



## Frequency of common MDR1 gene variants in a Polish population

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### Abstract:

P-glycoprotein (P-gp) is a transmembrane transporter playing an important role in drug efflux. There is growing evidence that P-gp activity may be related to haplotypes of *MDR1* gene. In the current study, the frequencies of common functional polymorphisms in *MDR1* gene (2677G > A,T and 3435C > T) were evaluated using PCR-RFLP and allele-specific amplification, in a group of 204 healthy individuals of Caucasian origin from Poland. It was found that the frequencies of the studied single nucleotide polymorphisms were similar to those reported for other Caucasian populations, and were as follows: 2677G-3435C – 0.453, 2677G-3435T – 0.143, 2677T-3435C – 0.015, 2677T-3435T – 0.370, 2677A-3435C – 0.008, 2677A-3435T – 0.011. The results of our study may give the basis for predicting pharmacokinetic and pharmacodynamic effects of many commonly used drugs in the Polish population.

### Key words:

pharmacogenetics, MDR1, P-glycoprotein, genetic polymorphism, MDR1 haplotypes

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**Abbreviations:** ABC – ATP-binding cassette, AUC – area under curve, LD – linkage disequilibrium, MDR1 – multidrug resistance-1, PCR-RFLP – polymerase chain reaction – restriction fragment length polymorphism, PBMCs – peripheral blood mononuclear cells, P-gp – P-glycoprotein, SNP – single nucleotide polymorphism

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### Introduction

P-glycoprotein (P-gp) is a transmembrane transporter playing an important role in drug efflux. This product of human *MDR1* (*ABCB1*) gene was primarily associated with resistance to multiple cytotoxic anticancer agents due to its overexpression in tumor cells [27]. Subsequently, P-gp was found to be widely expressed in most of blood-tissue barriers, including the liver,

kidneys, intestine, blood-brain barrier, endothelium, placenta, ovaries, testes, salivary glands. Additionally, P-gp has been detected in hematopoietic stem cells, PBMCs and macrophages, suggesting its physiological function [6, 26, reviewed in 20]. P-gp transports a wide range of chemically divergent substrates, including a variety of pharmacologically distinct agents, i.e. anticancer, antihypertensive agents, antiarrhythmics, glucocorticoids, antiviral drugs, antibiotics, immunosuppressants, antidepressants, neuroleptics, opioids and many others [20]. The broad substrate specificity and tissue localization of P-gp suggest that this transporter plays a significant role in bioavailability, distribution and excretion of many drugs. Experiments with P-gp deficient mice revealed that in animals lacking the transporter expression, oral absorption and brain accumulation of drugs was greatly increased, leading to toxic concentrations [20]. Additionally, knockout

mice were also more prone to colitis, probably caused by accumulation of bacterial toxins in the intestine [21]. Because of its crucial role in pharmacokinetics, many studies were undertaken focusing on factors responsible for interindividual differences in P-gp expression and activity, i.e. P-gp inhibitors, inducers and also a potentially important role of genetic factors. Recently, genetic variations of *MDR1* gene have been studied very extensively, reporting over 50 single nucleotide polymorphisms (SNPs) and 3 insertion/deletion polymorphisms naturally occurring in different populations [14]. Among many variants, 3435C > T in exon 26 and 2677G > T/A in exon 21 (Ala893Ser/Thr) were the most extensively evaluated in relation to P-gp expression. *MDR1* 3435C > T is a silent polymorphism, not resulting in a change in amino acid sequence. Unexpectedly, it was associated with altered P-gp expression and substrate drug pharmacokinetics [12], and more recently also with increased risk of some diseases (renal tumor, Crohn's disease, ulcerative colitis, Parkinson's disease, HIV infection) [20]. However, results of subsequent studies with different P-gp substrates and in ethnically different populations were discrepant, as some researchers reported lower P-gp expression in 3435TT homozygotes, some found higher activity of the transporter in 3435TT subjects, and some did not observe any difference [9, 12, 13, 16, 20]. Although several hypotheses have been raised to clarify influence of the silent polymorphism on altered P-gp function, molecular background of this effect is still unclear. Recently, it has been reported that a silent 3435C > T SNP may change mRNA stability, thus it can be considered as a determinant of *MDR1* expression [28]. Multiple studies have demonstrated linkage disequilibrium between 3435C > T polymorphism in exon 26 and other SNPs, especially 2677G > T/A in exon 21, suggesting that the functional effects may be rather haplotype-dependent. Indeed, superiority of haplotype analysis in predicting pharmacokinetics of some drugs and a risk of disease development were recently demonstrated [5, 16, 23]. Available data indicate that the frequencies of *MDR1* haplotypes differ in various populations. In the current study, the authors attempted to evaluate the frequencies of *MDR1* 2677G > T/A and 3435C > T alleles and haplotypes in a Polish population, as haplotype determination may prove to be important when the *in vivo* functional consequences of *MDR1* variants are finally assessed.

## Material and Methods

Two hundred four unrelated healthy Polish subjects of Caucasian origin from West Pomeranian region of the country, 111 males and 93 females, aged from 21 to 72 years (mean 45.5) were included in this study after giving informed consent. The Ethics Committee of the Pomeranian Medical University in Szczecin, Poland approved protocol of the study.

Genomic DNA was extracted from 450 µl of whole blood samples using a non-organic and non-enzymatic extraction method [11]. To analyze 2677G > A/T polymorphism, an allele-specific PCR method was developed. The following set of primers was used: FA: 5'-TGA AAG ATA AGA AAG AAC TAG AAG GTA-3', FG: 5'-TGA AAG ATA AGA AAG AAC TAG AAG GTG-3', FT: 5'-TGA AAG ATA AGA AAG AAC TAG AAG GTT-3' – forward sequence-specific primers, FK: 5'-AGC AAA TCT TGG GAC AGG AA-3' – a forward internal control primer and RR: 5'-AGT CCA AGA ACT GGC TTT GC-3' – a common reverse primer. Three separate amplification reactions were performed for each DNA sample for detection of 2677G, 2677T and 2677A alleles, using Eppendorf Mastercycler (Hamburg, Germany). Amplification mix (15 µl) contained 0.7 µM of an applicable allele-specific primer, 0.2 µM of a control primer, 0.7 µM of a reverse primer, 60–120 ng of genomic DNA, 0.5 U of RedTaq Polymerase (Sigma) in 1 × enzyme-specific buffer (containing magnesium chloride at final concentration of 1.1 mM) and deoxynucleoside triphosphates (Sigma), 200 µM of each. The temperature profile was: initial denaturation at 94°C for 4 minutes, 8 cycles at 57/72/94°C for 30 seconds, followed by 25 cycles at 55/72/94°C for 30 seconds with a final extension step at 72°C for 7 minutes. The PCR products were analyzed in 2% agarose gels, stained with ethidium bromide. Amplified control DNA fragment (353 bp) was followed by allele-specific fragment (222 bp) in a presence of the detected allele. Genotyping results were confirmed by sequencing of DNA fragments, obtained using FK and RR primers, for each genotype variant detected (2677GG, 2677GT, 2677TT, 2677TA and 2677GA, respectively). Genotyping for the presence of 3435C > T SNP was performed using previously described PCR-RFLP method with *Sau3AI* endonuclease [22].

Genotype and allele frequencies were calculated by direct counting and then dividing by the number of

**Tab. 1.** Frequencies of common SNPs in MDR1 gene in Polish compared with other populations

Population studied	Reference	2677G > A/T			3435C > T	
		G	T	A	C	T
Polish Caucasians n = 204	this study	0.595	0.385	0.020	0.475	0.525
German Caucasians n = 461	4	0.564	0.416	0.019	0.461	0.539
British Caucasians n = 190	2	ND	ND	ND	0.480	0.520
American Caucasians n = 100	18	0.500*	0.464*	0.036	0.439	0.561
Russian Caucasians n = 290	8	0.548	0.419	0.033	0.457	0.543
Spanish Caucasians n = 408	3	ND	ND	ND	0.520	0.480
African Americans n = 100	18	0.895**	0.100**	0.005	0.798	0.202**
Ghanians n = 206	2	ND	ND	ND	0.830	0.170**
Beninese n = 111	1	0.991**	0.009**	0*	0.860	0.140**
Japanese n = 117	13	0.440**	0.360	0.200**	0.620	0.480**
Chinese n = 104	24	0.505*	0.437*	0.058*	0.596	0.404**
Polish Caucasians, Central Poland n = 122	15	ND	ND	ND	0.623	0.377**
Polish Caucasians, West Pomerania n = 139	24	0.576	0.414	0.011	0.478	0.522
Polish Caucasians, Silesia n = 188	19	ND	ND	ND	0.505	0.495

$\chi^2$  test, only significant values are indicated; ND – not determined; \*  $p < 0.05$ ; \*\*  $p < 0.01$

subjects or the number of chromosomes to produce genotype and allele frequencies, respectively. The data were tested for their fit to Hardy-Weinberg equilibrium by calculating expected frequencies of genotypes and comparing them to the observed values using a  $\chi^2$  test (Statistica 6.0, Statsoft). The  $\chi^2$  test (with Yate's correction when applicable) was used to compare the observed allele and genotype frequencies to published data for other populations. EH program (Jurg Ott, Rockefeller University, New York) was used to test linkage disequilibrium between two analyzed loci and estimate MDR1 2677–3435 haplotype frequencies.

3435CT – 51.0% (n = 104) and C3435TT – 27.0% (n = 55). Frequencies of the studied alleles are given in Table 1. The genotype frequency distribution for MDR1 2677 and 3435 loci in the studied population did not show a significant deviation from Hardy-Weinberg equilibrium. On the basis of the observed frequencies of MDR1 2677 and 3435 combined genotypes, a mathematical analysis was performed to estimate frequencies of 2677–3435 haplotypes in a Polish population. A strong linkage disequilibrium (LD) between these two loci was found, resulting in haplotype frequencies significantly different from expected in case of lack of such an association (Table 2).

## Results

In 204 subjects studied, 38.7% (n = 79) were homozygous for 2677G allele, 17.6% (n = 36) for 2677T allele, and 39.7% (n = 81) were 2677GT heterozygotes. Eight subjects (4.0%) carried rare 2677A allele, 4 of them were 2677GA heterozygotes (2.0%), 4 – 2677TA heterozygotes (2.0%). The following MDR1 3435 genotype frequencies were observed: 3435CC – 22.0% (n = 45),

## Discussion

The MDR1 gene spans almost 100 kbp in human genome, containing over 50 SNPs identified in Caucasians up to date. Only few of them occur with significant frequency, and most of them are silent or localized in gene introns. Among commonly observed SNPs (frequency over 10%), only 2677G > T/A leads to amino acid change (Ala893Ser, Thr) in Caucasian

**Tab. 2.** Linkage disequilibrium in *MDR1* gene: comparison of *MDR1* 2677–3435 haplotype frequencies expected in case of no association between two loci and estimated on the basis of genotyping results

Haplotype	Symbol	Frequency expected in case of no association	Frequency estimated on the base of genotyping results	p value*
2677G–3435C	11	0.283	0.453	<10 <sup>-5</sup>
2677G–3435T	12	0.312	0.143	<10 <sup>-5</sup>
2677T–3435C	21	0.183	0.015	NS
2677T–3435T	22	0.202	0.370	<10 <sup>-5</sup>
2677A–3435C	31	0.009	0.008	<10 <sup>-5</sup>
2677A–3435T	32	0.010	0.011	NS

\*  $\chi^2$  test with Yate's correction when applicable; NS – not significant

population, which may result in altered P-gp function and activity [4]. The current study confirms previous observations that there is a tight linkage disequilibrium between 3435 and 2677 loci, which is one of the explanations of association of *MDR1* 3435 variants with different functional phenotypes. Our results give the evidence that the frequencies of *MDR1* alleles, haplotypes and thus genotypes determined, are very similar to the respective frequencies observed in other European populations (Table 1, Table 3) [2, 3, 4, 8, 16, 18]. The frequency of 3435T variant, most often associated with a decreased P-gp activity, is significantly higher compared to populations of African and Asian origin [1, 2, 13, 18, 25]. The results of the current study, like three other including Polish subjects [7, 19, 24], are contradictory to observations reported

by Jamroziak et al. [15], who had suggested an increased frequency of 3435C allele in a Polish population. As loci 2677 and 3435 are in LD, increased frequency of 3435C allele would have to be followed by more frequent occurrence of 2677G allele, which was also not reported [24]. On the basis of these facts, one can assume that higher frequency of 3435C allele determined in a group of 122 individuals was probably accidental [15].

The distribution of alleles in locus 2677 was also similar to most European populations [3, 4, 8]. The frequency of 2677G allele, considered as wild-type, was significantly lower than that observed in Africans and African Americans, whereas 2677A allele seems to be more frequent in Asians and probably absent in Africans [1, 13, 25].

To our knowledge, this is the first attempt to estimate *MDR1* 2677–3435 haplotype frequencies in a Polish population. There is growing evidence that functional effects in P-gp activity may be related to haplotypes and diplotypes of *MDR1* gene. It has recently been shown that haplotype 2677G–3435T was associated with significantly higher and haplotype 2677G–3435C with lower AUC values after oral digoxin administration, and haplotype analysis was superior to SNP analysis in predicting *MDR1* phenotype [16]. In another study with fexofenadine as a probe drug, 1236T–2677T–3435T haplotype carriers represented higher P-gp activity compared to non-carriers [17]. On the basis of our results, one can expect that genetically determined variations in P-gp activity in Poles would be similar to those observed in other populations of Caucasian origin, and on the other hand, probably different when compared to Africans and Asians [1, 10, 18]. There are still some discrepan-

**Tab. 3.** *MDR1* 2677–3435 haplotype frequencies in Polish compared with other populations

Population studied	Reference	2677G–3435C	2677G–3435T	2677T–3435C	2677T–3435T	2677A–3435C	2677A–3435T
Polish Caucasians n = 204	this study	0.453	0.143	0.015	0.370	0.008	0.011
German Caucasians n = 687**	16	0.433	0.133	0.018	0.416	ND	ND
Russian Caucasians*** n = 290	8	0.422	0.126	0.012	0.407	0.017	0.016
American Caucasians n = 100	18	0.383	0.127	0.034	0.420	0.026	0.010
African Americans n = 100	18	0.783*	0.117	0.015	0.085*	0.005	0
Beninese n = 111	1	0.853*	0.138	0.005	0.005*	0	0
Japanese n = 69	10	0.420	0.022*	0.022	0.412	0.116*	0.008

$\chi^2$  test, only significant values are indicated; ND – not determined; \* p < 0.01; \*\* sum-up of several studies; \*\*\* estimated on the basis of the provided results

cies among results from different studies, mainly due to ethnic differences, the use of different P-gp substrates, with different specificity for drug-metabolizing enzymes and other transporters and different haplotype determination techniques (mathematical modeling or direct molecular haplotype determination). Additionally, most of findings suggested rather slight influence of MDR-1 gene polymorphism on P-gp expression. MDR-1 expression can be induced and significantly increased by many environmental factors, which makes analysis of influence of genetic factors on drug pharmacokinetics even more complex. However, in the analysis of the findings some factors should be considered: only two most common SNPs (of over 50 reported) were genotyped. The 2677G > T/A SNP in exon 21, potentially influences MDR1 activity, as it alters amino acid sequence (Ala893Ser/Thr). The other one is a silent polymorphism 3435C > T in exon 26. Recently, it has been reported that 3435C > T SNP may change mRNA stability [28]. Other genetic determinants of MDR1 gene expression and/or P-gp activity might also exist, however, on the basis of present knowledge, 2677G > T/A and 3435C > T can be considered as two main genetic factors implicated in MDR1 expression. However, the authors believe that finally the consensus in the matter of the influence of genetic factors on P-gp function will be reached and this study will be of some relevance in predicting *MDR1* phenotype and pharmacokinetics as well as pharmacodynamic effects of many commonly used drugs in the Polish population.

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