Ann Hematol (2006) 85: 291–300
DOI 10.1007/s00277-005-0072-2

ORIGINAL ARTICLE

Kathrin Seidemann · Marion Book · Martin Zimmermann · Ulrike Meyer · Karl Welte · Martin Stanulla · Alfred Reiter

MTHFR 677 (C→T) polymorphism is not relevant for prognosis or therapy-associated toxicity in pediatric NHL: results from 484 patients of multicenter trial NHL-BFM 95

Received: 16 June 2005 / Accepted: 16 December 2005 / Published online: 7 February 2006
© Springer-Verlag 2006

Abstract We analyzed the relationship of genetic variation within the methylenetetrahydrofolate reductase gene (*MTHFR 677* C→T) with clinical characteristics, outcome, and therapy-related toxicity in pediatric non-Hodgkin's lymphoma (NHL) in our multicenter trial NHL-BFM 95. In this trial, high-dose methotrexate (MTX) infusion regimens were randomized (4- vs 24-h infusion) in patients with B-cell lymphoma; MTX was applied as 24-h infusion in all patients with lymphoblastic lymphoma and anaplastic large cell lymphoma. Toxicity data were collected per patient and therapy course according to National Cancer Institute Common Toxicity Criteria (NCI-CTC). The genotypes in 484 pediatric patients were distributed as follows: *MTHFR 677* CC, 206 patients (42.6%); *MTHFR 677* CT, 214 patients (44.2%); and *MTHFR 677* TT, 64 patients (13.2%). Lymphoblastic lymphoma was significantly associated with homozygosity for the *MTHFR 677* T allele. No association of *MTHFR 677* genotype with clinical characteristics (sex, age, and tumor stage), outcome, or therapy-related toxicity could be detected. Therefore, we conclude that the *MTHFR 677* C→T polymorphism does not appear to influence outcome or therapy-associated toxicity in pediatric patients with NHL treated on BFM protocols.

Keywords *MTHFR* · Polymorphism · Toxicity · Pediatric · NHL · BFM

K. Seidemann (✉) · M. Book · M. Zimmermann · K. Welte · M. Stanulla
Department of Pediatric Hematology and Oncology, Hannover Medical School,
Carl-Neuberg-Str. 1,
30625 Hannover, Germany
e-mail: kseidemann@web.de
Tel.: +49-511-5323220
Fax: +49-511-5329029

U. Meyer · A. Reiter
NHL-BFM Study Center, Department of Pediatric Hematology and Oncology, University Children's Hospital,
Justus Liebig University Giessen,
Giessen, Germany

Introduction

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism and DNA synthesis. By interconverting methyltetrahydrofolate to methylenetetrahydrofolate, MTHFR recruits one-carbon fragments necessary for the methylation of deoxyuridine monophosphate (dUMP) to deoxythymidylate monophosphate (dTMP), which is necessary for DNA synthesis. MTHFR is therefore suggested to play an important role in rapidly growing cells [45]. Several single nucleotide polymorphisms (SNPs) within the *MTHFR* gene have been described, resulting in variant enzyme activity. The two most frequent *MTHFR* polymorphisms involve base exchanges at nucleotide position 677 (C→T, leading to an amino acid exchange from alanine to valine) and at position 1298 (A→C, leading to an amino acid exchange from glutamine to alanine). The *MTHFR 677* (C→T) polymorphism was first described in 1995 and leads to the expression of a thermolabile form of MTHFR with reduced enzyme activity [8]. The frequency of the *MTHFR 677* T allele varies significantly among different populations [3, 24, 26, 39]. In Europe, 8–18% of the Caucasian population are homozygous for the *MTHFR 677* T allele; approximately 40% are heterozygous [3, 17, 30].

Homozygosity for the *MTHFR 677* T allele has been associated with various disorders such as neural tube defects, preeclampsia, cleft lip and palate, Down syndrome, vascular disease, coronary heart disease, and increased susceptibility to thromboembolic complications [11, 22, 25, 28, 37, 45]. Due to the clinical impact of mutations in the *MTHFR* gene, especially in rapidly growing cells, these polymorphisms are of interest in the pathogenesis and treatment of lymphoid malignancies. Data on the association of *MTHFR 677* polymorphisms with risk of acute lymphoblastic leukemia (ALL) or non-Hodgkin's lymphoma (NHL) are still controversial. Whereas some authors described a decreased risk for ALL or NHL in adult patients with the *MTHFR 677* TT genotype, other authors did not confirm these findings [5, 10, 18, 35, 47]. It has been hypothesized that changes in the

availability of methylenetetrahydrofolate secondary to altered MTHFR activity might reduce the frequency of misincorporation of uracil into DNA, thus reducing the risk of DNA double-strand breaks as potential key event in the pathogenesis of malignant disease. On the other hand, reduced MTHFR activity might result in hypomethylation of DNA promoter regions, leading to increased expression of proto-oncogenes, thus increasing the risk of malignant disease [5, 10, 18, 35, 47].

As suggested by a limited number of studies, genetic variations might also be of relevance in treatment, prognosis, and therapy-related toxicity of lymphoid malignancies. NHL is of special interest in this respect because methotrexate (MTX)—a specific inhibitor of dihydrofolate reductase—is one of the key chemotherapeutic agents in the therapy of NHL. The introduction of therapy regimen containing high-dose MTX significantly improved outcome especially in the treatment of high-grade B-cell malignancies in pediatric patients [29, 33]. MTX acts via inhibition of dihydrofolate reductase, thus leading to intracellular deprivation of tetrahydrofolate, jeopardizing DNA synthesis and cell division. Intracellular tetrahydrofolate deprivation might partially be compensated by MTHFR, which converts methylenetetrahydrofolate into methyltetrahydrofolate, a precursor for tetrahydrofolate that can be demethylated independent of dihydrofolate reductase. Prognosis of patients with NHL and decreased MTHFR activity receiving MTX might therefore be better compared to patients with normal MTHFR activity in the setting of MTX-related tetrahydrofolate deprivation. On the other hand, patients carrying the *MTHFR 677* T allele might be at risk for increased toxicity of therapy regimen containing high-dose MTX due to more profound folate deprivation in tissues with high cell turnover such as gut mucosa or mononuclear cells, thus predisposing patients to more severe orointestinal and infectious toxicity [29, 33, 48]. In fact, genetic polymorphisms of the *MTHFR* gene have been associated with differences in MTX-related toxicity after bone marrow transplantation and in patients with rheumatoid arthritis receiving low-dose MTX over a long period of time [7, 42, 44, 46].

So far, the potential role of *MTHFR* polymorphisms in pediatric patients with NHL has not been examined. The present study, as part of the population-based multicenter therapy trial NHL-BFM 95, examines the impact of the *MTHFR 677* C→T polymorphism in pediatric patients with NHL regarding diagnostic entities, prognosis, and therapy-related toxicity.

Patients and methods

Patients

From April 1996 to March 2001, 791 eligible patients up to 18 years of age with newly diagnosed NHL were enrolled in multicenter therapy trial NHL-BFM 95 from centers in Austria, Germany, and Switzerland. Informed consent was obtained from all patients' parents or guardians. Diagnosis was based on histopathology, cytology, immunology, and immunohistochemistry. NHL subtypes originally diagnosed according to the updated Kiel classification for NHLs were reclassified on the basis of the World Health Organization (WHO) classification of hematological malignancies [13, 38]. B-ALL was diagnosed if the bone marrow smears showed at least 25% of blasts with typical FAB L3 morphology and immunology [2, 13, 31]. Tumor slides of all patients were reviewed by central reference pathology and/or cytology.

Of 791 patients, 484 (61.2% of the study population) were successfully genotyped for the *MTHFR 677* C→T polymorphism. The genotyped cohort of patients did not differ from the remaining study population regarding clinical characteristics, prognostic factors, distribution of risk factors for failure, therapy, or outcome (data not shown).

Therapy

Patients with lymphoblastic lymphoma or peripheral T-cell lymphoma were treated in therapy group Non-B and received chemotherapy according to stage and therapy response as described previously, with only minor modifications. After induction, patients received consolidation/extra-compartment therapy consisting of four courses of high-dose MTX (5 g/m^2) as i.v. infusion over 24 h as described previously [Fig. 1; only therapy branches SR (standard risk) and MR (medium risk) are shown] [32]. Patients with B-cell lymphoma and B-ALL were treated in therapy group B-NHL/B-ALL and were stratified in four therapy branches of different therapy intensity according to stage and tumor mass at diagnosis as previously described (Fig. 1) [33]. Patients with anaplastic large cell lymphoma (ALCL) were treated in therapy group ALCL and were stratified in three therapy branches according to stage (Fig. 1) [34]. Therapy for B-cell NHL and ALCL consisted of two to six 5-day courses of polychemotherapy, including MTX, with dose adjusted to risk group (doses ranging from 0.5 to 5 g/m^2). Radiotherapy was not part of the protocol.

In trial NHL-BFM 95, duration of MTX infusion was randomized in therapy group B-NHL/B-ALL for patients with B-NHL within each therapy branch: Patients received MTX either as a 4-h infusion or as a 24-h infusion. MTX serum concentration was measured at 24, 36 42, and 48 h from the start of MTX i.v. infusion. Leucovorin rescue (racemic folinic acid) was given i.v. in a dose of 30 mg/m^2 at 42 h, and leucovorin rescue was given i.v. in a dose of 15 mg/m^2 at 48 and 54 h from the start of MTX infusion as previously described [33].

Treatment success was determined by event-free survival (EFS). Events were defined as follows: death due to any cause, tumor progression, and second malignancy. Progression was defined as growth of an incompletely resolved tumor or as recurrence proven by biopsy.

Toxicity data and MTX levels were collected from treating centers by standardized questionnaires. Therapy-

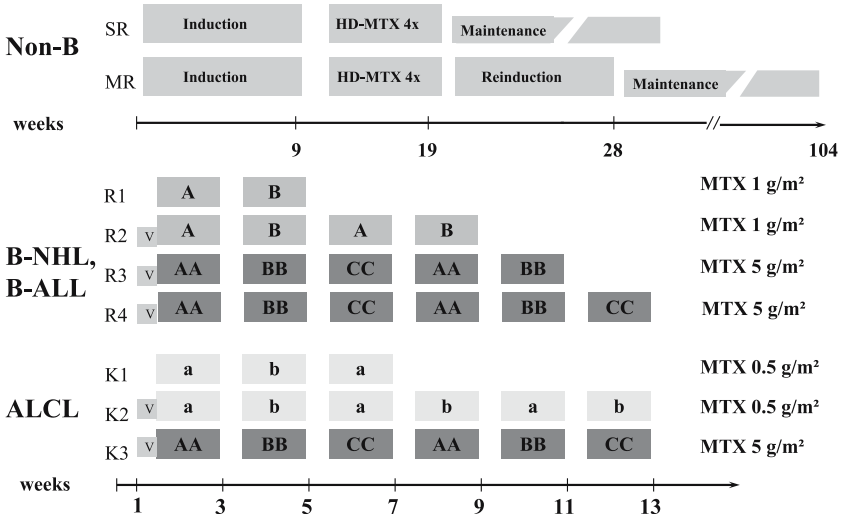

Fig. 1 Therapy strategy and MTX dosages in trial NHL-BFM 95. Patients with lymphoblastic lymphoma and peripheral T-cell lymphoma were treated in therapy group Non-B, patients with B-cell lymphoma (Burkitt's lymphoma, B-ALL, and diffuse large B-cell lymphoma) in therapy group B-NHL/B-ALL, patients with anaplastic large cell lymphoma in therapy group ALCL. Stratification of therapy intensity and dosages of methotrexate (MTX) are shown for every therapy branch

related toxicity was documented according to NCI/CTC criteria after each therapy course.

Genotyping

Genomic DNA was isolated from all available tumor-free bone marrow or peripheral blood smears from patients enrolled in trial NHL-BFM 95. Genotyping the *MTHFR 677* (C→T) polymorphism was performed using conventional polymerase chain reaction (PCR) (Expand High Fidelity PCR System, Roche Diagnostics, Mannheim, Germany) followed by restriction fragment length polymorphism (RFLP) analysis. A 198-bp region of exon 4 of the *MTHFR* gene was amplified using the following primer pairs: forward 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and reverse 5′-AGGACGGTGCGGTGAGAGTG-3′. Amplification was followed by restriction enzyme digestion with *Hinf* I (New England Biolabs, Frankfurt, Germany). The PCR product representing the *MTHFR 677* C allele remained uncut and was visualized on short fragment agarose gel electrophoresis as a single band of 198 bp. Heterozygous patients showed three bands of 198, 175, and 23 bp in length. Patients homozygous for the *MTHFR 677* T allele showed two bands of 175 and 23 bp in length. A random sample of 10% of the patients was genotyped twice without any discordances found in results regarding genotype.

Statistical analysis

Correlation of *MTHFR* genotype with clinical characteristics [age, sex, subtype of NHL, stage, serum lactate dehydrogenase (LDH) at diagnosis, therapy-related toxicity, and outcome] was performed using the χ^2 or Fisher's exact test. Analysis of genotype distribution among patients with NHL was performed by comparing observed genotype frequencies to previously published data from 257 healthy German individuals (published by Koch et al. (n=153 patients) [17] and by Reinhardt et al. (n=104 patients) [30]). Prognostic relevance of different parameters was examined by stepwise Cox regression analysis [6, 12]. Analysis of EFS was performed according to Kaplan and Meier, with differences compared by the log-rank test [15, 23]. EFS was calculated from the date of diagnosis to the first event or to the date of last follow-up. Relapse, death in continuous complete remission, and second malignancy were evaluated as events, and failure to achieve remission was evaluated as an event on day 1.

Statistical analyses were performed using the SAS program (SAS-PC, version 6.12; SAS Institute Inc., Cary, NC). Follow-up was actualized as of 1 January 2005.

Results

Patients

Of a total number of 791 pediatric patients enrolled in trial NHL-BFM 95 between April 1996 and March 2001, 484 patients (61.2% of the total study population) were genotyped for the *MTHFR 677* (C→T) polymorphism. Clinical characteristics of the genotyped study population (e.g., age, sex, tumor load at diagnosis, events, and clinical prognostic factors) did not differ from the entire study population (data not shown).

Of 484 genotyped patients, 33 were noneligible and were not included in analyses of EFS and treatment results for the following reasons: patient had received chemotherapy prior to diagnosis of NHL (n=8), NHL was second malignancy (n=1), patient suffered from HIV infection (n=1) or severe combined immunodeficiency (n=8), NHL occurred after transplantation (n=8), patient was not treated according to protocol NHL-BFM 95 or was treated in

Table 1 Frequency of genotypes of *MTHFR 677* in pediatric NHL entities

	Genotype						p[a]
	677 CC		677 CT		677 TT		
	n	%	*n*	%	*n*	%	
All patients (*n*=484)	206	42.6	214	44.2	64	13.2	
Lymphoma entity							
Lymphoblastic (*n*=99)	36	36.4	45	45.5	18	18.1	0.04
Burkitt/B-ALL (*n*=218)	92	42.2	95	43.6	31	14.2	0.26
Diffuse large B (*n*=65)	31	47.7	28	43.1	6	9.2	0.96
ALCL (*n*=67)	32	47.8	29	43.3	6	8.9	0.95
PTCL and other (*n*=35)	15	42.9	17	48.5	3	8.6	0.74
Healthy population[b] (*n*=257)	124	48.3	107	41.6	26	10.1	

No differences can be discerned regarding the distribution of genotypes for *MTHFR* 677 within different NHL entities. The distribution of genotypes within the study population was similar to that in healthy German controls[b] as described by Koch et al. (*n*=153 patients) [17] and by Reinhardt et al. (*n*=104 patients) [30]

ALCL Anaplastic large cell lymphoma, *PTCL* peripheral T-cell lymphoma

[a] *p* values refer to the comparison the distribution of genotypes among the study population and healthy German individuals

[b] Comparison with healthy Caucasian population in Germany

wrong therapy group due to initial misdiagnosis of NHL subtype (*n*=4), therapy was discontinued for nonmedical reasons (*n*=1), and treating institution was not participating in trial NHL-BFM 95 (*n*=2).

Of 484 genotyped patients, 99 patients (20.5%) were suffering from lymphoblastic lymphoma, 218 patients (45.0%) from Burkitt's lymphoma or B-ALL, 65 patients (13.4%) from diffuse large B-cell lymphoma, 67 patients (13.9%) from ALCL, 7 patients (1.4%) from peripheral T-cell lymphoma, and 28 patients (5.8%) from other NHL entities. The distribution of NHL entities among the genotyped population did not differ from the total study population of trial NHL-BFM 95.

Genotype analysis, clinical risk factors, and diagnostic entities

Of 484 genotyped patients, 206 patients (42.6%) had the *MTHFR 677* CC genotype, 214 patients (44.2%) were heterozygous, and 64 (13.2%) patients were homozygous for the *MTHFR 677* T allele. In the entire group, the distribution of genotypes was similar to the frequency of these genes observed in healthy Caucasian populations (Table 1) [17, 26, 30].

Clinical characteristics and prognostic factors such as sex, age, serum LDH, stage at diagnosis, central nervous system (CNS) disease, or MTX infusion regimen (in

Table 2 Clinical characteristics of 484 pediatric patients with NHL by *MTHFR 677* genotype

Clinical characteristic	Number (=100%)[a]	Genotyped patients (*N*=484)						*p* (univariate)
		677 CC (*n*=206, 42.6%)		677 CT (*n*=214, 44.2%)		677 TT (*n*=64, 13.2%)		
		n	%	*n*	%	*n*	%	
Age								
<10 years	263	112	42.6[a]	116	44.1[a]	35	13.3[a]	0.99
≥10 years	221	94	42.5	98	44.4	29	13.1	
Sex								
Male	340	142	41.8	153	45.0	45	13.2	0.85
Female	144	64	44.4	61	42.4	19	13.2	
LDH								
<1000 U/l	395	167	42.3	172	43.5	56	14.2	0.49
≥1000 U/l	82	34	41.5	40	48.8	8	9.7	
Not determined	7	5		2		0		
Stage[b]								
I	46	20	43.5	21	45.6	5	10.9	0.38
II	92	42	45.6	40	43.5	10	10.9	
III	230	85	37.0	109	47.4	36	15.6	
IV	68	37	54.4	23	33.8	8	11.8	
B-ALL	47	22	46.8	20	42.6	5	10.6	

[a] Percentages given in this table refer to sum of patients with same clinical characteristic (rows)

[b] Stage was not determined in one patient

patients with B-cell neoplasms only) of 484 genotyped patients were not associated with *MTHFR 677* genotype (Table 2).

In patients with lymphoblastic lymphoma (n=99), 36 patients (36.4%) were homozygous for the *MTHFR 677* C allele, 45 patients (45.5%) were heterozygous, and 18 patients (18.1%) had the *MTHFR 677* TT genotype. Among 218 patients with Burkitt's lymphoma or B-ALL, 92 patients (42.2%) had the *MTHFR 677* CC genotype, 95 patients (43.6%) were heterozygous, and 31 patients (14.2%) were homozygous for *MTHFR 677* T. Of 65 patients with diffuse large cell lymphoma, 31 patients (47.7%) were homozygous for the *MTHFR 677* C allele, 28 patients (43.1%) were heterozygous, and 6 patients (9.2%) were homozygous for the *MTHFR 677* T allele. In patients with ALCL, distribution of genotypes was similar: 32 patients (47.8%) were homozygous for the *MTHFR 677* C allele, 29 patients (43.3%) were heterozygous, and 6 patients (8.9%) were homozygous for the *MTHFR 677* T allele. In patients with B-cell lymphomas or ALCL, these differences in distribution of genotypes were not significant and very similar to the genotype frequency observed in the Caucasian population in Germany [17, 30]. However, in patients with lymphoblastic lymphoma, significantly more patients were homozygous for the *MTHFR 677* T allele (p=0.04), suggesting a potential pathogenetic role of this polymorphism in pediatric lymphoblastic lymphoma (Table 1). Whether this finding merely represents a false-positive result or indeed suggests a potential pathogenetic role of this polymorphism in pediatric lymphoblastic lymphoma remains an open question requiring further discussion and additional studies.

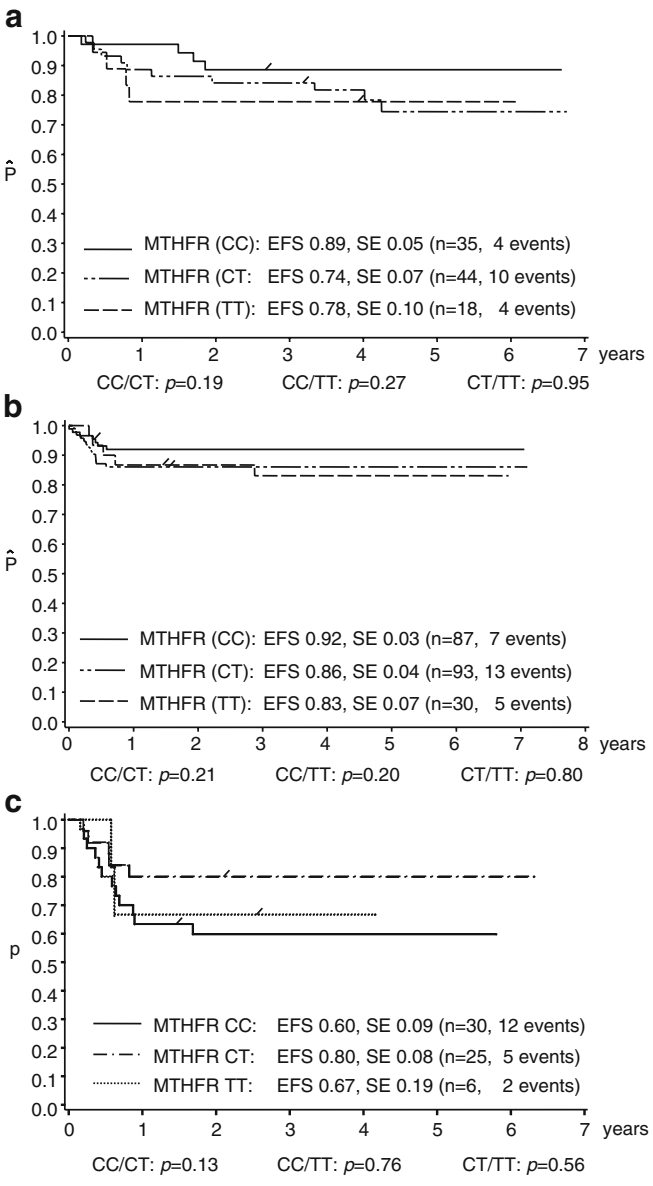

Fig. 2 **a**–**c** Event-free survival at 5 years by NHL entity and *MTHFR 677* genotype. **a** Event-free survival at 5 years according to *MTHFR 677* genotype in lymphoblastic NHL (n=97 patients*). **b** Event-free survival at 5 years according to *MTHFR 677* genotype in Burkitt's lymphoma and B-ALL (n=210 patients*). **c** Event-free survival at 5 years according to *MTHFR 677* genotype in ALCL (n=61 patients*). Differences in outcome between *MTHFR 677* genotypes and different lymphoma entities are not significant (p>0.05). *Only patients eligible for outcome analysis are included. Numbers of patients in these analyses therefore differ from numbers given in the tables demonstrating clinical and biological features. Angular tic marks mark the duration of the shortest follow-up. *SE* indicates standard error. p indicates p value of log-rank test in comparison of differences in outcome (Kaplan–Meier)

MTHFR 677 (C→T) polymorphism and EFS

Event-free survival at 5 years was analyzed with regard to *MTHFR 677* genotype for larger diagnostic subgroups of patients. Results with regard to genotype for patients with lymphoblastic lymphoma are shown in Fig. 2a, for patients with Burkitt's lymphoma or B-ALL in Fig. 2b, and for patients with ALCL in Fig. 2c. No association could be found between *MTHFR 677* genotype and EFS at 5 years in any subgroup of patients.

Events in association with *MTHFR 677* (C→T) polymorphism are demonstrated in Table 3. Tumor progression (i.e., relapse or growth of incompletely resolved tumor) was the most frequent event and was evenly distributed in groups of *MTHFR 677* genotype. No predisposition of a certain genotype to relapse or progression could be observed (Table 3).

MTHFR 677 (C→T) polymorphism and therapy-associated toxicity

The association of genotype and therapy-associated toxicity was analyzed by calculating the percentage of therapy courses with maximum toxicity (grade III or IV according to NCI/CTC criteria) regarding all MTX-containing courses. With regard to the very small number of patients with organ-related toxicity (hepatic, renal, or neurological toxicity), we focused on the most frequent and relevant toxicities: fever, infection, stomatitis, and diarrhea. No association was found between *MTHFR 677* genotype and

Table 3 Events in 451[a] eligible pediatric patients with NHL by *MTHFR-677* genotype

	MTHFR 677 genotype		
	CC (*n*=191, 32 events)	CT (*n*=198, 31 events)	TT (*n*=62, 12 events)
Early death from tumor complication	1	0	0
Death unrelated to tumor	5	6	1
Tumor progression	24	23	11
Second malignancy	2	2	0

Events are shown according to *MTHFR 677* genotype. No differences in number or types of events could be discerned between genotypes for *MTHFR 677*. Tumor progression was defined as growth of an incompletely resolved tumor or as recurrence proven by biopsy

[a]Of 484 genotyped patients, 33 were noneligible and were not included in analyses of treatment results for the following reasons: prior chemotherapy (*n*=8), NHL as second malignancy (*n*=1), HIV infection (*n*=1), immunodeficiency (*n*=8), posttransplant NHL (*n*=8), treatment protocol other than NHL BFM 95 (*n*=4), discontinuation of therapy for nonmedical reasons (*n*=1), and treating institution was not participating in trial NHL-BFM 95 (*n*=2)

therapy-related toxicity, independent of therapy group and therapy branch. Detailed data on percentages of courses with toxicity grades III/IV are only demonstrated for patients treated in therapy group B-NHL/B-ALL in Table 4. However, results were similar in therapy group Non-B and therapy group ALCL (data not shown).

In patients with B-NHL, the MTX infusion regimen was randomized in two branches with different infusion times (24 vs 4 h) with identical Leucovorin rescue. Therefore, we carefully looked for *MTHFR 677* genotype-associated differences in therapy related toxicity in both MTX randomization arms. Data are shown in Table 4a and b. However, for neither MTX infusion regimen (4 vs 24 h) could a correlation of toxicity data with *MTHFR 677* genotype be observed.

Because toxicity grades were generally low in our study population, we analyzed toxicity of the first—and most toxic—therapy course separately. However, no differences regarding toxicity grades of the first course between groups of patients with *MTHFR 677* genotype could be found

Table 4 Percentage of patients with grade III/IV toxicity in MTX-containing therapy courses in patients with B-NHL

	CC		CT		TT		*P*
	%	*n*[a]	%	*n*	%	*n*	
Infection							
A/B (MTX 1 g/m^2)							
MTX 4 h	5.7	22	7.1	28	2.1	12	0.73
MTX 24 h	8.9	44	3.5	36	0.0	8	0.23
AA/BB (MTX 5 g/m^2)							
MTX 4 h	6.5	23	12.6	31	6.3	8	0.33
MTX 24 h	9.1	34	9.8	35	12.5	10	0.55
Stomatitis							
A/B (MTX 1 g/m^2)							
MTX 4 h	3.4	22	9.8	28	6.8	11	0.49
MTX 24 h	17.1	44	15.1	36	40.6	8	0.11
AA/BB (MTX 5 g/m^2)							
MTX 4 h	27.5	23	25.8	31	12.5	8	0.45
MTX 24 h	35.5	34	36.4	35	42.5	10	0.81
Diarrhea							
A/B (MTX 1 g/m^2)							
MTX 4 h	2.3	22	1.8	28	4.2	12	0.48
MTX 24 h	4.1	43	0.7	36	0.00	8	0.70
AA/BB (MTX 5 g/m^2)							
MTX 4 h	10.9	23	6.7	31	3.1	8	0.92
MTX 24 h	2.9	34	7.1	35	5.0	10	0.44

Percentage (median) of patients with B-NHL and toxicity grades III and IV is shown in relation to genotype and MTX randomization arm (4- vs 24-hour infusion time). Results are shown separately for therapy branches R1 and R2 (dose of MTX 1 g/m^2) and branches R3 and R4 (5 g/m^2)

Leucovorin rescue was identical in both MTX infusion regimens. Only the clinically most relevant toxicities infection, stomatitis, and diarrhea are shown in this table

[a]Number of patients with available toxicity data

(data not shown). Regarding therapy-related deaths and their possible association with *MTHFR* 677 genotype, we also did not find any association with *MTHFR 677* genotype: Of 13 patients who died of toxic complications, 6 were homozygous for the *MTHFR 677* C allele, 6 patients were heterozygous, and only 1 patient was homozygous for the variant allele *MTHFR 677* T. To exclude differences in MTX levels as possibly confounding variable in toxicity analyses, we compared median MTX levels in both MTX randomization arms in therapy group B-NHL/B-ALL. MTX levels did not differ significantly by *MTHFR 677* genotype, independent of therapy branch (i.e., MTX dose) and infusion regimen (4 vs 24 h) (data not shown).

In summary, careful toxicity analyses did not show any association of *MTHFR 677* genotype with therapy-related toxicity in neither therapy group nor MTX infusion regimen.

Discussion

This is the first study so far describing the potential impact of the *MTHFR 677* (C→T) polymorphism in a population-based cohort of unselected pediatric patients suffering from NHL. Patients enrolled in trial NHL-BFM 95 comprise approximately 90% of all newly diagnosed cases of pediatric NHL in Germany, therefore rendering the presented data epidemiologically representative.

The frequency of *MTHFR 677* genotypes observed in the whole study population was similar to that described in healthy Caucasian populations [17, 24, 26, 30, 39]. The distribution of *MTHFR 677* genotype within different diagnostic subgroups of NHL did not show any significant differences for patients with B-cell lymphomas or ALCL. Therefore, a pathogenetic role of the *MTHFR 677* (C→T) polymorphism in these pediatric NHL entities seems unlikely.

Currently available data on the association of *MTHFR* genotype with risk of lymphoid malignancies are controversial; most studies focus on ALL rather than NHL. Whereas some studies in adult patients observed a decreased risk for ALL in patients homozygous for the *MTHFR 677* T allele, other authors described an increased risk or did not find any association [5, 10, 18, 35]. Other studies describe a positive association of *MTHFR 677* genotype with certain subentities of childhood ALL (*MLL* gene rearrangement positives), suggesting a protective role of the *MTHFR 677* T allele [47]. These findings on associations between *MTHFR 677* genotype and ALL are of special interest in the view of the fact that a positive association of the *MTHFR 677* (TT) genotype with lymphoblastic lymphoma could be observed in trial NHL-BFM 95, suggesting that alterations in folate metabolism may play a role in the pathogenesis of this subtype of pediatric NHL that shares many clinical, immunological, and pathological features with ALL. However, interpretation of data regarding positive associations of *MTHFR 677* polymorphisms with ALL or lymphoblastic NHL remains difficult. Small patient numbers and heterogeneous composition of patient groups may conceal positive associations. On the other hand, positive associations in the view of small patient numbers may merely represent false-positive findings. Other confounding factors such as complex gene–environment interactions due to differences in diet, folate intake, or nutritional vitamin substitution may significantly influence findings: results of a Canadian study on the association of *MTHFR 677* polymorphisms with pediatric ALL showed a positive correlation of *MTHFR 677* (TT) with reduced risk for ALL only in patients born before 1996, i.e., prior to regular folate substitution during pregnancy, suggesting that folate status and nutritional factors significantly influence the impact of genetic risk factors [18].

Regarding the potential association of *MTHFR* genotype with NHL other than lymphoblastic NHL, only very few studies have been published comprising heterogeneous groups of patients or NHL subentities [10, 23, 36, 41]. While some authors do not find an association, others describe a protective effect of the *MTHFR 677* TT genotype [10, 23, 41]. One recently published large population-based study on 1,593 patients found an increased risk of follicular lymphoma—a type of NHL that is almost exclusively observed in adult patients—and diffuse large cell lymphoma in adult patients being homozygous for the mutated allele [36].

In NHL-BFM trials, clinical risk factors for relapse were identified in different therapy groups and have been described in detail previously [32–34]. Neither of these clinical risk factors for relapse or other adverse events nor EFS were associated with *MTHFR 677* genotype. Therefore, *MTHFR 677* genotype does not seem to be a prognostic parameter in pediatric NHL or NHL subentities. No data have been published so far regarding the association of *MTHFR 677* and outcome in pediatric NHL. Recent evidence from studies on homogeneously stratified and treated pediatric patients with ALL suggests that *MTHFR 677* genotype may also play a role in therapy in patients with lymphoblastic NHL, an entity closely related to pediatric ALL [1]. However, we were unable to discern such an effect of genotype on outcome in lymphoblastic NHL. On the other hand, this negative association of *MTHFR 677* genotype and outcome in lymphoblastic NHL may be due to the small number of patients in this group (n=97) as compared to the results published by Aplenc et al. (n=520 patients with ALL) [1].

Methotrexate-related orointestinal toxicity is one of the most important side effects of modern polychemotherapy regimen for the treatment of pediatric NHL. Genetic polymorphisms causing reduced MTHFR activity might aggravate MTX-induced therapy toxicity. To our knowledge, data of the presented study comprise the largest cohort of homogeneously treated patients with NHL regarding therapy-associated toxicity and *MTHFR 677* genotype. In neither therapy group (Non-B, B-NHL/B-ALL, or ALCL) was homozygosity for the *MTHFR 677* T allele associated with increased therapy-related toxicity.

In therapy group B-NHL/B-ALL, MTX infusion time was randomized (4- vs 24-h infusion with identical

Leucovorin rescue) in all therapy branches (1 g/m^2 dose of MTX in R1 and R2, 5 g/m^2 dose of MTX in R3 and R4). Short-term infusion of MTX was associated with significantly diminished mucocutaneous toxicity in all therapy branches [48]. However, relapses were significantly more frequent in patients with high tumor load and advanced tumor stages (therapy branches R3 and R4) having received MTX over a short period of time. It was concluded from these findings that the duration of MTX exposure plays an important role in tumor control in pediatric B-cell NHL [48]. Polymorphisms in the *MTHFR* gene with reduced enzyme activity and prolonged depletion of intracellular folate could possibly influence therapy-related toxicity in different MTX infusion regimens. Therefore, we carefully looked for differences in the distribution of *MTHFR 677* genotype in both randomization arms. However, in patients with B-cell neoplasms who were homozygous for the *MTHFR 677* T allele, therapy-related toxicity did not differ from other genotypes in neither MTX infusion regimen, independent of the dose of MTX (1 vs 5 g/m^2); *MTHFR 677* genotypes were evenly distributed between randomization arms. The higher rate of MTX-related toxicity after a 24-h infusion compared to a 4-h infusion in this group of patients could therefore not be attributed to *MTHFR 677* genotype.

To our knowledge, only few other studies have been published examining the impact of *MTHFR 677* (C→T) polymorphism on therapy-related toxicity in pediatric ALL. In one study examining 61 patients, toxicity grades for hepatic and myeloid toxicity were more pronounced in patients homozygous for the *MTHFR 677* T allele after prolonged administration of MTX during maintenance therapy [4]. Two other studies examining the impact of *MTHFR* 677 polymorphism on the outcome on 520 and 201 pediatric patients with ALL did reveal an association of genotype *MTHFR* C677T with outcome, but not with therapy-related toxicity [1, 19]. Studies examining the association of MTX-related neurological toxicity after application of MTX in relation to *MTHFR 677* genotype did not provide any evidence for a significant role of the *MTHFR 677* genotype in MTX-associated neurotoxicity or intellectual performance after ALL therapy [16, 20]. Data on therapy-related toxicity of MTX-containing therapy regimen in other malignant diseases (i.e., breast cancer) showed increased toxicity in patients homozygous for the *MTHFR 677* T allele. However, numbers were small, and patient samples were heterogeneous [40].

In contrast to these studies on MTX-related toxicity and *MTHFR* genotype in patients with malignant disease, other studies on adult patients receiving MTX in low dosages for prophylaxis of graft-versus-host disease after bone marrow transplantation or for treatment of rheumatoid arthritis described significant differences in therapy-associated toxicity depending on the *MTHFR 677* genotype [7, 44, 46]. The reason for these discrepancies, despite much lower dosages of MTX in rheumatoid or other autoimmune disease compared to high-dose MTX therapy of pediatric NHL, is not clear. One reason might be the shorter period of time during which cells are folate-depleted during NHL therapy. Secondly, the use of Leucovorin in the treatment of pediatric NHL might be efficient enough to counteract MTX-related folate depletion in rapidly growing normal tissue cells, thus blunting a potential negative effect of reduced MTHFR activity. It has also been shown that folinic acid counteracts the reduction in enzyme activity of MTHFR in vitro by stabilizing the thermolabile form of the enzyme [9]. In vivo studies showed that folate levels greater than 15.4 nmol/l appeared to neutralize the effect of *MTHFR 677* genotype [14]. Although folate levels have not been measured in the present study, folinic acid rescue might have been sufficient to counteract reduced MTHFR activity of patients with *MTHFR 677* TT genotype. Additional intravenous folate substitution with parenteral nutrition used in pediatric patients suffering from severe orointestinal mucositis might sufficiently counteract potential effects of *MTHFR 677* genotype. Furthermore, recent experimental studies on rats have shown that administration of growth hormone reduces MTX-associated mortality and morbidity by influencing cell regeneration and reducing apoptosis [27]. These effects might play a role in differences regarding toxicity and *MTHFR 677* genotype between pediatric and adult patients receiving MTX.

In summary, this is the first study examining the *MTHFR 677* (C→T) polymorphism in a large population-based cohort of pediatric patients with NHL regarding its association with outcome, therapy-related toxicity, and MTX-related toxicity in different randomized MTX infusion regimens. In conclusion, *MTHFR 677* genotype appears to be associated with lymphoblastic lymphoma in pediatric patients. However, regarding outcome and therapy-related toxicity, the *MTHFR 677* (C→T) polymorphism does not appear to play a role in pediatric NHL or NHL subentities. Also, *MTHFR 677* genotype does not seem to influence MTX-related therapy toxicity independent of MTX infusion.

Appendix

Reference laboratories for histopathology and immunohistochemistry

R. Parwaresch, Lymph Node Registry founded by the Society of German Pathologists, Institute of Hematopathology, University of Kiel; A. Feller, Institute of Pathology, University of Lübeck; M.L. Hansmann, Institute of Pathology, University of Frankfurt; P. Möller, Institute of Pathology, University of Ulm; H.K. Müller-Hermelink, Institute of Pathology, University of Würzburg; H. Stein, Institute of Pathology, University of Berlin; I. Simonitsch, Institute of Pathology, University of Vienna, Austria

Immunophenotyping

W.-D. Ludwig, Berlin, Germany; W. Knapp, Vienna, Austria; F. Niggli, Zürich, Switzerland

Cytomorphology

A. Reiter, Giessen, Germany; W. Haas, Vienna, Austria; F. Niggli, Zürich, Switzerland

Principal investigators

R. Mertens (Aachen), R. Angst (Aarau), A. Gnekow (Augsburg), R. Dickerhoff (St. Augustin), P. Imbach (Basel), G.F. Wuendisch (Bayreuth), W. Dörffel (Berlin-Buch), G. Henze (Berlin-Charité), U. Bode (Bonn), W. Eberl (Braunschweig), H.-J. Spaar (Bremen), I. Krause (Chemnitz), J.-D. Thaben (Coburg), D. Moebius (Cottbus), W. Wiesel (Datteln), B. Ausserer (Dornbirn), H. Breu (Dortmund), G. Weiβbach (Dresden), W. Kotte (Dresden), U. Göbel (Düsseldorf), W. Weinmann (Erfurt), J.D. Beck (Erlangen), W. Havers (Essen), G. Müller (Feldkirch), B. Kornhuber (Frankfurt), C. Niemeyer (Freiburg), F. Lampert (Gieβen), M. Lakomek (Göttingen), C. Urban (Graz), H. Reddemann (Greifswald), S. Burdach (Halle), G. Janka-Schaub (Hamburg), K. Welte (Hannover), B. Selle/A. Kulozik (Heidelberg), C. Tautz (Herdecke), N. Graf (Homburg), F.M. Fink (Innsbruck), F. Zintl (Jena), G. Neβler (Karlsruhe), H. Wehinger (Kassel), R. Schneppenheim (Kiel), H. Messner (Klagenfurt), M. Rister, (Koblenz), F. Berthold (Köln), W. Sternschulte (Köln), C. Schulte-Wissermann (Krefeld), M. Domula (Leipzig), I. Mutz (Leoben), K. Schmitt (Linz), O. Stoellinger (Linz), L. Nobile (Locarno), P. Bucsky (Lübeck), H. Ruetschele (Ludwigshafen), U. Caflisch (Luzern), U. Mittler (Magdeburg), P. Gutjahr (Mainz), O. Sauer (Mannheim), C. Eschenbach (Marburg), W. Tillmann (Minden), K.-D. Tympner (München-Harlaching), R.J. Haas (München), C. Bender-Götze (München), S. Müller-Weihrich (München), H. Jürgens (Münster), O. Schofer (Neunkirchen), A. Jobke (Nürnberg), G. Eggers (Rostock), R. Geib-König (Saarbrücken), H. Grienberger (Salzburg), H. Haas (Schwarzach), R. Schumacher (Schwerin), F.-J. Göbel (Siegen), R. Ploier (Steyr), A. Feldges (St. Gallen), J. Treuner (Stuttgart), H. Rau (Trier), D. Niethammer (Tübingen), K.-M. Debatin (Ulm), D. Franke (Vechta), H. Gadner (Wien), F. Haschke (Wien), J. Weber (Wiesbaden), D. Dohrn (Wuppertal), J. Kühl (Würzburg), F. Niggli (Zürich).

Acknowledgements We thank E. Odenwald for expert work in cytological diagnosis and U. Meyer for excellent data management. We especially thank all doctors and nurses in participating hospitals for their continuous care for sick children and their excellent cooperation with study center NHL-BFM. Supported by the Deutsche Krebshilfe, Bonn (grant no. M 109/91/Re1) and by the Verein zur Förderung der Behandlung krebskranker Kinder Hannover e.V.

References

1. Aplenc R, Thompson J, Han P, La M, Zhao H, Lange B, Rebbeck T (2005) Methylenetetrahydrofolate reductase polymorphisms and therapy response in pediatric acute lymphoblastic leukemia. Cancer Res 15:2482–2487
2. Bennett JM, Catorsky D, Daniel MT et al (1976) Proposal for the classification of the acute leukemias. French–American–British (FAB) Cooperative Groups. Br J Heamatol 33:451–458
3. Botto LD, Yang Q (2000) 5,10-methylentetrahydrofolate reductase gene variations and congenital anomalies: a HuGE review. Am J Epidemiol 151:862–877
4. Chiusolo P, Reddiconto G, Casorelli I, Laurenti L, Sora F, Mele L, Leone G, Sica S (2002) Preponderance of methylenetetrahydrofolate reductase C677 homozygosity among leukemia patients intolerant to methotrexate. Ann Oncol 13:1915–1918
5. Chiusolo P, Reddiconto G, Cimino G, Sica S, Fiorini A, Farina G, Vitale A, Sorà F, Laurenti L, Bartolozzi F, Fazi P, Mandelli F, Leone G (2004) Methylenetetrahydrofolate reductase genotypes do not play a role in acute lymphoblastic leukemia pathogenesis in the Italian population. Haematologica 89:139–144
6. Cox DR (1972) Regression models and life tables. J R Stat Soc 34:187–220
7. Evans WE (2002) Differing effects of methylenetetrahydrofolate reductase single nucleotide polymorphisms on methotrexate efficacy and toxicity in rheumatoid arthritis. Pharmacogenetics 12:181–182
8. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Metthews RG, Boers GHJ, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R (1995) A candidate genetic risk factor for vascular disease: a common mutation in methyleneterahydrofolate reductase. Nature Genet 10:111–114
9. Frosst P, Zhang Z-X, Pai A, Rozen R (1996) The methylenetetrahydrofolate reductase (MTHFR) gene maps to distal mouse chromosome 4. Mamm Genome 7:864–869
10. Gemmati D, Ongaro A, Scapoli GL, Della Porta M, Reverberi R, Caruso A, Pasello M, Pellati A, De Mattei M (2004) Common gene polymorphisms in the metabolic folate and methylation pathway and the risk of acute lymphoblastic leukemia and non-Hodgkin's lymphoma in adults. Cancer Epidemiol Biomarkers Prev 13:787–794
11. Goyette P, Rozen R (2000) The thermolabile variant 677C-T can further reduce activity when expressed in CIS with severe mutations for human methylenetetrahydrofolate reductase. Hum Mutat 16:132–138
12. Greenwood M (1926) A report on the natural duration of cancer. In: Reports on public health and medical subjects. Ministry of Health, Her Majesty's Statistical Office, London, pp 1–26
13. Harris NL (1997) Principles of the revised European–American Lymphoma Classification (from the International Lymphoma Study Group). Annals Oncol 8:11–16
14. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R (1996) Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. Circulation 93: 7–9
15. Kaplan EL, Meier P (1958) Non-parametric estimation from incomplete observations. J Am Stat Assoc 53:457–481
16. Kishi K, Griener J, Cheng C, Das S, DCook E, Pei D, Hudson M, Rubnitz J, Sandlund JT, Pui C-H, Relling MV (2003) Homocysteine, pharmacogenetics, and neurotoxicity in children with leukemia. J Clin Oncol 21:3084–3091
17. Koch MC, Stegmann K, Ziegler A, Schroter B, Ermert A (1998) Evaluation of thr MTHFR C677T allele and the MTHFR gene in a German spina bifida population. Eur J Pediatr 157:487–492
18. Krajinovic M, Lamothe S, Labuda D, Lemieux-Blanchard E, Théorêt Y, Moghrabi A, Sinnett D (2004) Role of MTHFR genetic polymorphisms in the susceptibility to childhood acute lymphoblastic leukemia. Blood 103:252–257

19. 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
20. Krajinovic M, Robaey P, Chiasson S, Lemieux-Blanchard E, Rouillard M, Primeau M, Bournissen FG, Moghrabi A (2005) Polymorphisms of genes controlling homocysteine levels and IQ score following the treatment for childhood ALL. Pharmacogenomics 6:293–302
21. Mantel N (1966) Evaluation of survival data and two new rank order statistics arising in its consideration. Cancer Chemother Rep 50:163–170
22. Martinelli M, Scapoli L, Pezzetti F, Carinci F, Stabellini G, Bisceglia L, Gombos F, Tognon M (2001) C677T variant form at the MTHFR gene and CL/P: a risk factor for mothers? Am J Hum Genet 98:357–360
23. Matsuo K, Suzuki R, Hamajima M, Ogura M, Kagami Y, Taji H, Kondoh E, Maeda S, Asakura S, Kaba S, Nakamura S, Seto M, Morishima Y, Tajima K (2001) Association between polymorphisms of folate- and methionine-metabolizing enzymes and susceptibility to malignant disease. Blood 97:3205–3209
24. Mogk RL, Rothenmund H, Evans JA, Carson N, Dawson AJ (2000) The frequency of the C677T substitution in the methylenetetrahydrofolate reductase gene in Manitoba. Clin Genet 58:406–408
25. Mornet E, Muller F, Lanvoise-Furet A, Delezoide A-L, Col J-Y, Soimon-Bouy B, Serre J-L (1997) Screening the C677T mutation on the methylenetetrahydrofolate reductase gene in French patients with neural tube defects. Hum Genet 100: 512–514
26. Ogino S, Wilson RB (2003) Genotype and haplotype distributions of MTHFR 677 C>T and 1298 A>C single nucleotide polymorphisms: a meta-analysis. J Hum Genet 48:1–7
27. Ortega M, Gomez-de-Segura IA, Vazquez I, Lopez JM, de Guevara CL, De-Miguel E (2001) Effects of growth hormone plus a hyperproteic diet on methotrexate-induced injury in rat intestines. Acta Oncol 40:615–621
28. Papapetrou C, Lynch SA, Burn J, Edwards YH (1996) Methylenetetrahydrofolate reductase and neural tube defects. Lancet 348:58–58
29. Patte C, Leverger G, Rubie H, Bertrand Y, Coze C, Méchinaud F, Lutz P, Michon J, Baruchel A, Courbon B (1993) High cure rate in B-cell (Burkitt's) leukemia in the LMB 89 protocol of the SFOP (French Pediatric Oncology Society) (abstract). Proc ASCO 12:317
30. Reinhardt D, Sigusch HH, Vogt SF, Farker K, Muller S, Hoffmann G (1998) Absence of association between a common mutation in the methylenetetrahydrofolate reductase gene and the risk of coronary artery disease. Eur J Clin Invest 28:20–23
31. Reiter A, Schrappe M, Parwaresch R, Henze G, Müller-Weihrich S, Sauter S, Sykora K-W, Ludwig W-D, Gadner H, Riehm H (1995) Non-Hodgkin's lymphomas of childhood and adolescence: results of a treatment stratified for biologic subtypes and stage—a report of the Berlin–Frankfurt–Münster Group. J Clin Oncol 13:359–372
32. Reiter A, Schrappe M, Ludwig W-D, Tiemann M, Parwaresch R, Zimmermann M, Schirg E, Henze G, Schellong G, Gadner H, Riehm H (1999) Intensive ALL-type therapy without local radiotherapy provides a 90% event-free survival for children with T-cell lymphoblastic lymphoma: a BFM Group report. Blood 95:416–421
33. Reiter A, Schrappe M, Tiemann M, Ludwig W-D, Yakisan E, Zimmermann M, Mann G, Chott A, Ebell W, Klingebiel T, Graf N, Kremens B, Müller-Weihrich S, Plüss H-J, Zintl F, Henze G, Riehm H (1999) Improved treatment results in childhood B-cell neoplasms with tailored intensification of therapy: a report of the Berlin–Frankfurt–Münster Group Trial NHL-BFM 90. Blood 94:3294–3306
34. Seidemann K, Tiemann M, Schrappe M, Yakisan E, Simonitsch I, Janka Schaub G, Dörffel W, Zimmermann M, Mann G, Gadner H, Riehm H, Reiter A (2001) Short-pulse B-non-Hodgkin lymphoma type chemotherapy is efficacious treatment for pediatric anaplastic large cell lymphoma: a report of the Berlin–Frankfurt–Munster Group Trial NHL-BFM 90. Blood 97:3699–3706
35. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, Morgan G (1999) Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. Proc Natl Acad Sci USA 96:12810–12815
36. Skibola CF, Forrest MS, Coppedé F, Agana L, Hubbard A, Smith MT, Bracci PM, Holly EA (2004) Polymorphisms and haplotypes in folate metabolizing genes and risk of non-Hodgkin lymphoma. Blood 104:2155–2162
37. Sohda S, Arinami T, Hamada H, Yamada N, Hamaguchi H, Kubo T (1997) Methylenetetrahydrofolate reductase polymorphism and pre-eclampsia. J Med Genet 34:525–526
38. Stansfeld AG, Diebold J, Kapanci Y, Noel H, Rilke F, Kelenyi G, Sundstrom C, Lennert J, van Unnik JA, Mioduszewska O (1988) Updated Kiel classification for lymphomas. Lancet 1:292–293
39. Stevenson M, Schwartz CE, Du Y-Z, Adams MJ (1997) Differences in methylenetetrahydrofolate reductase genotype frequencies between whites and blacks. Am J Hum Genet 60:229–230
40. Toffoli G, Veronesi A, Boiocchi M, Crivellari D (2000) MTHFR gene polymorphism and severe toxicity during adjuvant treatment of early breast cancer with cyclophosphamide, methotrexate, and fluouracil (CMF). Ann Oncol 11:373–374
41. Toffoli G, Rossi D, Gaidano G, Cecchin E, Boiocchi M, Carbone A (2003) Methylenetetrahydrofolate reductase genotype in diffuse large cell lymphomas with and without hypermethylation of the DNA repair gene O6-methylguanine DNA methyltransferase. Int J Biol Markers 18:218–221
42. Ulrich CM, Storb R, Schubert MM, Potter JD (2001) MTHFR polymorphism, DNA repair capacity, and methotrexate toxicity. Blood 98:2283–2283
43. Ulrich CM, Robien K, Sparks R (2002) Pharmacogenetics and folate metabolism—a promising direction. Pharmacogenomics 3:299–313
44. Urano W, Taniguchi A, Yamanaka H, Tanaka E, Nakajima H, Matsuda Y, Akama H, Kitamura Y, Kamatani N (2002) Polymorphisms in the methylenetetrahydrofolate reductase gene were associated both with the efficacy and the toxicity of methotrexate used for the treatment of rheumatoid arthritis, as evidenced by single locus and haplotype analysis. Pharmacogenetics 12:183–190
45. van der Put NM, Steegers-Theunissen RPM, Frosst P, Trijbels FJM, Eskes TKA, van den Heuvel LP, Mariman ECM, den Heyer M, Rozen R, Blom HJ (1995) Mutated methylenetetrahydrofolate reductase as a risk factor for spina bifida. Lancet 346:1070–1071
46. van Ede AE, Laan RF, Blom HJ, Huizinga TW, Haagsma CJ, Giesendorf BA, de Boo TM, van der Putte LB (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
47. Wiemels JL, Smith RN, Taylor GM, Eden OB, Alexander FE, Greaves MF (2001) Methylenetetrahydrofolate reductase (MTHFR) polymorphisms and risk factor of molecularly defined subtypes of childhood acute leukemia. Proc Natl Acad Sci USA 98:4004–4009
48. Wössmann W, Seidemann K, Mann G, Zimmermann M, Burkhardt B, Oschlies I, Ludwig WD, Klingebiel T, Graf N, Gruhn B, Jürgens H, Niggli F, Parwaresch R, Gadner H, Riehm H, Schrappe M, Reiter A (2005) The impact of the methotrexate administration schedule and dose in the treatment of children and adolescence with B-cell neoplasms: A report of the BFM Study Group NHL-BFM 95. Blood 105:948–958