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The likelihood approach for potential role of "GABRG2 (C588T, C315T) gene polymorphisms" on the poor response to carbamazepine therapy in Pakhtun population of Pakistan

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Abstract

Background: Gamma-aminobutyric acid A receptor, gamma 2 gene (GABRG2) encode the GABAA receptor which is responsible for fast neuronal inhibition. Polymorphisms in GABGR2 gene affect the clinical response of anti-epileptic drugs (AEDs). Therefore, we carried out an updated study to find the association GABRG2 gene polymorphisms with carbamazepine (CBZ) non-responsive therapy in the Pakhtun population.

Methods: A clinical prospective cohort study was conducted in 79 CBZ treated patients upon consent after the approval of Khyber Medical University Advanced Study and Research Board. Blood sample were taken at optimal dose of CBZ at base line, third and sixth months of the treatment. Blood level of CBZ was measure through reverse phase high performance liquid chromatography (HPLC). Restriction fragment length polymorphisms techniques were used to genotype GABRG2 gene in these patients. CBZ responses were evaluated on three and six months of study by measuring the decrease in frequency of seizure per week.

Results: The average maximum dose of CBZ was 455 ± 133 mg/day at baseline, 479 ± 142 mg/day at third month and 495 ± 133 mg/day at sixth month of the treatment. CBZ level was found within therapeutic range (4-12 mg/L) without any significant ($P > .5$) variations among the CC, CT and TT genotypes of GABRG2 (C588T and C315T) gene. But the poor clinical response during CBZ treatment was linked ($P < .05$) with CT and TT genotypes of GABRG2 (C588T and C315T) gene in Pakhtun Population.

Conclusion: A poor response to CBZ was found in variant genotypes (CT and TT) of GABRG2 (C588T and C315T) gene in Pakhtun Population.

Abbreviations: AEDs = anti-epileptic drugs, CBZ = carbamazepine, GABRG2 = Gamma-aminobutyric acid A receptor, gamma 2 gene, KP = Khyber Pakhtunkhwa, SNPs = single nucleotide polymorphisms, VPA = valproic acid.

Keywords: Asia, carbamazepine, control response, GABRG2, Pakistan, poor response, valproic acid

1. Introduction

Epilepsy is a heterogenous nature brain disease, phenotypically exhibited by episodic seizures occurring within 24 hours.[1] Carbamazepine (CBZ) is the anti-epileptic drug (AEDs) that is mostly used in generalized epilepsies in low developed countries.[2] Variable response to CBZ has been observed from different studies in various populations.[3] Pharmacoresistant epilepsy is attributed to different factors such as complex molecular, morphological, and functional alterations. Current published data demonstrates that problems in pharmacoresistance in epilepsy are the leading paradigm shifting to novel exciting therapies. Some patients show poor response to medical treatment and lead to pharmacoresistant epilepsy.[4] Different studies suggest that genetic abnormalities are linked with the resistant epilepsy.[4] This problem is highlighted by Argumosa and Herranz that poor controlled epilepsy was 2.7 times more expensive compared to controlled epilepsy.[5] Variable response of CBZ may be due to alteration in gene encoding pharmacokinetic enzymes or encoding different AEDs binding receptors and ethnicity of individuals.[6] However, Single nucleotide polymorphisms (SNPs) in gene is considered one of the main factor in genetic resistant epilepsy.[7,8] It has been

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The patients gave consent to publish their data. Their confidentiality is maintained.

The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

The study was approved by the Ethics Board of the Khyber Medical University, Peshawar via approval no: DIR/KMU-EB/AC/000047 that complied with Helsinki's declaration.

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observed that these SNP is associated with resistant epilepsy due to abnormal conformational changes in ion channels and AEDs receptors.[9] Gamma-aminobutyric acid A receptor, gamma 2 gene (GABRG2) gene has attracted the attention of many scientists and has been explored in different population to find the link with different types of epilepsies. GABRG2 gene encodes γ subunit $GABA_A$ receptor which is associated with fast neuronal inhibition by Gamma-aminobutyric acid (GABA) neurotransmitter.[10,11–13] It has been believed that crucial neuronal conduction is attributed to GABRG2 gene in brain.[14] Most AEDs like benzodiazepines, phenobarbital, gabapentin and topiramate bind with GABA receptor to show it pharmacological activities.[15] Data suggest that variable response in AEDs may be due to GABRG2 gene polymorphisms. Important mutations in GABRG2 genes are C588T and C315T which are responsible for different types of epilepsies.[16] However, few studies are available to find out the effect of GABARG2 gene polymorphisms on pharmacoresistance to AEDs.[17–20] The mechanisms underlying pharmacoresistance to AEDs is not still properly explored on scientific background.[21]

Therefore, this study is designed to find the link of GABRG2 (C588T, C315T) gene polymorphisms with pharmacoresistance to CBZ in Pakhtun population of Pakistan.

2. Methods

2.1. Participants recruitment

Epileptic patients (N = 79) properly diagnosed were enrolled upon a written consent from Outpatient Department of Neurology of Lady Reading Hospital, Peshawar. The study was approved by Advanced Study and Research Board and Ethical Board of Khyber Medical University, Peshawar via no: "DIR/KMU-EB/AC/000047". At baseline blood were taken for GABRG2 gene genotyping and at third and sixth month of the treatment for blood level of CBZ.

2.2. Protocol of the study

Study was a single center hospital based prospective study. Demographic and clinical features were recorded in a questionnaire at base line of the study. At the third and sixth months of treatment, the patients were followed up on, and the clinical outcome was assessed (Fig. 1). Clinical response was measured at third and sixth month of the treatment by measuring the reduction in frequency of seizure per week. Patients' reported outcomes (PRO) and clinician reported outcomes (CliRO) were used to determine clinical outcomes in epilepsy patients.[22,23]Clinical results were documented on a standard established proforma in the context of local languages as a reduction in seizure frequency or duration.

2.3. Extraction of genomic DNA and GABRG2 gene genotyping

DNA was extracted from whole blood using kit method (NucleoSpin® Blood, Germany). Forward primer "5-CAAATGTGGTGAATTAGTAACTGG-3 and reverse primer 5-TCACATTTTCTCTCAAACATGC-3" was used for the amplification of exon 3 of GABRG2 (C315T) gene. Restriction enzyme *Bas*MI was used to digest the amplified products for genotyping. Furthermore, forward primer "5-AATCACCTTTTATTCTAATGGTC-3 and reverse primer 5-CAGTGAAGGCAACTTACTAAGA-3" used to amplify exon 5 of GABRG2 (C588T) gene using gradient PCR. The resultant product was restricted with digestive enzyme *Apo*I. 5% agarose was used to run the products of each exon and fragments were evaluated with 50Pb ladder.

2.4. Measurement of plasma level of CBZ

The blood level of CBZ was determined using reverse phase high performance liquid chromatography (LC-20AT Shimdzu Kyoto, Japan) at the third and sixth month of treatment. CBZ ware extricated by adding 200 µL plasma to a solution of 50 µL of ammonium hydroxide (25%) (BDH Laboratory Supplies England) and 5mL of HPLC grade chloroform (Scharlab S.L. Spain). After appropriate mixing with a mechanical shaker for 20 minutes, the sample was centrifuged at 3000 rpm for 10 minutes using PLC-05 (Taiwan). The organic (chloroform) layer was evaporated after transferring to another tube at 50 ^{0}C in water bath. After drying the remaining were dissolved in the mobile phase (methanol: demineralized water: glacial acetic acid 100%) at a ratio of 65:34:1 (v/v/v). Nylon membrane filter with Micropore 0.45 µm were used to filter the reconstituted extract.[24] About 20 µL reconstituted sample was injected into injection port of HPLC and flow rate of mobile phase through a C18 column (SEA 18, 5 µm 25 × 0.46 Mediterranean) was adjusted at 0.8 mL/min. Detection of CBZ was performed was observed on 220 nm.

2.5. Data analysis

GraphPad prism 6 was used to analyze the data. Results were shown in tables and figures. The data were presented in frequencies, mean ± SD. One way ANOVA followed by Tukey test was used to compare the blood level of CBZ at third and sixth month of the treatment among all genotypes of GABRG2 gene. Chi^2 test was used to find out the relationship of poor CBZ with variant genotypes of GABRG2 gene. Results were considered as statistically significant if ($P > .05$).

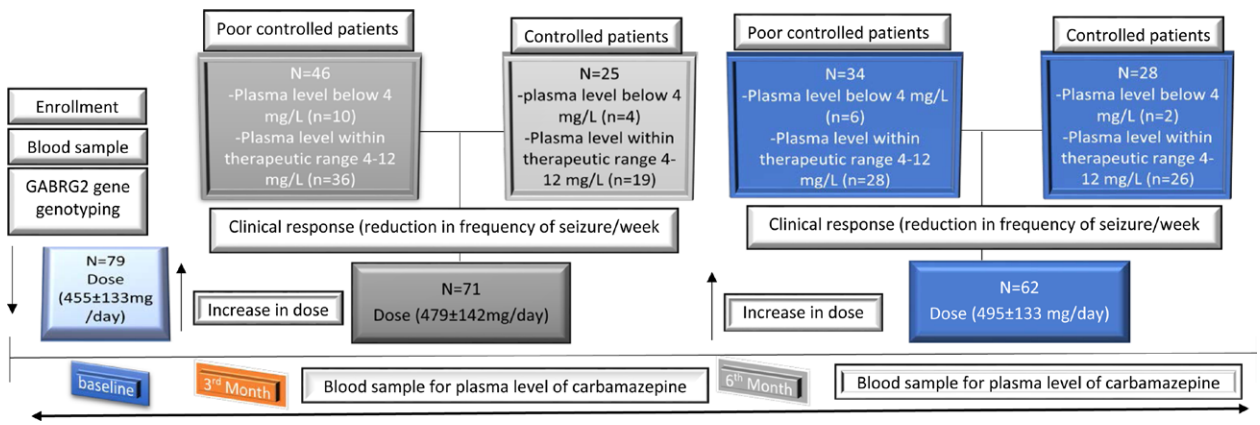

Figure 1. Timeline of study with CBZ dose and their therapeutic response. CBZ = carbamazepine.

3. Results

3.1. Clinical and demographic characteristics of epileptic patients

Mean age of the patients was 18.1 ± 8.2 year was at baseline, 17.84 ± 6.3 year at third month and 18.08 ± 7.5 year at sixth month of the treatment (Table 1). The frequency of female patients was high 41, 37, 34 (53%, 53.1%, 51.9%) female patients than male patients 37, 34, 28 (47%, 47.9%, 45.1%) at baseline, 3rd, and 6th month of therapy (Table 1). Similarly, the frequency of generalized tonic clonic epilepsy was high in the enrolled patients (Table 1). The daily mean dose of CBZ was 455 ± 133 mg/day, 479 ± 142 mg/day and 495 ± 133 mg/day at baseline, third, and sixth month of the treatment. The observed clinical outcome was depicted in Figure 1.

3.2. Blood level of CBZ versus GABRG2 gene polymorphisms

The blood level of CBZ at third and sixth month of the treatment in different genotypes are presented in Figure 2. There was no significant ($P < .05$) difference in the blood level of CBZ in different genotypes of GABRG2 gene at third and sixth month of the treatment. Its mean that blood level was in therapeutic range and has no association with clinical outcome of CBZ (Fig. 2).

3.3. Measurement of the potential of GABRG2 (C588t, c315t) genotypes in CBZ treatment

Poor response to CBZ was observed in 46 patients at third month and 34 patients at sixth month of the treatment (Tables 2 and 3). It has found that variant genotypes of GABRG2 (588CT and 588TT) gene show an association ($\chi^2 = 9.9$, $P = .01$) with poor response to CBZ at third month of the treatment (Table 2). Similarly, again variant genotypes of GABRG2 (588CT and 588TT) gene showed an association ($\chi^2 = 11.2$, $P = .01$*) with poor response to CBZ at sixth month of the treatment (Table 3).

In addition to, poor response to CBZ were more likely than those in responsive group to have 315CT and 315TT genotypes of GABRG2 gene ($\chi 2 = 9.4$, $P = .02$*) (Table 2) at third month of CBZ treatment. More, poor response to CBZ were also more likely ($\chi 2 = 8.8$, $P = .03$*) to happen in variant genotypes of GABRG2 (315CT, 315TT) gene at sixth month of CBZ treatment (Table 3).

4. Discussion

Our study delved the impact of GABRG2 (C588T and C315T) gene polymorphism on the clinical response of CBZ treatment in epileptic patients of Pakhtun population of Khyber Pakhtunkhwa, Pakistan. The clinical outcome was measure at third and sixth month of the treatment in the context of reduction of frequency of seizure per week. Our

Table 1

Demographic and clinical features of epileptic patients on CBZ treatment.

Variables		Patients treated with CBZ as monotherapy		
		Baseline (n = 79)	3rd month (n = 71)	6th month (n = 62)
Gender	Male, *n* (%)	37 (47)	34(47.9)	28 (45.2)
	Female, *n* (%)	42 (53)	37 (52.1)	34 (54.8)
Age (year)	Mean (range)	18.1 ± 8.2 (1-42)	17.84 ± 6.3 (1-42)	18.08 ± 7.5 (1-42)
Types of seizures	Generalized tonic clonic seizure, *n* (%)	52 (70.9)	48 (62.0)	44 (77.4)
	Generalized tonic seizure, *n* (%)	4 (5.1)	4 (7.0)	3 (1.6)
	Atonic seizure, *n* (%)	3 (3.8)	2 (7.0)	2 (3.2)
	Simple partial seizure, *n* (%)	3 (3.8)	3 (7.0)	3 (4.8)
	Complex partial seizure, *n* (%)	5 (6.3)	4 (4.2)	3 (4.8)
	Secondary generalized complex seizure, *n* (%)	12 (7.6)	10 (7.0)	7 (9.7)
Mean daily dose ± SD (mg/day), (200-800)		455 ± 133	479 ± 142	495 ± 133

CBZ = carbamazepine, SD = standard deviation.

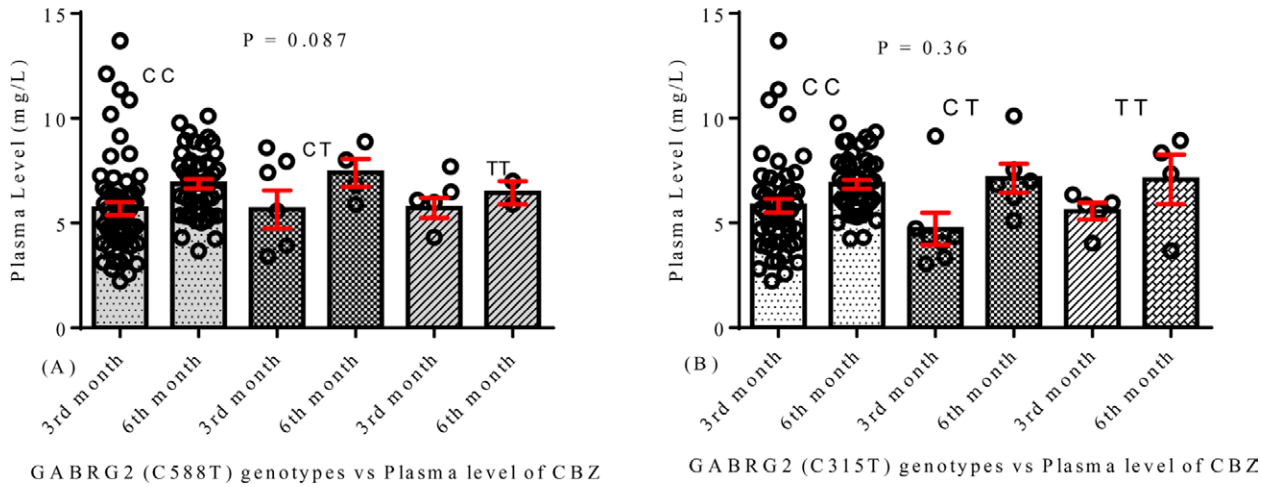

Figure 2. Plasma levels of CBZ vs GABRG2 gene genotypes. (A) Plasma level of CBZ vs GABRG2 (C588T) genotypes. (B) Plasma level of CBZ vs GABRG2 (C315T) genotypes. Data represent mean ± SEM (CC vs CT, CC vs TT at 3rd and 5th month of therapy) (One-way ANOVA, Tukey post hock test). CBZ = carbamazepine, GABRG2 = Gamma-aminobutyric acid A receptor, gamma 2 gene.

Table 2

Association of GABRG2 (C588T and C315T) gene polymorphisms with CBZ treatment at third month (N = 71).

N = 71	Poor seizure-controlled patients (n = 46)	Controlled patients (n = 25)
Variables		
Number (%)	46 (65)	25 (35)
Mean plasma level (mg/L)	5.1 ± 2.0	5.6 ± 2.2
Genotypes		
588CC	22	18
588CT	20	4
588TT	4	3
315CC	16	19
315CT	24	4
315TT	6	2

GABRG2 (C588T) Gene. Heterozygous (588CT) genotypes were more likely frequent in CBZ resistant patients compared to CBZ responsive patients in Pakhtun population of KP ($\chi^2 = 9.9$, $P = .01$) * comparing total patients of CBZ resistant versus total patients who are responsive to CBZ therapy. However, homozygous mutant (588TT) genotypes were less likely frequent in CBZ therapy resistant patients as compared to CBZ responsive patients ($\chi 2 = 3.1$, $P = .37$) comparing resistant patients to CBZ therapy

GABRG2 (C315T) Gene. Heterozygous (315CT) genotypes were more likely frequent in CBZ resistant patients compared to CBZ responsive patients in Pakhtun population of KP ($\chi^2 = 9.4$, $P = .02$) * comparing total patients of CBZ resistant versus total patients who are responsive to CBZ therapy. However, homozygous mutant (315TT) genotypes were less likely frequent in CBZ therapy resistant patients as compared to CBZ responsive patients ($\chi^2 = 6.7$, $P = .07$) comparing resistant patients to CBZ therapy. CBZ = carbamazepine, GABRG2 = Gamma-aminobutyric acid A receptor, gamma 2 gene.

Table 3

Association of GABRG2 (C588T and C315T) gene polymorphisms with CBZ treatment at sixth month (N = 62).

N = 62	Poor seizure-controlled patients (n = 34)	Controlled patients (n = 28)
Variables		
Number (%)	34 (55)	28 (45)
Mean plasma level (mg/L)	4.7 ± 1.9	5.5 ± 1.6
Genotypes		
588CC	10	24
588CT	18	3
588TT	6	1
315CC	12	20
315CT	16	6
315TT	3	2

GABRG2 (C588T) Gene. Heterozygous (588CT) genotypes were more likely frequent in CBZ resistant patients compared to CBZ responsive patients in Pakhtun population of KP ($\chi 2 = 11.2$, $P = .01$) * comparing total patients of CBZ resistant Vs total patients who are responsive to CBZ therapy. However, (588TT) genotypes were less likely frequent in CBZ therapy resistant patients as compared to CBZ responsive patients ($\chi 2 = 5.5$, $P = .13$) comparing resistant patients to CBZ therapy.

GABRG2 (C315T) Gene. Heterozygous (315CT) genotypes were more likely frequent in CBZ resistant patients compared to CBZ responsive patients in Pakhtun population of KP ($\chi 2 = 8.8$, $P = .03$) * comparing total patients of CBZ resistant Vs total patients who are responsive to CBZ therapy. However, (315TT) genotypes were less likely frequent in CBZ therapy resistant patients as compared to CBZ responsive patients ($\chi 2 = 7.5$, $P = .06$) comparing resistant patients to CBZ therapy. CBZ = carbamazepine, GABRG2 = Gamma-aminobutyric acid A receptor, gamma 2 gene.

study suggests that frequency generalized tonic clonic seizure is high compared to other types of epilepsies. Furthermore, the frequency of epilepsy is high in young population. It is evident that single nucleotide polymorphism affect the pharmacokinetics (drug metabolizing enzymes) and pharmacodynamics (sodium channels, GABA receptors,) of drugs.[18,25] It is confirmed from the results that clinical response of CBZ was not affected by its blood level because the blood level was within the reference range (4-12 mg/L) among all genotypes of GABRG2 gene. This evidence is supported by the fact that GABAA receptor is important target of many drugs (gabapentin and topiramate).[15] GABRG2 gene is not involved in the metabolism of CBZ. The other factors that can affect the clinical response of CBZ are dose and compliance with the drug use. But the CBZ is used at its optimal dose and the compliance was assured through counting the number of tablets within the allotted time. However, it has been found that poor response of CBZ was associated with variant (588CT, 588TT, 315CT and 315TT) genotypes of GABRG2 gene at third month of the therapy. The same observations were found that variant (588CT, 588TT, 315CT and 315TT) genotypes of GABRG2 gene at sixth month of the therapy. These observations are supported by Bethmann et al study that an alteration in GABAA receptor subunit has a relationship with AEDs resistance.[16] Another study also coincide with my observations that GABRG2 gene polymorphisms modulate drug response to therapy because of variation in the structure and inhibitory action of the GABA receptor.[26] The binding of neurotransmitters with GABA receptor is crucial for normal brain physiological functions which are affected by polymorphisms in GABRG2 gene.[14] Therefore, we may come across that GABRG2 genes polymorphisms, has a role in variable response to CBZ therapy. However, the variable response to CBZ is multifactorial and these above factors play an important role in pharmacogenomics of CBZ.

To control the genotype quality and minimize the false positive results a replication stage using alternative standard method was used.[27] It is recommended that further multicenter studies are required to extrapolate the find to clinical practice in term of precise personalized medicine.

5. Conclusion

The variant genotypes (CT and TT) GABRG2 (C588T and C315T) gene is associated with poor response to CBZ in Pakhtun Population.

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Author contributions

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Writing – review & editing: Shakir Ullah, Syed Wadood Ali Sha, Feras Almarshad.

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