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Research Article

Inherited Variants is a Genetic Determinant of Mercaptopurine/Methotrexate Intolerance in Children With Acute Lymphoblastic Leukemia

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Abstract

Objectives: Acute lymphoblastic leukemia (ALL) is the most common malignancy in children with distinctive features across its different subtypes. Although the etiology of ALL has not been fully elucidated, it has been shown that the onset of ALL is partly due to genetic factors. Genes related to drug transport, metabolism, detoxification and DNA repair could influence the cytotoxic effects associated with chemotherapy, including those involved in the transport (e.g., ABCB1, ABCC2, MTHFR and SLCO1B3), and transporter of folate (SLC19A1) of MTX and 6-MP. Likewise, genes involved in DNA-binding transcription factor activity and RNA polymerase II cis-regulatory region sequence-specific DNA binding (NR3C1) and DNA repair (TYMS) have previously been linked with cytotoxicity of 6-MP and/or MTX.

Methods: In the current study, we therefore aimed to assess prior associations for 17 selected genetic variants in 5 genes, in a large cohort of 41 ALL patients treated with mercaptopurine (6-MP) and methotrexate (MTX) combination therapy or mono-therapy of whom detailed clinical toxicity datas were available.

Results: Among the 17 variants in 5 genes evaluated according to the results of our study, the polymorphisms in rs2893881 (G/A) in ARID5B were closely related to therapy toxicity. To our knowledge, the effect of ARID5B variants on childhood ALL has not been studied in Turkey. Several genome-wide and candidate gene association studies have reported strong associations between ARID5B SNPs and risk of ALL and toxicity to therapeutic drugs in different populations.

Conclusion: Finally, one of the most significant findings from this study is that ARID5B germline SNPs related to ALL treatment toxicity. To our knowledge, this is the first report to describe the relationship between ARID5B and ALL treatment response in the context of a preliminary ALL clinical trial. Further investigation of ARID5B variation in line with different ALL treatment regimens is required to improve its value as a prognostic marker.

Keywords: ARID5B, childhood leukemia, single nucleotide polymorphism

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Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy, accounting for approximately 30% of all childhood malignancies and representing 80% of pediatric leukemias.[1] The prognosis of the disease has improved remarkably over the past 30 years, with a 5-year survival of 90%.[2] A further increase in survival will be with

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a reduction in drug-induced toxicity, resulting in fewer interruptions to chemotherapy and fewer relapses of the disease.[3,4] Therefore, individual-specific therapy is needed to prevent drug-induced toxicity and improve survival of high-risk patients.

The success of treatment is considered in part to be the 18-24 months of adequate maintenance therapy necessary to prolong the remission achieved in the early stages of treatment. Mercaptopurine (6-MP) and methotrexate (MTX) are the main components of ALL maintenance therapy.[5] For both drugs, the mechanism exerts its antileukemic effect by inhibiting denovo purine synthesis (DNPS). The combination of these two drugs at low doses results in a synergistic antileukemic effect.[6] MTX inhibits dihydrofolate reductase, which is necessary for purine synthesis.[7] 6-MP is a prodrug that is metabolized by enzymatic steps of the purine rescue pathway: the anabolic pathway via hypoxanthine guanine phosphoribosyl transferase (HPRT) produces 6-thioinosine monophosphate followed by 6-thioguanine nucleotides (6-TGN).[8,9] 6-TGN is phosphate coupled in nucleic acids or 6 thioinosine triphosphate (TITP); this is a process that can be reversed by inosine triphosphate pyrophosphatase (ITPA). Catabolic pathways include xanthine oxidase (XO), which inactivates 6-MP to 6-thiouric acid, and thiopurine S-methyltransferase (TPMT), which metabolizes 6-MP to 6-methylmercaptopurine (6 MMPN), which inhibits DNPS.[10] It is known that in patients receiving 6-MP, variability in red blood cell (RBC) concentrations of 6-TGN and 6-MMPN can alter efficacy and adverse effects, with a high risk of myelosuppression in patients with homozygous TPMT deficiency.[11,12] However, single nucleotide polymorphisms (SNPs) in genes encoding enzymes involved in 6-MP metabolism may also affect the efficacy and toxicity of this pathway.[13]

Genes related to drug transport, metabolism, detoxification and DNA repair could influence the cytotoxic effects associated with chemotherapy, including those involved in the transport (e.g., ABCB1, ABCC2, MTHFR and SLCO1B3), and transporter of folate (SLC19A1) of MTX and 6-MP. Likewise, genes involved in DNA-binding transcription factor activity and RNA polymerase II cis-regulatory region sequence-specific DNA binding (NR3C1) and DNA repair (TYMS) have previously been linked with cytotoxicity of 6-MP and/or MTX.

In the current study, we therefore aimed to assess prior associations for 17 selected genetic variants in 5 genes, in a large cohort of 41 ALL patients treated with mercaptopurine (6-MP) and methotrexate (MTX) combination therapy or mono-therapy of whom detailed clinical toxicity datas were available.

Methods

Briefly, patients diagnosed with ALL between 2019 and 2021 and treated or followed up at the hospital were included for peripheral whole blood and clinical data collection. Forty one children with ALL, included in the European Organization for Research and Treatment of Cancer (EORTC) 58951 protocol, were enrolled in this study.

The control group consisted of 20 randomly selected, thrombocytopenia patients, unrelated volunteers. They were recruited from August 2019 to July 2021. Personal information was obtained through a direct interview with the volunteers, or with parents or legal tutors for individuals younger than 18 years old. The control group patients were thrombocytopenic patients and they were selected from those who did not have hepatotoxicity.

The institutional review committee (Trakya University Faculty Of Medicine, TUMF Scientific Research Ethics Committee Directive TUTF-BAEK 2017/153 Edirne, Turkey) approved the study and informed consent fromparents or guardians was collected for all children. Maintenance therapy consisted of daily oral 6-MP (25-50 mgm^2) and weekly oral MTX (10-20 mgm^2). Treatment information (such as drug toxicity and doses) was collected from consolidation and continuation phases only, since MP with MTX is the backbone of these two treatment phases.

The dose of MP reached during continuation phase thatwas needed to maintain the white blood cell count (WBC) above 1,500 per µl and the absolute neutrophil count (ANC) above 300 per µl in order to be able to deliver continuous daily doses of MP and weekly MTX doses without interruption was used for evaluation of MP intolerance. During this phase, patients treated on the intermediate- or high-risk protocol were started on 50 mg/m2 MP daily until continuation week 20, when the dose was increased to 75 mg/m^2 daily. Those who were on the low-risk protocol arm were started on MP 75mg/m^2 daily.

DNA was extracted from peripheral blood using a Qiagen (Gemantown, MD) DNA isolation kit according to the manufacturer's guidelines and stored at -20°C until analysis. Overall 17 SNPs in 5 genes were genotyped with an individual call rate >95% and an overall success rate >98,5%. Genotyping for NUDT15 rs116855232, rs2838958, MTHFR rs1801133, rs1801131 ve ABCB1 rs1045642, SLCO1B1 rs4149056, rs11045879, rs4149081, ARID5B rs6479778, rs2893881, rs4948488, rs2393782, rs10821938, rs7923074, rs6479779, rs17215180 were performed using light SNP assays on a Roche Lightcycler (Roche Diagnostics, Switzerland). Ten percent of samples were genotyped twice and the results showed 100% reproducibility.

Statistical Analysis

We calculated median values and inter-quartile ranges for all continuous variables, while frequencies and percentages were calculated for categorical variables. Genotype frequencies were tested for Hardy-Weinberg equilibrium using a 1°-of-freedom χ^2-test and considered significant at $p<0.05$. Each of the variants were correlated with toxicity events (i.e., the primary objective) using binary logistic regression, while assuming an additive genotypic model. Per-allele odds ratios (OR) and their respective 95% confidence intervals (CI) are reported. All tests were two-sided and statistical significance was set at p=0.05. Statistical analyses were performed using SPSS version 26 (SPSS for Windows, Rel. 26.0.0. 2022. Chicago, Illinois, USA: SPSS Inc.) Regression analyses were performed without correction for covariates and after correction for relevant covariates, including age and BMI at the time of treatment, number of administered cycles and treatment regimen. For anemia, an additional covariate was included, i.e., use of erythropoiesis stimulating agents (ESAs), whereas for neutropenia and febrile neutropenia, use of colony stimulating factors (CSFs) was included as an additional covariate.

Results

Between January 2019 and December 2021 (pre-specified period of 2 years), we recruited 41 ALL patients treated with 1–8 cycles MP/MTX combination therapy. Of all recruited patients, 99% was Caucasian. The demographic and clinical characteristics of these 41 patients are summarized in Table 1. Hematological toxicity was analyzed in 41 patients. Patient, disease and toxicity characteristics are summarized in Table 1. Minor allele frequencies (MAF) were similar to those reported previously in Caucasians and adhered to Hardy-Weinberg equilibrium.

Association with Hepatotoxicity

Among the 41 patients eligible for the hematological toxicity analysis, we observed significant associations for 1 variant. After correction for relevant covariates (as explained in the statistical methods), this variant were still significantly associated with toxicity. ARID5B(rs2893881) p-value = 0,009255.

Discussion

This is the first report on the association of MTHFR, ABCB1, SLCO1B1, ARID5B and NUDT15 polymorphisms with MP/MTX dose intolerance in Turkish patients with ALL. We correlated MP and MTX induced toxicity with genetic variation in ARID5B (rs2893881) polymorphism involved in pharmacokinetics of these chemotherapeutics or DNA repair, and observed various correlations supporting a role for ARID5B in mediating toxicity and therapy outcome. Most other studies assessing similar correlations have been performed in same populations, and typically evaluated only few variants. Our study evaluates a more systematically-selected panel of 17 variants in ALL patients, of which 41 were evaluable for hematologic toxicity (Table 2).

The soluble transporter organic anion transporter 1B1 (SLCO1B1) is an MTX transporter primarily found in hepatocytes. Three SNPs in SLCO1B1, rs11045879, rs4149081 and rs4149056 have been associated with MTX clearance and severe gastrointestinal toxicity during consolidation therapy between regimens.[14] However, in our study, no significant correlation was found in terms of therapy toxicity and these SNPs. rs11045879 (T/C) p-value = 0.441, rs4149081 (G/A) p-value = 0.441, rs4149056 (T/C) p-value = 0.13.

Methylenetetrahydrofolate reductase (MTHFR) is the most studied gene in MTX metabolism. It mediates the conversion of 5,10-methylene-tetrahydrofolate to 5-methyl tetrahydrofolate, which acts as a methyl donor for the conversion of homocysteine to methionine.[15] Two SNPs, C677T (rs1901133) resulting in the replacement of alanine with valine at codon 222 (Ala222Val), and A1298C (rs1801131), resulting in replacement of glutamic acid with alanine at codon 429 (Glu429Ala), were associated with increased MTX as a result of decreased MTHFR activity.[16,17] Some case reports have shown that variant C677T (rs 1901133) induces neurotoxicity and liver toxicity, while some other publications

Table 1. Demography and hematologic parameters of the patients

Parameters	ALL (n=41)	Control (n=20)
Age (years)	6.54	6.93
Gender		
Female	16	8
Male	25	12
Immunologic		
Subtype (Lineage)		
B ALL	31	
T ALL	7	
NHL	3	
Thrombocytopenia		20
MP dose m^2	36.21	
MTX dose m^2	15.21	16 mg /a week
Toxicity		
Alt	276	21
Ast	137	23.2
Treatment interrupt	2.21	-
Outcome		
Remission	26	20
Recurrence	15	-

Table 2. Genotypic and allelic frequencies of the 5 genes and 17 SNPs

SNPs	41 patients n (%)	20 controls (%)
MTHFR rs1801131 T/G	18 (43.9)	10 (50)
T/T	17 (41.46)	8 (40)
G/G	6 (14.63)	2 (10)
rs1801133 G/A	16 (39.02)	9 (45)
G/G	18 (43.9)	11 (55)
A/A	7 (17.07)	-
NUDT15 rs116855232 C/T	3 (7.31)	-
C/C	38 (92.68)	20 (100)
rs2838958 G/A	16 (39.02)	8 (40)
A/A	9 (21.95)	8 (40)
G/G	7 (17.07)	4 (20)
ABCB1 rs1045642 G/A	13 (31.7)	10 (50)
A/A	7 (17.07)	5 (25)
G/G	11 (26.82)	5 (25)
SLCO1B1 rs4149056 T/C	8 (19.51)	2 (10)
C/C	-	1 (5)
T/T	30 (73.17)	17 (85)
rs11045879 T/C	12 (29.26)	3 (15)
T/T	28 (68.29)	16 (80)
C/C	1 (2.43)	1 (5)
rs4149081 G/A	12 (29.26)	3 (15)
G/G	28 (68.29)	16 (80)
A/A	1 (2.43)	1 (5)
ARID5B rs6479778 C/T	21 (51.21)	7 (35)
C/C	20 (48.78)	13 (65)
rs2893881 G/A	7 (17.07)	7 (35)
A/A	20 (48.78)	13 (65)
G/G	14 (34.14)	-
rs4948488 C/T	19 (46.34)	6 (30)
T/T	20 (48.78)	14 (70)
C/C	2 (4.87)	-
rs2393782 G/C	18 (43.9)	4 (20)
G/G	21 (51.21)	16 (80)
C/C	2 (4.87)	-
rs10821938 A/C	21 (51.21)	7 (35)
A/A	11 (26.82)	4 (20)
C/C	9 (21.95)	9 (45)
rs7923074 C/A	22 (53.65)	6 (30)
A/A	11 (26.82)	5 (25)
C/C	8 (19.51)	9 (45)
rs6479779 C/G	17 (41.46)	10 (50)
C/C	12 (29.26)	3 (15)
G/G	12 (29.26)	7 (35)
rs17215180 C/T	14 (34.14)	11 (55)
C/C	18 (43.9)	4 (20)
T/T	9 (21.95)	5 (25)

have failed to confirm this association.[18, 19, 20] Chiusolo et al. found that the C677T (rs1901133) and A1298C (rs1801131) alleles were not significant predictors of relapse-free survival or EFS in Thai pediatric ALL patients (n=76), but were associated with increased susceptibility for hematopoietic and hepatotoxicity at doses of 15-30 mg/m^2 reported. These divergent results make it difficult to draw any strong conclusions about the role of MTHFR SNPs in determining MTX toxicity and response. The variability may be due to differences in treatment protocols between different studies, as well as other irregular factors such as inconsistent MTX doses, other SNPs, or ethnic differences between Asian and Northern European patient populations.

In terms of MTHFR polymorphisms, no significant difference was found in our study in terms of the regions examined and toxicity effects. MTHFR rs1801133 (G/A) p-value = 0,4231, rs1801131 (T/G) p-value = 0,8167.

J Gregers et al investigated the relationship between ABCB1 1199G4A polymorphism and the risk of recurrence in childhood ALL. The marked increase in relapse rates for high-risk ALL patients with variant 1199GA is likely because high-risk patients had greater exposure to P-gp substrates (glucocorticosteroids, vincristine, anthracyclines, and alkylating agents) than low-risk patients. In addition to these findings, the 1199GG genotype was also associated with a superior outcome among patients with acute myeloid leukemia.[21-24] In the study of Kimchi-Sarfaty et al, ABCB1 2677T/T or 3435T/T genotypes were associated with an increased risk of recurrence/resistance in ALL. ABCB1 2677G4T/A exhibits an Ala-to-Ser/Thr change in exon 21, while 3435C4T is a synonymous polymorphism in exon 26 that affects transport by changing substrate specificity.[24]

Although these studies reported different results, no significant finding was obtained between ABCB1 polymorphism and drug toxicity in our study.

With the replacement of MP in the treatment of ALL, this has significantly contributed to the improvement of malignancy and to a significant increase in survival. However, thiopurines have a limited therapeutic index with dose-limiting toxicities in hematopoietic tissues, especially in children with ALL. Fixed-dose MP titration is difficult in clinical practice and may adversely affect the overall treatment outcome.

Yang et al. identified the investigated variants for TPMT as the strongest predictor of MP tolerance, but more importantly, they identified NUDT15 as another critical gene associated with susceptibility to MP intolerance resulting from extreme toxicity. They detected a significant correlation between the copy number of the T allele in the NUDT15 SNP rs116855232 and the tolerated MP dose, indicating a gene dosage effect. Patients with the homozygous TT genotype were hypersensitive to MP and only tolerated 8,3% of the MP dose planned in the protocol.

NUDT15 genotyping can identify the group of children at risk for ALL and therefore may be of importance for clinical practice. Although patients with the NUDT15 SNPs heterozygous genotype also required a larger MP dose reduction compared to wild-type patients, there was a relatively wide variation in the final tolerated dose and additional studies with larger sample sizes to fully characterize MP tolerance for this patient group will be necessary.[25]

In our study, in addition to the polymorphism region in this study, we also analyzed a different region in NUDT15. We searched for NUDT15 rs116855232 (C/T), rs2838958 (G/A) variations. However, in our study, a significant correlation was found between the variations in NUDT15 and therapy toxicity, perhaps due to the small number of patients or ethnicity differences. The determined values were evaluated as NUDT15 rs116855232 (C/T) p-value = 0,5419 rs2838958 (G/A) p-value = 0,2556.

Genome-wide association (GWA) studies in populations of European descent have shown that ARID5B single-nucleotide polymorphisms (SNPs) are associated with childhood ALL. Several retrospective follow-up studies have confirmed the association of ALL risk with ARID5B genetic variations.[26-28] Childhood ALL disease shows substantial variation in disease incidence across geographic regions, with a higher prevalence in Europe than reported in Asian populations.[29] In different populations, it is well differentiated by genetic polymorphisms related to race and ethnicity, which, in addition to environmental risk factors, can cause these incidence differences.[30,31] To understand the differences in susceptibility to ALL and to comprehend the causes and mechanism of ALL progression, it is critical to examine the effects of genetic variation in different ethnicities.

To our knowledge, the effect of ARID5B variants on childhood ALL has not been studied in Turkey. Several genome-wide and candidate gene association studies have reported strong associations between ARID5B SNPs and risk of ALL and toxicity to therapeutic drugs in different populations.[32] Previously, GWA studies have been conducted in populations of European, Hispanic, and African American ancestry. Therefore, the aim of this study was to determine the possible association of ARID5B gene SNPs and haplotypes with ALL in Turkish children of Balkan and Asian descent. We evaluated the relationship between ARID5B SNPs (rs6479778, rs2893881, rs4948488, rs2393782, rs10821938, rs7923074, rs6479779, rs17215180) and drug toxicity in ALL in Turkish children. The main findings in our study are that ARID5B rs2893881 (G/A) p-value= 0,009255 polymorphisms are associated with an increased risk of ALL therapeutic drugs toxicity. Our study showed no association between SNPs rs6479778, rs4948488, rs2393782, rs10821938, rs7923074, rs6479779, rs17215180 and ALL treatment toxicity, but we found that this SNP was associated with the risk of ALL in Turkish children. About 15% of ALL patients suffer from relapse after treatment, and minimal residual disease (MRD) is considered one of the strongest prognostic factors. Of note, risk alleles for ALL susceptibility of ARID5B SNPs are associated with worse treatment outcome. Most ALL relapse-associated SNPs were associated with MRD status at the end of remission induction therapy, and some remained prognostic even after adjustment for MRD.[27]

B-cell progenitors and bone marrow cellularity are reduced in homozygous Arid5b knockout mice, confirming the role of ARID5B in B-cell lineage development. It has been found that ARID5B gene inhibits B-lymphocyte development and contributes to leukemogenesis with its abnormal expression during fetal life. It has been suggested that ARID5B variations may affect the function of this gene during maturation of B-progenitor cells, leading to an increased risk of B-ALL.[33] While exactly how ARID5B is linked to ALL is unknown, it is safe to hypothesize that it may be involved in the epigenetic regulation of gene expression in hematopoietic stem cells and early lymphoid progenitors such as other AT-rich DNA-binding proteins.[34] According to the results of our study, we have shown that ARID5B rs2893881 (G/A) change is also associated with therapy toxicity. ARID5B, which was previously shown to be associated with the development of childhood ALL, was also revealed to be associated with the development of toxicity during treatment.

Finally, one of the most significant findings from this study is that ARID5B germline SNPs related to ALL treatment toxicity. To our knowledge, this is the first report to describe the relationship between ARID5B and ALL treatment response in the context of a preliminary ALL clinical trial. Further investigation of ARID5B variation in line with different ALL treatment regimens is required to improve its value as a prognostic marker. Interestingly, we have previously presented a mechanism where methotrexate metabolites of the ARID5B SNP genotype (i.e., methotrexate with polyglutamate) are associated with leukemic cell accumulation, plausibly suggesting that ARID5B is associated with overall relapse.[35] Together, these results point to the possibility that leukemogenesis and antileukemic drug response mechanisms may converge in common pathways.

Conclusion

Further studies with a larger sample size are warranted to confirm our results. Also, functional studies are needed to determine the causative variants of ARID5B gene. Under-

standing how these and other SNP variations affect the overall structure and function of the ARID5B protein will be of significant value in improving risk-directed therapies and disease outcomes in childhood leukemia.

As ARID5B SNPs are closely associated with the onset and outcome of childhood ALL, previously published findings warrant comprehensive genetic and functional studies to uncover molecular mechanisms and evaluate the diagnostic and therapeutic significance of ARID5B for ALL, which we outlined in our study.

Disclosures

Ethics Committee Approval: The institutional review committee (Trakya University Faculty Of Medicine, TUMF Scientific Research Ethics Committee Directive TUTF-BAEK 2017/153 Edirne, Turkey) approved the study.

Competing interests: The authors declare that they have no competing interests.

Data Availability: The datasets generated during and/or analysed during the current study are available in the Emine Ikbal Atli repository emine.ikbal@gmail.com

Consent to participate: Informed consent was obtained from all individual participants included in the study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – T.E., E.I.A.; Design – E.I.A.; Supervision – H.G.; Materials – E.A.; Data collection &/or processing – E.I.A.; Analysis and/or interpretation – D.E.; Literature search – T.E., E.I.A.; Writing – E.I.A.; Critical review – S.D., S.Y.

References

1. Rubnitz JE, Pui CH. Recent advances in the treatment and understanding of childhood acute lymphoblastic leukaemia. Cancer Treat Rev 2003;29:31–44. [CrossRef]
2. Pui CH, Pei D, Sandlund JT, Ribeiro RC, Rubnitz JE, Raimondi SC, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. N Engl J Med 2009;360:2730–41.
3. Kishi S, Cheng C, French D, Pei D, Das S, Cook EH, et al. Ancestry and pharmacogenetics of antileukemic drug toxicity. Blood 2007;109:4151–7. [CrossRef]
4. Rivera GK, Evans WE, Kalwinsky DK, Mirro J, Ochs J, Dow LW, et al. Unexpectedly severe toxicity from intensive early treatment of childhood lymphoblastic leukemia. J Clin Oncol 1985;3:201–6. [CrossRef]
5. Schmiegelow K, Schrøder H, Gustafsson G, Kristinsson J, Glomstein A, Salmi T, et al. Risk of relapse in childhood acute lymphoblastic leukemia is related to RBC methotrexa-te and mercaptopurine metabolites during maintenance chemotherapy. Nordic Society for Pediatric Hematology and Oncology. J Clin Oncol 1995;13:345–51. [CrossRef]
6. Dervieux T, Hancock M, Evans W, Pui CH, Relling MV. Effect of methotrexate polygluta-mates on thioguanine nucleotide concentrations during continuation therapy of acute lymphoblastic leukemia with mercaptopurine. Leukemia 2002;16:209–12. [CrossRef]
7. Chabner BA, Allegra CJ, Curt GA, Clendeninn NJ, Baram J, Koizumi S, et al. Polyglutama-tion of methotrexate. Is methotrexate a prodrug? J Clin Invest 1985;76:907–12.
8. Bostrom B, Erdmann G. Cellular pharmacology of 6-mercaptopurine in acute lymphob-lastic leukemia. Am J Pediatr Hematol Oncol 1983;15:80–6.
9. Elion GB. The purine path to chemotherapy. Science 1989;244:41–7.
10. Cheok MH, Evans WE. Acute lymphoblastic leukaemia: a model for the pharmacogeno-mics of cancer therapy. Nat Rev Cancer 2006;6:117–29. [CrossRef]
11. Colombel JF, Ferrari N, Debuysere H, Marteau P, Gendre JP, Bonaz B, et al. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. Gastroenterology 2000;118:1025–30. [CrossRef]
12. McLeod HL, Krynetski EY, Relling MV, Evans WE. Genetic polymorphism of thiopurine methyltransferase and its clinical relevance for childhood acute lymphoblastic leukemia. Leukemia 2000;14:567–72. [CrossRef]
13. Sahasranaman S, Howard D, Roy S. Clinical pharmacology and pharmacogenetics of thi-opurines. Eur J Clin Pharmacol 2008;64:753–67. [CrossRef]
14. Trevino LR, Shimasaki N, Yang W, Panetta JC, Cheng C, Pei D, et al. Germline genetic va-riation in an organic anion transporter polypeptide associated with methotrexate phar-macokinetics and clinical effects. J Clin Oncol 2009;27:5972–8. [CrossRef]
15. Kodidela S, Suresh Chandra P, Dubashi B. Pharmacogenetics of methotrexate in acute lymphoblastic leukaemia: why still at the bench level? Eur J Clin Pharmacol 2014;70:253–60.
16. Kantar M, Kosova B, Cetingul N, Gumus S, Toroslu E, Zafer N, et al. Methylenetetrahyd-rofolate reductase C677T and A1298C gene polymorphisms and therapy-related toxicity in children treated for acute lymphoblastic leukemia and non-Hodgkin lymphoma. Leuk Lymphoma 2009;50:912–7. [CrossRef]
17. D'Angelo V, Ramaglia M, Iannotta A, Crisci S, Indolfi P, Francese M, et al. Methotrexate toxicity and efficacy during the consolidation phase in paediatric acute lymphoblastic le-ukaemia and MTHFR polymorphisms as pharmacogenetic determinants. Cancer Che-mother Pharmacol 2011;68:1339–46.
18. Vagace JM, de la Maya MD, Caceres-Marzal C, Gonzalez de Murillo S, Gervasini G. Cent-ral nervous system chemotoxicity during treatment of pediatric acute lymphoblastic leu-kemia/lymphoma. Crit Rev Oncol Hematol 2012;84:274–86. [CrossRef]
19. Tanaka Y, Manabe A, Nakadate H, Kondoh K, Nakamura K, Koh K, et al. Methylenetet-rahydrofolate reductase gene haplotypes affect toxicity during maintenance therapy for child-

hood acute lymphoblastic leukemia in Japanese patients. Leuk Lymphoma 2014;55:1126–31. [CrossRef]
20. Kishi S, Griener J, Cheng C, Das S, Cook EH, Pei D, et al. Homocysteine, pharmacogene-tics, and neurotoxicity in children with leukemia. J Clin Oncol 2003;21:3084–91. [CrossRef]
21. Chiusolo P, Reddiconto G, Farina G, Mannocci A, Fiorini A, Palladino M, et al. MTHFR polymorphisms' influence on outcome and toxicity in acute lymphoblastic leukemia pa-tients. Leuk Res 2007;31:1669–74. [CrossRef]
22. Gregers J, Gréen H, Christensen IJ, Dalhoff K, Schroeder H, Carlsen N, et al. Polymorp-hisms in the ABCB1 gene and effect on outcome and toxicity in childhood acute lymp-hoblastic leukemia. Pharmacogenomics J 2015;15:372–9 [CrossRef]
23. Green H, Falk IJ, Lotfi K, Paul E, Hermansson M, Rosenquist R, et al. Association of ABCB1 polymorphisms with survival and in vitro cytotoxicty in de novo acute myeloid le-ukemia with normal karyotype. Pharmacogenomics J 2010;12:111–8.
24. Kimchi-Sarfaty C, Oh JM, Kim IW, Sauna ZE, Calcagno AM, Ambudkar SV, et al. A "silent" polymorphism in the MDR1 gene changes substrate specificity. Science 2007;315:525–8.
25. Yang JJ, Landier W, Yang W, Liu C, Hageman L, Cheng C, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphob-lastic leukemia. J Clin Oncol 2015;33:1235–42. [CrossRef]
26. Prasad RB, Hosking FJ, Vijayakrishnan J, Papaemmanuil E, Koehler R, Greaves M, et al. Verification of the susceptibility loci on 7p12.2, 10q21.2, and 14q11.2 in precursor B-cell acute lymphoblastic leukemia of childhood. Blood 2010;115:1765–7. [CrossRef]
27. Treviño LR, Yang W, French D, Hunger SP, Carroll WL, Devidas M, et al. Germline geno-mic variants associated with childhood acute lymphoblastic leukemia. Nat Genet 2009;41:1001–5.
28. Gutiérrez-Camino Á, López-López E, Martín-Guerrero I, Sánchez-Toledo J, García de An-doin N, Carboné Bañeres A, et al. Intron 3 of the ARID5B gene: a hot spot for acute lymphoblastic leukemia susceptibility. J Cancer Res Clin Oncol 2013;139:1879–86. [CrossRef]
29. Linet MS, Brown LM, Mbulaiteye SM, Check D, Ostroumova E, Landgren A, et al. Interna-tional long-term trends and recent patterns in the incidence of leukemias and lympho-mas among children and adolescents ages 0-19 years. Int J Cancer 2016;138:1862–74. [CrossRef]
30. Li JZ, Absher DM, Tang H, Southwick AM, Casto AM, Ramachandran S, et al. Worldwide human relationships inferred from genome-wide patterns of variation. Science 2008;319:1100–4.
31. Bolufer P, Barragan E, Collado M, Cervera J, López JA, Sanz MA. Influence of genetic polymorphisms on the risk of developing leukemia and on disease progression. Leuk Res 2006;30:1471–91. [CrossRef]
32. Al-Absi B, Noor SM, Saif-Ali R, Salem SD, Ahmed RH, Razif MF, et al. Association of ARID5B gene variants with acute lymphoblastic leukemia in Yemeni children. Tumour Biol 2017;39:1010428317697573.
33. Xu H, Cheng C, Devidas M, Pei D, Fan Y, Yang W, et al. ARID5B genetic polymorphisms contribute to racial disparities in the incidence and treatment outcome of childhood acu-te lymphoblastic leukemia. J Clin Oncol 2012;30:751–7.
34. Yang W, Treviño LR, Yang JJ, Scheet P, Pui CH, Evans WE, et al. ARID5B SNP rs10821936 is associated with risk of childhood acute lymphoblastic leukemia in blacks and contributes to racial differences in leukemia incidence. Leukemia 2010;24:894–6. [CrossRef]
35. Yokota T, Kanakura Y. Role of tissue-specific AT-rich DNA sequence-binding proteins in lymp-hocyte differentiation. Int J Hematol 2014;100:238–45. [CrossRef]