GENETIC ASPECTS OF PREVENTION IN PEDIATRIC HEMATOONCOLOGY

PhD thesis

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List of publications

Papers on which the thesis is based

I. 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. **IF: 2.562**

II. Bartyik K, Gabor KM, Ivanyi B, Nemeth 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

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. Subgroups of Paediatric Acute Lymphoblastic Leukaemia Might Differ Significantly in Genetic Predisposition to Asparaginase Hypersensitivity. PLoS One 2015;10(10):e0140136. **IF: 3.234**

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ABBREVIATIONS

ABC: absolute blast count
ADR: adverse drug reaction
ALL: acute lymphoblastic leukemia
AML: acute myeloid leukemia
ara-C: cytosine arabinoside, 1-b-D-arabinofuranosylcytosine, cytarabine
ara-CDP: cytosine arabinoside-diphosphate
ara-CMP: cytosine arabinoside-monophosphate
ara-CTP: cytosine arabinoside-triphosphate
ara-U: 1-B-D-arabinofuranosyl uracil
ara-UMP: arabinofuranosyl uracil-monophosphate
BFM group: Berlin-Frankfurt-Münster group
BLM-syndrome: Bloom -syndrome
BM: bone marrow
CDA: cytidine deaminase
CI: confidence intervals
CMPK1: cytidine monophosphate kinase 1
CNS: central nervous system
CREBBP: cAMP-response element binding protein - binding protein
CTCAE: Common Terminology Criteria for Adverse Events
DCK: deoxycytidine kinase
DCTD: deoxycytidine-monophosphate deaminase
dCTP: deoxycytidine-triphosphate
EBV: Ebstein-Barr virus
EFS: event-free survival
FC: flow cytometry
hCNT1: human concentrative nucleoside transporter member 1
hCNT3: human concentrative nucleoside transporter member 3
hENT1: human equilibrative nucleoside transporter member 1
GPT: glutamate pyruvate transaminase
HATs: histone acetyltransferases

Hb: hemoglobin

HR: high risk

Ht: hematocrit

LCLs: lymphoblastoid cell lines

LR: low risk

6-MP: 6-mercaptopurin

M: morphology

MR: medium risk

MRD: minimal residual disease

NDPs: nucleoside diphosphate kinases

NHL: non-Hodgkin lymphoma

NR: non-responder

NT5C2: 5'nucleotidase II

OR: odds ratio

OS: overall survival

PCR: polymerase chain reaction

PGR: prednisone good response

PPR: prednisone poor response

RECQL4: recQ protein-like 4

RFHs: recQ-family helicase

RTS: Rothmund-Thomson Syndrome

RRM1 and RRM2: ribonucleotide reductase holoenzyme 1 and 2 subunits

RSTS: Rubinstein-Taybi syndrome

SCT: stem cell transplantation

SLC28A1: solute carrier family 28 member 1

SLC28A3: solute carrier family 28 member 3

SLC29A1: solute carrier family 29 member 1

SNPs: single nucleotide polymorphisms

SR: standard risk

WBC: white blood cell

WRN-syndrome: Werner syndrome

I. THESIS

1. We examined at first time the impact of single nucleotide polymorphisms of cytosine arabinoside metabolic genes on drug toxicity and survival in acute childhood leukaemia: *CDA* rs1048977, *DCK* rs12648166, rs4694362, *DCTD* rs4742, *SLC28A3* rs7853758, rs7867504, and *SLC29A1* rs9394992, rs324148.

2. We found two SNPs of the *DCK* gene, rs12648166 and rs4694362, which were associated with altered risk to leukopenia at the allele, genotype and haplotype levels. None of the SNPs influenced thrombocytopenia, anaemia, infection or the survival of the patients.

3. We present a case of Rothmund-Thomson syndrome associated with aggressive biphenotype, biclonal EBV-associated cutan lymphoma first in the literature. The patient was 3 years old at diagnosis, any kind of lymphoma had not been described at this young age in conjunction with RTS previously.

4. We present 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.

5. We think over diagnosis and management of rare genetic cancer predisposition syndromes in the light of elevated risk to malignancies and emphasize the importance of genetic testing of these disorders.

II. INTRODUCTION

II.1. Pharmacogenetics, personalized medicine, predictive medicine

Cancer genetics is increasingly becoming integrated into the practice of modern pediatric oncology. The knowledge regarding genetic background of malignancies resulted in major evolution in diagnosis, treatment, prognosis and disease-prevention. [Garber 2005] In my study I have been looking for possibilities of genetic aspects of prevention in pediatric oncology: 1. lowering drug toxiticy with the identification of drug metabolism at the level of the genes and 2. preparing for, delaying or even preventing development of potential malignancies with the investigation of determining mutations in cancer predisposition syndromes.

Patients respond in different ways to the same medication. Drug metabolism, therapeutic and adverse effects might be influenced by gender, age, drug interaction, organ function, but it is estimated that genetics can account for 20 to 95 percent of this variability. [Kalow et al. 1998] Pharmacogenetics is the study of genetic background influencing drug effects and aims to use this knowledge for improving therapeutic response and reducing drug toxicity. Recent studies in cancer chemotherapy investigate genetic variation in key genes of cytostatic drugs' metabolic pathway and look for the associations of these polymorphisms with clinical features. [Stuart, Scott 2011].

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 outcome and toxicity. To obtain personalization individual toxicity and survival profiles are required. Knowledge of the known polymorphisms in the patients' genes responsible for serious side-effects or reduced outcome, creates the opportunity of modifying drug dosage to avoid severe toxicity and improve survival. [Kovács 2007]

Knowing of the genetic background of a disease, may allow us to prevent or delay the development of expectable symptoms, release the severity of potential outcome and avoid consecutive morbidity. The aim of predictive medicine is to explore someone's predisposition for a disease, and with appropriate information and regular check-ups enable the patient to live a proactive life to reduce morbidity and mortality. [http://www.quackwatch.org/01QuackeryRelatedTopics/Tests/gpm.html].

In numerous congenital malformations malignancies occur more frequently. Possessing the proper diagnosis – with specific care, aimed attention, careful examination and follow-up – these tumors can be diagnosed in time and enables us to prepare for the potential outcome. Moreover, in some cases, development of cancer may get preventable (like sun screening and frequent dermatologic check-up in case of skin cancer or stool blood test and imaging studies in case of gastrointestinal tumors). [Roelfsema et al. 2005; Capell et al. 2009; Gandon 2014]. Understanding the genetic background, the mechanisms of the evolution of underlying condition and tumor formation, helps us in this effort.

II.2. Pharmacogenetic study of cytosine arabinoside

II.2.1. Childhood acute lymphoblastic leukemia

II.2.1.1. Symptoms, diagnosis, prognosis

Acute lymphoblastic leukemia (ALL) is the most common childhood hematologic malignancy, representing more than a quarter of all pediatric cancers and almost the three-quarter of all cases of pediatric leukemia. 50 to 70 children diagnosed in every year in Hungary [Garami et al. 2014]. There is a peak incidence among children aged 2 to 5 years. Males are affected more often than females except in infants, the difference increases with puberty [Conter et al. 2004].

ALL is clonal disease of lymphoblasts, the normal control of proliferation is damaged and the cells are also stucked at any point of the early stages of normal lymphoid differentiation. It is also generally accepted that tumorgenesis results from complex interaction between inherited genetic background and specific environmental exposure. [Lautner-Csorba et al. 2012; Pui et al. 2004]

ALL signs and symptoms mostly emerge rapidly, that reflect bone marrow infiltration as hemorrhage (thrombocytopenia), recurring infections (neutropenia), or pallor, fatigue and lethargy (anaemia). Pain in the limbs due to leukemia infiltration of the periosteum, bone and joints, or due to the expansion of the marrow cavity by the abnormal cells is often the initial symptom. Less common sings are of any organ or tissue infiltrated by leukemia cells include headache, vomiting, oliguria and anuria. Even life-threatening incidences such as hyperacute infection, bleeding, elevated intracranial pressure or episode of respiratory distress may also note as the first symptom of the disease. Enlargement of the spleen and the liver, lymphadenopathy, rarely testicular involvement may be also present at diagnosis. Symptoms of central nervous system (CNS) involvement are rarely presented at initial diagnosis but if so, these are more common in T-lineage and mature B cell ALL [Ribera et al. 2009; Conter et al. 2004].

ALL is a biologically heterogeneous disorder; complete morphologic, immunologic, cytogenetic, biochemical, and molecular genetic characterizations of leukemic cells are needed to establish the exact diagnosis. The subtypes of ALL needs altered doses of chemotherapeutic drugs or different medication to the proper therapy, as well the prognosis of the disease depends on several factors.

Prognosis is determined by three main factors according to the ALL IC-BFM Protocol 2009:

(i) *ALL subtypes:*

t(9;22) [BCR/ABL], t(4;11) [MLL/AF4] and hypodiploidy ≤ 45 , T-cell immunphenotype are negative predictors

t(12;21)(p13;q22)[TEL-AML1] or Down syndrome, hyperdiploidy are predictors of positive outcome.

(ii) *Initial clinical data of the patients:*

Age 1 to 6 and initial white blood cells (WBC) count lesser than 20000/μL account better;

CNS manifestation is responsible for poorer prognosis

(iii) *Response to therapy:*

Peripheral blast count on the therapeutic day 8, bone marrow (BM) status and minimal residual disease (MRD) on the day 15 and 33, duration of the first remission and site of relapse influence the outcome. Prednisone poor responders (PPR ≥1000 blasts/μL in the peripheral blood count on day 8), M2 (BM with 5% - 25%) or M3 (BM with ≥ 25% blasts) flow cytometric

assessment of (FC) MRD 0,1% - 10% or >10% on the day 15 and 33 mean worse recovery options, versus prednisone good responders (PGR <1000 blasts/μL in the peripheral blood count on day 8), bone marrow status M1 (BM with < 5% blasts), FC MRD < 0,1% on the day 15 and 33.

Using combined chemotherapy, pediatric ALL is a well curable disease. In Hungary, approximately 85% of patients with ALL survive 5 years after therapy [Garami et al. 2014].

II.2.1.2. Therapy

Hungary joined to the Berlin-Frankfurt-Münster (BFM) Study Group, therefore Hungarian children with leukemia receive therapy according to the actual ALL-BFM Protocol. The protocols used in the treatment of our study patients (ALL-BFM Protocol 1990, 1995, ALL IC-BFM Protocol 2002), as well as the current protocol (ALL IC-BFM Protocol 2009) are based on the stratification shown in **Table 1.** Three distinct risk groups are derived based on prognostic factors detailed above: standard-risk/low-risk (SR/LR), medium-risk/intermediate-risk (MR/IR) and high-risk (HR). Infants are treated with INTERFANT-06 Protocol.

During BFM treatment protocols patients undergo induction, early intensification, consolidation, reinduction and maintenance therapy. SR and MR risk group's children are treated mainly with the same medicaments, differences can be found only in treatment dosage and duration. HR branch therapy varies mainly in consolidation phase: patients get intensive blocks of chemotherapy. The main drugs in the SR/MR protocols are prednisolone, dexamethasone, vincristine, daunorubicin, doxorubicin, asparaginase, methotrexate, cyclophosphamide, cytarabine, 6-mercaptopurine, 6-thioguanine. In the HR protocol this medication is completed with ifosphamide and etoposide.

HR, T-immunophenotype, or CNS leukemia positive patients receive cranial irradiation as central nervous system preventive therapy.

Allogenic stem cell transplantation (SCT) is indicated as first-line therapy in specific circumstances shown in **Table 2.**

The completion of the therapy, without SCT, lasts for about two and a half years.

Table 1. ALL IC-BFM 2009 Classification [ALL IC-BFM 2009]

STANDARD-RISK (SR)	HIGH-RISK GROUP (HR)	MEDIUM-RISK GROUP (IR)
PB day 8: < 1000 blasts/µL *and* age ≥ 1 yr – < 6 yr *and* initial WBC < 20000/µL *and* if available FC MRD < 0,1% or M1/ M2 marrow on day 15 *and* no M2/M3 marrow on day 33 *All criteria must be fulfilled.*	1. IR and, if available FC MRD >10% or M3 marrow on day 15 2. SR if available FC MRD >10% 3. PB on day 8: ≥ 1,000 ABC/µL 4. M2 or M3 marrow on day 33 5. Translocation t(9;22) [BCR/ABL] or t(4;11) [MLL/AF4] 6. Hypodiploidy ≤ 45 *At least one criterion must be fulfilled.*	All patients who are not stratified to SR or HR are medium- risk patients.

ABC: absolute blast count (% blasts x WBC), PB: Peripheral blood, FC MRD: flow cytometric assessment of minimal residual disease, WBC: white blood cell

Bone marrow status (% blasts) M1: < 5, M2: ≥ 5 – < 25, M3: ≥ 25; M: morphology

Table 2. Indications for allogenic SCT [ALL IC-BFM 2009].

INDICATIONS		MFD SCT
NR^ d33		+
PPR	+ T-ALL	+
	+ pro B-ALL	+
	+ WBC > 100,000/µL	+
	+ t(9;22) or BCR/ABL	+
	+ t(4;11) or MLL/AF4*	+
PGR	+ t(9;22) or BCR/ABL	+
HR	+ M3 d15	+

ALL: acute lymphoblastic leukemia, HR: high risk, MFD matched family donor, NR: non responder, PGR: prednisone-good response (blasts < 1,000/µL), PPR: prednisone-poor response (blasts ≥ 1,000/µL), SCT: stem cell transplantation, WBC: white blood cells

* Infants < 1 yr only

^NR: M3 and FC MRD >10%

HR patients due only to M3 at day 15 are not eligible for SCT

II.2.2. Cytarabine

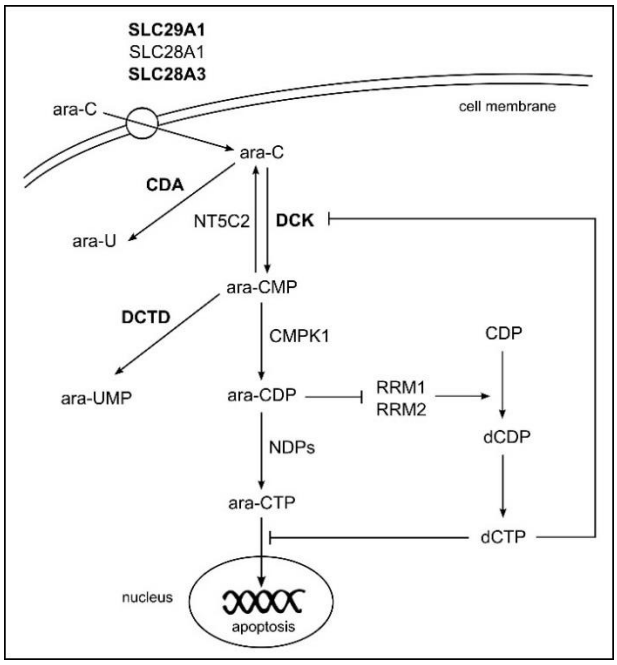
II.2.2.1. Metabolism, effects, toxicity

The Berlin-Frankfurt-Münster (BFM) Study Group used the nucleoside analogue cytarabine (ara-C, cytosine arabinoside, 1-b-D-arabinofuranosylcytosine) in 1981 for the first time in combination with methotrexate, cyclophosphamide and doxorubicin [Bowman et al. 1996; Reiter et al. 1992; Rivera 1994]. 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.

The major toxicities of ara-C at standard dose are myelosuppression, mucositis and infection [Peters 2006]. 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. There is a high interpatient variability of sensitivity and toxicity to ara-C, therefore 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 [Lamba et al. 2007; Hartford et al. 2009; Young et al. 2013]. Ara-C requires active cellular uptake via nucleoside transporters (Fig. 1). The primary transporters are solute carrier family 29 member 1 (SLC29A1, previous name is human equilibrative nucleoside transporter member 1, hENT1) which transports 80% of the drug, and solute carrier family 28 member 1 (SLC28A1, previous name is human concentrative nucleoside transporter member 1, hCNT1) [Gray et al. 2004; Cros et al. 2004; Sarkar et al. 2005; Young et al. 2013]. The expression of solute carrier family 28 member 3 (SLC28A3, previous name is human concentrative nucleoside transporter member 3, hCNT3) was slightly increased in H9-ara-C cells selected with high-dose ara-C [Sarkar et al. 2005]. Inside the cell, ara-C is metabolized by the same pathway as other nucleoside analogues; e.g. gemcitabine, decitabine, and clofarabine [Cros et al. 2004]. Conversion of ara-C into cytosine arabinoside-monophosphate (ara-CMP) by deoxycytidine kinase (DCK) is the rate-limiting step for further phosphorylation [Lamba et al. 2007; Hartford et al. 2009]. 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) [Cros et al. 2004; Emadi and Karp 2012]. 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-alfa [Kufe et al. 1984; Cros et al. 2004; Lamba 2009; Emadi and Karp 2012]. 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 [Graham and Whitmore 1970; Lamba 2009]. Ara-CMP is dephosphorylated by 5'nucleotidase II (NT5C2), thereby preventing the production of ara-CTP [Dumontet et al. 1999; Lamba 2009]. Several feedback mechanisms influence the metabolism of ara-C, for example, deoxycytidine-triphosphate (dCTP) is a potent feedback inhibitor of DCK [Hubeek et al. 2005]. Intracellular dCTP pools are regulated by ribonucleotide reductase holoenzyme (consisting of RRM1 and RRM2 subunits) (see **Figure 1.)** [Shao et al. 2006].

Figure 1. Schematic description of ara-C transport and metabolism



Bold letters indicate genes that are examined 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, CDP: cytidine-diphosphate, cytidine-diphosphate 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 and RRM2: ribonucleotide reductase M 1 and 2, SLC28A1 and SLC28A3: solute carrier family 28 member 1 and 3, SLC29A1: solute carrier family 29 member 1

Several in vitro studies have verified, that intracellular level of ara-CTP is determined by cellular sensitivity to ara-C [Lamba et al. 2007; Hartford et al. 2009]. In vivo observations have demonstrated association between achieving complete remission and intracellular levels of ara-C [Dumontet et al. 2009].

II.2.2.2. Pharmacogenetics. The examined SNPs in our study

Numerous studies reported that SNPs play a significant role in modifying pharmacokinetics and pharmacodynamics of pyrimidine antagonists thus the development of adverse effects [Maring et al.2005]. Many trials investigate cytarabine and gemcitabine, a nucleoside analogue with a very similar metabolic pathway to that of cytarabine. Gemcitabine is used mainly in solid tumors therapy [Alvarellos et al 2014]. We examined ara-C pharmacogenetics: we looked for association between SNPs of 5 genes: *CDA, DCK*, *DCTD, SLC28A3, SLC291* and ara-C toxicity. These genes are coding enzymes and transporter molecules important in ara-C transport and metabolism.

Cytidine deaminase

CDA is the predominant ara-C degradation enzyme [Hubeek et al. 2006]. Although CDA residual activity in serum seems to be predictive of toxicity [Maring et al 2005; Ciccolini et al. 2010] and resistance [Steuart et al. 1971; Yusa et al. 1992] in adults after ara-C and other nucleoside analogues based chemotherapy, some authors have found genotype screening failed to identify *CDA* T435C, G208A and A76C SNPs associated with the occurrence of gemcitabine toxicity [Ciccolini et al. 2010; Mercier et al. 2007]. However many others showed direct connection of these polymorphisms and the adverse drug reactions (ADRs) and clinical outcome [Sugiyama et al. 2007; Tibaldi et al. 2008]. *CDA* G208A polymorphism identified in the Japanese population proved to result in significantly lower CDA activity and increased sensitivity to ara-C [Yue et al. 2003], but was not present in Caucasians or African–Americans [Gilbert et al. 2006]. *CDA* A79C was found to lower the activity of the enzyme, hence a decreased rate of ara-C metabolism [Kirch et al. 1998]. *CDA* A76C resulting in decreased enzyme activity was also led to an increased risk of treatment related mortality with ara-C therapy in children for AML [Bhatla et al. 2009]. A76C was also found to be responsible for life-threatening consequences in lymphoma patients treated with ara-C [Banklau et al. 2010]. Moreover, *CDA* C111T and A76C

haplotype showed significant association with gemcitabine toxicity and overall survival (OS) [Capizzi et al. 1991].

Deoxycytidine kinase

DCK (deoxycytidine kinase) is required for the pharmacologic activity of several clinically important anticancer nucleoside analogues. 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 catalyses the conversion of ara-C to ara-CMP [Chottiner et al. 1991]. 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 [Lamba et al. 2007; Sarkar et al. 2005]. 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 [Lamba et al. 2007].

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) [Hartford et al. 2009]. 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 [Hartford et al. 2009]. These data provide evidence that genetic variation within the *DCK* gene can affect function of the protein.

Deoxycytidine-monophosphate deaminase

DCTD is another pyrimidine inactivation enzyme, although its role in the pathogenesis of ara-C sensitivity is still poorly identified. *DCTD* Asn58Asp has decreased activity in in vitro assays [Kirch et al. 1998]. Substantial role for DCTD in the metabolism of ara-C in T-lymphoblastic leukemia have been demonstrated [Capizzi et al. 1991, Fridland et al. 1987], while others found no association between long/small cell lung cancer survival and DCTD expression treated with gemcitabine [Ueno et al. 2007]. *DCTD* T-47C was reported to be in weak association with OS in pancreatic cancer patients [Okazaki et al. 2010].

Solute carrier family 28 member 3 and solute carrier family 29 member 1

The activity of nucleotide transporters plays a role in ara-C sensitivity [Hubeek et al. 2005; Stam et al. 2003]. The *SLC29A1* promoter region haplotype containing the C1345G, G1050A, and G706C SNPs was reported to influence gene expression [Myers et al. 2006]. While other in vitro studies exploring *SLC29A1* haplotype have found no functional significance [Kim et al. 2006; Osato et al. 2003]. In pancreatic cancer treated with gemcitabine *SLC28A3* A25G showed significance with hematologic toxicity and combined genotype effect of *SLC28A3* C-69T and *SLC29A1* T-549C on ADRs was detected. Combined genotype effect of *SLC29A1* C913T, *DCK* C-1205T, *CDA* A-76C and *DCTD* T-47C on OS also was confirmed [Okazaki et al. 2010]. In pancreatic cancer *SLC29A1* A201G have been suggested to affect patient outcome and toxicity [Tanaka et al. 2010]. So far there are not published in vivo studies on the influence of the SNPs on nucleoside transporters in patient treated with ara-C.

II.3. Rare hereditary cancer predisposition syndromes

II.3.1. Hereditary syndromes disposed to malignancies

Numerous inherited mutations of genes are associated with heightened susceptibility to specific malignancies. 5% to 10% of all cancers belongs to a cancer predisposition hereditary syndrome. Identification of underlying genetic aberrations, revealing gene penetrance, which 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. [Garber, 2005; Gerstenblith, 2010]

II.3.2. Rothmund-Thomson syndrome

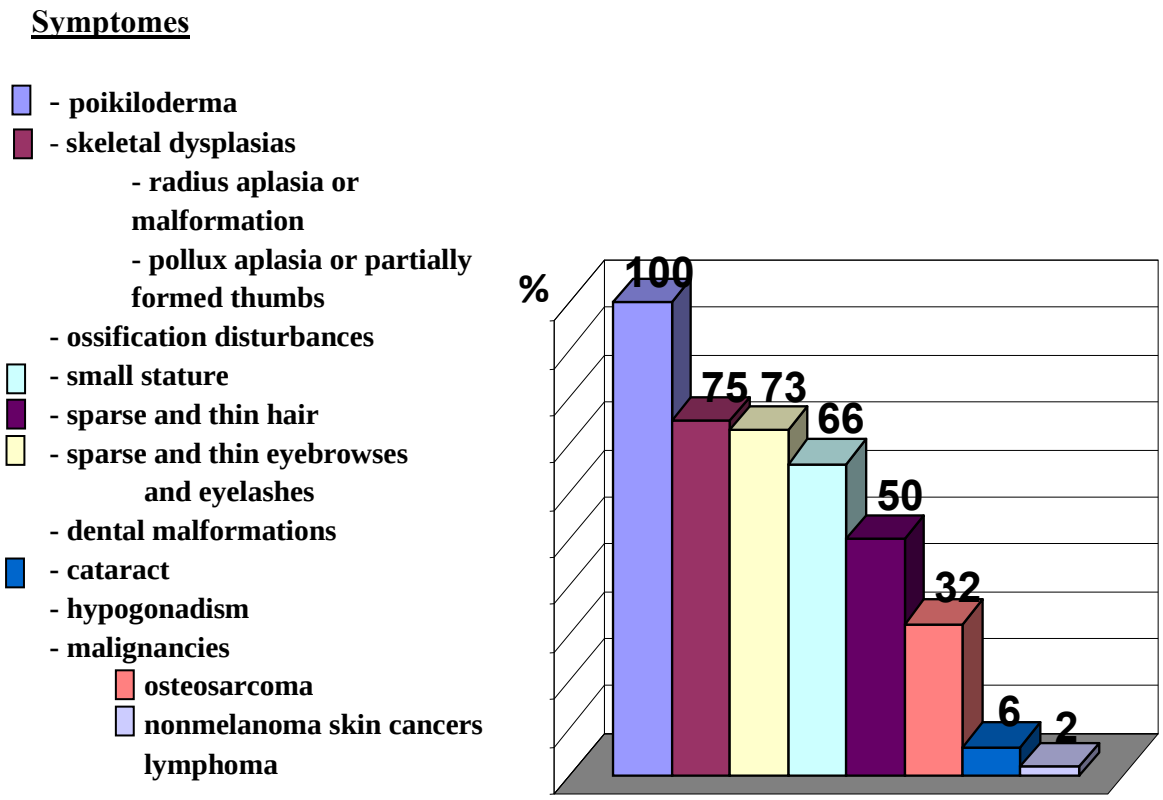
Rothmund-Thomson syndrome (RTS) was described first in 1868 by Rothmund [Rothmund 1868] and in 1936 by Thomson [Thomson 1936]. Up to nowadays approximately 300 cases have been reported in the literature [Wang et al. 2006]. There are two types of RTS. Type I is characterised by poikiloderma, ectodermal dysplasia and juvenile cataracts while type II is featured by poikiloderma, congenital bone defects and an increased risk of cancer [Wang et al. 2003].

Patients generally present skin rash, small stature, and skeletal dysplasias. Other cutaneous symptoms like photosensitivity, poikiloderma, hyperkeratosis, alopecia, other abnormalities such as dystrophic teeth, nails, juvenile cataract, short stature, hypogonadism, congenital bone defects, soft tissue contractures, and mental retardation can be present [Larizza et al. 2010].

The characteristic skin findings are diagnostic hallmarks of the syndrome. More than 90% of patients develop initial skin manifestations during the first year of life, usually from month 3 to 6. The acute phase begins in early infancy as red patches or oedematous plaques, sometimes with blistering. The cheeks are usually first involved, later spread to other areas of the face, the extremities, and the buttocks. Over months to years, the rash enters a chronic stage characterized by poikiloderma (atrophy, telangiectasias, and pigmentary changes). Photosensitivity is a feature in more than 30% of cases. Acral

hyperkeratotic lesions on the elbows, knees, hands, and feets can be seen at puberty. [Vennos, James 1995]. Palmar keratoderma has also been reported [Popadić et al. 2006]. Patients may have sparse hair, eyebrowses and eyelashes, and dystrophic or atrophic nails. Dental abnormalities include microdontia, failure of eruption. Juvenile cataracts have been reported in as many as 40% to 50% of patients aged 4 to 7 years. Patients usually have short stature, which ranges from dwarfism to a small build. About one half of the patients have skeletal abnormalities, most frequently a characteristic facies with frontal bossing, saddle nose, and micrognathia. Small hand and feet disproportionate to the patient's body size are observed in 20% of patients. Approximately 10% of patients have absent or malformed radii, and 5% of patients have absent or partially formed thumbs [Rothmund 1868; Larizza et al. 2010; Vennos, James 1995; Irvine et al. 2010] (see **Figure 2).**

Figure 2. Frequency of symptoms in Rothmund-Thomson syndrome.



RTS has been grouped with other genetic cancer predisposition disorders that fall into the class of DNA repair or chromosomal instability disorders. In the backgound of type II RTS homozygous or compound heterozygous mutations in the *RECQL4* gene were found, while type I RTS is negative for *RECQL4* mutation [Kitao et al. 1999]. The protein

encoded by the *RECQL4* gene (*RecQ protein-like 4*, cytogenetic location at 8q24.3) is a member of a protein family called RecQ helicases (RecQ-family helicases, RFHs), which maintain the structure and integrity of DNA [http://www.ncbi.nlm.nih.gov/projects/mapview/mapsearch.cgi?taxid=9606]. Unwinding double-stranded DNA into single-stranded DNA RFHs are the key enzymes for replicating DNA in preparation for cell division, and for repairing damaged DNA enable it for homologous recombination [Croteau et al. 2012]. In human there are five isoforms of RFHs (RECQL1–5), loss-of function mutations of three of them are responsible for genomic instability leading to autosomal genetic syndromes, premature aging and predisposition of malignancy [Capell et al. 2009]. BLM (RECQL2)-helicase got its name after Bloom-syndrome, WRN(RECQL3)-helicase was named after Werner-syndrome. Mutation of *RECQL4* can lead to Rothmund-Thomson, Baller-Gerold and RAPADILLINO syndrome [Hanada et al. 2007].

According to recent studies RECQL4 has no helicase-activity and promote independently DNA-dependent unwinding [Xu et al. 2009]. Also seems to hasnot To date more than 39 mutations were already found in RTS patients, which lead to the lack of or shortened, non-functional version of the protein [Larizza et al. 2006]. The RECQL4 protein is active in several types of cells before and after birth, particularly in cells of the developing bones and skin, and enterocytes [Dietschy et al. 2007].

Patients are particularly prone to developing osteosarcoma as well as nonmelanomatosus skin cancers (squamosus cell carcinoma, malignant fibrosus histiocytoma) [Rothmund 1868; el-Khoury et al. 1997; Padhy et al. 2010; Green, Rickett 1998; Macura et al. 1998; Howell, Bray 2008; Stinco et al 2008; Piquero-Casals et al. 2002; Capell et al. 2009]. Different types of lymphoma, such as large cell anaplastic T cell lymphoma at the age of 9 years, diffuse large cell B lymphoma and nasopharyngeal non-Hodgkin's lymphoma has been also described [Simon et al. 2010]. Other tumors, like meningeoma [Pencovich et al. 2012], malignant fibrosus histiocytoma [Ilhan et al. 1995] and myelodysplasia [Carlson et al. 2011] may appear. Usually the disease tends to progress during the first year of life, than becomes static so that patients may have a normal lifespan with a good quality of life. The mortality from neoplastic disease during the second or third decade is very significantly increased [Pencovich et al. 2012; Ilhan et al. 1995; Carlson et al. 2011; Castori et al. 2012; Pianigiani et al. 2011; Broom et al. 2006; Marín-Bertolín et al. 1998]. Patients with DNA repair or chromosomal instability disorders have well-known

increased sensitivity to DNA damaging agents including ionizing and ultraviolet radiation [Dahele et al. 2004]. In RTS conflicting results are obtained on this sensitivity [Vennos al. 1992].

II.3.3. Rubinstein-Taybi syndrome

Rubinstein-Taybi syndrome (RSTS) is a condition characterized by a specific pattern of physical features and developmental disabilities. RSTS was first described in 1963 by Jack Rubinstein and Hooshang Taybi [Rubinstein, Taybi 1963]. Short stature even from birth, moderate to severe intellectual disability, distinctive facial features, broad thumbs and first toes are characteristic. The facial features include small jaws, small 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. At smiling, patients' face deforms to a typical grimace with furling eyes. Vertebral deformations, hypermobility of joints, funnel chest, scoliosis and lordosis also occurs more frequently. Cardiac and urinary defects, coloboma, cataract and cryptorchidism are often present. Many of these children have eating difficulties and delayed speech and language development. Susceptibility for infection often leads to hearing impairments. Moreover, people with this condition have an increased risk of developing noncancerous and cancerous tumors, including keloids, certain kinds of brain tumors and cancer of blood-forming tissue (leukemia). [Rubinstein, Taybi 1963; Bonioli et al. 1993; Roelfsema et al. 2005]

The condition occurs in an estimated 1 in 100,000 to 125,000 new-borns, it is equally present in males and females [Bartholdi et al. 2007].

RSTS is considered to be inherited in an autosomal dominant manner, but the vast majority of cases results from a *de novo* heterozygous mutation. Damage to the short arm of chromosome 16 (16p 13.3) (*CREBBP* gene) and damage on chromosome 22 (22q13) (*EP300* gene) have also been detected about half of the people with RSTS [Petrij et al 1995; Bartsch et al. 2010]. More than hundred mutations, mainly in *CREBBP* – range from point mutations to very large deletions, translocations and pericentric insertions – are published to date; these mutations seem imfluence clinical outcome [Bartsch et al. 2010; Chrivia et al. 1993].

CREBBP (cAMP-response element binding protein-binding protein) and *EP300* (E1A-associated cellular p300 transcriptional co-activator protein) genes encode

homologous transcriptional co-activator proteins. These proteins are thought to be the bridge between the DNA-binding transcription factor and the RNA polymerases [Kwok et al. 1994; Lundblad et al. 1995]. CREBBP and P300 also act as histone acetyltransferases (HATs). Histone-tail acetylation is an epigenetic modification that serves to control transcription, deregulation of histone modification are substantial for the tumorgenesis [Johanessen, 2015]. In addition CREBBP and P300 serves as cofactors to several transcription factors and modulate p53, that also increase tumor incidence. [Ogryzko et al. 1996]. Despite the homology of the two proteins, CREBBP and EP300 slightly differ in the structure of the protein, in the subcellular localisation and expression patterns during oocyte development and embryogenesis [Kasper et al. 2006; Kwok et al. 2006; Yao et al. 1998]. Experiments with cell-lines and *CREBBp* and *EP300* knockout mice revealed differences in the role of these proteins [Kasper et al. 2006; Yao et al. 1998; Ugai et al. 1999]. Furthermore, skeletal features of RSTS caused by *EP300* mutations seem to be milder [Bartholdi et al 2007]. Still, there are no references that CREBBP and p300 differ in their roles as HATs and there is a large overlap in the range of other proteins they can acetylate [Roelfsema et al. 2005].

III. 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, rs4694362, *DCTD* rs4742, *SLC28A3* rs7853758, rs7867504, and *SLC29A1* rs9394992, 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 biphenotype, biclonal 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 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 *CDA* rs1048977 (C111T), *DCK* rs12648166 (A9846G), rs4694362 (C1205T), *DCTD* rs4742 (T47C), *SLC28A3* rs7853758 (C69T), rs7867504 (A25G), and *SLC29A1* rs9394992 (C913T), rs324148 (T549C) SNPs and cytarabine toxicity?

2. Can we find any association of haplotype blocks *DCK* rs12648166 (A9846G), rs4694362 (C1205T), *SLC28A3* rs7853758 (C69T), rs7867504 (A25G) and *SLC29A1* rs9394992 (C913T), rs324148 (T549C) and cytarabine toxicity?

3. Can we find any association between the *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) SNPs and overall and event-free survival in our population?

4. Can we find any association of haplotype blocks *DCK* rs12648166 (A9846G) and rs4694362 (C1205T), *SLC28A3* rs7853758 (C69T) and rs7867504 (A25G) and *SLC29A1* rs9394992 (C913T) and rs324148 (T549C) and overall and event-free survival in our population?

5. Can we indentify any causative mutation at our RSTS patient?

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

IV. PATIENTS AND METHODS

IV.1. Pharmacogenetic study of cytosine arabinoside

IV.1.1. 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 3**. 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. A part of the DNA samples were stored in the „Biobank for Childhood acute lymphoid leukemia, osteosarcoma and testicle tumors" at the Department of Genetics, Cell- and Immunobiology, Semmelweis University. 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. 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.

Ara-C was administered in the intensification (Protocol 1/II.) and reintensification (Protocol 2/II.) phases. The course of the dosing was daily doses of 75 mg/m2 intravenously 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 every day 60 mg/m2/day 6-mercaptopurine (6-MP) orally in Protocol 1. and 50 mg/m2/day thioguanin (TG) in Protocol 2.; and 12 mg/two weeks methotrexate intrathecally. Two days before the first ara-C administration, the patients were given a single dose of intravenous cyclophosphamide

(1 g/m2). HR patients of the ALL-BFM 1990 and 1995 protocols got ara-C therapy for two weeks only in the reintensification phase.

Table 3. Characteristics of patients during the examined period

Variable		**Patients**
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 x 10^9/L)	31 (22)
	Grade 3-4 (<2.0 x 10^9/L)	109 (78)
Thrombocytes (10^{-9}/L)	Median (range)	77 (5-416)
Thrombopenia (%)	Grade 1-2 (>50 x 10^9/L)	103 (73)
	Grade 3-4 (<50 x 10^9/L)	38 (27)
Hemoglobin (g/l)	Median (range)	75 (40-125)
Anemia (%)	Grade 1-2 (>80)	53 (36)
	Grade 3-4 (<80)	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%

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

Ara-C was administered in the intensification (Protocol 1/II.) and reintensification (Protocol 2/II.) phases. The course of the dosing was daily doses of 75 mg/m2 intravenously 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 every day 60 mg/m2/day 6-

mercaptopurine (6-MP) orally in Protocol 1. and 50 mg/m2/day thioguanin (TG) in Protocol 2.; and 12 mg/two weeks methotrexate intrathecally. Two days before the first ara-C administration, the patients were given a single dose of intravenous cyclophosphamide (1 g/m2). HR patient of the ALL-BFM 1990, 1995 protocols got ara-C therapy for two weeks only in the reintensification phase.

We investigated the first two weeks of ara-C therapy after the cyclophosphamide administration at every patient in the intensification phase except HR patients of the ALL-BFM 1990, 1995 protocols, who's ara-C therapy was investigated in the reintensification phase. During our studied period all the patients got 8 times 75 mg/m2 doses of ara-C intravenously, a continuous 60 mg/m2/day 6-MP or 50 mg/m2/day TG orally and 1 dose 12 mg MTX intrathecally.

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 2/3/4) were monitored in the patients' medical records. Adverse drug reactions were graded according to Common Terminology Criteria for Adverse Events v3.0 (CTCAE). Toxicity data were collected up to the next ara-C administration or in lack of following ara-C regimen up to the recovery of the indicator values (leukocytes, hemoglobin, thrombocytes, creatinine and GTP activity).

The 5-year event-free survival (EFS) was calculated from the date of diagnosis to the date of relapse. Survival data of patients were received from the National Pediatric Cancer Registry of Hungary.

IV.1.2. SNP selection

We selected 8 SNPs of 5 genes: *CDA* rs1048977 (C111T), *DCK* rs12648166 (A9846G), rs4694362 (C1205T), *DCTD* rs4742 (T47C), *SLC28A3* rs7853758 (C69T), rs7867504 (A25G), and *SLC29A1* rs9394992 (C913T), 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. 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 4.**

Table 4. The studied SNPs, distribution of genotypes and alleles in ALL children

Gene	rs number	Chr	Function
CDA	rs1048977	1p36.2	Thr145Thr
DCK	rs12648166	4q13.3	intron
	rs4694362		intron
DCDT	rs4742	4q35.1	Val116Val
SLC28A3	rs7867504	9q21.3	Thr89Thr
	rs7853758		Leu461Leu
SLC29A1	rs324148	6p21.1	intron
	rs9394992		intron

Chr: chromosome, MAF: minor allele frequency, SNP: single nucleotide polymorphism

IV.1.3. DNA extraction

DNA was isolated from peripheral blood taken during remission phase using Qiagen isolation kits (QIAmp DNA Blood Maxi Kit, Qiagen, Hilden, Germany) appropriately to the manufacturer's instructions. 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 (Roche) according to the 2.7 protocol, but we needed to deviate from the description: we drew the abraded cells in 200µl PBS and incubated on 55 ˚C for two hours is 40µl Proteinase K, then we followed the method in accordance to the instructions.

III.1.4. Genotyping

The SNPs were genotyped using the fluorescence-based competitive allele-specific KASPTM by Design genotyping assays (LGC Genomics, Teddington, UK) according to the manufacturer's instructions. This system uses two allele-specific primers (one for each SNP allele, each primer contains a unique unlabelled tail sequence at the 5' end); one common reverse primer. We used two 5' fluor-labelled oligos, one labelled with FAM, one with HEX (these oligo sequences are designed to interact with the sequences of the tails of the allele-specific primers) and two oligos, with quenchers bound at the 3' ends (these oligo sequences are complementary to those of the fluor-labelled oligos and therefore also

complementary to the tails of the allele-specific primers). These quenched oligos therefore bind their fluor- labelled complements and all fluorescent signal is quenched until required. In the initial stage of PCR, the appropriate allele-specific primer binds to its complementary region directly upstream of the SNP (with the 3' end of the primer positioned at the SNP nucleotide). The common reverse primer also binds and PCR proceeds, with the allele-specific primer becoming incorporated into the template. During this phase, the fluor-labelled oligos remain bound to their quencher-bound complementary oligos, and no fluorescent signal is generated. As PCR proceeds further, one of the fluor-labelled oligos, corresponding to the amplified allele, also gets incorporated into the template, and is hence no longer bound to its quencher-bound complement. As the fluorophore is no longer quenched, the appropriate fluorescent signal is generated and detected. If the genotype at a given SNP is homozygous, only one of the possible fluorescent signals will be generated. If the individual is heterozygous, the result will be a mixed fluorescent signal. (KASP version 4.0 SNP Genotyping Manual). PCR reactions were carried out using a 7900HT Fast Real-Time PCR System (Life Technologies, Grand Island, NY). Samples with known genotypes were used in every measurement for technical control.

IV.1.5. Statistical methods

A Hardy-Weinberg equilibrium analysis for genotype distribution and differences in allele distribution between the groups was carried out using a Chi^2 goodness-of-fit test using an online application (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). A significant violation of Hardy-Weinberg equilibrium 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 r2) 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.

IV.2. Patient with Rothmund-Thomson syndrome

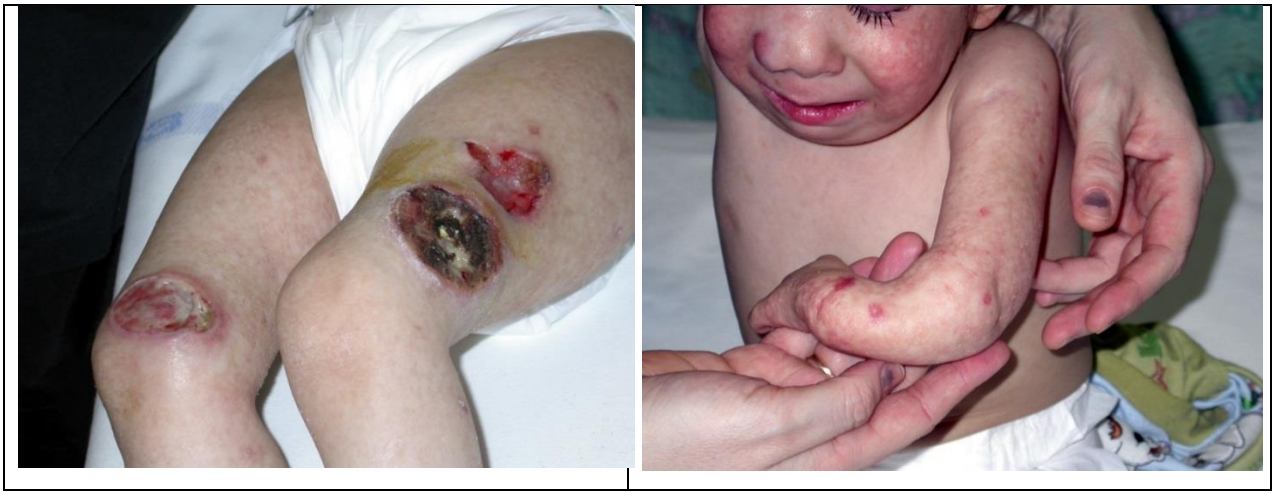
Our patient was born in 40 weeks of gestation, at weight 2430 gr. At birth she had multiplex anomalies: palatoschisis, skeletal abnormalities (aplasia radii, hypoplastic right and left thenar and thumbs, pes equinus on both side), ectopy renis and additional pneumothorax. Further dental malformations, growth retardation, cranial dysostosis with saddle nose and facial dysmorphism, sparse scalp hair, eyebrows and eyelashes, telangiectasia, dystrophic nails and photosensitivity, mild mental retardation were observable). Her family history was negative. Chromosome examination showed 46 (XX), normal karyotype. Examinations had not revealed any metabolic disease. 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.

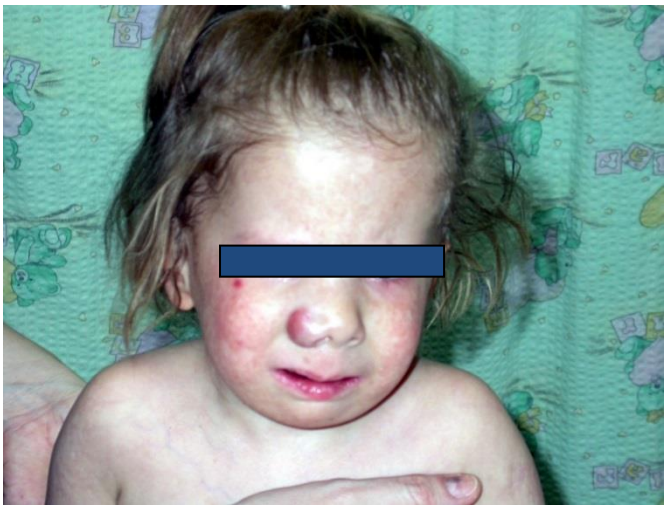
The parents did not bring her to supervision, even did not used intensificated sun protection. They presented with the girl at her age 3 and half at our clinic with delayed gain in weight and diarrhoea. At this time painful hyperemic, compact swelling on her right nasal wing and 3 round, 6 cm in diameter ulcerative lesions on her limbs were present. The parents noticed first the lesions on her limb as maculopapolosus nodes 2 months earlier, which have been treated initially as pyoderma gangrenosum at the dermatology ambulance. Than the lesions became soon purulent, her therapy was completed first with azithromycin, then exchanged to clarithromycin. 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**.** The nasal mess had appeared two weeks before with nose-dripping followed by swelling of the nose wing, which increased after the girl had fallen and hit that

area. She was examined by an otolaryngologist, a CT scan have raised the suspicion of ethmoiditis and sinusitis maxillaris (see **Figure 3.**).

Figure 3. Skin lesions at the time of diagnosis of cutan T-cell lymphoma.

Written consent of publication of the clinical pictures was obtained from the patient's parents





IV.3. Patient with Rubinstein-Taybi syndrome

IV.3.1. Patients

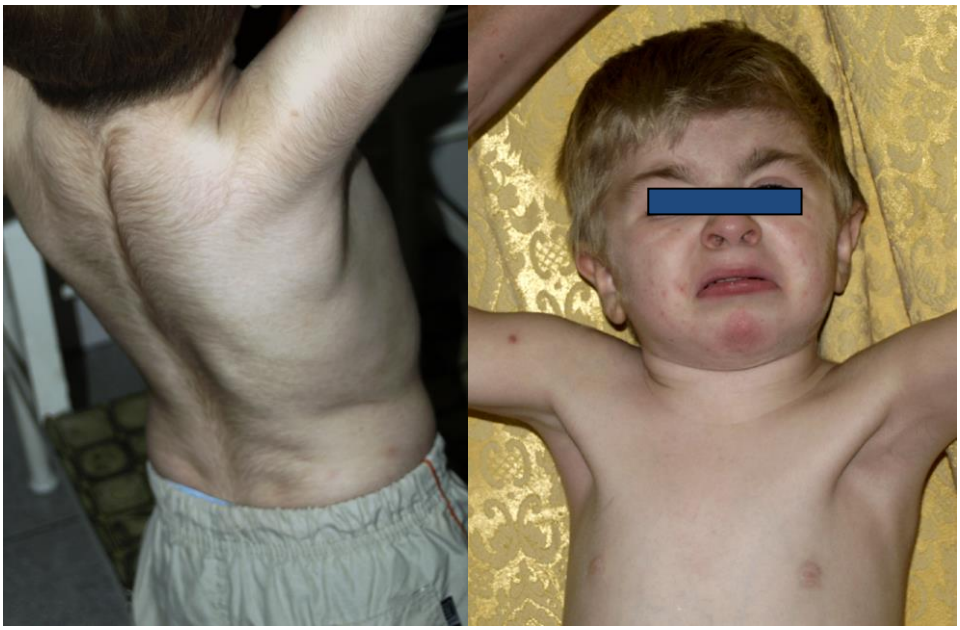
Our patient came to our pediatric clinic at the month 3 for investigation with suspected metabolic disease. At his arrival mild hepatomegaly, unilateral criptorhism, inspiratory stridor, pes equinovarus, trichosis and minor facial anomalies, like epicanthus, extended hair to the forehead, low-set ears were observable. His family history was negative. Due to elevated blood ammonia, lactate and liver-enzyme level he was treated with the diagnosis hyperammoniemia and lactate acidosis. The clinical appearance have raised the suspect of Cornelia de Lange syndrome. Other tests for metabolic diseases, EEG, cranial X-ray and babygram, abdominal sonography were negative, his motor and mental development seemed normal. Chromosome examination showed 46 (XY), normal karyotype. The hyperammoniemia resolved in a month, the lactate serum level normalized at his age 3. Because of recurrent upper respiratory infections and consecutive inhibited nasal respiration and hear-loss adenotomia and grommet insertion happened, when he was 3 years old. At this age his microcephalus, wide nasal bridge and underdeveloped speech became well-marked, though neurogic examination didn't reveal any neurogic disease. He also had orhidopexia. At age 4, he could walk alone, but his speed-reflexes was underdeveloped, he spoke only some worlds and had enuresis without encopresis. Recurrent upper respiratory infections, mental retardation and nanosomy was observable. At this time some other dysmorphic signs became markable: brachydactilia, broad thumbs and first toes, synophris, arched palate, prominent nose and down-slanting eyes (see **Figure 7.**). On the ground of these marks Rubinstein-Taybi syndrome has been diagnosed. C*REBBP* analysis has not been performed considering the characteristic disease morphology and the tardiness (heterogeneous genetic background of the syndrome). We planned follow-up half a year.

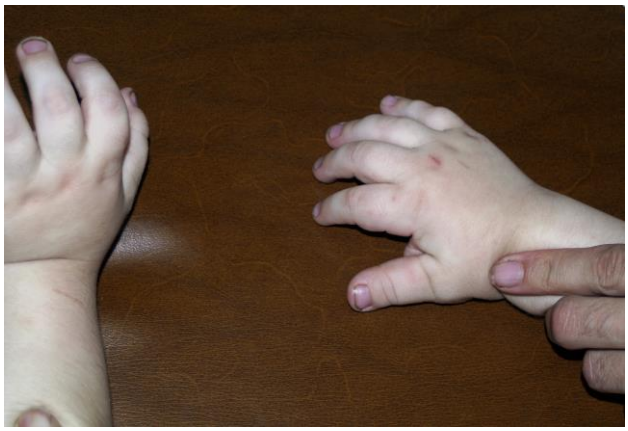
By his age 6, his intellectual status mildly improved, but he used furthermore amphigoric speech and pointing to make himself understood. Considering his underlying condition, that RSTS is disposed to malignancies, especially brain tumors, routine cranial MRI scan was performed, which revealed two lesions, one in the vermis and the other in the left hemisphere with mild compression of the forth ventricle. The lesions 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.

Figure 7. Features of our patient with RSTS.

Written consent of publication of the clinical pictures was obtained from the patients' parents





IV.3.2. Genotyping. Clinical exome sequencing

After obtaining written informed consent, genomic DNA was extracted from peripheral blood leukocytes by standard protocol. For clinical exome sequencing a total of 60 ng of genomic DNA was used for library preparation and sequenced with Trusight One

clinical exome kit (Illumina) on Illumina MiSeq platform. The clinical exome kit covers the coding region of 4813 clinically relevant, disease-associated genes.

The 150 bp paired reads were aligned to the GRCh37.75 human reference genome by Burrows Wheel Aligner (BWA v0.7.9a) software. The variants were called by Genom Analysis Toolkit Haplotype Caller (GATK v3.5) best practice; annotated by SnpEff and VariantStudio softwares. Variants were filtered based on severity and frequency against public variant databases including dbSNP, ClinVar, ExAC, EVS and an in-house clinical exome database of 148 unrelated Hungarian patients.

V. RESULTS

V.1. Pharmacogenetic study of cytosine arabinoside

V.1.1. Genotype and allele frequencies

The 8 SNPs were genotyped in the patient population; the minor allele and genotype frequencies are presented in **Table 5.** The genotype distributions were in Hardy-Weinberg equilibrium for all SNPs.

Table 5. Distribution of genotypes and alleles in ALL children in the studied SNPs.

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

V.1.2. 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 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, p0.0044). No association of leukopenia with the other polymorphisms was observed, neither significant association was found with thrombocytopenia in the investigated population (see **Table 6.**).

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.

V.1.3. 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 7.**

Table 6. 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

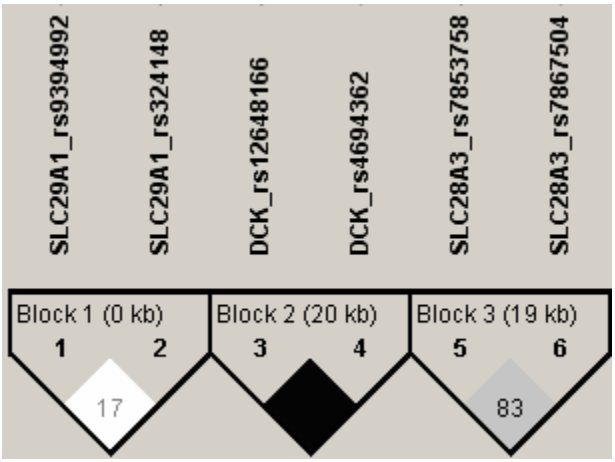
Table 7 Association of haplotype with leukopenia and thrombocytopenia.

Gene	SNPs	Haplotypes	*Leukopenia during the fist twoo weeks of intensification phase of chemotherapy*					*Thrombocytopenia during the fist twoo weeks of intensification phase of chemotherapy*				
			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

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 (see **Figure 7.**).

Figure 7. 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

V.1.4. 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 [Garami et al. 2014]. The SNPs seemed to have no significant influence on the survival of our pediatric ALL population.

V.2. Patient with Rothmund-Thomson syndrome

V.2.1. Histology

Nasal endoscopy and biopsy from the nasal nodule was performed. Histological findings showed biphenotype, biclonal, Ebstein-Barr virus (EBV)-associated cutan lymphoma (see **Figures 4–7)**. Initial investigation in addition to protocol could not reveal systemic presence of the disease, flow cytometry of bone marrow was tumor-free. Peripheral blood smears showed mild anaemia (Htc: 0.29, Hb: 92 g/l), liver and kidney function was normal. Bacterial findings resulted from secretion of the lesions were *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Streptococcus pyogenes*. Antibiotic therapy was given (ceftriaxone, aminoglycoside, clindamycin).

Figure 4. Histology 1**.** Hematoxylin eosin staining

Németh István, Medical University of Szeged, Pathology Institute

Pleiomorf cellproliferation

- atypical blasts ➨
- atypical mitoses →

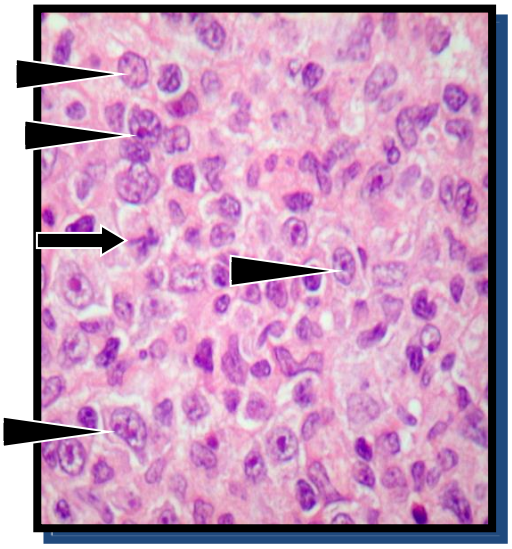
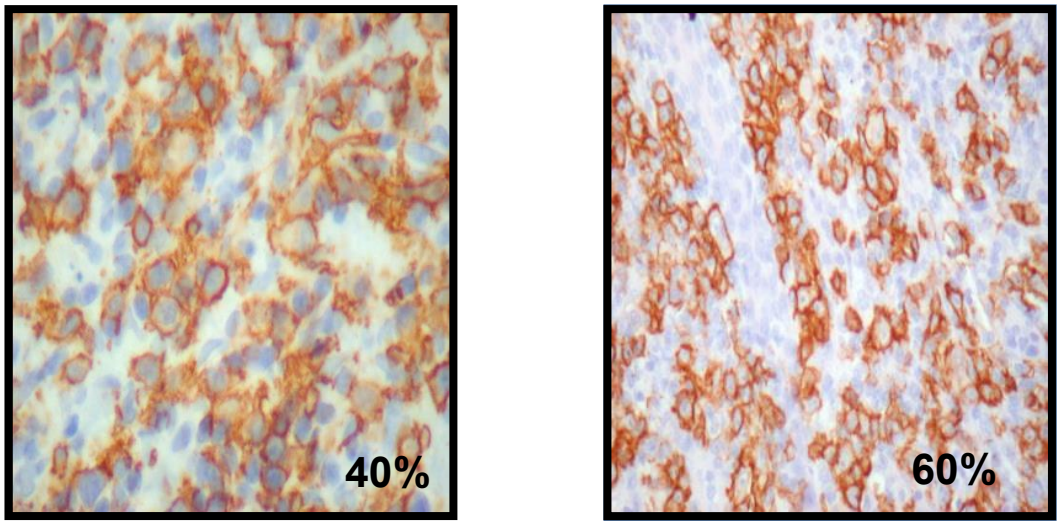


Figure 5. Histology 2. Immunofenotype

Németh István, Medical University of Szeged, Pathology Institute



T – cell markers:
CD2+, CD3+
CD8+, TIA-1+
CD4-, CD56-

B-cell markers
CD20+

Figure 6. Histology 3. EBV immunostaining

Németh István, Medical University of Szeged, Pathology Institute

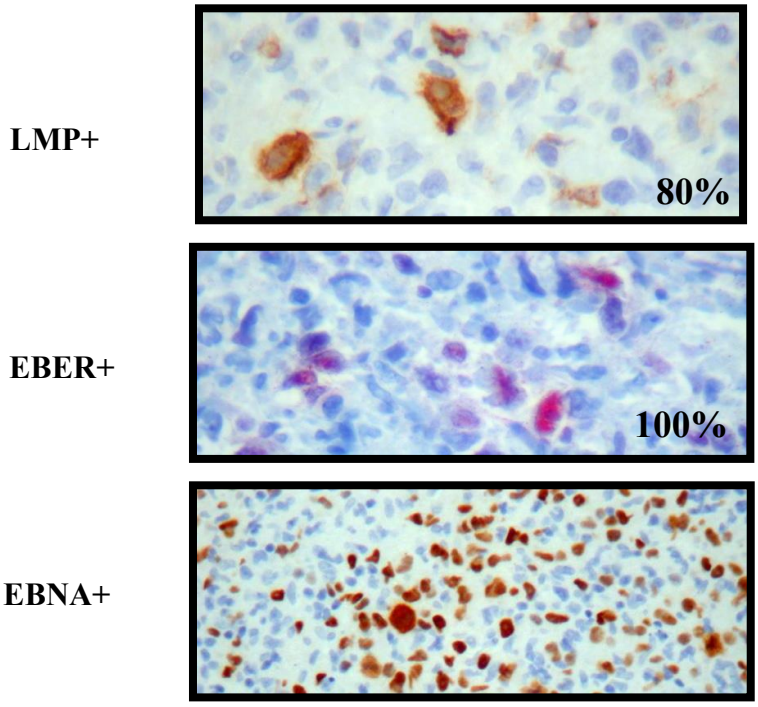
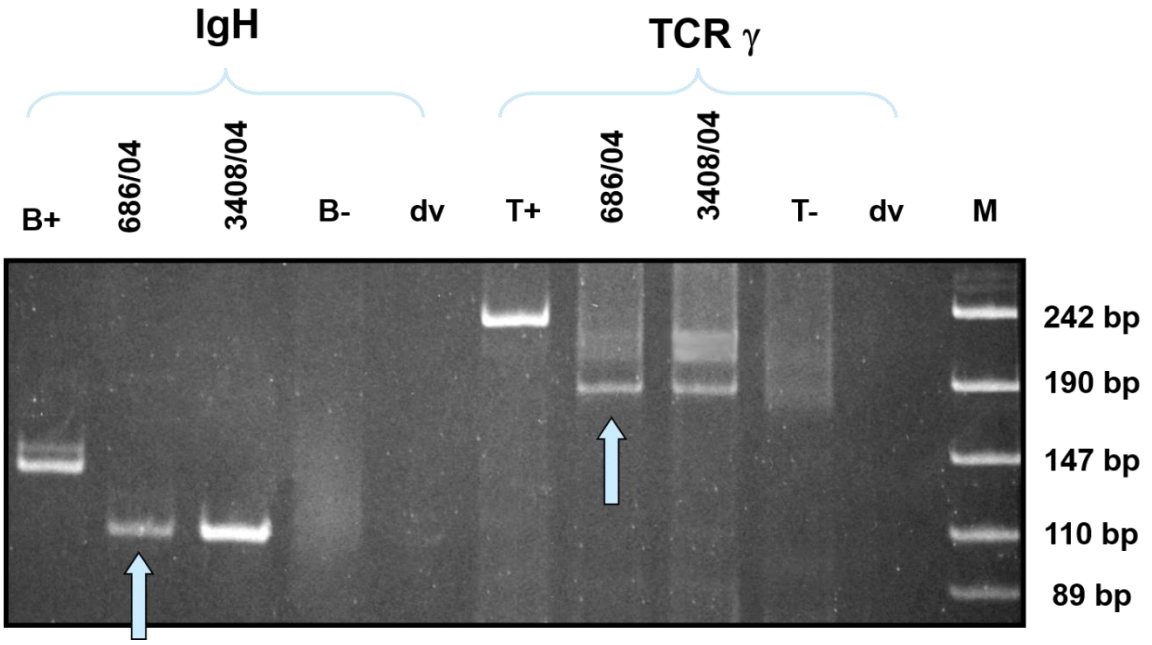


Figure 7. IgH and TCR γ clonal rearrangement



V.3.1. Clinical outcome

We started 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 (prednisolone 60 mg/m2/day, vincristine 2 mg/m2/ week, daunorubicin 20 mg/m2/week, asparaginase 10,000 U/m2 2×/week) 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 proper catheter heparinisation.

V.3. Patient with Rubinstein-Taybi syndrome

V.3.1. Histology

Subtotal removal of the patients brain tumor happened, the histology revealed grade IV. medulloblastoma with focal neural differentiation and calcification, which referred the tumor long-standing origin. Immunophenotype was: CD99+ (diffuse), synaptophisin++

(diffuse), chromogranin A+++ (diffuse), Ki67+++ (>70%), nestin++ (focal), CD56+++, S100 -, vimentin-, HEMA-, MPO-.

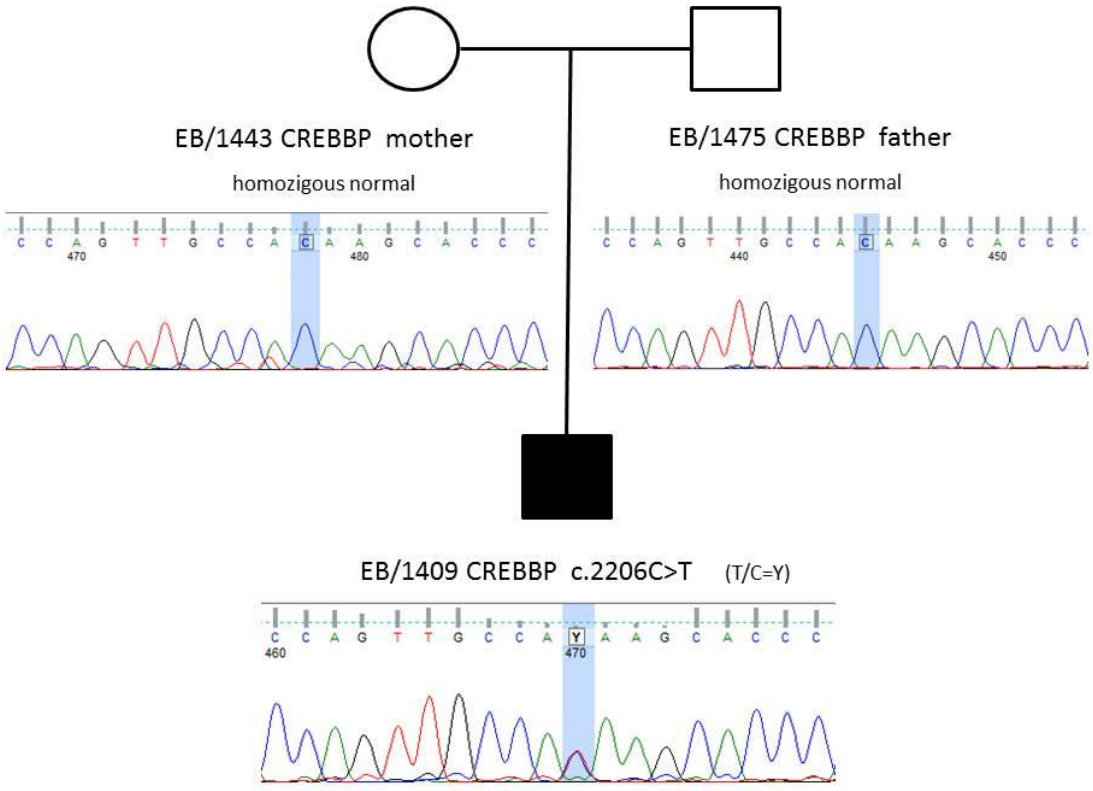
V.3.2. Genotype

Searching for possible causative variations of Rubinstein-Taybi syndrome associated genes we identified variants in the *CREBBP* and *EP300* genes. We excluded all identified EP300 variants as possible causative mutations based on their too high minor allele frequency /NM_001429.3:c.2053+8G>T (MAF=3,43), NM_001429.3:c.2989A>G (MAF=21,06), NM_001429.3:c.*10_*12delGTA (MAF=23,03), NM_001429.3:c.3183T>A (MAF=33,42) and NM_001429.3:c.2242-17_2242-16delTTinsTTT (MAF=35,81)/ in public (ExAc, HGMD, Exome Variant Server) or own 148 unrelated Hungarian control databases. We have also identified two *CREBBP* variations in the sample, one of them NM_004380.2:c.3370-4dupT (rs75459669) is a classified benign variant (ClinVar MAF=24,83) while the other is unique, so far not reported NM_004380.2:c.2206C>T variant causing a nonsense (STOP) NP_004371.2:p.Gln736Ter mutation (see **Figure 8.**). This mutation must be extremely rare, since has not been found in the available public genom databases.

Figure 8. Integrative Genomics Viewer view of the identified *CREBBP* NM_004380.2:c.2206C>T mutation (reverse strand shown) in the index patient.

We have confirmed the presence of the above mutation by targeted Sanger sequencing in the index patient. Patient was confirmed with a heterozygous *de novo* *CREBBP* mutation since both parents have proven to be homozygous normal (see **Figure 9**).

Figure 9. Targeted Sanger resequencing of exon 12 of *CREBBP* gene in the index patient's family.



V.3.1. Clinical outcome

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.

VI. DISCUSSION

VI.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. The relatively small sample size is a limitation of this study. It is not possible to detect minor associations, also the detected associations on the small cohort would result in difficulty in interpreting 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.

Several studies investigated the influence of the genetic background of the patients on treatment response, side effects and patient survival [Gervasini et al. 2012; Mahlknecht et al. 2009], but only a few studies have focused on the *DCK* SNPs examined in our study (rs12648166 and rs4694362). One of those studies analysed 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: *DCK* rs4694362 was associated with neutropenia, and patients with the TT genotype had a higher risk for having grade 3–4 neutropenia [Tanaka et al. 2010]. They also investigated *DCK* rs12648166, but found no association. Another study analysed 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 [Okazaki et al. 2010]. 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 [Kim et al 2013].

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 30 UTR of *DCK* found by Lamba et al. [Lamba et al. 2007], 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 30 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 [Xu et al. 2012]. Polymorphisms in the 50 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 [Shi et al. 2004]. This result could attributed to the genes described above that may be more responsible for the side effects of the treatment.

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.

VI.2. Rare hereditary cancer predisposition syndromes

VI.2.1. Patient with Rothmund-Thomson syndrome

Altough malignancies often develop in RTS syndrome, lymphomas are rare. Furthermore our patient is the first reported case who had 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. In the development of this entity both the DNA instability in RTS and the EBV infection might play an important role.

Our patient has not been brought back for check-ups and her parents even didn’t use advanced sun-protection by her. Her cutan nodular, later 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 (our recommendation is half a year) by a specialist (dermatologist/clinical geneticist suggested) to manage the patient and to reveal novel symptoms is indispensable. Spotting signs for potential cancers – skin cancers, osteosarcomas, lymphomas, but also any other tumors! – is of especial significance. Regular ophthalmologic, endocrine, orthopedic, dental and may other specialist-visits are necessary. Physiotherapy can help patients in the everyday, special education might be required. Genetic examination is recommended if the diagnosis is not evidenced clinically and to make the tumor-risk and prognosis more accurate. It also helps the family for further family planning.

VI.2.2. Patient with Rubinstein-Taybi syndrome

Although we didn't know our patient's proper diagnosis for years, the child remained in our view. Regarding to his signs regular check-up has been planned and had doned. In parallel with the appearance of characteristic symptoms of RSTS the proper diagnosis has been set up. With the indentification of causative *CREBBP* variation the diagnosis of RSTS was confirmed.

Considering his underlying condition, that RSTS is disposed to malignancies, especially brain tumors, cranial MRI scan was performed, which revealed his brain lesions. At the turnout of the patients first symptoms of elevated intracranial pressure, repeated MRI was done without delay and therapy started at an early stage, the patient recovered.

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

RSTS patients require regular (according to our practice: 6 month) supervision at the caring specialist doctor (clinical geneticist suggested). Endocrine, orthopedic, cardiology, pulmonology, ophthalmology and other professional controls required in accordance with the symptoms. Increased attention for malignancies (especially blood-forming and brain tumors, keloids), blood-count half a year and routine cranial MRI a year recommended. Physiotherapy, special education should 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.

VI.2.3. Diagnosis and management of patients with rare hereditary syndromes prone to malignancies

Adequate management of children with genetic syndromes disposed to malignancies is of great importance. For lack of exact diagnosis, appropriate follow-up, treatment and prevention are hard to achieve. Rare diseases may misdiagnosed and mistreated time and again. Initial signs and symptoms may not be markable, and when manifestations arise, patients may not get to a specialist. On the other hand, proper diagnosis is often hard to set up since the lack of characteristic appearance especially at younger age and the overlapping features of similar syndromes. Genetic test may provide evidence, but it can't give solution for all the cases. In numerous diseases genetic background is not yet discovered. Another problem of genetic identification that a significant portion of these conditions are of multi-gene origins. Lack of positive result does not exclude the diagnosis: the examination of all the known mutations is not always feasible and undescribed aberrations also can stay in the background. In addition the costs of the genetic investigations in most of the time are non-negligible. We also have to deal with ethical aspects.

Exact diagnosis allows us to do predictive medicine: suitable management in light of the expected outcome. Hence, developement of the diagnostic possibilities is of great importance. Revealing the genetic background not only permit the precise diagnosis in doubtful cases, and make certain the clinically presumptive syndromes, but helps to know more accurately the prognosis, and 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 potential tumor development. Noticing in time the forming tumor is extremely important for adequate treatment and positive outcome. Avoiding misdiagnosis, referral to the caring specialist would be necessary of every novel entity detected by the patient (parents) or doctors.

To avoid the pitfalls of diagnostics and disease-surveillance, some special thoughts 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. Couching could be courses at university, post gradual training lectures, publications.
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. Doctors have to emphasize the importance of potential tumor prevention and early recognition of cancer signs. Brochures with these information might be helpful, which should be kept by the patients visiting any doctor.

VII. SUMMARY OF OUR FINDINGS

1. We examined first the impact of the following SNPs of cytosine arabinoside metabolic genes in acute childhood leukaemia in association with ara-C toxicity and survival: *CDA* rs1048977, *DCK* rs12648166 and rs4694362, *DCTD* rs4742, *SLC28A3* rs7853758 and rs7867504, and *SLC29A1* rs9394992 and rs324148.

2. We found rs12648166 and rs4694362 SNPs of the *DCK* gene associated with altered risk to leukopenia at the allele, genotype and haplotype levels. None of the SNPs influenced thrombocytopenia, anemia, infection and the survival of the patients.

3. We presented a case of Rothmund-Thomson syndrome associated with aggressive biphenotype, biclonal EBV-associated cutan lymphoma first in the literature. The patient was 3 years old at diagnosis, any kind of lymphoma had not been described at this young age in conjunction 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.

VIII. CONCLUSIONS

1. Confirming the correlation of *DCK* gene rs12648166 and rs4694362 with 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 interindividual 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: give suitable treatment and follow-up, lower cancer risk, improve outcome and help family-planning. Genetic testing helps in diagnosis, surveillance, proper tumor-risk, prognosis and family planning. With presenting two disease-history and thinking over the diagnostics and management of rare cancer predisposition hereditary diseases we may ministrative for other practitioners with patients suffering from the same syndromes.

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