

CLINICAL RESEARCH

## MDR1 Gene C3435T and C1236T Polymorphisms among Patients with Pharmaco-resistant Epilepsy and Healthy Individuals

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### Abstract

MDR1 gene C3435T and C1236T single-nucleotide polymorphisms (SNPs) have been studied in 59 Uzbek patients with epilepsy aged from 1 to 40 years. The patients were resistant to anticonvulsant drugs in therapeutic doses with no remission attained. The disease duration was about two years. The DNA samples were isolated from peripheral blood of patients and healthy individuals. The study found a statistically significant difference in the frequency of the TT genotype of the MDR1 gene C3435T polymorphism, which was associated both with rapid and slow drug metabolism. In the TT genotype group, the share of the patients resistant to the therapy was almost 4.8 times higher than in the control group. Despite high OR=1.9, there were statistically insignificant differences in the frequency of C1236T SNP. The 3435C – 1236T haplotype of MDR1 gene was associated with an increase the risk of drug-resistance development in epileptic patients.

**Keywords:** MDR1 gene; C3435T and C1236T single-nucleotide polymorphisms; pharmaco-resistant epilepsy.

### Introduction

Epilepsy is one of the common neurological diseases, its frequency being from 1% to 2% of people in the entire population. One of the most urgent and unsolved problems of current epileptology is the development of pharmaco-resistant epilepsy (PRE), the frequency of which is nearly 30% [1-3].

The mechanisms of pharmaco-resistance are still insufficiently studied. One of the causes of PRE forming is that some genetic factors affect the bioavailability of drugs, in particular those factors that contribute to disorders in drug metabolism and lead to low effectiveness of anti-epileptic drugs (AED). The key participants in the AED metabolism systems are the enzymes of the xenobiotics biotransformation system and drug transporters [1,4,5]. Among protein-transporters, glycoprotein P coded by the multi-drug resistance (MDR1) gene plays the major role in the processes of AED absorption,

distribution and excretion [4,6-8]. The MDR1 gene is located in 7q21.1 chromosome and codes 170 kDa glycoprotein [9,10]. By now, many polymorphous loci of the MDR1 gene have been studied. However, many researchers give preference to C3435T (rs1045642) and C1236T (rs1128503) polymorphisms [11-13]. C1236T SNP is located in exon 12, C3435T SNP in exon 26. The presence of these polymorphisms in the MDR1 gene is supposed to lead to a change in glycoprotein P expression [14-17].

Nowadays, the population profiles of these SNPs of the MDR1 gene need further research. The same is true of the effect of genotypic variants of these markers on the risk of PRE development. The data in the literature are still contradictory [7,11,13,18-21].

Introduction of molecular research methods to clinical practice to reveal PRE will allow performing individual selection of AEDs and their doses, as well as choosing the tactics of therapeutic and prevention interventions for PRE patients.

**The aim** of this research was to study C3435T and C1236T SNPs of the MDR1 gene and evaluate their association with development of drug-resistance in epileptic

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patients being treated with AEDs.

The research involved 59 PRE patients (31 males и 28 females), Uzbeks, aged from 1 to 40 years, observed at the hospital of Tashkent Medical Academy. *The criteria* for PRE were the duration of the disease over two years and application of more than two AEDs in maximum permissible doses with no remission attained (valproic acid, carbamazepin, topiromat).

The control group comprised 60 apparently healthy individuals of Uzbek nationality with no epileptic syndromes and similar diseases.

The DNA from peripheral blood was isolated by the standard phenol technique with some modifications. Genotyping of MDR1 gene C1236T and C3435T SNPs was made by the real-time polymerase chain reaction (PCR) using thermocycler Rotor-Gene 6000 (Corbett Research, Australia) and the Genotechnology LLC sets (Moscow, Russia) according to the instructions of the manufacturer.

The following sequence primers were used:

C1236T:

F: 5'-TTC GAA GAG TGG GCA CAA ACC-3'

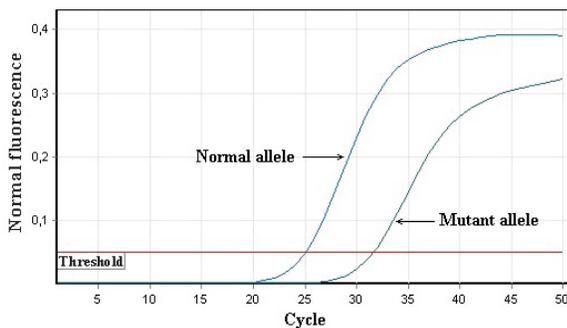
R: 5'-CAG CCA CTG TTT CCA ACC AGG-3';

C3435T:

F: 5'-CTG AAT GTT CAG TGG CTC CGA-G-3'

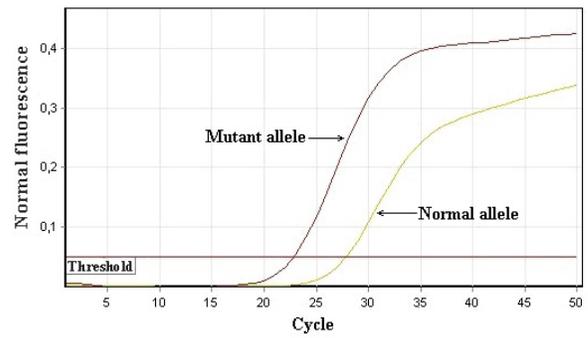
R: 5'-AAG GTA ACA ACT AAC CCA AAC AGG-3'.

The PCR real time conditions for C1236T и C3435T SNPs were as follows: denaturation at 95° C -10 min (1 cycle), 95 °C- 15 sec, 60°C -15 sec (50 cycles) and 62°C - 40 sec. Interpretation of the results was made in the Yellow channel (Figures 1-2).

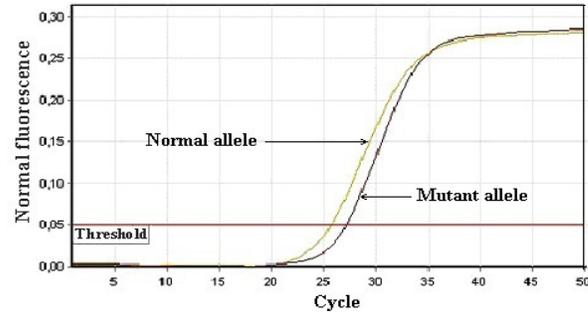


**Figure 1.** Normal genotype of C3435T u C1236T polymorphisms of MDR1 gene.

Statistical analysis was performed using a statistical software package, OpenEpi (ver. 9.3). Chi square ( $\chi^2$ ) or Fischer's exact test (two sided) was used to compare the association between the genotypes and alleles in relation to the cases, and test for deviation of genotype distribution from Hardy–Weinberg equilibrium. P values of < 0.05 were considered statistically significant. The odds ratio (OR) and their 95 % confidence intervals (CI) were calculated to estimate the strength of the association. The predictive efficiency (AUC-classifier) of the genetic markers under study was determined by the standard formula:  $AUC = (SE + SP)/2$ .



a)



b)

**Figure 2.** Homozygous and heterozygous genotypes of C3435T and C1236T polymorphism of MDR1 gene.

## Results

We analyzed the predictive efficiency of the investigated SNPs (Table 1). The indicators of sensitivity and specificity for C1236T SNP reached the mean values, namely SE=0.66 and SP=0.53.

**Table 1.**  
Indicators of the markers predictive efficiency

Genetic marker of MDR gene	SE	SP	AUC	OR (95%CI)	*P
1236 T/C	0.66	0.53	0.60	3.9 (0.46-33.31)	0.2
3435 T/C	0.81	0.6	0.70	4.8; 95%CI= 1.013, 22.48	0.03

SE – sensitivity; SP – specificity; AUC – prediction efficiency; \*P – precise Fisher's test.

The estimated AUC (0.60) also demonstrates the mean level of efficiency by the marker classifier as an independent gene candidate.

The predictive value of C3435T SNP turned out to be high. The high level of specificity (SP = 0.81) and the mean level of sensitivity (SP=0.6) may indicate a rather independent predictive value of the marker on the risk of PRE development (AUC = 0.70).

Tables 2 and 3 show the frequencies of alleles and genotypes of C3435T and C1236T SNPs. All allele frequencies

were in Hardy-Weinberg equilibrium.

Both in the control group and in the group of PRE patients, the heterozygous CT genotype was the most common genotype of C1236T SNP, which was quite unexpected, with a frequency of 40.0% for the control group and 54.2% for the group of PRE patients ( $\chi^2=2.4$ ;  $P=0.1$ ; OR=1.8; 95% CI: 0.859-3.679). The homozygous TT genotype occurred also with a high frequency – 53.3% in the control group and 33.9% in the PRE patients group ( $\chi^2=4.6$ ;  $P=0.03$ ; OR=0.4; 95% CI: 0.2141-0.9406). The frequency of homozygous CC genotype was 6.7% in the control group and 11.8% in the PRE patients group ( $\chi^2=1.0$ ;  $P=0.3$ ; OR=1.9; 95% CI: 0.5213-6.813). Despite high OR=1.9, there were statistically insignificant differences in the frequency of this genotype. Probably, this is due to the small sample or demonstrates a rather high distribution of this polymorphism in our population.

**Table 2.**  
*Distribution of alleles and genotypes of 1236 C/T polymorphism of gene MDR1 in the groups of patients and controls*

Group	n	Allele frequency				Genotype distribution frequency					
		T		C		T/T		T/C		C/C	
		n	%	n	%	n	%	n	%	n	%
PRE patients	59	72	61.0	46	39.0	20	33.9	32	54.2	7	11.8
Control	60	88	73.3	32	26.7	32	53.3	24	40.0	4	6.7

The same comparative analysis of C3435T SNP has been made between the PRE patients and controls (Table 3). All genotype variants of this SNP were present in both groups. In the group of PRE patients, the following genotypes were observed: CC – in 18.6%, CT – in 55.9%, TT – in 25.4% of cases. In the control group: CC – in 60.0%, CT – in 33.3%, TT – in 6.6% of cases.

**Table 3.**  
*Distribution of alleles and genotypes of C3435T polymorphism of gene MDR1 in the groups of patients and control*

Group	n	Allele frequency				Genotype distribution frequency					
		C		T		C/C		C/T		T/T	
		n	%	n	%	n	%	n	%	n	%
PRE patients	59	55	46.6	63	53.4	11	18.6	33	55.9	15	25.4
Control	60	92	76.6	28	23.3	36	60.0	20	33.3	4	6.6

The obtained findings are in agreement with the data of some researchers [11-13] and do not agree with the findings of others [19-21]; this result suggests population peculiarities of these SNPs and ethnic specificity of the AED resistance development. According to our data, the presence of T allele of C3435T SNP increases the risk of PRE development in Uzbek patients ( $\chi^2=22.8$ ;  $P=0.000002$ ; OR=3.8; 95% CI: 2.157-6.566); TT homozygous carriers showed the highest percent

of PRE ( $\chi^2=7.8$ ;  $P=0.005$ ; OR=4.8; 95%CI: 1.479-15.4).

We analyzed the risk of PRE development in simultaneous carriers of different allelic variants of studied SNPs. The 1236C–3435T haplotype of the MDR1 gene in the PRE patient group was found six times more often than in the control group (79.7% vs. 40.0%, respectively;  $\chi^2=21.3$ ;  $P=0.0002$ ; OR=6.5; 95% CI: 2.842 - 15.07).

## Conclusion

The obtained findings suggest the heterogeneous nature of PRE formation with the participation of studied SNPs. C3435T SNP is associated with PRE to AED. Carriage of T allele, especially the TT genotype, is associated with a higher risk of PRE. The 1236C–3435T haplotype of the MDR1 gene may be an additional risk factor for PRE development.

## Competing interests

The authors declare that they have no competing interests.

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