GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 00, Number 00, 2012

Pp. 1–
DOI: 10.1089/gtmb.2011.0225



# Role of *MDR1* C3435T and *GABRG2* C588T Gene Polymorphisms in Seizure Occurrence and *MDR1* Effect on Anti-Epileptic Drug (Phenytoin) Absorption

Shivani Ponnala,[1,2] Jaideep Ray Chaudhari,[3] Momin Abdul Jaleel,[3] Dilnawaz Bhiladvala,[3] Prabhakar Rao Kaipa,[2] Undurti N. Das,[4] and Qurratulain Hasan[5]

*Aims:* To assess the role of MDR1 and gamma-aminobutyric acid receptor–gamma 2 sub unit (GABRG2) gene polymorphism in seizure susceptibility in generalized seizure (GS) and febrile seizure (FS) patients and to evaluate MDR1 C3435T gene polymorphism's role in absorption of the anti-epileptic drug, phenytoin (PHT) in a cohort of patients. *Methods:* One hundred twenty-seven cases of seizure (86 GS and 41 FS) patients were analyzed for MDR1 C3435T and GABRG2 C588T gene polymorphisms using restriction fragment length polymorphism-polymerase chain reaction. Serum PHT levels were analyzed. *Results:* The T allele of MDR1 C3435T and GABRG2 C588T gene polymorphism was significantly associated with GS in the Indian population ($p < 0.05$) compared with controls. From the data in GS, CT and TT genotype carriers of the MDR1 gene and TT genotype carriers of the GABRG2 gene had more recurrent seizures compared with others. MDR1 T allele carriers in the seizure reoccurrence (SR) group of GS and FS were high compared with the well-controlled seizure group (with no seizures after treatment). TT genotype carriers in SR group were high in FS (with regard to MDR1 gene polymorphism) and GS (with regard to GABRG2 gene polymorphism) compared with a well-controlled seizure group. MDR1 C3435T gene polymorphism affects serum PHT levels ($p < 0.015$). Association of dose PHT ratio and genotype groups of MDR1 C3435T gene polymorphism showed a significant association ($p < 0.05$). MDR1*CC genotype was more common in cases with low serum PHT levels.In addition, it is evident that CT and TT genotype carriers have a high percentage of SR with elevated serum PHT levels. *Conclusions:* Our results show that the MDR1 3435T and GABRG2 588T alleles play a role in seizure occurrence. Moreover, the MDR1 3435T allele also affects PHT absorption. We suggest MDR1 C3435T and GABRG2 C588T genotyping would be of value in order to lower the risk of concentration-dependent drug toxicity and for better patient management.

## Introduction

Epilepsy is a common chronic neurological condition that is characterized by recurrent unprovoked seizures. Two most common seizure types are generalized seizures (GS) affecting adults and febrile seizures (FS) affecting children.

MDR1 functions in an energy-dependent manner by exporting substances from the inside of cells to the outside. MDR1 and other transporters form an important class of proteins for regulating pharmacokinetics. MDR1 exports a number of structurally unrelated drugs (Marzolini *et al.*, 2004; Pauli-Magnus and Kroetz, 2004). They play an important role in selective absorption and elimination of endogenous substances and xenobiotics, including drugs (Kim, 2002). Decreased expression of MDR1 due to alteration in the gene is known to affect the response of antiepileptic drugs (AEDs) (Amara and Sonders, 1998).

Neurotransmitters are chemicals that transmit signals between neurons as well as between neurons and other cell types (Bear *et al.*, 2011). Gamma-aminobutyric acid (GABA) and glutamate are the two major neurotransmitters of the brain. The majority of receptors are αβγ and αβδ isoforms, α1β2γ2 subunit combination is the most abundant in the brain (Sieghart *et al.*, 1999; Whiting, 2003). The importance of specific aspects of GABR function in the maintenance of central synaptic inhibition is well documented (Baulac *et al.*, 2001; Wallace *et al.*, 2001). Impaired GABAergic function contributes to certain forms of epilepsy, schizophrenia, alzheimer's

[1]Department of Cancer Biology and Pharmacology, University of Illinois College of Medicine at Peoria, Peoria, Illinois.
[2]Department of Genetics, Osmania University, Hyderabad, India.
[3]Department of Neurology and Neuro Surgery, Kamineni Hospitals, Hyderabad, India.
[4]UND Life Sciences, Shaker Heights, Ohio.
[5]Department of Genetics, Kamineni Hospitals, Hyderabad, India.

Table 1. Demographic Details of Individuals Included in the Study

| Case type | No of cases (n = 227) | Sex Males (%) Females (%) | Mean age (Range in years) Males Females |
|---|---|---|---|
| Seizure type | | | |
| GS | 86 | 73 (84.8) | 41 ± 19 (5–78) |
| | | 13 (15.1) | 42 ± 19 (16–69) |
| FS | 41 | 30 (73.17) | 2.40 ± 1.52 (0.83–6) |
| | | 11 (26.82) | 2.32 ± 1.11 (0.6–5) |
| Controls | | | |
| Head injury | 49 | 45 (91.83) | 38.57 ± 13.62 (11–65) |
| | | 4 (8.33) | 44.25 ± 14.56 (28–63) |
| Healthy individuals | 51 | 35 (68.62) | 40.41 ± 12.87 (22–78) |
| | | 19 (37.25) | 33.32 ± 12.32 (16–62) |

GS, generalized seizures; FS, febrile seizure.

disease, and other neurological disorders. GABRG2 (a exonic gamma-aminobutyric acid receptor–gamma 2 sub unit) C588T gene polymorphism has been associated with susceptibility to variety of seizures (Chou *et al.*, 2007; Kang *et al.*, 2009; Kumari *et al.*, 2010).

The present study aimed at evaluating whether the MDR1 C3435T gene polymorphism plays a role in seizure occurrence in GS and FS and to understand its effect on clinical pharmacokinetics of phenytoin (PHT) in patients. It also aimed at evaluating the GABRG2 C588T gene polymorphism to understand the susceptibility to GS and FS in our population.

## Materials and Methods

### *Patient recruitment*

A total of 127 cases were included for the study groups just mentioned. Of these, 41 infants/children with FS below the age of 5 years were selected based on the clinical symptoms and electroencephalogram findings. Another 86 cases with GS were recruited (generalized tonic seizures, generalized clonic seizures, and generalized tonic clonic seizures) from the neurology department. The cases with GS were included based on the clinical symptoms, magnetic resonance imaging, electro-encephalogram, and computer tomography scan findings. The epilepsies and epileptic syndromes were diagnosed based on the revised "Classification of Epilepsies and Epileptic syndromes of the International League Against Epilepsy" (International League Against Epilepsy, 1989). Fifty-one randomly selected healthy volunteers and 49 patients with head injury cases without any history of neurological diseases or a family history of any neurological problem were included as normal controls. Clinical, personal, and family history of the patients were obtained in a well-designed proforma. Table 1 shows demographic details of the cases studied. The institutional ethical committee approved this study.

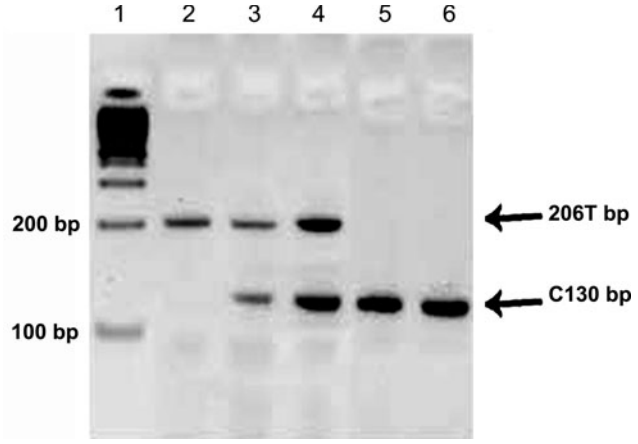


**FIG. 1.** Three percent ethidium bromide stained agarose gel showing MDR 1 polymerase chain reaction (PCR) products after digestion with *Mbo*I restriction enzyme (Inverted image). Lane 1, 100 bp DNA ladder; Lane 2, PCR product showing single band (206 bp) indicating homozygous T allele; Lanes 3 and 4, PCR product showing two bands indicating heterozygosity (206 and 130 bp); Lanes 5 and 6, PCR product after *Mbo*I digestion showing 130 bp indicating homozygous C allele.

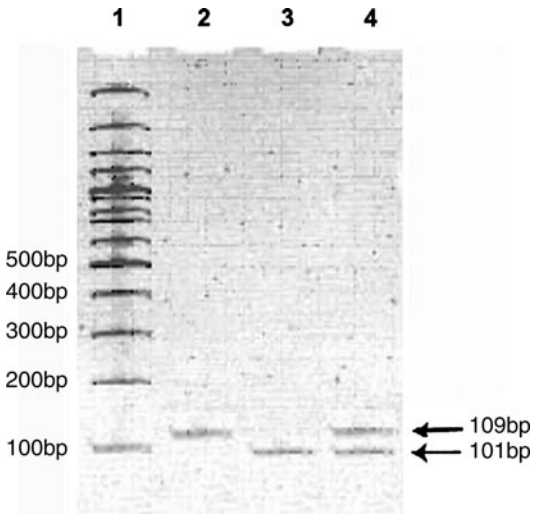


**FIG. 2.** Fifteen percent polyacrylamide gel electrophoresis image showing ethidium bromide stained bands of *Tas*I restriction enzyme digested PCR product with 109 and 101 bp bands. (Inverted image). Lane 1, 100 bp DNA ladder; Lane 2, *Tas*I digested product showing single band (109 bp) indicating C588 allele; Lane 3, digested PCR product with 101 bp indicating homozygous 588T allele; Lane 4, digested PCR product two bands indicating heterozygous CT alleles in the patient.

Table 2. Genotype and Allele Frequencies Distribution of MDR1 C3435T Gene Polymorphism in Cases with Generalized Seizures, Febrile Seizures, and Controls

| MDR1 C3435T | GS no. (%) (n = 86) | FS no. (%) (n = 41) | Controls no. (%) (n = 100) |
|---|---|---|---|
| Genotype | | | |
| CC | 9 (10.46) | 4 (12.19) | 16 (16) |
| CT | 44 (51.16) | 24 (58.53) | 48 (48) |
| TT | 33 (38.37) | 13 (30.23) | 36 (36) |
| Allelic frequency | | | |
| C | 0.36 | 0.39 | 0.40 |
| T | 0.64 | 0.61 | 0.60 |

Table 3. Analysis of MDR1 C3435T Genotypes and Alleles Among Two Patient Groups and Controls

| *Genotype* | *Generalized seizures vs. control OR (95% CI); chi square* p*-value* | *Febrile seizures vs. control OR (95% CI); chi square* p*-value* |
|---|---|---|
| CC vs. CT | 0.6136 (95% CI = 0.2462 to 1.5297); $\chi^2$ $p > 0.05$ | 0.5997 (95% CI = 0.1908 to 1.8278); $\chi^2$ $p > 0.05$ |
| CC vs. TT | 0.6136 (95% CI = 0.2389 to 1.5763); $\chi^2$ $p > 0.05$ | 0.8754 (95% CI = 0.2630 to 2.8281); $\chi^2$ $p > 0.05$ |
| TT vs. CT | 1.0000 (95% CI = 0.5353 to 1.8680); $\chi^2$ $p > 0.05$ | 0.6753 (95% CI = 0.3023 to 1.5001); $\chi^2$ $p > 0.05$ |
| Recessive model | | |
| CC vs. CT + TT | 1.1069 (95% CI = 0.6097 to 2.0096); $\chi^2$ $p > 0.05$ | 0.7654 (95% CI = 0.3543 to 1.6440); $\chi^2$ $p > 0.05$ |
| Co dominant model | | |
| CT vs. CC + TT | 1.1349 (95% CI = 0.6375 to 2.0205); $\chi^2$ $p > 0.05$ | 1.4946 (95% CI = 0.7252 to 3.0005); $\chi^2$ $p > 0.05$ |
| Dominant model | | |
| TT vs. CC + CT | 0.6136 (95% CI = 0.2562 to 1.4695); $\chi^2$ $p > 0.05$ | 0.6475 (95% CI = 0.2340 to 2.0052); $\chi^2$ $p > 0.05$ |
| Allele | | |
| C vs. T | 0.8455 (95% CI = 0.5552 to 1.2875); $\chi^2$ $p > 0.05$ | 1.0002 (95% CI = 0.6051 to 1.6998); $\chi^2$ $p > 0.05$ |
| T vs. C | 1.1828 (95% CI = 0.7767 to 1.8013); $\chi^2$ $p > 0.05$ | 0.9214 (95% CI = 0.5705 to 1.5957); $\chi^2$ $p > 0.05$ |

OR, odds ratio; CI, confidence interval.

### *Blood collection*

A 1 mL peripheral blood sample with 0.1 mL heparin was collected in a syringe. DNA was isolated from all these samples according to the standard procedure followed in the lab; and stored at −20°C until further use (Ponnala *et al.*, 2009).

For serum PHT analysis, 3 mL of plain blood sample was collected in a sterile plain vacutainer from 108 cases 2 h after drug intake. The sample was left for about 10 min at room temperature and then spun down in a centrifuge for 10 min at 1000 rpm. The serum was then separated and analyzed for PHT levels immediately by the homogenous-particle-enhanced turbidimetric inhibition immunoassay technique.

### *MDR1 C3435T and GABRG2 C588T gene polymorphism analysis*

MDR1 C3435T gene was amplified by using 5′ TTGA TGGCAAAGAAATAAAGC 3′ (forward) and 5′ CTTACA TTAGGCAGTGACTCG 3′ (reverse) primers and GABRG2 gene by using 5′ CCATCTTATGTTTAATATCTTTCT 3′ (forward) and 5′AATCAGAAAGACTGTAGGTGAGG 3′ (reverse) primers (Kananura *et al.*, 2002). polymerase chain reaction (PCR) was set up using the PCR cycle (95°C for 5 min, [95°C for 30 s, 56°C for 30 s, and 72°C for 30 s] × 30 cycles, 72°C for 10 min). The wild-type allele of MDR1 is C, and the mutant is T. The C3435T substitution generates a *Mbo*I restriction recognition sequence (Cat No. ER0811; Fermentas), and restriction digestion gives two fragments of 206 and 130 bp. Normal homozygotes were identified by the presence of a 130 bp fragment, mutated homozygotes by the presence of a 206 bp fragment, and heterozygotes by the presence of both fragments (Fig. 1). The nucleotide change from C to T at 588 position in the GABRG2 gene creates a restriction site for the *Tas*I restriction enzyme (Cat No. ER1351; Fermentas). The wild-type allele of GABRG2 is C, and the mutant is T. The C588T substitution generates a *Tas*I restriction recognition sequence, and restriction digestion gives fragments of the following sizes: 109 + 36 + 17 + 6 bp (C allele) and 101 + 36 + 17 + 6 + 8 bp (T allele). However, < 100 bp fragments are not seen on the gel (Fig. 2).

### *Statistical analysis*

All the data was analyzed using the SPSS for Windows 9.0 (Version 9) and MedCalc Version 7.4.3.0 (Windows 98/NT/Me/2000/XP). Chi square ($\chi^2$) analysis, correlation coefficient values, and odds ratio (OR) test have been used to analyze the data.

Table 4. Distribution of Allele and Genotype Frequencies of MDR1 C3435T Gene Polymorphism in Different Populations

| | *Cases* | *Allele* | | *Genotype* | | | |
|---|---|---|---|---|---|---|---|
| *Population* | *N* | *C* | *T* | *CC* | *CT* | *TT* | *References* |
| Caucasian, United Kingdom | 190 | 0.48 | 0.52 | 0.24 | 0.48 | 0.28 | Hoffmeyer *et al.* (2000) |
| Caucasian, Germany | 188 | 0.52 | 0.48 | 0.28 | 0.48 | 0.24 | Hoffmeyer *et al.* (2000) |
| African American | 88 | 0.84 | 0.16 | 0.68 | 0.31 | 0.01 | Hoffmeyer *et al.* (2000) |
| Chinese | 132 | 0.53 | 0.47 | 0.32 | 0.42 | 0.26 | Hoffmeyer *et al.* (2000) |
| Japanese | 114 | 0.61 | 0.39 | 0.35 | 0.53 | 0.12 | Hoffmeyer *et al.* (2000) |
| South west Asians | 89 | 0.34 | 0.66 | 0.15 | 0.38 | 0.47 | Hoffmeyer *et al.* (2000) |
| Chinese | 98 | 0.46 | 0.54 | 0.24 | 0.44 | 0.32 | Balram *et al.* (2003) |
| Malays | 99 | 0.48 | 0.52 | 0.25 | 0.46 | 0.28 | Balram *et al.* (2003) |
| Indian | 93 | 0.38 | 0.62 | 0.18 | 0.39 | 0.43 | Balram *et al.* (2003) |
| Indian | 227 | 0.38 | 0.62 | 0.12 | 0.53 | 0.35 | Present study |

Table 5. MDR1 C3435T Genotype and Allele Frequency of Generalized Seizures and Febrile Seizure Cases in Seizure Reoccurrence

| *Generalized seizures* *Genotype frequency* | *SR (%)* N = *39* | *Controlled seizures of GS (%)* N = *47* | p-*Value SR vs. controlled seizures* |
|---|---|---|---|
| CC | 2 (5.1) | 7 (14.89) | 0.056 |
| CT | 21 (53.84) | 24 (51.06) | |
| TT | 16 (41.02) | 16 (34.04) | |
| Allele frequency | | | |
| C | 0.32 | 0.4 | OR = 1.43 |
| T | 0.68 | 0.6 | 95% CI = 0.76 to 2.69 |
| *Febrile seizures* *Genotype frequency* | *SR (%)* N = *17* | *Controlled seizures (%)* N = *24* | p-*Value SR vs. controlled* |
| CC | 2 (11.76) | 2 (8.33) | 0.415 |
| CT | 9 (52.94) | 15 (62.50) | |
| TT | 6 (35.29) | 7 (29.16) | |
| Allele frequency | | | |
| C | 0.38 | 0.4 | OR = 1.12 |
| T | 0.62 | 0.6 | 95%CI = 0.42 to 2.89 |

SR, seizure reoccurrence.

## Results

### *MDR1 C3435T gene polymorphism*

In this study, the distribution and analysis of genotype and allele frequencies of MDR1 C3435T gene polymorphism of GS and FS cases were assessed along with the controls (Table 2). Table 2 shows a higher percentage of CT and TT genotype carriers of MDR1 C3435T polymorphism in GS compared with the controls. However, CT genotype carriers were high in FS cases compared with the controls. Statistical analyses for genotype models showed no significant association ($p > 0.05$) (Table 3).

T allele frequency was higher in GS compared with FS and controls (Table 2). Pooled allele frequency of MDR1 C3435T gene polymorphism was 0.38 of C allele and 0.62 T allele in the cohort studied, which was similar to the earlier reported frequency in the Indian population (Table 4). In the present study group, the MDR1 C3435T genotype frequency followed Hardy–Weinberg equilibrium (Calculated $\chi^2 = 1.14 < 3.84$ at 5% level significance for first degree of freedom).

Variations in drug absorption, drug targets, and metabolism between individuals are involved in uncontrolled seizures. Therefore, the present study was undertaken to determine the impact of MDR1 C3435T gene polymorphism in seizure reoccurrence (SR) of GS and FS types (Table 5). 45.34% (39/86) cases of GS and 41.46% (17/41) cases of FS had SR. From the data, CT and TT genotype carriers of SR of GS were high compared with others (Table 5). However, in FS cases, TT genotype carriers in the SR group were high compared with the well-controlled seizure group (Table 5). Allele frequency of SR and controlled seizures of GS and FS was not statistically significant (OR = 1.43 95% confidence interval [CI] = 0.76 to 2.69 and OR = 1.18 95% CI = 0.48 to 2.86 respectively). However, T-allele carriers in the SR group of GS and FS were high compared with well-controlled seizures (Table 5).

MDR1 plays a critical role in the development of resistance to anticancer drugs, antiviral agents, and anticonvulsants (Anglicheau *et al.*, 2003; Sakaeda *et al.*, 2003). In the present study, pharmacoresistant epilepsy indicated by SR was evaluated in GS and FS types. We observed well-controlled GS compared with the SR group, which showed $\chi^2 = 5.739$, $p < 0.05$, indicating the protective role of the MDR1 C3435 allele in controlling SR (Table 5). SR observed in CT and TT genotype carriers of GS and TT genotype carriers of FS cases could have a high risk of developing seizures compared with others. This is the first study from the Indian population

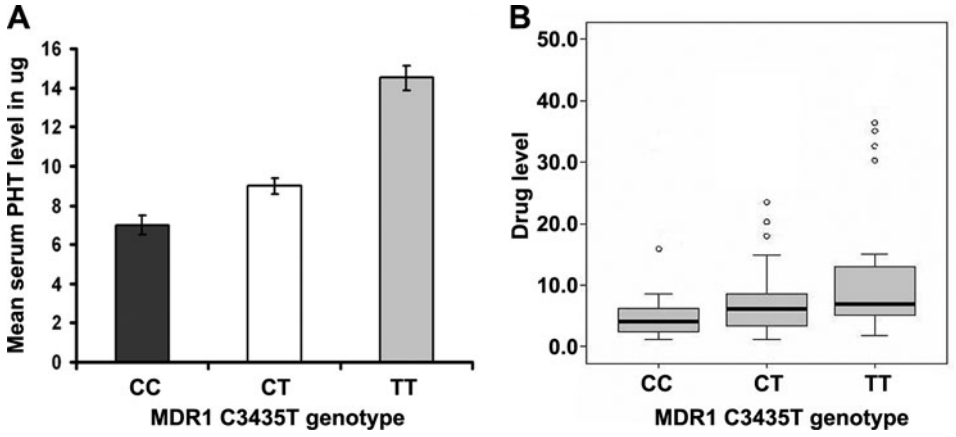


**FIG. 3.** **(A)** Mean serum phenytoin (PHT) level in three different genotypes of MDR1 gene. **(B)** Box plot graph showing interindividual differences in genotype groups of MDR1 gene and horizontal line showing mean PHT level. Dots show individuals with high serum PHT levels.

Table 6. Genotype and Allele Frequencies Distribution of GABRG2 C588T Gene Polymorphism in Cases with Generalized Seizures, Febrile Seizures, and Controls

| | *GS no. (%)* (n = *86*) | *FS no. (%)* (n = *41*) | *Controls no. (%)* (n = *100*) |
|---|---|---|---|
| Genotype | | | |
| CC | 67 (77.90) | 32 (78.04) | 87 (87) |
| CT | 15 (17.44) | 8 (19.51) | 12 (12) |
| TT | 4 (4.65) | 1 (2.4) | 1 (1) |
| Allele frequency | | | |
| C | 0.87 | 0.88 | 0.93 |
| T | 0.13 | 0.12 | 0.07 |

where the role of MDR1 gene polymorphism and its contribution to seizure occurrence in GS and FS was assessed.

Further, evaluation of 108 patients for serum PHT levels along with MDR1 C3435T gene polymorphism showed a gradual increase in mean serum PHT levels from homozygous C allele, heterozygous CT, and homozygous T allele (Fig. 3A). In this study, 12.03% (13/108) CC, 49.07% (53/108) CT, and 38.88% (42/108) TT genotype carriers were present. Genotype and serum PHT level analysis in cases showed that the MDR1 C3435T gene polymorphism affects serum PHT levels (Pearson Correlation 0.230 $p < 0.015$). A non-parametric Kruskal–Wallis test was performed to compare the dose PHT ratio and genotype groups of MDR1 C3435T. It showed a significant association ($\chi^2 = 8.14$; $p = 0.01$). The MDR1*CC genotype was more common in cases with low PHT levels. The mean serum PHT levels in SR genotype groups were 8.65, 9.51, and 17.54 μg/mL for CC, CT, and TT genotypes, respectively. Inter-individual variation in serum PHT levels with genotype groups of MDR1 C3435T polymorphism was also observed in this study (Fig. 3B). In this study, cases were evaluated for SR in individual MDR1 C3435T genotype groups; 15.38% (2/13) CC, 30.18% (16/53) CT, and 45.23% (19/42) TT genotype carriers had SR. From the data, it is evident that CT and TT genotype carriers have a high percentage of SR and also elevated serum PHT levels; this may suggest that the presence of the T allele plays a role in SR by MDR1 over-expression.

### *GABRG2 C588T gene polymorphism*

In this analysis, 23/86 (26.74%) cases of GS and 7/41 (17.48%) cases of FS had a positive family history of seizures. The distribution and analysis of genotype and allele frequencies of GS and FS cases was assessed along with controls (Table 6). Statistical analyses carried out with different genotype models indicate no association with both seizure types studied (Table 7). The allele distribution was statistically significant with the "T" allele being associated with GS in our population ($p < 0.05$) (Table 7). When the allele frequencies of GS and FS were combined and compared with controls, it showed a significant association of the T allele with seizures ($p < 0.05$). Table 8 shows the association of the GABRG2 C588T gene polymorphism in different seizure types. Distributions of genotype and allele frequency of GABRG2 C588T polymorphism in controls in the present study were similar to the frequency reported by Kananura *et al.* (2002) in the German population (Table 8). However, a slightly higher percentage of cases was observed in TT genotype carriers (Table 6).

In this study, the association of the GABRG2 C588T gene polymorphism was assessed in GS and FS patients with SR (Table 9). About 45.34% (39/86) GS and 41.46% (17/41) FS cases had SR. TT genotype carriers were high in the recurrent seizure group of GS compared with the well-controlled GS (with no seizures after treatment); however, this was not statistically significant (Table 9). Compared with GS, in FS, CT genotype carriers were high in the recurrent seizure group (Table 9). However, none of the SR cases of FS carried the TT genotype. SR cases when compared with controlled seizures cases of both GS and FS did not show a significant change in distribution of genotype or allele frequency (Table 9).

## Discussion

Epilepsy is a common neurological disorder affecting 1% of people worldwide, it is estimated that 40% of them have a genetic basis. In the Indian population, the prevalence is 5.5 per 1000 (Das *et al.*, 2006). The MDR1 transporter is a P-glycoprotein that regulates exchange of xenobiotics between plasma/brain in normal brain; however, in the epileptic brain, MDR1 levels are abnormal in the epileptic region leading to altered penetration/distribution of drugs (Sisodiya *et al.*, 2002; Marchi *et al.*, 2004). The mechanism of pharmacoresistance

Table 7. Analysis of GABRG2 C588T Genotypes and Alleles Among Two Patient Groups and Controls

| *Genotype* | *Generalized seizures vs. control OR (95% CI); chi square* p-*value* | *Febrile seizures vs. control OR (95% CI); chi square* p-*value* |
|---|---|---|
| CC vs. CT | 0.6161 (95% CI = 0.2705 to 1.4034); | 0.5517 (95% CI = 0.2066 to 1.4731); |
| CC vs. TT | 0.1925 (95% CI = 0.0210 to 1.7627); $\chi^2$ $p > 0.05$ | 0.3678 (95% CI = 0.0223 to 6.0564); $\chi^2$ $p > 0.05$ |
| TT vs. CT | 3.2000 (95% CI = 0.3148 to 32.534); $\chi^2$ $p > 0.05$ | 1.5016 (95% CI = 0.0815 to 27.608); $\chi^2$ $p > 0.05$ |
| Dominant model | | |
| CC vs. CT + TT | 0.5269 (95% CI = 0.2430 to 1.1427); $\chi^2$ $p > 0.05$ | 0.5313 (95% CI = 0.2072 to 1.3622); $\chi^2$ $p > 0.05$ |
| Co dominant model | | |
| CT vs. CC + TT | 1.5493 (95% CI = 0.6817 to 3.5211); $\chi^2$ $p > 0.05$ | 1.7778 (95% CI = 0.6672 to 4.7368); $\chi^2$ $p > 0.05$ |
| Recessive model | | |
| TT vs. CC + CT | 4.8293 (95% CI = 0.5293 to 44.054); $\chi^2$ $p > 0.05$ | 2.4750 (95% CI = 0.1511 to 40.541); $\chi^2$ $p > 0.05$ |
| Allele | | |
| C vs. T | 0.4876 (95% CI = 0.2425 to 0.9804); $\chi^2$ $p > 0.05$ | 0.5419 (95% CI = 0.2303 to 1.2754); $\chi^2$ $p > 0.05$ |
| T vs. C | 2.0508 (95% CI = 1.0205 to 4.1234); $\chi^2$ $p > 0.05$ | 1.8452 (95% CI = 0.7840 to 4.3428); $\chi^2$ $p > 0.05$ |

Table 8. Association of GABRG2 C588T Gene Polymorphism in Different Seizure Types of Studies

| *Population Studied* | *Type of seizure studied* | *Association compared with controls* | *Genotype frequency* | | | *Allele frequency* | | *Reference* |
|---|---|---|---|---|---|---|---|---|
| Germany | IAE | Higher percentage of cases carried T allele | 0.61 | 0.35 | 0.04 | 0.79 | 0.21 | Kananura *et al.* (2002) |
| | Control | | 0.68 | 0.27 | 0.05 | 0.81 | 0.19 | |
| Chinese | FS | Positive association with C allele | 0.17 | 0.53 | 0.30 | 0.43 | 0.57 | Chou *et al.* (2007) |
| | Control | | 0.11 | 0.39 | 0.50 | 0.30 | 0.70 | |
| Indian | GS | Positive association with T allele | 0.77 | 0.17 | 0.05 | 0.87 | 0.13 | Present study |
| | FS | Higher percentage of cases carried T allele | | | | | | |
| | Control | | 0.87 | 0.12 | 0.01 | 0.93 | 0.07 | |
| Nigeria | Healthy controls | | 0.59 | 0.37 | 0.03 | 0.78 | 0.22 | Hapmap (2003) |
| Japan | | | 0.25 | 0.40 | 0.34 | 0.45 | 0.55 | Hapmap (2003) |
| China | | — | 0.18 | 0.38 | 0.44 | 0.37 | 0.63 | Hapmap (2003) |
| Utah, Europe | | | 0.20 | 0.53 | 0.27 | 0.47 | 0.53 | Hapmap (2003) |

IAE, idiopathic absence epilepsy.

in epilepsy is poorly understood and accounts for much of the economic burden of epilepsy (Regesta and Tanganelli, 1999). The MDR1 C3435T gene polymorphism was reported to alter the drug response in humans and contribute to pharmacoresistant epilepsy. In view of this, in the present study of GS, FS patients along with controls were assessed for the MDR1 C3435T gene polymorphism.

The MDR1 C3435T gene polymorphism in the present study followed Hardy–Weinberg equilibrium, similar to the earlier reported data from India (Balram *et al.*, 2003; Ramasamy *et al.*, 2006). A higher percentage of T-allele carriers in SR of GS and FS cases are observed. Presence of the T allele might lead to over-expression of MDR1 in endothelial cells in the blood brain barrier. This was supported by the earlier observation where 3435T allele was associated with over-expression of MDR1 (Sakaeda *et al.*, 2002). Another study from Japan reported higher MDR1 mRNA expression in the TT genotype of MDR1 C3435T variant (Nakamura *et al.*, 2002). Active efflux of AEDs (substrates of MDR1) from endothelial cells back into the blood may cause lowered levels of these AEDs in the brain and could result in recurrent seizures. An earlier report by Regesta and Tanganelli (1999) showed that decreased drug uptake in the brain because of altered expression of MDR1 transporters affects the transport of drug across the blood-brain barrier and results in drug resistance. Other researchers also reported MDR1 3435T allele and TT genotype association with drug-resistant epileptic patients with recurrent seizures in the Asian population (Seo *et al.*, 2006; Kwan and Brodie, 2007).

CT and TT genotype carriers of GS and TT genotype carriers of FS cases could have a higher risk of developing seizures compared with others. This is one of the very few studies from the Indian population, wherein the role of the MDR1 gene polymorphism and its contribution to seizure occurrence in GS and FS were assessed. MDR1 3435T was associated with drug-resistant epilepsy when haplotype analysis was performed (Hung *et al.*, 2005; Seo *et al.*, 2006). Ethnic difference with regard to MDR1 C3435T gene polymorphism was observed, and this may indicate the role of other modifier genes influencing the expression

Table 9. GABRG2 C588T Genotype and Allele Frequency of Generalized Seizures and Febrile Seizure Cases in Seizure Reoccurrence

| *GABRG2 C588T* | *SR in GS no. (%)* (n=*39*) | *Controlled seizures of GS no. (%)* (n=*47*) | $\chi^2$ p-*Value* |
|---|---|---|---|
| Genotype | | | |
| CC | 31 (79.48) | 36 (76.59) | |
| CT | 5 (12.82) | 10 (21.27) | 0.06 |
| TT | 3 (7.69) | 1 (2.12) | |
| Allele frequency | | | |
| C | 0.86 | 0.87 | OR=1.08 |
| T | 0.14 | 0.13 | (95% CI=0.48 to 2.45) |
| *GABRG2 C588T* | *SR in FS no. (%)* (n=*17*) | *Controlled seizures of FS no. (%)* (n=*24*) | $\chi^2$ p-*Value* |
| Genotype | | | |
| CC | 13 (76.47) | 19 (79.16) | |
| CT | 4 (23.52) | 4 (16.66) | 0.33[a] |
| TT | 0 | 1 (4.16) | |
| Allele frequency | | | |
| C | 0.88 | 0.875 | OR=1.04 |
| T | 0.12 | 0.125 | (95% CI=0.80 to 1.36) |

[a]Statistical analysis of genotype frequency was performed after applying Yates.

of MDR1 (Siddiqui *et al.*, 2003; Tan *et al.*, 2004; Shahwan *et al.*, 2007).

The high interpatient variability in MDR1 expression can cause substantial variation in both the rate and extent of absorption of orally administered drugs that are MDR1 substrates, including the AEDs. PHT is the most widely used anticonvulsant drug worldwide and it exhibits nonlinear pharmacokinetics. Tishler *et al.* (1995) demonstrated in resected glia that over-expression of MDR1 resulted in PHT drug resistance. We show here that MDR1 C3435T gene polymorphism regulates serum PHT levels in epileptic patients ($p < 0.01$). An understanding of the physiological role of C3435T genetic alteration in MDR1 transporter will help improve the therapeutic efficacy of AEDs (Sukhai and Piquette-Miller, 2000).

Over-expression of MDR1 in the endothelial cells of brain may efflux the drug back from endothelial cells into the blood, leading to inadequate levels of PHT in the brain and, hence, precipitate seizures. Effluxed PHT drug in the blood may also result in high serum levels of PHT as shown by elevated serum levels in CT and TT genotype carriers in the present study. CC genotype carriers showed low serum PHT levels, this might be explained by influx of drug into the brain and no active participation of MDR1 in effluxing the drug back into blood. These results corroborate the Hoffmeyer and coworkers (2000) published article showing the effect of the MDR1 3435T allele on bioavailability of digoxin. However, Allabi *et al.* (2005) showed the association of the 3435T allele with low levels of serum PHT. Some studies showed that the MDR1 C3435T gene polymorphism was not associated with refractory epilepsy (Leschziner *et al.*, 2006; Dericioglu *et al.*, 2008). Further, it was observed that heterozygotes were highest with 15.74% (17/108) followed by 11.11% (12/108) of MDR1 TT genotype carriers. The overall results of the polymorphism studies carried out show that the MDR1 3435T allele plays a role in seizure occurrence of GS and FS. MDR1 C3435T gene polymorphism showed good correlation with the serum PHT levels, indicating a role in drug response of AEDs. Thus, the association of MDR1 3435T allele association with serum PHT levels observed in the present study is of clinical significance in the management of epileptic patients.

GABA is the main inhibitory neurotransmitter, and major AEDs used clinically act as GABAergic neurotransmission enhancers (Korpi *et al.*, 2002). Disruption of GABAergic neurotransmission mediated by GABA has been implicated in epilepsy, and the nature of the mutant channel may modulate the response to a given treatment (Wallace *et al.*, 2001; Bowser *et al.*, 2002). In GABRG2, two reported mutations have been associated with generalized epilepsy with FS plus and two others with FS and childhood absence epilepsy (Baulac *et al.*, 2001; Wallace *et al.*, 2001; Ito *et al.*, 2006). The present study was conducted to evaluate the GABRG2 C588T gene polymorphism in GS and FS patients to assess whether it confers any susceptibility to seizures.

Allele frequency of control population in the present study was similar to the German and Nigerian population. However, Japanese, Chinese, and European populations carried a high frequency of the T allele. From the results of the present study, it was evident that the GABRG2 588T allele was associated with seizure occurrence. To the best of our knowledge, this is the first report from the Indian population that assesses FS association with GABRG2 C588T gene polymorphism.

Approximately 30% of children with FS experience one or more SRs during subsequent bouts of fever. In 30% of patients with epilepsy, the seizures persist despite regular and adequate AED and carefully monitored treatment (Regesta and Tanganelli, 1999). Hence, it is clinically important to understand the underlying mechanism of SR for proper management of patients. As shown earlier in this study that the T allele was associated with seizure occurrence, TT and CT genotype carriers of SR group of GS and FS, respectively, are at a higher risk of developing recurrent seizure. As yet, there are no studies reporting the role of GABRG2 C588T gene polymorphism in association with SR.

From our study, we recommend dose adjustment based on MDR1 C3435T genotyping at the induction of therapy, and GABRG2 C588T genotyping would help in better patient management.

## Acknowledgments

The authors thank Shellee Abraham for the preparation of this article.

## Disclosure Statement

The authors declare no competing financial interests.

## References

Allabi AC, Gala JL, Horsmans Y (2005) CYP2C9, CYP2C19, ABCB1 (MDR1) genetic polymorphisms and phenytoin metabolism in a Black Beninese population. Pharmacogenet Genomics 15:779–786.

Amara SG, Sonders MS (1998) Neurotransmitter transporters as molecular targets for addictive drugs. Drug Alcohol Depend 51:87–96.

Anglicheau D, Verstuyft C, Laurent-Puig P, *et al.* (2003) Association of the multidrug resistance-1 gene single-nucleotide polymorphisms with the tacrolimus dose requirements in renal transplant recipients. J Am Soc Nephrol 14:1889–1896.

Balram C, Sharma A, Sivathasan C, *et al.* (2003) Frequency of C3435T single nucleotide MDR1 genetic polymorphism in an Asian population: phenotypic-genotypic correlates. Br J Clin Pharmacol 56:78–83.

Baulac S, Huberfeld G, Gourfinkel-An I, *et al.* (2001) First genetic evidence of GABA(A) receptor dysfunction in epilepsy: a mutation in the gamma2-subunit gene. Nat Genet 28:46–48.

Bear MF, Connors BW, Paradiso MA (2011) Neuroscience: Exploring the Brain. Lippincott, Williams & Wilkins, Hagerstown, MD.

Bowser DN, Wagner DA, Czajkowski C, *et al.* (2002) Altered kinetics and benzodiazepine sensitivity of a GABAA receptor subunit mutation [gamma 2(R43Q)] found in human epilepsy. Proc Natl Acad Sci U S A 99:15170–15175.

Chou IC, Lee CC, Tsai CH, *et al.* (2007) Association of GABRG2 polymorphisms with idiopathic generalized epilepsy. Pediatr Neurol 36:40–44.

Das SK, Biswas A, Roy T, *et al.* (2006) A random sample survey for prevalence of major neurological disorders in Kolkata. Indian J Med Res 124:163–172.

Dericioglu N, Babaoglu MO, Yasar U, *et al.* (2008) Multidrug resistance in patients undergoing resective epilepsy surgery is not associated with C3435T polymorphism in the ABCB1 (MDR1) gene. Epilepsy Res 80:42–46.

Hapmap (2003) Available at www.hapmap.org/hapmap populations.html.

Hoffmeyer S, Burk O, von RO, *et al.* (2000) Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity *in vivo*. Proc Natl Acad Sci U S A 97:3473–3478.

Hung CC, Tai JJ, Lin CJ, *et al.* (2005) Complex haplotypic effects of the ABCB1 gene on epilepsy treatment response. Pharmacogenomics 6:411–417.

International League Against Epilepsy (1989) Proposal for revised classification of epilepsies and epileptic syndromes. Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 30:389–399.

Ito M, Yamakawa K, Sugawara T, *et al.* (2006) Phenotypes and genotypes in epilepsy with febrile seizures plus. Epilepsy Res 70 Suppl 1:S199–S205.

Kananura C, Haug K, Sander T, *et al.* (2002) A splice-site mutation in GABRG2 associated with childhood absence epilepsy and febrile convulsions. Arch Neurol 59:1137–1141.

Kang JQ, Shen W, Macdonald RL (2009) The GABRG2 mutation, Q351×, associated with generalized epilepsy with febrile seizures plus, has both loss of function and dominant-negative suppression. J Neurosci 29:2845–2856.

Kim RB (2002) Transporters and xenobiotic disposition. Toxicology 181–182:291–297.

Korpi ER, Grunder G, Luddens H (2002) Drug interactions at GABA(A) receptors. Prog Neurobiol 67:113–159.

Kumari R, Lakhan R, Kalita J, *et al.* (2010) Association of alpha subunit of GABAA receptor subtype gene polymorphisms with epilepsy susceptibility and drug resistance in north Indian population. Seizure 19:237–241.

Kwan P, Brodie MJ (2007) Emerging drugs for epilepsy. Expert Opin Emerg Drugs 12:407–422.

Leschziner G, Jorgensen AL, Andrew T, *et al.* (2006) Clinical factors and ABCB1 polymorphisms in prediction of antiepileptic drug response: a prospective cohort study. Lancet Neurol 5:668–676.

Marchi N, Hallene KL, Kight KM, *et al.* (2004) Significance of MDR1 and multiple drug resistance in refractory human epileptic brain. BMC Med 2:37.

Marzolini C, Paus E, Buclin T, *et al.* (2004) Polymorphisms in human MDR1 (P-glycoprotein): recent advances and clinical relevance. Clin Pharmacol Ther 75:13–33.

Nakamura T, Sakaeda T, Horinouchi M, *et al.* (2002) Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. Clin Pharmacol Ther 71:297–303.

Pauli-Magnus C, Kroetz DL (2004) Functional implications of genetic polymorphisms in the multidrug resistance gene MDR1 (ABCB1). Pharm Res 21:904–913.

Ponnala S, Rao KP, Chaudhury JR, *et al.* (2009) Effect of polyunsaturated fatty acids on diphenyl hydantoin-induced genetic damage *in vitro* and *in vivo*. Prostaglandins Leukot Essent Fatty Acids 80:43–50.

Ramasamy K, Sisy SS, Chandrasekaran A (2006) Allele and genotype frequency of MDR1 C3435T in Tamilian population. Drug Metab Pharmacokinet 21:506–508.

Regesta G, Tanganelli P (1999) Clinical aspects and biological bases of drug-resistant epilepsies. Epilepsy Res 34:109–122.

Sakaeda T, Nakamura T, Okumura K (2002) MDR1 genotype-related pharmacokinetics and pharmacodynamics. Biol Pharm Bull 25:1391–1400.

Sakaeda T, Nakamura T, Okumura K (2003) Pharmacogenetics of MDR1 and its impact on the pharmacokinetics and pharmacodynamics of drugs. Pharmacogenomics 4:397–410.

Seo T, Ishitsu T, Ueda N, *et al.* (2006) ABCB1 polymorphisms influence the response to antiepileptic drugs in Japanese epilepsy patients. Pharmacogenomics 7:551–561.

Shahwan A, Murphy K, Doherty C, *et al.* (2007) The controversial association of ABCB1 polymorphisms in refractory epilepsy: an analysis of multiple SNPs in an Irish population. Epilepsy Res 73:192–198.

Siddiqui A, Kerb R, Weale ME, *et al.* (2003) Association of multidrug resistance in epilepsy with a polymorphism in the drug-transporter gene ABCB1. N Engl J Med 348:1442–1448.

Sieghart W, Fuchs K, Tretter V, *et al.* (1999) Structure and subunit composition of GABA(A) receptors. Neurochem Int 34:379–385.

Sisodiya SM, Lin WR, Harding BN, *et al.* (2002) Drug resistance in epilepsy: expression of drug resistance proteins in common causes of refractory epilepsy. Brain 125:22–31.

Sukhai M, Piquette-Miller M (2000) Regulation of the multidrug resistance genes by stress signals. J Pharm Pharm Sci 3:268–280.

Tan NC, Heron SE, Scheffer IE, *et al.* (2004) Failure to confirm association of a polymorphism in ABCB1 with multidrug-resistant epilepsy. Neurology 63:1090–1092.

Tishler DM, Weinberg KI, Hinton DR, *et al.* (1995) MDR1 gene expression in brain of patients with medically intractable epilepsy. Epilepsia 36:1–6.

Wallace RH, Marini C, Petrou S, *et al.* (2001) Mutant GABA(A) receptor gamma2-subunit in childhood absence epilepsy and febrile seizures. Nat Genet 28:49–52.

Whiting PJ (2003) The GABAA receptor gene family: new opportunities for drug development. Curr Opin Drug Discov Dev 6:648–657.

Address correspondence to:
*Shivani Ponnala, Ph.D.*
*Department of Cancer Biology and Pharmacology*
*University of Illinois College of Medicine at Peoria*
*One Illini Drive*
*Peoria, IL 61656*

*E-mail:* sponnala@uic.edu