Impact of Single Nucleotide Polymorphisms of Cytarabine Metabolic Genes on Drug Toxicity in Childhood Acute Lymphoblastic Leukemia

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Background. Cytarabine (cytosine arabinoside, ara-C) is a chemotherapeutical agent used in the treatment of pediatric acute lymphoblastic leukemia (ALL). Adverse drug reactions, such as interpatient variability in sensitivity to ara-C, are considerable and may cause difficulties during chemotherapy. Single nucleotide polymorphisms (SNPs) can play a significant role in modifying nucleoside-drug pharmacokinetics and pharmacodynamics and thus the development of adverse effects. Our aim was to determine whether polymorphisms in genes encoding transporters and enzymes responsible for the metabolism of ara-C are associated with toxicity and clinical outcome in a patient population with childhood ALL. ***Procedure.*** We studied 8 SNPs in the *CDA*,*DCK*, *DCTD*, *SLC28A3*, and *SLC29A1* genes in 144 patients with childhood acute lymphoblastic leukemia treated according to ALLIC BFM 1990, 1995 and 2002 protocols. ***Results.*** *DCK* rs12648166 and *DCK* rs4694362 SNPs were associated with hematologic toxicity (OR = 2.63, CI 95% = 1.37–5.04, $P = 0.0036$ and OR = 2.53, CI 95% = 1.34–4.80, $P = 0.0044$, respectively). ***Conclusions.*** Our results indicate that *DCK* polymorphisms might be important genetic risk factors for hematologic toxicity during ALL treatment with ara-C. Individualized chemotherapy based on genetic profiling may help to optimize ara-C dosing, leading to improvements in clinical outcome and reduced toxicity. Pediatr Blood Cancer 2014;9999:1–7

Key words: cytarabine; single nucleotide polymorphism; toxicity; childhood acute lymphoblastic leukemia; *DCK*

INTRODUCTION

Using combined chemotherapy, pediatric ALL (acute lymphoblastic leukemia) is a very curable disease. In Hungary, approximately 85% of patients with ALL survive 5 years after therapy [1]. The Berlin–Frankfurt–Münster (BFM) group first used the nucleoside analogue cytarabine (cytosine arabinoside, 1-β-D-arabinofuranosylcytosine, ara-C) in 1981 in combination with methotrexate, cyclophosphamide and doxorubicin [2–4]. However, the therapeutic agents used in the treatment of this disease are highly toxic and induce serious side effects. The major toxicities of ara-C at standard doses are myelosuppression, mucositis and infection [5]. Cytopenias as the result of myelosuppression can rapidly become life threatening or affect the quality of life, often leading to interruptions in chemotherapy and a subsequent increase in the risk of relapse. Because there is a high interpatient variability of sensitivity and toxicity to ara-C, understanding the background of this variance could provide an opportunity to identify patients at increased risk of adverse reactions. Genetic variations in the key genes involved in the transport and metabolism of ara-C may play an important role in these interpatient differences [6–8].

Ara-C requires active cellular uptake via nucleoside transporters (Fig. 1). The primary transporters are SLC29A1 (solute carrier family 29 member 1, previous name is equilibrative nucleoside transporter, hENT1) which transports 80% of the drug, and SLC28A1 (solute carrier family 28 member 1, previous name is human concentrative nucleoside transporter, hCNT1) [8–11]. The expression of SLC28A3 (solute carrier family 28 member 3, previous name is human concentrative nucleoside transporter, hCNT3) was slightly increased in H9-ara-C cells selected with high-dose ara-C [11]. Inside the cell, ara-C is metabolized by the same pathway as other nucleoside analogs; e.g., gemcitabine, decitabine, and clofarabine [9]. Conversion of ara-C into cytosine arabinoside-monophosphate (ara-CMP) by deoxycytidine kinase (DCK) is the rate-limiting step for further phosphorylation [6,7]. Cytidine monophosphate kinase 1 (CMPK1) converts ara-CMP into cytosine arabinoside-diphosphate (ara-CDP). Several nucleoside diphosphate kinases (NDPs) take part in the conversion of ara-CDP to cytosine arabinoside-triphosphate (ara-CTP) [9,12]. The intracellular conversion of ara-C into the active derivate ara-CTP is indispensable to exert its cytotoxic effect, which occurs in the S-phase of the cell cycle. Ara-CTP is incorporated into the DNA, competitively inhibiting DNA synthesis and DNA-polymerase-α [9,12–14]. Ara-C and ara-CMP are degraded by cytidine deaminase (CDA) and deoxycytidine-monophosphate deaminase (DCTD) into the non-toxic metabolite 1-B-D-arabinofuranosyl-uracil (ara-U) and arabinofuranosyl-uracil-monophosphate (ara-UMP), respectively [14,15]. Ara-CMP is dephosphorylated by 5′ nucleotidase II (NT5C2), thereby preventing the production of ara-CTP [14,16]. Several feedback mechanisms influence the metabolism of ara-C, For example, deoxycytidine triphosphate (dCTP) is a potent feedback inhibitor of DCK [17]. Intracellular dCTP pools are regulated by ribonucleotide reductase holoenzyme (consisting of RRM1 and RRM2 subunits) (Fig. 1) [18].

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Grant sponsor: NKTH (National Research and Technology); Grant number: TECH_08-A1/2-2008-0120; Grant sponsor: OTKA (Hungarian Scientific Research Fund); Grant number: PD 109200; Grant sponsor: Beam of Hope Foundation of Region Southern Alföld of Hematologic and Oncologic children, Hungary (Reménysugár a Dél-alföldi Hematológiai és Onkológiai Gyermekbetegekért Alapítvány)

Conflict of interest: Nothing to declare.

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Received 7 July 2014; Accepted 5 November 2014

DOI 10.1002/pbc.25379
Published online in Wiley Online Library (wileyonlinelibrary.com).

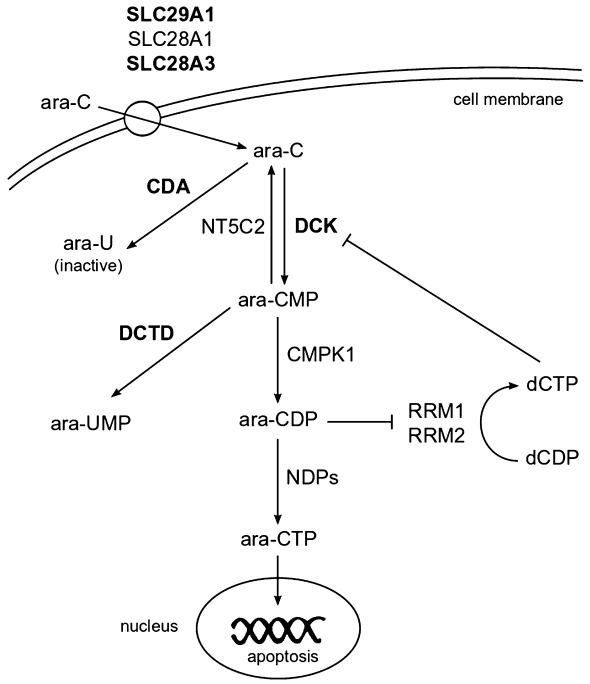

Fig. 1. Schematic description of ara-C transport and metabolism. Bold letters indicate the genes investigated in this study. Abbreviations: *Ara-C* cytosine arabinoside; *ara-CMP* cytosine arabinoside-monophosphate; *ara-CDP* cytosine arabinoside-diphosphate; *ara-CTP* cytosine arabinoside-triphosphate; *ara-U* arabinofuranosyl-uracil; *ara-UMP* arabinofuranosyl-uracil-monophosphate; *CDA* cytidine deaminase; *DCK* deoxycytidine kinase; *CMPK1* cytidine monophosphate kinase 1; *DCTD* deoxycytidylate deaminase; *dCDP* deoxycytidine diphosphate; *dCTP* deoxycytidine triphosphate; *NDPs* nucleoside diphosphate kinases; *NT5C2* 5′nucleotidase; *RRM1, RRM2* ribonucleotide reductase M 1,2; *SLC28A1, SLC28A3* solute carrier family 28 member 1, 3; *SLC29A1* solute carrier family 29 member 1.

Several *in vitro* studies have verified that the intracellular level of ara-CTP is determined by cellular sensitivity to ara-C [6,7]. *In vivo* observations have revealed an association between complete remission and intracellular levels of ara-C [16]. In recent years, numerous SNPs (single nucleotide polymorphisms) in genes of the ara-C metabolic pathway were identified as factors modifying nucleoside-drug pharmacokinetics and pharmacodynamics [19]. These genetic alterations might have clinical consequences [20–22].

Our aim was to test the hypothesis that genetic polymorphisms in ara-C transport and metabolism affect the hematologic toxicity and outcome of patients with childhood ALL treated with ara-C. Eight SNPs of the candidate genes *CDA*, *DCK*, *DCTD*, *SLC28A3* and *SLC29A1* were studied. These genes could form the molecular basis of the interpatient variability observed in intracellular ara-CTP concentration, toxicity to ara-C and survival after leukemia.

METHODS

Patients

In this retrospective study, 144 patients with childhood acute lymphoblastic leukemia diagnosed between 1991 and 2007 were enrolled. A detailed description of the study population may be found in Table I. The patients received chemotherapy following the ALL BFM 1990, 1995 or ALL IC BFM 2002 protocols at two Hungarian children oncology centers: the 2nd Department of Pediatrics, Semmelweis University, Budapest, and the Department of Pediatrics, Faculty of Medicine, University of Szeged. Following the protocol, cases were classified into three risk-groups based on initial clinical, pathological and genetic characteristics and response to early therapy as low risk (LR), medium risk (MR) and high risk (HR). Children with co-morbidities that may affect clinical outcome and toxicity were excluded from this study. We followed the patients for at least 5 years or until the date of death. All study subjects belonged to the Hungarian population. Written informed consent was requested from the guardians of the patients prior to their inclusion in the study. The study was approved by the Ethics Committee of the Hungarian Medical Research Council and conducted according to the principles of the Declaration of Helsinki.

The chemotherapy regimen is described in detail in our previous article [23]. In brief, ara-C was administered in the intensification and reintensification phases. We investigated ara-C toxicity during the intensification phase only. In every protocol and risk group, the patients were treated 8–16 times with 75 mg/m^2 doses of ara-C intravenously. The course of the dosing was daily doses of 75 mg/m^2 for 4 days repeated for 2 or 4 weeks according to the ALL BFM 1990/1995 and ALL IC BFM 2002 protocols. During the therapy, patients received 60 mg/m^2 6-mercaptopurine (6-MP) orally daily; and one or two doses of methotrexate intrathecally. Two days before the first ara-C administration, the patients were given a single dose of intravenous cyclophosphamide (1 g/m^2). Leukopenia, thrombocytopenia, anemia, nephrotoxicity (characterized by creatinine levels), hepatotoxicity (determined from glutamate pyruvate transaminase, [GPT] activity), encephalopathy (defined as any neurological symptoms) and infections (characterized by antibiotic usage and fever grade 2–4) were monitored in the patients' medical records. These adverse drug reactions were graded according to Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Detailed description of the toxicities are in Table I. Toxicity data during the weeks of the ara-C containing cycles plus the following two-week-long break before commencing the next therapeutic regimen were collected. The 5-year event-free survival (EFS) was calculated from the date of diagnosis to the date of relapse.

SNP Selection, DNA Extraction and Genotyping

We selected 8 SNPs in the *CDA*, *DCK*, *DCTD*, *SLC28A3*, and *SLC29A1* genes according to the following criteria: (i) the minor allele frequency of the SNP is greater than 10% among Caucasians; (ii) synonymous or intronic SNPs; and (iii) SNPs that have been associated with cancer risk or clinical outcome in previous investigations. The genes, nucleotide substitutions, function (such as encoding amino acid changes), and reference SNP identification numbers of the 8 SNPs evaluated in this study are summarized in Table II. DNA was isolated from peripheral blood taken during remission using Qiagen isolation kits (QIAmp DNA Blood Maxi Kit, Qiagen, Hilden, Germany).

The SNPs were genotyped using the fluorescence-based competitive allele-specific KASP^{TM} by Design genotyping assays (LGC Genomics, Teddington, UK) according to the manufacturer's instructions. PCR reactions were carried out using a 7900HT Fast

TABLE I. Characteristics of Patients

Variable		Data
Gender (%)	Male (%)	65 (45)
	Female (%)	79 (55)
Age at diagnosis	Mean (± SD)	6.7 (± 8.1)
	Median (range)	2 (0.5–17.5)
Risk (%)	LR (%)	36 (25)
	MR (%)	97 (67)
	HR (%)	11 (8)
White blood cells (10^{-9}/L)	Median (range)	1.3 (0.2–4.3)
Leukopenia (%)	Grade 1–2 ($>2.0 \times 10^{-9}$/L)	31 (22)
	Grade 3–4 ($<2.0 \times 10^{-9}$/L)	109 (78)
Trombocytes (10^{-9}/L)	Median (range)	77 (5–416)
Thrombopenia (%)	Grade 1–2 ($>50 \times 10^{-9}$/L)	103 (73)
	Grade 3–4 ($<50 \times 10^{-9}$/L)	38 (27)
Hemoglobin (g/l)	Median (range)	75 (40–125)
Anemia (%)	Grade 1–2 ($>80 \times 10^{-9}$/L)	53 (36)
	Grade 3–4 ($<80 \times 10^{-9}$/L)	91 (64)
Antibiotics usage (%)	No	99 (69)
	Yes	45 (31)
Fever (%)	No	89 (62)
	Grade 2–4 (≥39.0°C)	55 (38)
Survival	OS (5 year)	87.1%
	EFS (5 year)	83.5%

LR, low risk; MR, medium risk; HR, high risk; OS, overall; EFS, event free survival.

Real-Time PCR System (Life Technologies, Grand Island, NY). Samples with known genotypes were used in every measurement for technical control.

Statistical Methods

A Hardy–Weinberg equilibrium (HWE) analysis for genotype distribution and differences in allele distribution between the groups was carried out using a χ^2 goodness-of-fit test using an online application (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). A significant violation of HWE was considered when $P < 0.05$. Unadjusted logistic regression and multi-adjusted logistic regression models were applied to obtain odds ratios (OR) and 95% confidence intervals (95% CI) to estimate the risk for each polymorphism to toxicity. To assess the effect of the genetic background on blood counts, multi-adjusted general linear model procedures were used. Gender (male–female) and age (years) at diagnosis were used as potential cofactors. Three genotype groups were analyzed separately when the number of patients was sufficient in each group ($n > 5$). A Bonferroni correction considering multiple testing for the 8 SNPs was performed ($P < 0.00625$ was considered as significant).

Linkage disequilibrium (indicated with D' and r^2) and estimated haplotype frequencies in cases and controls were calculated using Haploview 4.1 software (http://www.broad.mit.edu/mpg/haploview/). Haplotype blocks were generated for all genes with at least two SNPs (*DCK*, *SLC28A3*, *SLC29A1)*. The haplotype-specific odds ratio (OR) was estimated using logistic regression. The survival rates were estimated with the Kaplan–Meier method. Statistical analysis was performed using IBM SPSS Statistics 21 (IBM Corporation, Armonk, NY) and MedCalc 10.0.2.0 (MedCalc Software, Ostend, Belgium) software.

TABLE II. The Studied SNPs, Distribution of Genotypes and Alleles in ALL Children

					Genotype (%)		
Gene	Rs number	Chr.	Function	Minor allele (MAF)	11	12	22
CDA	rs1048977	1p36.2	Thr145Thr	T (0.31)	70 (50)	51 (37)	18 (13)
DCK	rs12648166	4q13.3	intron	A (0.40)	48 (36)	67 (50)	20 (15)
	rs4694362		intron	C (0.40)	49 (36)	66 (49)	21 (15)
DCDT	rs4742	4q35.1	Val116Val	C (0.30)	69 (51)	52 (38)	15 (11)
SLC28A3	rs7867504	9q21.3	Thr89Thr	C (0.31)	60 (45)	64 (48)	9 (7)
	rs7853758		Leu461Leu	A (0.13)	102 (77)	28 (21)	3 (2)
SLC29A1	rs324148	6p21.1	intron	T (0.21)	83 (61)	48 (35)	5 (4)
	rs9394992		intron	T (0.30)	68 (49)	59 (42)	13 (9)

Chr, chromosome; MAF, minor allele frequency; SNP, single nucleotide polymorphism; The genotype groups are indicated by: 11 = homozygote for the frequent allele; 12 = heterozygote; 22 = homozygote for the rare allele.

RESULTS

Genotype and Allele Frequencies

The 8 SNPs were genotyped in the patient population; the minor allele and genotype frequencies are presented in Table II. The genotype distributions were in Hardy–Weinberg equilibrium for all SNPs.

Association Between SNPs and Toxicity

Leukopenia, thrombocytopenia, anemia, nephrotoxicity, hepatotoxicity, encephalopathy and infections were monitored in our childhood acute lymphoblastic leukemia patient cohort. None of the patients had nephrotoxicity. Hepatotoxicity was detected in three patients, but with certainly due to other causes, such as hepatotropic virus infection. They were excluded from our patient cohort. One patient had encephalopathy after exposure to ara-C. Because of these small numbers, it was not possible to analyze these toxicities in relation to the genotypes. Leukopenia, thrombocytopenia, anemia and infections were studied in association with the allele and genotype frequencies of the polymorphisms. The alleles of two SNPs in the *DCK* gene were associated with leukopenia. Patients carrying the rs12648166 G and rs4694362 T alleles had a higher risk of grade 3/4 leukopenia (OR = 2.25, 95% CI = 1.27–3.99, $P = 0.005$; and OR = 2.24, 95% CI = 1.26–3.97, $P = 0.0053$, respectively).

After the analysis of genotype distribution, two SNPs associated with severe leukopenia were identified in the univariate and in multi-adjusted models (Table III). More patients had leukopenia with the *DCK* rs12648166 GG genotype (41%) compared to patients with the AA genotype (12%) (OR = 2.63, 95% CI = 1.37–5.04, $P = 0.0036$). Patients with the DCK rs4694362 TT genotype were more susceptible to leukopenia compared to patients with the CC genotype (42 vs. 12%) (OR = 2.53, 95% CI = 1.34–4.80, $P = 0.0044$).

No association of leukopenia with the other polymorphisms was observed, neither significant association was found with thrombocytopenia in the investigated population (Table III). Anemia, infections, total number of white blood cells, total number of thrombocytes and hemoglobin counts were also studied in relation to polymorphism, but no associations were observed.

Haplotype Association With Toxicity

Haplotype analyses were carried out to determine the association of haplotype blocks of the genes and ara-C side effects, such as leukopenia and thrombocytopenia. The estimated haplotype frequencies are shown in Table IV for patients with or without these side effects. There were significant differences in the frequencies of the haplotypes of the *DCK* gene. The GT haplotype was more frequent in patients with grade 3/4 leukopenia than among other haplotypes (65% vs. 43%; OR = 2.37, 95% CI = 1.34–4.21, $P = 0.0031$), while the AC haplotypes were less frequent in patients with grade 3/4 leukopenia than other haplotypes (35% vs. 57%; OR = 0.41, 95% CI = 0.23–0.73, $P = 0.0025$). Adverse effects did not differ among haplotype blocks of the other genes.

The linkage disequilibrium coefficients (D' and r^2) between the alleles were also calculated (Fig. 2). A strong linkage was found between the two SNPs (rs12648166 and rs4694362) of the *DCK*

TABLE III. Association of Genotype With Leukopenia and Thrombocytopenia

		Grade III/IV leukopenia during the induction phase of chemotherapy						Grade III/IV thrombocytopenia during the induction phase of chemotherapy					
		Univariate results			Multivariate results			Univariate results			Multivariate results		
Gene	SNP	*P* value	OR	(CI 95%)	*P* value	OR	(CI 95%)	*P* value	OR	(CI 95%)	*P* value	OR	(CI 95%)
CDA	rs1048977	0.76	1.10	0.60–1.99	0.75	1.11	0.60–2.04	0.53	1.19	0.68–2.09	0.67	1.13	0.64–2.01
DCK	rs12648166	0.0035	2.63	1.38–5.04	0.0036	2.63	1.37–5.04	0.30	1.36	0.76–2.43	0.28	1.39	0.77–2.49
	rs4694362	0.0041	2.55	1.35–4.81	0.0044	2.53	1.34–4.80	0.53	1.20	0.69–2.08	0.49	1.22	0.70–2.13
DCTD	rs4742	0.84	0.94	0.51–1.73	0.90	0.96	0.52–1.78	0.81	0.93	0.53–1.66	0.86	0.95	0.53–1.70
SLC28A3	rs7853758	0.03	2.29	1.06–4.92	0.02	2.61	1.17–5.84	0.59	1.27	0.54–3.02	0.45	1.43	0.57–3.61
	rs7867504	0.22	1.53	0.78–3.01	0.19	1.59	0.79–3.19	0.55	1.22	0.63–2.38	0.43	1.32	0.67–2.61
SLC29A1	rs324148	0.90	1.05	0.51–2.16	0.97	1.01	0.49–2.09	0.20	1.64	0.78–3.46	0.23	1.59	0.75–3.37
	rs9394992	0.47	0.79	0.42–1.50	0.44	0.78	0.41–1.48	0.25	0.71	0.40–1.26	0.17	0.67	0.37–1.19

OR, odds ratio; CI, confidence interval.

TABLE IV. Association of Haplotypes With Leukopenia and Thrombocytopenia

			Grade III/IV leukopenia during the induction phase of chemotherapy					Grade III/IV thrombocytopenia during the induction phase of chemotherapy				
Gene	SNPs	Haplotypes	Grade 1/2	Grade 3/4	OR	95% CI	*P* value	Grade 1/2	Grade 3/4	OR	95% CI	*P* value
DCK	rs12648166–rs4694362	AC	57%	35%	0.41	0.23–0.73	0.0025	59%	64%	1.18	0.69–2.03	0.55
		GT	43%	65%	2.37	1.34–4.21	0.0031	40%	37%	0.86	0.50–1.49	0.60
SLC28A3	rs7853758 – rs7867504	GT	59%	70%	1.66	0.93–2.97	0.09	66%	73%	1.35	0.76–2.41	0.31
		GC	19%	20%	1.02	0.50–2.08	0.95	21%	16%	0.73	0.36–1.48	0.39
		AC	18%	10%	0.49	0.22–1.09	0.08	12%	12%	1.02	0.45–2.30	0.96
		AT	4%	–				2%	–			
SLC29A1	rs9394992 – rs324148	CC	52%	53%	1.14	0.65–1.98	0.65	53%	54%	1.04	0.62–1.77	0.88
		TC	25%	25%	0.95	0.49–1.80	0.87	23%	30%	1.43	0.79–2.57	0.23
		CT	22%	15%	0.59	0.29–1.19	0.14	19%	9%	0.43	0.19–1.02	0.06
		TT	–	7%				5%	7%	1.68	0.59–4.79	0.33

OR, odds ratio; CI, confidence interval.

gene ($D' = 1$, $r^2 = 0.98$), but only a slight or no linkage could be detected between the SNPs of *SLC28A3* ($D' = 0.83$, $r^2 = 0.23$) and *SLC29A1* ($D' = 0.17$, $r^2 = 0.01$), respectively.

Survival and Genotype Association With Survival

Overall (OS) and event-free survivals (EFS) were studied in our population, and the relationship of the genotypes with the overall and event-free survival rate of our population was determined. The 5-year OS was 87.1% and the 5-year EFS was 83.5%, which are comparable to the Hungarian survival rate [1]. The SNPs seemed to have no significant influence on the survival of our pediatric ALL population.

DISCUSSION

Treatment of patients with acute lymphoblastic leukemia is very effective, but has serious side effects. In this study, we investigated 8 polymorphisms in 5 genes responsible for the transport and metabolism of cytosine arabinoside in relationship with ara-C side effects, leukopenia, thrombocytopenia, anemia and infections. Two SNPs of the *DCK* gene, rs12648166 and rs4694362, were associated with altered risk to leukopenia at the allele, genotype and haplotype levels. None of the SNPs influenced thrombocytopenia, anemia, infection or the survival of the patients.

The relatively small sample size is a limitation of this study. It is not possible to detect minor associations; also the detected associations on small cohort would result in difficulty in interpretating the results. The identified associations must be replicated in independent patient cohorts and will need validation on larger populations. Also, it has to be mentioned that patients who died before the period of sample collection are underrepresented in our cohort. Apart from this, sample selection was random.

DCK (deoxycytidine kinase) is required for the pharmacologic activity of several clinically important anticancer nucleoside

Fig. 2. Linkage disequilibrium analysis of the SNPs. Pairwise linkage disequilibrium is expressed as r^2 and D' (both from 0 to 1). The value of r^2 is indicated by the shade of the boxes, where the denser shade represents a higher linkage ($r^2 = 0$ is white, $0 < r^2 > 1$ are shades of grey and $r^2 = 1$ is black). D'x 100 is indicated in the boxes as numbers when $D' < 1$.

analogs. It plays a key role as the first enzyme in the activation of ara-C to the active metabolite ara-CTP with phosphorylation because it catalyzes the conversion of ara-C to ara-CMP [24]. Its activity is also a major determinant of ara-C resistance because the expression of the *DCK* gene in ara-C resistant cells was reduced 60% compared to the level in human lymphoid cells. The reduced mRNA level was correlated with a lower DCK protein level and reduced protein activity (31.4%). As a consequence, resistant cells accumulated <1% ara-CTP [7,11]. Several other studies have investigated the potential function of SNPs of the *DCK* gene. Sequencing the promoter region and exons of *DCK* in lymphoblastoid cell lines from European origin, Lamba et al. identified several polymorphisms, such as I24V (rs66878317), A119G (rs66472932), and P122S (rs67437265), with different enzymatic activity than the wild-type protein. In addition, one SNP (35708 C<T rs4643786) in the 3′ UTR region was associated with lower DCK mRNA expression in the cell lines. They also investigated the potential effect of *DCK* SNPs on the level of the active metabolite ara-CTP in patients with acute myeloid leukemia (AML) who were treated with ara-C. They found that rs4643786 was associated with significantly lower intracellular ara-CTP concentrations [7].

To identify genetic determinants that contribute to ara-C toxicity, Hartford et al. conducted a study in which they examined SNPs in the *DCK* gene and applied a whole-genome pharmacogenomic analysis on lymphoblastoid cell lines (LCLs) derived from different populations (African or European) [6]. There was strong correlation between DCK mRNA and protein expression, and a higher DCK mRNA level was significantly correlated with cytotoxicity and sensitivity to ara-C. Studying the contribution of SNPs in the *DCK* gene to sensitivity to ara-C, they found that lymphoblastoid cell lines heterozygous for SNP 70 (I24V, rs66878317) were more sensitive to ara-C and contained more ara-CTP compared to the homozygous cell lines [6]. These data provide evidence that genetic variation within the *DCK* gene can affect function of the protein.

Several studies investigated the influence of the genetic background of the patients on treatment response, side effects and patient survival [25,26], but only a few studies have focused on the *DCK* SNPs examined in our study (rs12648166 and rs4694362). One of those studies analyzed genetic variation in gemcitabine metabolic and transporter genes that were associated with toxicity and efficacy of gemcitabine-based therapy in patients with locally advanced pancreatic cancer [27]. Gemcitabine is a nucleoside analog with a very similar metabolic pathway to that of cytarabine (https://www.pharmgkb.org/pathway/PA2036#PGG) [28]. *DCK* rs4694362 was associated with neutropenia, and patients with the TT genotype had a higher risk for having grade 3–4 neutropenia [27]. They also investigated *DCK* rs12648166, but found no association. Another study analyzed patients with pancreatic cancer treated with gemcitabine, and an association was found between genotype and tumor response to preoperative treatment for both of the SNPs (rs12648166 and rs4694362), but only patients with the rs4694362T allele had a higher risk for neutropenia [29]. The SNP rs4694362 of the *DCK* gene was a significant prognostic factor for overall survival in patients with AML from Korea; having at least one T allele was significantly associated with better survival time compared to the CC genotype [30].

Nevertheless, some studies detected associations between DCK function or ara-C toxicity and SNPs near *DCK* rs4694362, which were associated with ara-C toxicity in our population. One of these is rs4643786 in the 3′ UTR of *DCK* found by Lamba et al. [7], which might be in linkage disequilibrium with rs4694362, because the two SNPs are very close to each other (approximately 1400 bp). The rs72552079 in the 3′ UTR region of the *DCK* which is approximately 1800 bp from rs4694362, seems to influence the outcome of the therapy because carrying at least one T allele in rs72552079 is associated with a better response to the therapy [31].

Polymorphisms in the 5′ regulatory region of *DCK* also might have biological and clinical effects. For example, Chinese patients with a -360CC/-201CC genotype had less DCK mRNA, lower transcriptional activation activity and a poor response to chemotherapy [32].

Genome-wide association (GWA) studies investigating the genetics of chemotherapeutic susceptibility of ara-C in lymphoblastoid cell lines have not identified the ara-C metabolizing enzymes (*SLC29A1*, *DCK*, *CDA*) [33–36]. This result could attributed to the genes described above that may be more responsible for the side effects of the treatment.

Personalized dosing of chemotherapeutic agents based on the patient's genetic background can decrease life-threatening toxicities and side effects [37]. Our results may contribute to a better understanding of the pharmacogenetic background of cytarabine toxicity in patients with childhood acute lymphoblastic leukemia. Better elucidation of the pharmacogenetics of interindividual differences could help to individualize chemotherapy and thus potentially improve outcome.

ACKNOWLEDGMENTS

This study was supported by NKTH (National Research and Technology) TECH_08-A1/2-2008-0120: (C Szalai, P Antal); OTKA (Hungarian Scientific Research Fund): PD 109200 (ÁF Semsei); and the Beam of Hope Foundation of Region Southern Alföld of Hematologic and Oncologic children, Hungary (Reménysugár a Dél-alföldi Hematológiai és Onkológiai Gyermekbetegekért Alapítvány). We are grateful to the National Pediatric Cancer Registry of Hungary, Dr. Zsuzsanna Jakab for providing survival data for the patients.

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