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Frequencies of the C1236T, G2677T/A, and C3435T Polymorphisms of the MDR1 Gene and their Haplotypes in Uzbekistan

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Abstract: P-glycoprotein (P-gp) is encoded by the MDR1 gene and is involved in the pharmacokinetics of drugs. The best-known MDR1 gene polymorphisms are 1236C>T, 3435C>T, and 2677G>T/A, which are associated with protein folding, changes in mRNA levels, and drug pharmacokinetics. The aim of this study was to identify the frequency of distribution of functional polymorphisms C1236T, C3435T, G2677T/A of the MDR1 gene and their haplotypes in Uzbekistan. For this research, peripheral blood samples were obtained from 218 apparently healthy people from Uzbekistan. MDR1 gene polymorphisms were genotyped by Polymerase Chain Reaction (PCR) and restriction analysis (RFLP). The results of genotyping of the MDR1 gene C1236T variant in 21 volunteers showed that the frequency of the T allele was 58.7%, and the C allele was 41.3% (P = 0.377891). The frequency of the occurrence of the alleles for polymorphism 2677G>T/A was as follows: G, 94.5%; A, 5.5% (P>0.005). For variant 3435C>T, the frequency of the C allele was 43% and the frequency of the T allele was 57% (P = 0.561084). This study also evaluated the simultaneous presence of the C1236T, C3435T, and G2677T/A polymorphisms of the MDR1 gene. The most frequently occurring combination of genotypes was CT/GG/CT (26.1%) and TT/GG/TT (17.4%). The frequency of the common haplotype CGC, representing the simultaneous appearance of all wild-type alleles in the tested MDR1 SNPs, was 6.4% (n = 14) in people with a normal genotype. However, the simultaneous presence of polymorphic nucleotides for all polymorphisms (TAT) was not found. The research made it possible to establish the distribution of allele frequencies of the MDR1 gene of C3435T and C1236T polymorphisms among the samples of our study in Uzbekistan. The frequency occurrence of the CT and TT genotypes for both polymorphisms is higher than that of the CC genotype. Although the obtained results are insufficient to assess the frequency of distribution of polymorphisms in all Uzbekistan residents, further research will be focused on the study of these polymorphisms in a wide range to confirm the reliability of the results.

Keywords: Pharmacogenetics, Glycoprotein-P, MDR1, Polymorphism, Genotype, Population

Introduction

ABC transporters (ATP-binding cassettes) are members of the superfamily of ATP-binding transmembrane proteins that transport various substrates, including carcinogens, metabolites, anticancer and cytotoxic drugs, across biological membranes (Zhai *et al*., 2012). One of the most important members of the ABC transporter superfamily is glycoprotein P (P-gp), which is an ATP-dependent transmembrane protein (ABCB1) involved

in the transport of lipophilic exogenous and endogenous substrates out of the cell (Schinkel *et al*., 1997). P-gp is involved in a wide range of physiological processes, including proliferation, differentiation, immune response regulation, and apoptosis (Balcerczak *et al*., 2010), and is expressed in the apical membranes of various cell types (Sharom, 2011).

P-gp is involved in drug pharmacokinetics and this participation is determined by its polyspecificity concerning a wide range of substrates, with molecular weights from 330 Da to 4000 Da (Wessler *et al*., 2013).

Presumably, P-gp has multiple xenobiotic binding sites, which allows export-oriented transport of compounds of various chemical natures (Juliano and Ling, 1976; Zhao *et al*., 2015). The interaction of P-gp with medicinal compounds can modulate its activity: P-gp inducers increase the functional activity of the protein by changing the pharmacokinetics of substrates and thereby reduce the effectiveness of pharmacotherapy, whereas P-gp inhibitors reduce its functional activity, increasing the risk of developing adverse bodily reactions to drugs (Yakusheva *et al*., 2011; 2014; Yakusheva and Chernykh, 2012).

It should be noted that P-gp inhibition is a promising target for overcoming the barrier of multidrug resistance in tumors (Iakusheva *et al*., 2014).

In humans, P-gp is encoded by the MDR1 and MDR2 (multidrug resistance) genes, which are involved in the development of drug resistance (Kramer *et al*., 1995).

Systemic screening of the MDR1 gene revealed approximately 50 Single-Nucleotide Polymorphisms (SNPs), which, to one degree or another, can impact the function and expression of P-gp (Yan-Hong *et al*., 2006; Tamura *et al*., 2012; Uludag *et al*., 2014; Subhani *et al*., 2015).

Most research focuses on three SNPs: 3435C>T in exon 26 and 1236C>T in exon 21, which are synonymous and 2677G>T/A in exon 21, which is a nonsynonymous three-allelic polymorphism responsible for the amino acid substitution of Ala to Ser/Thr at position 893. These SNPs are in high linkage disequilibrium (Tang *et al*., 2002) and their allelic variations occur with different frequencies among populations and subpopulations of different ethnic or racial origins (Cascorbi, 2006). The SNP 1236C>T correlates with a glycine residue located on the outer surface of the N-terminal domain. The SNP 3435C>T correlates with an isoleucine residue located in the inner regions of the C-terminal domain. This nonsynonymous variation is associated with altered P-gp activity (Naumovska *et al*., 2014). Numerous results support that SNP variations are associated with protein folding, changes in mRNA levels, and drug pharmacokinetics (Green *et al*., 2006; Kimchi-Sarfaty *et al*., 2007a).

Correlations between the presence of SNPs and susceptibility to cancer, ulcerative colitis, schizophrenia, Crohn's disease, Alzheimer's disease, Parkinson's disease, and HIV infection, acute myeloid leukemia, (Hoffmeyer *et al*., 2000; Kim *et al*., 2005; Dulucq *et al*., 2008; Ekhart *et al*., 2009; Vivona *et al*., 2012; Rubis *et al*., 2012; Ait Boujmia *et al*., 2020), as well as their course, progression, and response to drug therapy, are the subject of numerous scientific studies (Johne *et al*., 2002; Chowbay *et al*., 2003; Yi *et al*., 2004; Crettol *et al*., 2006; Green *et al*., 2006; Tsai *et al*., 2008). Although other researchers have confirmed that, the C1236T polymorphism of the MDR1 gene is not associated with the occurrence of B-cell non-Hodgkin's lymphoma in the Han East Chinese population (Zhang *et al*., 2022). A study by Al-Ghafari *et al*. (2020) did not reveal an association between C1236T polymorphism and the risk of developing CRC (colorectal cancer).

Thus, the determination of the P-gp genotype can serve as a new prognostic marker for identifying subpopulations with an increased risk of certain diseases while also opening up the possibility of developing new approaches to personalized drug therapy. In this study, we characterized the C3435T, G2677T/A, C1236T polymorphisms of the MDR1 gene in the samples of 218 volunteers living in Uzbekistan. As far as we know, research on this topic has not been conducted in Uzbekistan. Therefore, we designed this investigation to study the frequency of genotypes among healthy adults living in Uzbekistan, which allowed us to compare them with the frequencies of other major ethnic groups in the SNP database and use statistically significant variants as markers for population genetic studies. According to the results of population genetic analysis, these polymorphisms can be used as biomarkers in pharmacogenetic testing.

Aim. This study aimed to determine the distribution frequency of the MDR1 gene functional polymorphisms C1236T, C3435T, and G2677T/A and their haplotypes in Uzbekistan.

Materials and Methods

The research involved 218 healthy Uzbek volunteers aged 18 to 80 years. All participants were informed about the purpose and objectives of the study, based on which a form for voluntary informed consent was signed for study participation. This study was approved by the Ethics Committee of the Ministry of Health of the Republic of Uzbekistan under the number No. 3/1-1023 (March 30, 2019). According to the results of the survey, persons suffering from chronic and hereditary diseases were excluded from the study. A peripheral blood sample, 5 mL in volume, was obtained from the cubital vein into a sterile vacuum tube. Molecular genetic studies were carried out at the Institute of Biophysics and Biochemistry at the National University of Uzbekistan and the Center for Advanced Technologies under the Ministry of

Innovative Development of the Republic of Uzbekistan. Genomic DNA was isolated from the peripheral blood samples using the DiatomPrep100 kit (Isogene, Russia). The genotyping of MDR1 gene polymorphisms was performed by Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism (RFLP) analysis. The amplification of PCR products was carried out on a Veriti Thermal Cycler (Applied Biosystems, USA) amplifier using an Isogene Gen Pak PCR-Core lyophilic reagent kit (Isogene, Russia). To identify the C3435T, G2677T/A, and C1236T polymorphisms of the MDR1 gene were used the primers are shown in Table 1. The primer sequences were obtained from a previously published article (Yan *et al*., 2017). However, the described conditions for PCR amplification of the G2677T/A, C3435T, and C1236T, polymorphisms of the MDR1 gene were not confirmed in the course of our study (Yan *et al*., 2017).

The PCR program included the following parameters: Initial denaturation at 94°C for 4 min; followed by a denaturation cycle at 94°C for 25 sec, annealing at 55-57°C for 35 sec (C1236T-55°C, C3435-57°C, G2677T/A - 55°C) and elongation at 72°C for 35 sec for 40 cycles; and finally elongation at 72°C for 4 min. Electrophoretic analysis of the PCR products for the C3435T, G2677T/A, and C1236T polymorphisms showed that their lengths corresponded to the expected lengths (558, 485, 548 bp, respectively). The PCR products of the MDR1 gene C3435T, G2677T/A, and C1236T polymorphisms were digested using the corresponding restriction enzymes BstMBI, RsaI, and BsuRI. Genotypes were identified through gel electrophoresis of the amplified DNA fragments after restriction (Fig. 1 to 3).

The genotype and allele frequencies of the C3435T, G2677T/A, and C1236T polymorphisms of the MDR1 gene were obtained based on the results of the PCR-RFLP method. Genotype distribution was assessed by Hardy-Weinberg Equilibrium (HWE) using the $\chi 2$ test.

Table 1: Primers for specific amplification of the MDR1 gene for the C3435T, G2677T/A and C1236T polymorphisms

Gene	Name of primers		Nucleotide sequence (5'-3')
MDR1	C1236T rs1128503	Forward	GTTCACTTCAGTTACCCATCTCG
		Reverse	CGTGGTGGCAAACAATACAGG
	G2677T/A rs2032582	Forward	TCAGCATTCTGAAGTCATGGAA
		Reverse	TTAGAGCATAGTAAGCAGTAGGGAGT
	C3435T rs1045642	Forward	GTGTGCTGGTCCTGAAGTTG
		Reverse	TGGAGCCTCAAGCCTATAGC

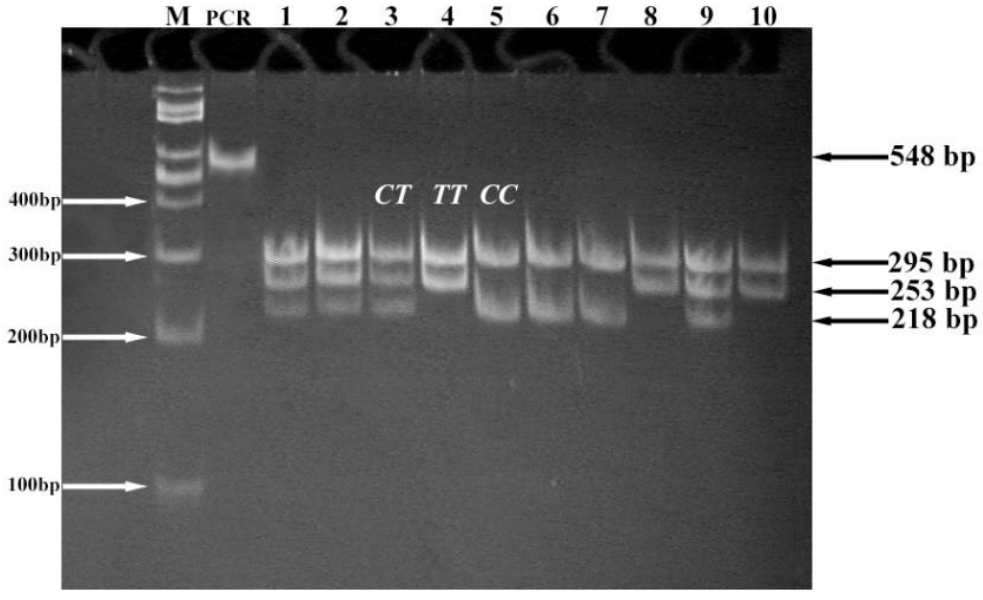

Fig. 1: Electrophoresis of the PCR products of the C1236T polymorphism of the MDR1 gene. Genotype CC, 218 and 295 bp; genotype CT, 218, 253 and 295 bp; genotype TT, 253 and 295 bp; M, molecular weight marker (100 bp); PCR, PCR product (548 bp)

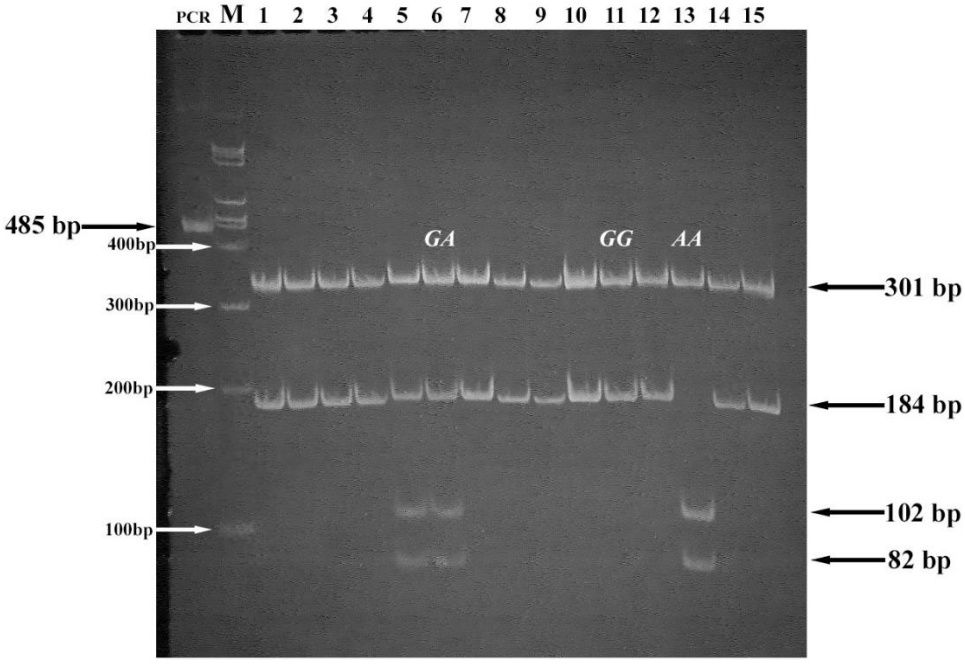

Fig. 2: Electrophoresis of the PCR products of the G2677T/A polymorphism of the MDR1 gene. Genotype GG, 184 and 301 bp; genotype GA, 82, 102, 184 and 301 b.p.; genotype AA, 82, 102 and 301 b.p. M, molecular weight marker (100 bp); PCR, PCR product (485 bp)

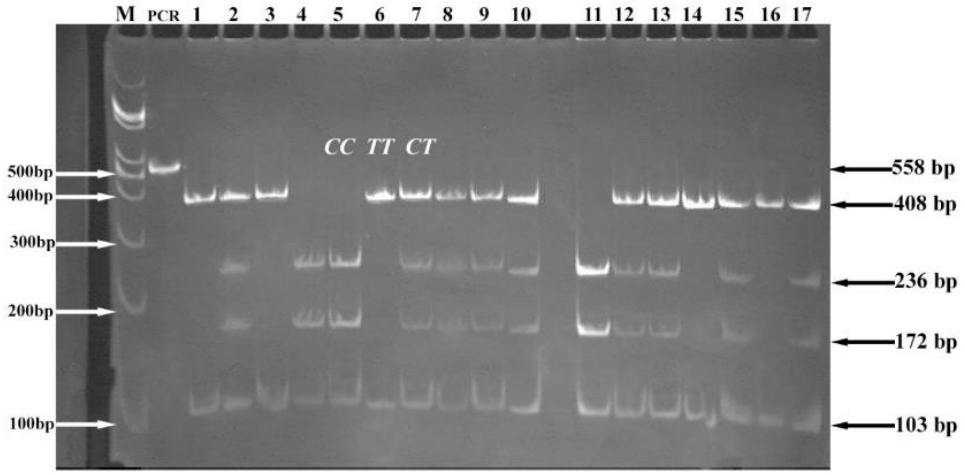

Fig. 3: Electrophoresis of the PCR products of the C3435T polymorphism of the MDR1 gene. Genotype CC, 103, 172, and 236 bp; genotype CT, 103, 172, 236, and 408 bp; genotype TT, 103 and 408 bp. M, molecular weight marker (100 bp); PCR, PCR product (558 bp)

Results

In the present study, we analyzed the genotypes for the MDR1 gene polymorphic variants 1236C>T (rs1128503), 2677G>T/A (rs2032583), 3435C>T (rs1045642) among 218 healthy volunteers living in Uzbekistan.

The results of the genotyping of the MDR1 gene polymorphisms C3435T, G2677T/A, and C1236T are presented in Table 2.

It was found that the frequency of the T allele for the polymorphism C1236T was 17% higher than the frequency of the C allele and the frequencies of the heterozygous CT genotype and the TT genotype were 3.2 times and 2 times higher, respectively than the frequency of the homozygous CC genotype. A decrease in P-gp expression has been observed for the CT and TT genotypes (Hoffmeyer *et al*., 2000). An analysis of the distribution of genotype frequencies showed a deviation

from Hardy-Weinberg equilibrium (P = 0.377891). Our results allowed us to conclude that the T allele of the C1236T polymorphism of the MDR1 gene is more common in this group of people.

An analysis of the distribution of genotype frequencies for the 2677G>T/A polymorphism showed a deviation from Hardy-Weinberg equilibrium (P>0.005). Thus, taking into account that the nonsynonymous 2677G>T/A mutation of the MDR1 gene was detected among clinically healthy volunteers, as well as the potential phenotypic effects of this variant through altered activity and expression of P-gp, we can recommend the 2677G>T/A polymorphism of the MDR1 gene for use in pharmacogenetic testing as a molecular biomarker for drug susceptibility.

The genotyping results for the C3435T polymorphism showed that the frequency of the T allele was 14% higher than that of the C allele. The frequencies of the CT genotype and the TT genotype were 2.9 times and 1.8 times higher, respectively than the frequency of the CC genotype. An analysis of the distribution of genotype frequencies showed a deviation from Hardy-Weinberg equilibrium (P = 0.561084). Based on the data obtained, it was shown that the 3435TT genotype had a twofold decrease in expression compared to the CC genotype. The homozygous T allele is associated with a more than twofold decrease in duodenal P-gp expression levels compared to the CC genotype (Hoffmeyer *et al*., 2000).

This research also evaluated the simultaneous presence of the C1236T, G2677T/A, and C3435T polymorphisms of the MDR1 gene. The results of this evaluation showed that the most frequent combinations of genotypes were CT/GG/CT (26.1%) and TT/GG/TT (17.4%) (Fig. 4).

However, the simultaneous presence of polymorphic variants for all polymorphisms (TT/AA/TT) was absent. The frequency of the common CGC haplotype, representing the simultaneous occurrence of all wild-type alleles in the tested MDR1 SNPs, was 6.4% (n = 14) in people with a normal genotype. This finding means that in 6% of volunteers, xenobiotics and cytotoxins can be released outside the cell with maximum efficiency, while in 94% of volunteers, a decrease in the level of P-gp expression can be observed, which leads to its deficiency in the apical cell membranes, a decrease in the intensity of toxin elimination and xenobiotics into tissue fluid, bile and urine and as a result, tissue intoxication.

Table 2: Results of the genotyping of the MDR1 gene polymorphisms C1236T, G2677T/A and C3435T

Polymorphism	Genotype	Genotype frequency, %	Allele frequency, %		P-value
C1236T	CC	16.0	C – 41.3	T – 58.7	0.377891
	CT	51.0			
	TT	33.0			
2677G>T/A	GG	91.0	G – 94.5	A/T – 5.5	>0.005
	GA/T	7.0			
	AA/TT	2.0			
C3435T	CC	17.4	C – 43.0	T – 57.0	0.561084
	CT	51.0			
	TT	31.6			

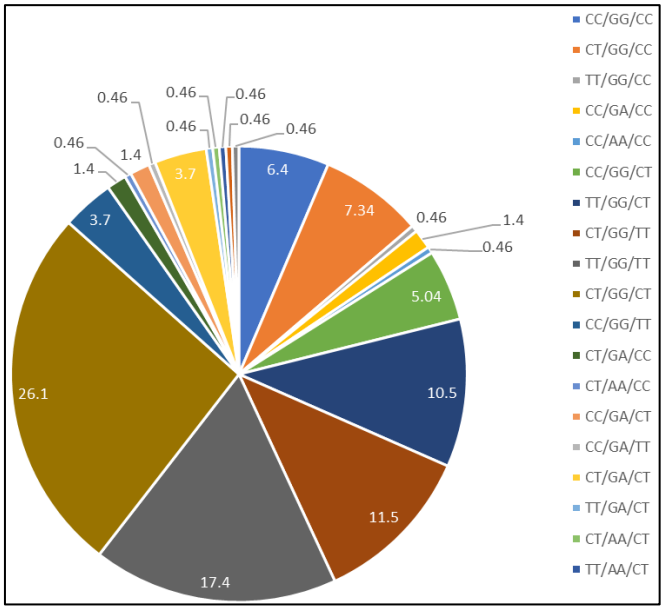

Fig. 4: Frequency of combination of genotypes of the MDR1 gene polymorphisms C3435T, G2677T/A and C1236T in the Uzbek population (n = 218)

An analysis of the distribution of the main MDR1 gene polymorphisms C1236T, G2677T/A, and C3435T demonstrates wide variability in different populations. A haplotype containing three wild-types or reference variants (CGC) is most common in the African American population at 60.9%, followed by Caucasians at 36.5% (Kim *et al*., 2001). In contrast to African Americans, in the Chinese and Turkish populations, the frequency of the wild-type haplotype is relatively rare, at 23.163 and 25%, respectively (Tang *et al*., 2002; Gumus-Akay *et al*., 2010). The frequency of the wild-type haplotype (CGC) in Uzbekistan (6.4%) is very low compared to that in other populations.

The frequency of a polymorphic haplotype (TTT) is 27% in the Caucasian population, 6.5% in the African American population (Kim *et al*., 2001), 38.923% in the Chinese population, and 33.7% in the Turkish population (Tang *et al*., 2002; Gumus-Akay *et al*., 2010). The occurrence of a polymorphic haplotype (TTT) in Uzbekistan was not revealed.

Discussion

The expression of P-gp, a product of the MDR1 gene, is an important factor affecting the bioavailability of many cardiovascular and anticancer drugs with a narrow therapeutic window. Genetic variation in the MDR1 gene affects the pharmacokinetics of various drugs, leading to changes in drug efficacy and side effects (Shen and Cheng, 2019; Zhao *et al*., 2021).

MDR1 polymorphisms impact the pharmacodynamic and pharmacokinetic profiles of drug substrates and directly affect the prognosis and efficacy of drug therapy for several life-threatening diseases (Pauli-Magnus and Kroetz, 2004).

The C1236T polymorphism (Gly412Gly) is associated with variability in response to different P-gp substrates. It has been shown that people with the TT genotype have decreased expression of the MDR1 gene (Eichelbaum Eichelbaum *et al*., 2004). Decreased expression of the MDR1 gene in hepatocytes, intestinal epithelial cells, and renal epithelial cells leads to a decrease in the amount of P-gp in the cytoplasmic membranes of these cells and, consequently, to more complete absorption and delayed excretion of the drug substrate P-gp.

According to the NCBI SNP (Database, 2021), the average frequency of the T allele in the world population is 42%. The frequency of the C allele in Americans is 40% and in Europeans, it is 42%. In contrast, in the African population, the frequency of the C allele (14%) is lower than that in the American and European populations. The frequency of the T allele in the American population is 60%, which is slightly lower than that in Europeans (58%) and in the African population, the frequency of the T allele is 86%. Thus, the distribution frequencies of the C (41.3%) and T (58.7%) alleles in the Uzbek population are comparable to those in the American and European populations. The presented statistical indicators of our research may differ with an increase in the sample for the study of population genetics. However, most articles present the results of studies conducted with a small number of samples.

The first MDR1 polymorphism identified was G2677T/A (Mickley *et al*., 1998). Among all SNPs, G2677T/A is the only polymorphism that results in an amino acid substitution (Ala893Ser/Thr) with three possible variants at the same gene locus. This nonsynonymous polymorphism has a major impact on both ATPase activity and PGP substrate specificity for various drugs (Sakurai *et al*., 2007).

According to the SNP database (Ensembl genome browser https://asia.ensembl.org/Homo_sapiens/Variation/Population?db = core; r = 7:87530745-87531745; v = rs2032583;vdb = variation; vf = 729606037), the frequency of the 893-serine-containing 2677T allele is 33% in the world population. The frequency of 893Ala/Ala homozygotes (2677 GG genotype) exceeds 98% in African populations; in Americans and Europeans, this figure is slightly lower (57%) and in Asians, it varies between 36 and 47%. The frequency of the occurrence of the 893-threonine-containing 2677A allele in different ethnic groups varies from 0 to 13%. In a study by Afsar *et al*. (2019), it has been shown that the Pakistani population had a higher prevalence of the 2677G>T/A polymorphism of the MDR1 gene compared to other residents. In numerous detection studies, potential phenotypic associations with G2677T/A provided evidence for the association of the 893Ser allele with altered P-gp activity and expression (Hitzl *et al*., 2001; Goto *et al*., 2002).

A conserved polymorphism in exon 26 (C3435T) is the most common SNP of the MDR1 gene. Despite being silent, this SNP affects P-gp function by changing its substrate specificity (Kimchi-Sarfaty *et al*., 2007b). It was found that the response to drug treatment correlated with the frequency, allele variability, and genotype of C3435T. In a study by Talaat *et al*. (2018), it was noted that the CT genotype of the C3435T polymorphism may be associated with an unfavorable outcome of acute lymphoblastic leukemia.

The frequency of the 3435C allele was found to be 43% in Portuguese individuals, 46% in Russians, and 48% in Malaysians; the frequency of the occurrence of the 3435T allele in the above populations was 57, 54, and 52%, respectively. The frequency of the C allele was found to be 52% in Spaniards, 55% in Saudis, 83% in Kenyans, 53% in Chinese individuals, and 61% in Japanese individuals; the frequency of the occurrence of the T allele in the above populations was 48, 45, 17, 47 and 39%, respectively (Sakaeda *et al*., 2004).

The comparative analysis of data showed that the frequency of the distribution of alleles of the C3435T polymorphism (C-43, T-57%) among volunteers living in Uzbekistan was comparable to that in the Portuguese, Russian, and Malaysian populations but different from that in the Spanish, Chinese, Japanese, Kenyan populations.

Conclusions. In the present study, for the first time, population data on the MDR1 gene were obtained and the distribution frequency of its C1236T, G2677T/A, and C3435T polymorphisms in a sample of apparently healthy volunteers living in Uzbekistan was estimated.

Among clinically healthy volunteers, polymorphisms C1236T and C3435T of the MDR1 gene have a high frequency of occurrence. The present study has some limitations, including the small sample size, which has affected the ability to perform certain statistical analyses. Therefore, further studies involving more samples are needed to confirm the reliability of the results of the present study. After that, these polymorphisms can be recommended for use as molecular genetic markers in pharmacogenetic testing of the population of Uzbekistan.

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Author's Contributions

S.B. Nurmatova: Conducted experiments in Fig. 1, 2, and 3 and wrote a draft of the article.

A. A. Abdurakhimov: Processed the statistical data of the manuscript.

O. S. Charishnikova: Participated in experiments and wrote a literature review of the manuscript.

Sh. U. Turdikulova: Designed and supervised the experiment, and final review of the article.

D. A. Dalimova: Supervised the execution of the study, and edited the latest version of the manuscript.

Ethics

This study was approved by the Ethics Committee of the Ministry of Health of the Republic of zbekistan under the number No. 3/1-1023 (March 30, 2019).

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