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SHORT COMMUNICATION

The interleukin-10 gene promoter polymorphism −1087AG does not correlate with clinical outcome in non-Hodgkin's lymphoma

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The Interleukin 10 (IL-10) gene is highly polymorphic, and the IL-10$_{-1087AG}$ (rs1800896) gene variation is the only so far studied intensively in association with certain diseases. Conflicting data have been published about an association of IL-10$_{-1087AG}$ gene variation with lower rates of complete remission and lower overall survival (OS) in patients with diffuse large B-cell lymphoma. To further investigate this in malignant lymphoma, we established the IL-10 genotypes in patients from the NHL-B1/ B2 studies from the German High-Grade Non-Hodgkin's Lymphoma Study Group. In our study, allele frequencies of lymphoma patients are comparable as in healthy controls. No increase of IL-10$_{-1087G}$ alleles was found. In addition we did not find any difference in OS or event-free survival between patients with IL-10$_{-1087AA}$ and the other genotypes. Comparable results were obtained for the IL-10 loci at −3538 (A/T), −1354 (A/G), −824 (C/T) and −597 (A/C) (rs1800890, rs1800893, rs1800871 and rs1800872).
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With conventional chemotherapy, long-term remission can be achieved in approximately 50% of patients with disseminated 'aggressive' lymphoma. The disease incidence is increasing, but aetiologic factors contributing to this phenomenon remain still largely unknown. Although it is a curable disease, many patients do not achieve complete remission (CR), or they relapse after conventional chemotherapy. Tumour- and host-related parameters are likely to reflect some underlying biologic mechanisms and differences in the response to therapy may be related to genetic factors of the host.

One suggestion is that deregulated components of the immune system may be linked to the incidence and clinical course of lymphomas. Cytokines are major mediators of inflammation and deregulated expression of defined subsets of cytokines was found to be associated with lymphoid malignancies. Therefore, attempts to clarify the mechanisms involved in immune system deregulation in lymphoma should contribute to a better understanding of the clinical course of these malignancies.

Interleukin 10 (IL-10) is an important immunoregulatory cytokine in man and is part of a balanced network of immunoregulatory factors, where it also stimulates proliferation of certain B-cell malignancies or suppresses the immune response against lymphomas.[1] IL-10 is produced by a number of cells including normal and neoplastic B cells, stimulated monocytes/macrophages and subsets of T cells. IL-10 has been implicated in certain infectious diseases, autoimmunity, transplantation tolerance and tumorigenesis.[2–15] Polymorphisms in the IL-10 5′-flanking region genetically affect interindividual differences in IL-10 production.[16–21] Increased IL-10 plasma levels and poor outcome of some lymphoma entities have been shown, suggesting an role for IL-10 in lymphoma development.[3,4,9,22]

Recently Lech-Maranda and co-workers reported that homozygocity for −1087A (rs1800896 A/G) of the *IL-10* promoter is associated with lower rates of complete remission and lower overall survival (OS) in patients with diffuse large B-cell lymphoma (DLBCL), whereas Berglund and co-workers showed an absence of such an association.[22,23] In a recent epidemiological multicenter study, evidence was provided that for carriers of the IL-10$_{-3538A}$ allele (rs1800890 A/T) the risk of NHL is doubled, but this was not verified by a follow-up study.[24,25]

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To further investigate IL-10 promoter gene variations in malignant lymphoma, we established the IL-10$_{-1087}$ genotype, in comparison to IL-10$_{-3538}$, IL-10$_{-1354}$, IL-10$_{-824}$ and IL-10$_{-597}$ genotypes (rs 1800893, 1800871 and 1800872, respectively) in patients from the NHL-B1/ B2 studies from the German High-Grade Non-Hodgkin's Lymphoma Study Group (DSHNHL) and compared it with OS and event-free survival (EFS).[26,27] We used for this analysis a homogeneous, equally treated cohort of patients with non-Hodgkin's Lymphoma (NHL). Patients were eligible if they had previously untreated, biopsy-confirmed, aggressive NHL according to the Revised European-American Lymphoma Classification (translated into the World Health Organization (WHO) classification). Patients were excluded if the diagnosis of aggressive lymphoma was not confirmed (i.e., no pathology review was available) or if the diagnosis had to be changed into indolent lymphoma or no lymphoma at all by a panel of expert hematopathologists who conducted a blinded central pathology review. The patients had mandatory baseline examinations that included clinical examination, laboratory tests, chest radiograph, abdominal sonography, computed tomography of chest and abdomen, and a bone marrow biopsy. All patients included in this study received anthracycline-containing regimens, consisting of CHOP/CHOEP (cyclophosphamide, adriamycin, vincristine, etoposide, prednisone).[26,27] Complete remission (CR) was defined as the disappearance of all disease manifestations for at least 2 months after the final restaging. EFS was determined from the onset of treatment until either disease progression, initiation of salvage therapy; or additional (off-protocol) treatment, relapse, death or in case of no event. Overall survival (OS) was determined from the onset of treatment until the last follow-up evaluation or death from any cause. Within 5 years the OS was 60.2% (95% confidence interval: 54.8:65.5) for all and 61.8% (95% confidence interval: 55.3:68.3) for the DLBCL subgroup. The EFS was respectively 49.4% (95% confidence interval: 44.1:54.8) and 49.7 (95% confidence interval: 43.0:56.4). Further clinical characteristics of the patients enrolled in the study are described in detail in Pfreundschuh *et al*.[26,27] and Wojnowski *et al*.[28]

We also assessed the allele frequencies in 193 ethnically matched healthy controls. The frequency of IL-10$_{-1087}$ alleles and corresponding genotypes were not significant different between patients and controls (0.285 vs 0.271% for −1087AA; $P=0.516$). The same was found when only DLBCL-patients were analysed (0.285 vs 0.305%; $P=0.344$) (see also Table 1). The frequency of IL-10$_{-1354}$, IL-10$_{-824}$ and IL-10 IL-10$_{-597}$ alleles and corresponding genotypes were not significant different between patients and controls ($P=0.273$, 0.855 or 0.543, respectively) (Table 2). Analysing the IL-10$_{-3538}$ gene variation significant differences between healthy controls and lymphoma patients can be visualized: genotype and allele based analysis showed *P*-values of 0.008 and 0.015, respectively, suggesting a risk for aggressive NHL for carriers of the A-allele as described by Rothman *et al*.[24] Using Fishers exact test the odds ratio is 1.62 (95% confidence interval: 1.12;2.38). However performing multivariate testing (Bonferoni, FDR-method) these differences at IL-10$_{-3538}$ were not longer significant. For the DLBCL subgroup no significant differences for the analysed IL-10 loci were found. Overall our data did not show differences in allele

Table 1 Genotype frequency of the IL10 promoter gene variation at −1087 in 409 NHL patients (including 256 patients with DLBCL) and 193 control subjects

	Controls	*Lymphoma patients*	*DLBCL*
Number of individuals	193	409	256
Genotypes (%)			
IL-10 –1087AA	55 (28.5%)	111 (27.1%)	78 (30.5%)
IL-10 –1087AG	90 (46.6%)	210 (51.3%)	129 (50.4%)
IL-10 –1087GG	48 (24.9%)	88 (21.5%)	49 (19.1%)
		$P=0.516$	$P=0.344$

Abbreviations: DLBCL, diffuse large B-cell lymphoma; IL-10, interleukin 10; NHL, non-Hodgkin's lymphoma.
Analysis of the IL-10 gene variation was performed as described in Mörmann *et al*. or using an TaqMan-assay for the IL-10$_{-1087}$ gene variation as described in http://snp500cancer.nci.nih.gov/snp.cfm?both_snp_id=IL10-03.[21,26,27] Sixty-seven percent of the patients were older than 60 years (median 64; range 23–75), whereas in the DLBCL subgroup ($n=256$) 69% were older than 60 years (median 65; range 23–75).

Table 2 Genotype frequency of the IL10 promoter gene variations at −3538, −1354, −824 and −597 in NHL and control subjects

Genotypes	*−3538 AA; AT; TT %*	N	*−1354 AA; AG; GG %*	N	*−824 CC; CT; TT %*	N	*−597 AA; AC; CC %*	N
Control	14, 52, 34	193	26, 47, 27	192	55, 40, 5	202	5, 40, 55	202
Patients	11, 44, 45	367	21, 50, 29	353	55, 40, 5	418	5, 40, 55	412
Minor genotype	AA		AA		TT		AA	
Minor allele frequency	0.402		0.492		0.25		0.25	
pCAT	0.008		0.224		0.823		0.437	
Chi allele	0.015		0.273		0.855		0.543	
PCHI 11+12 vs 22	0.008		0.557		0.823		0.437	
PCHI 11 vs 12+22	0.284		0.224		0.897		0.938	

Abbreviations: IL-10, interleukin 10; NHL, non-Hodgkin's lymphoma.
Analysis of the IL-10 gene variation was performed as described in Mörmann *et al*.[21] pCAT: *P*-value using the Freidlin test (genotype based association); Chi allele: *P*-value of the respective allele-based comparison; PCHI: *P*-values when comparing one genotype against the others together; 11 represents the minor genotype, 12 the respective heterozygous genotype and 22 the major genotype; *N* – number of individuals.

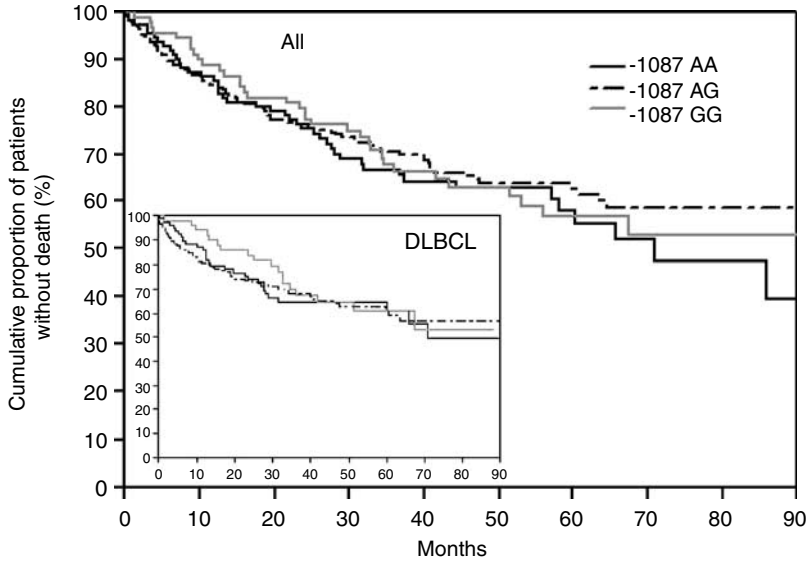

Figure 1 OS for the 409 patients with NHL in comparison to the IL-10 –1087 genotype. Inset presents the same data for the DLBCL group with $n = 256$.

frequencies between lymphoma patients and healthy controls.

Thus, in our study allele frequencies of lymphoma patients are comparable as in healthy controls. No increase of IL-10_{-1087G} alleles was found. In addition, we did not find any difference in OS (all $P = 0.704$, DLBC $P = 0.892$) or EFS (all $P = 0.822$, DLBC 0.864) between patients with IL-$10_{-1087AA}$ genotype and the other genotypes, calculated in different ways as presented by Lech-Maranda *et al*. or Berglund *et al*. (Figure 1). No significant differences in the clinical presentation were detected between the genotype groups compared when sex, the factors of international prognostic index (IPI) (age > 60, LDH > N, ECOG > 1, stage III/IV, extranodal involvement >1) or the IPI score were taken into consideration. There were no differences according to the complete remission rate between the genotype groups. No associations were found between the prognostic variables, EFS, or OS intervals and alleles/genotypes for the other analysed gene loci (OS: −3538 $P = 0.302$; −1354 $P = 0.703$; −824 $P = 0.665$; −597 $P = 0.294$; EFS: −3538 $P = 0.521$; −1354 $P = 0.695$; −824 $P = 0.902$; −597 $P = 0.397$).

In our study, 67.0% of all patients were older than 60 years with a range between 23 and 75 years (69.5% >60 years within the DLBCL-group). IPI low/low intermediate, CS III/IV, extranodal sites, bulky disease and B symptoms were comparable in our cohort to those described by Lech-Maranda *et al*., percentage of LDH > N was lower in our group. In addition after adjusting the analysis for the prognostic factors from the IPI no significant differences between the genotype groups were observed.

At this stage the precise role of *IL-10* promoter gene variations, both individually or as part of defined proximal or distal haplotypes in determining IL-10 expression is still a subject awaiting answers. The IL-10 gene is highly polymorphic, and the IL-10_{-1087} gene variation is the most intensively studied variation in this cytokine gene promoter. Furthermore in most studies published so far the SNPs –1087, −824 and –597 or the so-called proximal haplotypes GCC/ATA/ACC were found to be related to the IL-10 production capacity *in vitro*, where GCC was described as an IL-10 high producer haplotype.[16,18–21] The mechanism behind this is still unkown, probably because of the close proximity of −1087AG to the IL-10.G microsatellite. The IL-10.R microsatellite in close proximity of −3538AT may also affect IL-10 expression levels. First reports on structural changes of the chromatin within the *IL10* gene in differentiated Th1 and Th2 cells or macrophages emphasize the surprising diversity of mechanisms used to regulate cytokine gene expression at the chromatin level and might be opening the chance to understand the mechanism of interindividual differences in IL-10 expression.[29–34] DNase I-hypersensitive sites on a conserved 40-kb region between the *IL-19* and *IL-10* genes in different murine T-cell populations revealed three enhancer elements, which function in T cells *in vitro*.[35]

The role of inherited factors in the extend of IL-10 deregulation in malignant disorders is still controversy. The data obtained so far indicate that additional, probably larger studies are required, also in order to confirm initial results. This will significantly contribute to the understanding of the role of IL-10 in lymphoma development. Some studies revealed that the *IL-10* promoter region was associated with a poor prognosis of aggressive NHL. However, as in our study in the majority of single SNP analysis this effect is not. This may suggest that additional not yet identified gene variations within the chromosomal region 1q31/32 around the *IL-10* gene are important for our understanding of the role of inherited factors in lymphomas. In addition it is relevant to analyse the relation of respective gene variations in this chromosomal area to intergenic RNAs and their role in regulating the expression of IL-10 family members 35. Follow-up analysis will need to analyse additional gene variations across 1q31/32 and other immunological important cytokines as suggested recently by Lan *et al*.[36]

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