GENETIC ASPECTS OF PREVENTION IN PEDIATRIC HEMATOONCOLOGY

Summary of PhD thesis

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SUMMARY

INTRODUCTION: There is an impressing development of genetics in preventive pediatric hematooncology.

Cytosine arabinoside (1-β-D-arabinofuranosylcytosine, ara-C, cytarabine) is one of the chemotherapeutical agents used in the treatment of pediatric acute lymphoblastic leukemia (ALL). Adverse drug reactions (ADR) of ara-C are considerable and generate difficulties during chemotherapy. Single nucleotide polymorphisms (SNPs) can play a significant role in modifying nucleoside-drug pharmacokinetics and pharmacodynamics and thus the development of interpatient variability in sensitivity and toxicities to ara-C adverse effects.

Rothmund-Thomson syndrome (RTS) is a rare genetic disease with characteristic bone and skin findings. Cells from patients with RTS demonstrate genomic instability, mutations in *RECQL4* gene with susceptibility to osteosarcoma and skin cancers. Rubinstein-Taybi syndrome (RSTS) is an infrequent condition characterized by a specific pattern of physical features and developmental disabilities. Damage to *CREBBP* gene and *EP300* gene have been detected in the background of the disease.

OBJECTIVE: 1. To determine whether polymorphisms in genes coding transporters and enzymes are responsible for the metabolism of ara-C are associated with toxicity and clinical outcome in our childhood ALL patient population. 2. To indentificate genetic background of the RTS patient to confirm diagnosis and give more precise prognosis. 3. To present the RTS and RSTS and draw attention to the importance of proper diagnosis and follow-up of genetic diseases disposed to malignancies in order to achieve better management and outcome of these disorders.

PATIENT AND METHODS: We studied 8 SNPs in the *CDA*, *DCK*, *DCTD*, *SLC28A3* and *SLC29A1* genes in 144 childhood acute lymphoblastic leukemia patients treated according to ALL-BFM 1990 and1995 and ALL IC-BFM 2002 protocols. Case-studies: 1. A girl, diagnosed with RTS soon after birth, developed cutan lymphoma at her age three. 2. A boy, diagnosed with at his age four, developed medulloblastoma at his age ten.

RESULTS: *DCK* rs12648166 and *DCK* rs4694362 SNPs were associated with altered risk to leukopenia at the allele, genotype and haplotype levels. Case 1. We presented a case of Rothmund-Thomson syndrome associated with aggressive biphenotype, biclonal EBV-associated cutan lymphoma first in the literature and the lymphoma risk existence at the early age of three in conjunction with RTS. Insufficient check-ups and lack of essential sun-protection may have played a role in her premature death. Case 2. Due to proper follow-up an early diagnosis and successful treatment of the RSTS patient's brain tumor happened. We indentified a novel *CREBBP* variant confirming the exact diagnosis.

CONCLUSIONS: 1. With the knowledge of genetic background of toxicity, individualized chemotherapy based on genetic profiling may help to optimize ara-C dosing, leading to reduced toxicity. 2. We presented, that bi-phenotype bi-genotype EBV-associated cutan lymphoma can be associated with RTS. We also presented, that lymphoma can arise at an early age of three in RTS. 3. Regarding to the formation of brain tumor of our patient, the newly identified *CREBBP* variant contributes to the growing database of possible predisposing mutations. 4. In case of hereditary syndromes prone to malignancies exact diagnosis helps to do predictive medicine: to give proper treatment and follow-up, lower cancer risk, improve outcome and help family-planning. With presenting two instructive disease-history and think-over the diagnosis and management of rare cancer predisposition malignancies we may ministrative for other practitioners with patients suffering from the same syndromes.

INTRODUCTION

Understanding genetic background, opportunities of prevention in pediatric oncology has a remarkable improvement. In my study I have been looking for possibilities of genetic aspects of prevention in pediatric oncology.

1. Pharmacogenetic study of cytosine arabinoside

A potential possibility in cancer therapy improvement may be achieved by personalization of chemotherapy: individual modification according to the patients' characteristics to optimize therapy. One way of individualization is to identify pharmacogenetic variants in the genes of chemotherapeutic agents influencing toxicity and outcome.

Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy with 50-70 newly diagnosed children in every year in Hungary. Using combined chemotherapy, pediatric ALL is a very curable disease. In Hungary, approximately 85% of patients with ALL survive 5 years after therapy. Our chemotherapy treatment in Hungary is according to the actual ALL-BFM Protocol. With on-going modifications the combined chemotherapy became very effective over time. However, the therapeutic agents used in the treatment are highly toxic and induce serious side effects, 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.

The nucleoside analogue ara-C is an important part of the therapy, the major toxicities at standard dose are myelosuppression, mucositis and infection. 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.

Numerous studies reported SNPs of nucleoside-drug metabolization playing a significant role in modifying pharmacokinetics and pharmacodynamics of pyrimidine antagonist thus the development of adverse effects. We examined 8 SNPs of 5 genes: *CDA* (cytidine deaminase), *DCK* (deoxycytidine kinase), *DCTD* (deoxycytidine-monophosphate deaminase), *SLC28A3* (solute carrier family 28 member 3) and *SLC29A* (solute carrier family 29 member 1). CDA is the predominant ara-C degradation enzyme, numerous studies have found association between SNPs (*CDA* G208A, *CDA* A79C, *CDA* A76C and *CDA* C111T) and the metabolism of nucleoside antagonists, while others failed to identify some polymorphisms predictive to toxicity (*CDA* T435C, G208A and A76C). DCK plays a key role as the first enzyme in the activation of several clinically important anticancer nucleoside analogues. It catalyses the conversion of ara-C to ara-CMP. Lower DCK level is correlated with ara-C

resistance, and several studies identified SNPs with different enzymatic activity influencing the toxicity in cell lines (I24V, A119G 2, P122S, 35708 C<T). DCTD is another pyrimidine inactivation enzyme, *DCTD* T47C was reported to be in weak association with OS. The activity of nucleotide transporters plays a role in ara-C sensitivity. *SLC29A1* C1345G, G1050A, T-549C, G706C, A201G; *SLC28A3* A25G, C-69T was reported to influence toxicity and survival.

2. Cancer prone hereditary syndromes

A number of inherited mutations of genes are associated with heightened susceptibility to specific malignancies. Identification of underlying genetic aberrations, revealing gene penetrance (predict associated tumor risk) allows preventive oncology. Genetic diagnosis helps us working out strategies for reducing the risk of associated cancer development and surveillance for malignancies and also predicts clinical outcome. Genetic counselling to help family planning also becomes available.

Rothmund-Thomson syndrome (RTS) is a rare genetic disease with characteristic congenital skeletal-dysplasias (radius, patella and pollux aplasia), short stature, sparse hair, cataract, mental deficiency and photosensitivity leading in all patients to poikiloderma. Cells from patients with RTS demonstrate genomic instability, mutations in the *RECQL4* gene, a member of a protein family called RecQ helicases, which maintain the structure and integrity of DNA. Consequently, RTS patients are particularly prone to developing osteosarcoma as well as nonmelanomatosus skin cancers. Different types of lymphoma and other malignancies have been also described.

Rubinstein-Taybi syndrome (RSTS) is a condition characterized by short stature, moderate to severe intellectual disability, distinctive facial features (small jaws and mouth with crowded teeth and high, arched palate, prominent nose with a low hanging columella, thick scalp hair extending onto the forehead and down-slanting eyes), broad thumbs and first toes, cardiac and urinary defects. Susceptibility for infection often leads to hearing impairments. Several damage to *CREBBP* and *EP300* gene have been detected in the background of this syndrome. CREBBP and EP300 proteins are thought to be co-activators between the DNA-binding transcription factor and the RNA polymerases, and also act as histone-acetyltransferases. Deregulation of histone modification leads to loss of transcription control, hence people with this condition have an increased risk of developing noncancerous and cancerous tumors, including keloids, certain kinds of brain tumors and leukemia.

AIMS AND QUESTIONS

Aims

1. 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. Eight SNPs of the candidate genes *CDA*, *DCK*, *DCTD*, *SLC28A3* and *SLC29A1* were studied (*CDA* rs1048977, *DCK* rs12648166 and rs4694362, *DCTD* rs4742, *SLC28A3* rs7853758 and rs7867504 and *SLC29A1* rs9394992 and rs324148). These genes could form the molecular basis of the interpatient variability observed in intracellular ara-CTP concentration, subsequently the toxicity to ara-C and survival after leukemia.

2. To present the hazard of aggressive bi-phenotype, bi-genotype EBV-associated cutan lymphoma in Rothmund-Thomson syndrome and the lymphoma risk existence at the early age of three in conjunction with RTS

3. To present a well-managed patient suffering from Rubinstein-Taybi syndrome developed brain-tumor. To indentificate genetic background of the patient to confirm diagnosis and give more precise prognosis.

4. To emphasize the importance of early exact diagnosis in hereditary syndromes with cancer predisposition - the importance of determining genotype.

5. To think over the proper follow-up with the aim of identifying the appearance of the expected malignancies in time, even in particular cases to avoid the development of these tumors.

Questions

1. Can we find any association between the examined SNPs and cytarabine toxicity?

2. Can we find any association of the examined haplotype blocks and cytarabine toxicity?

3. Can we find any association between the examined SNPs and ALL childhood-patient survival?

4. Can we find any association of the examined haplotype blocks and ALL childhood-patient survival?

5. Can be achieved better outcome of genetic diseases dispose to malignancies with an exact and in a timely manner set up diagnosis? Can we attain more sufficient tumor surveillance with proper patient-information and follow-up?

PATIENTS AND METHODS

1. Pharmacogenetic study of cytosine arabinoside

Patients

In this retrospective study, 144 patients with childhood acute lymphoblastic leukemia diagnosed between 1991 and 2007 were enrolled. The patients received chemotherapy following the ALL BFM 1990, 1995 or ALL IC BFM 2002 protocols at two Hungarian children oncology centres: 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 standard risk (SR), 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.

We investigated the first two weeks of ara-C therapy at every patient in the intensification (Protocol 1/II.) phase except HR patients of the ALL-BFM 1990, 1995 protocols, who's ara-C therapy was investigated in the reintensification (Protocol 2/II.) phase. During our studied period all the patients got unitedly 8 times 75 mg/m2 doses of ara-C intravenously, a continuous 60 mg/m2/day 6-MP or 50 mg/m2/day mercaptopurine/tioguanine orally and 1 dose 12 mg methotrexate intrathecal. Cytopenia, leukopenia, thrombocytopenia, anemia, nephrotoxicity, hepatotoxicity, encephalopathy and infections were monitored in the patients' medical records. Toxicity data were collected up to the next ara-C administration or in lack of following ara-C regimen up to the recovery of these markers. Adverse drug reactions were graded according to Common Terminology Criteria for Adverse Events v3.0 (CTCAE). The 5-year event-free survival (EFS) was calculated from the date of diagnosis to the date of relapse. Survival (EFS and overall survival – OS) data of patients arrived from the National Pediatric Cancer Registry of Hungary.

SNP selection

We selected 8 SNPs of 5 genes: *CDA* rs1048977 (C111T), *DCK* rs12648166 (A9846G) and rs4694362 (C1205T), *DCTD* rs4742 (T47C), *SLC28A3* rs7853758 (C69T) and rs7867504 (A25G) and *SLC29A1* rs9394992 (C913T) and rs324148 (T549C) from the related literature 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.

DNA extraction

DNA was isolated from peripheral blood taken during remission phase using Qiagen isolation kits. From children who died before the sample-collection, we extract DNA from preserved bone-marrow smears. For the DNA isolation we used High Pure PCR Template Preparation Kit.

Genotyping

The SNPs were genotyped using the fluorescence-based competitive allele-specific KASP assays. Samples with known genotypes were used in every measurement for technical control.

Statistical methods

A Hardy-Weinberg equilibrium analysis for genotype distribution and differences in allele distribution between the groups was carried out using an x2 goodness-of-fit test using an online application. 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 analysed 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. Haplotype blocks were generated for all genes with at least two SNPs (*DCK*, *SLC28A3* and *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 and MedCalc 10.0.2.0 software.

2. Patient with Rothmund-Thomson syndrome

Our patient had multiplex anomalies at birth: palatoschisis, skeletal abnormalities (aplasia radii, hypoplastic right and left thenar and thumbs, pes equinus on both side), ectopy renis and additional pneumothorax. Further dental malformations, growth retardation, cranial dysostosis with saddle nose and facial dysmorfism, sparse scalp hair, eyebrows and eyelashes, telangiectasia, dystrophic nails and photosensitivity, mild mental retardation were observable. When she became half year old, poikilodermatous rash appeared on her face and her limbs, RTS was diagnosed. She was planned to come check-ups for 6 months and her parents' attention was attracted for increased sun protection to avoid skin tumors. The parents did not bring her to supervision, even did not used intensificated sun protection. They presented with the girl at her age 3 and half at our clinic with delayed gain in weight. At this time painful hyperemic, compact swelling on her right nasal wing and 3 round, 6 cm in diameter ulcerative lesions on her limbs were present, which have been treated initially as pyoderma gangrenosum at the dermatology ambulance. In spite of administered antibiotics and steroid there was not improvement, moreover, progression of the lesions eventuated, and red papules on her arms also appeared**.**

3. Patient with Rubinstein-Taybi syndrome

Our patient came to our pediatric clinic at the month 3 for investigation with mild hepatomegaly, unilateral criptorhism, inspiratory stridor, pes equinovarus, trichosis and minor facial anomalies, like epicanthus, extended hair to the forehead, low-set ears. Due to elevated blood ammonia, lactate and liver-enzyme level he was treated with the diagnosis hyperammoniaemia and lactate acidosis. On the ground of the clinical appearance the possibility of Cornelia de Lange syndrome also aroused, nevertheless genetic examination has never done. At this age 3 microcephaly, wide nasal bridge, orhidopexia and underdeveloped speech became well-marked. Recurrent upper respiratory infections and consecutive inhibited nasal respiration and hear-loss were also present. At his age 4 other dysmorphic signs became noticeable: brachydctily, broad thumbs and first toes, synophris, arched palate, prominent nose and down-slanting eyes and he cold not walk alone. On the ground of these marks Rubinstein-Taybi syndrome has been diagnosed. With the indentification of causative *CREBBP* variation the diagnosis of RSTS was confirmed. We planned follow-up half a year.

Considering his underlying condition, that RSTS is disposed to malignancies, especially brain tumors, MRI scan was performed, which revealed two lesions. The terimes impressed low grade astrocytoma, the neurosurgeon suggested observation. Five month later ataxia and vomiting occurred, MRI scan showed remarkable enlargement of the vermis tumor with liquor-stop.

RESULTS

1. Pharmacogenetic study of cytosine arabinoside

Genotype and allele frequencies

The 8 SNPs were genotyped in the patient population; the minor allele and genotype frequencies are presented in **Table 1**. The genotype distributions were in Hardy-Weinberg equilibrium for all SNPs.

Table 1. The studied SNPs, distribution of genotypes and alleles in ALL children

Gene	rs number	Chr.	Function	MAF	Genotype (%)		
					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

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 hepatotrop 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 analyse 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. More patients had Gr. 3/4 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 severe leukopenia compared to patients with the CC genotype (42 vs. 12%) (OR=2.53, 95% CI=1.34-4.80, P=0.0044).

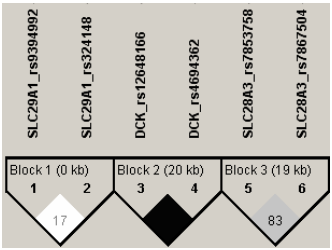
No association of leukopenia with the other polymorphisms was observed, neither significant association was found with thrombocytopenia in the investigated population. **Table 2.** Anemia, infections, total number of white blood cells, total number of thrombocytes and hemoglobin counts were also studied 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. 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 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. See **Table 3.**

The linkage disequilibrium coefficients (D' and r^2) between the alleles were also calculated. A strong linkage was found between the two SNPs (rs12648166 and rs4694362) of the *DCK* 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. (**Figure 1.**)

Figure 1. Linkage disequilibrium analysis



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 whereby the more dense shade represents the 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.
DCK: deoxycytidine kinase, *SLC28A3*: solute carrier family 28 member 3, *SLC29A1*: solute carrier family 29 member 1

Table 2. Association of genotype with leukopenia and thrombocytopenia

Gene	SNP	*Grade III/IV leukopenia during the first two weeks of intensification*						*Grade III/IV thrombocytopenia during the first two weeks of intensification*					
		Univariate results			Multivariate results			Univariate results			Multivariate results		
		p value	OR	(CI 95%)	p value	OR	(CI 95%)	p value	OR	(CI 95%)	p value	OR	(CI 95%)
CD4	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

Table 3. Association of haplotype with leukopenia and thrombocytopenia

Gene	SNPs	Haplotypes	*Leukopenia during the first two weeks of intensification*					*Thrombocytopenia during the first two weeks of intensification*				
			Grade 1/2	Grade 3/4	OR	95% CI	p value	Grade 1/2	Grade 3/4	OR	95% CI	p value
DCK	rs1264816 – 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

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. The SNPs seemed to have no significant influence on the survival of our pediatric ALL population.

2. Patient with Rothmund-Thomson syndrome

Biopsy from the nasal nodule was performed. Histological findings showed bifenotype, biclonal, Ebstein-Barr virus associated cutan lymphoma without systemic appearance.

We initiated our patients chemotherapy with NHL BFM SR non-Hodgkin lymphoma protocol. At the beginning of the treatment, central vein catheter was implanted into jugular vein. The skin necrosis were improved in 2 weeks. On the 3rd week of the treatment, during induction phase in relatively good condition she suddenly died at home. Dissection proved thrombosis in central vein catheter and in the sinus sagittalis superior, in spite of the catheter heparinisation.

3. Patient with Rubinstein-Taybi syndrome

We identified with a novel heterozygous *de novo* *CREBBP* NM_004380.2:c.2206C>T nonsense mutation in the background.

Subtotal removal of the patients brain tumor have done, the histology revealed grade IV. medulloblastoma with focal neural differentiation and calcification, which referred the tumor long-standing origin.

He got chemotherapy according to the Hungarian Brain Tumor Therapy Protocol MBL2008 HR Subtotal Resected or PNET or Anaplastic or Metastatic Tumor protocol. From four years on he is free from tumor. He comes for check-ups regularly.

DISCUSSION

1. Pharmacogenetic study of cytosine arabinoside

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.

Several studies investigated the influence of the genetic background of the patients on treatment response, side effects and patient survival, but only a few studies have focused on the *DCK* SNPs examined in our study (rs12648166 and rs4694362). Tanaka et al. have found *DCK* rs4694362 was associated with neutropenia, and patients with the TT genotype had a higher risk for having grade 3/4 neutropenia. They also investigated *DCK* rs12648166, but found no association. Okazaki et al. examined both of the SNPs, but only patients with the rs4694362T allele had a higher risk for neutropenia. The SNP rs4694362 of the *DCK* gene was a significant prognostic factor for overall survival in patients with AML in an investigation by Kim et al. According to Lamba, rs4643786, which might be in linkage disequilibrium with rs4694362 seems to influence the outcome of the therapy.

Integration of information from genetic polymorphisms into current therapy would present an opportunity to increase our possibilities to avoid serious side-effects thus influence the therapeutic outcome in ALL patients.

2. Cancer prone hereditary syndromes

Patient with Rothmund-Thomson syndrome

Although malignancies often develop in RTS, lymphomas are rare. Furthermore our patient is the first reported case with aggressive biphenotype, biclonal, EBV-associated lymphoma associated with RTS. Moreover, any kind of lymphoma had not described previously at this young age in conjunction with RTS.

Our patient has not been brought back for the concerted check-up and her parents even didn't use advanced sun-protection by her. Her cutan nodular then ulcerative lesions on her limbs were misdiagnosed and mistreated. Presumably an augmented protection from sunshine and more carefully observation could have prevented the formation of the lymphoma. An earlier exact diagnosis might have been treated with less aggressive treatment.

Once a patient is diagnosed with RTS, protection from sunshine is extremely important to prevent further skin lesions and avoid cutaneous malignancies. Close

follow-up (half a year) by a specialist (dermatologist, clinical geneticist, oncologist) to reveal novel symptoms, especially signs for potential cancers – skin cancers, osteosarcomas and lymphomas (but also any other tumors!) are indispensable in the patient management. Regular ophtalmology, endocrine, orthopedic, dental and may other specialists visits are necessary. Physiotherapy, special education can help patients in the everyday. Genetic examination is recommended if the diagnosis is not evidenced clinically and to make the prognosis more accurate. It also helps the family for further family planning.

Patient with Rubinstein-Taybi syndrome

Although we didn't know the exact diagnosis from the beginnings, the child remained in our view. Regarding to the signs and symptoms regular follow-ups have been planned and his parents had good compliance. With the after-years appearance of characteristic symptoms of RSTS the proper diagnosis has done. Considering his underlying condition, that RSTS is disposed to malignancies, especially brain tumors, cranial MRI scan was performed, which revealed his lesions. At the turnout of the patients first symptoms of elevated intracranial pressure, repeated MRI was done without delay and therapy had performed in time.

Regarding to the formation of brain tumor of our patient, our newly identified *CREBBP* variant contributes to the growing database of possible cancer predisposition mutations.

RSTS patients require regular (6 month) check-ups at the caring specialist doctor (clinical geneticist suggested). Endocrine, orthopedic, cardiology, pulmonology, ophtamology controls required in accordance with the symptoms. Increased attention for malignancies (especially blood-forming and brain tumors, keloids), blood-count half a year, routine cranial MRI a year recommended. Physiotherapy, special education can supplement the patients' management. Determining genetic background can help in case of doubtful diagnosis, in identification of outcome and potential tumors and in planning parenthood.

Diagnosis and management of patients with cancer prone hereditary syndromes

Proper management of children with genetic syndromes prone to malignancies is of great importance. Exact diagnosis allows us to do predictive medicine: suitable management in light of the expected outcome. Revealing the genetic background not only permit the precise diagnosis in doubtful cases, but helps to know more accurately the prognosis, not least cancer risk. Moreover knowledge of mutations allows us to give the patient and his family more exact help in the further family planning.

Regular follow-up (anamnesis, physical examination, imaging) are indispensable, in particular attention to the expectable tumor development. Noticing in

time the forming tumor is extremely important for adequate treatment and positive outcome.

To avoid the pitfalls of diagnostics and disease-surveillance, some special steps might be worth considering.

1. More oriented education would be necessary for physicians (mostly pediatric and family doctors) emphasizing the significance of early detection of signs and symptoms of genetic syndromes and referral to a specialist in favour of exact diagnosis and better outcome.
2. Usage of algorithms could help in diagnosis and following patients.
3. Proper education for parents would be essential: detailed information of disease, prognosis, expectable symptoms, and accentuation of life-threating complications and cancer predisposition. Brochures with these information might be helpful.

SUMMARY OF OUR FINDINGS

1. We examined at first time in acute childhood leukaemia the following SNPs: *CDA* rs1048977, *DCK* rs12648166 and rs4694362, *DCTD* rs4742 and *SLC28A3* rs7853758, rs7867504 and *SLC29A1* rs9394992 and rs324148 in assiciation with ara-C toxicity.

2. We found two SNPs of the *DCK* gene, rs12648166 and rs4694362 in 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.

3. We presented with the first time a case of a three year old patient with Rothmund-Thomson syndrome associated with aggressive biphenotype, biclonal, EBV-associated cutan lymphoma. Moreover, any kind of lymphoma had not described at this young age in conjuction with RTS previously.

4. We presented a Rubinstein-Taybi syndrome patient developed medulloblastoma, identified with a novel heterozygous *de novo CREBBP* NM_004380.2:c.2206C>T mutation in the background. This variant may possibly predispose medulloblastoma in this syndrome.

CONCLUSIONS

1. Confirming the correlation of *DCK* gene rs12648166 and rs4694362 and leukopenia caused by ara-C therapy 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 interpersonal differences can help to individualize chemotherapy and thus potentially improve outcome.

2. There is a hazard of aggressive biphenotype, biclonal, EBV-associated cutan lymphoma in RTS and the risk of any form of lymphoma at this young age in connection with RTS.

3. Regarding to the formation of brain tumor of our patient, the newly identified *CREBBP* variant contributes to the growing database of possible tumor, especially medulloblastoma predisposing mutations.

4. In case of rare hereditary syndromes prone to malignancies exact diagnosis helps to do predictive medicine: to give proper treatment and follow-up, lower cancer risk, improve outcome. Genetic testing helps in diagnosis, surveillance and family planning. With presenting two instructive disease-history and think-over the diagnostics and management of rare cancer predisposition malignancies we may ministrative for other practitioners with patients suffering from the same syndromes.

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LIST OF PUBLICATIONS

Papers on which the thesis is based

1. Gabor KM, Schermann G, Lautner-Csorba O, Rarosi F, Erdelyi DJ, Endreffy E, Berek K, Bartyik K, Bereczki C, Szalai C, Semsei AF: Impact of single nucleotide polymorphisms of cytarabine metabolic genes on drug toxicity in childhood acute lymphoblastic leukemia. Pediatric Blood & Cancer 2015;62(4):622-8 doi: 10.1002/pbc.25379 [PubMed :25557962]. **IF:2.562**
2. Bartyik, KM. Gabor KM, Iványi B, Németh I, Karg E: Rothmund-thomson syndrome and cutan T-cell lymphoma in childhood. Open Journal of Pediatrics, 2013;3:270-273

Other paper related to the topic of the thesis

1. Kutszegi N, Semsei ÁF, Gézsi A, Sági JC, Nagy V, Csordás K, Jakab Z, Lautner-Csorba O, Gábor KM, Kovács GT, Erdélyi DJ, Szalai C, 2015. Subgroups of Paediatric Acute Lymphoblastic Leukaemia Might Differ Significantly in Genetic Predisposition to Asparaginase Hypersensitivity. PLoS One 10(10):e0140136. doi: 10.1371/journal.pone.0140136. [PubMed 26457809]. **IF: 3.234**

Other papers

1. Gábor Krisztina; Antal Andrea; Dibó György; Kéri Szabolcs; Janka Zoltán; Vécsei László; Benedek György Eseményfüggő potenciálok vizsgálata Parkinson-kórban és essentialis tremorban. Ideggyógyászati Szemle, 1999;52(7-8):289-293.
2. Antal A, Dibo G, Keri S, Gabor K, Janka Z, Vecsei L, Benedek G. P300 component of visual event-related potentials distinguishes patients with idiopathic parkinson's disease from patients with essential tremor. J Neural Transm. 2000;107:787–797. [PubMed: 11005544]. **IF: 1.785**
3. Müller Judit, Koós Rozália, Garami Miklós, Hauser Péter, Borgulya Gábor, Schuler Dezsô, Benyó Gábor, Magyarosy Edina, Galántai Ilona, Milei Krisztina, Török Katalin, Bárdi Edit, Hunyadi Katalin, Gábor Krisztina, Masáth Péter, Bodnár László, a Magyar Gyermekonkológiai Hálózat és Kovács Gábor: Gyermekkori Langerhans-sejtes histiocytosissal szerzett magyarországi tapasztalataink. Hungarian Oncology; 2004;48:289-295.