacogenetic studies exploring association analysis of *SCN1A* and *GABRA1* variants with drug response and therapeutic optimization parameters (dose and drug levels) in patients treated with first-generation AEDs

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
1	Tate et al. 2005 [34]	Caucasian (European)	Dose	**Dose** *Maximum dose*: Maximum dose to which patients were exposed to during their regular treatment.	Various types	PHT CBZ	*SCN1A*	rs590478, rs8191987, rs3812718, rs2126152	425 PHT 281 CBZ	Significant association of rs3812718 with maximum dose in CBZ as well as PHT patients (Trend: A>AG>GG)
2	Tate et al. 2006 [41]	Asian (Chinese)	Dose Drug levels	**Dose** *Maintenance dose:* Dose which has not been changed for two or more consecutive visits in the history of the patient's treatment. *Maximum dose:* Maximum dose to which patients were exposed to during their regular treatment.	Various types	PHT	*SCN1A*	rs3812718	168	Significant association of rs3812718 with PHT serum levels at maintenance dose (Trend: A>AG>GG)
3	Abe et al. 2008 [39]	Asian (Japanese)	Drug response Dose	**Drug response** *Drug responsive:* No seizures for a minimum of one year after receiving AED(s). *Drug resistant*: Uncontrolled seizures over a year despite attempts to treat with three or more different AEDs. **Dose** *Maintenance dose*: Latest dose in the drug-responsive patient. *Maximum dose*: Highest dose during the study period.	Various types	Various drugs	*SCN1A*	rs3812718	221 117 drug responsive 104 drug resistant	AA genotype was associated with drug resistance in CBZ patients. Trend for maximum or maintenance dose of CBZ: AA>GA+GG
4	Kwan et al. 2008 [42]	Asian (Han Chinese)	Drug response	**Drug response** *Drug responsive:* No seizure for at least a year up to the date of recruitment on a stable dose of AED treatment. *Drug resistant:* An average of one seizure or more per month over the preceding year, despite treatment with two or more AEDs at therapeutic dosages and/or serum drug concentrations.	Various types	Various drugs	*SCN1A*	rs1020853, rs2298771, rs3812718, rs10188577, rs4667866, rs13405797, rs1461197, rs2169312	471 272 drug responsive 199 drug resistant	No association

(continued)

Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
5	Zimprich et al. 2008 [36]	Caucasian (European)	Dose	**Dose** *Maintenance dose*	FE	CBZ	*SCN1A*	rs3812718	369	No association
6	Jang et al. 2009 [43]	Asian (Korean)	Drug response	**Drug response** *Drug responsive:* In patients treated with single AED, no seizures for at least one year, up to the date of the last follow-up visit. *Drug resistant:* Occurrence of at least four unprovoked seizures over the year before recruitment with trials of more than two appropriate AEDs at maximal tolerated doses or patients who had undergone surgical treatment for seizure control.	Various types	Various drugs	*SCN1A*	1 SNP (Nonsynonymous variant, exon 16)	400 200 drug responsive 200 drug resistant	No association
7	Lakhan et al. 2009 [44]	Asian (North Indian)	Drug response	**Drug response** *Drug responsive*: No seizures for at least one year from the last follow-up visit. *Drug resistant*: Occurrence of at least four seizures over a period of one year with three appropriate AEDs at maximum tolerated doses or patients who had undergone surgery for seizure control.	Various types	Various drugs	*SCN1A*	rs2298771	336 219 drug responsive 117 drug resistant	No association
8	Kumari et al. 2010 [32]	Asian (North Indian)	Drug response	**Drug response** *Drug responsive:* No seizures for at least one year from the last follow-up visit. *Drug resistant:* Occurrence of at least four seizures over a period of one year with three appropriate AEDs at maximum tolerated doses or patients who had undergone surgery for seizure control.	Various types	Various drugs	*GABRA1*	rs2279020	381 259 drug responsive 122 drug resistant	GG genotype and G allele were associated with drug resistance.

(continued)

Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
9	Sanchez et al. 2010 [45]	Caucasian (Spanish)	Drug response	**Drug response** *Drug responsive:* No seizures for at least one year from the last follow-up visit. *Drug resistant*: Occurrence of at least four seizures over a period of one year with three appropriate AEDs at maximum tolerated doses or patients who had undergone surgery for seizure control.	Various types	Various drugs	*SCN1A*	rs2298771, rs3812718	289 178 drug responsive 111 drug resistant	No association
10	Kim et al. 2011 [33]	Asian (Korean)	Drug response	**Drug response** *Drug responsive:* No seizure for at least one year, up to the date of the last follow-up visit. *Drug resistant:* Occurrence of at least four unprovoked seizures over the year before recruitment with trials of more than two appropriate AEDs at maximal tolerated doses or patients who had undergone surgery for seizure control.	Various types	Various drugs	*SCN1A*, *GABRA1*	rs2298771 (*SCN1A*); rs12658835, rs35166395 (*GABRA1*)	400 200 drug responsive 200 drug resistant	Interactive association of rs12658835 and rs35166395 (GABRA1), rs2228622 (EAAT3), and rs2304725 (GAT3) with drug resistance
11	Manna et al. 2011 [38]	Caucasian (Italian)	Drug response and dose	**Drug response** *Drug responsive*: No seizure for at least the previous two years. *Drug resistant:* Persistence of seizures during the previous two years with a frequency of at least one seizure/month, despite current or previous treatment with three or more appropriate AEDs, either alone or in combination, and at the highest tolerated dose. For patients who had undergone epilepsy surgery, drug resistance was defined as at least one seizure/month during the two years that	FE	Various drugs		rs3812718	883 482 drug responsive 401 drug resistant	No association

(continued)

Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
				preceded surgery, provided that at the time of surgery the patient met the above criteria for pharmacoresistance. **Dose** *Dose ratio*: Prescribed daily dose (PDD)/defined daily dose (DDD) ratios, where DDD is the average maintenance daily dose in adults.						
12	Haerian et al. 2012 [46]	Asian (Malaysian and Hong Kong !Chinese)	Drug response	**Drug response** *Drug responsive:* No seizure for at least one year during monotherapy. *Drug resistant:* Occurrence of seizures over a period of one year during treatment with monotherapy at maximally tolerated therapeutic dosages.	Various types	VP	*SCN1A*	rs3812718	583 306 drug responsive 277 drug resistant	No association
13	Hung et al. 2012 [47]	Asian (Han Chinese)	Dose Drug levels	**Dose** *Maintenance dose*: Dosage that had not been changed for at least one year under good compliance and good seizure control (freedom from seizures for a minimum of three times the longest pre-intervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever was longer. **Dose and drug levels** *Concentration/dose ratio (CDR):* Mean steady-state serum concentration/ daily dose.	Various types	CBZ	*SCN1A*	rs2298771, rs3812718	234	rs3812718 A allele and AA genotype were associated with higher CBZ dosages and lower ln(concentration/ dose ratios)
14	Sterjev et al. 2012 [48]	Caucasian (Macedonian)	Drug response Dose Drug levels	**Drug response** *Drug responsive:* No seizure for at least one year of treatment with CBZ. Drug resistant: Occurrence of at least four seizures over one year of treatment with CBZ.	Various types	CBZ	*SCN1A*	rs3812718	147 82 drug responsive 65 drug resistant	No association with drug response, association of rs3812718 with dose in drug-responsive patients (Trend: A>AG>GG)

(continued)

Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
				Dose *Maintenance dose*: Dose that has not been changed for two or more consecutive visits in the history of the patient's treatment. *Dose ratio:* Prescribed daily dose (PDD)/defined daily dose (DDD) ratios, where DDD is the average maintenance daily dose in adults. **Dose and drug levels** *Index of comparison:* CBZ daily dose/CBZ plasma level						
15	Zhou et al. 2012 [49]	Asian (Han Chinese)	Drug response Dose Drug levels	**Drug response** *Good response:* Seizure free (SF) during 24-month follow-up period; *poor response:* combination of 75%, 50–75%, and <50% SF during 24-month follow-up period.	FE	CBZ	*SCN1A, GABRA1*	rs2298771, rs3812718, rs4667869, rs11692675, rs1020853, rs10497275, rs7577411, rs1813502 (*SCN1A*); rs12658835, rs2290732, rs35166395 (*GABRA1*)	448	Poor response was associated with rs2298771 AG+GG from 3 months to 15 months of follow-up. Maintenance doses and serum levels of CBZ in carriers of the rs3812718 AA genotype of were significantly higher than those of GG genotype from 3 to 12 months of follow-up,
16	Balan et al. 2013 [50]	Asian (South Indian)	Drug response	**Drug response** *Drug responsive:* No seizure for at least one year on AED therapy. *Drug resistant:* Unresponsive to at least two monotherapy trials and one duo-therapy trial, each of at least six-month duration, and had seizure frequency of at least 12 per year for at least two years.	JME, MTLE-HS	Various drugs	*GABRA1*	rs2279020	441 201 drug responsive 240 drug resistant	No association
17	Haerian et al. 2013 [51]	Asian (Malaysian, Hong Kong Chinese)	Drug response	**Drug response** *Drug responsive:* No seizure for at least one year during treatment with monotherapy. *Drug resistant:* Occurrence of seizures over a period of one year while undergoing treatment with monotherapy at maximally tolerated therapeutic dosages.	Various types	CBZ VP	*SCN1A*	rs10182473, rs1020853, rs2298771, rs10930195	1504 702 drug responsive 802 drug resistant	No association

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Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
18	Hung et al. 2013 [52]	Asian (Han Chinese)	Drug response	**Drug response** *Drug responsive:* No seizure for a minimum of three times the longest pre-intervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever was longer. *Drug resistant:* Failure to achieve seizure freedom with at least two appropriately prescribed AEDs under maximum tolerated doses	Various types	Various drugs	*GABRA1*	Gene wide tagging SNPs (including rs6883877, rs1157122, rs6892782, rs10068980)	721 349 drug responsive 371 drug resistant	Interactive association of rs6883877 (GABRA1), GABRA2 (rs511310), and GABRA3 (rs4828696) with drug resistance.
19	Kumari et al. 2013 [37]	Asian (North Indian)	Drug response	**Drug response:** *Drug responsive:* No seizure for at least one year from the last follow-up visit. *Drug resistant*: Occurrence of at least four seizures over a period of one year with three appropriate AEDs at maximum tolerated doses or patients who had undergone surgery for seizure control.	Various types	CBZ	*SCN1A*	rs3812718	484 294 drug responsive 170 drug resistant	No association
20	Yun et al. 2013 [40]	Asian (Chinese)	Drug response Dose Drug levels	**Drug response** *Drug responsive:* Response to treatment in a one-year study. *Drug resistant:* Poor response to treatment in a 1-year study. **Dose** *Maintenance dose* **Dose and Drug levels** *Index of comparison:* Steady-state plasma concentrations of CBZ were adjusted by the dose and body weight	Various types	CBZ	*SCN1A*	rs3812718	83 59 drug response 34 drug resistant	No association

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Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
21	Wang et al. 2014 [53]	Asian (Han Chinese)	Drug response	**Drug response** *Seizure free:* 100% decrease in the proportion of seizures between first three and last three months of the one-year study. *Nonseizure free:* Less than 100% decrease in the proportion of seizures between first three and last three months of the one-year study.	FE	CBZ	*SCN1A*	rs3812718, rs2298771	351 194 seizure free 157 nonseizure free	rs2298771 G allele and GG+GA were associated with nonseizure free patients
22	Yip et al. 2014 [54]	Caucasian (Australian)	Drug response	**Drug response** *Drug responsive:* No seizure for at least a year, up to the date of the last follow-up visit, in patients with epilepsy treated with sodium channel blocking AEDs. *Drug resistant:* Occurrence of at least four seizures over the year before recruitment with trials of two or more sodium channel blocking AEDs at maximal tolerated doses.	Various types	Various drugs	*SCN1A*	rs1813502, rs1461195, rs1020853, rs6432860, rs1972445, rs10188577, rs7607543, rs11686142, rs1461197	519 161 drug responsive 358 drug resistant	rs10188577 CT was associated with drug resistance
23	Daci et al. 2015 [55]	Caucasian (Kosovo)	Drug response Dose Drug levels	**Drug response** *Drug responsive:* Seizure free for at least one year during monotherapy treatment. *Drug resistant:* Occurrence of at least four seizures over a period of one year of monotherapy treatment. **Dose and drug levels** *Concentration/dose ratios (CDRs):* Maintenance dose-adjusted concentrations of CBZ, CBZE and CBZD and CBZE: CBZ, CBZD: CBZ, and CBZD: CBZE ratios were used as parameters for the evaluation of CBZ metabolism.	Various types	CBZ	*SCN1A*	rs2298771, rs3812718	145 99 drug responsive 46 drug resistant	No association with drug response; rs3812718 AA showed significantly lower levels of plasma CBZ compared to GG.
24	Ma et al. 2015 [56]	Asian (Han Chinese)	Dose Drug levels	**Dose** *Maintenance dose:* Dose that had not been changed for at least one year in association	Various types	CBZ	*SCN1A*	rs2298771, rs3812718	166	rs3812718 A allele and rs3812718A-rs2298771A haplotype were associated with a higher

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Table 4 (Continued)

	Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A/ GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A/ GABRA1* variants
				with good compliance and seizure control. Good seizure control was defined as freedom from seizures for a minimum of 3-fold the longest pre-intervention interseizure interval (determined from seizures occurring within the past 12 months) or 12 months, whichever was longer. **Dose and Drug levels** *Concentration/dose ratios (CDRs)*: Plasma CBZ Css/CBZ maintenance dose. The CBZE: CBZ ratio (at trough).						adjusted CBZ maintenance dose and a lower CBZ natural logarithmic concentration–dose ratio (lnCDR).
25	Namazi et al. 2015 [57]	Asian (Iranian)	Dose Drug levels	**Dose** *Minimum Dose*, Maximum Dose, *Mean Dose* **Drug levels** CBZ, CBZ-E **Dose and Drug levels** CBZ concentration–dose	Various types	CBZ	*SCN1A*	rs2298771	70	No association
26	Zhou et al. 2015 58]	Asian (Han Chinese)	Drug response	**Drug response** *Drug responsive:* Complete seizure freedom for a minimum of three times the longest interseizure interval before treatment, or one year (the longer). *Drug resistant:* Failure of continuous seizure freedom after adequate treatment of two tolerated and appropriately used AEDs (monotherapy or in combination).	Various types	Various drugs	*SCN1A*, *GABRA1*	rs3812718 (*SCN1A*); rs2279020 (*GABRA1*)	391 235 drug responsive 156 drug resistant	No association
27	Present study	Asian (North Indian)	Drug response Dose Drug levels	**Drug response** *No seizure:* Patients who remained seizure free in the last ten months, despite appropriate AED treatment, after attaining steady-state AED levels in the first two months of the study. *Recurrent seizures:* Patients with one or more seizures in the last	Various types	PHT CBZ VP PB	*SCN1A*, *GABRA1*	rs6735544, rs1381105, rs4667869, rs10188577, rs8191987, rs3812718, rs6432860, rs10197430, rs2298771, rs10497276, rs1813502 (*SCN1A*); rs11575999,rs11576001, rs12658835, rs35166395, rs7735530, rs12188495, rs7732641, rs1350372,	379 225 No seizure 154 Recurrent seizures 94 on PHT 168 on CBZ 85 on VP 32 on PB	GABRA1 diplotype rs12658835A-rs7735530G/rs12658835A-rs7735530G and SCN1A diplotype rs6432860A-rs3812718C/ rs6432860A-rs3812718C were associated with recurrent seizures in patients treated with PHT

(continued)

Table 4 (Continued)

Author and year	Population (Ethnicity)	Outcome variable	Definition of outcome	Epilepsy syndrome	AEDs	*SCN1A*/ *GABRA1*	Genetic variants	Total No. of patients	Association with *SCN1A*/ *GABRA1* variants
			ten months despite appropriate AED treatment, after attaining steady-state AED levels in the first two months of the study. **Dose** *Maintenance dose,* *Maximum dose* **Dose and Drug levels** *Concentration/dose ratios (CDRs):* AED Css/AED maintenance dose, AED Css/AED maximum dose.				rs1157122, rs2279020, rs2290732, rs998754 (*GABRA1*)		monotherapy. SCN1A diplotype rs6432860A-rs3812718C/rs6432860A-rs3812718C was also associated with lower dose-adjusted drug levels in patients treated with PHT monotherapy.

AED, antiepileptic drug, CBZ, carbamazepine, CBZE, carbamazepine-10,11-epoxide, CBZD, carbamazepine-10,11-trans dihydrodiol, Css, steady-state drug concentration, FE, focal epilepsy, JME, juvenile myoclonic epilepsy, MTLE-HS, mesial temporal lobe epilepsy with hippocampal sclerosis, PB, phenobarbitone, PHT, phenytoin, VP, valproate.

marker haplotypic and diplotypic combination (rs6432860_rs3812718, AC) of exonic SNP (rs6432860) and intronic splice variant (rs3812718) was observed to be significantly higher in patients with "recurrent seizures." Of the associated haplotypes, rs6432860 is a synonymous SNP and was in LD with another significantly associated SNP rs2298771 (Ala1067Thr) which may alter structure and function of SCN1A. The intronic SNP rs3812718 (IVS5-91G>A) is known to be a functional variant and disrupts the consensus 5′ splice donor site of a highly conserved alternative exon (5N) resulting in altered proportion of neonate and adult exon 5 transcripts in adult brain tissue. The study by Tate *el al.* was the first to describe the significant association of IVS5-91G>A with maximum dose and drug concentration at maintenance dose of PHT as well as CBZ [34,35]. However, another study of CBZ dosage in Austrian population failed to reveal the significant association [36]. Additionally, other studies also failed to replicate the associations [28,37]. Later the IVS5-91AA genotype was also reported to be associated with CBZ-resistant epilepsy without any underlying influence on dose [38]. On the contrary, a recent study on Chinese patients revealed significant association of AA genotype with higher dose-adjusted CBZ concentration; however, it did not influence CBZ-resistant phenotype [39]. Another report by Zhou et al. observed that patients with AA genotype had higher maintenance dose and serum levels as compared to GG genotype carriers. Additionally, this study also revealed significant association of GG genotype with higher retention rates of CBZ. The report has also showed significant association of the variant with CBZ tolerability. Although our study did not find significant association of IVS5-91G>A with therapy optimization parameters, we did observe significant association of another variant rs6432860 from the same gene with drug levels. It was observed that genotypic distribution of rs6432860 had significant influence on dose-adjusted drug levels at maximum PHT dose in the order AA<GA<GG (P = 0.042). And lastly, both the SNPs (rs6432860 and IVS5-91G>A) were observed to be significantly associated with therapy optimization parameters (dose and drug levels), when present in diplotypic combination. We observed a significant association of *SCN1A* diplotype rs6432860|rs3812718 (AC) with lower dose-adjusted drug levels at maximum PHT dose (P-value = 0.021). Being a drug target, significant association of *SCN1A* may not affect the metabolism of drug directly rather it may alter the structure or function of the ion channel. The altered sensitivity could further lead to a change in the dose requirement for efficacious treatment which may then indirectly result in the altered serum drug levels [34]. In fact, association of drug levels with drug targets is considered to be more informative and stronger as compared to dose, because drug levels rule out the possibility of underlying pharmacokinetic variability. A comprehensive list of all pharmacogenetic studies exploring *GABRA1* and *SCN1A* along with their findings in different worldwide populations has been further summarized in Table 4 [30,34–36,38–56].

In summary, present study reports significant overrepresentation of *GABRA1* and *SCN1A* variants in patients with "recurrent seizures" who were on PHT monotherapy. Association of *GABRA1* variants further supports mode of action of PHT through α1 subunit of *GABRA1*. Detailed functional analysis of *GABRA1* genetic architecture is required to highlight the exact mechanism by which *GABRA1* influences drug response. *In silico* analysis

supported by functional experiments may also further highlight the possible binding sites and affinity of PHT for *GABRA1* receptor. Furthermore, association of *SCN1A* variants also highlights its role in poor response to PHT monotherapy, possibly by modulating serum drug levels. Although we did observe several significant associations supported by functional relevance, our study has its own limitations. In an attempt to achieve a homogenous patient pool in terms of phenotype and drug therapy, we adopted a stringent inclusion and exclusion criteria. We further excluded all heterogeneous complex phenotypes and multitherapy patients from the final analysis. This has resulted in small patient size which is the major limitation of the present study. Hence, some of the modest associations reported (*P*-values >0.01) in the present study may turn out to be false positive. On the other hand, we did observe several strong and well-powered associations. Despite being significant, results of the present study may be viewed cautiously and replication of these associations needs to be demonstrated in larger cohorts of similar and different ethnicities.

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Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

The following supplementary material is available for this article:

Table S1 Gene wise summarized status of SNPs for final genotype phenotype correlation.
Table S2 Allele frequencies of single nucleotide polymorphisms among patients with epilepsy and healthy controls.
Table S3 Association of variants in all epilepsy and drug groups.
Table S4 Effects of GABRA1 diplotypes, genotypes and model on PHT maintenance doses, maximum doses and corresponding adjusted serum PHT concentrations.
Table S5 Allelic and genotypic frequencies of studied SNPs in all epilepsy patients and different subgroups stratified by drug type.