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Polymorphism of the Human *MDR1* Gene in Siberian and Central Asian Populations

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Abstract—The multidrug resistance gene *MDR1* (*ABCB1*) codes for a P-glycoprotein that acts as an ATP-dependent transporter and is involved in removing drugs, xenobiotics, and peptides from the cell. *MDR1* is expressed in the brain, kidneys, liver, and gastrointestinal tract. The P-glycoprotein is thought to play a role in individual resistance to xenobiotics and infections. Several polymorphisms of *MDR1* are associated with the level of its expression and resistance to various neurodegenerative and gastrointestinal diseases. The allele and haplotype frequencies, genetic differentiation, and linkage disequilibrium for five *MDR1* single nucleotide polymorphisms (3435C/T, 2677G/T/A, 1236C/T, +139C/T, and –1G/A) were studied in the Russian, Tuvinian, and northern and southern Kyrgyz populations. Significant genetic differences were observed between Russians and northern Kyrgyz and between Tuvinians and northern Kyrgyz. The linkage disequilibrium pattern was characterized by high population specificity.

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Key words: human populations of Siberia and Central Asia, linkage disequilibrium, single-nucleotide polymorphism, multidrug resistance gene

INTRODUCTION

The P-glycoprotein encoded by the multidrug resistance gene *MDR1* (*ABCB1*) acts as a transmembrane transporter of various lipophilic agents (xenobiotics, drugs, peptides, and virus particles) [1–5]. *MDR1* (209,660 bp) is on chromosomes 7q21.1, contains 29 exons, and is transcribed to produce a 4916-nt RNA [6, 7]. The P-glycoprotein belongs to the family of ATP-dependent membrane transporters, consists of 1280 amino acid residues, and is 141,462 Da. Its function is to transfer drugs and other compounds from the cell into the intercellular space. The P-glycoprotein has been associated with cancer cell resistance to chemotherapy [8, 9] and is implicated in multiple drug resistance, which correlates with the amplification of a nucleotide sequence termed *mdr* [10–12]. Cancer cells with an elevated level of the P-glycoprotein are resistant to various anticancer drugs [13]. The P-glycoprotein is synthesized in the intestine, kidneys, liver, and vascular endothelium of the brain [14–17].

The functions and expression pattern of the P-glycoprotein suggest its role in resistance to various toxins. Acting in various organs, the P-glycoprotein facilitates xenobiotic excretion with urine and bile and through the gastrointestinal tract [18].

Many works have focused on the functional polymorphism of *MDR1*, analyzing the correlation of various single nucleotide polymorphisms (SNPs) with the level of *MDR1* expression and the transporting activity of the P-glycoprotein [5, 19, 20]. SNPs have been tested for the effect on the P-glycoprotein ability to remove drugs from the cell, which is important for chemotherapy of cancer. Some *MDR1* SNPs have been associated with neurodegenerative and gastrointestinal diseases [13, 21, 22]. Another aspect of *MDR1* functioning is important from the viewpoint of human evolutionary genetics. There is evidence that *MDR1* is involved in human resistance to various infections, conferring resistance to bacterial toxins and viruses [3, 4, 23]. Infections have been the most potent factor of natural selection in *Homo sapiens* populations throughout their history (including modern). Hence, the genetic variation of *MDR1* in modern populations is, to a great extent, a product of natural selection.

The objectives of this work were to estimate the genotype and haplotype frequencies for five *MDR1* SNPs and to study the linkage disequilibrium pattern in several geographic and ethnic groups (Russians, Tuvinians, and northern and southern Kyrgyz).

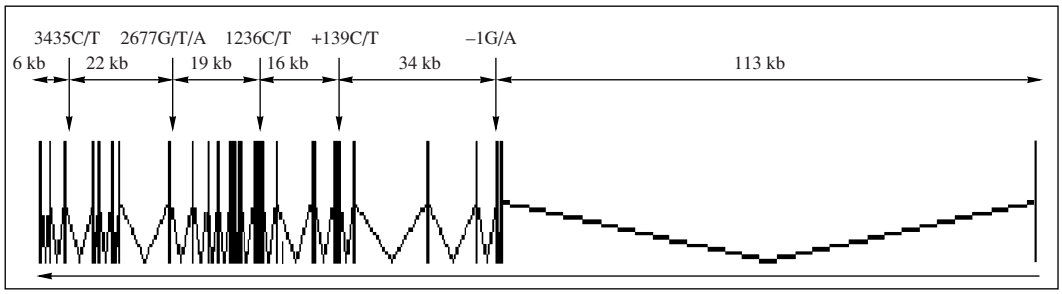

Fig. 1. Map of *MDR1* and its SNPs.

EXPERIMENTAL

Sample. We examined four populations: Russians (N = 90) from Tomsk; Tuvinians (N = 142) from the village of Bai-Taiga, Tyva Republic; southern Kyrgyz (N = 44) from Osh; and northern Kyrgyz (N = 41) from Bishkek, Kyrgyzstan. The total sample included 317 people.

SNPs. We examined five SNPs: 3435C/T (rs1045642), 2677G/T/A (rs2032582), 1236C/T (rs1128503), +139C/O (rs1202168), and –1G/A (rs2214102), which are regularly distributed in the *MDR1* coding region (Fig. 1). The 3435C/T and 1236C/T sites are in exons 26 and 12, respectively, corresponding to cDNA positions 3435 and 1236. Although substitution C/T in these sites does not change the amino acid residue, 3435C/T has been associated with the level of *MDR1* expression and the function of the P-glycoprotein [5]. Site 2677G/T(A) is in exon 21; substitutions G/A and G/T cause substitution of Ser or Thr for Ala, respectively [22]. Site 1G/A is in a noncoding region of exon 2 [5, 24]. Site +139C/T is in intron 6 [5].

Genotyping employed the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis. PCR and digestion with restriction enzymes followed a published protocol [24]. The product was electrophoretically resolved in 2 or 3% agarose gel, stained with ethidium bromide, and visualized in transmitted UV light.

Statistical analysis. Genetic diversity and interpopulation differentiation were evaluated by AMOVA, using the ARLEQUIN software package. The linkage and block structure of haplotypes were analyzed with the Haploview 3.2 program. A dendrogram of genetic relationships of the populations was plotted using the PHYLIP program.

RESULTS AND DISCUSSION

We examined five *MDR1* SNPs distributed through a region of about 100 kb, including all coding exons.

The allele frequencies, heterozygosities, and the significance level for the SNPs are summarized in Table 1. The allele frequency distributions of all SNPs in all populations obeyed the Hardy–Weinberg equilibrium. High heterozygosity was observed in most cases with the exception of the –1G/A locus, which was virtually monomorphic in all populations. This agreed with published data [19, 20, 24, 25].

Genetic differentiation of the populations with respect to the allele frequencies of the five *MDR1* SNPs was estimated with the F_{st} statistics at 1.18%. The results obtained for each SNP in the total sample are shown in Fig. 2. Genetic differentiation was significant with respect to 3435C/T, 2677G/T(A), and 1236C/T and nonsignificant with respect to +139C/T and –1G/A ($P < 0.01$). The difference between these two groups of SNPs is that the former is in the coding and the latter is in the noncoding region of *MDR1*. Hence, it is possible to assume that SNPs of the first group are adaptive or are linked to other adaptive polymorphic sites: their variation is probably limited by selection and, consequently, interethnic differences are lower than in the case of nonfunctional haplotypes.

Analysis of the interpopulation differentiation with respect to the *MDR1* allele frequencies revealed significant differences ($P < 0.05$) between Russians and

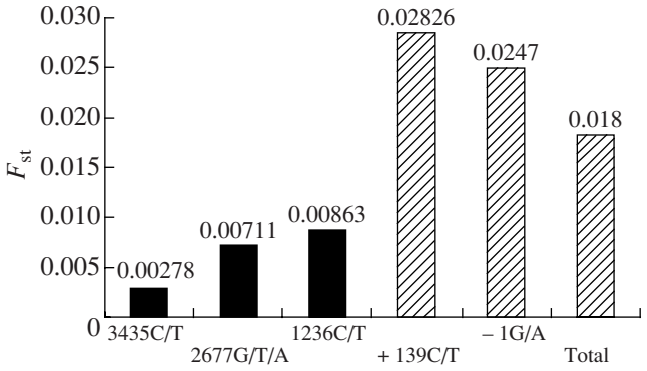

Fig. 2. Total genetic differentiation of the total sample by all *MDR1* SNPs examined. Crosshatched columns show significant differences ($P < 0.05$).

Table 1. Allele frequencies, heterozygosity, and significance level for five *MDR1* SNPs

SNP, population	Allele frequency			Observed heterozygosity	Expected heterozygosity	Significance level*
C3435T	C	T				
Russians	0.43	0.57		0.44	0.49	0.3940
Tuvinians	0.43	0.57		0.46	0.49	0.4810
Northern Kyrgyz	0.39	0.61		0.60	0.47	0.1690
Southern Kyrgyz	0.49	0.51		0.42	0.50	0.3670
G2677T(A)	G	T	A			
Russians	0.51	0.47	0.02	0.51	0.50	1.0000
Tuvinians	0.47	0.43	0.09	0.56	0.49	0.1050
Northern Kyrgyz	0.57	0.36	0.08	0.43	0.48	0.6770
Southern Kyrgyz	0.59	0.35	0.06	0.42	0.46	0.7530
C1236T	C	T				
Russians	0.55	0.45		0.48	0.50	0.9070
Tuvinians	0.46	0.54		0.57	0.50	0.1130
Northern Kyrgyz	0.40	0.60		0.43	0.49	0.5910
Southern Kyrgyz	0.43	0.57		0.54	0.49	0.7200
+139C/T	C	T				
Russians	0.55	0.45		0.51	0.49	1.0000
Tuvinians	0.54	0.46		0.49	0.50	0.9000
Northern Kyrgyz	0.78	0.22		0.30	0.35	0.5800
Southern Kyrgyz	0.56	0.44		0.48	0.49	1.0000
–1G/1	G	A				
Russians	0.97	0.03		0.07	0.07	1.0000
Tuvinians	1.00	0.00		0.00	0.00	1.0000
Northern Kyrgyz	0.97	0.03		0.05	0.05	1.0000
Southern Kyrgyz	0.96	0.04		0.08	0.08	1.0000

* Significance level for the correspondence to the Hardy–Weinberg equilibrium.

northern Kyrgyz (F_{st} = 3.313%) and between Tuvinians and northern Kyrgyz (F_{st} = 2.574%).

The haplotype frequencies for the five SNPs were computed using the expectation maximization (EM) algorithm implemented in the ARLEQUIN software package. The results are summarized in Table 2.

A combination of the alleles of four diallelic loci and one triallelic locus theoretically yields 48 haplotypes. Of these, 29 were observed in the populations examined. We found 19 haplotypes in Russians, 20 in Tuvinians, 16 in southern Kyrgyz, and 14 in northern Kyrgyz. The haplotypes occurring at a frequency of more than 4% in any group were regarded as major haplotypes (mhs). The threshold of 4% was chosen according to the approach used [25]. In total, 13 mhs (mh1–mh13) were observed, including five in Russians, eight in Tuvinians, nine in southern Kyrgyz, and seven in northern Kyrgyz. Four mhs (mh2, mh8, mh12, and mh13) were common for all populations and together accounted for more than 61% of all chromosomes of the total sample. The highest frequencies were observed for mh2 and mh13, each occurring at a frequency of 21%.

Despite the difference in haplotype number, Kyrgyz and Tuvinians displayed similarly high haplotype diversity (0.87 and 0.89, respectively) and similar frequencies of the most common haplotypes. Substantial genetic diversity of these populations has been observed with other markers, including Y-chromosome haplotypes and several autosomal markers [26].

Table 2. Haplotype frequencies of *MDR1* in the Russian, Tuvinian, northern Kyrgyz, and southern Kyrgyz populations

Main haplotype (mh)	Haplotype*	Population, haplotype frequency			
	1 2 3 4 5	Russians	Tuvinians	southern Kyrgyz	northern Kyrgyz
	C A C C A			0.74	
mh1	**C A C C G****	1.58	3.05	2.69	**7.50**
	C A C T G		3.34		1.25
	C A T C G		0.64		
	C G C C A	1.59		2.32	
mh2	**C G C C G**	**28.85**	**16.65**	**22.44**	**15.94**
mh3	**C G C T G**	3.80	**6.83**		2.76
	C G T C G	3.25	1.24	3.30	1.87
mh4	**C G T T G**	1.83	**4.19**	**11.10**	**4.20**
mh5	**C T C C G**	0.38		**4.05**	
	C T C T G		0.42		
mh6	**C T T C G**	1.36	**4.02**		2.72
mh7	**C T T T G**	0.51	3.64	**5.40**	
	T A C C G		1.24	1.67	
	T A C T G			1.02	
	T A T C G		0.89		
mh8	**T G C C G**	**7.93**	**10.40**	**7.92**	**10.86**
	T G C T A	0.52		1.02	
	T G C T G	2.70	2.06		
	T G T C A	0.59			1.25
mh9	**T G T C G**		2.66	**4.21**	**15.61**
mh10	**T G T T G**		**4.92**	**6.87**	
	T T C C A				1.25
mh11	**T T C C G**	**4.21**	0.43		1.68
	T T C T A	0.55			
	T T C T G	3.16	1.71		
mh12	**T T T C G**	**5.53**	**12.66**	**4.74**	**18.81**
	T T T T A	1.50			
mh13	**T T T T G**	**30.17**	**19.01**	**20.51**	**14.29**
Total haplotypes		19	20	16	14
Haplotype diversity *H*		0.81	0.89	0.87	0.87

Notes: * SNPs: 1, 3435C/T; 2, 2677G/T/A; 3, 1236C/T; 4, +139C/T; 5, –1G/A.
** Haplotypes shown in bold and shadowed occur at a frequency of more than 4%.

This fact probably reflects the contribution of originally different (ancient Caucasian and Mongoloid) components to the gene pools of these populations.

Russians displayed lower haplotype diversity (H = 0.81) with a higher number of the observed genotypes, which was explained by the high (about 30%) frequencies of two mhs (mh2 and mh13).

To compare our findings with data on other populations, mh2 is similarly widespread in the Chinese, Malay, Hindu, Caucasian, and Afro-American popula-

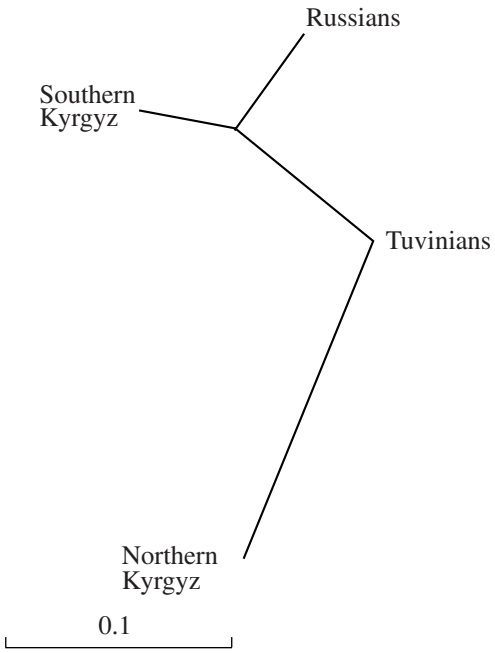

Fig. 3. Tree of Siberian and Central Asian populations with genetic distances computed from the *MDR1* haplotype frequencies.

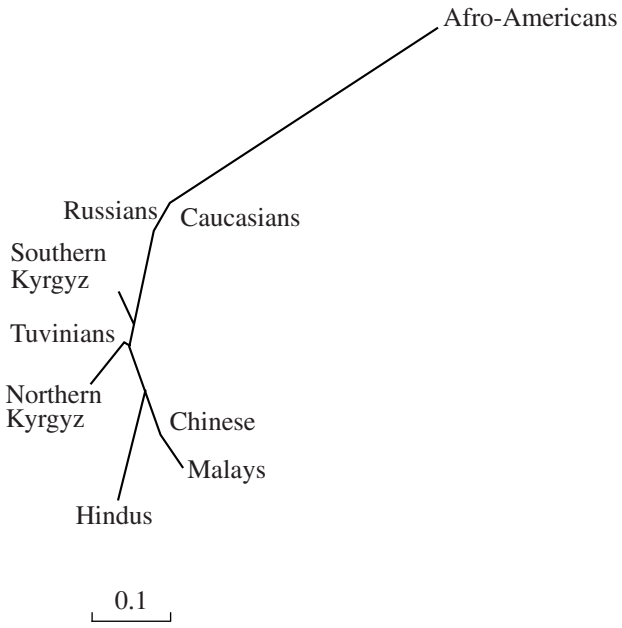

Fig. 4. Tree of world populations with genetic distances computed from *MDR1* haplotype frequencies.

tions and mh13 is widespread in the same populations with the exception of Afro-Americans.

A tree of genetic distances between the four populations examined was constructed on the basis of the *MDR1* haplotype frequencies (Fig. 3). Our findings testify to a high genetic differentiation of the Kyrgyz. Northern Kyrgyz are closer to Tuvinians than to southern Kyrgyz on our tree. In addition, Russians, Tuvinians, and southern Kyrgyz cluster somewhat separately from northern Kyrgyz.

Based on the haplotype frequencies of three SNPs (3435C/T, 2677G/T(A), and 1236C/T), we constructed a tree of genetic distances for our populations, Malays, Chinese, Hindus, Caucasians, and Afro-Americans [25] (Fig. 4). Our populations cluster separately on the tree. The closest group is Caucasians, which agrees with the ethnic history and geography of migrations of Russians, Tuvinians, and Kyrgyz. Generally, the topology of the tree reflects the structure of genetic diversity in populations, assuming a recent African origin for modern humans.

The linkage disequilibrium patterns of the five *MDR1* SNPs are shown in Fig. 5. The patterns were population-specific. Extended linked blocks were not detected in any population. We observed only some blocks of two closely spaced SNPs with a high linkage coefficient ($D' > 0.85$). However, the composition of the blocks differed among the populations.

The closest linkage was observed for 3435C/T and 2677G/T(A) in Russians ($D' = 0.87$), 2677G/T/A and 1236C/T in Tuvinians ($D' = 0.86$), and 1236C/T and +139C/T in southern Kyrgyz ($D' = 0.93$). It is noteworthy that different linkage disequilibrium patterns of the locus under study were found even in the two originally close populations of southern and northern Kyrgyz.

It is clear that the specificity of linkage disequilibrium patterns in human populations requires close investigation in the context of genetic studies of high-incidence disorders and genetic mapping based on association testing in population samples.

To summarize, the allele frequencies of the five SNPs in our populations agree with those observed in other human populations. In particular, this is true for –1G/A, which displays an extremely low polymorphism. Significant differences in SNP allele frequencies ($P < 0.05$) were observed between Russians and northern Kyrgyz and between Tuvinians and northern Kyrgyz. Although the haplotype distribution was population-specific, haplotypes 3435/G2677/C1236/C+139/G–1, T3435/G2677/C1236/C+139/G–1, T3435/T2677/T1236/C+139/G–1, and T3435/T2677/T1236/T+139/G–1 occurred at high frequencies (4.74–30.17%) in all ethnic groups examined. The linkage disequilibrium pattern proved to differ among the populations. Some *MDR1* SNPs displayed

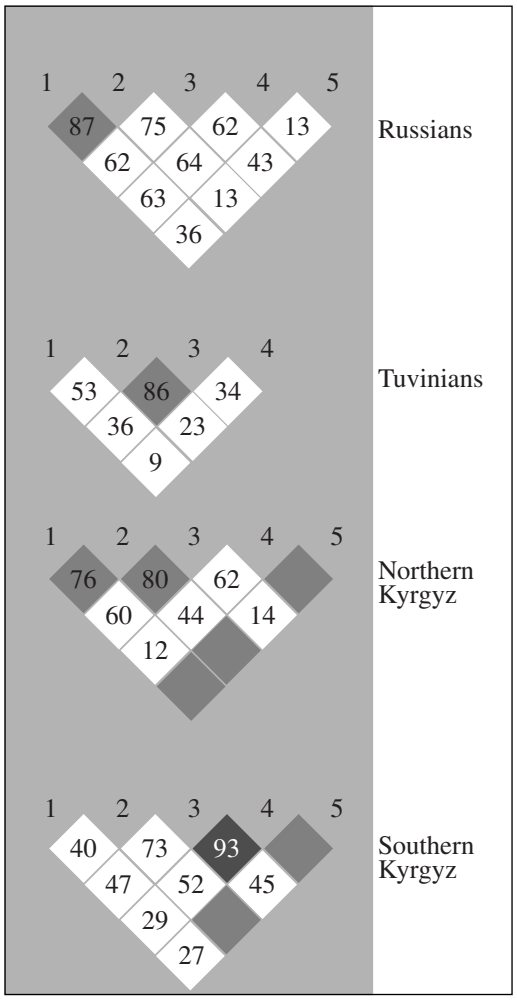

Fig. 5. Patterns of linkage disequilibrium at *MDR1* in Siberian and Central Asian populations. The linkage coefficient *D*' is indicated in the cells. SNPs: 1, 3435C/T; 2, 2677G/T/A; 3, 1236C/T; 4, +139C/T; 5, –1G/A. The linkage is strong (dark gray), considerable (gray), or weak (white). The absence of *D*' in a cell indicates that its computation was impossible because of the low frequency of the rare allele of –1G/A.

close linkage, but the composition of the linkage blocks was population-specific.

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