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Research of Polymorfic Locus (430 C/T) of CYP2c9/*2 Gene Distribution Frequencies among Patients with Epilepsy

Nodira Miratalievna Tuychibaeva[1,*], Parakhat Rustamovna Alimkhodzhaeva[1], Kodirjon Tuhtabaevich Boboev[2]

[1]Department of Nervous Diseases, Tashkent Medical Academy, Tashkent, Uzbekistan
[2]Department of Molecular Medicine and Cell Technologies, Scientific Research Institute of Hematology and Blood Transfusion of the Ministry of Health of the Republic of Uzbekistan, Tashkent, Uzbekistan

Abstract The aim of the study was to establish the association of polymorphism (430 C/T) of the CYP2C9/*2 gene with the risk of developing pharmacoresistant epilepsy. The study included patients with pharmacoresistant and controlled forms of epilepsy. There was a tendency to associate the T allele and the heterozygous C/T genotype with the pharmacoresistant form of epilepsy.

Keywords Polymorphism (430 C/T), CYP2C9/*2 gene, Pharmacoresistant epilepsy, Allele, Genotype

1. Introduction

The problem of treating a pharmacoresistant form of epilepsy is the subject of a study by various authors [4a], but so far an effective method of treatment and prediction of this pathology has not been found. The basis of the treatment of epilepsy is a long-term lifelong intake of antiepileptic drugs [3]. The criterion of effective antiepileptic therapy can be considered the complete absence or minimization of side effects and complications of therapy [1,2,12,13].

One of the most effective ways to improve drug therapy is a personalized approach to treatment, requiring the study of the molecular genetic basis of the disease. As you know, one of the key roles in drug metabolism is played by cytochrome P450 [6,11,14,18,17]. In this regard, the study of the cytochrome P450 2C9 gene (CYP2C9) is of considerable interest to researchers, in which polymorphic changes are attributed to the development of undesirable complications against the background of pharmacotherapy for epilepsy and, inter alia, when taking valproic acid [8,5,9,10,15,19,21].

In this regard, the study of one of the poorly studied polymorphisms (430 C/T) of the CYP2C9/*2 gene is of interest for studying the etiopathogenetic basis of pharmacoresistant epilepsy [7].

* Corresponding author:
alteam2201@gmail.com (Nodira Miratalievna Tuychibaeva)
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2. Main Body

2.1. The Purpose of Our Research

The purpose of this study was to establish the association of polymorphism (430 C/T) of the CYP2C9/*2 gene and the risk of developing pharmacoresistant epilepsy.

2.2. Material and Methods of Study

The study included a total of 382 subjects, including the main group consisting of 124 people, including 77 patients with pharmacoresistant epilepsy (1a-subgroup) and 47 patients with controlled epilepsy (1b-subgroup). The control group consisted of 134 healthy individuals.

The diagnosis was established on the basis of clinical and anamnestic examination and laboratory studies. In patients with an established diagnosis in the main group and conditionally healthy individuals in the control group, a study was made of the distribution of polymorphic locus (430 C/T) in the CYP2C9/*2 gene.

DNA was isolated from venous blood using a DNA sorb and "Ribo-Sorb" kit (AmpliSens®, Russia). The concentration and purity of the isolated DNA was determined on a NanoDrop 2000 instrument (USA).

Genotyping of polymorphism (430 C/T) in the CYP2C9/*2 gene was performed by PCR-RFLP using an Applied Biosystems 2720 instrument.

Statistical processing of the research results was performed using the statistical software package "OpenEpi 2009, Version 2.3" and "DoctorStat 2013, Version 1.9".

2.3. Results of the Study

As can be seen from the results of the analysis performed in the distribution of alleles and polymorphism genotypes (430 C/T) in the CYP2C9/*2 gene, there were certain differences (Fig. 1, 2).

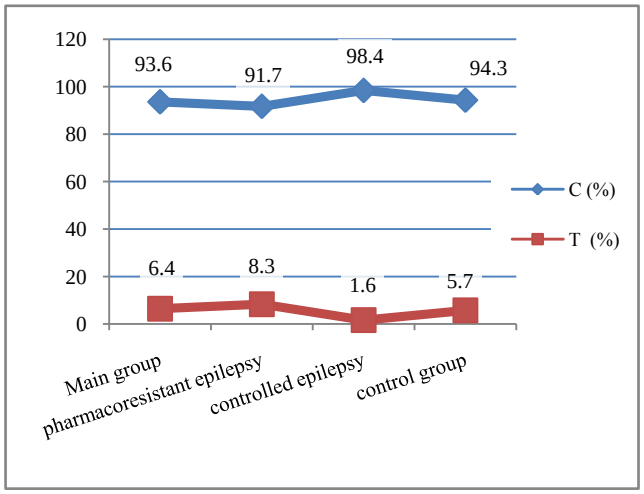

Figure 1. The frequency of distribution of polymorphism alleles (430 C/T) in the CYP2C9/*2 gene in patient groups and controls

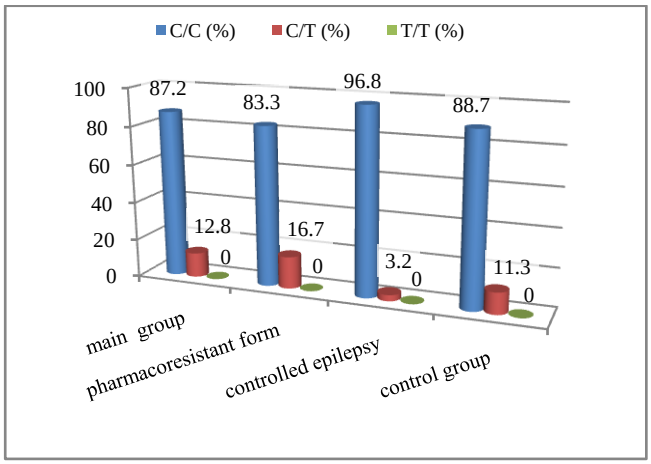

Figure 2. The frequency of distribution of genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene in the patient group and the control group

Table 1. presents the frequency of distribution of alleles and genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene in groups of patients with epilepsy and among healthy individuals. From table 1. can be seen that the differences in the distribution of alleles and polymorphism genotypes (430 C/T) in the CYP2C9/*2 gene were not statistically significant. Allele C in the control group was extremely insignificant and statistically insignificantly prevailed in the control sample, being practically at the same level as its detection rate among patients with epilepsy ($\chi2$ = 0.102; P = 0.750; RR = 0.992; OR = 0.8759; 95% CI: 0.3775-1.9952).

For the T allele, on the contrary, the statistically insignificant, insignificant, only 1.1 times, was characteristic for the prevalence of its detection rate among patients of the main group ($\chi2$ = 0.102; P = 0.750; RR = 1.133; OR = 1.142; 95% CI: 0.501- 2,649).

A study of the distribution of the C/C genotype, the detection rate of which was almost at the same level, which showed its very insignificant and statistically unreliable prevalence in the control group ($\chi2$ = 0.024; P = 0.878; RR = 0.983; OR = 0.970; 95% CI: 0.656-1.434).

Table 1. The frequency of distribution of alleles and genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene in group of patients with epilepsy and in the control group

Groups	n	Alleles and genotypes				
		C	T	C/C	C/T	T/T
Main group	abs	204	14	95	14	0
	%	93.6	6.4	87.2	12.8	0
Control group	abs	183	11	86	11	0
	%	94.3	5.7	88.7	11.3	0
χ^2		0.1018	0.1018	0.0236	0.1018	-
p		0.7497	0.7497	0.8780	0.7497	-
RR		0.992	1.1326	0.983	1.1326	-
OR		0.8759	1.1417	0.9699	1.1417	-
95% CI:	Lower	0.3775	0.5012	0.6563	0.5012	-
	Upper	1.9952	2.6489	1.4339	2.6489	-

A study of the frequency distribution of the C/T genotype demonstrated its insignificant 1.1 times, the prevalence among patients with epilepsy, which was statistically insignificant ($\chi2$ = 0.102; P = 0.750; RR = 1.133; OR = 1.142; 95% CI: 0.501-2.649).

The results of studies in the main and control groups allowed us to estimate the contribution of the CYP2C9/*2 gene to the development of epilepsy as a whole. When analyzing the frequency distribution of polymorphism alleles (430 C/T) of the CYP2C9/*2 gene, it was established that there are no significant differences in their distribution between the main and control groups.

The distribution of alleles and genotypes of the polymorphic locus among patients with a pharmacoresistant form of epilepsy and a group of healthy individuals was also studied (Table 2).

The results showed that the C allele is more pronounced, 1.6 times, but still statistically insignificant prevailed among patients with pharmacoresistant epilepsy ($\chi2$ = 0.9602; P = 0.3271; RR = 0.9718; OR = 0,6612; 95% CI: 0.2807-1.5398). the frequency of detection of the T allele was also 1.6 times higher in the group of patients with pharmacoresistant epilepsy relative to the control group, however, this predominance was also not statistically significant ($\chi2$ = 0, 0.9602; P = 0.3271; RR = 1.4697; OR = 1.5124; 95% CI: 0.6495-3.5619).

The C/C genotype, on the contrary, was more often detected in the population group, where its detection rate was statistically insignificant 1.3 times exceeded its occurrence

in the control group ($\chi2$ = 0.250; P = 0.617; RR = 0.940; OR = 0.897; 95% CI: 0.585-1.375). Similarly, 1.3 times, the frequency of occurrence of the C/T genotype prevailed among healthy individuals compared with the group of patients with pharmacoresistant epilepsy ($\chi2$ = 0, 0.9602; P = 0.3271; RR = 1.4697; OR = 1.5124; 95% CI: 0.6495-3.5619).

Table 2. The frequency of distribution of alleles and polymorphism genotypes (430 C/T) in the CYP2C9/*2 gene in groups of patients with pharmacoresistant epilepsy and in the control group

Groups	n	Alleles and genotypes				
		C	T	C/C	C/T	T/T
Pharmaco-resistant epilepsy	abs	143	13	65	13	0
	%	91.7	8.3	83.3	16.7	0
Control group	abs	183	11	86	11	0
	%	94.3	5.7	88.7	11.3	0
χ^2		0.9602	0.9602	0.2500	0.9602	-
p		0.3271	0.3271	0.6171	0.3271	-
RR		0.9718	1.4697	0.9399	1.4697	-
OR		0.6612	1.5124	0.8970	1.5124	-
95% CI:	Lower	0.2807	0.6495	0.5848	0.6495	-
	Upper	1.5398	3.5619	1.3749	3.5619	-

Table 3. presents the frequency distribution of alleles and genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene among patients with a controlled form of epilepsy and among healthy individuals. Thus, allele C, being practically at the same level in the main group and in the population sample, was statistically unreliable and extremely insignificantly prevailed among patients with controlled epilepsy ($\chi2$ = 1.7311; P = 0.1883; RR = 1.043; OR = 3.6667; 95% CI: 0.6047-80.869).

Table 3. The frequency of distribution of alleles and genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene in groups of patients with controlled epilepsy and in the control group

Groups	n	Alleles and genotypes				
		C	T	C/C	C/T	T/T
Controlled epilepsy	abs	61	1	30	1	0
	%	98,4	1,6	96,8	3,2	0
Control group	abs	183	11	86	11	0
	%	94,3	5.7	88.7	11.3	0
χ^2		1,7311	1,7311	0,3121	1,7311	-
p		0,1883	0,1883	0,5764	0,1883	-
RR		1,0430	0,2845	1,0915	0,2845	-
OR		3,6667	0,2727	1,1773	0,2727	-
95% CI:	Lower	0,6047	0,0124	0,6602	0,0124	-
	Upper	80,869	1,6537	2,0945	1,6537	-

The study of the distribution of the T allele of the polymorphism (430 C/T) of the CYP2C9/*2 gene showed its predominance in the population sample 3.5 times, which was not statistically significant ($\chi2$ = 1.7311; P = 0.1883; RR = 0, 2845; OR = 0.2727; 95% CI: 0.01224-1.6537).

In the group of patients with controlled epilepsy, an insignificant and statistically insignificant prevalence of the C/C genotype detection rate was found (χ^2=0.3121; p=0.5764; RR=1.0915; OR=1.1773; 95% CI: 0, 6602-2.0945).

Also, the prevalence of the C/T genotype detection rate in the control sample was found to be 3.5 times higher than the prevalence among patients with the controlled form of C/T epilepsy (χ^2=1.7311; p=0.1883; RR=0,2845; OR=0.2727; 95% CI: 0.01224-1.6537).

The results of a comparative analysis of the distribution of alleles and genotypes among patients with pharmacoresistant and controlled forms of epilepsy were presented in Table 4. The incidence of allele C in both subgroups was practically at the same level, with an extremely insignificant and statistically unreliable prevalence among patients with a controlled form of epilepsy ($\chi2$ = 3.334; P = 0.068; RR = 0.932; OR = 0.180; 95% CI: 0.008-1.070) while the frequency of detecting the T allele, on the contrary, was already 3.5 times more significant, but still statistically insignificantly prevailed among patients with a pharmacoresistant form of epilepsy ($\chi2$ = 3.344; P = 0.068; RR = 5.167; OR = 0.180; 95% CI: 0.935-120,800).

The C/C genotype was statistically insignificant, almost 1.2 times more often detected among patients with a controlled form of epilepsy ($\chi2$ = 0.815; P = 0.3667; RR = 0.8611; OR = 0.7619; 95% CI: 0, 4207-1.3839). The proportion of patients with pharmacoresistant epilepsy in whom the C/T genotype was found, on the contrary, 5.2 times, but still statistically insignificantly exceeded the proportion of healthy individuals with this identified genotype ($\chi2$ = 3.3343; P = 0.0678; RR = 5.1667; OR = 5.5455; 95% CI: 0.9346-120.8).

Table 4. The frequency of distribution of alleles and genotypes of polymorphism (430 s / t) in the CYP2C9/*2 gene in groups of patients with pharmacoresistant epilepsy and with controlled epilepsy

Groups	n	Alleles and genotypes				
		C	T	C/C	C/T	T/T
Pharmaco-resistant epilepsy	abs	143	13	65	13	0
	%	91,7	8,3	83,3	16,7	0
Controlled epilepsy	abs	61	1	30	1	0
	%	98,4	1,6	96,8	3,2	0
χ^2		3,3343	3,3343	0,8150	3,3343	-
p		0,0678	0,0678	0,3667	0,0678	-
RR		0,9317	5,1667	0,8611	5,1667	-
OR		0,1803	5,5455	0,7619	5,5455	-
95% CI:	Lower	0,0083	0,9346	0,4207	0,9346	-
	Upper	1,0700	120,80	1,3839	120,80	-

According to the results of the analysis, in the main group (table 5), the observed and expected frequencies did not have significant differences. The observed and expected distribution frequencies of all the studied genotypes of this polymorphism, according to the analysis, did not have

statistically significant differences (p = 0.493). An extremely insignificant deviation was found in the distribution of the theoretically calculated frequency of occurrence of the C/C genotype from the actually observed, which practically had no significant differences, being almost at the same level (χ^2 = 0.002), C/T = (χ2 = 0.057) = (χ2 = 0.412).

Moreover, if the homozygous C/C genotype was marked by a very slight prevalence of the expected detection frequency (χ^2 = 0.002), then for the heterozygous genotype, on the contrary, the inconsequential prevalence of the actually observed frequency (χ^2 = 0.057) was observed. distribution of alleles and genotypes of polymorphism (430 C/T) in the CYP2C9/*2 gene in groups of patients with pharmacoresistant epilepsy and with controlled epilepsy.

Table 5. The expected and observed frequency distribution of polymorphism genotypes (430 C/T) of the CYP2C9/*2 gene in the main group, in accordance with the Hardy-Weinberg equilibrium

Genotypes		Genotype distribution:		χ^2	p
Name	n	observed	expected		
C/C	95	0,87	0,88	0,002	
C/T	14	0,13	0,12	0,057	
T/T	0	0,00	0,00	0,412	
total	109	1,00	1,00	0,471	0,493
Total*2=	218				

Even less pronounced differences were found when analyzing the distribution of polymorphism genotypes (430 C/T) in the CYP2C9/*2 gene in the control sample (table 6).

Table 6. The expected and observed frequency distribution of polymorphism genotypes (430 C/T) of the CYP2C9/*2 gene in the control group, in accordance with the Hardy-Weinberg equilibrium

Genotypes		Genotype distribution:		χ^2	p
Name	n	observed	expected		
C/C	86	0,79	0,79	0,001	
C/T	11	0,10	0,10	0,034	0,571
T/T	0	0,00	0,00	0,286	
total	97	0,89	0,89	0,322	
Total*2=	194				

A similar situation could be observed in the study of the heterozygous genotype C/T (χ2 = 0.034), as well as in the analysis of the total distribution of theoretically calculated and actually expected frequencies of the genotypes of the studied polymorphism (χ2 = 0.322; p = 0.571). A similar situation could be observed in the study of the heterozygous genotype C/T (χ2 = 0.034), as well as in the analysis of the total distribution of theoretically calculated and actually expected frequencies of the genotypes of the studied polymorphism (χ2 = 0.322; p = 0.571).

The analysis of gene diversity by polymorphism 430 (C/T) in the CYP2C9/*2 gene was also carried out (table. 7).

In the main group, a relatively higher degree of heterozygosity for the studied polymorphism was found, which may indicate moderate gene diversity.

Table 7. Differences between expected and observed frequencies of heterozygosity

Groups	Heterozygosity		D*
	Observed (h_{obs})	Expected (h_{exp})	
Main	0.13	0.12	+ 0.069
Control	0.10	0.10	+ 0.060

At the same time, in the control group, the observed and expected heterozygosity were at the same level $h_{obs} = h_{exp}$ = 0.10. The obtained results corresponded to the values of the coefficient of relative deviation (D*), which in the main and population samples corresponded to +0.069 and +0.060, respectively, which indicated a sufficient number of heterozygotes whose level corresponded to a moderate.

The results obtained as a whole, despite the absence of statistically significant deviations in the distribution of the mutant allele, still, indirectly, confirm the viability of continuing research in the chosen direction. The revealed tendency to the association of a pathological polymorphic locus with a pharmacoresistant form of epilepsy does not contradict the literature data [15,19,21].

Studies on the effect of polymorphisms of the CYP2C9 gene also indicate its possible effect on various pharmacokinetic and pharmacodynamic changes in drug metabolism [16,20]. Thus, the results of the research can help in the development of personalized antiepileptic pharmacotherapy, which requires further research in this direction.

3. Conclusions

Thus, despite the absence of significant differences in the distribution of alleles and polymorphism genotypes (430 C/T) in the CYP2C9/*2 gene among patients with epilepsy, there was a tendency to associate the T allele and the heterozygous C/T genotype with the drug-resistant form of the disease.

At the same time, detection of the homozygous C/C genotype was statistically insignificantly associated with a controlled form of epilepsy, which may indicate the possible presence of its protective properties.

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