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THE ROLE OF CYP3A4*1B POLYMORPHISM IN THE DEVELOPMENT OF PHARMACORESISTANTEPILEPSY IN UZBEK POPULATION

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ABSTRACT--*Depending on the assessment method, data on the prevalence of epilepsy may vary, however, this neurological disorder is considered one of the most common. [1]. With appropriate treatment with antiepileptic drugs, about 60-70% of patients can be relieved of seizures, however, despite the progress made in the treatment of epilepsy and the emergence of the latest antiepileptic drugs, from 30 to 40% of patients are pharmacoresistant[2,3,4]. Pharmacoresistant epilepsy is defined as the inability to achieve a stable remission of seizures when using two well-tolerated and correctly selected antiepileptic drugs (in mono- or polytherapy mode) [5]. In addition to relapsing seizures, patients with drug-resistant epilepsy often experience psychosocial problems, an increased risk of injury, and a risk of sudden unexpected death (SUDEP) [4,6].*

KEYWORDS-- *cyp3a4*1b polymorphism, pharmacoresist, antepilepsy, population.*

I. INTRODUCTION

The pharmacoresistant course of epilepsy often causes, simultaneously with the selection of therapy, to search for the cause of resistance. According to some reports, 20-30% of patients with refractory seizures have an incorrect diagnosis [7]. Treatment may be ineffective due to the choosing of an ineffective antiepileptic drug due to an incorrectly classified type of seizure or form of epilepsy. However, neither clinical nor morphological factors can adequately explain the existence of pharmacoresistant forms of this neurological disorder.One of the explanations of DREdevelopment is involvement of P450 (CYP450), including proteins CYP2C9, CYP2C19, CYP2D6, CYP3A4, CYP3A5, CYP1B1, CYP2E1, etc.[5,8, 9, 10].

A significant role in the metabolism of antiepileptic drugs, including carbamazepine, oxcarbazepine, clonazepam, diazepam, phenobarbital, phenytoin, tiagabine, zonisamide, and others plays the enzyme CYP3A4, for the activity of which a clear personalized variability was shown (idiosyncrasy [11,8,12,13]. The CYP3A4 enzyme is encoded by the CYP3A4 gene, which is part of the P450 - CYP3 cytochrome gene cluster, located at the 7q21.1 chromosomal locus [14]. Many allelic variants of polymorphism are known

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(https://www.pharmvar.org/htdocs/archive/cyp3a4.htm), however, the frequency distribution of these variants is quite low, and therefore their functional significance is poorly understood [15].

The wild-type allele, CYP3A4 * 1, also known as CYP3A4 * A or CYP3A4 * W, characteristic of the general population, encodes an enzyme with normal enzymatic activity. The most common polymorphism CYP3A4 is CYP3A4 * 1B (rs2740574), which is characterized by A> G in the 5' promoter region of the gene. Clinical studies have shown that CYP3A4 * 1B polymorphism may affect the expression of CYP3A4 or its enzymatic activity. [16]. The frequency of this allele varies by population, ranging from 0.0% among Chinese to 54.6% among African Americans [17, 18, 19].

Data on the effect of this CYP3A4 polymorphism in the development of pharmacoresistant form of epilepsy are quite contradictory [20]. Studies by some authors show a significant role of the allelic variant of CYP3A4 * 1B as a risk factor for the development of drug resistance to antiepileptic drugs [21], whereas other researchers have not found any association of the polymorphism with DRE [22, 23]. It is noteworthy that researchers who did not confirm the significance of polymorphism carried out associative studies in the Asian population, where the frequency of occurrence of CYP3A4 * 1B is extremely low.

The aim of our study was to assess the relationship of the polymorphism of the CYP3A4 * 1B gene (rs2740574) with the occurrence of drug-resistant epilepsy in patients in Uzbekistan.

II. MATERIALS AND METHODS

As the object of study, we used DNA isolated from the peripheral blood of patients with epilepsy (main group, n = 109) and relatively healthy individuals (control group, n = 97). Patients with a neurological disorder were divided into two independent subgroups: patients with pharmacologically controlled epilepsy (n = 31), and patients with pharmacoresistant epilepsy (n = 78).

Carbamazepine and valproic acid were used to treat the patients.DNA samples were obtained using commercial kits for the isolation of nucleic acids (AmpliPrim RIBO-prep, Moscow) according to the manufacturer's instructions. For genotyping, the polymorphic CYP3A4 * 1B variant (rs2740574) was chosen. Genotyping of CYP3A (−392A> G) was performed using a PCR kit (SNP Express, NPF LITEH, Moscow) with the separation of amplification products in a 2% agarose gel in Tris acetate buffer. Amplification was performed using GeneAmp PCR-system 2720 thermal cyclers (Applied Biosystems, USA).

Distribution of genotypes were checked by Hardy-Weinberg equilibrium ("Genetics of Population", http://wbiomed.curtin.edu.au/genepop). As a calculation tool, the OpenEpi 2009, Version 2.3 application package was used.

III. RESULTS

Our study of the distribution of allelic and genotypic variants of the rs2740574 (−392A> G) polymorphism of the CYP3A4 gene in the population control group showed that the mutant G allele met with a frequency of 1.6%, while the wild allele frequency was 98.4%; genotype A / A was found with a frequency of 96.9%, genotype A / G

with a frequency of 3.1%, while the genotype G / G was not detected in the sample of conditionally healthy individuals we studied (Table 1).

A comparative assessment of the frequency of allelic variants in the control group and in the group of patients with epilepsy (main group) did not reveal a significant difference in wild (A: 98.4 % and 97.2 %; χ2=0,6986; P=0.4033; RR=0.9878; 95% CI 0.3923-2.487; OR=0,5550; 95% CI 0.1122-2.2635), and the mutant one (G: 1.6 % and 2.7 %; χ2=0.6986; P=0.4033; RR=1.7798; 95% CI 0.7069-4.4811; OR=1.8019; 95% CI 0.4418-8.9137). The absence of a statistically significant difference in the frequency of alleles of polymorphism (−392A> G) of the CYP3A4 gene was also shown by us when comparing with the control a group of patients with epilepsy controlled by taking pharmacological drugs (A: 9.84 % and 96.8 %; χ 2=0.6920; P=0.4055; RR=0.9829; 95% CI 0.1147-8.4196; OR=0.4712; 95% CI 0.0688-4.0544; G: 1.6 % and 6.4 %; χ 2=0.6920; P=0.4055; RR=2.0860; 95% CI 0.2435-17.8689; OR=2.1222; 95% CI 0.2466-145282) and the groups of patients with DRE (A: 98.4 % and 97.4 %; χ2=0.4569; P=0.4991; RR=0.9897; 95% CI 0.2756-3.5545; OR=0.5969; 95% CI 0.110-2.9340; G: 1.6 % and 2.6 %; χ2=0.4569; P=0.4991; RR=1.6581; 95% CI 0.4617-5.9551; OR=1.6754; 95% CI 0.3408-9.0873). Comparison of allele frequency between the samples of patients with various forms of epilepsy (drug-responding and drug-resistant) also did not reveal any reliable difference (A: 96.8 % and 97.4 %; χ 2=0.0726; P=0.7876; RR=1.0068; 95% CI 0.3281-3.0892; OR=1,2667; 95%CI 0,1585-7,3173; G: 6.4 % and 2,6 %; χ 2=0,0726; P=0,7876; RR=0.7949; 95% CI 0.2591-2.4390; OR=0.7895; 95% CI 0.1367-6.3076) (Table 1).

An analysis of the distribution of the genotypic variants of polymorphism (−392A> G) of the CYP3A4 gene showed that the A / A genotype was predominant in all groups. In the control sample, its frequency was 96.9%. The A / G genotype was found in 3.5% of the subjects from the control group, while the G / G genotype was not detected by us in any of the examined healthy individuals.

A comparative assessment of the frequency of genotypes in the control group and in the main group of patients with epilepsy did not reveal a significant difference in genotype A / A (96.9 % and 94.5 %; χ^2=0.0598; P=0.8068; RR=0.9751; 95%CI0.6818-1.3946; OR=0.9528; 95%CI 0.6463-1.4050), genotype A/G (3.1 % and 5.5 %; χ^2=0.6986; P=0.4033; RR=1.7798; 95%CI0.7069-4.4811; OR=1.8019; 95%CI 0.4418-8.9137).Study of the frequency of genotypic variants of polymorphism (−392A> G) of the CYP3A4 gene in groups of relatively healthy individuals and patients with epilepsy controlled by pharmacological preparations (A/A: 96.9 % and 93.5 %; χ^2=0.0531; P=0.8178; RR=0.9653; 95%CI0.4120-2.2617; OR=0.9349; 95%CI 0.5243-1.6628; A/G: 3.1 % and 6.4 %; χ^2=0.6920; P=0.4055; RR=2.0860; 95%CI0.2435-17.8689; OR=2.1222; 95%CI 0.2466-14.5282), as well as in the groups of controls and DRE patients (A/A: 96.9 % and 94.9 %; χ^2=0.0359; P=0.8498; RR=0.9790; 95%CI0.6188-1.5488; OR=0.960; 95%CI 0.6287-1.4658; A/G: 3.1 % and 5.1 %; χ^2=0.4569; P=04991; RR=1.6581; 95%CI0.4617-5.9551; OR=16754; 95%CI 0.3408-9.0873) did not found out any statisticallysignificantdifference.A comparative assessment of the frequency of alleles between groups of patients with pharmacologically controlled and pharmacoresistant epilepsy, in turn, did not reveal a significant difference in the indicators (A/A: 93.5 % and 94.9 %; χ^2=0.0078; P=0.9297; RR=1.0141; 95%CI0.7303-1.4081; OR=1.0269; 95%CI 0.5677-1.8620; A/G: 6.4 % and 5.1 %; χ^2=0.0726; P=0.7876; RR=07949; 95%CI 0.2591-2.4390; OR=0.7895; 95%CI 0.1367-6.3076) (Table 1).

Table 1: The distribution frequency of alleles and genotypes of rs2740574 polymorphism (−392A> G) of the CYP3A4 gene in groups of patients with epilepsy and control

Group	Allele frequency				Genotype frequency					
	A		G		A/A		A/G		G/G	
	n	%	n	%	n	%	n	%	n	%
Main group (all patients with epilepsy), n=109	212	97.2	6	2.7	103	94.5	6	5.5	0	0.0
Patients with drug-resistant epilepsy, n=78	152	97.4	4	2.6	74	94.9	4	5.1	0	0
Patients with controlled epilepsy , n=31	60	96.8	2	6.4	29	93.5	2	6.4	0	0.0
Control group, n=97	191	98.4	3	1.6	94	96.9	3	3.1	0	0.0

Our assessment of the correspondence between the expected and observed frequencies of the genotypes A / A, A / G, and G / G CYP3A4 polymorphism (−392A> G) showed the absence of a statistically significant deviation both in the population control group and in the main group of patients with epilepsy (Table 2), which indicates the existence of Hardy-Weinberg equilibrium in the population.

Table 2: Observed and expected frequency of the genotypes of rs2740574 polymorphism (−392A> G) of the CYP3A4 gene in the group of patients with epilepsyand in the control group (according toHardy-Weinberg equilibrium)

Group	Genotype	Frequency	Genotype frequency		χ^2	P
		n	Observed	Expected		
Maingroup (n=109)	A/A	103	0.940	0.950	0.000	
	A/G	6	0.055	0.050	0.004	
	G/G	0	0.000	0.000	0.076	
	Σ	109	1.000	1.000	**0.080**	**0.777**
	Σ*2	218				
Control group (n=97)	A/A	94	0.860	0.860	0.000	
	A/G	3	0.031	0.031	0.001	
	G/G	0	0.000	0.000	0.021	
	Σ	97	0.890	0.890	**0.022**	**0.882**

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	Σ*2	194				

An analysis of the heterozygosity of the polymorphism (−392A> G) of the CYP3A4 gene in the sample under study showed that the expected and observed heterozygosity in the population control group were the same. In the main group of patients with epilepsy, the observed heterozygosity was higher than expected, while the F_{is} index was negative (–0.100), which indicates an excess of heterozygotes. However, the difference between H_{obs} and H_{exp} was not statistically significant (Tables 2, 3).

Table 3: The difference between the expected and observed heterozygosity frequencies

Group	Heterosygosis (H)		F	D	F_{is}
	H_{obs}	H_{exp}			
Main	0.055	0.050	0.010	+ 0.100	– 0.100
Controls	0.031	0.031	0	0	0

$F = H_{obs} – H_{exp}$

D – relativedeviationofexpectedheterosygosisfrom the observed one:

$D = (H_{obs} - H_{exp}) / H_{exp}$; D = -Fis

$F_{is} = (H_{exp} - H_{obs}) / H_{exp}$

F_{is} «–/+» – «excess/deficit» of heterozygotes

[TongD., ChenY., 2013; KurdistaniZ.K., SaberiS., 2015]

IV. DISCUSSION

Our study of the distribution frequency of allelic and genotypic variants of the rs2740574 (−392A> G) polymorphism of the CYP3A4 gene showed the prevalence of the CYP3A4 * 1 allele and the wild homozygous A / A genotype in all groups of the sample we studied, both among population control individuals and among patients with epilepsy. The frequency of the functionally unfavorable CYP3A4 * 1B allele was relatively low, varying from 1.6% in the control group to 6.4% in the group of patients with drug-controlled epilepsy. However, the absence of statistically significant significance of intergroup differences in the frequency of the polymorphic allele CYP3A4 * 1B precludes its associative relationship with the etiology of epilepsy in general, and with the development of a pharmacoresistant form of this neurological disorder.

The frequency of the heterozygous genotype A / G (alleles of CYP3A4 * 1 / CYP3A4 * 1B) was also the lowest among control subjects (3.1%) and reached its maximum value in the group of patients with pharmacological controlled epilepsy (6.4%). Moreover, the significance of the difference in the frequency of the heterozygous genotype between the groups did not have statistical significance, which may indicate the absence of an association of this polymorphic genotypic variant with resistance to antiepileptic drugs.

It should be noted that the functionally unfavorable homozygous genotype G / G (alleles of CYP3A4 * 1B / CYP3A4 * 1B) was not found in the sample under study, which did not allow us to evaluate its frequency and

associative relationship with pharmacoresistant epilepsy. Apparently, this fact is associated with the rare occurrence of this genotypic variant polymorphism (−392A> G) of the CYP3A4 gene in the Uzbek population. Since the ethnic population of Uzbekistan is mainly represented by people of Asian origin, this explains our data on the low frequency of allelic and genotypic polymorphic variants rs2740574, which correspond to the data of other researchers [17,18,19].Considering that the decrease in the functional activity of the product of the polymorphic gene CYP3A4 is of great importance for the effectiveness of biotransformation of drugs in the body, it is possible that the presence of two mutant alleles plays a role in the development of a pharmacoresistant form of epilepsy. However, even if such a relationship exists, in our study, as well as in studies of other authors conducted in Asian populations [22,23]failed to revealpolymorphism associations (−392A> G) of gene *CYP3A4*cwith DREdue toits extremely low frequency or absence in the samples of the homozygous mutant genotype. For the same reason, it can be argued that in Asian populations this polymorphism is not significant for the development of a pharmacoresistant form of epilepsy.

Since the population frequency of polymorphic gene genotypes is a variable parameter and may depend on a number of factors (gene drift, geographical, social or other isolation, mutations, migrations, panmixia, selection), an important task in studying the associative relationship with genetic determinants is to assess the compliance with the expected (exp) and the observed (obs) frequencies of genotypes of Hardy-Weinberg equilibrium. Our study showed a correspondence of the frequency of the observed polymorphism genotypes (−392A> G) of the CYP3A4 gene to the frequency of the expected genotypes, which indicates the existence of Hardy-Weinberg equilibrium in the population under study.

It is known that the relative level of theoretically expected heterozygosity (H_{exp}) is an indicator of genetic diversity, while the observed heterozygosity (H_{obs}) can be used to judge the measure of genetic variability (polymorphism) of a population. An analysis of the heterozygosity of the CYP3A4 gene (−392A> G) in the sample we studied showed that both H_{exp} and H_{obs} were characterized by low values and had no statistically significant differences, which is an indicator of the low genetic diversity of rs2740574 polymorphism in our population. The absence in our study of a significant difference between the expected and observed frequencies of genotypes can also testify to the selective neutrality of this polymorphism.

V. CONCLUSION

Thus, the data of our study showed the absence of an associative relationship between the polymorphism rs2740574 (−392A> G) of the CYP3A4 gene and the pharmacoresistant form of epilepsy in patients in the Uzbek population. The results of our study suggest that this polymorphism does not contribute to the development of drug resistance to antiepileptic drugs in people of Central Asian origin.Further studies should be carried out ina larger number of patients.

REFERENCES

1. Helmers S.L., Thurman D.J., Durgin T.L., … Descriptive epidemiology of epilepsy in the U.S. population: A different approach //Epilepsia. 2015 Jun;56(6):942-8. //doi: 10.1111/epi.13001. Epub 2015 Apr 29.

2. Berg A.T., Shinnar S., Levy S.R., … Early development of intractable epilepsy in children: a prospective study // Neurology. 2001 Jun 12;56(11):1445-52.
3. Lipatova L.V., Skoromets T.A., Gromov S.A., … Experience of Using Vagus Nerve Stimulation to Treat Drug Resistant Epilepsy // Neurology, Neuropsychiatry, Psychosomatics. 2014; (1S):18–21. // DOI: http://dx.doi.org/10.14412/2074-2711-2014-1S-18-21
4. Henning O., Lossius M.I., Lima M., … Refractory epilepsy and nonadherence to drug treatment // Epilepsia Open. 2019 Nov 6;4(4):618-623. // doi: 10.1002/epi4.12367. eCollection 2019 Dec.
5. BobylovaM.Yu., MukhinK.Yu. Perampanel in the treatment of drug-resistant epilepsy in children // RUSSIAN JOURNAL of CHILD NEUROLOGY. 2017. V.12. №4. P.7-20. // DOI: 10.17650/2073-8803-2017-12-4-7-20
6. May T.W., Israel C.W. [Sudden unexpected death in epilepsy (SUDEP) : Epidemiology, cardiac and other risk factors]. [Article in German] // HerzschrittmachertherElektrophysiol. 2019 Sep;30(3):274-286. // doi: 10.1007/s00399-019-00643-0.
7. Paolicchi J.M. The spectrum of nonepileptic events in children // Epilepsia. 2002;43 Suppl 3:60-4.
8. Emich-Widera E., Likus W., Kazek B., … CYP3A5*3 and C3435T MDR1 polymorphisms in prognostication of drug-resistant epilepsy in children and adolescents // Biomed Res Int. 2013;2013:526837. // doi: 10.1155/2013/526837. Epub 2013 Aug 1.
9. Silva-Alves M.S., Secolin R., Carvalho B.S., … A Prediction Algorithm for Drug Response in Patients with Mesial Temporal Lobe Epilepsy Based on Clinical and Genetic Information //PLoS One. 2017 Jan 4;12(1):e0169214. // doi: 10.1371/journal.pone.0169214. eCollection 2017.
10. López-García M.A., Feria-Romero I.A., Serrano H., … Influence of genetic variants of CYP2D6, CYP2C9, CYP2C19 and CYP3A4 on antiepileptic drug metabolism in pediatric patients with refractory epilepsy //Pharmacol Rep. 2017 Jun;69(3):504-511. // doi: 10.1016/j.pharep.2017.01.007. Epub 2017 Jan 19.
11. Ikeda T. Drug-induced idiosyncratic hepatotoxicity: prevention strategy developed after the troglitazone case // Drug MetabPharmacokinet. 2011;26(1):60-70. // Epub 2010 Dec 17.
12. Zahno A., Bouitbir J., Maseneni S., … Hepatocellular toxicity of clopidogrel: mechanisms and risk factors //Free Radic Biol Med. 2013 Dec;65:208-216. // doi: 10.1016/j.freeradbiomed.2013.06.007. Epub 2013 Jun 11.
13. Tolosa L., Jiménez N., Pérez G., … Customized in vitro model to detect human metabolism-dependent idiosyncratic drug-induced liver injury //Arch Toxicol. 2018 Jan;92(1):383-399. // doi: 10.1007/s00204-017-2036-4. Epub 2017 Jul 31.
14. Inoue K., Inazawa J., Nakagawa H., … Assignment of the human cytochrome P-450 nifedipine oxidase gene (CYP3A4) to chromosome 7 at band q22.1 by fluorescence in situ hybridization //Jpn J Hum Genet. 1992 Jun;37(2):133-8.
15. Abdel-Kahaar E., Winter S., Tremmel R., … The Impact of *CYP3A4*22* on Tacrolimus Pharmacokinetics and Outcome in Clinical Practice at a Single Kidney Transplant Center // Front Genet. 2019 Sep 26;10:871. // doi: 10.3389/fgene.2019.00871. eCollection 2019.
16. Op den Buijsch R.A., Christiaans M.H., Stolk L.M., … Tacrolimus pharmacokinetics and pharmacogenetics: influence of adenosine triphosphate-binding cassette B1 (ABCB1) and cytochrome (CYP) 3A polymorphisms // Fundam Clin Pharmacol. 2007 Aug;21(4):427-35.

17. Ball S.E., Scatina J., Kao J., … Population distribution and effects on drug metabolism of a genetic variant in the 5' promoter region of CYP3A4 //Clin PharmacolTher. 1999 Sep;66(3):288-94.
18. Sata F., Sapone A., Elizondo G., … CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity //Clin PharmacolTher. 2000 Jan;67(1):48-56.
19. Kotowski M.J., Bogacz A., Bartkowiak-Wieczorek J., … Effect of Multidrug-Resistant 1 (MDR1) and CYP3A4*1B Polymorphisms on Cyclosporine-Based Immunosuppressive Therapy in Renal Transplant Patients //Ann Transplant. 2019 Feb 25;24:108-114. // doi: 10.12659/AOT.914683.
20. Jaramillo N.M., Galindo I.F., Vázquez A.O., … Pharmacogenetic potential biomarkers for carbamazepine adverse drug reactions and clinical response //Drug Metabol Drug Interact. 2014;29(2):67-79. // doi: 10.1515/dmdi-2013-0046.
21. Puranik Y.G., Birnbaum A.K., Marino S.E., … Association of carbamazepine major metabolism and transport pathway gene polymorphisms and pharmacokinetics in patients with epilepsy //Pharmacogenomics. 2013 Jan;14(1):35-45. // doi: 10.2217/pgs.12.180.
22. Yun W., Zhang F., Hu C., … Effects of EPHX1, SCN1A and CYP3A4 genetic polymorphisms on plasma carbamazepine concentrations and pharmacoresistance in Chinese patients with epilepsy //Epilepsy Res. 2013 Dec;107(3):231-7. // doi: 10.1016/j.eplepsyres.2013.09.011. Epub 2013 Sep 30.
23. He X.J., Jian L.Y., He X.L., … Association of ABCB1, CYP3A4, EPHX1, FAS, SCN1A, MICA, and BAG6 polymorphisms with the risk of carbamazepine-induced Stevens-Johnson syndrome/toxic epidermal necrolysis in Chinese Han patients with epilepsy // Epilepsia. 2014 Aug;55(8):1301-6. // doi: 10.1111/epi.12655. Epub 2014 May 23.