PROSPECTIVE STUDY ON GENETIC DETERMINANTS OF CARBAMAZEPINE RESPONSE; THE CYP3A5-RS15524 GENE POLYMORPHISM IN PASHTUN PATIENTS WITH EPILEPSY

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ABSTRACT

Objective: To evaluate the impact of CYP3A5-rs15524 polymorphism on the clinical response to carbamazepine (CBZ) monotherapy in Pashtun patients with epilepsy.

Materials and Methods: A longitudinal study was carried out among Pashtun patients with epilepsy in Khyber Pakhtunkhwa from October 2020 to April 2022. Participants were recruited from Lady Reading Hospital, while laboratory work was performed at the Pharmacology Department of Khyber Medical University, Peshawar. Patients were genotyped for the CYP3A5-rs15524 polymorphism using Sanger sequencing, with variants analyzed via Finch TV. The study correlated each genotype with clinical response and serum CBZ levels, which were measured using reversed-phase HPLC during each follow-up visit.

Results: A sample of 223 patients was analyzed, including 63.2% males and 36.8% females. Generalized tonic-clonic seizures comprised 82.5% of cases, while partial seizures accounted for 17.5%. Highest and lowest plasma levels of carbamazepine across both follow-ups were observed in AA & AG genotypes, respectively. In 3rd month, the non-responder and responder rates were 18.4% and 31.3% for AA, 44.6% and 16.9% for AG, and 63.6% and 18.2% for GG genotypes. In the 6th month, the rates were 9.5% and 53.1% for AA, 32.3% and 32.3% for AG, and 27.3% and 54.5% for GG, demonstrating highly significant differences in clinical response. Genotype analyses showed a significant improvement in CBZ response in subsequent follow-up.

Conclusion: Our findings suggest that CYP3A5-rs15524 polymorphism may impact the clinical response to carbamazepine in Pashtun patients with epilepsy. Clinical pharmacogenetic research can greatly enhance personalized treatment within our study population.

Keywords: CYP3A5-rs15524, Carbamazepine, Clinical responses, Single nucleotide polymorphism

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INTRODUCTION

Epilepsy is a chronic disorder defined by a persistent vulnerability to spontaneous seizures triggered by any neurological insult, along with neurobiological, cognitive, and social repercussions.[1] Epilepsy affects 50 million people worldwide. Asia, with a population of over 4 billion people, half of the global population, anticipates having around 23 million individuals living with epilepsy. The incidence of epilepsy in the region fluctuates from 1.5 to 14 per 1000 inhabitants. [2, 3] Although epidemiological statistics in Pakistan are scarce, epilepsy is estimated at 9.99 per 1000 people. [4]

While the majority of patients have a positive therapeutic outcome, about 30% continue to suffer from seizures despite having carefully adjusted antiepileptic drug (AED) treatment. [5] Carbamazepine (CBZ) is often prescribed to prevent partial seizures and alleviate generalized tonic-clonic seizures and trigeminal neuralgia. [6,

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[7] A major portion of CBZ is extensively metabolized in the liver, less than 5% of the administered drug is eliminated without alteration. [8] This biotransformation is primarily facilitated by the cytochrome P450 (CYP3A4 & CYP3A5) enzymes. The CYP3A4/5 genes, which encode CYP3A4/5 enzymes, are highly polymorphic. [9] Genetic consortia like PharmGKB and CPIC guidelines have placed several CYP3A4/5 gene polymorphisms in the third level of evidence. This warrants further research of the said polymorphisms to establish a definite phenotype.

By deciphering the therapeutic response to CBZ in epilepsy patients in the context of single nucleotide polymorphisms (SNPs), health services could be optimized, and healthcare providers would be able to successfully configure treatments.

This will allow the prediction of real-time resistance to drugs. The important role of CYP3A5-rs15524 in this setting was the key consideration in its choice. The single nucleotide polymorphism CYP3A5*1D (rs15524) in chromosome 7 is positioned 99245914 in the 3-untranslated region (UTR). Polymorphisms in UTRs may impact the mRNA stability, thus modulating the expression and function of the enzymes. [10]

Genetic traits have profound effects on pharmacokinetics in general. In our previously published study, we examined the pharmacokinetic features of CBZ relative to the CYP3A5-rs15524 polymorphism and revealed that this genetic variation strongly impacts CBZ metabolism. [11] Building upon the previous research, the present investigation aimed to investigate the clinical outcomes of CBZ treatment in Pashtun epilepsy patients, with a particular emphasis on CYP3A5-rs15524. We aspire to enhance the effectiveness of personalized treatment strategies for epilepsy.

MATERIALS AND METHODS

Approved by the Ethics Board of Khyber Medical University (Dir./KMU-EB/PS/000807), this longitudinal study comprising two follow-ups and adhering to the Helsinki Statement was conducted in Khyber Pakhtunkhwa from October 2020 to April 2022. With a confidence interval of 95% and a margin of error of 7.5%, the sample size was calculated as 180 using the web-based Open Epi. To confirm the results' validity and consider follow-up withdrawals, the pool of subjects was set at 223.

Participants were recruited from the neurology department of Lady Reading Hospital, Peshawar. After obtaining informed consent, individuals of either gender, aged between 5 and 70 years, who were recently diagnosed with epilepsy and prescribed carbamazepine monotherapy, were included. History, general physical examination, and complete laboratory tests were recorded using the University's Advanced Studies and Research Board-approved proforma. Those who were unwilling to enroll, had co-morbidities, impaired renal or hepatic function, were pregnant, or were taking drugs that interact with carbamazepine were excluded.

DRUG THERAPY AND ASSESSMENT OF CLINICAL EFFICACY

Patients received standard carbamazepine monotherapy (10–20 mg/kg) manufactured by a multinational pharmaceutical company in compliance with the BPS assays for tablet dosage forms. Due to the disparity in dosages among patients, the steady-state concentration was set to comply with both body weight and dose. Since, due to auto-induction, CBZ plasma levels could take 8 to 12 weeks to achieve a plateau, the first follow-up was scheduled for 3rd month and the second for 6th month. [12]

Before initiating CBZ therapy, the monthly seizure rate of the selected patients was recorded using a structured proforma listing all details about demographics and seizures. Treatment response was determined at three-month intervals by evaluating a decline in seizure frequency. Patients were categorized into three groups based on their response: Responders, who experienced a seizure reduction of more than 97%; Partial responders, with a reduction greater than 50%; and Non-responders, who had less than a 50% reduction, no improvement, or a decline in condition. [13]

Treatment adherence was confirmed by calculating the number of tablets left on the strip. [12] At the first follow-up, the CBZ dose was tailored as per response and severity of the disease status.

MONITORING OF DRUG LEVELS

At each follow-up visit, the CBZ plasma levels were computed through reversed-phase HPLC (LC-20AT), equipped with the UV detector (SPD-20A/20AV, Shimadzu Kyoto), having a mobile phase of demineralized water, methanol, and glacial acetic acid in a volumetric ratio of 65:34:1 at pH 5.6. [12]

The sample was injected at 0.8 ml/min using a C18 column with a UV range of λ_{max} 220 nm. Diclofenac sodium was used as an internal standard. The approach was verified by assessing intra-day and inter-day coefficients of

variance and percentage recovery. [14]

GENETIC ANALYSIS OF STUDY POPULATION

At the baseline visit, blood samples were collected, and DNA was extracted using the salting-out method. [15] After adding elution buffer, genomic DNA was stored at -200°C (Servizo Assistanza, Model KFDC 350, Italia). The purity and concentration of extracted DNA were validated by a nanodrop spectrophotometer. The targeted region of the CYP3A5 gene was amplified using a gradient thermocycler.

The respective forward and reverse primers (ACGAGTCCACAAGAATTTGTCT, TCTGGGGACAGCTTTCTTG) were generated with the help of the UCSC genome database (https://genome.ucsc.edu/cgi-bin/hgPcr). The PCR products were run on a 2% agarose gel for confirmation. [16] The targeted gene was sequenced employing Sanger sequencing on a Seq Studio™ system.

The sequencing products were enhanced using the BigDye X-terminator™ kit, and the evaluation was concluded with the application of a capillary electrophoresis. The sequences of the specific gene location were inspected using Finch TV, as shown in Fig. 1. Analysis was carried out to explore the distribution of genotypes in Pashtuns concerning the global population. The precision of the analysis was validated using the Hardy-Weinberg equilibrium test.

STATISTICAL ANALYSIS

Data analysis was conducted using SPSS version 22, with graphs created in Microsoft Excel. Categorical data were analyzed using frequencies and percentages, while numerical data were expressed as means and standard deviations. Chi-square and Fisher's exact tests were used to examine associations between genotypes and factors like gender, seizure types, and clinical outcomes. ANOVA with the Bonferroni post hoc test was applied to compare CBZ plasma levels across genotypes. P value less than 0.05 was considered statistically significant.

RESULTS

A total of 223 individuals were analyzed, with an average age of 30.7 years. The sample consisted of 63.2% males and 36.8% females. Among these participants, 27.8% reported a positive family history of seizures. The types of seizures recorded included generalized tonic-clonic (GTC) seizures, which comprised 82.5% of the cases, and partial seizures, which accounted for 17.5%.

DISTRIBUTION PATTERN OF GENOTYPES

The distribution of genotypes for CYP3A5-rs15524 shows that AA was the most prevalent genotype found in 147 (65.9%) subjects, followed by the AG genotype present in 65 (29.1%) individuals, while the GG genotype was the least common, observed in 11 (4.9%) subjects in the studied population. This distribution aligns closely with what is expected under Hardy-Weinberg equilibrium.

DISTRIBUTION OF SEIZURE TYPES ACROSS GENDERS

As illustrated in Fig. 2, males with the AA genotype predominantly experienced generalized tonic-clonic seizures (GTC), whereas females typically reported partial seizures. However, statistical analysis revealed no significant associations between the seizure type and genotype ($P = 0.73$) and between the gender and genotype ($P = 0.09$).

CBZ LEVELS AND CLINICAL RESPONSES IN CONTEXT OF CYP3A5 GENOTYPES

Table 1 presents CBZ plasma levels across different genotypes. In both follow-ups, individuals with the AA genotype exhibited the highest plasma levels, while those with the AG genotype had the lowest. Significant mean differences in plasma concentrations were observed between AA vs. AG and AA vs. GG, with p-values less than 0.001.Additionally, Table 1 shows the relationship between CYP3A5 genotypes and clinical responses at the

Table No 1: Association of CBZ levels and clinical responses with CYP3A5 genotypes

Follow-Up	Genotype	*CBZ Level (Mean ± SD)	Non-Responders	Partial Responders	Responders
First Follow-up	AA^{ref}	5.6 ± 1.7	27 (18.4%)	74 (50.3%)	46 (31.3%)
	AG	3.8 ± 1.2	29 (44.6%)	25 (38.5%)	11 (16.9%)
	GG	4.8 ± 1.7	7 (63.6%)	2 (18.2%)	2 (18.2%)
Second Follow-up	AA^{ref}	6.3 ± 1.8	14 (9.5%)	55 (37.4%)	78 (53.1%)
	AG	4.5 ± 1.4	21 (32.3%)	23 (35.4%)	21 (32.3%)
	GG	5.5 ± 1.9	3 (27.3%)	2 (18.2%)	6 (54.5%)

*CBZ levels are expressed in mg/L

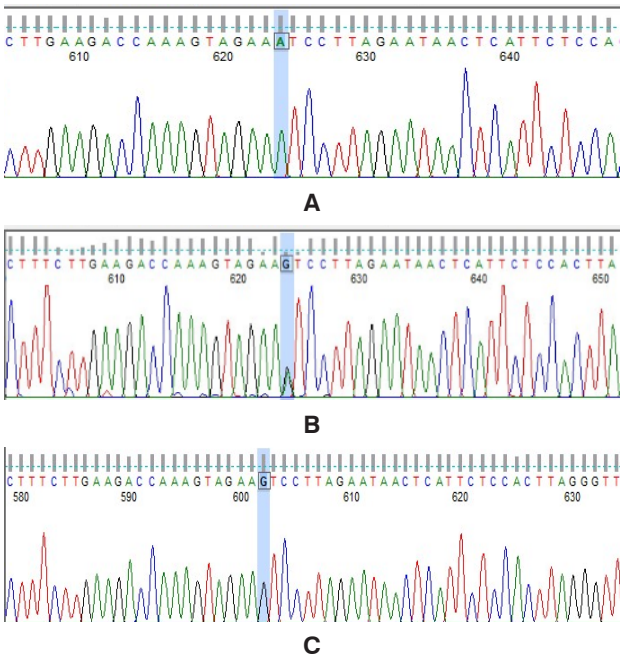

Fig 1: Electropherograms of CYP3A5-rs15524. a. Wild Type (AA) b. Heterozygous Type (AG) c. Homozygous Mutant (GG)

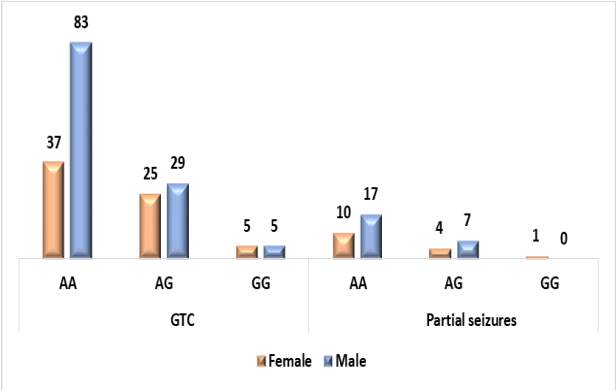

Fig 2: Correlation of gender with seizure type in perspective of CYP3A5 genotypes

3rd and 6th months of CBZ therapy, with all associations being statistically significant (p-values <0.05). At the first follow-up, the GG genotype had the highest proportion of non-responders (63.6%), while the AA genotype had the highest proportion of responders (31.3%). By the second follow-up, the AG genotype was more associated with non-responders (32.3%), while both GG and AA genotypes had the highest proportions of responders (54.5% and 53.1%, respectively).

A comparative analysis across follow-ups showed a significant improvement in CBZ response during the second follow-up. The proportion of non-responders decreased by 8.9%, 12.3%, and 36.3% for the AA, AG, and GG genotypes, respectively. At the same time, the frequency of responders increased by 21.8%, 15.4%, and 36.3% for the AA, AG, and GG genotypes, with the greatest improvement in GG genotype carriers. Additionally, carriers of the AG genotype had lower CBZ plasma levels and a higher proportion of non-responders compared to other genotypes.

DISCUSSION

A key challenge in antiepileptic therapy is finding an optimal dose that ensures complete seizure control while minimizing adverse effects for each patient. Since 1974, carbamazepine (CBZ) has been a first-line anti-epileptic drug for partial epilepsy due to its efficacy. The CYP3A5 enzyme is implicated in carbamazepine's major metabolic route and pharmacokinetics. Therapeutic drug monitoring is needed to improve therapy results because of its limited therapeutic range (4–12 mg/l) and notable inter-individual variability in dosage and side effects. [17]

In this study, we investigated the potential association between genetic mutations in CBZ-metabolizing enzymes and the variability in response among Pashtun patients with epilepsy. The CYP3A5-rs15524 gene polymorphism plays an important role in this context. [7] Very little literature is available regarding this selected gene polymorphism and its impact on clinical outcomes in response to CBZ therapy in patients with epilepsy. The current study offers crucial information that can facilitate further investigation in this uncharted field. Researchers have addressed the CYP3A5-rs15524 polymorphism in association with analgesics, particularly opioids, as well as other drugs, for example, amlodipine[10], tacrolimus[19], sirolimus, and midazolam. [21]

The CYP3A5-rs15524 polymorphism affects tacrolimus levels in Chinese patients with myasthenia gravis, influencing dosage adjustments and treatment efficacy. Meng et al. reported a mutant allele association with increased tacrolimus levels as well as better myasthenia gravis control among the Chinese population. [19] However, this report does not align with our findings, where the mutant allele was observed to be associated with lower drug levels and poorer clinical responses. Wang et al. found a significant association between the concentration-to-dose ratio (CDR) and plasma levels with CYP3A5-rs15524 genotypes in patients receiving CBZ polytherapy but not in those on CBZ monotherapy. [7] On the contrary, we detected a correlation between CYP3A5-rs15524 genotypes and CBZ levels in monotherapy individuals.

Finasteride (5 α reductase inhibitor) is majorly metabolized in the liver by the CYP3A family, includ-

ing CYP3A5. Chau et al. reported higher levels of finasteride among homozygous mutant allele carriers of CYP3A5-rs15524, a finding not in line with our results. [22] We found higher levels of CBZ among wild-type carriers. AL-Eitan et al. reported the distribution of CYP3A5-rs15524 genotypes among Jordanian population, where AA genotype carriers had 109 (43%) good responders and 145 (57%) poor responders, GA had 14 (37.8%) good responders and 23 (62.1%) poor responders, while GG had 1 (25%) good responder & 3 (75%) poor responders. This was in line with our results with the increased frequency of responders among AA carriers in the first follow-up. However, the data of non-responders in our study contrasted with their findings. [23] These differences highlight potential population-specific variations in the genotype-response relationship, underscoring the need for further investigation into genetic factors influencing drug response in different populations.

Genotype frequencies for generalized epilepsy and partial epilepsy in our cohort differ significantly from AL-Eitan et al. They reported genotype frequencies of AA, AG, and GG at 84.9%, 14.5%, and 0.6% for generalized epilepsy, while our investigation revealed diminished AA frequencies and higher AG and GG frequencies at 65.2%, 29.3%, and 5.4%, respectively. For partial epilepsy, they reported 87.9%, 9.7%, and 2.4%, compared to our 69.2%, 28.2%, and 2.5%.[23] Our ethnic group might be carrying the mutant allele G more frequently than others, presenting population-specific gene diversity.

CONCLUSION

The genetic background of epilepsies is heterogeneous, and there is a limited association established to date. This study suggests that the CYP3A5-rs15524 polymorphism may influence clinical response to carbamazepine therapy in epilepsy patients. In our cohort, individuals with the variant allele G had lower plasma levels and a higher proportion of non-responders. Therefore, genotyping could serve as a reliable biomarker for predicting treatment response. These findings highlight the significance of personalized medicine in enhancing treatment outcomes and patient care.

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Authors Contribution:

Following authors have made substantial contributions to the manuscript as under

Authors	Conceived & designed the analysis	Collected the data	Contributed data or analysis tools	Performed the analysis	Wrote the paper	Other contribution
Jamil A	✓	×	✓	×	✓	×
Ali N	✓	✓	×	✓	✓	×
Adil MI	×	✓	×	×	✓	×
Ullah S	✓	✓	✓	×	✓	✓
Faisal MS	×	✓	×	×	✓	×
Awan B	✓	✓	×	✓	✓	×
Khan SA	×	✓	×	×	✓	×

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Ethical Approval:

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