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Polymorphisms within glutathione S-transferase genes in pediatric non-Hodgkin's lymphoma

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Background and Objectives. Glutathione S-transferases (GSTs) are involved in the metabolism of a number of cancer chemotherapeutic agents. Certain members within the GST superfamily exhibit phenotypically relevant genetic polymorphisms which have been associated with outcome in hematologic malignant disease.

Design and Methods. In the present study we genotyped a cohort of 169 pediatric non-Hodgkin's lymphoma (NHL) patients with available specimens from the NHL-BFM trials 86 and 90 conducted by the Berlin-Frankfurt-Münster (BFM) study group to assess a potential association of phenotypically relevant glutathione S-transferase polymorphisms (*GSTM1*, *GSTT1*, *GSTP1* codon 105) with treatment outcome in this patient group.

Results. Treatment failure in patients with mature B-cell NHL was significantly less likely to occur in patients carrying at least one *GSTM1* allele in comparison to those with a homozygous deletion of *GSTM1*. This protective effect mediated by the presence of *GSTM1* was even more pronounced within the subset of therapy group B patients at highest clinical risk of treatment failure (B-ALL, disease stage IV, disease stage III with unresected abdominal tumor, and LDH activity ≥ 500 U/L). Of all events in therapy group B, 87.5% occurred in this high risk group. Within this subset, the multivariate relative risk reached 4.98 (95% CI = 1.27–19.52; p= 0.021).

Interpretation and Conclusions. Our results suggest that genetic variation at the *GSTM1* locus may be of clinical importance in pediatric NHL and may be a potential candidate for indicating future treatment stratification strategies.

Key words: non-Hodgkin's lymphoma, prognostic factor, glutathione S-transferase, polymorphism.

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Glutathione S-transferases (GSTs) are involved in the metabolism of a number of cytotoxic cancer chemotherapeutic agents.[1,2] Certain members of the GST enzyme superfamily exhibit phenotypically relevant genetic polymorphisms. The genes coding for the isozymes GSTM1 and GSTT1, for example, are homozygously deleted in 40 to 60% and 15 to 30% of the Caucasian population, respectively, resulting in so-called double null genotypes that correlate with non-conjugator phenotypes of GSTM1 and GSTT1.[3,4] For the GSTπ class isozyme GSTP1, single nucleotide polymorphisms within its coding region leading to amino acid exchanges have been described (Ile105Val; Ala114Val).[5-7] The coding region polymorphisms in *GSTP1* have been suggested to confer different catalytic activities.[8,9] Until now, only a few studies on the association of GST genotypes and treatment outcome in pediatric lymphoid malignancies have been reported.[10-12] While Chen *et al.* did not observe any significant associations between GST genotypes (*GSTM1* and *GSTT1*) and outcome of childhood acute lymphoblastic leukemia (ALL) treated in three consecutive trials at St. Jude Children's Research Hospital,[10] results from two case-control studies conducted by our group recently demonstrated that polymorphisms within GST genes may be associated with the initial response to treatment and risk of relapse in childhood ALL treated according to Berlin-Frankfurt-Münster (BFM) study group protocols.[11,12] However, to date, no study on the association of GST genetic polymorphisms with the clinical course of NHL of childhood and adolesence has been reported. Thus, we conducted a study on 169 pediatric patients with NHL to evaluate a potential relationship between GST genotype and treatment failure (non-response/recurrence) in this disease group.

Design and Methods

Patients

The present study utilizes data and patients' specimens derived from the Berlin–Frankfurt–Münster study group trials NHL-BFM 86 and 90 on the treatment of pediatric NHL, which are described in detail elsewhere.[13-15] Patients in therapy group non-B (lymphoblastic lymphoma, peripheral T-cell lymphoma, NHL not classified T-cell, NHL with no available classification and immunophenotype at mediastinal localization) were treated according to an acute lymphoblastic leukemia (ALL)-type treatment strategy while patients in therapy group B (mature B-cell NHL, including B-ALL) and those with anaplastic large cell lymphoma received a short pulse-type therapy. The ALL-type therapy included prednisone, dexamethasone, vincristine, daunorubicin, doxorubicin, L-asparaginase, cyclophosphamide, cytarabine, 6-mercaptopurine, 6-thioguanine, and methotrexate. In the pulsatile treatment regimen dexamethasone, vincristine, vindesine, doxorubicin, ifosfamide, cyclophosphamide, etoposide, cytarabine, and methotrexate were applied.[13-15] Only evaluable study patients were included in the present analysis. Of the entire evaluable study population (n = 950 patients) enrolled in the NHL-BFM 86 and 90 trials, 169 subjects (17.8%) with available spare bone marrow smears for DNA extraction and genotype analysis were identified. NHLs were classified according to the updated Kiel classification. The corresponding entities of the revised European–American classification of lymphoid neoplasms (REAL classification) are included in Table 1. Tumor response was evaluated after each course of therapy. Follow-up studies were performed at 4- to 6-week intervals during the first 1.5 years. In patients with bone marrow (BM) or/and central nervous system (CNS) involvement, control punctures of bone marrow or/and cerebrospinal fluid (CSF) were performed only until the bone marrow or the CNS, respectively, was cleared from blasts. Treatment failure was defined as a recurrence of lymphoma proven by biopsy or regrowth of an incompletely resolved tumor. The diagnosis of isolated progression in the BM was based on 25% or more blasts in the BM. The diagnosis of isolated progression in the CNS was based on the appearance of blasts in the CSF.

Genotype analysis

Genotype analyses for *GSTM1*, *GSTT1*, and *GSTP1* codon 105 polymorphisms were essentially performed as described previously.[16,17]

Table 1. Characteristics of 169 pediatric non-Hodgkin´s lymphoma (NHL) patients analyzed for polymorphisms within the glutathione S-transferase genes *GSTM1*, *GSTT1* and *GSTP1* (codon 105) compared to the entire evaluable patient population from therapy trials NHL-BFM 86 and NHL-BFM 90.*

	No. of subjects and prevalence (%)	
	Study sample (n = 169)	*NHL-BFM 86/NHL-BFM 90 (n = 950)*
Trial		
NHL-BFM 86	44 (26.0)	302 (31.8)
NHL-BFM 90	125 (74.0)	648 (68.2)
Sex		
male	121 (71.6)	711 (74.8)
female	48 (28.4)	239 (25.2)
Age at diagnosis (years)		
< 3	9 (5.3)	48 (5.1)
3 - 9	90 (53.3)	498 (52.4)
10 - 14	55 (32.5)	325 (34.2)
15 - 18	15 (8.9)	79 (8.3)
Lymphoma classification		
Lymphoblastic		
T-cell	27 (16.0)	155 (16.3)
Precursor B-cell	4 (2.4)	29 (3.1)
Immunophenotype n.a.†	2 (1.2)	17 (1.8)
Burkitt´s lymphoma	73 (43.2)	376 (39.6)
B-ALL	18 (10.7)	97 (10.2)
Diffuse large B-cell lymphoma		
Centroblastic	8 (4.7)	56 (5.9)
Immunoblastic	4 (2.4)	11 (1.2)
Large mediastinal B-cell lymphoma	2 (1.2)	17 (1.8)
PTCL#	4 (2.4)	17 (1.8)
ALCL§	15 (8.9)	105 (11.1)
NHL not classified		
T-cell	–	13 (1.4)
B-cell	7 (4.1)	39 (4.1)
Immunophenotype n.a.†	5 (3.0)	14 (1.5)
Other entities	–	4 (0.4)
Stage		
I	19 (11.2)	98 (10.3)
II	34 (20.1)	194 (20.4)
II	82 (48.5)	459 (48.3)
IV/ B-ALL	34 (20.1)	199 (20.9)
LDH (U/L)		
< 500	78 (46.2)	467 (49.2)
500 - 1000	26 (15.4)	132 (13.9)
1000	34 (20.1)	120 (12.6)
not examined	31 (18.3)	231 (24.3)

**Patients with pre-existing neoplastic diseases, pre-existing immunologic or hematologic disorders, genetic syndromes, and relevant deviations from therapy protocol were excluded. †Not available; #Peripheral T-cell lymphoma; §Anaplastic large-cell lymphoma.*

Statistical analysis

Statistical analyses included calculation of frequencies of characteristics and common factors known to be associated with treatment outcome followed by investigation of interrelationships between GST genotypes and their associations with clinical risk factors (e.g. sex, age at diagnosis, lactate dehydrogenase (LDH) activity) using Wilcoxon or χ^2 tests. Differences in the distribution of categorical variables between the study sample and the

Table 2. Distribution of *GSTM1*, *GSTT1*, and *GSTP1* genotypes and their association with the occurrence of treatment failure in the whole study sample and separately in all patients of therapy group B (mature B-NHL and B-ALL) as well as patients of therapy group B with high clinical risk of treatment failure*.

	No. of subjects and prevalence (%)		*Univariate risk analysis*			*Multivariate risk analysis§*		
	Treatment failure	*No treatment failure*	*RR*	*95% CI*	*p*	*RR*	*95% CI*	*p*
Whole study sample								
GSTM1								
Present	15 (46.9)	69 (50.4)	1.00†			1.00†		
Null	17 (53.1)	68 (49.6)	1.18	0.59-2.37	0.637	1.71	0.72-4.04	0.223
GSTT1								
Present	29 (90.6)	113 (82.5)	1.00†			1.00†		
Null	3 (9.4)	24 (17.5)	0.51	0.15-1.67	0.262	0.39	0.08-1.81	0.229
GSTP1 codon 105								
Ile/Ile or Ile/Val	27 (84.4)	113 (82.5)	1.00‡			1.00‡		
Val/Val	5 (15.6)	24 (17.5)	0.81	0.31-2.11	0.668	0.50	0.14-1.97	0.350
Therapy group B								
GSTM1								
Present	5 (31.2)	46 (46.0)	1.00†			1.00†		
Null	11 (68.8)	54 (54.0)	1.82	0.63-5.23	0.268	3.84	1.17-12.56	0.026
GSTT1								
Present	15 (93.8)	83 (83.0)	1.00†			1.00†		
Null	1 (6.2)	17 (17.0)	0.33	0.04-2.50	0.284	0.25	0.03-2.18	0.211
GSTP1 codon 105								
Ile/Ile or Ile/Val	16 (100)	85 (85.0)		–			–	
Val/Val	–	15 (15.0)		–			–	
Therapy group B: patients with high clinical risk of treatment failure								
GSTM1								
Present	3 (21.4)	18 (54.5)	1.00†			1.00†		
Null	11 (78.6)	15 (45.5)	3.96	1.10-14.21	0.035	4.98	1.27-19.52	0.021
GSTT1								
Present	13 (92.9)	26 (78.8)	1.00†			1.00†		
Null	1 (7.1)	7 (21.2)	0.30	0.04-2.32	0.250	0.33	0.04-.2.84	0.799

**Treatment failure:non-response to chemotherapy or lymphoma recurrence; definition of high clinical risk of treatment failure: all cases of B-ALL, all patients at disease stage IV, all patients at disease stage III with unresected abdominal tumor and LDH activity ≥ 500 U/L. †Reference category. ‡Reference category was a combined category of GSTP1 Ile^{105}/Ile^{105} and Ile^{105}/Val^{105} genotypes. §Co-variates for the whole study sample: NHL-BFM trial, therapy group, sex, age at diagnosis, disease stage, LDH activity; Co-variates for therapy group B: NHL-BFM trial, sex, age at diagnosis, disease stage, LDH activity RR:relative risk; 95% CI:95% confidence interval.*

entire patient population were analyzed by χ^2 or Fisher's exact test. The association between GST genotypes and treatment failure (non-response to treatment or lymphoma recurrence) was examined by use of Cox regression analysis to calculate relative risks (RR) and their 95% confidence intervals (CI). Co-variates in multivariate analyses were variables known to be associated with disease outcome.

Results

Table 1 displays the distribution of the patients' demographic and clinical characteristics in the present study sample in comparison to the entire patient population of the NHL-BFM 86 and 90 trials. Compared to the entire patient population, there was a slightly higher percentage of patients with LDH levels equal to or above 500 U/L in the present study sample. With regard to other variables displayed in Table 1, the observed distributions were similar between the two groups. Thus, our present study sample reflects a representative subgroup of the entire patient population of the NHL-BFM 86 and 90 trials.

Table 2 shows the prevalences of GST genotypes and their associations with treatment failure. None of the GST genotypes showed significant increases in risk or protective effects when analyzed within the complete study sample. However, when the analysis was restricted to patients within therapy group B (mature B-cell NHL including B-ALL), treatment failure was less likely to occur in patients carrying at least one *GSTM1* allele in comparison to in patients with a homozygous deletion of *GSTM1* (multivariate RR: 3.84; 95% CI = 1.17 – 12.56; $p = 0.026$; see Table 2). This protective effect mediated by the presence of *GSTM1* was even more

pronounced within the subset of therapy group B patients at highest clinical risk of treatment failure (B-ALL, disease stage IV, disease stage III with unresected abdominal tumor, and LDH activity > 500 U/L). It was in this high-risk group that 87.5% of all the events in therapy group B occurred. Within this subset, the multivariate RR reached 4.98 (95% CI = 1.27–19.52; $p = 0.021$). In therapy group non-B (29 patients of whom 9 exhibiting treatment failure), no associations between the *GSTM1* genotype and treatment failure could be observed (*data not shown*). With regard to the *GSTT1* and the *GSTP1* genotypes, statistically significant effects on treatment failure were not observed in the overall study sample or in one of the therapy groups (Table 2; *data not shown for non-B*). However, results for *GSTT1* and *GSTP1* genotypes should be viewed carefully as the number of treatment failures was small and prevalences of the potential low-risk genotypes (*GSTT1* double null, *GSTP1* Val^{105}/Val^{105}) were low. Thus, potential effects may have been missed due to a lack of study power. The same issue also has to be considered with regard to therapy group non-B.

Discussion

In previous studies by others and our group on GST genotypes and treatment outcome in childhood ALL, no significant associations were observed for the *GSTM1* genotype.[10-12] However, Chen *et al.* observed a tendency to higher central nervous system (CNS) relapse-free survival at five years among patients with the *GSTM1* double null genotype ($p = 0.054$) in a study of childhood ALL patients treated in three consecutive trials at St. Jude Children's Research Hospital.[10] Furthermore, in a case-control study of relapsed vs. non-relapsed successfully treated patients with B-cell precursor ALL, we found a suggestive protective effect of the *GSTM1* double null genotype (odds ratio = 0.5; $p = 0.078$).[11] Of interest, differential effects on disease-free survival at different treatment intensities for *GSTM1* were recently observed in a study on GST genotypes in childhood acute myeloid leukemia (AML).[18] In this study, patients with the *GSTM1* double null genotype did significantly better than those displaying at least one GSTM1 allele (59 vs. 33%; $p < 0.05$) when on a standard timed regimen, while on an intensified regimen no differences in disease-free survival could be observed (59 vs 62%).

Therapeutic approaches of the BFM group to pediatric ALL and B-cell NHL differ considerably, with ALL treated on a more continuous and B-cell NHL on a more dose-intensive pulsatile schedule.[13–15,19] Thus, the observed differential results for *GSTM1* could suggest that different disease entities and/or different treatment schedules may be differentially modulated by GSTs. Although the numbers of patients were too small to draw conclusive results, such a hypothesis would be supported by the finding that in our present study, the *GSTM1* genotype was not associated with treatment failure in therapy group non-B which was treated similarly to ALL. Further support for such a hypothesis comes from the above-mentioned study in childhood AML.[18]

To our knowledge, the only other study suggesting a significant beneficial effect of the presence of *GSTM1* on treatment outcome is a study in epithelial ovarian cancer by Howells and colleagues.[20] These authors hypothesized that less effective detoxification of oxidative stress may result in an increase of DNA damage to key genes (e.g. *p53*). For the interpretation of our results, a mechanism through which differences in the modulation of intensive oxidative stress during chemotherapy lead to varying degrees of genotoxicity associated with differences in the potential for clonal evolution sounds intriguing, as well. This is particularly so because *GSTM1* has been suggested to play a cytoprotective role in lymphoid cells.[21] Larger studies including more patients are needed to confirm the results presented in this study and to lead to a better understanding of the complex association patterns of host genetic variation with different clinical endpoints in hematologic neoplasias.

Contributions and Acknowledgments

While all authors contributed to requirements a to c of the WAME criteria of medical research authorship, more specifically AR, MSch, KW and MSt had the major input designing the study and were involved in the interpretation of the results. BOD and RB were mostly involved in acquisition of available bone marrow smears, DNA isolation, and genotyping. MZ, BOD, MSt, and AR were involved in the statistical analysis. MSt and BOD wrote the first draft of the paper, which was critically revised by all other authors to derive the final version of the paper. All authors approved the final version of the paper. We thank all the participants of the NHL-BFM 86 and 90 studies for their co-operation.

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Disclosures

Conflict of interest: none.

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Peer Review Outcomes

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Dr. Franco Locatelli, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Locatelli and the Editors. Manuscript received July 20, 2001; accepted April 11, 2002.

What is already known on this topic

A few data on the association on GST genotypes and treatment outcome in pediatric lymphoid malignancies are available. However, no study has specifically focused on non-Hodgkin's lymphoma.

What this study adds

This is the first study investigating the role played by GST genotypes on outcome of children with non-Hodgkin's lymphoma.

Potential implications for clinical practice

This study could be useful for identifying risk groups of patients and designing of new protocols for childhood B-cell non-Hodgkin's lymphoma.

Franco Locatelli, Associate Editor