Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v31i1.4034

DECODING THE GENOME: *CYP3A5* rs15524 POLYMORPHISM AND HOW IT AFFECTS PLASMA CARBAMAZEPINE LEVELS IN PATIENTS WITH EPILEPSY IN KHYBER PAKHTUNKHWA

Ayesha Jamil[1], Niaz Ali[2]*, Muhammad Saleh Faisal[3]*, Gulmakay Zaman[4], Muhammad Sami Khan[5]

[1,2]Department of Pharmacology, Institute of Basic Medical Sciences, Khyber Medical University, Peshawar-Pakistan
[1]Department of Pharmacology, Khyber Girls Medical College, Peshawar-Pakistan
[3]Department of Pharmacology, Khyber Medical College, Peshawar-Pakistan
[4]Kabir Medical College and Naseer Teaching Hospital, Peshawar-Pakistan
[5]Peshawar Medical College, Peshawar-Pakistan

***Corresponding Author:** Niaz Ali
*Professor, Department of Pharmacology, Khyber Medical University, Peshawar-Pakistan
dr.niazali@kmu.edu.pk
***Corresponding Author:** Muhammad Saleh Faisal
*Assistant Professor, Department of Pharmacology, Khyber Medical College, Peshawar-Pakistan
drsalehfaisal@gmail.com

ABSTRACT

Background: Epilepsy is an unusually prevalent and intricated neurological condition afflicting millions of individuals. Carbamazepine (CBZ), one of the important anti-epileptic drugs is metabolized by several enzymes, including CYP3A5, to produce its active metabolite, carbamazepine 10–11 epoxide. Variability between patients in the pharmacokinetics of CBZ is indicative of metabolic differences. The prevalence of the single nucleotide polymorphism *CYP3A5* (rs15524) and the study of the pharmacokinetic parameters of CBZ in the context of the gene polymorphism among the Pashtun ethnic group of Khyber Pakhtunkhwa remains vacant. Nevertheless, this study examined the impact of genotypes of rs15524 on the CBZ pharmacokinetic variables.

Methods: A total of 223 patients were genotyped for *CYP3A5* (rs15524) employing Sanger sequencing. Finch TV was used for the identification of gene polymorphisms, and HPLC explored their impact on plasma levels of CBZ.

Results: In *CYP3A5* (rs15524), the prevalence of wild genotype AA_{ref} was 65.9%, heterozygous AG genotype was 29.1%, and homozygous mutant GG was 4.9%. The values observed were in line with the Hardy-Weinberg equation. Generalized tonic-clonic (GTC) seizure was the most prevalent type. Maximum GTC was recorded among AA_{ref} carriers in an age range of 21-30 years. Maximum doses of CBZ were utilized by heterozygous AG carriers across both follow-ups with an uplift of 175.38 ± 264.36 mg/day in the 2nd follow-up. The association between *CYP3A5* (rs15524) and doses utilized, was statistically significant (p-value-<0.001). Carriers of AA_{ref} genotype indicated the highest plasma

levels of CBZ and concentration dose ratio (CDR) across both follow-ups. There was an uplift of 0.74 ± 2.49 mg/L and a drop of 0.50 ± 1.70 mg/L per mg/kg/day of plasma levels and CDR, respectively among AA_{ref} genotype in the 2nd follow-up.

Conclusion: Genotypes of the selected gene revealed a statistically significant association with CBZ pharmacokinetics.

Keywords: Single Nucleotide Polymorphism, CYP3A5, Epilepsy, Carbamazepine, Sanger sequencing

INTRODUCTION

Epilepsy, a highly prevalent and complex neurological disorder that impacts millions of individuals globally, has a genetic component that defies the conventional understanding of its origin. Genomic breakthroughs have shifted our perspective, suggesting epilepsy to be a varied symptom in contrast to a distinct condition (1). Initially relying on family research and subsequently benefiting from the progress in gene sequencing techniques, computational methods, and the formation of extensive collaborative projects, it has become evident that the influence of genetics in epilepsy is significantly more substantial than previously acknowledged (2).

One of the medications that is most frequently used to treat partial and generalized tonic-clonic seizures is carbamazepine (CBZ)(3,4). Specific shifts in CBZ levels of plasma have been observed in epileptic patients. At present, anti-seizure drugs operate as the primary means to alleviate seizures caused by epilepsy. Variations in gene expression emerge to be a key component in the patients' diverse responses to anti-seizure drugs such as carbamazepine (5). CYP3A4, CYP3A5, and CYP2C8 enzymes primarily metabolize CBZ (6). It is also partially metabolized to 2-hydroxy-CBZ and 3-hydroxy-CBZ by CYP3A4 and CYP2B6, with CBZ-10, 11-epoxide being the pharmacologically active metabolite (7,8). All these enzymes are under the control of encoding genes. Variations in encoding genes influence how epileptic patients respond to medications. Absorption, transport, metabolism, and elimination shape the potency and outcome of a drug. Variations in genetics can impact an individual's response to a drug at all these phases. Concisely, the effects of drugs are significantly influenced by the body's reaction to them, and genetic variations can affect individual responses. A Clear understanding of these differences is of the utmost importance for the treatment of epilepsy.

Research in pharmacogenetics enhances personalized medication approaches (8,9). Several factors, like gene polymorphisms, race, ethnicity, gender, multiple medical conditions, and drug interactions, are associated with inter-patient variability (10). The sequences of genes that encode for CYP3A5 enzymes are extensively polymorphic (11). Research has shown that when administered as monotherapy, *CYP3A4/5* SNPs significantly correlate with plasma levels of CBZ (3). *CYP3A5* resides on 7q22.1 of chromosome 7 and codes 502 amino acids. The rs15524 is a common variant of the *CYP3A5* gene. It is positioned at 3'UTR (12). As per genetic consortiums, several SNPs have been designated 3rd level of evidence in the context of the CBZ metabolism. Level 3 evidence suggests that the impact of genotypes on health outcomes has been understudied and more research on varied ethnic groups is required. If repeated, such experiments may yield fresh insights. Understanding CBZ plasma levels and SNPs will improve healthcare and clinicians' ability to customize treatment. This will also enable the prediction of real-time drug resistance. *CYP3A5* (rs15524) being one of them led to the selection of this SNP.

Moreover, the polymorphism of this gene has never been researched in patients with epilepsy in the Khyber Pakhtunkhwa region of Pakistan. Carbamazepine is commonly used in Pakistan, despite superior anti-epileptics. It is affordable for most people with epilepsy with tight financial resources. The present study analyzes the prevalence of *CYP3A5* (rs15524) genotypes along with its impact on plasma levels, concentration-to-dose ratio (CDR), and doses of CBZ utilized at 3 months and 6 months follow-up intervals in patients with epilepsy in Khyber Pakhtunkhwa.

MATERIAL AND METHODS

Sample selection

This longitudinal study following Helsinki's statement, was conducted after approval from the Ethics board of Khyber Medical University (Dir./KMU-EB/PS/000807). Comprising of two follow-ups, the research continued from October 2020 to April 2022 in Khyber Pakhtunkhwa. The sample size was calculated to 180 through Web-based OpenEpi, with a 7.5% margin of error, and a confidence interval of 95%. In consideration of follow-up withdrawals and preserving the validity of the study's results, the initial number of participants was set at 223. Patients were enrolled from the neurology department of Lady Reading Hospital. Newly diagnosed patients with epilepsy who were prescribed carbamazepine monotherapy were selected. Those who were not willing to participate, with co-morbidities, compromised renal or hepatic profiles, pregnant or consuming CBZ-interacting drugs were excluded. A standardized proforma approved by the University's Advanced Studies and Research Board was employed for the documentation of patients' demographics, disease and extensive laboratory tests.

Drug Therapy

Patients were prescribed Carbamazepine monotherapy (Baseline Pharmacopoeia standard tests were passed for tablet dosage versions of manufacturer of a multinational pharmaceutical company). As patient doses varied, the steady-state concentration was regulated by dose and body weight. Doses ranging from 10 to 20 mg/kg were prescribed to the patients in accordance with the severity of the disease and maintenance of required plasma concentrations. In the first follow-up (3^{rd} month) as per the severity of the disease status of each patient, either the dose of CBZ was escalated or the patients were prescribed along with CBZ another anti-epileptic medication.

Blood Sampling

At the baseline visit, samples were collected and preserved at 8°C for DNA extraction. Research conducted by Ullah et. al demonstrated a shift in CBZ levels till 10-12 weeks (10,13). Keeping in view the time of fluctuations, samples were collected in case of both follow-ups at an interval of 3 months. CBZ requires 5.9 hours ± 1.8 hours to achieve its highest plasma concentration (C-max), therapeutic drug monitoring was executed at the time required to achieve maximum plasma concentration (T-max).

DNA Extraction and Genotyping

The salting-out approach was employed for extracting DNA. Genomic DNA was preserved at -20°C following the addition of elution buffer. The purity and concentration of isolated DNA were confirmed using a Nanodrop spectrophotometer. A Gradient Thermo-cycler was used to amplify the *CYP3A5* gene. As indicated in Table 1, primers were constructed with the assistance of the UCSC genome database. The PCR results were performed on a 2% Agarose gel (14).

Table 1. Primers of *CYP3A5* (rs15524)

Primer	Sequence
Forward	ACGAGTCCACAAGAATTTGTCT
Reverse	TCTGGGGACAGCTTTCTTG

The targeted gene was Sanger sequenced on Seq Studio TM. The manufacturer's instructions were followed for sequencing the amplified products. With the assistance of BigDye X-terminator TM kit, the sequenced products were optimized. After establishing a medium run-on electrophoresis and loading the samples, analysis was concluded. Analysis of genotype distribution was conducted in the context of global population and validated by the Hardy-Weinberg equilibrium test using an online calculator (15). The sequences were examined for the presence of single nucleotide polymorphisms using Finch TV, as illustrated in Figure 1.

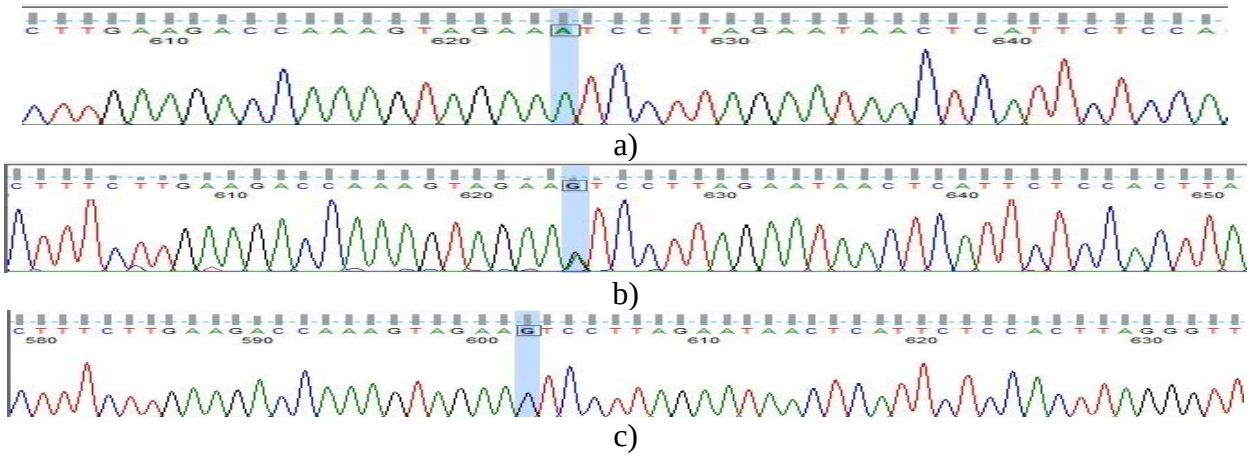

Figure 1 a). Wild Type (AA) **b).** Heterozygous Mutant (AG) **c).** Homozygous Mutant (GG)

Therapeutic monitoring of CBZ plasma levels

A few modifications as per protocols of Ullah et al. were made to the HPLC LC-20AT equipped with the UV detector SPD-20A/20AV (Shimadzu Kyoto, Japan). Plasma concentrations of CBZ were measured via reversed-phase high-performance liquid chromatography (13). Plasma was mixed with acetonitrile (3:1) for deproteinization. After centrifugation, the organic layer was discarded and remnant was dried. The dry extract was reconstituted with a mobile phase comprised of demineralized water, methanol, and glacial acetic acid in a ratio of 65:34:1. (v/v/v) set at a pH of 5.6. The sample was injected at 0.8 ml/min flow rate, using a C18 column, with a UV range of λ_{max} 220 nm to recognize CBZ. Diclofenac sodium was employed as an internal standard. The process was validated by measuring metrics like coefficient of variance (CV) and % recovery on an intra-day and inter-day basis (16).

Statistical Analysis

Numerical data were characterized using means and standard deviations, while categorical data were presented through frequencies and percentages. To identify associations between genotypes and CBZ levels, statistical methods such as One-way ANOVA, followed by Bonferroni post hoc analysis, were utilized. Statistical significance was indicated by a p-value below 0.05. Data analysis was conducted using SPSS version 22, and Microsoft Excel was employed for graph generation.

RESULTS

Population Demographics

Table 2 reveals the demographic information of the study population. There was a considerable predominance of males as compared to females, comprising 63.2% and 36.8%, respectively. Two types of epilepsies were encountered. GTC, being more prevalent, accounted for 82.5% of the cases and the remainder of cases accounted for partial seizures. Positive family history was reported only among 27.8% of the cases. Moreover, only 3.1% of the patients had affected parents while 24.6% had siblings with epilepsy.

Table 2. Demographics of the study population

Gender	Type of seizure	Family History	Effected parents	Effected siblings
Female	Partial	Positive	Yes	Yes
82	39	62	7	55
Male	GTC	Negative	No	No
141	184	161	216	168

Distribution of Genotypes

Figure 2 illustrates the genotypic data acquired, revealing that 65.9% of individuals with epilepsy carried the wild genotype (AA_{ref}). Heterozygous (AG) was reported among 29.1% of individuals whereas 4.9% carried homozygous mutant (GG) genotype.

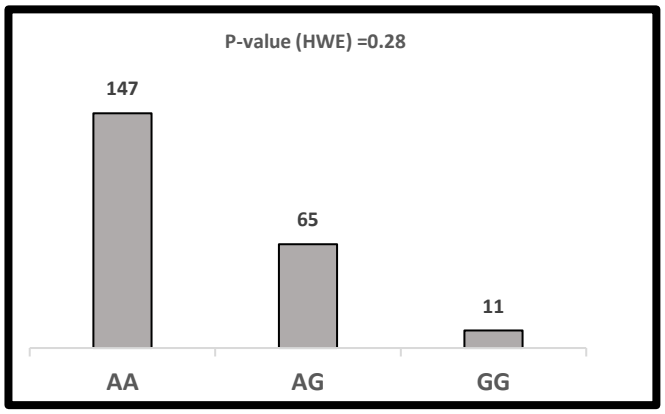

Figure 2. Frequency of Genotypes of *CYP3A5*

Distribution of seizure types across different age categories and genders in perspective of *CYP3A5* genotypes

Table 3 indicates maximum cases of GTC seizures among AA_{ref} carriers between an age range from 21-30 years. Similarly, partial seizures were most prevalent across the age range from 21-30 years among AA_{ref} carriers. GTC seizure frequency was maximum (17%) among males with AA_{ref} genotype, whereas the frequency of partial seizures was maximum (21%) among female carriers of the AA_{ref} genotype. But statistically, there were no significant associations revealed between *CYP3A5* genotypes and age, gender, and seizure types as suggested by p-values greater than 0.05.

Table 3. Association of Seizure types with Age & Gender among different genotypes

		Type of Epilepsy					
		GTC			**Partial Seizures**		
		AA	AG	GG	AA	AG	GG
Age (Years)	Less than 10	4	0	1	0	0	0
	10-20	23	18	3	5	4	0
	21-30	38	16	3	12	3	1
	31-40	28	14	1	2	2	0
	41-50	13	3	1	4	1	0
	51-60	6	2	0	2	0	0
	61-70	6	0	1	0	0	0
	More than 70	2	1	0	2	1	0
Gender	Female	37	25	5	10	4	1
	Male	83	29	5	17	7	0

*≤ 0.05 is significant

P-value: Age vs. genotype = 0.18

P-value: Seizure type vs. genotype = 0.73

P-value: Gender vs. genotype = 0.09

Association between *CYP3A5* gene Polymorphism and CBZ dose requirements

Analysis of data in Table 4 showed that individuals carrying the heterozygous variant (AG) required the highest doses of CBZ across both follow-ups. In contrast, carriers of the homozygous mutant (GG) genotype necessitated relatively lower doses. The association of *CYP3A5* genotypes with CBZ dose requirements was statistically significant across both follow-ups with p-values <0.001. Although both the follow-ups exhibited the same pattern, the mean levels in the second follow-up were significantly higher. Carriers of AG genotype utilized a 28.7% higher dose in the second follow-up.

Table 4. CBZ dose requirements among different *CYP3A5* genotypes

	Genotype	[a] Mean ± SD	*P-value	95% CI
1st Follow-up	AA_{ref}	299.32 ± 131.6	< 0.001	277.86 to 320.78
	AG	433.85 ± 178.7	< 0.001	389.55 to 478.15
	GG	327.27 ± 100.9	< 0.001	259.48 to 395.06
2nd Follow-up	AA_{ref}	452.38 ± 191	< 0.001	421.2 to 483.5
	AG	609.23 ± 195	< 0.001	560.9 to 657.5
	GG	563.64 ± 215	< 0.001	418.7 to 708.5

***** Chi-Square test
[a] CBZ Dose in mg/day

Association of *CYP3A5* gene Polymorphism with CBZ plasma levels and its CDR

As depicted in Table 5, the highest plasma levels of CBZ and CDR values were among carriers of AA_{ref} genotype whereas lower levels were among heterozygous (AG) carriers. Although the highest plasma levels were among AA_{ref} carriers in both the follow ups, there was an uplift of 0.74 ± 2.49 mg/L among them in the 2nd follow-up. The p-values generated by ANOVA indicated a statistically significant association of *CYP3A5* genotypes with CBZ plasma levels and CDR. The overall findings of this study endorse the hypothesis that drug levels are influenced by the selected mutation.

Table 5. Plasma levels and CDR of CBZ among different *CYP3A5* genotypes

Variable	Genotype	1st Follow-up			2nd Follow-up		
		Mean ± SD	95% CI	*P-value	Mean ± SD	95% CI	*P-value
Plasma levels (mg/L)	AA_{ref}	5.6 ± 1.7	5.39 to 5.96		6.34 ± 1.82	6.04 to 6.6	
	AG	3.8 ± 1.27	3.57 to 4.20	< 0.001	4.58 ± 1.4	4.23 to 4.9	< 0.001
	GG	4.87 ± 1.7	3.67 to 6.07		5.56 ± 1.9	4.23 to 6.8	
CDR (mg/L per mg/kg/day)	AA_{ref}	2.32 ± 1.21	2.12 to 2.52		1.82 ± 1.2	1.62 to 2.02	
	AG	1.08 ± 0.70	0.90 to 1.25	< 0.001	0.88 ± 0.61	0.73 to1.03	< 0.001
	GG	1.73 ± 1.13	0.97 to 2.49		1.28 ± 1.19	0.47 to 2.08	

*****ANOVA followed by Bonferroni Post hoc analysis

DISCUSSION

Epilepsy is characterized by recurrent and irregular seizures that arise from aberrant electrical activity in the brain. These seizures lead to motor neuropathy. The clinical signs associated with these seizures may vary from mild sensory disruptions to convulsive disorders (3,17). Epilepsy is a significant worldwide health issue due to its adaptability to individuals of all ages, nationalities, and geographic regions (18). A wide range of thorough studies have been conducted to explore the prevalence of epilepsy in diverse contexts. Consistent results have been witnessed, suggesting a rate ranging from 4 to 10 per one thousand individuals. In Pakistan, an equivalent prevalence of epilepsy has been recorded (19,20). Based on the analysis of data about demographic information, our study identified 30.2% more males with epilepsy than females. Ullah et al., L. Forsgren et al., and S. Al Rajeh et al. reported findings that were comparable to this observation (21–23). On the contrary, Mohammadi et al. found that females presented a greater prevalence of epilepsy than males (24). A positive family history was a crucial factor in our demographic analysis of epilepsy patients. A small fraction of epilepsy patients had a positive family history. Our results contradicted the results of Asadi-Pooya et al. who reported a positive association between a family history and epilepsy risk (25).

The main goal of this study was to explore how the genetic differences in the *CYP3A5* (rs15524) gene affect people with epilepsy taking CBZ as a monotherapy for the control of epilepsy. This gene is of immense importance since the CYP3A5 enzyme engages in the main metabolic pathway and

pharmacokinetics of CBZ. It also contributes to the development of drug-resistant epilepsy. The *CYP3A5* (rs15524) polymorphism has been previously explored in the context of analgesics like opioids (26) and other pharmaceuticals such as amlodipine (27), tacrolimus, and sirolimus (28). We calculated the prevalence of the mutant allele rs15524 to be 19.5%. Addressing *CYP3A5* rs15524, the prevalence was 25.4% in other South Asian nations and 26.1% in East Asians, according to available data https://www.ncbi.nlm.nih.gov/snp/rs15524. P. Wang et al. established a noteworthy correlation between CDR, plasma levels, and *CYP3A5* rs15524 genotypes in the CBZ-polytherapy group, but not in the CBZ monotherapy group. Our results were inconsistent with P. Wang et al. findings (3). The literature lacks an in-depth review of the associations between CBZ pharmacokinetics and pharmacogenomics, which emphasizes the vitality of the current study.

CONCLUSION

The study revealed that *CYP3A5* (rs15524) could potentially influence the CBZ pharmacokinetics among patients with epilepsy. The carriers of the AA_{ref} genotype exhibited the highest plasma concentration and the lowest dose requirement of CBZ. Heterozygous genotype carriers showed the greatest dose utilization of CBZ. However, further research with larger patient cohorts is essential to validate findings and gain a more thorough understanding of the clinical implications of CBZ pharmacogenomics.

Acknowledgment

The authors are pleased to express gratitude to Khyber Medical University for furnishing the essential infrastructure to conduct this research.

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. Thijs RD, Surges R, O'Brien TJ, Sander JW. Epilepsy in adults. Lancet [Internet]. 2019;393(10172). Available from: http://dx.doi.org/10.1016/S0140-6736(18)32596-0
2. Perucca P, Bahlo M, Berkovic SF. The Genetics of Epilepsy. Annu Rev Genomics Hum Genet. 2020;21:205–30.
3. Wang P, Yin T, Ma HY, Liu DQ, Sheng YH, Wang C, et al. Effects of CYP3A4/5 and ABCB1 genetic polymorphisms on carbamazepine metabolism and transport in Chinese patients with epilepsy treated with carbamazepine in monotherapy and bitherapy. Epilepsy Res [Internet]. 2015;117:52–7. Available from: http://dx.doi.org/10.1016/j.eplepsyres.2015.09.001
4. Brodie J, Dichter A. antiepileptic. 1997;
5. Puranik YG, Birnbaum AK, Marino SE, Ahmed G, Cloyd JC, Remmel RP, et al. Association of carbamazepine major metabolism and transport pathway gene polymorphisms and pharmacokinetics in patients with epilepsy. Pharmacogenomics. 2013;14(1):35–45.
6. Lu Q, Huang YT, Shu Y, Xu P, Xiang DX, Qu Q, et al. Effects of CYP3A5 and UGT2B7 variants on steady-state carbamazepine concentrations in Chinese epileptic patients. Med (United States). 2018;97(30):1–9.
7. Kerr BM, Thummel KE, Wurden CJ, Klein SM, Kroetz DL, Gonzalez FJ, et al. Human liver carbamazepine metabolism. Role of CYP3A4 and CYP2C8 in 10,11-epoxide formation. Biochem Pharmacol. 1994;47(11):1969–79.
8. Zhao GX, Zhang Z, Cai WK, Shen ML, Wang P, He GH. Associations between CYP3A4, CYP3A5 and SCN1A polymorphisms and carbamazepine metabolism in epilepsy: A meta-analysis. Epilepsy Res [Internet]. 2021;173(March):106615. Available from: https://doi.org/10.1016/j.eplepsyres.2021.106615
9. Ferraro TN, Buono RJ. The relationship between the pharmacology of antiepileptic drugs and human gene variation: An overview. Epilepsy Behav [Internet]. 2005 Aug [cited 2017 Sep

23];7(1):18–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15979945
10. Shakir S, Ali N, Nazish H. Carbamazepine verses valproic acid as monotherapy in epileptic patients. J Coll Physicians Surg Pakistan. 2018;28(5):357–60.
11. Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, et al. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet [Internet]. 2001;27(4):383–91. Available from: https://doi.org/10.1038/86882
12. Bains RK, Kovacevic M, Plaster CA, Tarekegn A, Bekele E, Bradman NN, et al. Molecular diversity and population structure at the Cytochrome P450 3A5 gene in Africa. BMC Genet. 2013;14.
13. Ullah S, Ali N, Khan A, Ali S, Nazish HR, Uddin Z. Epilepsy control with carbamazepine monotherapy from a genetic perspective. BMC Pharmacol Toxicol. 2018;19(73):1–10.
14. Riemann K, Adamzik M, Frauenrath S, Egensperger R, Schmid KW, Brockmeyer NH, et al. Comparison of manual and automated nucleic acid extraction from whole-blood samples. J Clin Lab Anal. 2007;21(4):244–8.
15. Emmert EAB. A Review of Germs Make Me Sick Germs Make Me Sick; BergerMelvin; (1995). Illustrated by HafnerMarylin. Harper Collins, New York, NY. 32 pages. . J Microbiol Biol Educ. 2010;11(2):186–186.
16. GANESH† DG and GL. HPLC Method for Determination of Gliclazide in Human Serum. Asian J Chem. 2009;21(6):4258–64.
17. N'guyen The Tich S, Péréon Y. [Epidemiology of drug-resistant epilepsy]. Rev Neurol (Paris). 2004 Jun;160 Spec N:5S31-5.
18. Thurman DJ, Beghi E, Begley CE, Berg AT, Buchhalter JR, Ding D, et al. Standards for epidemiologic studies and surveillance of epilepsy. Epilepsia. 2011;52(SUPPL. 7):2–26.
19. Sander JW. The epidemiology of epilepsy revisited. [Curr Opin Neurol. 2003] - PubMed result. Curr Opin Neurol. 2003;16(2):165–70. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12644744.
20. Khatri IA, Iannaccone ST, Ilyas MS, Abdullah M, Saleem S. Epidemiology of Epilepsy in Pakistan: Review of literature. Vol 53, Journal of Pakistan Medical Association. 2003. p. 594–7.
21. Ullah S, Ali N, Khan A, Ali S, Nazish HR. The epidemiological characteristics of epilepsy in the province of Khyber Pakhtunkhwa, Pakistan. Front Neurol. 2018;9(NOV):6–11.
22. Al Rajeh S, Awada A, Bademosi O, Ogunniyi A. The prevalence of epilepsy and other seizure disorders in an Arab population: A community-based study. Seizure. 2001;10(6):410–4.
23. Forsgren L, Beghi E, Õun A, Sillanpää M. The epidemiology of epilepsy in Europe - A systematic review. Eur J Neurol. 2005;12(4):245–53.
24. Mohammadi MR, Ghanizadeh A, Davidian H, Mohammadi M, Norouzian M. Prevalence of epilepsy and comorbidity of psychiatric disorders in Iran. Seizure. 2006;15(7):476–82.
25. Asadi-Pooya AA, Hojabri K. Risk factors for childhood epilepsy: a case–control study. Epilepsy Behav [Internet]. 2005;6(2):203–6. Available from: https://www.sciencedirect.com/science/article/pii/S1525505004003610
26. Freiermuth CE, Kisor DF, Lambert J, Braun R, Frey JA, Bachmann DJ, et al. Genetic Variants Associated With Opioid Use Disorder. Clin Pharmacol Ther. 2023;113(5):1089–95.
27. Liang H, Zhang X, Ma Z, Sun Y, Shu C, Zhu Y, et al. Association of CYP3A5 Gene Polymorphisms and Amlodipine-Induced Peripheral Edema in Chinese Han Patients with Essential Hypertension. Pharmgenomics Pers Med [Internet]. 2021 Dec 31;14:189–97. Available from: https://www.tandfonline.com/doi/abs/10.2147/PGPM.S291277
28. Tamashiro EY, Felipe CR, Genvigir FDV, Rodrigues AC, Campos AB, Hirata RDC, et al. Influence of CYP3A4 and CYP3A5 polymorphisms on tacrolimus and sirolimus exposure in stable kidney transplant recipients. Drug Metab Pers Ther. 2017;32(2):89–95.