ORIGINAL ARTICLE

POLYMORPHISM OF BIOTRANSFORMATION GENES AND RISK OF RELAPSE IN CHILDHOOD ACUTE LEUKEMIA

Gra OA[1,2], Kozhekbaeva ZhM[1,2,3], Makarova OV[4], Samochatova EV[4], Nasedkina TV[1,*]

***Corresponding Author:** Tatyana V. Nasedkina, Department of Biological Microarray, Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, 119991 Moscow, Russia; Tel.: +7-499-135-62-59; Fax: +7-499-135-14-05; E-mail: nased@biochip.ru, nased@eimb.ru

ABSTRACT

Leukemia is a hematological malignancy that involves bone marrow. Polymorphism of biotransformation genes plays an important role in primary childhood leukemia and affects the incidence and character of acute leukemia relapse. A biochip designed to assess some polymorphisms of biotransformation genes was used to determine the frequency of the polymorphic variants of *CYP1A1*, *CYP2D6*, *GSTT1*, *GSTM1*, *MTHFR*, *MTRR*, *NQO1, CYP2C9*, *CYP2C19* and *NAT2* in 332 children with acute lymphoblastic leukemia (ALL) and 71 children with acute myeloblastic leukemia (AML). The *CYP1A1 *1/*2A*, *GSTT1* non null and *GSTM1* non null genotypes were more frequent in patients with primary leukemia than in relapse. Analysis of the *NAT2* genotype frequency revealed a characteristic genotype for each type of leukemia, which prevailed in patients with relapse: the genotype *341C/–, 481T/–, 590G/G, 857G/G* prevailed in ALL patients with relapse, and the genotype *341T/T, 481C/C, 590A/–* in AML patients with relapse when compared with patients having primary ALL or AML, respectively. Thus, the polymorphisms of *CYP1A1*, *GSTT1*, *GSTM1* and *NAT2* genes can be considered as markers for risk of relapse in childhood acute leukemia and can be used for the prognosis and individualization of standard therapy.

Key words: Childhood acute leukemia; Polymorphism; Predisposition; Relapse; Microarray

INTRODUCTION

Leukemia is a hematological malignancy that involves bone marrow. This disease is socially important, as it is among the major causes of death in children. Acute lymphoblastic leukemia (ALL) is the most common form, accounting for about 80% of all acute leukemia cases. Acute myeloblastic leukemia (AML) accounts for 15% of leukemia cases in children [1,2]. The causes of the acute leukemias have not yet been identified. The current concept of their etiology and pathogenesis suggests an important role for chromosome aberrations, which may be inherited or induced by external factors [3].

Childhood acute leukemia is a clinically heterogeneous disease with highly variable response to therapy and prognosis. Almost all current prognostic factors are characteristics of leukemia cells in both primary acute leukemia and in relapse [3,4]. Individual features of drug metabolism are not pres-

[1] Engelhardt Institute of Molecular Biology, Russian Academy of Sciences, Moscow, Russia
[2] Vavilov Institute of General Genetics, Russian Academy of Sciences, Moscow, Russia
[3] University of Miami Miller School of Medicine Institute for Human Genomics, Miami, Florida, USA
[4] Research Institute of Pediatric Hematology, Russian Federation Ministry of Health and Social Development, Moscow, Russia

ently considered during choice of anti cancer therapy. However, several studies have shown that the activities of some drug-metabolizing enzymes can modulate to a considerable extent the individual efficacy of cancer therapy [5,6].

All xenobiotics, including therapeutic agents, are degraded and eliminated from the body by a system of enzymes encoded by specific genes. Most of these genes are polymorphic, and some polymorphic forms have a changed activity. The most important are phase I biotransformation enzymes (cytochromes of the P450 family, such as CYP1A1, CYP2D6, CYP2C9, and CYP2C19), which activate xenobiotics to yield genotoxic intermediates, and phase II enzymes [glutathione S-transferases (GSTs), arylamine N-acetyltransferases (NATs), *etc.*], which convert the genotoxic compounds to non toxic ones [7,8]. In addition, several genes determine the response to anti cancer drugs. For instance, the products of methylene tetrahydrofolate reductase (*MTHFR*), methionine synthase reductase (*MTRR*) and thiopurine-S-methyltransferase (*TPMT*) genes, participate in metabolism of the anti cancer agents 5-fluorouracil, methotrexate, and thiopurine derivatives [6,9,10].

Polymorphism of phase I and II enzymes play an important role in primary childhood leukemia [8,11-14]. Moreover, polymorphisms of the biotransformation genes affect the incidence and features of acute leukemia relapse [6,10,15-20]. Since genotype frequencies vary among populations, it is useful to test the polymorphisms of the xenobiotic detoxification genes for association with the efficacy of therapy.

A biochip that assesses several polymorphisms of biotransformation genes has been designed in our laboratory [7,21,22]. It permits assessment of 24 mutations of 11 genes: *CYP1A1* (4887C>A, 4889A>G, and 6235T>C), *CYP2D6* (1934G>A and DelA2637), *GSTT1* (deletion), *GSTM1* (deletion), *MTHFR* (677C>T and 1298A>C), *MTRR* (66A>G), *NQO1* (609C>T), *CYP2C9* (430C>T and 1075A>C), *CYP2C19* (681G>A), *NAT2* (341T>C, 481C>T, 590G>A, and 857G>A), and *TPMT* (238G>C, 292G>T, 460G>A, 644G>A, 681T>G and 719A>G). The relation of 6-MP therapy to TPMT-genotype was studied previously and showed that ALL patients with a heterozygous TPMT-genotype were characterized by lower tolerance for standard doses [6,23]. In the present study, we set out to identify the markers for the prognosis and the efficacy of therapy for children with acute leukemia, in the genetic polymorphisms of key enzymes involved in the metabolism of xenobiotics and anti cancer agents. Using the biochip, we determined the frequency of the polymorphic alleles of *CYP1A1*, *CYP2D6*, *GSTT1*, *GSTM1*, *MTHFR*, *MTRR*, *NQO1, CYP2C9*, *CYP2C19* and *NAT2* in 403 children with acute leukemia. After the patients were classified by type of leukemia, the frequency of the allelic variants of the genes were compared in children diagnosed with primary leukemia or with leukemia relapse.

MATERIALS AND METHODS

Patients. We examined 332 children, including 258 diagnosed with primary ALL and 74 with ALL relapse. Their mean age was 6 years (from 3 months to 16 years; 57.8% males). We also examined 71 children with AML, including 41 diagnosed with primary AML and 30 with AML relapse. Their mean age was 8 years (from 1 to 17 years; 47.9% males). All patients were residents of the European part of Russia and originated from the East Slavonic population. The parents of all patients gave their informed consent according to the Declaration of Helsinki; the study was approved by the authorities of the Engelhardt Institute of Molecular Biology, Moscow, Russia. Diagnosis was made according to the 1997 World Health Organization (WHO) classification [23]. Blood/bone marrow specimens were obtained for diagnosis of primary disease, relapse, or remission in the oncohematology divisions of the Russian Children's Clinical Hospital (Moscow), Morozov Children's Clinical Hospital (Moscow), and the Moscow Regional Oncological Dispensary (Balashikha) from 2000 to 2004. The ALL and AML patients received scheduled therapy according to the protocol ALL-MB-02 or AML-2000 (Moscow-Minsk), respectively. The drugs administrated are summarized in Table 1.

Specimens. DNA samples were isolated from whole blood or bone marrow leukocytes, using a Wizard genomic DNA purification system (Promega, Madison, WI, USA).

Oligonucleotide Synthesis and Biochip Preparation. The oligonucleotides to be immobilized in a

Table 1. General characteristics of the chemotherapy

Protocols	Medication
ALL-MB-02	**Induction:** Dexamethasone (prednisone), vincristine, daunorubicin **Intensification** *(three blocks)*: 6-mercaptopurine, methotrexate, L-asparaginase, daunorubicin **Continuation:** 6-mercaptopurine, methotrexate
AML-2000	**Induction** *(two blocks)*: *First block:* cytarabine, vepesid, daunorubicn *Second block:* cytarabine, mitoxantrone **Intensification** *(three blocks)*: *First block:* cytarabine, vepesid *Second block:* cytarabine, L-asparaginase *Third block:* cytarabine, vepesid, mitoxantron

biochip were synthesized on a 394 automated DNA/RNA synthesizer (Applied Biosystems, Carlsbad, CA, USA) according to the standard phosphoramidite procedure [24]. The 3' end of the oligonucleotides was linked to a spacer with a free amino group, which was introduced during synthesis with the use of a 3'-Amino-Modifier C7 CPG 500 (Glen Research, Sterling, VA, USA). The nucleotide sequences of the immobilized nucleotides [7,21] are available on request. Biochips were obtained by photoinducible copolymerization of the oligonucleotides with the components of polyacrylamide gel [25].

Multiplex Polymerase Chain Reaction (PCR). DNA fragments of all genes were amplified in two rounds of nested multiplex PCR except for the *GSTT1* and *GSTM1* genes, which were amplified in one round of PCR. The primers were designed using the Oligo 6 program (Molecular Biology Insights, Cascade, CO, USA). The target mutations and the primer sequences [7,21] are available on request. Five parallel multiplex reactions were run. The primer combinations and the PCR conditions were as described [7, 21].

The fluorescently-labeled products of the second rounds of multiplex PCR were hybridized to the biochip. The hybridization mixture (30 µL) contained 25% formamide (Serva, Heidelberg, Germany), 5× SSPE (Promega), and the PCR product (3 µL from each multiplex reaction), and denatured at 95°C for 5 min, quickly chilled in ice for 1 min, and applied onto the biochip. The biochip was incubated at 37°C overnight, washed with 1× SSPE at room temperature for 10 min and then dried.

Image Analysis. The fluorescent signal from cells of the microchip was detected using a portable biochip analyzer supplied with a CCD camera and Imageware software (Biochip-IMB, Moscow, Russia) [21-22].

Sequencing. Specimens with different polymorphic variants of *CYP1A1* were sequenced using a 3100 ABI PRISM™ genetic analyzer (Applied Biosystems).

Statistical Analysis. This was performed using the GraphPad InStat program (San Diego, CA, USA). Genotype frequency distributions were tested for correspondence to the Hardy-Weinberg equilibrium by the $\chi 2$ test and proved to obey it for all biotransformation genes. For each genotype we estimated odds ratios (OR) and 95% confidence intervals (95% CI). The OR value is widely adopted in epidemiological studies to estimate the probability to have the disease for the carrier of a particular genotype (see also [7]). To calculate the OR in case-control study the 2×2 contingency table is used.

	Patient Group N_1	Control Group N_2
Number of subjects with genotype 1	a	c
Number of patients without genotype 1	b	d

The $OR = \frac{a/b}{c/d}$, where $a = n_1$, $b = N_1 - n_1$, $c = n_2$, $d = N_2 - n_2$; N_1 and N_2 are the sample sizes; and n_1 and n_2 are the numbers of individuals with the given character in the two samples. The polymorphic variants of investigated genes were estimated for non-random association with disease recurrence by Fisher's exact test (double-sided). The null hypothesis is that each two values in row and columns are random. The null hypothesis is rejected at critical level of confidence 5%, if the calculated probability

$$p = \frac{(a+b)!(a+c)!(b+d)!(c+d)!}{N!a!b!c!d!}$$

is less than 0.05.
The OR values below are given with a 95% confidence interval; p = 0.05 was used as a threshold to estimate the statistical significance of difference in genotype frequencies.

RESULTS

The biochip addressing the biotransformation gene polymorphisms was used to examine the DNA of 403 children with acute leukemia. The genotype frequencies in children with primary acute leukemia or relapse are summarized in Tables 2 to 11.

Distribution of CYP1A1 Genotypes. The *CYP1A1* genotype frequency was studied in patients with primary leukemia or relapse (Table 2). We observed both common (**1/*1*, **1/*2A*, **1/*2B* and **1/*4*) and rare allele combinations such as **2B/*4*, **2B/*2B*, and **2A/*2A*). Genotype frequency of the *CYP1A1* genotype **1/*2A* was higher in patients with ALL or AML relapse than in those with primary acute leukemia (Table 3). The difference was significant for patients with ALL (OR = 2.29, 95% CI = 1.09-4.80, p = 0.0322). The group of relapsed patients (not separated according to leukemia variant) showed a significant increase in frequency of the *CYP1A1* genotype **1/*2A* when compared with primary leukemia patients (OR = 2.11, 95% CI = 1.11-4.02, p = 0.0291). Because the *CYP1A1 *2A/*2A* genotype is very rare (genotype frequency is 0.4 in patients with primary ALL and 0.0 in all other groups), it was not taken into account.

Distribution of GSTT1 Genotypes. An opposite tendency was observed for *GSTT1*: the null genotype was far less frequent in ALL or AML relapse than in primary leukemia. The difference was significant in the case of ALL (OR = 0.48, 95% CI = 0.26-0.90, p = 0.0227; Table 4). The null genotype frequency in patients with relapse (not separated according to leukemia variant) was 1.8-fold less than in primary leukemia patients (OR = 0.55, 95% CI = 0.33-0.92, p = 0.0265).

Combined Analysis of CYP1A1 Genotype *1/*2A and GSTs Genotypes. Patients with ALL and/or AML relapse had a higher frequency of *CYP1A1* genotype **1/*2A* in combination with the *GSTT1* non null genotype, *GSTM1* non null genotype, or the double *GSTT1/GSTM1* non null genotype when compared with primary leukemia patients (Table 5). The patients with relapse also had a significantly higher frequency of *CYP1A1* genotype **1/*2A* and the double *GSTT1/GSTM1* non null genotype when compared with those with primary leukemia (OR = 3.48, 95% CI = 1.23-9.84, p = 0.0293).

Distribution of NAT2 Genotypes. The *NAT2* allele frequency distribution in patients with ALL and AML revealed a characteristic genotype for each type of leukemia, whose presence increases the risk of relapse. Patients with ALL relapse had

Table 2. The *CYP1A1* genotypes in children with acute leukemia

	Genotypes, *n* (%)						
Diagnosis (n)	***1/*1**	***1/*2A**	***1/*2B**	***1/*4**	***2B/*4**	***2B/*2B**	***2A/*2A**
ALL (258)	199 (77.1)	22 (8.5)	19 (7.4)	12 (4.6)	4 (1.6)	1 (0.4)	1 (0.4)
ALL relapse (74)	53 (71.6)	13 (17.6)	2 (2.7)	4 (5.4)	1 (1.35)	1 (1.35)	0 (0.0)
AML (41)	29 (70.7)	5 (12.2)	5 (12.2)	2 (4.9)	0 (0.0)	0 (0.0)	0 (0.0)
AML relapse (30)	22 (73.3)	5 (16.7)	2 (6.7)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)

Table 3. Frequency of the *CYP1A1* genotype **1/*2A* in children with primary leukemia or relapse

	Allele Frequency, *n* (%)				
CYP1A1 (n)	****1/*2A***	Other genotypes	OR	95% CI	***p***
ALL (258)	22 (8.5)	236 (91.5)	1.0	–	–
ALL relapse (74)	13 (17.6)	61 (82.4)	**22.9**[a]	**1.09-4.80**	**0.0322**
AML (41)	5 (12.2)	36 (87.8)	1.0	–	–
AML relapse (30)	5 (16.7)	25 (83.3)	1.44	0.38-5.50	0.733
ALL plus AML (299)	27 (9.0)	272 (91.0)	1.0	–	–
ALL plus AML relapse (104)	18 (17.3)	86 (87.2)	**2.11**[a]	**1.11-4.02**	**0.0291**

[a] Statistically significant data are shown in bold.

Table 4. The *GSTT1* genotypes in children with acute leukemia

	Genotype, *n* (%)				
GSTT1	**[–/–]**	**[+/–], [+/+]**	**OR**	**95% CI**	***p***
ALL (258)	89 (34.5)	169 (65.5)	1.0	–	–
ALL relapse (74)	15 (20.30	59 (79.7)	**0.48**[a]	**0.26-0.90**	**0.0227**
AML (41)	13 (31.7)	28 (68.3)	1.0	–	–
AML relapse (30)	8 (26.7)	22 (73.3)	0.78	0.28-2.22	0.793
ALL plus AML (299)	102 (34.1)	197 (65.9)	1.0	–	–
ALL plus AML relapse (104)	23 (22.1)	81 (77.9)	**0.55**[a]	**0.33-0.92**	**0.0265**

[–/–]: null genotype (absence of functionally competent alleles); [+/–], [+/+]: presence of one or two functionally competent alleles.
[a] Statistically significant data are shown in bold.

a higher frequency of heterozygotes and homozygotes for the *NAT2* mutations (heterozygous and homozygous variants) *341C/–* and *481T/–*, and for their combination, when compared with primary leukemia patients (data not shown). An opposite tendency was observed for polymorphic variants 590G>A and 857G>A: the heterozygous and homozygous variants *590A/–* and *857A/–*, and their combination, were less frequent in ALL relapse than in primary leukemia. In total, patients with ALL relapse showed a significantly higher frequency of genotype *341C/–, 481T/–, 590G/G, 857G/G* when compared with primary leukemia patients (OR = 1.64, 95% CI = 0.97-2.78, p = 0.074).

The patients with AML relapse had a lower frequency of heterozygous and homozygous variants *341C/–* and *481T/-*, and their combination, when compared with primary leukemia patients (data not shown). They also had a higher frequency of the heterozygous and homozygous variant *590A/-* when compared with primary leukemia patients. In total, patients with AML relapse had a significantly higher frequency of genotype *341T/T, 481C/C, 590A/–* when compared with primary leukemia patients (OR = 2.37, 95% CI = 0.86-6.54, p = 0.125).

Combined Analysis of NAT2 and GSTs Genotypes. The *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* in combination with the *GSTT1* and/or *GSTM1* non null genotype occurred in patients with ALL relapse at a significantly higher frequency than in those with primary leukemia (Table 6). Patients with AML relapse also displayed a higher frequency of *NAT2* genotype *341T/T, 481C/C, 590A/–* in combination with the *GSTT1* and/or *GSTM1* non null genotype when compared with primary leukemia patients. The difference was significant for the com-

Table 5. Combination of the *CYP1A1* genotype **1/*2A* with the *GSTT1* and/or *GSTM1* non null genotype in children with acute leukemia

Genotype CYP1A1 *1/*2A	**Genotype, *n* (%)**				
Plus *GSTT1*	***CP1A1 *1/*2A GSTT1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (258)	16 (6.2)	242 (93.8)	1.0	ı	–
ALL relapse (74)	10 (13.5)	64 (86.5)	**2.36**[a]	**1.02-5.46**	**0.0494**
AML (41)	3 (7.3)	38 (92.7)	1.0	–	–
AML relapse (30)	3 (10.0)	27 (90.0)	1.41	0.26-7.15	0.692
ALL plus AML (299)	19 (6.4)	280 (93.6)	1.0	–	–
ALL plus AML relapse (104)	13 (12.5)	91 (87.5)	2.11	1.00-4.43	0.057

Genotype *CYP1A1 *1/*2A*	**Genotype, *n* (%)**				
Plus *GSTM1*	***CP1A1 *1/*2A GSTM1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (258)	8 (3.1)	250 (96.9)	1.0	–	–
ALL relapse (74)	6 (8.1)	68 (91.9)	2.76	0.93-8.22	0.093
AML (41)	2 (4.9)	39 (95.1)	1.0	–	–
AML relapse (30)	3 (10.0)	27 (90.0)	2.17	0.34-13.86	0.644
ALL plus AML (299)	10 (3.3)	289 (96.7)	1.0	–	–
ALL plus AML relapse (104)	9 (8.7)	95 (91.3)	2.74	1.08-6.94	0.055

Genotype *CYP1A1 *1/*2A*	**Genotype, *n* (%)**				
Plus *GSTT1* and *GSTM1*	***CP1A1 *1/*2A GSTT1* [+/–], [+/+] *GSTM1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (258)	6 (2.3)	252 (97.7)	1.0	–	–
ALL relapse (74)	5 (6.8)	69 (93.2)	3.04	0.90-10.27	0.072
AML (41)	1 (2.4)	40 (97.6)	1.0	–	–
AML relapse (30)	3 (10.0)	27 (90.0)	4.44	0.44-45.04	0.304
ALL plus AML (299)	7 (2.3)	292 (97.7)	1.0	–	–
ALL plus AML relapse (104)	8 (7.7)	96 (92.3)	**3.48**[a]	**1.23-9.84**	**0.0293**

[a] Statistically significant data are shown in bold.

Table 6. Combination of the *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* with the *GSTT1* and/or *GSTM1* non null genotype in children diagnosed with primary acute lymphoblastic leukemia or acute lymphoblastic leukemia relapse

NAT2 genotype 341C/–, 481T/–, 590G/G, 857G/G	**Genotype, *n* (%)**				
Plus *GSTT1*	***341C/–, 481T/–, 590G/G, 857G/G GSTT1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (258)	56 (21.7)	202 (78.3)	1.0	–	–
ALL relapse (74)	26 (35.1)	48 (64.9)	**1.95**[a]	**1.11-3.43**	**0.022**
Plus *GSTM1*	*341C/–, 481T/–, 590G/G, 857G/G GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (258)	36 (14.0)	222 (86.0)	1.0	–	–
ALL relapse (74)	19 (25.7)	55 (74.3)	**21.3**[a]	**1.14-4.00**	**0.0213**
Plus *GSTT1* and *GSTM1*	*341C/–, 481T/–, 590G/G, 857G/G GSTT1* [+/–], [+/+] *GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (258)	29 (11.2)	229 (88.8)	1.0	–	–
ALL relapse (74)	16 (21.6)	58 (78.4)	**2.18**[a]	**1.11-4.28**	**0.033**

[a] Statistically significant data are shown in bold.

Table 7. Combination of the *NAT2* genotype *341T/T, 481C/C, 590A/–* with the *GSTT1* and/or *GSTM1* non null genotype in children diagnosed with primary acute myeloblastic leukemia or acute myeloblastic leukemia relapse

NAT2 genotype 341T/T, 481C/C, 590A/–	**Genotype, *n* (%)**				
Plus *GSTT1*	***341T/T, 481C/C, 590A/– GSTT1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
AML (41)	6 (14.6)	35 (84.3	1.0	–	–
AML relapse (30)	11 (36.7)	19 (63.3)	**3.38**[a]	**1.08-10.57**	**0.048**
Plus *GSTM1*	*341T/T, 481C/C, 590A/– GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
AML (41)	5 (12.2)	36 (87.8)	1.0	–	–
AML relapse (30)	7 (23.3)	23 (76.7)	2.19	0.62-7.74	0.337
Plus *GSTT1* and *GSTM1*	*341T/T, 481C/C, 590A/– GSTT1* [+/–], [+/+] *GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
AML (41)	3 (7.3)	38 (92.7)	1.0	–	–
AML relapse (30)	7 (23.3)	23 (76.7)	3.86	0.91-16.41	0.084

[a] Statistically significant data are shown in bold.

Table 8. Frequency of the *CYP1A1* genotype **1/*2A* and *GSTT1* genotypes in boys diagnosed with primary leukemia or relapse. Combination of *CYP1A1* genotype **1/*2A* with the *GSTT1* non null genotype in boys with acute leukemia

	Genotype, *n* (%)				
Genotype *CYP1A1*	***1/*2A**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (144)	12 (8.3)	132 (91.7)	1.0	–	–
ALL relapse (48)	10 (20.8)	38 (79.2)	**2.90**[a]	**1.16-7.22**	**0.033**
Plus *GSTT1*	[–/–]	[+/–], [+/+]	OR	95% CI	*p*
ALL (144)	47 (32.6)	97 (67.4)	1.0	–	–
ALL relapse (48)	7 (14.6)	41 (85.4)	**0.35**[a]	**0.15-085**	**0.0163**
AML (16)	5 (31.5)	108 67.5)	1.0	–	–
AML relapse (18)	3 (16.7)	15 (83.3)	0.44	0.09-2.25	0.429
ALL plus AML (160)	52 (32.5)	108 (67.5)	1.0	–	–
ALL plus AML relapse (66)	10 (15.2)	56 (84.8)	**0.37**[a]	**0.18-0.79**	**0.0085**
*CYP1A1 *1/*2A* plus *GSTT1*	*CYP1A1 *1/*2A GSTT1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (144)	10 (6.9)	134 (93.1)	1.0	–	–
ALL relapse (48)	9 (18.8)	39 (81.3)	**3.09**[a]	**1.17-8.15**	**0.0254**

[–/–]: null genotype (absence of functionally competent alleles); [+/–], [+/+]: presence of one or two functionally competent alleles.
[a] Statistically significant data are shown in bold.

bination of the *NAT2* genotype with the *GSTT1* non null genotype (OR = 3.38, 95% CI = 1.08-10.57, p = 0.048; Table 7).

Distribution of the CYP1A1 Genotype *1/*2A and GSTs Genotypes in Boys with Acute Leukemia. Combinations of rhe CYP1A1 Genotype *1/*2A and GSTs Genotypes. Analysis of the *CYP1A1* genotype frequency showed that boys with ALL relapse had a significantly higher frequency of *CYP1A1* genotype **1/*2A* when compared with boys with primary leukemia (OR = 2.90, 95% CI = 1.16-7.22, p = 0.033; Table 8). Boys with ALL or AML relapse had a lower frequency of the *GSTT1* null genotype when compared with boys with primary leukemia. The difference was significant only for ALL (OR = 0.35, 95% CI = 0.15-0.85, p = 0.0163). The frequency of the *GSTT1* null genotype in boys with relapse (not separated according to leukemia variant) was 2.7-fold lower than in boys with primary leukemia (OR = 0.37, 95% CI = 0.18-0.79, p = 0.0085). There was a significant increase in frequency of *CYP1A1* genotype **1/*2A* in combination with *GSTT1* non null genotype for boys with ALL relapse when compared with boys with primary ALL (OR = 3.09, 95% CI = 1.17-8.15, p = 0.0254).

Distribution of the CYP1A1 Genotype *1/*2A and GSTs Genotypes in Girls with Acute Leukemia. Combinations of the CYP1A1 Genotype *1/*2A and GSTs Genotypes. Girls with ALL or AML relapse had a higher frequency of *CYP1A1* genotype **1/*2A* when compared with girls with primary leukemia (Table 9). The patients with ALL or AML relapse had a lower frequency of the *GSTM1* null genotype when compared with girls with primary leukemia. The difference was significant only for ALL (OR = 0.37, 95% CI = 0.15-0.90, p = 0.0303). Frequency of the *GSTM1* null genotype in girls with acute leukemia relapse (not separated according to leukemia variant) was 2.4-fold lower than in those with primary leukemia (OR = 0.41, 95% CI = 0.19-0.85, p = 0.0175). Girls with leukemia relapse had a higher frequency of the *CYP1A1* genotype **1/*2A* in combination with the *GSTM1* non null genotype when compared with girls with primary leukemia (Table 9).

Table 9. Frequency of the *CYP1A1* genotype **1/*2A* and *GSTM1* genotypes in girls diagnosed with primary acute leukemia or relapse. Combination of the *CYP1A1* genotype **1/*2A* with the *GSTM1* non null genotype in girls with acute leukemia

	Genotype, *n* (%)				
CYP1A1	****1/*2A***	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (114)	10 (8.8)	104 (91.2)	1.0	–	–
ALLl relapse (26)	3 (11.5)	23 (88.5)	1.36	0.34-5.32	0.709
AML (25)	2 (8.0)	23 (92.0)	1.0	–	–
AML relapse (12)	4 (33.3)	8 (66.7)	5.75	0.88-37.64	0.073
ALL plus AML (139)	12 (8.6)	127 (91.4)	1.0	–	–
ALL plus AML relapse (38)	7 (18.4)	31 (81.6)	2.39	0.87-6.57	0.134
GSTM1	[–/–]	[+/–], [+/+]	OR	95% CI	*p*
ALL (114)	67 (58.8	47 (41.2)	1.0	–	–
ALL relapse (26)	9 (34.6)	17 (65.4)	**0.37**[a]	**0.15-0.90**	**0.0303**
AML (25)	15 (60.0)	10 (40.0)	1.0	–	–
AML relapse (12)	5 (41.7)	7 (58.3)	0.48	0.12-1.93	0.482
ALL plus AML (139)	82 (59.0)	57 (41.0)	1.0	–	–
ALL plus AML relapse (38)	14 (36.8	24 (63.2)	**0.41**[a]	**0.19-0.85**	**0.0175**
*CYP1A1 *1/*2A* plus *GSTM1*	*CYP1A1 *1/*2A GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (114)	4 (3.5)	110 (96.5)	1.0	–	–
ALL relapse (26)	2 (7.7)	24 (92.3)	2.29	0.40-13-25	0.309
AML (25)	1 (4.0)	24 (96.0)	1.0	–	–
AML relapse (12)	2 (16.7)	10 (83.3)	4.80	0.39-59.18	0.241
ALL plus AML (139)	5 (3.6)	134 (96.4)	1.0	–	–
ALL plus AML relapse (38)	4 (10.5)	34 (89.5)	3.15	0.80-12.38	0.101

[–/–]: null genotype (absence of functionally competent alleles); [+/–], [+/+]: presence of one or two functionally competent alleles.
[a] Statistically significant data are shown in bold.

The NAT2 Genotype Distribution in Girls with Acute Lymphoblastic Leukemia. Girls with ALL relapse had a higher frequency of heterozygous and homozygous variants 341C/– and *481T/–* of *NAT2* and a lower frequency of heterozygous and homozygous variants *590A/-* and *857A/-* when compared with these with primary ALL (data not shown). Girls with ALL relapse showed a higher frequency of genotype *341C/–, 481T/–, 590G/G, 857G/G* when compared with those with primary leukemia (OR = 1.71, 95% CI = 0.73-4.04, p = 0.267).

The NAT2 Genotype Distribution in Boys with Acute Myoloblastic Leukemia. Boys with AML relapse had a lower frequency of heterozygous and homozygous variants *341C/–* and *481T/–* of *NAT2* and their combinations when compared with those with primary AML (Table 10). An opposite tendency was observed for the *NAT2* polymorphic variant 590G>A, the frequency of the heterozygous and homozygous variant *590A/-* in AML relapse being higher than in primary leukemia. Boys with AML relapse had a higher frequency of genotype *341T/T, 481C/C, 590A/-* when compared with those with primary AML (OR = 2.40, 95% CI = 0.55-10.39, p = 0.297).

Table 10. The *NAT2* genotypes in boys diagnosed with primary acute myeloblastic leukemia or acute myeloblastic leukemia relapse

NAT2	**Genotype, *n* (%)**				
T341C	***C/–***	***T/T***	**OR**	**95% CI**	***p***
AML (16)	13 (81.3)	3 (18.8)	1.0	–	–
AML relapse (18)	7 (38.9)	11 (61.1)	**0.15**[a]	**0.03-0.71**	**0.0173**
C481T	T/–	C/C	OR	95% CI	*p*
AML (16)	11 (68.8)	5 (31.3)	1.0	–	–
AML relapse (18)	7 (38.9)	11 (61.1)	0.30	0.07-1.20	0.101
T341C plus *C481T*	*341C/–, 481T/–*	Other genotypes	OR	95% CI	*p*
AML (16)	13 (81.3)	3 (18.8)	1.0	–	–
AML relapse (18)	7 (38.9)	11 (61.1)	**0.15**[a]	**0.03-0.71**	**0.0173**
G590A	*A/–*	*G/G*	OR	95% CI	*p*
AML (16)	7 (43.8)	9 (56.3)	1.0	–	–
AML relapse (18)	10 (55.6)	8 (44.4)	1.61	0.41-6.24	0.732
T341C plus *C481T* plus *G590A*	*341T/T, 481C/C, 590A/–*	Other genotypes	OR	95% CI	*p*
AML (16)	4 (25.0)	12 (75.0)	1.0	–	–
AML relapse (18)	8 (44.4)	10 (55.6|)	2.40	0.55 10.39	0.297

[a] Statistically significant data are shown in bold.

Table 11. Combination of *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* with the *GSTT1* and/or *GSTM1* non null genotype in girls diagnosed with primary acute lymphoblastic leukemia or acute lymphoblastic leukemia relapse

NAT2 genotype 341C/-, 481T/-, 590G/G, 857G/G	**Genotype, n (%)**				
Plus *GSTT1*	***341C/–, 481T/–, 590G/G, 857G/G GSTT1* [+/–], [+/+]**	**Other genotypes**	**OR**	**95% CI**	***p***
ALL (114)	24 (21.1)	90 (78.9)	1.0	–	–
ALL relapse (26)	10 (38.5)	16 (61.5)	2.34	0.94-5.82	0.077
Plus *GSTM1*	*341C/–, 481T/–, 590G/G, 857G/G GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (114)	13 (11.4)	101 (88.6)	1.0	–	–
ALL relapse (26)	9 (34.6)	17 (65.4)	**4.11**[a]	**1.52-11.11**	**0.0067**
Plus *GSTT1* and *GSTM1*	*341C/–, 481T/–, 590G/G, 857G/G GSTT1* [+/–], [+/+] *GSTM1* [+/–], [+/+]	Other genotypes	OR	95% CI	*p*
ALL (114)	11 (9.6)	103 (90.4)	1.0	–	–
ALL relapse (26)	7 (29.6)	19 (73.1)	**3.45**[a]	**1.19-10.03**	**0.0448**

[a] Statistically significant data are shown in bold.

Combined Analysis of NAT2 and GSTs Genotypes in Girls with Acute Lymphoblastic Leukemia. The *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* in combination with the *GSTT1* and/or *GSTM1* non null genotype occurred in girls with ALL relapse at a higher frequency than in those with primary ALL (Table 11), the difference being significant for combinations of the *NAT2* genotype with the *GSTM1* non null genotype or the double *GSTT1/ GSTM1* non null genotype.

Combined Analysis of NAT2 and GSTs Genotypes in Boys with Acute Myoloblastic Leukemia. Boys with AML relapse had a higher frequency of *NAT2* genotype *341T/T, 481C/C, 590A/–* in combination with the *GSTT1* non null genotype when compared with those with primary leukemia (OR = 5.60, 95% CI = 0.97-32.21, p = 0.063). For *CYP1A1* (4887C>A and 4889A>G), *CYP2D6* (1934 G>A and DelA2637), *MTHFR* (677C>T and 1298A>C), *MTRR* (66A>G), *NQO1* (609C>T), *CYP2C9* (430C>T and 1075A>C), and *CYP2C19* (681G>A) no significant difference in genotype frequencies was observed between the patients with relapse and those with primary leukemia.

DISCUSSION

Our results demonstrate that particular variant alleles of the biotransformation genes are associated with a higher risk of relapse in childhood acute leukemia. Most of the regularities we observed agree with the published data [16-20]. For instance, the association of polymorphic variant *CYP1A1*2A* with poor therapeutic prognosis has been shown in children with ALL (OR = 3.08, 95% CI = 1.62-5.88, p = 0.0011) [16]. An increase in frequency of the *CYP1A1* genotype **1/*2A* in children with ALL relapse can be explained by synthetic glucocorticoids (such as dexamethasone and prednisolone) being an important component of the ALL treatment protocol and in induction therapy. They cause lymphopenia and involution of lymphoid tissue that lead to immunosuppression. Published data demonstrate that they can potentiate the activity of CYP1A1 by action through the glucocorticoid-responsive elements [16,26]. The presence of a polymorphism in the *CYP1A1* gene increases the enzymatic activity and, leads to increased concentration of intermediate genotoxic metabolites and of total mutagenic activity [14,19]. Since formation of additional mutations may cause resistance of cancer cells towards therapy, it is likely that the *CYP1A1 *1/*2A* genotype may decrease the efficacy of therapy and promote development of relapse [16].

We also observed that patients with the *GSTT1* and/or *GSTM1* null genotype have a lower risk of recurrence when compared with those with one or two functional alleles [17,18,20]. The GSTs are involved in metabolism of many anti tumor drugs, catalyzing conjugation of intermediate metabolites with reduced glutathione. In addition, they can determine resistance to chemotherapeutic agents [27]. Thus, ALL children carrying the *GSTT1* null genotype have been observed to respond well to induction therapy with prednisone, while carriers of at least one functional *GSTT1* allele displayed glucocorticoid resistance, a poor response to therapy, and a higher relapse rate [20]. The total 5-year survival in high-risk ALL has been shown to be substantially higher in children carrying the *GSTM1* null genotype than in carriers of the non null genotype (p = 0.03) [17]. Our results suggest that, in carriers of the *GSTT1* and/or *GSTM1* null genotype, lack of GSTT1 and/ or GSTM1 enzymes leads to an accumulation of cytotoxic drugs, with enhancement of their efficacy and longer relapse-free survival.

The ALL-MB-02 treatment protocol does not include oxazaphosphorines. The cytostatics cyclophosphamide and ifosfamide are widely used for treating ALL according to the ALL-BFM protocol and exert an immunosuppressive effect by inhibiting proliferation of lymphocyte clones involved in the immune response. The GSTs catalyze conjugation of cyclophosphamide with glutathione [28]. Also among children with steroid-dependent nephropathic syndrome, carriers of the *GSTM1* null genotype have a far greater relapse-free survival when compared with carriers of one or two functional *GSTM1* alleles [29]. Thus, the *GSTM1* null genotype improves the efficacy of cyclophosphamide: lack of GSTM1 allows accumulation of the cytotoxic and immonosuppressing metabolite 4-OH-cyclophosphamide which is a substrate of the enzyme GSTM1 [30].

An important component of induction therapy for ALL is the anthracyclines (*e.g.,* daunorubicin and doxorubicin), which exert an immunosuppressive effect. It has been shown [31] that daunorubicin *in vitro* causes induction of GSTs, which may account

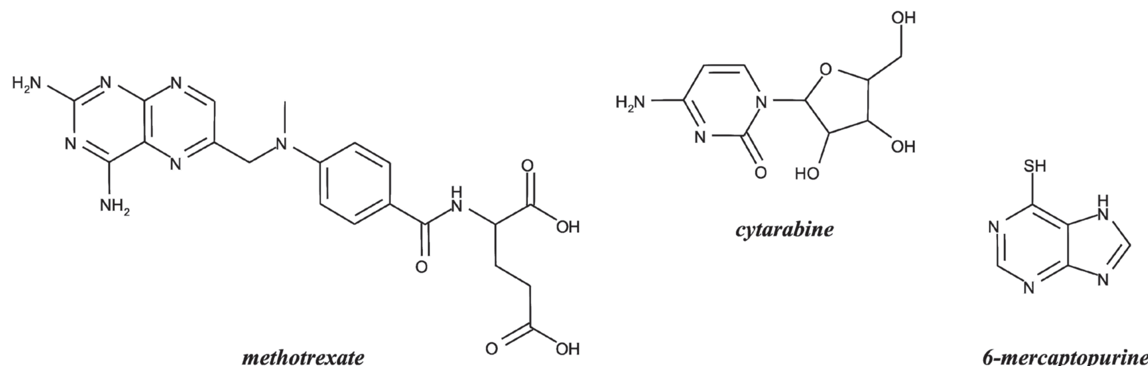

Figure 1. Structural formulas of methotrexate, cytarabine, and 6-mercaptopurine.

for the resistance to cytotoxic effects. However by increasing the efficacy of the anthracyclines, the *GSTs* null genotypes may increase the risk of certain adverse reactions. Moreover, among AML patients with the *GSTT1* null genotype, those receiving low doses of anthracyclines had a better prognosis than those who received high-dose therapy [32].

We found that the presence of both the *CYP1A1* genotype **1/*2A* and the *GSTT1* and/or *GSTM1* non null genotype has an additive effect on the risk of acute leukemia relapse. The polymorphic variant *CYP1A1*2A* has been associated with a higher risk of acute leukemia relapse, whereas the *GSTT1* and/or *GSTM1* null genotype exerts a protective effect [16-20]. Our results suggest that therapy is far more effective in patients carrying no *CYP1A1*2A* allele, increasing CYP1A1 activity, neither *GSTT1* and/or *GSTM1* functional alleles, where the lack of function favors the accumulation of cytotoxic metabolites and strengthens the therapeutic effect.

The NATs catalyze the addition of the acetyl group to the terminal nitrogen of arylhydrazines and arylamine-containing drugs and carcinogens [33]. The NAT2 is the main acetylating enzyme, while NAT1 acetylates fewer arylamines. Three main slow alleles which cause a slow NAT2 acetylation phenotype: *NAT2*5* (T341C, C481T), *NAT2*6* (G590A), and *NAT2*7* (G857A), and one fast (wild type) allele *NAT2*4*, form the basis for comparing genetic and biochemical data. A combination of a fast and a slow allele yields an intermediate acetylation phenotype [34,35]. According to published data, the *NAT2* polymorphic variants that determine the slow acetylator phenotype decrease either the activity or the stability of the enzyme. For instance, substitution of C for T at position 341 causes the substitution $Ile^{114}Thr$ and reduces the N-acetylation rate, while the substitutions $Arg^{197}Gln$ and $Gly^{286}Glu$ result in a less stable enzyme [33-36].

The *NAT2* alleles can modulate the risk of various tumors [37-39], including acute leukemia [11]. We observed two characteristic *NAT2* genotypes: *341C/–, 481T/–, 590G/G, 857G/G* for ALL relapse and *341T/T, 481C/C, 590A/–* for AML relapse. Since the intermediate/slow acetylator phenotype occurred at a higher frequency in both cases, the difference in genotype could be determined by the structure of NAT2 substrates. Since methotrexate and cytarabine are aryl and heterocyclic amines they may most probably be substrates for NAT2 [11,40] (Figure 1). Both are employed in acute leukemia: methotrexate for ALL in the consolidation phase, and cytarabine throughout AML therapy. NAT2 may metabolize 6-mercaptopurine (Figure 1) and its analogs used in the ALL therapy, but these are metabolized mainly by thiopurine-S-methyltransferase [6,22]. Thus, we speculate that the intermediate/slow acylator phenotype promotes accumulation of these drugs and their reactive metabolites in childhood acute leukemia. These metabolites cause multiple chromosome aberrations in bone marrow cells [41,42], which, with other adverse factors, lead to relapse.

An interesting finding is that patients with ALL relapse showed a significant increase in the frequency of the *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* in combination with *GSTT1* and/or *GSTM1*

non null genotype when compared with primary leukemia patients, while patients with AML relapse displayed a higher frequency of the *NAT2* genotype *341T/T, 481C/C, 590A/–* in combination with the *GSTT1* and/or *GSTM1* non null genotype. This leads us to suggest that for carriers of "non null" *GSTT1* and/or *GSTM1* in combination with *NAT2* genotype *341C/–, 481T/–, 590G/G, 857G/G* (in children with ALL) or *341T/T, 481C/C, 590A/–* (in children with AML), therapy is less effective than for carriers of the other genotypes. The presence of functionally significant alleles *GSTT1* and/or *GSTM1* causes effective detoxification of anti cancer drugs by the enzymes GSTT1 and/or GSTM1, and their elimination with a decrease in their effectiveness, while "unfavorable" *NAT2* genotype favors accumulation of genotoxic agents which can provoke chromosomal aberrations in bone marrow cells and thus lead to relapse.

The analysis of genotype frequency for *CYP1A1*, *GSTs* and *NAT2* genes in subgroups of patients with acute leukemia, divided according to their gender (boys and girls, separately), showed the same regularities as in total group of patients. It is worth noting that the differences found in the group of boys were more pronounced that those in the group of girls. The results are in good agreement with the data that boys not only more frequently develop the disease, but usually have a poorer prognosis due to higher risk of relapse [43-45]. The reason is not clearly understood, but it is likely that due to sexual difference in the metabolic work of biotransformation enzymes the unoptimized anti cancer therapy becomes more critical for boys than for girls [43].

To summarize, we have shown that a wide range of *CYP1A1* allele combinations can be identified using the biochip designed to assess the biotransformation genes. The *CYP1A1*, *GSTT1*, *GSTM1* and *NAT2* polymorphic alleles are prognostic for increased risk of acute leukemia relapse in children. Our method can be employed in a search for other variant alleles that affect the risk of leukemia relapse and in clinical diagnosis aimed at individualizing therapy.

ACKNOWLEDGMENTS

This study was supported by the Russian Foundation for Basic Research (project 06-04-49771). Thanks are due to T.E. Ivashchenko and B.S. Baranov for DNA from normal controls, to Drs. D.V. Prokopenko and R.A. Yurasov (EIMB RAS, Moscow, Russia) for help in data processing, and to Drs. S.A. Surzhikov and S.V. Pan'kov (EIMB RAS, Moscow, Russia) for manufacturing the biochips.

REFERENCES

1. Wood ME, Philips GK. Hematology/Oncology Secrets. 3rd ed. Philadelphia: Hanley & Belfus Inc., 2003.

2. Chan KW. Acute lymphoblastic leukemia. Curr Probl Pediatr Adolesc Health Care 2002; 32(2): 40-49.

3. Ansari M, Krajinovic M. Pharmacogenomics of acute leukemia. Pharmacogenomics 2007; 8(7): 817-834.

4. Seeger K, Adams HP, Buchwald D, Beyermann B, Kremens B, Niemeyer C, Ritter J, Schwabe D, Harms D, Schrappe M, Henze G. TEL-AML1 fusion transcript in relapsed childhood acute lymphoblastic leukemia. The Berlin-Frankfurt-Munster Study Group. Blood 1998; 91(5): 1716-1722.

5. Relling MV, Yanishevski Y, Nemec J, Evans WE, Boyett JM, Behm FG, Pui CH. Etoposide and antimetabolite pharmacology in patients who develop secondary acute myeloid leukemia. Leukemia 1998; 12(3): 346-352.

6. Chupova NV, Samochatova EV, Rudneva AE, Solopova GG, Nasedkina TV, Fedorova OE, Glotov AS, Zemliakova VV, Krynetski EYu, Krynetskaia NF, Ribeiro RC, Evans WE, Rumyantsev AG. Genetic polymorphism of thiopurine-S-methyltransferase and treatment of childhood acute lymphoblastic leukemia. Haematol Transfusiol (in Russian with English abstract) 2005; 6: 3-8.

7. Gra OA, Glotov AS, Nikitin EA, Glotov OS, Kuznetsova VE, Chudinov AV, Sudarikov AB, Nasedkina TV. Polymorphisms in xenobiotic-metabolizing genes and the risk of chronic lymphocytic leukemia and non-Hodgkin's lymphoma in adult Russian patients. Am J Hematol 2008; 83(4): 279-287.

8. Gra OA, Glotov AS, Kozhekbaeva ZhM, Makarova OV, Nasedkina TV. Genetic polymorphism of GST, NAT2, and MTRR and susceptibility to childhood acute leukemia. Mol Biol (Mosk) 2008; 42(2): 187-197.

9. Sohn KJ, Croxford R, Yates Z, Lucock M, Kim YI. Effect of the methylenetetrahydrofolate reductase

C677T polymorphism on chemosensitivity of colon and breast cancer cells to 5-fluorouracil and methotrexate. J Natl Cancer Inst 2004; 96(2): 134-144.

10. Stanulla M, Schaeffeler E, Flohr T, Cario G, Schrauder A, Zimmermann M, Welte K, Ludwig WD, Bartram CR, Zanger UM, Eichelbaum M, Schrappe M, Schwab M. Thiopurine methyltransferase (TPMT) genotype and early treatment response to mercaptopurine in childhood acute lymphoblastic leukemia. JAMA 2005; 293(12): 1485-1489.

11. Krajinovic M, Richer C, Sinnett H, Labuda D, Sinnett D. Genetic polymorphisms of N-acetyltransferases 1 and 2 and gene-gene interaction in the susceptibility to childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev 2000; 9(6): 557-562.

12. Krajinovic M, Labuda D, Richer C, Karimi S, Sinnett D. Susceptibility to childhood acute lymphoblastic leukemia: influence of CYP1A1, CYP2D6, GSTM1, and GSTT1 genetic polymorphisms. Blood 1999; 93(5): 1496-1501.

13. Smith MT, Wang Y, Skibola CF, Slater DJ, Lo Nigro L, Nowell PC, Lange BJ, Felix CA. Low NAD(P)H: quinone oxidoreductase activity is associated with increased risk of leukemia with MLL translocations in infants and children. Blood 2002; 100(13): 4590-4593.

14. Infante-Rivard C, Labuda D, Krajinovic M, Sinnett D. Risk of childhood leukemia associated with exposure to pesticides and with gene polymorphisms. Epidemiology 1999; 10(5): 481-487.

15. Takanashi M, Morimoto A, Yagi T, Kuriyama K, Kano G, Imamura T, Hibi S, Todo S, Imashuku S. Impact of glutathione S-transferase gene deletion on early relapse in childhood B-precursor acute lymphoblastic leukemia. Haematologica 2003; 88(11): 1238-1244.

16. Krajinovic M, Labuda D, Mathonnet G, Labuda M, Moghrabi A, Champagne J, Sinnett D. Polymorphisms in genes encoding drugs and xenobiotic metabolizing enzymes, DNA repair enzymes, and response to treatment of childhood acute lymphoblastic leukemia. Clin Cancer Res 2002; 8(3): 802-810.

17. Rocha JC, Cheng C, Liu W, Kishi S, Das S, Cook EH, Sandlund JT, Rubnitz J, Ribeiro R, Campana D, Pui CH, Evans WE, Relling MV. Pharmacogenetics of outcome in children with acute lymphoblastic leukemia. Blood 2005; 105(12): 4752-4758.

18. Stanulla M, Schrappe M, Brechlin AM, Zimmermann M, Welte K. Polymorphisms within glutathione S-transferase genes (GSTM1, GSTT1, GSTP1) and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study. Blood 2000; 95(4): 1222-1228.

19. Voso MT, D'Alo F, Gumiero D, Guidi F, Hohaus S, Leone G. The CYP1A1*2a allele is an independent prognostic factor for acute myeloid leukemia. Haematologica 2005; 90(7): 982-984.

20. Anderer G, Schrappe M, Brechlin AM, Lauten M, Muti P, Welte K, Stanulla M. Polymorphisms within glutathione S-transferase genes and initial response to glucocorticoids in childhood acute lymphoblastic leukemia. Pharmacogenetics 2000; 10(8): 715-726.

21. Glotov AS, Nasedkina TV, Ivashchenko TE, Iurasov RA, Surzhikov SA, Pan'kov SV, Chudinov AV, Baranov VS, Zasedatelev AS. Biochip development for polymorphism analysis in biotransformation system genes. Mol Biol (Mosk) 2005; 39(3): 403-412.

22. Nasedkina TV, Fedorova OE, Glotov AS, Chupova NV, Samochatova EV, Maiorova OA, Zemlyakova VV, Roudneva AE, Chudinov AV, Yurasov RA, Kozhekbaeva JM, Barsky VE, Krynetskiy EY, Krynetskaia NF, Cheng C, Ribeiro RC, Evans WE, Roumyantsev AG, Zasedatelev AS. Rapid genotyping of common deficient thiopurine S-methyltransferase alleles using the DNA-microchip technique. Eur J Hum Genet 2006; 14(9): 991-998.

23. Harris NL, Jaffe ES, Diebold J, Flandrin G, Muller-Hermelink HK, Vardiman J, Lister TA, Bloomfield CD. The World Health Organization classification of neoplasms of the hematopoietic and lymphoid tissues: report of the Clinical Advisory Committee Meeting, Airlie House, Warrenton,VA, USA, November 1997. Hematol J 2000; 1(1): 53-66.

24. Surzhikov SA, Timofeev EN, Chernov BK, Golova JB, Mirzabekov AD. Advanced method for oligonucleotide deprotection. Nucleic Acids Res 2000; 28(8):E29.

25. Rubina AY, Pan'kov SV, Dementieva EI, Pen'kov DN, Butygin AV, Vasiliskov VA, Chudinov AV, Mikheikin AL, Mikhailovich VM, Mirzabekov AD. Hydrogel drop microchips with immobilized DNA: properties and methods for large-scale production. Anal Biochem 2004; 325(1): 92-106.

26. Celander M, Weisbrod R, Stegeman JJ. Glu-

cocorticoid potentiation of cytochrome P4501A1 induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin in porcine and human endothelial cells in culture. Biochem Biophys Res Commun 1997; 232(3): 749-753.

27. Tew KD. Glutathione-associated enzymes in anticancer drug resistance. Cancer Res 1994; 54(16): 4313-4320.

28. Yuan ZM, Smith PB, Brundrett RB, Colvin M, Fenselau C. Glutathione conjugation with phosphoramide mustard and cyclophosphamide. A mechanistic study using tandem mass spectrometry. Drug Metab Dispos 1991; 19(3): 625-629.

29. Vester U, Kranz B, Zimmermann S, Buscher R, Hoyer PF. The response to cyclophosphamide in steroid-sensitive nephrotic syndrome is influenced by polymorphic expression of glutathion-S-transferases-M1 and -P1. Pediatr Nephrol 2005; 20(4): 478-481.

30. Zhang J, Tian Q, Yung Chan S, Chuen Li S, Zhou S, Duan W, Zhu YZ. Metabolism and transport of oxazaphosphorines and the clinical implications. Drug Metab Rev 2005; 37(4): 611-703.

31. Papageorgiou G, Iliadis S, Botsoglou N, Dioudis C, Goulas A, Fletouris D, Dimitriadou-Vafiadou A. Lipid peroxidation of rat myocardial tissue following daunomycin administration. Toxicology 1998; 126(2): 83-91.

32. Autrup JL, Hokland P, Pedersen L, Autrup H. Effect of glutathione S-transferases on the survival of patients with acute myeloid leukaemia. Eur J Pharmacol 2002; 438(1-2): 15-18.

33. Pompeo F, Brooke E, Kawamura A, Mushtaq A, Sim E. The pharmacogenetics of NAT: structural aspects. Pharmacogenomics 2002; 3(1): 19-30.

34. Goldenkova-Pavlova IV, Bruskin SA, Abdeev RM, Markarova EV, Bigvava SG, Radkevich LA, Kozhekbaeva ZhM, Glotov AS, Gra OA, Zasedatelev AS, Nasedkina TV, Kurdanov KhA, Piruzian ES. Comparative analysis of N-acetylation polymorphism in humans as determined by phenotyping and genotyping. Genetika 2006; 42(8): 1143-1150.

35. Kozhekbaeva ZhM, Glotov AS, Gra OA, Golden-kova-Pavlova IV, Bruskin SA, Agafonova EE, Markarova EV, Abdeev RM, Korsunskaia IM, Piruzian AL, Barskiĭ VE, Zasedatelev AS, Nasedkin TV. Identification of functionally significant mutations in NAT2 gene using biological microchips. Mol Biol (Mosk) 2007; 41(4): 725-733.

36. Leff MA, Fretland AJ, Doll MA, Hein DW. Novel human N-acetyltransferase 2 alleles that differ in mechanism for slow acetylator phenotype. J Biol Chem 1999; 274(49): 34519-34522.

37. Hein DW. N-acetyltransferase 2 genetic polymorphism: effects of carcinogen and haplotype on urinary bladder cancer risk. Oncogene 2006; 25(11): 1649-1658.

38. Borlak J, Reamon-Buettner SM. N-acetyltransferase 2 (NAT2) gene polymorphisms in colon and lung cancer patients. BMC Med Genet 2006; 7: 58.

39. Chiou HL, Wu MF, Chien WP, Cheng YW, Wong RH, Chen CY, Lin TS, Lee H. NAT2 fast acetylator genotype is associated with an increased risk of lung cancer among never-smoking women in Taiwan. Cancer Lett 2005; 223(1): 93-101.

40. Meyer UA, Zanger UM. Molecular mechanisms of genetic polymorphisms of drug metabolism. Annu Rev Pharmacol Toxicol 1997; 37: 269-296.

41. Acar H, Caliskan U, Demirel S, Largaespada DA. Micronucleus incidence and their chromosomal origin related to therapy in acute lymphoblastic leukemia (ALL) patients: detection by micronucleus and FISH techniques. Teratog Carcinog Mutagen 2001; 21(5): 341-347.

42. Emoto Y, Kisaki H, Manome Y, Kharbanda S, Kufe D. Activation of protein kinase Cδ in human myeloid leukemia cells treated with 1-β-D-arabinofuranosylcytosine. Blood 1996; 87(5): 1990-1996.

43. Chessells JM, Richards SM, Bailey CC, Lilleyman JS, Eden OB. Gender and treatment outcome in childhood lymphoblastic leukaemia: report from the MRC UKALL trials. Br J Haematol 1995; 89(2): 364-372.

44. Schroeder H, Garwicz S, Kristinsson J, Siimes MA, Wesenberg F, Gustafsson G. Outcome after first relapse in children with acute lymphoblastic leukemia: a population-based study of 315 patients from the Nordic Society of Pediatric Hematology and Oncology (NOPHO). Med Pediatr Oncol 1995; 25(5): 372-378.

45. Moorman AV, Richards SM, Martineau M, Cheung KL, Robinson HM, Jalali GR, Broadfield ZJ, Harris RL, Taylor KE, Gibson BE, Hann IM, Hill FG, Kinsey SE, Eden TO, Mitchell CD, Harrison CJ. Outcome heterogeneity in childhood high-hyperdiploid acute lymphoblastic leukemia. Blood 2003; 102(8): 2756-2762.