Cancer Chemother Pharmacol (2011) 68:1339–1346
DOI 10.1007/s00280-011-1665-1

ORIGINAL ARTICLE

Methotrexate toxicity and efficacy during the consolidation phase in paediatric acute lymphoblastic leukaemia and MTHFR polymorphisms as pharmacogenetic determinants

Velia D'Angelo · Maria Ramaglia · Adriana Iannotta · Stefania Crisci · Paolo Indolfi · Matteo Francese · Maria Carmen Affinita · Giulia Pecoraro · Addolorata Napolitano · Claudia Fusco · Matilde Oreste · Cristiana Indolfi · Fiorina Casale

Received: 22 February 2011 / Accepted: 27 April 2011 / Published online: 18 May 2011

Abstract
Purpose Folate-metabolizing single-nucleotide polymorphisms (SNPs) are emerging as important pharmacogenetic prognostic determinants of the response to chemotherapy. With high doses of methotrexate (MTX) in the consolidation phase, methylenetetrahydrofolate reductase (MTHFR) polymorphisms could be potential modulators of the therapeutic response to antifolate chemotherapeutics in identifying a possible correlation with the outcome. This study aims to analyse the potential role of the MTHFR C677T and A1298C genetic variants in modulating the clinical toxicity and efficacy of high doses of MTX in a cohort of paediatric ALL patients ($n = 151$) treated with AIEOP protocols.
Methods This work includes DNA extraction by slides and RFLP-PCR.
Results The first observation relative to early toxicities (haematological and non-haematological), after the first doses of MTX in all protocols, was an association between the 677T and 1298C carriers and global toxicity. We found that in the 2 g/m^2 MTX group, patients harbouring 677TT homozygously exhibited a substantial 12-fold risk of developing toxicity. In this study, we demonstrate that the MTHFR 677TT variant is associated with an increased risk of relapse when compared to other genotypes. The Kaplan–Meier analysis showed that the 677TT variant had a lower 7-year DFS(disease-free survival) probability compared to the 677C carrier genotype (log-rank test $P = 0.003$) and OS (overall survival) and also confirms the lower probability of survival for patients with the 677TT variant (log-rank test, $P = 0.006$).
Conclusions Our study provides further evidence of the critical role played by folate pathway enzymes in the outcome of ALL, possibly through the interference of MTX.

Keywords Leukaemia · SNPs · Methotrexate · MTHFR

V. D'Angelo (✉) · M. Ramaglia · A. Iannotta · P. Indolfi · M. Francese · M. C. Affinita · G. Pecoraro · A. Napolitano · C. Fusco · M. Oreste · C. Indolfi · F. Casale
Pediatric Department, Second University of Naples, Naples, Italy
e-mail: velia.dangelo@unina2.it

S. Crisci
Hematology Oncology Department, UOSC Hematology Oncology G Pascale Foundation, Naples, Italy

Introduction

Despite remarkable progress in antitumoral therapy, there is still much heterogeneity in the pharmacological response in the treatment of childhood ALL (acute lymphoblastic leukaemia), the most frequent malignancy in the paediatric population. Genetic polymorphisms and haplotypes have been shown to be among the most important factors influencing the pharmacokinetics (PK) and pharmacodynamics (PD) of many drugs [1]. Methotrexate (MTX), a key component of ALL treatment, blocks the conversion of dihydrofolates to tetrahydrofolates, the biologically active folate cofactors [2], increases serum homocysteine, induces a low folate level and, by affecting the intracellular folate pool, influences the activity of the enzyme methylenetetrahydrofolate reductase (MTHFR) [3, 4].

This enzyme catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of folate and the carbon moiety required for the conversion of homocysteine to methionine [5].

Two common polymorphisms, a 677C → T transition causing an alanine to valine amino acid substitution at codon 222 (Ala222Val) and an 1298A to C transversion causing a glutamic acid to alanine replacement at codon 429 (Glut429Ala), leading to 30–60% reduction in enzyme activity [6], occur in the MTHFR gene [7]. Patients with the C677T variant have impaired remethylation of homocysteine to methionine and subsequent hyperhomocysteinaemia [3]. Although 1298A → C has been associated with reduced MTHFR activity, neither the homozygous nor heterozygous state is associated with a change in homocysteine or folate levels [8].

However, it appears that individuals heterozygous for both 677C → T and 1298A → C have a phenotype similar to that of 677TT homozygotes [9].

An alteration in reduced folate pools, derived from inherited changes in MTHFR activity, may have a significant effect on the response of malignant and non-malignant cells to MTX, whose activity depends on cellular composition of folate [10].

Consequently, patients with decreased MTHFR activity are at an increased risk of MTX-related toxicity, and despite being widely used in the treatment of several diseases, many aspects about its pharmacology are still not clear [11]. Also, there is significant inter-individual variability in the patients' response to the treatment. High-dose MTX treatment, typical of paediatric ALL treatment protocols, often causes hepatic toxicity and bone marrow suppression [12–14]. This MTX-associated toxicity could alter the response to therapy of paediatric ALL and thus lead to a higher number of relapses. Moreover, several studies have shown an increased MTX toxicity and higher risk of relapse in ALL patients with the MTHFR C677T variant allele, both in adults [2, 15] and in children [16, 17]. On the other hand, the MTHFR A1298C polymorphism was not associated with either altered risks of relapse or toxicity in ALL children [2].

Other studies have reported that MTHFR variants are associated with MTX-related toxicity and survival both in adults [18] and in children with ALL [19]. Such research demonstrates a significant association with MTHFR variants, yet other authors reported neither association nor the contrary [20].

All of these studies have drawn upon the relatively small and heterogeneous sample sets of multicenter institutions.

This study aims to analyse the potential roles of the MTHFR C677T and A1298C genetic variants in modulating the clinical toxicity and efficacy of high doses of MTX in paediatric ALL patients. In particular, we studied the frequency of the MTHFR genetic variants C677T and A1298C in a cohort of homogeneous children treated with the same MTX doses and schedule, according to the Associazione Italiana Ematologia Oncologia Pediatrica (AIEOP) protocols. The ultimate objective was to identify new insights for a personalized therapy based on the human genotype in order to establish the optimum drug dose on leukaemia cells.

Materials and methods

Study population

The patients ($n = 178$) who enrolled in this study were all children of Caucasian European origin with ALL and were being treated at the Oncology Paediatric Department of the Second University of Naples. According to their clinical characteristics at diagnosis, the patients were subclassified as standard/intermediate or high risk. The multi-agent chemotherapeutic protocols used were AIEOP ALL 91', '95 and '00 [21]. All protocols had a consolidation phase with high doses of MTX (2 g/m^2 if the patient was subclassified as standard/intermediate risk or 5 g/m^2 if high risk or T cell enrolled in '95 protocol) followed by leucovorin rescue treatment in addition to daily oral 6-mercaptopurine (25 mg/mq). Twenty-three cases were not included in the study due to technical reasons (i.e. unavailable records or inadequate laboratory samples). Therefore, 151 patients (103 male/47 female) were included in the analysis. It is known that the incidence of paediatric ALL shows a 1.2-fold on higher male predominance [22]. In our series, higher 2-fold male rate probably is related to non-consecutive patients' selection. The median age at diagnosis was 5 years (range 3 months to 15 years). The diagnosis was established by the cytological examination of bone marrow smears according to the French-American-British (FAB) group classification and by immunophenotyping [23, 24]. The immunophenotype was assessed by flow cytometry (FACScan; Becton–Dickinson,) using monoclonal antibodies (MoAbs) against CD2, CD3, CD4, CD5, CD7, CD8, CD11b, CD13, CD14, CD15, CD19, CD20, CD22, CD33, CD34, CD41, HLA-DR and CD10 [25].

Haematological (leukopenia, anaemia, trombocytopenia) and non-haematological (hepatic, gastrointestinal and nervous system) toxicities were graded according to WHO criteria [26]. In this study, we chose only grades 3 or 4 of toxicity according to WHO criteria [26].The study was approved by the regional ethical review board at the Second University of Naples and performed in compliance with the Helsinki Declaration. Informed consent was obtained from parents and local participating institutions.

Laboratory analysis

DNA was isolated from peripheral whole blood or from archived bone marrow slides stored in histoteque or frozen

at −20°C. The extraction was performed by Salting Out methods for archived slides stored in histoteque and by Qiagen DNA Blood Mini Kit for other samples. DNA extraction from stored slides was optimized for maximal DNA yields according to literature data [27, 28].

The MTHFR genotype was performed using the polymerase chain reaction/restriction fragment length polymorphism method (PCR-based RFLP) [9, 29]. The reaction mixture containing 100–200 ng of DNA, 100 μM dNTP, 1,5 unit Taq DNA Polymerase Stoffel fragment (Applied Biosystem), 100 ng of the specific primers for the C677T polymorphism (forward primer 5′-TGA AGG AGA AGG TGT CTG CGG GA-3′ and reverse primer 5′-AGG ACG GTG CGG TGA GAG TG-3′) and the A1298C variant (forward primer 5′-GGG AGG AGC TGA CCA GTG CAG-3′ and reverse primer 5′-GGG GTC AGG CCA GGGGCA G-3′), and 1 mmol/L MgCl2, 50 mmol/L TRIS–HCl pH 8.8 in a 50-μl reaction volume was analysed.

Amplification reactions were performed using the following protocol for C677T on a 2400 GeneAmp PCR System (Applied Biosystem): 95°C for 10 min, following 4 cycles of 94° C for 1 min, 65°C for 1 min and 72°C for 1 min and then 23 cycles at 94°C for 30 s, 65°C for 30 s, 72°C for 45 s. The 198-bp polymerase chain reaction (PCR) products were digested with the HinfI restriction enzyme for 24 h at 37°C and the DNA fragment size separated on 12% of non-denaturing polyacrylamide gel. The DNA fragments were visualized by silver staining. The wild-type genotype (677CC) produces a single band at 198 bp; the heterozygote (677CT) produces a band of 175 bp, a fragment of 23 bp and a band at 198 bp; and the homozygote (677TT) only produces a band of 175-bp and a 23-bp fragments.

The protocol for the A1298C variants was 5' at 94°C following 30 cycles at 94°C for 30 s, 59°C for 13 s and 72°C for 17 s. The 138-bp PCR products were digested with Fnu4HI restriction enzymes for 24 h at 37°C and the DNA fragment size separated on 12% of non-denaturing gel. The DNA fragments were visualized by silver staining. The wild-type genotype (1298AA) produces a single band at 138 bp; heterozygous (1298AC) produces a band of 119 bp, a fragment of 19 bp and a band at 138 bp; and homozygous (1298CC) only produces a band of 119- and a 19-bp fragments. A negative control was used as an undigested amplified sample for each polymorphism.

Statistical analysis

Odds ratios (OR) and 95% confidence intervals (95% CI) were used to estimate the risk of developing global and different kind of toxicity. The differences in the frequency of MTHFR polymorphisms between children treated with the two different MTX dosages and across categories of different prognostic factors (gender, age, WBC count, ALL immunophenotype, and risk group) were calculated by multivariate logistic regression analysis.

Univariate analysis of MTHFR genotypes and relapse was done with the chi-squared test.

Then, the patients with the MTHFR polymorphisms, represented as dichotomous variables, were tested according to their influence on relapse, disease-free survival (DFS) and overall survival (OS). Survival differences (OS and DFS), estimated at 7 years by the Kaplan–Meier analysis, were assessed using a log-rank test. In the OS analysis, all deaths were considered treatment failures. OS was calculated for all patients from diagnosis to date of death or, if alive, date of last follow-up. In the DFS analysis, relapse and death in remission as a result of any cause were considered treatment failures. DFS was calculated for all patients that obtained complete remission from the date of remission to relapse, death or date of last follow-up, if alive in complete remission. Results were expressed as probability (percent) and 95% confidence intervals (CI.)

The hazard ratio (HR; with a 95% CI) for genetic MTHFR variants was estimated by the Cox regression analysis and categorized according to relapse risk prediction with the common prognostic factors. All P values < 0.05 were considered statistically significant.

Results

Main clinical characteristics, MTX toxicity and outcome

Table 1 shows clinical characteristics and the MTX toxicity of the patients. The whole group consisted of 151 paediatric patients, of which 88 cases (58.3%) were defined as standard/intermediate risk and 63 (41.7%) as high risk. In the consolidation phase, 78 patients were treated with 2 g/m^2 of MTX and the other 73, whose 10 with immunophenotype T cell, with 5 g/m^2 of MTX with the same protocol schedule according to the relative risk and immunophenotype classification.

Overall, at a 7-year follow-up (median = 65 months 95% C.I 39.7–81.6), 117 patients (67.5%) were alive and well in continuous complete remission. Forty-nine patients (32.5%) relapsed. The estimated 7-year DFS and OS rates for all patients were 67.5 and 77.5%, respectively.

Concerning MTX toxicity among the whole group of patients, 103 (68.2%) developed grade 3-4 toxicity (haematological/non-haematological/combined). The global pattern of toxicities was 44 patients (29.1%) developed haematological, 30 (19.9%) non-haematological toxicity (predominantly hepatic) and 29 (19.2%) combined toxicity.

Table 1 Main characteristics and outcome of the patients

Clinical characteristics	Number of the patients (%)
Gender	
Male	103 (68.2)
Female	48 (31.7)
Age (years)	
≤2	22 (14.5)
>2	129 (85.4)
WBC*	
≤20.000/mm^3	62 (41.0)
>20.000/mm^3	89 (58.9)
FAB phenotype	
L1	45 (29.8)
L2	106 (70.2)
Immunophenotype	
B cell	120(79.4)
T cell	31 (20.5)
MTX toxicity	
Haematological	44 (29.1)
No haematological	30 (19.9)
Combined	29 (19.2)
No toxicity	48 (31.7)
Disease status	
Relapsed	49 (32.5)
No relapsed	102 (67.5)
Risk index	
Standard/intermediate	88 (58.3)
High	63 (41.7)
MTX doses	
2 g/m^2	78 (53.6)
5 g/m^2	73 (46.4)

*WBC White blood cell

Analysing this pattern of toxicities according to the different MTX dosage, there was no difference in toxicity distribution between standard/intermediate and high-risk group. In more detail, among the 50 patients treated with 2 g/MTX 23 showed haematological, 10 non-haematological toxicity and 17 combined toxicity, whereas among the 53 patients treated with 5 g/MTX 21 presented haematological, 20 non-haematological and 12 combined toxicity, respectively ($P = 0.5$).We considered meaningless the toxicity results of 6-mercaptopurine related to the low daily dose of the drug.

MTX toxicity related to the MTHFR polymorphisms:

Genotype distribution shows no statistical differences between risk index stratification and two different MTX dosages (Table 2).

Haplotype analysis was carried out: two haplotypes (AC CT and AC CC), accounted for 47.7%, and three (CC TT, CC CT and CC CC) haplotypes had a frequency of 13.8%. The analysis yielded nine main haplotypes. We had no statistically significant results among our haplotypes, risk of relapse and MTX toxicity.

Therefore, we performed an analysis of MTX toxicity by comparing the MTHFR genotypes with global and other types of toxicity. There proved to be no differences among all patients stratified by the MTHFR C677T and A1298C genotypes and other kinds of toxicity.

When the global toxicity and MTHFR C677T genotypes in the two MTX treatment groups (2 g/m^2 vs. 5 g/m^2) were analysed, the toxicity risk was significantly over-expressed among 677TT homozygotes. In more detail, among the patients treated with MTX 2 g, the 677TT genotype (19 cases) had a 12-fold higher risk of developing toxicity than patients with other genotypes (OR = 12.2; 95% CI; 2.54–58.9; $P = 0.001$). Concerning the different kinds of toxicity in the 677TT genotype group, only the occurrence of non-haematological toxicity was significantly over-represented in the MTX/2 g treatment group (11 vs. 3), with a 13-fold greater risk with than the MTX/5 g group (OR = 13.44; 95% CI; 2.21–81.77; $P = 0.002$) (Table 3).

No significant association was found between the MTHFR A1298C variant and the risk of toxicity (data not shown).

Clinical outcome and MTHFR polymorphisms

The C677T genotypes showed a higher risk of adverse events when the whole group was stratified according to

Table 2 Genotype distribution respect to MTX dosage and risk index

MTHFR genotype	Total cases	MTX Dosage		*P*	Risk index		*P*
		2 g	5 g		Standard/intermediate	High	
677CC	48	21	27		22	26	
677CT	71	38	33	0.1	46	25	0.1
677TT	32	19	13		20	12	
1298AA	43	23	20		24	19	
1298AC	83	40	43	0.7	48	35	0.6
1298CC	25	15	10		16	9	

Table 3 MTHFR C677T genotypes and risk of toxicity in relation to different MTX dosage

MTHFR genotype	Total cases $n = 151$	2 g n (%) Tox	5 g n (%) Tox	OR* (95% CI)	P
677CC	48	14 (29.2)	20 (41.6)	Reference	
677CT	71	22 (31)	29 (40.8)	0.6 (0.26–1.26)	
677TT	32	14 (43.7)	4 (12.5)	12.2 (2.54–58.9)	0.001
Haematological					
677CC	17	8 (47)	9 (53)	Reference	
677CT	23	10 (43)	13 (56.5)	0.59 (0.184–1.89)	
677TT	4	3 (75)	1 (25)	9.00 (0.367–220.9)	NS
Non-haematological					
677CC	17	6 (35.3)	11 (64.70)	Reference	
677CT	28	12 (42.8)	16 (57.1)	0.562 (0.19–1.62)	
677TT	14	11 (78.6)	3 (21.4)	13.44 (2.21–81.77)	0.002

OR values were computed considering the number of MTHFR 677CC cases as the reference

* OR values were computed comparing the number of MTHFR C677T carriers cases versus the number of cases with the two different MTX dosage for each toxicity. P values above 0.100 are not shown

Table 4 MTHFR polymorphisms and relapses

MTHFR genotype	Total cases $n = 151$	No relapse n (%)	Relapse n (%)	P
677CC	48	32 (66.6)	16 (33.3)	
677CT	71	55 (77.5)	16 (22.5)	0.008
677TT	32	15 (46.8)	17 (53.1)	
1298AA	43	30 (69.7)	13 (30.2)	
1298AC	83	54 (65)	29 (35)	0.7
1298CC	25	18 (72)	7 (28)	

MTHFR genotypes ($P = 0.008$). No significant differences were found between patients stratified according to the A1298C variant ($P = 0.7$) (Table 4). In detail, the 677TT genotype was at a higher risk of relapse compared to other genotypes (OR = 2.9; 95% CI = 1.23–6.9; $P = 0.01$).

The Kaplan–Meier analysis, comparing DFS curves at a 7 year of follow-up, shows that the 677TT genotype had a lower probability of DFS compared to cases with 677C carriers (63.7% vs. 36.2%) (log-rank test, $P = 0.03$, Fig. 1). The estimated 7-year OS for all patients, stratified by the C677T polymorphism, confirms the lower probability of survival for patients with the 677TT variant respect to patients with 677CC and 677CT (76% vs. 48%) (log-rank test, $P = 0.006$, Fig. 2).

No significant differences in DFS and OS rate were found between patients stratified according to the A1298C variant (log-rank test $P = 0.6$).The Cox model, containing gender, age, initial WBC count, PDN response, FAB phenotype, immunophenotype, type of toxicity, MTX dosage, risk index and MTHFR C677T variants, confirmed an increased hazard ratio of relapse among patients with the MTHFR 677TT carriers vs other genotypes (HR 2.62; 95% CI; 1.32–5.21; $P = 0.001$).

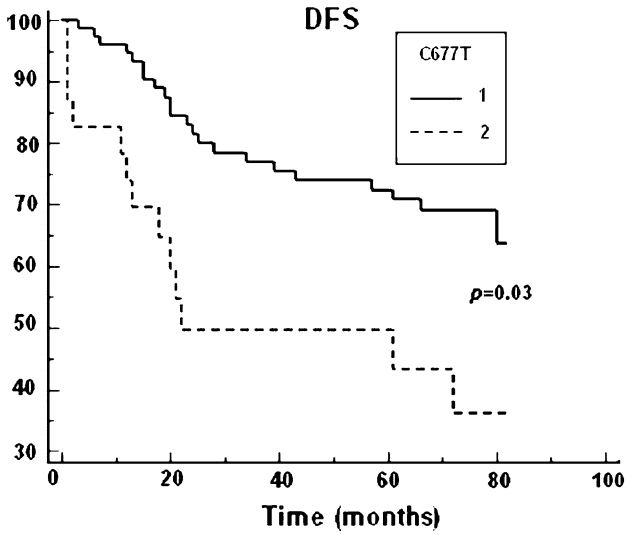

Fig. 1 Kaplan–Meier analysis of disease-free survival (DFS) and MTHFR C677 > T genotype. 1 (CC + CT carriers) versus 2 (TT carriers)

Discussion

The great inter-individual variability in drug effects and efficacy is one of the major issues in the clinical management of paediatric patients with cancer, and the severe toxicity is often the treatment's major limitation. The most common reason for failure in childhood ALL is the relapse. Folate-metabolizing single-nucleotide polymorphisms (SNPs) are emerging as important pharmacogenetic prognostic determinants of the response to chemotherapy in both haematological and solid cancers [20].

In the present study, we found that the effects of MTHFR SNPs showed severe grades of toxicity when

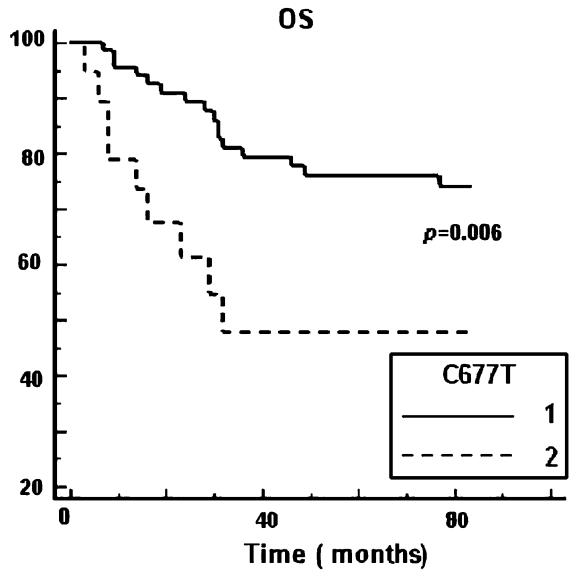

Fig. 2 Kaplan–Meier analysis of overall survival (OS) stratified by MTHFR C677 > T genotype. 1 (CC + CT carriers) versus 2 (TT carriers)

compared to the different MTX dosages. Specifically, in the 2 g MTX group, patients harbouring 677TT homozygously exhibited a substantial 12-fold risk of developing toxicity respect to other genotypes. Whereas the global toxicity in our study group is more evident in 5 g MTX group, we hypothesized that the major risk to develop toxicity among 2 g MTX dosage and 677TT carriers is mainly associated with the MTHFR reduced activity. Concerning the different kinds of toxicity, our analysis shows that patients who carry the 677TT genotype had about a 13-fold increased risk of developing non-haematological toxicity in the 2 g/MTX subgroup when compared to patients with other genotypes. We hypothesized that the combined effects of MTX treatment and MTHFR reduced activity brings on alteration in the reduced folate pool and higher homocysteine concentration that both interfere with antitumoral drug activity and its relative toxicity. This consideration is based on the following evidence: first, an acute increase in homocysteine levels was observed after MTX administration [30, 31]; secondly, the MTHFR C677T polymorphisms also caused an increase in homocysteine levels [29]; thirdly, homocysteine seems to have a role in hepatic toxicity by raising liver enzyme levels [32].

Our results are in contrast with a study where the MTHFR C677T allele does not increase the risk of MTX-induced toxicities in ALL children receiving high-dose MTX [33]. Aplenc et al. [19] also reported that MTHFR C677T is not associated with MTX toxicities in a large cohort of ALL patients. These studies analysed the effect of MTX dosage in maintenance therapy. For this reason, and differently from our study, the results could therefore be influenced by the cumulative toxic effects of several drugs and a minor dosage of MTX. Thus, the role of MTHFR variants found in our cohort of patients could be ascribable to their effects in higher MTX doses. In fact, reduced MTHFR activity prolongs high-dosage MTX action with more toxic effects on ALL patients, and this explains our toxicity results. Using lymphoblasts originating from ALL patients who suffered from MTX-related toxicity, Taub et al. [17] found that patients with the TT genotype exhibited greater in vitro MTX sensitivity when compared to patients with other genotypes. Another study conducted on a Thai population of children with ALL (74 cases) during the consolidation phase with 1.5 g/m^2 of MTX showed that only the MTHFR 1298CC genotype was significantly associated with a decreased risk of myelosuppression [34]. Moreover, MTHFR polymorphisms are associated with the elimination of MTX, as demonstrated in higher serum concentrations of MTX 48 h after the start of infusion in TT genotype patients [12, 35].

In accordance with other studies in paediatric ALL patients and haematological malignancies [20, 36], we did not observe significant differences in toxicity between patients with the A1298C variant.

Moreover, we evaluated the influence of folate polymorphisms, directly involved in the MTX pharmacological pathway, in the risk of relapse. The study shows that the MTHFR 677TT variant is associated with an increased risk of relapse when compared to other genotypes. The Kaplan–Meier analysis confirms that the 677TT variant had a lower probability of DFS and OS when compared to cases with 677C carriers.

Our results are also in accordance with Chiusolo and Ongaro's findings in adult ALL patients treated with methotrexate-based maintenance therapy [15, 18]. Two childhood ALL studies showed that the 677T variant is associated with lower EFS, but in patients who received a weekly 20 mg/m^2 of MTX during interim maintenance and maintenance therapy [16, 19]. Our results can be explained by the fact that 677TT patients conserve 5,10-methylenetethaydrofolate more in their cells than carriers of other genotypes. This condition may counteract the folate-depleting effect of MTX and affect both MTX toxicity and the outcome of patients [20].

About haplotype analysis, the number of our patients studied produced very little subgroups considering the two different MTX dosages, the toxicity and relapses. Probably, for this reason, we had no statistically significant results by our analysis. Polymorphisms in drug-metabolizing enzymes, transporters and/or pharmacological targets of drugs may profoundly influence the dose–response relationship among individuals. For some drugs, although retrospective data suggest that these polymorphisms are frequently associated

with adverse drug reactions or failure of efficacy, the clinical utility of such data remains unproven. Therefore, there is an urgent need for prospective data to determine whether genotype analysis before treatment can improve the safety and toxicity of the therapy.

Understanding pharmacogenomics and its application to the complex interaction between drug, host and disease, will lead to better selection methods in order to improve our ability to predict the toxicity and response to therapy.

In conclusion, our study provides further evidence of the critical role played by folate pathway enzymes in ALL outcome, possibly through the interference of MTX.

The confirmation of these results in a larger prospective study could draw recommendations with respect to dose adjustment and establish the efficacy of MTX treatment according to MTHFR polymorphisms.

Acknowledgments The work was supported by Luciano Gaudio, Professor of Genetics Dept Biological Sciences, University of Naples 'Federico II' Chair.

References

1. Yong WP, Innocenti F, Ratain MJ (2006) The role of pharmacogenetics in cancer therapeutics. Br J Clin Pharmacol 62:35–46
2. Chiusolo P, Reddiconto G, Casorelli I et al (2002) Preponderance of methylenetetrahydrofolate reductase C677T homozygosity among leukemia patients intolerant to methotrexate. Annal Oncol 13:1915–1918
3. Bagley PJ, Selhub J (1998) A common mutation in the methylenetetrahydrofolate reductase gene is associated with an accumulation of formylated tetrahydrofolates in red blood cells. Proc Natl Acad Sci USA 95:13217–13220
4. Calvert H (1999) An overview of folate metabolism: features relevant to the action and toxicities of antifolate anticancer agents. Semin Oncol 26:3–10
5. Narayanan S, McConnell J, Little J, Sharp L, Piyathilake C, Powers H, Basten G, Duthie S (2004) Associations between two common variants C677T and A1298C in the methylenetetrahydrofolate reductase gene and measures of folate metabolism and DNA stability (Strand Breaks, Misincorporated uracil, and DNA Methylation Status) in human lymphocytes in vivo. Cancer Epidemiol Biomarkers Prev 13:1436–1443
6. Kim HN, Kim YK, Lee IK, Yang DH, Lee JJ, Shin MH, Park KS, Choi JS, Park MR, Jo DY, Won JH, Kwak JY, Kim HJ (2009) Association between polymorphisms of folate-metabolizing enzymes and hematological malignancies. Leuk Res 33(1):82–87
7. Bottiger AK, Hurtig-Wennlof A, Sjostrom M, Yngve A, Nilsson TK (2007) Association of total plasma homocysteine with methylenetetrahydrofolate reductase genotypes 677C > T, 1298A > C, and 1793G > A and the corresponding haplotypes in Swedish children and adolescents. Int J Mol Med 19:659–665
8. Toffoli G, Russo A, Innocenti F et al (2003) Effect of methylenetetrahydrofolate reductase 677C ≥ T polymorphism on toxicity and homocysteine plasma level after chronic methotrexate treatment of ovarian cancer patients. Int J Cancer 103:294–299
9. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R (1998) A second genetic polymorphism in methylentetrahydofolaten reductase (MTHFR) associated with decreased enzyme activity. Mole Gene Metabol 64:169–172
10. Rosenblatt DS (2001) Methylenetetrahydrofolate reductase. Clin Invest Med 24:56–59
11. Kremer JM (2006) Methotrexate pharmacogenomics. Ann Rheum Dis 65:1121–1123
12. Imanishi H, Okamura N, Yagi M et al (2007) Genetic polymorphisms associated with adverse events and elimination of methotrexate in childhood acute lymphoblastic leukemia and malignant lymphoma. J Hum Genet 52:166–171
13. Costea I, Moghrabi A, Laverdiere C, Graziani A, Krajinovic M (2006) Folate cycle gene variants and chemotherapy toxicity in pediatric patients with acute lymphoblastic leukemia. Haematologica 91(8):1113–1116
14. Pui CH, Relling MV, Evans WE (2002) Role of pharmacogenomics and pharmacodynamics in the treatment of acute lymphoblastic leukaemia. Best Pract Res Clin Haematol 15:741–756
15. Chiusolo P, Reddiconto G, Farina G et al (2007) MTHFR polymorphisms: influence on outcome and toxicity in acute Lymphoblastic leukemia patients. Leuk Res 31:1669–1674
16. Krajinovic M, Lemieux-Blanchard E, Chiasson S, Primeau M, Costea I, Moghrabi A (2004) Role of polymorphisms in MTHFR and MTHFD1 genes in the outcome of childhood acute lymphoblastic leukemia. Pharmacogenomics 4:66–72
17. Taub JW, Matherly LH, Ravindranath Y et al (2002) Polymorphisms in methylenetetrahydrofolate reductase and methotrexate sensitivity in childhood acute lymphoblastic leukemia. Leukemia 16:764–765
18. Ongaro A, De Mattei M, Della Porta M, Rigolin G, Ambrosio C, Di Raimondo F, Pellato A, Masieri F, Caruso A, Catozzi L, Gemmati D (2009) Gene polymorfisms in folate metabolizing enzymes in adult acute lymphoblastic leukemia: effects on methotrexate- related toxicity and survival. Haematologica 94(10):1391–1398
19. Aplenc R, Thompson J, Han P et al (2005) Methylenetetrahydrofolate reductase polymorphisms and therapy response in paediatric acute lymphoblastic leukemia. Cancer Res 65(6): 2487–2582
20. De Mattia E, Toffoli G (2009) G C677T and A1298C MTHFR polymorphisms, a challenge for antifolate and fluoropyrimidine-based therapy. Eu J Cancer 60:1–19
21. Masera G, Conter V, Rizzari M et al (1999) AIEOP non-B ALL trials. J Ped Haem Onc 6:101
22. Pizzo AP, Poplack DG (2006) Principles and practice of pediatric oncology. Lippincot-Williams and Wilkins, Philadelphia
23. Bennet J, Catovsky D, Daniel MT, Flandring G, Galton DAG, Gralnick HR, Sultanj C (1976) French-American-British (FAB) cooperative group proposals for the classification of acute leukaemia's. Br J of Haematol 33:451
24. Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C (1981) The morphological classification of acute lymphoblastic leukemia: concordance among observers and clinical correlation. Br J of Haematol 9(6):1012
25. Borowitz M, Schuster J, Land V et al (1999) Myeloid—antigen expression in childhood acute lymphoblastic leukemia. N Eng J Med 1325:1378–1382
26. World Health Organization (WHO) an book For Reporting Results of Cancer Treatment WHO (1979) Offset publication no. 48. World Health Organization, Geneva
27. Aplenc R (2002) Differential bone marrow aspirate DNA yields from commercial extraction kits. Leukemia 16:1865–1866
28. Boyle E, Steinbuchn M, Tekautz T, Gutman J, Robinson J, Perentesis J (1998) Accuracy of DNA amplification from archival haematological slides for use in genetic biomarker studies. Cancer Epidemiol 7:1127–1131
29. Frosst P, Blom HJ, Milos R et al (1995) A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 10:111–113

30. Refsum H, Wesenbrg F, Ueland PM (1991) Plasma homocysteine in children with acute lymphoblastic leukemia: changes during a chemotherapeutic regimen including methotrexate. Cancer Res 51:828–835
31. Van Ede AE, Laan RF, Blom HJ, Boers GH, Haagsma CJ, Thomas CM et al (2002) Homocysteine and folate status in methotrexate-treated patients with rheumatoid arthritis. Rheumatology 41:658–665
32. Van Ede AE, Laan RF, Blom HJ, Huizinga TW, Haagsma CJ, Giesendorf BA et al (2001) The C677T mutation in the methylenetetrahydrofolate reductase gene: a genetic risk factor for methotrexate-related elevation of liver enzymes in rheumatoid arthritis patients. Arthritis Rheum 44:2525–2530
33. Shimasaki N, Mori T, Torii C, Sato R, Shimada H, Tanigawara Y, Kosaki K, Takahashi T (2008) Influence of MTHFR and RFC1 polymorphisms on toxicities during maintenance chemotherapy for childhood acute lymphoblastic leukemia or lymphoma. J Pediatr Hematol Oncol 30(5):347–352
34. Pakakasama S, Kanchanakamhaeng K, Kajanachumpol S, Udomsubpayakul U, Sirachainan N, Thithapandha A, Hongeng S (2007) Genetic polymorphisms of folate metabolic enzymes and toxicities of high dose methotrexate in children with acute lymphoblastic leukaemia. Ann Hematol 86(8):609–611 (Epub 2007 Feb 24)
35. Kantar M, Kosova B, Cetingul N, Gumus S, Toroslu E, Zafer N, Topcuoglu N, Aksoylar S, Cinar M, Tetik A, Eroglu Z (2009) Methylenetetrahydrofolate reductase C677T and A1298C gene polymorphisms and therapy-related toxicity in children treated for acute lymphoblastic leukemia and non-Hodgkin lymphoma. Leuk Lymphoma 50(6):912–917
36. Ongaro A, De Mattei M (2008) Folate-pathway gene variants in cancer: haematological malignancies. Transworld Research Network Editor Donato Gemmati, Kerala, pp 51–94