ONCOLOGY

MDR1 Gene $C^{1236}T$ and $C^{6+139}T$ Polymorphisms in the Russian Population: Associations with Predisposition to Lymphoproliferative Diseases and Drug Resistance

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Study of *MDR1* polymorphism in intron 6 and exon 12 of healthy individuals and patients with chronic lymphoproliferative diseases showed that the presence of mutant 6+139T allele is a factor determining resistance to lymphoproliferative diseases. Comparison of genotyping results in 53 patients and the data on the efficiency of drug therapy showed no significant associations of $C^{6+139}T$ and $C^{1236}T$ genotypes with drug resistance.

Key Words: *MDR1/P-glycoprotein; genetic polymorphism; lymphoproliferative diseases; drug resistance*

MDR1 gene product P-glycoprotein is an ATP-dependent enzyme realizing transmembrane transport of endogenous compounds and xenobiotics from the cell and thus protecting the body from many toxic compounds including antitumor drugs [3,4]. The function of P-glycoprotein can be a mechanism of multiple drug resistance and a cause of inefficient chemotherapy in malignant tumors [9]. By the present time about 50 single nucleotide substitutes are known in *MDR1* gene, the majority of them are silent mutations or situates in the intron regions [7]. Until present, the functional and clinical significance of mutations in the encoding part of the gene is known only for silent $C^{3435}T$ mutation [5] and for mutations in the 5'-regulatory region. These mutations are associated with expression of *MDR1* mRNA and level of P-glycoprotein [8].

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The functions of the gene non-protein-encoding sequences are not yet known exactly, but we know that introns can modify gene expression by, *e. g.* participating in transcription or splicing [6]. The study of the gene polymorphism not only in the encoding and regulatory parts of the gene, but in the intron regions will clear out the significance of these mutations for protein function and associations of various genotypes with the risk of certain diseases and with various clinical manifestations.

Previously we studied two *MDR1* polymorphisms in exons 21 ($G^{2677}T$, Ala893Ser) and 26 ($C^{3435}T$, I1e1142I1e) in patients with lymphoproliferative diseases (LPD) and showed association of $G^{2677}T$ and $C^{3435}T$ genotypes in these patients with the risk of drug resistance [1].

Here we studied the distribution of single nucleotide substitutes in *MDR1* gene intron 6 ($C^{6+139}T$) and exon 12 ($C^{1236}T$, Glu412Glu) in healthy europeoids of West Siberia and patients with LPD and analyzed association between the genotypes and predisposition to the disease and formation of drug resistance in LPD.

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MATERIALS AND METHODS

The study was carried out on blood samples from healthy subjects and patients with LPD (chronic lympholeukemia and non-Hodgkin's lymphoma) collected in Novosibirsk Regional Clinical Hospital. Both groups were balanced by age and sex and consisted of only europeoids.

Control group (n=59) consisted of patients of traumatological department examined before discharge (53% men and 47% women aged 17-81 years, mean age 49.8 years). The criterion for selection into the control group was the absence of oncological diseases.

The group of patients (n=63) consisted of 68% men and 32% women aged 17-81 years (mean age 53.7 years). Chemotherapy was considered effective (tumor was sensitive to drugs) in 53 patients, if complete or partial remission was attained (decrease in lymphocyte counts in the blood and bone marrow, reduction of lymph nodes). The treatment was considered ineffective (drug resistance), when the clinical picture remained unchanged after chemotherapy or the patient died.

MDR1 polymorphism was detected by DNA restriction fragment length polymorphism analysis. Genome DNA was isolated from intact blood using standard kits (Laboratoriya Medigen Firm). DNA regions containing polymorphic sites were amplified using pairs of nucleotide sequences flanking *MDR1* gene sites in exon 12 and intron 6 [2] (Table 1) synthesized on an ASM-800 synthesizer (Biosset Firm). PCR was carried out in 25 μl PCR buffer containing 0.25 mM deoxynucleoside triphosphates, 5 pmol of each primer, 2 U Taq polymerase (Biosan), and 5-8 μl patient's DNA.

Amplification products (366 base pairs for exon 12 and 299 base pairs for intron 6) were subjected to endonuclease restriction using 5 U appropriate restrictase (Sibenzim Firm) for 16 h at 37°C. Restriction products were analyzed after electrophoresis in 8.5% PAAG and ethidium bromide staining.

MDR1 genotype association with one sign (disease or treatment efficiency) was evaluated by the chance ratio (CR), showing how much higher the probability to fall ill (or be drug resistant) was for an individual with a certain genotype in comparison with the probability to remain healthy (or be sensitive to drug therapy). CR was estimated using EpiInfo 6 software.

The significance of differences between the groups was evaluated using χ^2 test with Yates' correction and, if necessary (n<5), using Fisher bilateral test.

RESULTS

The incidence of alleles in *MDR1* gene intron 6 in healthy West-Siberian europeoids was close to that in the europeoid population of Germany (Table 2) and statistically did not differ from it [2]. The incidence of mutant T allele in exon 12 in West-Siberian europeoids was similar to that in Japanese population (0.48) [8] and significantly (χ^2=6.56, p=0.01) differed from that in the europeoid population of Germany [2].

Comparison of the incidence of intron 6 alleles and genotypes in normal subjects and LPD patients showed significant differences between the groups (χ^2=6.88, p=0.03), while the differences for exon 12 were insignificant (χ^2=4.53, p=0.1).

Analysis of associations of genetic variants with predisposition to LPD showed that C-allele in intron 6 is a factor of predisposition to LPD and hence, mutant T allele is a factor of resistance: individuals with at least one mutant allele 6+139T (heterozygotes and mutant homozygotes) are 2.44 times more resistant to LPD than $C^{6+139}C$ genotype carriers (Table 2), while mutant type homozygote carriers were even more resistant than individuals homozygotic by the 6+139C allele (CR=3.75, p<0.02).

A similar association between the presence of mutant allele and disease resistance was observed for $C^{1236}T$ mutation in exon 12, though neither for combined group of heterozygotes and mutant homozygotes, nor for the group of mutant homozygotes the effect of significance was achieved.

Analysis of associations between the genetic variants in intron 6 and exon 12 in LPD patients and the

TABLE 1. Primers for PCR Analysis and Restriction Endonucleases

Location/position in *MDR1* gene	Primer sequences for detection of nucleotide substitutes	Restriction endonuclease	Restriction site	Size of restriction products, base pairs*
Intron 6/$C^{6+139}T$	5' agg ttt cat ttt ggt gcc tg 3'	Ssp I	aat^att	299
	5' gaa caa aag gat gca cac gac aat 3'			(275 24)
Exon 12/$C^{1236}T$	5' tat cct gtg tct gtg aat tgc c 3'	Hae III	gg^cc	269 62 35
	5' cct gac tca cca cac caa tg 3'			(269 97)

Note. *Wild type allele fragments are shown; mutant allele fragments are shown in parentheses.

TABLE 2. Frequencies of *MDR1* Gene Alleles and Genotypes in Intron 6 ($C^{6+139}T$) and Exon 12 ($C^{1236}T$) in Normal Subjects (*n*=59) and LPD Patients (*n*=63) and Association with Predisposition to LPD

Location/position in gene in groups	Allele		Genotype				
	C	T	C/C	C/T	T/T	CR_1	CR_2
Intron 6/$C^{6+139}T$							
normal subjects	0.50	0.50	0.27	0.46	0.27	3.75	2.44
patients	0.67	0.33	0.47	0.40	0.13	$p<0.02$	$p<0.03$
Exon 12/$C^{1236}T$							
normal subjects	0.52	0.48	0.24	0.56	0.20	2.79	2.26
patients	0.64	0.36	0.41	0.46	0.13	$p<0.12$	$p<0.06$

Note. CR_1: number of wild type homozygotes to mutant homozygotes in LPD patients and normal subjects were taken for calculating the chance ratio; CR_2: number of wild type homozygotes to a combined group (heterozygotes and mutant homozygotes) in LPD patients and normal subjects were taken for estimating chance ratio.

TABLE 3. Association between *MDR1* Genotypes and Resistance to Chemotherapy in Patients with LPD (*n*=53)

Genotype; genotype combinations used for CR calculation		CR	Confidence interval	Significance of differences
C/C^{6+139}	T/T^{6+139}	5.87	0.69-50.96	$p<0.06$
	$C/T^{6+139}+T/T^{6+139}$	3.60	0.56-20.81	$p<0.18$
C/C^{1236}	T/T^{1236}	5.87	0.69-50.96	$p<0.06$
	$C/T^{1236}+T/T^{1236}$	3.60	0.56-20.81	$p<0.18$

results of chemotherapy (Table 3) showed that the risk of drug resistance for patients with $C^{6+139}C$ and $C^{1236}C$ genotypes was 5.87 times higher than for mutant homozygotes and 3.6 times higher than for patients with one or both mutant alleles. However, no statistical significance was attained for these associations in the examined group of 53 patients.

Hence, studies of *MDR1* genetic polymorphism and analysis of predisposition to LPD and drug resistance in LPD patients and our previous results [1] indicate that of the four most prevalent one-nucleotide substitutious in *MDR1* gene ($C^{6+139}T$, $C^{1236}T$, $G^{26677}T$, and $C^{3435}T$), only mutant T allele in intron 6 is associated with resistance to LPD; no reliable data on associations of mutations in exons 12, 21, and 26 with predisposition to LPD were obtained. The results of drug therapy of LPD patients depend on mutations in exons 21 and 26; patients with $T/T^{2677}+T/T^{3435}$ are at the highest risk of drug resistance [1]. No statistically significant associations between drug resistance and mutations in intron 6 and exon 12 were revealed.

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