Interleukin-10 Gene Polymorphisms are Associated With Freedom From Treatment Failure for Patients With Hodgkin Lymphoma

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Disclosures of potential conflicts of interest may be found at the end of this article.

Key Words. IL-10 • Polymorphisms • Hodgkin lymphoma • Cytokines • SNP

CME Learning Objectives

Discuss whether and how genetic variations influence clinical outcomes of Hodgkin lymphoma patients.

Evaluate evidence that proximal IL10 promoter gene variations are associated with clinical courses of Hodgkin lymphoma patients.

Compare host genetic variations from different cytokine/cytokine receptor gene variations.

ABSTRACT

Background. Hodgkin lymphoma (HL) is a lymphoid malignancy characterized by the production of various cytokines possibly involved in immune deregulation. Interleukin-10 (IL-10) serum levels have been associated with clinical outcome in patients with HL. Because host genetic variations are known to alter the expression and function of cytokines and their receptors, we investigated whether genetic variations influence clinical outcome of patients with HL.

Methods. A total of 301 patients with HL who were treated within randomized trials by the German Hodgkin Study Group were included in this exploratory retrospective study. Gene variations of IL-10 (IL-10^{-597AC}, rs1800872; IL-10^{-824CT}, rs1800871; IL-10^{-1087AG}, rs1800896; IL-10^{-3538AT}, rs1800890; IL-10^{-6208CG}, rs10494879; IL-10^{-6752AT}, rs6676671; IL-10^{-7400InDel}), IL-13 (IL-13^{-1069CT}, rs1800925; IL-13^{Q144R}, rs20541), and IL-4R (IL-4R^{I75V}, rs1805010; IL-4R^{Q576R}, rs1801275) were genotyped.

Results. Inferior freedom from treatment failure (FFTF) was found in patients harboring the IL-10^{-597AA}, IL-10^{-824TT}, or the IL-10^{-1087AA} genotype. In contrast, the IL-10^{-1087G-824C-597C} haplotype present in about 48% of analyzed HL patients is nominally significant for a better FFTF in a Cox-Regression model accounting for stage and treatment. No associations were observed between the other IL-10 gene variations, IL-13^{-1069CT}, IL-13^{Q144R}, IL-4R^{I75V}, IL-4R^{Q576R} and the clinical outcome of patients with HL.

Conclusions. Our study provides further evidence that proximal IL-10 promoter gene variations are associated with clinical course of patients with HL. However, treatment success and survival rates are already at a very high rate, supporting the need to design studies focusing on identification of predictors to reduce the side effects of therapy. *The Oncologist* 2013;18:80–89

Implications for Practice: Inter-individual genetic variations (polymorphisms) of host genes (patient genes) might be associated with different outcomes for cancer patients. This could be independent of the characteristics of the tumor itself. We asked if the course of Hodgkin Lymphoma patients is changed depending on the genetic background of the patient. As Hodgkin Lymphoma is characterized by an inflammatory microenvironment, we studied polymorphisms of three well-selected immune genes by molecular analysis. Our study provides evidence that a genetic variation of the IL-10 gene is associated with a worse clinical outcome in patients with HL. Although clinical practice is not changed based on these data, our study is a proof of concept and should stimulate further research in this area on a whole genome basis.

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INTRODUCTION

Hodgkin lymphoma (HL) is one of the most frequent lymphoid tumors in young adults. Over the last decades, therapies for HL have improved and have currently achieved long-term survival rates of up to 85%–90%. Nevertheless, observations of long-term survivors revealed a high incidence of chemotherapy-related secondary malignancies as well as a stagnant rate of relapses [1]. Therefore, it is important to define biological prognostic factors in addition to the clinical variables summarized in the International Prognostic Score (IPS) to more accurately predict outcome of patients with HL and adjust treatment regimens based on individual risk. Both sibling and family studies suggest that the host genetic background may include critical factors affecting susceptibility to HL, and therefore it may also comprise prognostic markers for the clinical outcome of patients with HL [2].

The presence of few multinuclear Hodgkin and Reed-Sternberg (HRS) cells surrounded by a reactive microenvironment consisting of nonclonal hematopoietic cells is the hallmark characteristic of HL. The production of various cytokines and chemokines by HRS cells are believed to recruit the microenvironment to the HRS cells and establish an immunologic favorable niche for the survival of HRS cells. Among those, cytokines like interleukin (IL)-10 or IL-13 have been implicated in the pathogenesis of HL [3].

IL-10 is of particular interest because of its immunomodulatory functions. It is able to inhibit production of proinflammatory cytokines and chemokines, expression of MHC class II molecules, and differentiation of dendritic cells, having thus mostly immunosuppressive activities [4]. Furthermore, IL-10 is able to upregulate antiapoptotic proteins like Bcl-2 in germinal center B-cells and could therefore be associated with increased survival of HRS cells [5]. The expression of IL-10 has been detected in 21%–36% of primary HL samples but is more frequently found in Epstein-Barr virus (EBV)-positive cases [3]. IL-10 seems to be produced mainly by HRS cells themselves but also by the reactive infiltrate and more specifically by regulatory T-cells surrounding the tumor cells. IL-13 is produced by HRS cells able to promote cell survival and proliferation in an autocrine fashion [3, 6].

Elevated serum levels of IL-10 have been detected in 30%–50% of patients with HL. Association with a poor survival prognosis was reported in patients with HL [7–9]. Increased IL-10 serum levels were also found in patients with HL with EBV-positive HRS cells in comparison to EBV-negative cases, but the effect of EBV infection on patient survival of the patients remains controversial [10, 11].

Increased IL-10 serum levels could be associated with a severe clinical course in patients with HL due to immune tolerance to the tumor cells. Clinical observations of hematopoietic stem cell transplantations underline the immunosuppressive effect of IL-10 and suggest that an increased IL-10 production may be associated with immunologic tolerance against the transplanted cells [12, 13]. These studies implicate that IL-10 could be one crucial factor forming the immunologic favorable niche for HRS cells by immunosuppression. Interindividual genetic variations in the regulatory region of cytokine genes are associated with differences in cytokine secretion. Nonsynonymous coding polymorphism within cytokine (as IL-10 or IL-13)

and cytokine receptor (for IL-13 the IL-4R α) genes have been associated with modified functionality [14].

Previous studies identified three regions 5' of the IL-10 gene locus showing frequently genetic variations. The region located most proximally with respect to the IL-10 transcriptional start site includes the single nucleotide polymorphisms (SNPs) IL-10_{-1087AG}, IL-10_{-824CT}, and IL-10_{-597AC}. A second more distal located region harbors the SNPs IL-10_{-3538AT}, IL-10_{-2812AG}, and IL-10_{-2726AC}. A third so-called far distal region locates the gene variations IL-10_{-7400InDel}, IL-10_{-6752AT} and IL-10_{-6208CG}. These regions are separated by the CA-dinucleotide repeat microsatellites IL-10.R and IL-10.G, respectively. Genetic variations of IL-10 have been intensively analyzed over the last decade in different diseases. Multiple associations between IL-10 production capacity and either the IL-10 microsatellite alleles, SNPs, or SNP haplotypes have been reported in a 7-kb 5'-flanking region of the IL-10 gene [15–21].

In lymphoid malignancies, an association of those IL-10 gene variations with susceptibility as well as clinical course of the disease has been proposed, but data is contradictory [22–26]. For HL, Munro et al. found no association of the IL-10 gene variations IL-10_{-597CA} and IL-10_{-1087AG} with the risk to develop HL within a cohort of 147 patients with HL [27]. Nieters et al. reported no association between the IL-10_{-1087AG} and IL-10_{-3538AT} SNPs with HL susceptibility in a cohort of 115 patients with HL who were part of a large lymphoma case-control study of the EpiLymph consortium [28]. An increased frequency of the IL-10_{-1087GG} genotype was found in EBV-positive HL cases compared to EBV-negative HL cases [29]. Hohaus et al. reported a negative impact of the IL-10_{-597AA} genotype and the TGCAA haplotype (consisting of IL-10_{-3538AT}, IL-10_{-2812AG}, IL-10_{-2726AC}, IL-10_{-1087AG} and IL-10_{-597AC}) on clinical outcome in 184 patients with HL [30]. More distal gene variations of IL-10, which have been shown to influence the clinical course of patients with non-Hodgkin lymphoma (NHL), have not been analyzed in patients with HL yet [22].

Based on the previously reported association of IL-10 serum levels and clinical outcome in patients with HL, we raised the question of whether IL-10 gene variations might predict treatment failure in a large cohort of clinically well-characterized patients with HL. We therefore genotyped seven IL-10 gene variations as well as two IL-13 gene variations and two gene polymorphism of the IL-13 receptor chain IL-4R in 301 patients with HL who were treated within the prospective randomized phase 3 trials HD13–15 of the German Hodgkin Study Group (GHSG).

PATIENTS AND METHODS

Available pretreatment samples were collected from 301 patients with HL who were included in the HD13, HD14, and HD15 trials of the GHSG, which began recruitment in 2003. The study was conducted in accordance with the Declaration of Helsinki. All patients gave written informed consent to the inclusion into the trials. Ethical approval from local authorities was granted for the study presented in this paper. HD13 and HD14 comprised patients with early favorable and unfavor-

able disease, respectively, whereas the HD15 trial was designed for patients with advanced-stage disease.

The patients' characteristics and histology are presented in Table 1. The majority of patients were between 20 and 50 years of age (77%) and 56% were men. In all, 53% of our patients were diagnosed with classical HL of the nodular sclerosis subtype. The median follow-up period of the entire cohort was 26.1 months and 28 treatment failures occurred during this period.

DNA Extraction and Genotyping Analyses

Blood samples of patients with HL were stored at -20°C until DNA extraction. Genomic DNA from blood samples was isolated using the Qiaamp Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The concentration and purity of extracted DNAs were determined by spectrophotometric analysis (NanoDrop 2000; Applied Biosystems, Darmstadt, Germany). The polymorphisms of IL-13 (-1069CT, rs1800925; Q144R, rs20541), IL-4R (I75V, rs1805010; Q576R, rs1801275), and IL-10 (-597AC, rs1800872; -824CT, rs1800871; -1087AG, rs1800896; -3538AT, rs1800890; -6208CG, rs10494879; -6752AT, rs6676671; -7400InDel) genes were analyzed. For the analysis of the genetic variation, an Applied Biosystems ABI 7900HT instrument was used and genotyping was performed as described recently [22, 25, 31, 32]. For quality control, 5% of the samples were genotyped in duplicate for each gene variation with an accordance of 100%.

Statistical Analysis

The primary outcome measure was freedom from treatment failure (FFTF), defined as the time from randomization until the first event or date of last known clinical status if no event occurred. Events were progression during therapy, administration of treatment in considerable excess of the protocol, failure to attain complete remission after protocol treatment, relapse, or death from any cause. For the univariate analysis, FFTF was analyzed using the Kaplan-Meier method and differences between subgroups (e.g., genotypes) were tested for significance using the log-rank test.

Additionally, a multivariate analysis was conducted using a Cox proportional hazards model for FFTF. The model was stratified by trial (HD13, HD14, HD15). The SNP was fitted as a two-level factor (AA vs. AC/CC or AA vs. AG/GG, respectively), whereas IPS was fitted as a linear covariate. When determining the IPS, a prognostic factor was assumed to be absent if the relevant data for that factor were missing or incomplete. Only the factors serum albumin, leukocyte count, and lymphocyte count had missing values in 12, 2, and 15 cases, respectively. Furthermore, we applied the Akaike's Information Criterion (AIC), quantifying the degree to which the model fits the data (Table 4). It takes into account both the residual deviations between fitted and observed values and the number of fitted parameters; smaller values indicate closer fit. Hardy-Weinberg equilibrium estimation was analyzed using GENEPOP software 3.4. Haplotypes within IL-10 were reconstructed from the genotypes data using the software PHASE (version 2.1.1).

We are aware of the problem of multiple comparisons between different combinations of heterozygous and homozygous groups within each genotype and haplotype in this study.

Table 1. Clinical characteristics and histopathology of the 301 analyzed patients with Hodgkin lymphoma

Patient characteristic	n	%
Trial		
HD13	81	27
HD14	100	33
HD15	120	40
Sex		
Male	170	56
Female	131	44
Age		
16–20 yrs	22	7
20–30 yrs	90	30
30–40 yrs	86	29
40–50 yrs	55	18
50–60 yrs	35	12
60–75 yrs	13	4
Stage		
I	30	10
II	169	56
III	69	23
IV	33	11
B symptoms		
A	188	62
B	113	38
WHO activity index		
0	214	71
1	75	25
2	12	4
Leukocytes before therapy ($10^3/\mu\text{L}$)		
Not available	2	
0–5	10	3
5–10	151	51
10–15	91	30
15–20	37	12
≥ 20	10	3
Histology		
Classic		
Nodular sclerosis	162	53
Mixed cellularity	76	24
Lymphocyte depleted	1	
Lymphocyte rich	12	4
Not specified (classic)	17	6
Lymphocyte predominant	9	3
Not specified	24	8

Therefore, we have chosen to report the most prominent aspects here and label this as an exploratory study.

RESULTS

Study Design

We analyzed allelic variations of IL-13 (-1069CT; Q144R), IL-4R (I75V; Q576R), and IL-10 (-597AC, -824CT, -1087AG, -3538AT,

-6208CG, -6752AT, -7400InDel) genes within our cohort of 301 patients with HL. All genotype frequencies conformed to Hardy-Weinberg equilibrium. Genotype distributions of the analyzed polymorphisms are shown in Table 2 and supplemental online Table 1. All *IL-10*_{-597A} alleles were in complete linkage to the *IL-10*_{-824T} alleles. To analyze the *IL-10* haplotypes with regard to the survival of patients with HL, the haplotypes were reconstructed using the PHASE software (Fig. 1) and nine different haplotypes were identified. The three haplotypes ATA, GCC, and ACC were found for the proximal polymorphisms *IL-10*_{-1087AG}, *IL-10*_{-824CT}, and *IL-10*_{-597AC}. Subsequently, both polymorphisms and haplotypes were correlated with probability of freedom from treatment failure (FFTF) of patients with HL—the main outcome measure in this investigation defined as the primary endpoint in the trial protocols.

Association of Proximal IL-10 Gene Variations with FFTF

Univariate analysis of the association of the gene variations with the FFTF probability revealed that the proximal polymorphisms of the 5'-flanking region of the *IL-10* gene are related to outcome of patients with HL. The 2-year FFTF rate of patients with *IL-10*_{-597AA} genotype was 71% versus 94% and 90% for patients with *IL-10*_{-597AC} or *IL-10*_{-597CC} genotypes, respectively ($p = .024$; Table 2).

Comparing homozygous carriers of the *IL-10*_{-597AA} genotype to all other genotypes (combined *IL-10*_{-597AC} and *IL-10*_{-597CC}), the FFTF rate was also significantly different ($p = .026$; Fig. 2A). In multivariate analysis adjusting for IPS, the hazard ratio for AA versus AC/CC was 2.70 (95% confidence interval [CI]: 0.93–7.81; $p = .067$). Due to the complete linkage of the *IL-10*_{-597AC} to the *IL-10*_{-824CT} gene variation, the same results were found for the *IL-10*_{-824CT} gene variation (Table 2). When comparing patients characterized by the homozygous *IL-10*_{-1087AA} genotype to all other genotypes (combined *IL-10*_{-1087AG} and *IL-10*_{-1087GG}), we found a lower FFTF rate in patients with *IL-10*_{-1087AA} genotypes (83% vs. 94%; $p = .033$; Fig. 2B).

In multivariate analysis, the hazard ratio for AA versus AG/GG was 2.18 (95% CI: 1.03–4.62; $p = .042$). In multivariate analysis adjusting for IPS and using the counts of homozygous minor allele carrier (AA) of *IL-10*_{-597AA} and *IL-10*_{-1087AA} as a combined linear covariate (values 0, 1, or 2), the hazard ratio per homozygote was 1.81 (95% CI: 1.09–3.01; $p = .022$).

These results suggest that patients characterized by the proximal *IL-10*_{-1087AA}, *IL-10*_{-824TT}, and/or *IL-10*_{-597AA} gene variations may have worse FFTF rates compared to other genotypes. In contrast, we did not find any association of FFTF with *IL-13* or *IL-4R* gene variations. Based on these results, haplotype analysis was restricted to proximal *IL-10* gene variations.

Table 3 displays the absolute and relative frequencies of clinical trial participation, sex, age, World Health Organization (WHO) activity index, stage, B-symptoms, baseline leukocyte count, and review pathology for the genotype subgroups AA and AC/CC of *IL-10*₋₅₉₇ and the subgroups AA and AG/GG of *IL-10*₋₁₀₈₇. The haplotype subgroups with and without GCC are identical to the subgroups AA and AG/GG of *IL-10*₋₁₀₈₇. Therefore, these haplotypes are not displayed. A higher frequency for the *IL-10*_{-597AA} was observed for patients within the HD15

trial (advanced stages), men, patients with WHO index >0, patients with B-symptoms, and lymphocyte predominant or lymphocyte-rich classical subtypes. However, none of these differences was statistically significant (i.e., no $p < .05$) with the exception of that with review pathology ($p = .019$) using Fishers exact test. For *IL-10*₋₁₀₈₇, no association with clinical or biological characteristics can be observed (no $p < .05$).

Favorable Prognostic Impact of the Proximal IL-10 Haplotype GCC in Patients with HL

To analyze a possible association with FFTF, patients with HL were stratified into four groups according to *IL-10* haplotypes (Fig. 2): homozygous GCC ($n = 69$), homozygous ACC ($n = 27$), homozygous ATA ($n = 16$), and all remaining heterozygous patients ($n = 189$). Comparison of these four groups showed nominally significant differences for FFTF rate ($p = .027$; Fig. 3A).

We next compared patients with at least one GCC allele ($n = 222$) to non-GCC carriers ($n = 79$), ACC allele carriers ($n = 138$) to non-ACC allele carriers ($n = 163$), and ATA allele carriers ($n = 130$) to non-ATA allele carriers ($n = 171$), respectively. Patients with HL characterized by at least one allele of the GCC (GCC/GCC; GCC/ACC; GCC/ATA) compared to patients without any GCC allele (ATA/ATA; ATA/ACC; ACC/ACC) had favorable FFTF rates (94% vs. 83%; $p = .033$; Fig. 3B). No association of the ATA ($p = .34$) or the ACC ($p = .33$) alleles with FFTF could be detected.

In a multivariate analysis adjusting for IPS, the three haplotype grouping factors GCC, ATA, and ACC (with the haplotype vs. without the haplotype in each case) were fitted together. The GCC haplotype ($p = .012$) and IPS ($p = .033$) were significant. The hazard ratio for the GCC haplotype was 3.30 (95% CI: 1.30–8.40; $p = .012$), whereas ATA ($p = .063$) and ACC ($p = .40$) showed borderline and nonsignificant results, respectively. Other partial or extended haplotype analyses showed no significant association with the outcome of our patients.

Comparing the model fit of the above-mentioned Cox regression models using the AIC, the best fit is given by the models including the combination of *IL-10*₋₅₉₇ and *IL-10*₋₁₀₈₇ as a single score, together with IPS. This is followed closely by the model with *IL-10*₋₁₀₈₇ and IPS. However, the inclusion of both SNPs as independent covariates did not improve the fit due to the strong linkage disequilibrium of both SNPs.

DISCUSSION

Cytokines and chemokines produced by HRS cells and the surrounding microenvironment are widely believed to be involved in the pathogenesis of HL by modulating immune functions and inflammatory responses [3, 33]. Amongst those cytokines, IL-10 seems to be most relevant for the shaping of a microenvironment favoring survival of HRS cells. Elevated IL-10 serum levels were even associated with a worse clinical outcome in patients with HL [7–9]. In a very recent study, our group evaluated the prognostic relevance of 30 cytokines, chemokines, and respective receptors in patients with advanced-stage HL [34]. In that study, only elevated IL-10 serum levels were predictive of early treatment failure in HL independently of clinical prognostic

Table 2. Genotype distribution of *IL-10* gene variations and freedom from treatment failure in patients with Hodgkin lymphoma

Polymorphism	Genotype	(n)	Genotype (frequency)	2-year FFTF rate (%)	<i>p</i>	Genotype	<i>p</i>
IL10 -597 (rs1800872)	CC	171	0.57	90	<i>.024</i>	AAvs. AC/CC	<i>.026</i>
	AC	114	0.38	94			
	AA	16	0.05	71			
IL10 -824 (rs1800871)	CC	171	0.57	90	<i>.024</i>	TTvs. TC/CC	<i>.026</i>
	CT	114	0.38	94			
	TT	16	0.05	71			
IL10 -1087 (rs1800896)	GG	69	0.23	93	<i>.09</i>	AAvs. AG/GG	<i>.033</i>
	AG	153	0.51	94			
	AA	79	0.26	83			
IL10 -3538 (rs1800890)	AA	45	0.15	91	<i>.18</i>	TTvs. AT/AA	<i>.072</i>
	AT	152	0.50	94			
	TT	104	0.35	86			
IL10 -6208 (rs10494879)	GG	51	0.17	92	<i>.25</i>	CCvs. CG/GG	<i>.097</i>
	CG	153	0.51	93			
	CC	97	0.32	86			
IL10 -6752 (rs6676671)	AA	105	0.35	86	<i>.18</i>	AAvs. AT/TT	<i>.078</i>
	AT	153	0.51	94			
	TT	43	0.14	91			
IL10 -7400	InIn	190	0.63	90	<i>.43</i>	DelDels.InDel/InIn	<i>.27</i>
	InDel	99	0.33	92			
	DelDel	12	0.04	100			

Significant *p* values are shown in italics.

Figure 1. Haplotypes of the analyzed gene variations in the interleukin-10 gene 5'-flanking region. The haplotype frequencies were estimated using the PHASE software applied to the patient cohort (*n* = 310) of the present study.

factors. Given the strong association of IL-10 serum levels and outcome in patients with HL and the knowledge that IL-10 expression depends on the host genetic background, we analyzed *IL-10* and other cytokine gene variations in germline DNA of patients with HL treated within prospective phase III trials of the GHSG.

Our results indicate that germline *IL-10* gene polymorphisms are associated with FFTF in patients with HL. Therefore, it might be concluded that the patients' genetic background influences the clinical course of HL. To our knowledge, this is the largest cohort of patients with HL analyzed to date (*n* = 301). Our explorative study showed that homozygous carriers of the proximal *IL-10*_{-1087A}, *IL-10*_{-824T} and *IL-10*_{-597A} alleles, respectively, have lower FFTF rates than the respective complementary group. It was also found that patients with HL characterized by the *IL-10*_{-1087G}, *IL-10*_{-824C} and

*IL-10*_{-597C} alleles (building the GCC haplotype) have a favorable outcome even after adjustment for IPS.

One drawback of our study cohort is that treatment failure rates in the GHSG trials (HD13–15) was already very low. Only about 10% of our study cohort experienced a treatment failure; thus, the present results might be due to unobserved confounding. However, no significant differences in genotype frequencies were found for any clinical or biological characteristics, with the exception of review pathology (*IL-10*_{-597AA} is associated with lymphocyte predominant and lymphocyte rich classical subtypes). Therefore, these factors are unlikely to act as confounders (LP/LRCHD subtypes have, on the contrary, a more favorable prognosis). Furthermore, our results were confirmed in a multivariate analysis stratified by GHSG trial and accounting for IPS.

Our data is in strong support of the findings published by Hohaus et al., who analyzed *IL-10* germline variations in a series of 184 Italian patients with HL [30]. In that study, carriers of the homozygous *IL-10*_{-597AA} genotype or the associated haplotypes had worse FFTF rates. The 5-year FFTF between the best and worst prognostic group differed by an impressive 57%, whereas the difference was only 19% in our analysis. Patients included in the previously published paper had mostly advanced-stage disease (63%), whereas most of patients studied in the GHSG had limited disease (60%). Moreover, overall FFTF rates were surprisingly low in the Italian cohort of patients, who were treated with a variety of different chemotherapy regimen, whereas treatment was very uniform in patients included within the prospective randomized trials of the

Figure 2. Freedom from treatment failure (FFTF) probabilities in patients with Hodgkin lymphoma in relation to interleukin (IL)-10 gene variations IL-10₋₅₉₇ (A) and IL-10₋₁₀₈₇ (B). **(A)** Comparison of patients homozygous for IL-10_{-597A} with other genotypes revealed a lower FFTF rate for the homozygous carrier ($p = .026$). **(B)** Patients characterized by IL-10_{-1087AA} had also a worse FFTF rate compared to other genotypes ($p = .033$). For 2-year FFTF rates, see Table 2. Abbreviations: FFTF, freedom from treatment failure; IL, interleukin.

GHSG. In summary, we were able to confirm an association between the patients' genetic background and the clinical course of the disease, but it remains unclear to what extent this will be clinically relevant.

Two lines of evidence have been reported regarding the influence of the *IL-10* gene variations onto IL-10 production. First, the most often cited *in vitro* studies provide evidence that the proximal GCC haplotype reflects a high IL-10 production capacity, whereas the ATA haplotype is correlated with a low IL-10 production capacity [15, 18–20]. The extended *IL-10* haplotype AAGCC consisting of *IL-10*_{-3538AT}, *IL-10*_{-2726AC}, *IL-10*_{-1087AG}, *IL-10*_{-824CT} and *IL-10*_{-597AC} SNPs is associated with a decreased IL-10 production capacity [17]. Second, *in vivo* studies of 400 healthy individuals provide evidence that the ATA haplotype is associated with increased IL-10 plasma levels and protection against primary EBV infection [35, 36]. Furthermore, hematopoietic stem cell transplant recipients characterized by a haplotype containing the *IL-10*_{-3538T}, *IL-10*_{-1087A}, *IL-10*_{-824T}, *IL-10*_{-597A} alleles have decreased risk of graft-versus-host disease and increased survival rates, probably as a re-

sult of a high IL-10 serum levels [12, 13, 37]. However, the mechanism behind the observed differences in the IL-10 production is still poorly understood. The close proximity of the proximal polymorphisms to the IL-10.G and the distal SNPs to the IL-10.R microsatellite, which may also affect IL-10 expression levels, has to be taken into account [18, 38].

Our study provides evidence that the GCC haplotype has a favorable impact on the survival of patients with HL. Given that high IL-10 serum levels are predictive for a severe clinical course of HL, it would indicate that patients with HL characterized by GCC alleles are IL-10 low producers *in vivo*. Recently, Hohaus et al. studied the IL-10 serum levels in a cohort of 95 patients with HL with regard to IL-10 gene variations [39]. Using a backward variable selection in this study, the *IL-10*_{-1087GG} and the *IL-10*_{-597AA} genotypes could be associated with increased IL-10 serum level in patients with HL, which is conflictive with regard to the three existing proximal haplotypes GCC, ACC, and ATA. To date, the *IL-10* genotype-phenotype relation is not clarified in patients with HL and current study cohorts do not allow combined analysis of *IL-10* geno-

Table 3. Clinical and biological characteristics of patients according to the genotype at position -1087 or -597 of the *IL-10* gene

	IL10–597				IL10–1087					
	AA		AC/CC		AA		AG/GG		All	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Trial										
HD13	3	19	78	27	18	23	63	28	81	27
HD14	3	19	97	34	24	30	76	34	100	33
HD15	10	63	110	39	37	47	83	37	120	40
Sex										
Female	3	19	128	45	32	41	99	45	131	44
Male	13	81	157	55	47	59	123	55	170	56
Age										
16–20 yrs	1	6	21	7	7	9	15	7	22	7
20–30 yrs	3	19	87	31	21	27	69	31	90	30
30–40 yrs	8	50	78	27	22	28	64	29	86	29
40–50 yrs	1	6	54	19	14	18	41	18	55	18
50–60 yrs	2	13	33	12	12	15	23	10	35	12
60–75 yrs	1	6	12	4	3	4	10	5	13	4
WHO activity index										
0	8	50	206	72	54	68	160	72	214	71
1	7	44	68	24	20	25	55	25	75	25
2	1	6	11	4	5	6	7	3	12	4
Clinical stage										
1	.	.	30	11	7	9	23	10	30	10
2	9	56	160	56	42	53	127	57	169	56
3	5	31	64	22	19	24	50	23	69	23
4	2	13	31	11	11	14	22	10	33	11
B symptoms										
0	7	44	181	64	44	56	144	65	188	62
1	9	56	104	36	35	44	78	35	113	38
Leukocytes										
Missing	.	.	2	1	.	.	2	1	2	1
<5	.	.	10	4	4	5	6	3	10	3
5–10	7	44	144	51	35	44	116	52	151	50
10–15	4	25	87	31	24	30	67	30	91	30
15–20	5	31	32	11	13	16	24	11	37	12
≥ 20	.	.	10	4	3	4	7	3	10	3
Review histology										
Not done	1	6	23	8	5	6	19	9	24	8
Lymphocyte predominant HL	2	13	7	2	4	5	5	2	9	3
Lymphocyte rich classic HL	3	19	9	3	4	5	8	4	12	4
Nodular sclerosis HL	6	38	156	55	38	48	124	56	162	54
Mixed cellularity HL	4	25	72	25	22	28	54	24	76	25
Lymphocyte depleted HL	.	.	1	0	.	.	1	0	1	0
Classic	.	.	17	6	6	8	11	5	17	6

Abbreviations: IL, interleukin; WHO, World Health Organization.

types, serum levels, and clinical outcome in large patient groups, which will be the challenge for the design of future studies.

In a cohort of 500 patients with aggressive NHL, we recently analyzed an association of far distal gene variations of

the 5'-flanking region of the *IL-10* gene with the clinical outcome. No impact of the proximal *IL-10* polymorphisms with regard to the clinical course of these NHL patients was found in our study [22]. Because high IL-10 serum levels are associated with poor prognosis in both HL and NHL patients, this might

Figure 3. Freedom from treatment failure (FFTF) probabilities in patients with Hodgkin lymphoma in relation to proximal interleukin (IL)-10 haplotypes formed by IL-10 SNPs IL-10_{-1087AG}, IL-10_{-824CT}, IL-10_{-597AC}. **(A)** Patients homozygous for the ATA, ACC, GCC and heterozygous patients (other) differ in FFTF rates ($p = .027$). **(B)** Patients with the GCC haplotype ($n = 222$) showed a lower risk of FFTF compared to non-GCC haplotype carriers ($n = 79$; $p = .033$). This result remained nominally significant in a multivariate analysis adjusted to stage and therapy ($p = .028$). Abbreviations: FFTF, freedom from treatment failure; IL, interleukin.

Table 4. Akaike's Information Criterion for the fitted Cox proportional hazards models

Covariates	AIC
IL10-597	253.47
IL10-597 IPS	252.04
IL10-1087	252.47
IL10-1087 IPS	250.80
IL10-597 IL10-1087 IPS	252.09
Combined (IL10-597 and IL10-1087) IPS	250.10
GCC ATA ACC IPS	251.23

Abbreviations: AIC, Akaike's Information Criterion; IL, interleukin; IPS, International Prognostic Score.

suggest differences in the regulation of the *IL-10* gene between Hodgkin and non-Hodgkin lymphoma entities [7–9, 23, 34, 40].

Distal and far distal gene variations may influence epigenetic modification, altering the *IL-10* and neighboring gene

loci such as *IL-19* to a more open conformation. Proximal gene variations could directly affect *IL-10* transcription by modifying transcription factor binding sites.

Lech-Maranda et al. reported a favorable impact of the proximal IL-10_{-1087G} allele on the survival of patients with diffuse large B-cell lymphoma (DLBCL), which could not be confirmed in our study of NHL and DLBCL cases, and a Swedish DLBCL cohort [23, 25, 26]. However, these three studies differ in the median age of the patients with DLBCL, whereas the cohort of Lech-Maranda et al. has included younger patients when compared to the two other studies. It remains to be elucidated if the impact of proximal gene variations on the clinical course of (mostly young) patients with HL might also be age dependent.

It has to be stressed that there is a well-established role for the Epstein-Barr virus in the pathogenesis of HL. More specifically, EBV protein latent membrane protein 1 (LMP1) is able to induce IL-10 production in B-cells and Burkitt lymphoma cells [41, 42]. EBV infection of HRS cells was also associated with increased IL-10 serum levels in patients with HL [10]. In

these EBV-positive cases, viral induction of IL-10 might have an impact on the relevance of the host *IL-10* polymorphisms, but it remains to be elucidated if the effects are potentiated or decreased. In our analyzed HL cohort, no immunohistochemical data about EBV status was available. Therefore, the impact of EBV infection of HRS cells on our presented results cannot be assessed.

We here provide additional evidence that proximal host *IL-10* gene polymorphisms are related to the clinical course of patients with HL. In view of recent publications identifying elevated IL-10 serum levels as a poor risk factor in HL, our results suggest that germline *IL-10* polymorphisms predict disease control in HL [7–9, 34]. It will be necessary to conduct concerted studies investigating the genotype-phenotype relation of IL-10 in patients with HL.

Although descriptive genetic data currently available is not sufficient to adapt treatment intensity based on individual genetic features in HL, it is tempting to speculate that genetic characteristics of both the host and the tumor will allow further risk stratification of the patients and thus change our therapeutic approach based on individual biologic factors.

CONCLUSION

This exploratory study in a large prospective cohort representative of patients with HL suggests an effect of *IL-10* SNPs on freedom from treatment failure in HL. Other studies are

needed to decipher the role of the host immunogenetic background with regard to HL outcomes, in particular for cases with high treatment success. These studies have to combine the parallel investigation of circulating levels of cytokines and their gene variations as well as clearly defined parameters of treatment side effects.

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