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REVIEW ARTICLE

The effect of polymorphisms of gamma-glutamyl hydrolase (GGH) gene on methotrexate-induced toxicity in acute lymphoblastic leukemia

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Abstract

Context: Acute lymphoblastic leukemia patients show differences in methotrexate-induced toxicity after treatment with this anti-cancer drug. Pharmacogenetics is an important determining factor for toxicity diversity. *Objective*: In this study, the effect of +452 CT and –401CT polymorphisms of Gamma-glutamyl hydrolase (GGH) gene on methotrexate serum levels and its associated toxicity in patients with acute lymphoblastic leukemia was assessed. Furthermore, the frequency of the above polymorphisms was investigated for the first time in Iran. *Material and methods*: The prevalence of these polymorphisms was assessed in 83 Iranian patients with ALL using PCR and RFLP. The relationship between the polymorphism and serum methotrexate levels and its toxicity was estimated by calculating the Odds Ratio. *Results*: No correlation was found between +452CT polymorphism and serum levels of methotrexate and methotrexate-related toxicity. –401CT polymorphism was found to be correlated with methotrexate-related toxicity leading to thrombocytopenia (95% CI = 0.009–0.019, odds ratio = 0.265) and leukopenia (95% CI = 0.021–0.042, odds ratio = 2.182) in consolidation phase of the treatment. *Discussion*: C allele polymorphism of −401 C/T allele is a risk factor of leukopenia and thrombocytopenia in patients treated with methotrexate. Moreover, our results suggested that the T allele had a supporting role in prevention of thrombocytopenia. *Conclusion*: Evaluation of patients for methotrexate-related polymorphism of GGH gene may be useful to determine the appropriate dose of methotrexate and reducing its toxic side effects.

Keywords

Acute lymphoblastic leukemia, bone marrow toxicity, gamma-glutamyl hydrolase (GGH) gene, methotrexate, polymorphisms, toxicity

History

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Introduction

Acute lymphoblastic leukemia (ALL) is a malignant disorder of T or B lymphocyte precursors. Although the disease has a higher prevalence in children, it can occur at any age (Jahedi et al., 2014). Proliferation and expansion of blast cells in bone marrow causes suppression of hematopoiesis and results in anemia, thrombocytopenia and neutropenia. ALL has various subtypes, and can be classified based on immunological, cytogenetic and molecular genetic methods (Einollahi et al., 2013). Almost 4000 new cases of ALL are diagnosed in USA annually, which include approximately 12% of total leukemia cases diagnosed in that country (Jemal et al., 2005). ALL is the most common malignancy in children under 15 years of age, and comprises 23% of the total malignancies and 76% of the leukemia in this age group. In contrast, it accounts for only 20% of acute leukemia in adults. The incidence of ALL is higher in boys than girls and is different in various geographic areas. Anemia, thrombocytopenia and excessive number of leukocytes in complete blood count are laboratory findings commonly observed at diagnosis time. Investigations show that several distinct genetic polymorphisms in metabolizing xenobiotics enzymes can cause ALL, through interaction with environmental, nutritional and foreign factors. Recent studies suggest that the folate pathway may also be involved in the risk of ALL, as folate supplements may reduce the risk of ALL (GLEIβNER et al., 2002; Lopez-Lopez et al., 2013). Factors related to the host (patient pharmacodynamics and pharmacogenetics) can affect the outcome of therapy (Evans et al., 1998; Wood et al., 2003). Similar doses of methotrexate reduced accumulation of active metabolites of these drugs in leukemic cells; because of increased drug clearance, inactivation or other factors are associated with poor improvement (Evans & Relling, 2004; Wood et al., 2003).

Methotrexate (MTX) is a major drug in treatment of ALL, which is used in high or medium doses in consolidation phase and in low dose in the maintenance phase. Methotrexate is a folate analogue used in treatment of lymphoid malignancies. Methotrexate inhibits Dihydrofolate reductase (DHFR) and

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Thymidylate synthase (TS) enzymes, disrupting DNA synthesis and inhibiting proliferation of normal and malignant cells in tissues such as bone marrow, liver, nervous system as well as in gastrointestinal disorders, kidney failure and mucositis (Cronstein, 1996; Evans et al., 1986). Gamma-glutamyl hydrolase (GGH) is a peptidase clearing polyglutamate from the cells, converting long chain to short chain methotrexate, ultimately reducing and eliminating methotrexate during the therapeutic process (Mancini et al., 2005; Vanasse et al., 1999). Several reports have indicated that single nucleotide polymorphisms (SNPs) play an important role in response to MTX treatment in ALL patients (Mancini et al., 2005; Pui et al., 2002). Catalytic and hydrolytic activity of GGH on poly-glutamine methotrexate in B or T cells is balanced in individuals with +452 C/T polymorphism at codon 127, while causing resistance to MTX in ALL patients with –401 C/T polymorphism (Evans & Relling, 2004; Wood et al., 2003). Overall, the functional differences between GGH and DHFR genes products may cause a risk of toxicity and side effects of MTX (Chave et al., 2003; Dervieux et al., 2004, 2006).

In this study, the relationship between –401 C /T and +452 C/T polymorphisms of GGH, (as the most important polymorphisms of GGH gene) and the risk of the disease recurrence was studied. If these polymorphisms affect methotrexate-associated cytotoxicity, methotrexate therapeutic dose can be adjusted in order to avoid its complications.

Materials and methods

Patients

Patients with acute lymphoblastic leukemia who attended in Ali Asghar Hospital (Tehran, Iran) were included to the study. These patients underwent consolidation treatment with moderate-dose methotrexate administration and maintenance phase with low-dose methotrexate administration. BFM 2002 treatment protocol was used for all the patients. According to this protocol, the patients receive 2 g/m^2 methotrexate every other week for four cycles during consolidation phase, and 20 mg/m^2 oral methotrexate weekly during maintenance phase. Laboratory and clinical data of patients were collected from patient records.

Collection of samples

Sample collection was performed after patient identification and explanation the importance of the research project to their parents. This study was performed according to the institutional ethical guidelines. Whole blood (5 ml) was drawn from each patient in EDTA anticoagulant.

Molecular Studies

Polymorphisms were analyzed using polymerase chain reaction analysis (PCR) and Restriction fragment length polymorphism (RFLP).

PCR for –401 C/T and +452 C/T

100 ng of extracted DNA was added to 1× PCR buffer containing 200 μM of each dNTP, 0.4 mM of each primer, 0.5 unit TaqDNA polymerase and 1.5 mM $Mgcl_2$ in the final volume of 25 ml. PCR conditions included a primary denaturation for 5 min at 95 °C, 40 cycles of 15 S at 94 °C, 45 S at 55 °C and 60 °C (60 °C for –401 C/T polymorphism and 45 S at 55 °C for +452 C/T polymorphism), 45 S at 72 °C and final extension for 10 min at 72 °C. Forward and reverse primers for analyzing –401 C/T polymorphism were (5′-CGCTGCCTGGTTACCAAACT-3′) and (5′-TGTTTACGTC GATGTGGACTTCAG-3′), respectively; and the PCR product was a 109 bp band of DNA. Forward and reverse primers for analyzing +452 C/T polymorphism reverse were (5′-GTGCCTATTTGGTTATGACA-3′) and (5′-CTACTTACTA ATCCTGCCCA-3′), respectively; and the PCR product was a 286 bp band DNA.

RFLP analysis for –401 C/T (rs3758149) and +452 C/T (rs11545078)

Bsl I and Ase I restriction enzymes are utilized for detecting –401 C/T and +452 C/T polymorphisms, respectively. Both enzymes were isolated from bacterium *Haemophilus influenza* RFL6; and the enzyme activity is maintained at a temperature −20 °C. Enzymatic digestion was performed using 2.5 units of Bsl I and Ase I enzymes at 50 °C and 37 °C, respectively. Enzymatic digestion products were analyzed on 3% agarose gel. Individuals with −401 C/C genotype showed 48 bp and 61 bp bands, those with −401 C/T genotype showed 48 bp, 61 bp and 109 bp bands, and individuals with −401 T/T genotype showed a 109 bp band. In +452 C/T genotype, the size of wild allele was 286 bp and that of mutated allele 109 bp and 177 bp. Incubation for 20 min at 80 C and 65 C disabled Bsl I and Ase I enzymes, respectively. The recognition site of Bsl I and Ase I restriction enzymes were as follow:

Bsl I recognition site: 5′... CCNNNNN$^{\downarrow}$NNGG... 3′
3′... GGNN$_{\uparrow}$NNNNNCC... 5′
Ase I recognition site: 5'... AT$^{\downarrow}$TAAT... 3′
3′ TAAT$_{\uparrow}$TA... 5′

Statistical analysis

For statistical analysis, chi-square and one-way ANOVA tests were carried out. Statistical analysis was done by SPSS version 17 and Open Epi version 2 (Tehran, Iran). Chi-square test and one-way ANOVA was performed to investigate the relationship between –401 C/T and +452 C/T polymorphisms of GGH gene, and various toxicities, and methotrexate level in serum, respectively. p value less than 0.05 was considered to be statistically significant. In addition, odd ratio (OR) was calculated with CI = 95% to evaluate the effect of –401 C/T and +452 C/T genotypes on methotrexate toxicity.

Results

Patient collection

In this study, 83 patients aged 2–13 years with B cell ALL were evaluated. They had passed consolidation and maintenance phases of the treatment, and were assessed for −401 C/T and +452 C/T polymorphisms of GGH gene (Figure 1). Genotype distribution and allele frequency of the −401C/T and +452 C/T polymorphisms of GGH in boys with ALL was

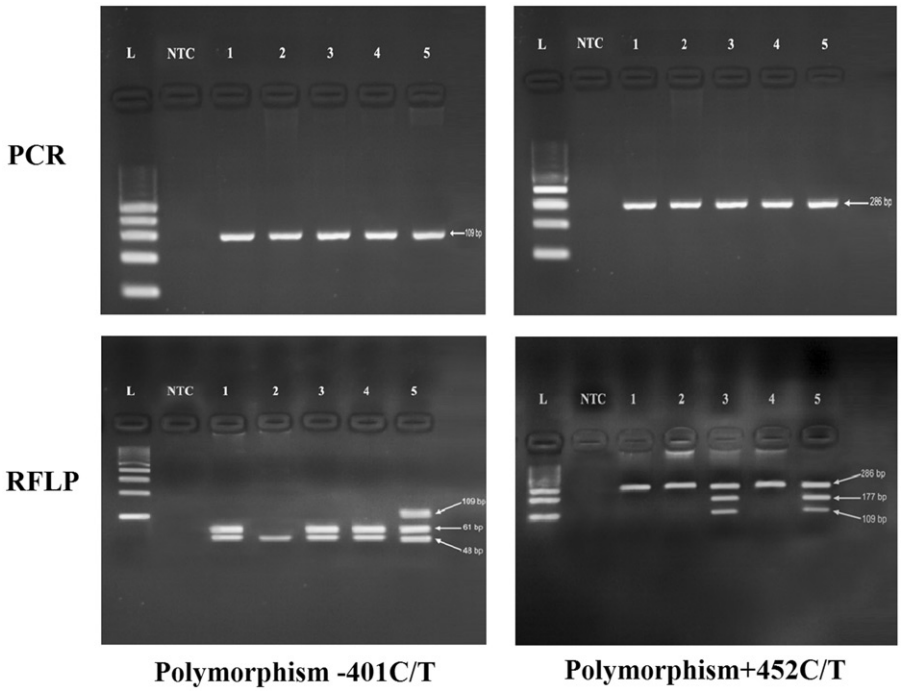

Figure 1. PCR and RFLP for −401 C/T and +452 C/T polymorphisms of GGH gene.

50.03% (49 individuals) and in girls was 40.96% (34 individuals). High-risk groups include high doses of methotrexate and low WBC and platelet counts.

Relation between GGH gene polymorphisms and risk of toxicity

Grading of leukopenia and thrombocytopenia was carried out based on CTCAE (Common Terminology Criteria for Adverse Events, 2006) (Table 1). In this study, all the toxic patients were analyzed versus non-toxic patients, regardless of their toxicity grade. The relationship between the incidence of toxicity (Grade 1–4) in consolidation and maintenance phases with C and T allele frequencies of −401C/T and +452 C/T polymorphisms were also analyzed, and the results are presented in the Tables 2 and 3.

In order to determine the effect of C and T alleles on the toxicity risk, each allele of C and T were studied separately. The homozygous C, homozygous T and heterozygous CT individuals with −401 C/TA and −401 C/TB polymorphism were studied separately in their consolidation and maintenance phases. Results indicated that there was no significant correlation between the composition of liver and bone marrow alleles and risk of toxicity (leukopenia, anemia and thrombocytopenia) which reflect no association between allele polymorphism −401 C/TB and risk of toxicity. In order to evaluate the effect of the presence or absence of C and T alleles on mentioned toxicities in −401 C/TA genotype, toxicity grades in patients with CC allele were compared to that in patients with CT+TT allele. As well, in −401 C/TB genotype, toxicity grades in patients with TT allele were compared to that in CC+ CT patients.

As it presented in Table 2, WBC and platelet counts were significantly different (PV <0.05) in patients with −401 C/T polymorphism in their consolidation phase of treatment. Moreover, patients may be also at border of anemia too (PV $=0.06$). Patients who received low-dose of oral methotrexate in maintenance phase of treatment showed no significant relationship between genotype and allele frequencies of −401C/T polymorphisms and risk of toxicity of bone marrow (including anemia, leukopenia and thrombocytopenia). Moderate dose (2 g/m^2) of methotrexate in consolidation phase of treatment resulted in significant association (PV <0.05) between genotype and allele frequencies of the polymorphism −401C/T and risk of toxicity in bone marrow (including leukopenia and thrombocytopenia). In contrast, there was no significant difference in anemia (p value $=0.06$).

As it demonstrated in Table 3, there is not any significant association between +452 C/T polymorphism and the risk of toxicity to the liver and bone marrow (leukopenia, anemia and thrombocytopenia) in both phases of consolidation and maintenance therapy.

Relation between GGH gene polymorphism and methotrexate serum levels

One-way ANOVA statistical analysis indicated no significant correlation between −401 C/T and +452 C/T polymorphisms and serum levels of methotrexate. Results are represented in Table 4. Although the mean of serum methotrexate levels in CC genotype was higher than CT genotype and in CT genotype higher than TT genotype, no significant association between methotrexate serum levels and GGH gene polymorphism was observed.

Discussion

In recent years, it has been tried to study proteins involved in drug metabolism as well as genetic variants of these proteins

Table 1. Grading of leukopenia and thrombocytopenia based on CTCAE.

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Hemoglobin	<10.0 g/dL	10.0–8.0 g/dL	8.0–6.5 g/dL	<6.5 g/dL	Death
Leukocyte	$<3.0 \times 10^9$/L	$2.0–3.0 \times 10^9$/L	$1.0–2.0 \times 10^9$/L	$<1.0 \times 10^9$/L	Death
Platelets	$<75.0 \times 10^9$/L	$50.0–75.0 \times 10^9$/L	$25.0–50.0 \times 10^9$/L	$<25.0 \times 10^9$/L	Death

Table 2. Relation between −401 C/T polymorphism Alleles and the risk of liver and bone marrow toxicity (leukopenia, anemia and thrombocytopenia) in consolidation and maintenance phase in ALL.

Phase	Toxicity	T Allele	C Allele	*p* Value
Consolidation	Liver	9 (10.84%)	30 (36.14%)	0.11
	Anemia	12 (14.45%)	37 (44.57%)	0.06
	Leukopenia	11 (13.25%)	39 (46.98%)	0.04
	Thrombocytopenia	10 (12.04%)	28 (33.73%)	0.01
Maintenance	Liver	13 (15.66%)	33 (39.75%)	0.52
	Anemia	7 (8.43%)	31 (37.43%)	0.73
	Leukopenia	51 (18.07%)	41 (49.39%)	0.23
	Thrombocytopenia	3 (3.61%)	23 (27.71%)	0.80

Table 3. Relation between +452 C/T polymorphism Alleles and the risk of liver and bone marrow toxicity (leukopenia, anemia and thrombocytopenia) in consolidation and maintenance phase in ALL.

Phase	Toxicity	T Allele	C Allele	*p* Value
Consolidation	Liver	0	40 (48.19%)	0.83
	Anemia	0	50 (60.24%)	0.98
	Leukopenia	0	52 (62.65%)	0.89
	Thrombocytopenia	0	39 (46.98%)	0.66
Maintenance	Liver	0	50 (60.24%)	0.67
	Anemia	0	37 (44.57%)	0.61
	Leukopenia	0	59 (71.08%)	0.93
	Thrombocytopenia	0	27 (32.53%)	0.96

Table 4. Relation between the polymorphism −401 C/T and + 452 C/T GGH gene and methotrexate serum levels in ALL.

Serum levels of methotrexate					
95% CI for Mean	SD	Mean	Quantity	Genotype	*p* Value
0.88–0.00	0.45	0.41	12	CC	0.53
0.48–0.29	0.17	0.39	35	CT	
0.38–0.21	0.12	0.30	22	TT	

in order to determine the pharmacokinetics and pharmacodynamics of chemotherapy for acute lymphoblastic leukemia. A good understanding of the effects and risk of toxicity of chemotherapy drugs can lead to a decrease in the risk of toxicity and side effects of these drugs and increase the possibility of successful treatment. Methotrexate is a main drug in ALL chemotherapy regimens; and like other chemotherapy drugs, it is associated with toxic side effects such as bone marrow suppression, renal toxicity, hepatotoxicity, neurotoxicity and toxicity in GI tract (Pui & Evans, 2006). The incidence of toxic effects is obviously different from one person to another even in the same dose of methotrexate. The toxic effects of this drug appear to be affected by several factors including individual genetics (Lopez-Lopez et al., 2013).

This study seems to be the first study evaluating the genotype frequency of −401C/T and +452 C/T polymorphisms of GGH gene in an Iranian ALL population. The frequency of C/C, C/T and T/T in −401 C/T polymorphism was 54.2, 27.7 and 18.1%, respectively; and the frequency of the C/C, C/T and T/T alleles in +542 C/T polymorphism was 74.69, 25.30 and 0.0%, respectively.

In this study, genotype and allele frequencies of −401C/T and +452 C/T polymorphisms of GGH gene and their association with toxicity in liver, gastrointestinal tract and bone marrow (including anemia, leukopenia, and thrombocytopenia) were separately evaluated. Based on the results, no association was observed between hepatic and gastrointestinal toxicities and −401 C/T and +452 C/T polymorphisms of GGH gene. According to the statistical analysis of −401 C/T polymorphism and its relationship with bone marrow cytotoxicity, it can be argued that the C allele polymorphism of −401 C/T allele is a risk factor of leukopenia and thrombocytopenia in patients treated with methotrexate. Moreover, our results suggested that the T allele had a supporting role in prevention of thrombocytopenia. Patients were in grade 3–4 of leukopenia and thrombocytopenia in consolidation phase of the treatment. In maintenance phase of the treatment compared to consolidation phase, no toxicity was observed in the bone marrow. In fact, the cell count was almost normal and even relative increase in white blood cells and platelets was observed in the patient's medical records. Based on our results, the patients in consolidation phase of the treatment were on the verge of anemia, and if the study was conducted on a higher number of these patients, the statistical value

could indicate anemia, and bone marrow cytotoxicity would include anemia in addition to leukopenia and thrombocytopenia. In separate statistical analysis of +452 C/T polymorphism and its association with bone marrow toxicity, it was found that this polymorphism has no role in the mentioned cytotoxicities. According to our study, the cited polymorphisms had no effect on serum level of methotrexate.

According to a study conducted by Koomdee et al. (2012) on 105 ALL children, GGH and DHFR genes have important role in MTX metabolism pathway. They observed that children with ALL who showed the polymorphisms of −401 C/T or T/T in their GGH gene had a high risk of leukopenia and thrombocytopenia, in comparison with the −401 C/C polymorphism. In contrast to their findings, our results indicated that C allele polymorphism of −401 C/T allele could deteriorate leukopenia and thrombocytopenia in patients treated with methotrexate.

In study accomplished by Organista-Nava et al. (2010) on 70 children with ALL in Mexico, the relationship of polymorphisms −401 C/T and +452 C/T of GGH gene and risk of recurrence was assessed. They found 83.10% risk of recurrence in polymorphism −401 T/T. In contrast to their findings, our study indicated no risk of recurrence in polymorphisms +452 C/T.

We could not find more related studies about polymorphisms −401 C/T and +452 C/T of GGH gene but many other studies performed to evaluate the association of the different gene polymorphisms that involved in methotrexate metabolism and serum levels of methotrexate and cytotoxicity (Csordas et al., 2014; Eissa & Ahmed, 2013; Liu et al., 2014; Sharifi et al., 2014; Shimasaki et al., 2008).

Shimasaki et al. (2008) evaluated the effect of A80G MTHFR polymorphisms on toxicity associated with high-dose of methotrexate in 15 children with ALL; and observed no correlation between A80G polymorphism and serum levels of methotrexate and liver toxicity. Sharifi et al. (2014) performed a study to evaluate the association of −24CT, 1249GA, and 3972CT ABCC2 gene polymorphisms with methotrexate serum levels and toxic side effects in children with ALL in Iran. They concluded that there was a reverse significant relationship between 3972T allele carriers and hepatotoxicity. Also, 1249A allele carriers had increased rate of gastrointestinal toxicity. No significant relationship was detected between −24CT polymorphism and methotrexate toxic side effects. There was no significant relationship between these three polymorphisms and methotrexate serum levels. Eissa & Ahmed (2013) studied relationship between C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene and risk of toxicity of methotrexate in adult patients with ALL. They came to the conclusion that, there is increased relapse and mortality rates and shorter survival in patients with 677 TT genotype than in those with CC and CT; whereas 1298 CC genotype patients had lower frequency of neutropaenia, hepatic toxicity and relapse than in those with AA and AC. In 2014, Liu et al figured out that the −24T allele in ABCC2 gene was significantly associated with higher risks of high-grade hematologic and non-hematologic MTX toxicities in childhood ALL (Liu et al., 2014). Csordas et al. (2014) findings confirm the association of novel genetic variations in folate-related and ARID5B genes with the methotrexate serum levels and acute toxicity of high-dose MTX in pediatric ALL.

Conclusion

No correlation was found between +452CT polymorphism and serum levels of methotrexate and methotrexate-related toxicity. −401CT polymorphism was found to be correlated with bone marrow methotrexate-related toxicity (thrombocytopenia and leucopenia) in consolidation phase of the treatment. These findings can be used in the future for prediction of methotrexate toxicity and its dose adjustment. It should be mentioned that the toxicity associated with these polymorphisms is different in most cases; however, these results need to be checked on larger groups and other ethnic groups.

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Declaration of interest

The authors report no conflicts of interest.

References

Chave KJ, Ryan TJ, Chmura SE, Galivan J. (2003). Identification of single nucleotide polymorphisms in the human γ-glutamyl hydrolase gene and characterization of promoter polymorphisms. Gene 319: 167–75.

Cronstein BN. (1996). Molecular therapeutics. Methotrexate and its mechanism of action. Arthritis Rheumatism 39:1951–60.

Csordas K, Lautner-Csorba O, Semsei AF, et al. (2014). Associations of novel genetic variations in the folate-related and ARID5B genes with the pharmacokinetics and toxicity of high-dose methotrexate in paediatric acute lymphoblastic leukaemia. Brit J Haematol 166: 410–20.

Dervieux T, Greenstein N, Kremer J. (2006). Pharmacogenomic and metabolic biomarkers in the folate pathway and their association with methotrexate effects during dosage escalation in rheumatoid arthritis. Arthritis Rheumatism 54:3095–103.

Dervieux T, Kremer J, Lein DO, et al. (2004). Contribution of common polymorphisms in reduced folate carrier and γ-glutamylhydrolase to methotrexate polyglutamate levels in patients with rheumatoid arthritis. Pharmacogenet Genomics 14:733–9.

Einollahi N, Alizadeh S, Dashti N, et al. (2013). Serum lipid profile alterations in acute leukemia before and after chemotherapy. IJBC 6: 3–9.

Eissa DS, Ahmed TM. (2013). C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene: effect on methotrexate-related toxicity in adult acute lymphoblastic leukaemia. Blood Coagul Fibrinolysis 24:181–8.

Evans WE, Crom WR, Abromowitch M, et al. (1986). Clinical pharmacodynamics of high-dose methotrexate in acute lymphocytic leukemia. New Engl J Medicine 314:471–7.

Evans WE, Relling MV. (2004). Moving towards individualized medicine with pharmacogenomics. Nature 429:464–8.

Evans WE, Relling MV, Rodman JH, et al. (1998). Conventional compared with individualized chemotherapy for childhood acute lymphoblastic leukemia. New Engl J Med 338:499–505.

Gleiβner B, Gökbuget N, Bartram CR, et al. (2002). Leading prognostic relevance of the BCR-ABL translocation in adult acute B-lineage lymphoblastic leukemia: a prospective study of the German Multicenter Trial Group and confirmed polymerase chain reaction analysis. Blood 99:1536–43.

Jahedi M, Shamsasenjan K, Sanaat Z, et al. (2014). Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia. Adv Pharm Bull 4:43.

Jemal A, Murray T, Ward E, et al. (2005). Cancer statistics, 2005. Cancer J Clinicians 55:10–30.

Koomdee N, Hongeng S, Apibal S, Pakakasama S. (2012). Association between polymorphisms of dihydrofolate reductase and gamma glutamyl hydrolase genes and toxicity of high dose methotrexate in children with acute lymphoblastic leukemia. Asian Pac J Cancer Prev 13:3461–4.

Liu Y, Yin Y, Sheng Q, et al. (2014). Association of ABCC2− 24C> t polymorphism with high-dose methotrexate plasma concentrations and toxicities in childhood acute lymphoblastic leukemia. PloS one 9: e82681.

Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, Garcia-Orad A. (2013). A systematic review and meta-analysis of MTHFR polymorphisms in methotrexate toxicity prediction in pediatric acute lymphoblastic leukemia. Pharmacogenomics J 13:498–506.

Mancini M, Scappaticci D, Cimino G, et al. (2005). A comprehensive genetic classification of adult acute lymphoblastic leukemia (ALL): analysis of the GIMEMA 0496 protocol. Blood 105:3434–41.

Organista-Nava J, Gómez-Gómez Y, Saavedra-Herrera MV, et al. (2010). Polymorphisms of the γ-glutamyl hydrolase gene and risk of relapse to acute lymphoblastic leukemia in Mexico. Leukemia Res 34:728–32.

Pu C.-H, Evans WE. (2006). Treatment of acute lymphoblastic leukemia. New Engl J Med 354:166–78.

Pui C.-H, Gaynon, PS, Boyett JM, et al. (2002). Outcome of treatment in childhood acute lymphoblastic leukaemia with rearrangements of the 11q23 chromosomal region. Lancet 359:1909–15.

Sharifi MJ, Bahoush G, Zaker F, et al. (2014). Association of-24CT, 1249GA, and 3972CT ABCC2 gene polymorphisms with methotrexate serum levels and toxic side effects in children with acute lymphoblastic leukemia. Pediatr Hematol Oncol 31:169–77.

Shimasaki N, Mori T, Torii C, et al. (2008). Influence of MTHFR and RFC1 polymorphisms on toxicities during maintenance chemotherapy for childhood acute lymphoblastic leukemia or lymphoma. J Pediatr Hematol/Oncol 30:347–52.

Vanasse GJ, Concannon P, Willerford DM. (1999). Regulated genomic instability and neoplasia in the lymphoid lineage. Blood 94: 3997–4010.

Wood AJ, Evans WE, Mcleod HL. (2003). Pharmacogenomics – drug disposition, drug targets, and side effects. New Engl J Med 348: 538–49.