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Thymidylate synthase gene polymorphism and its association with relapse in childhood B-cell precursor acute lymphoblastic leukemia

We investigated a tandem-repeat polymorphism within the promoter region of the thymidylate synthase gene in 40 matched pairs of relapsed and non-relapsed childhood B-cell precursor acute lymphoblastic leukemia patients. This polymorphism has previously been suggested to influence treatment outcome in childhood acute lymphoblastic leukemia. In our study, no association between thymidylate synthase genotype and risk of relapse was found.

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Expression of thymidylate synthase (TS) has been suggested to influence the effect of cancer chemotherapeutic agents such as methotrexate and 5-fluouracil. TS catalyzes the intracellular conversion of deoxyuridylate monophosphate to deoxythymidylate monophosphate, which makes it an essential enzyme in proliferating cells.[1,2] A genetic tandem-repeat polymorphism within the promotor region downstream of the cap site of the TS gene has been described.[3] Recently, the TS genotype of this tandem-repeat polymorphism was suggested to influence treatment outcome in a cohort of 205 French-Canadian children with acute lymphoblastic leukemia (ALL), including 32 who relapsed or died due to the disease.[4] In that study, patients homozygous for the variant TS allele (3R/3R) had a 4.6-fold higher risk of an event than did those expressing the 2R TS allele (2R/2R and 2R/3R) in multivariate analysis. In the same cohort, heterozygous patients (2R/3R) did not show an increased risk of an event compared to the risk in those homozygous for the 2R TS allele.

The present study was aimed at investigating the association of the above mentioned TS tandem-repeat polymorphisms and risk of relapse in a homogeneously treated group of patients with standard and intermediate risk childhood B-cell precursor ALL. The study was performed as a case-control study on 40 relapsed ALL patients with pre-B cell or common ALL immunophenotype, derived from the Berlin-Frankfurt-Münster trials ALL-BFM 86 and ALL-BFM 90. Cases were individually matched to successfully treated ALL patients. The minimum follow-up for the control subjects was 5 years. Matching criteria were gender, age at diagnosis, immunophenotype, initial white blood cell count and risk group. Our study could not consider genetic aberrations with prognostic impact on treatment outcome as potential confounding variables, because these data are not available for the majority of patients in trials ALL-BFM 86 and ALL-BFM 90. In particular, TEL/AML1 rearrangement was not investigated in these trials. All patients included were standard (SR) or intermediate risk (MR) patients. Treatment for SR and MR patients was similar in trials ALL-BFM 86 and ALL-BFM 90.[5,6] The cumulative dosage of methotrexate (MTX) applied was the same for all the patients in the current case-control study.[7] Polymerase chain reaction was performed using sense and antisense primers according to Horie *et al.*[3] Conditional logistic regression analysis to calculate odds ratios and their 95% confidence intervals (CI) was performed to assess the association of genotype with risk of ALL relapse. Genotypes were used as categorical variables in the analyses. The distribution of the patients' matching criteria are shown in Table 1.

The frequency of the 3R/3R TS genotype within the control sample of our study was similar to that observed in the control sample investigated by Krajinovic *et al.* The risk of relapse in the investigated subset of matched patients was not associated with the variant 3R/3R TS genotype, neither with reference

Table 1. Distribution of matching criteria in 80 children with acute lymphoblastic leukemia with (cases) and without (controls) relapse selected from trials ALL-BFM 86 and ALL-BFM 90.

	Cases (n=40) n (%)	*Controls (n=40) n (%)*
Sex		
male	26 (65.0)	26 (65.0)
female	14 (35.0)	14 (35.0)
Age at diagnosis		
<1 year	–	–
1 - <10 years	36 (97.5)	36 (97.5)
≥ 10 years	4 (10.0)	4 (10.0)
Initial WBC		
< 50,000/μL	37 (92.5)	37 (92.5)
≥ 50,000/μL	1 (2.5)	1 (2.5)
Immunophenotype		
common ALL	37 (92.5)	36 (90.0)
pre-B ALL	3 (7.5)	4 (10.0)
Risk group		
standard	13 (32.5)	13 (32.5)
intermediate	27 (67.5)	27 (67.5)

Table 2. Thymidylate synthase genotype in 40 matched pairs of children with standard and intermediate risk B-cell precursor ALL with and without relapse.

	Cases (n=40) n (%)	*Controls (n=40) n (%)*	*Odds ratio (95% CI)*	*p*
Genotype				
2R/2R	20 (50.0)	16 (40.0)	1*	
2R/3R	11 (27.5)	16 (40.0)	0.4 (0.13-1.94)	0.208
3R/3R	9 (22.5)	8 (20.0)	1.0 (–)	
2R/2R and 2R/3R	31 (77.5)	32 (80.0)	1*	
3R/3R	9 (22.5)	8 (20.0)	1.1 (0.70-2.98)	0.795

**Reference category.*

to the common 2R/2R genotype nor with reference to a combined category of 2R/2R and 2R/3R genotypes (Table 2). These results differ from those obtained by Krajinovic *et al.* However, the settings of both studies were different. Whereas our study evaluated a small, closely matched and relatively homogeneous sample of patients with B-cell precursor childhood ALL, Krajinovic performed a study in a heterogeneous cohort of 205 patients with various types of childhood ALL. Furthermore, the application of high-dose MTX might have been important. Our patients received 4 times 5 g/m^2 MTX in the extracompartment treatment protocol M, whereas the patients analyzed by Krajinovic *et al.* received a single dose of 4g/m^2 MTX during the induction phase. Therefore, one might speculate that the prognostic impact of the genetic tandem-repeat polymorphism within the promotor region of the TS gene might be overcome by higher doses of MTX. Nevertheless, larger studies are necessary to elucidate the significance of TS genotype for outcome in childhood ALL.

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Simultaneous expression of CD38 and its ligand CD31 by chronic lymphocytic leukemia B-cells: anecdotal observation or pathogenetic hypothesis for the clinical outcome?

A ligand for CD38 was identified as CD31, a molecule prevalently expressed by endothelial cells and selected lymphocyte populations. The finding that chronic lymphocytic leukemia (CLL) B-cells with high CD38 expression show marked propensity to apoptosis is apparently in contrast with its negative prognostic significance. Herein, we tried to explain this discrepancy showing that CD38 is co-expressed with its ligand CD31 on B-CLL cells.

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B-cell chronic lymphocyte leukemia (B-CLL) is a disorder typically characterized by a clonal expansion of CD5$^+$ CD23$^+$ monoclonal B-lymphocytes.[1] Human CD38 is a type II transmembrane glycoprotein widely expressed by different cell types.[2] Although many hypotheses have been proposed about its *in vivo* function, it is still difficult to construct a unifying model. For instance, CD38 blocks cell differentiation in the bone marrow B-cell compartment by activating apoptosis,[3] while CD38 signaling by agonistic monoclonal antibodies prevents apoptosis in human germinal center B-cells.[4] Different studies have analyzed CD38 expression in B-CLL. In particular, the evaluation of CD38 and IgD expression has been useful in distinguishing B-cells at various stages of differentiation from naive through to memory cells.[5] Moreover, it has been demonstrated that low CD38 levels predict a longer survival in B-CLL patients[6] and a longer time to progression.[7]

A ligand for CD38 has been identified as the platelet endothelial adhesion molecule-1 or CD31.[8] Interestingly, CD38 was recently demonstrated to parallel CD31 expression in myeloma cells with the exception of the leukemic form of multiple myeloma, in which neoplastic plasma cells lost CD31.[9]

This study demonstrates that the adhesion molecule CD31 was highly expressed by B-CLL, mostly in those cases display-

Table 1. Comparison between the expression of CD38 and CD31 on peripheral blood of B-CLL cells according to a cut-off value of 30%.

CD31	*CD38*		*Total*
	<30%	*>30%*	
<30%	11	6	17
>30%	5	21	26
Total	16	27	43