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Cytokine gene polymorphisms and progression-free survival in classical Hodgkin lymphoma by EBV status: Results from two independent cohorts

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Abstract

Background—Cytokines are important immune mediators of classical Hodgkin lymphoma (CHL) pathogenesis, and circulating levels at diagnosis may help predict prognosis. Germline single nucleotide polymorphisms (SNPs) in immune genes have been correlated with cytokine production and function.

Methods—We investigated whether selected germline SNPs in *IL10* (rs1800890, rs1800896, rs1800871, rs1800872), *TNFA* (rs1800629), *IL6* (rs1800795), *ILRN* (rs419598), *INFG* (rs2430561) and *CCL17* (rs223828) were associated with circulating levels of related cytokines at diagnosis and progression-free survival (PFS) in CHL. Patients were from France (GELA, *N* = 464; median age = 32 years) and the United States (Iowa/Mayo Specialized Program Of Research Excellence [SPOR], *N* = 239; median age = 38 years); 22% of 346 CHL cases with EBV tumor status were positive.

Results—There was no association with any of the SNPs with cytokine levels. Overall, there was no association of any of the SNPs with PFS. In exploratory analyses by EBV status, *TNFA* rs1800629 ($HR_{AA/AG} = 2.41$; 95% CI, 1.17–4.94) was associated with PFS in EBV-negative GELA patients, with similar trends in the SPORE patients ($HR_{AA/AG} = 1.63$; 95% CI, 0.61–4.40). In a meta-analysis of the two studies, *TNFA* ($HR_{AA/AG} = 2.11$; 95% CI, 1.18–3.77; $P = 0.01$) was statistically significant, and further adjustment for the international prognostic system did not alter this result.

Conclusions—This study showed that germline variation in *TNFA* was associated with CHL prognosis for EBV-negative patients, which will require confirmation. These results support broader studies on the differential impact of genetic variation in immune genes on EBV-positive vs. EBV-negative CHL pathogenesis.

Keywords

Hodgkin lymphoma; Cytokines; Polymorphism; *TNFA*; EBV

1. Introduction

Classical Hodgkin lymphoma (CHL) is a highly curable lymphoid malignancy. However, approximately 15% of localized and 30% of disseminated disease patients do not respond or relapse after optimal initial therapeutic strategy and may require adapted first-line treatment. Several scoring systems using conventional biological and clinical parameters have been developed for identifying prognostic groups of patients in order to adapt the therapeutic strategy [1]. While scoring systems are useful in clinical practice, they do not fully reflect the biological heterogeneity of HL. Recent gene expression profiling of Hodgkin Reed-Sternberg (HRS) cells suggests distinct molecular profiles among CHL [2]. HRS cells create an immunologically favorable environment, and interaction between neoplastic cells and reactive cells is mediated by cytokines, chemokines and soluble receptors [3].

The evaluation of cytokine levels at CHL diagnosis may provide prognostic information for patients [4], and increased serum levels of sCD30 [5–7], IL-10 [5, 6, 8, 9], IL-6 [5, 10, 11], TNF α [12], IL-1R α [5], IL-2R [11], BAFF [13] and CCL17 [14, 15] have been correlated with patient outcome, but results are conflicting and limited by small studies, evaluation of single or only a small number of cytokines, lack of replication, and lack of prospective study designs [4]. In multivariate analysis integrating classical biological and clinical data, sCD30, IL-10, IL-6, IL-2R and IL-1R α were each associated with outcome, with IL-6 showing the most consistent results [5, 11]. More recently, the prognostic significance of the immune microenvironment has been confirmed, with data showing a correlation between an increased number of tumor-associated macrophages and a shortened survival in CHL patients [16].

Germline polymorphisms have been described in immune genes, some of which appear to modify cytokine production *in vitro* and *in vivo* [17]. In the context of non-Hodgkin lymphoma (NHL), epidemiologic studies have suggested a role for germline single nucleotide polymorphisms (SNPs) in immune genes in disease risk, with the most robust (although not universal) association with the *TNFA* rs1800629 SNP [18–22]. Clinical studies

have also suggested that immune SNPs might also predict the disease prognosis. Two studies showed similar prognostic value for the *TNFA* SNP in diffuse large B-cell lymphoma (DLBCL) patients [23, 24] but conflicting results were reported for *IL10* promoter SNPs [24–28]. The prognostic value of SNPs in *IL4R*, *IL1A*, *IL8RB* in DLBCL and *IL8*, *IL2*, *IL12B*, *IL1RN* in follicular lymphoma remained to be validated in independent series [24, 29].

Relatively little is known about the role of cytokine gene polymorphisms in CHL. Five studies have explored the prognostic value of SNPs in *IL6*, *TNFA* and *IL10* genes [28, 30–33], with conflicting results for *IL6*–174G>C SNP [30, 31], consistent results for *IL10*–1082A>G and *IL10*–592>A SNPs [31, 33], and no association for the *IL10*–3575T>A SNP [28, 33] or the *TNFA*, *IL13* and *IL4R* SNPs [31, 33]. Herein, we investigated the association of cytokine gene polymorphisms in *IL10*, *TNFA*, *IL6*, *IL1RN*, *INFG* and *CCL17* with cytokine levels and patient outcome in two independent cohorts of CHL patients. Recent epidemiological studies suggest that genetic risk factors differ between EBV⁺ and EBV[–] CHL [34] and gene expression profiling studies have demonstrated that EBV status of the CHL tumor may delineate distinct biological entities with specific microenvironment and immune regulation [35]. Large studies have confirmed differences in clinical characteristics between EBV⁺ and EBV[–] CHL, with a poorer prognosis for EBV⁺ CHL patients older than 50 years old [36]. In this context, we analyzed the correlation between candidate immune SNPs and disease progression by EBV status, which to our knowledge has not been previously reported.

2. Materials and methods

2.1. Study populations

The first cohort consisted of 464 CHL patients enrolled between 1998 and 2002 and followed through 2009 as part of a prospective study of the GELA assessing the prognostic values of plasma cytokines and soluble receptors [5]. In accordance with French law, race or ethnicity could not be collected; however, given the national scope of this GELA study, the cohort likely reflects the genetic heterogeneity of the French population, which is dominated by Western-European ancestry, as shown by principal components analysis from genome-wide association studies in this population [37]. The second cohort consisted of 239 newly diagnosed CHL patients age 18 years and older prospectively enrolled from 2002 to 2009 and followed through 2011 in the University of Iowa/Mayo Clinic SPORE (Specialized Program of Research Excellence) Molecular Epidemiology Resource [38]. In the SPORE cohort, 202 patients were Caucasian, and the remainder were non-Caucasian ($n = 6$) or unknown/refused ($n = 31$). Patients in both cohorts were negative for human immunodeficiency virus. Both cohorts were systematically followed for disease progression, retreatment and death. A peripheral blood sample for serum, plasma and DNA were collected from all patients at diagnosis in the two studies, and DNA for genotyping was extracted using standard protocols. These studies were approved by the ethics committees of Dijon and Lyon University Hospitals for the GELA study and by the Human Subjects Institutional Review Board at Mayo Clinic and the University of Iowa for the SPORE study, and all patients provided written consent for participation.

2.2. Laboratory analysis

2.2.1. Genotyping—SNPs from candidate genes included *IL10* (rs1800890, rs1800896, rs1800871, rs1800872), *TNFA* (rs1800629), *IL6* (rs1800795) and *CCL17* (rs223828), which were all located in the promoter region; SNPs in *ILRN* (rs419598) and *INFG* (rs2430561) were localized in exon 5 (synonymous SNP) and intron 1, respectively. These SNPs were chosen based on i) their localization in regulatory regions of the genes (*IL10*, *TNFA*, *IL6*, *CCL17*); ii) previous studies showing *in vitro* functional effect of these SNPs on cytokine expression [17, 39]; iii) previous lymphoma studies showing that these SNPs might predict clinical outcome [23–33].

In GELA, genotyping used specific fluorescent dye-labeled (FAM and VIC) MGB probes (Applied Biosystems, Foster City, California, USA). Real-time PCR analysis was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems). All genotyping was performed in duplicate, and agreement was 100%. In the SPORE, the candidate SNPs were genotyped as part of a larger project using a custom Illumina Infinium array (Illumina, San Diego, CA). Standard genotyping quality control procedures were performed and included duplicate genotyping, dropping samples or SNPs with call rates <95%, and testing for Hardy–Weinberg equilibrium (HWE). We found >99.9% genotyping concordance among the 3502 samples with duplicates.

2.2.2. Measurement of cytokines—In GELA, data on plasma cytokine levels were available for IL-10, TNF α , IL-6 and IL-1R α , as previously described [5]. Briefly, plasma samples collected before any treatment (including corticosteroid administration) and were immediately stored at –80 °C in sterile EDTA-containing tubes. Levels were measured in a centralized referent laboratory in duplicate using ELISA kits obtained from Biosource (Nivelles, Belgium) for TNF α , R&D system (Minneapolis, NM, USA) for IL-1R α and Beckman-Coulter (Fullerton, CA, USA) for IL-6 and IL-10. The detection limit of the test was 5 pg/ml for IL-10, 3 pg/ml for TNF α , 3 pg/ml for IL-6 and 22 pg/ml for IL-1R α .

For the SPORE cohort, serum cytokines were measured from pre-treatment blood draws using a multiplex ELISA (Invitrogen, Camarillo, CA, USA). Thirty serum cytokines, including data on IL-10, TNF α , IL-6, IL-1R α , CCL17 reported here, were analyzed using the Luminex-200 system Version 1.7 (Luminex, Austin, Texas, USA) [11]. Data were acquired using STar Station software (Applied Cytometry, Dinnington, Sheffield, UK) and analysis was performed using the MasterPlex QT 1.0 system (MiraiBio, Alameda, CA). Cytokines measured below the limit of detection were assigned a value of (limit of detection/2). Inter-assay variation was assessed by inclusion of an internal control serum on all assay plates; the CV for the internal control log₂ cytokine values across plates ranged from 0.3% (RANTES) to 12.2% (IL-6).

2.2.3. Epstein Barr Virus (EBV) status of the tumor—Tumor samples were available for 242 of 464 GELA CHL patients (52%), and EBV status was determined by immunohistochemical detection of latent membrane protein-1 (LMP-1) in 186 patients (77%), by EBER *in situ* hybridization in 48 patients (20%) or by other techniques in the

remaining 8 patients (3%). Tumor samples were available 104 of the 239 SPORE CHL (48%), and EBV status was determined by EBER *in situ* hybridization.

2.3. Statistical analysis—Progression free survival (PFS) was measured from the date of initiation of therapy (GELA series) or the date of diagnosis (SPORE series) to the date of first relapse, progression, retreatment, or death (any cause); patients without an event were censored at date of last follow-up. Associations between cytokines and genotype were assessed using the Kruskal–Wallis test. Kaplan–Meier curves and Cox proportional hazards models were used to assess the association of each genotype with PFS in both a univariate fashion and after adjusting for the international prognostic score (IPS). A dominant genetic model for each candidate SNP was used based prior publications. Analyses were done independently for the two cohorts and then to increase power, a meta-analysis approach was used to combine results [40]. Analyses were performed using SAS version 9.2 (SAS Institute Inc., Cary, NC, USA) and R (<http://www.r-project.org/>).

3. Results

3.1. Patient characteristics and genotyping of immune SNPs—Clinical characteristics of the patients from the two cohorts are demonstrated in Table 1. In the GELA cohort, the median age was 32 years (range, 15–93), 57% were male, 44% had B-symptoms at diagnosis, 72% had an Ann Arbor stage I–II, and 73% had an IPS score of 0–2. Nodular sclerosis was the main CHL histologic subtype (384 patients, 83%). The EBV status of the tumor was established for 242/464 patients (52%), and was positive in 53 (22%) and negative for 189 (78%) patients. A total of 277 (60%) patients were treated with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine), 102 (22%) with EBVP (epirubicin, bleomycin, cyclophosphamide, vincristine, prednisone), 60 (13%) with BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) and 25 (5%) with other anthracycline-based regimens.

The SPORE cohort was similar to the GELA cohort. The median age was 38 years (range, 18–89), 52% were male, 39% had B-symptoms at diagnosis, 52% had an Ann Arbor stage I–II, and 50% had an IPS score of 0–2. Nodular sclerosis was also the most common subtype (161 patients, 67%). The EBV status of the tumor was established for 104/239 patients (48%), and was positive for 23 (22%) and negative for 81 (78%) patients. ABVD regimen was used in 214 (90%) patients, 12 (5%) patients received Stanford V protocol, three patients (1%) were treated by MOPP (caryolysine, vincristine, vinblastine, procarbazine) and 10 (4%) with other regimens.

Details of the genotype distributions are presented in Table 2. The minor allele frequencies (MAF) of the 9 immune SNPs were very similar between the two series, with the exception of *TNFA*, which had an MAF of 0.12 in GELA compared to 0.20 in the SPORE. Genotype distributions for each SNP by EBV status were consistent with the overall distributions in each cohort.

3.2. Correlation between genotypes and cytokine levels—Based on the results of the initial prospective studies of the prognostic value of cytokine levels [5, 11], we evaluated the correlation between immune SNPs and plasma cytokine levels in the GELA cohort and

serum cytokine levels in the SPORE cohort. IL-10, TNF α , IL-6 and IL-1R α were evaluated in both cohorts, while CCL17 was only evaluated in the SPORE. In GELA, there was no association of plasma cytokine levels and their respective genotypes for *IL10* (4 SNPs), *TNFA*, *IL6* and *IL1RN* (Table 3). In the SPORE cohort, there was also no association with serum cytokine for *IL10*, *TNFA*, *IL6*, *IL1RN* and their respective genotypes. Nor was there any association of *CCL17* genotypes with the serum level of this chemokine (Table 4). When we compared cytokine levels between EBV⁺ and EBV⁻ CHL, the median level of TNF α (36 vs. 27.5 pg/ml, $P = 0.005$) was higher in EBV⁺ compared to EBV⁻ CHL in the GELA. The finding for TNF α (serum) was not observed in the SPORE cohort. For other cytokines, we did not observe any difference of the median level between EBV⁺ and EBV⁻ CHL in the GELA or SPORE cohorts (data not shown).

3.3. Immune SNPs and outcome—After a median follow-up of 6.3 years (range, 0.3–8.2), the 6-year PFS was 81.5% (95% confidence interval [CI], 74.1–84.1) with 78 (17%) patients with a treatment failure (progression or relapse) in the GELA cohort. After a median follow-up of 5 years (range 0.04–9.75), the 6-year PFS was 72.9% (95% CI, 64.8–79.7) with 57 (24%) patients with a treatment failure in the SPORE cohort. The 6-year PFS was similar for EBV⁺ and EBV⁻ CHL (78.3% [68–91] vs. 82.5% [77–88], $P = 0.25$) in the GELA cohort, but EBV⁻ patients had a higher PFS rate compared to EBV⁺ CHL in the SPORE cohort (71.9% [58–89] vs. 29.0% [7–100], $P = 0.007$) noting that EBV⁺ CHL was based on only 23 cases and 11 events.

In both studies for all CHL, there was no association of any of the nine SNPs with PFS (Table 5). The association of SNPs with PFS was subsequently analyzed by tumor EBV status (Table 6). In EBV⁺ CHL, there were no associations of any SNPs with PFS in the GELA cohort, while in the SPORE cohort, there was an association of the *CCL17* with PFS (hazard ratio [HR]=6.97, 95%CI 1.54–31.46; $P = 0.01$), but this was based on a small number of events. In EBV⁻ CHL, *TNFA*–308G>A was associated with PFS in the GELA cohort (HR = 2.41, 95%CI 1.17–4.94; $P = 0.02$). In the SPORE cohort a trend in the same direction was observed (HR = 1.63, 95%CI 0.61–4.40), although this was not statistically significant ($P = 0.33$). Survival curves comparing PFS of EBV⁻ CHL patients with *TNFA*–308GG vs. AG + AA genotypes are shown in Fig. 1. In a meta-analysis of the two studies, the pooled estimate was statistically significant (HR = 2.11, 95%CI 1.18–3.77; $P = 0.01$), and further adjustment for IPS strengthened this result (HR = 2.52, 95%CI 1.39–4.58; $P = 0.002$).

4. Discussion

In these two prospective cohorts, we report that *TNFA*–308G>A was associated with PFS in EBV⁻ CHL, and these results were independent of the IPS. To our knowledge, no previous study has extensively assessed the relation between immune SNPs and CHL prognosis by integrating EBV status of the tumor. The prognostic value of *TNFA*–308G>A SNP was previously observed in diffuse large B-cell lymphoma (DLBCL) patients showing that *TNFA*–308A is associated with poor patient outcome [23, 24] in accordance with our findings observed in EBV⁻ CHL.

EBV⁺ and EBV⁻ CHL appear to represent distinct entities with respect to differences among clinical characteristics, outcome [36] biologic features [41] and etiology [42]. Genetic risk factors differ among EBV⁺ and EBV⁻ CHL, genetic variants within the HLA class I region were found to be more frequently associated with EBV⁺ CHL and those in class II region with EBV⁻ CHL [34]. These results are in accordance with findings showing that T-cell cytotoxic response against EBV is predominantly driven by HLA class I molecules. These data highlight the potential importance of genetic background in the development of either EBV⁺ or EBV⁻ CHL, and raises the hypothesis that different host immune SNPs are involved in the control of the EBV⁺ and EBV⁻ tumors and consequently affect the prognosis of patients differently. In addition, biologic studies have shown that immunologic response was different in EBV⁺ and EBV⁻ CHL with a Th1/antiviral response in EBV⁺ CHL [35] with some specificities for cytokine or chemokine profiles observed in EBV⁺ CHL [43]. In this context, the type of immune SNPs that modulate cytokine expression should be different in EBV⁺ and EBV⁻ CHL.

While we observed no significant results for EBV⁺ CHL, a major limitation is the small number of patients and events in this group (i.e., a total of 76 cases for the two cohorts combined). Our EBV⁻ results were derived from exploratory analyses, and we did not adjust of multiple testing. Thus, further studies are needed to replicate our results in EBV⁻ CHL as well as evaluate these associations in a sufficiently powered study of EBV⁺ CHL.

Analyses for the combined GELA and SPORE cohorts found no correlation between PFS and any of the nine candidate SNPs, confirming the results of previous studies for *IL10* [28, 32] *IL6* [30] and *TNFA* [31]. One study of 184 HL patients (46 in tumor tissue samples) observed an effect of *IL10*-592C>A and *IL6*-174G>C for freedom from treatment failure (FFTF), with *IL10*-592AA and *IL6*-174GG at higher risk of relapse [31]. A recent study on 301 HL patients confirmed the result for the *IL10*-592 SNP, showing that patients with *IL10*-592AA, *IL10*-819TT or *IL10*-1082AA genotypes had an inferior FFTF [33]. In DLBCL, the prognostic impact of promoter *IL10* SNPs is also conflicting [24–28]. To our knowledge, the prognostic role of SNPs in the *IL1RN*, *INFG* and *CCL17* genes for CHL patients have not been investigated previously, and we did not observe an association between these SNPs and patient outcome in the two cohorts.

The *TNFA* variant rs1800629 showed a difference of MAF between the GELA (0.125) and the SPORE (0.199) cohorts, and this remained similar for EBV⁻ and EBV⁺ CHL in each cohort. Technical error in genotyping is not a likely explanation, given the extensive quality controls. Cytokine SNPs can vary by race/ethnicity. In one population-based study, the *TNFA* MAF between African–American and Caucasian healthy women were 0.14 and 0.17, respectively [44]. In a NHL susceptibility study, *TNFA* MAFs were 0.18, 0.15, 0.10, 0.06 in White, Black, Hispanic and Asian NHL patients, respectively [22]. Unfortunately, we could not obtain data on the ethnicity by self-report in the French cohort (not allowed by law), so the role of racial/ethnic mixture on the French cohort is not known, but is not likely to be substantial since most patients are likely to be of Western-European ancestry. In one previous clinical study conducted in France for DLBCL patients, MAF of this variant was 0.17 as compared to 0.16 for healthy French controls [45] and 0.19 for DLBCL patients included in a USA study [24]. Thus, the differences in MAF between the two studies, which

are quite modest and are in the range of prior reports, may just be the result of sampling variability.

In this analysis, we also assessed the association of SNPs from cytokine genes described as functional in previous *in vitro* or *in vivo* studies [17] with circulating cytokines in the context of CHL. Globally, we did not observe a correlation between each SNP genotype and the median level of the related cytokines, either in GELA (plasma) or SPORE (serum) cohorts. The correlation between genotypes and cytokine levels has been mostly derived from *in vitro* studies, and cytokines were generally measured in the peripheral blood mononuclear cell (PBMC) supernatant with different leukocyte stimulation assays [17]. We could not confirm prior studies in CHL showing that *IL6*–174G>C, *IL10*–592C>A and *IL10*–1082A>G were associated with related cytokine levels [46, 47], or that a higher TNF α cytokine production is observed in NHL patients with *TNFA*–308A allele [23]. In fact, regarding our significant outcome results for *TNFA*–308G>A in EBV[–] CHL, *TNFA*–308A and *TNFA*–308GG patients had similar TNF α levels in two different analyzed supernatants.

One hypothesis to explain the difference of prognosis between *TNFA*–308A and *TNFA*–308GG patients is that the variant may influence the cytokine variability in the tumor microenvironment that is not reflected by the circulating TNF α levels. At a molecular level, SNPs in *TNFA* promoter could influence the binding of transcriptional factors as NF- κ B p50–p50 dimers or OCT-1 with potential consequences on gene expression [48, 49]. In the same chromosome 6 locus, there is a functional SNP in the promoter of inhibitory kappaB-like gene, *I κ BL*–63A>T (rs2071592) in complete linkage of disequilibrium with *TNFA*–308G>A [50]. However, there are no data whether the I κ BL protein has a similar function to I κ B. The HRS cell is characterized by a global cellular reprogramming with the deregulation of many transcriptional factors [51] and EBV participates to the down-regulation of the B-cell phenotype in HRS [52, 53]. Interestingly, a recent genome-wide transcriptional analysis of micro-dissected HRS cells demonstrated that relatively few transcriptional changes were driven by the presence of EBV in HRS cells, leading the authors to hypothesize that EBV might influence gene expression in the tumor microenvironment rather than impacting the HRS cells [54] as observed by Chetaille et al. with a Th1/antiviral response in EBV⁺ CHL [35]. In this context, it will be interesting to study whether germline SNPs in immune mediators affect the composition of reactive cells of tumor microenvironment, which is known to influence prognosis of patients [16, 55] and if EBV status further impacts these findings.

5. Conclusions

We found in two prospective cohorts of representative CHL patients that SNPs in the promoter of *TNFA* influenced progression free survival in patients with EBV[–] CHL, independent of IPS. Our study was limited by the small number of patients evaluated for EBV⁺ CHL, and lack of a controlled treatment trial setting for the SPORE. However, the two cohorts were very similar from a clinical perspective, and should be highly generalizable to CHL in Caucasian patients. The cytokine targets chosen in this study, although previously identified in several reports as good candidates for prognostication of CHL patients, are unlikely to fully reflect the complex network of immune mediators

involved in the pathogenesis of CHL. Given the complexity of immune interactions in CHL, further studies using whole-genome scans [34, 56] or sequencing in large cohorts are necessary to determine the prognostic impact of inheritable factors in this disease, integrating data related to EBV status with subsequently functional studies.

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Fig. 1.
 Progression free survival of EBV negative classical Hodgkin lymphoma patients by *TNFA* -308G>A polymorphism in GELA and SPORE cohorts.

Table 1

Clinical and biologic characteristics of classical Hodgkin lymphoma patients.

	GELA cohort				SPORE cohort			
	All	EBV ⁺	EBV ⁻	<i>p</i> ^a	All	EBV ⁺	EBV ⁻	<i>p</i> ^a
	<i>N</i> = 464	<i>N</i> = 53	<i>N</i> = 189		<i>N</i> = 239	<i>N</i> = 23	<i>N</i> = 81	
	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)		<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	
Median age	32	37	33	0.127	38	27	38	0.26
Range	15–93	17–89	15–93		18–89	21–73	18–81	
Age 45 years	101 (22)	13 (25)	46 (24)	0.98	92 (39)	5 (22)	30 (37)	0.17
Male	264 (57)	43 (81)	103 (54)	0.001	124 (52)	17 (74)	40 (49)	0.09
ECOG PS								
0–1	395 (97)	42 (98)	158 (95)	0.40	205 (86)	17 (74)	68 (84)	0.36
2–4	12 (3)	1 (2)	9 (5)		34 (14)	6 (26)	13 (16)	
Histological subtype								
Nodular sclerosis	384 (83)	39 (74)	161 (85)	0.07 ^b	161 (67)	14 (61)	62 (77)	0.06 ^b
Mixed cellularity	40 (9)	7 (13)	11 (6)		27 (11)	5 (22)	7 (9)	
Diffuse lymphocyte predominance	5 (1)	0 (0)	2 (1)		12 (5)	1 (4)	6 (7)	
Lymphocyte depletion	2 (<1)	1 (2)	0 (0)		0 (0)	0 (0)	0 (0)	
Unclassified	33 (7)	6 (11)	15 (8)		39 (17)	3 (13)	6 (7)	
Ann Arbor stage								
I–II	335 (72)	29 (55)	105 (56)	0.76	122 (52)	10 (45)	39 (49)	0.79
III–IV	129 (27)	24 (45)	84 (44)		113 (48)	12 (55)	41 (51)	
B symptoms	206 (44)	28 (53)	91 (48)	0.55	92 (39)	14 (61)	27 (33)	0.02
Hemoglobin level <10.5 g/dl	64 (14)	11 (21)	30 (16)	0.62	32 (14)	3 (14)	11 (14)	0.97
ESR 50	196 (44)	17 (34)	91 (51)	0.04	50 (34)	6 (43)	18 (38)	0.73
White-cell count 15,000/mm ³	85 (18)	4 (8)	41 (22)	0.01	30 (13)	2 (10)	15 (19)	0.31
Lymphocyte count <600/mm ³	40 (9)	3 (6)	21 (11)	0.20	29 (13)	6 (29)	12 (15)	0.13
Albumin level <40 g/l	191 (45)	22 (42)	88 (47)	0.15	103 (51)	9 (45)	34 (48)	0.82
Hasenclever score								
0	60 (14)	3 (6)	21 (12)	0.94	0 (0)	0 (0)	0 (0)	0.92
1	139 (33)	16 (33)	47 (28)		36 (15)	3 (13)	12 (15)	

	GELA cohort				SPORE cohort			
	All	EBV ⁺	EBV ⁻	<i>p</i> ^a	All	EBV ⁺	EBV ⁻	<i>p</i> ^a
	<i>N</i> = 464	<i>N</i> = 53	<i>N</i> = 189		<i>N</i> = 239	<i>N</i> = 23	<i>N</i> = 81	
	<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)		<i>N</i> (%)	<i>N</i> (%)	<i>N</i> (%)	
2	111 (26)	13 (27)	37 (22)		84 (35)	10 (44)	26 (32)	
3	64 (15)	11 (22)	33 (20)		64 (27)	3 (13)	23 (28)	
4	47 (12)	6 (12)	29 (18)		55 (23)	7 (30)	20 (25)	

EBV, Epstein Barr Virus; ECOG, Eastern Cooperative Oncology Group; PS, performance status.

^a *P*-value compares EBV⁺ and EBV⁻ classical Hodgkin lymphoma.

^b *P*-value compares nodular sclerosis and mixed cellularity histology subtype.

Table 2

Genotype distribution of immune SNPs in Hodgkin lymphoma, overall and by EBV status.

	Genotype	GELA cohort				SPORE cohort			
		All	EBV ⁺	EBV ⁻	P	All	EBV ⁺	EBV ⁻	P
		N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
<i>IL10</i> -3575T>A	N	448	49	182		239	23	81	
rs1800890	TT	200 (44)	22 (45)	86 (47)	0.95	112 (47)	13 (57)	36 (45)	0.24
	TA	204 (46)	23 (47)	81 (45)		97 (41)	9 (39)	35 (43)	
	AA	44 (10)	4 (8)	15 (8)		30 (12)	1 (4)	10 (12)	
<i>IL10</i> -1082A>G	N	459	52	186		239	23	81	
rs1800896	AA	154 (34)	17 (33)	69 (37)	0.84	82 (34)	11 (48)	27 (33)	0.35
	AG	232 (50)	27 (52)	91 (49)		107 (45)	8 (35)	40 (50)	
	GG	73 (16)	8 (15)	26 (14)		50 (21)	4 (17)	14 (17)	
<i>IL10</i> -819C>T	N	446	49	181		239	23	81	
rs1800871	CC	251 (56)	26 (53)	99 (55)	0.76	128 (54)	10 (43)	42 (52)	0.50
	CT	165 (37)	20 (41)	66 (36)		99 (41)	11 (48)	33 (41)	
	TT	30 (7)	3 (6)	16 (9)		12 (5)	2 (9)	6 (7)	
<i>IL10</i> -592C>A	N	447	49	182		239	23	81	
rs1800872	CC	251 (56)	26 (53)	99 (54)	0.77	128 (54)	10 (43)	42 (52)	0.50
	AC	166 (37)	20 (41)	67 (37)		99 (41)	11 (48)	33 (41)	
	AA	30 (7)	3 (6)	16 (9)		12 (5)	2 (9)	6 (7)	
<i>TNFA</i> -308G>A	N	464	53	189		239	23	81	
rs1800629	GG	357 (77)	41 (77)	148 (78)	0.61	154 (65)	14 (61)	54 (67)	0.58
	AG	98 (21)	12 (23)	38 (20)		75 (31)	8 (35)	25 (31)	
	AA	9 (2)	0 (0)	3 (2)		10 (4)	1 (4)	2 (2)	
<i>IL6</i> -174G>C	N	201	24	84		239	23	81	
rs1800795	GG	85 (42)	10 (42)	35 (42)	0.56	84 (35)	10 (43)	24 (29)	0.21
	CG	85 (42)	12 (50)	35 (42)		112 (47)	10 (43)	41 (51)	
	CC	31 (16)	2 (8)	14 (16)		43 (18)	3 (13)	16 (20)	
<i>ILIRN</i> +2018T>C	N	199	23	83		239	23	81	
rs419598	TT	109 (55)	13 (57)	45 (54)	0.88	132 (55)	17 (74)	44 (54)	0.13

	Genotype	GELA cohort				SPORE cohort			
		All	EBV ⁺	EBV ⁻	P	All	EBV ⁺	EBV ⁻	P
		N (%)	N (%)	N (%)		N (%)	N (%)	N (%)	
<i>INFG</i> +874A>T rs2430561	TC	78 (39)	9 (39)	32 (39)		88 (37)	4 (17)	29 (36)	
	CC	12 (6)	1 (4)	6 (7)		19 (8)	2 (9)	8 (10)	
	N	200	24	83		239	23	81	
	AA	54 (27)	7 (30)	24 (29)	0.89	61 (26)	6 (26)	20 (25)	0.58
	TA	97 (48)	12 (50)	38 (46)		129 (54)	15 (65)	48 (59)	
<i>CCL17</i> -431C>T rs223828	TT	49 (25)	5 (20)	21 (25)		49 (20)	2 (9)	13 (16)	
	N	198	24	82		239	23	81	
	CC	178 (90)	22 (92)	75 (91)	0.97	213 (89)	20 (87)	74 (91)	0.53
	TC	19 (10)	2 (8)	7 (9)		25 (11)	3 (13)	7 (9)	
	TT	1 (<1)	0 (0)	0 (0)		1 (<1)	0 (0)	0 (0)	

Table 3

Plasma cytokine levels by SNP genotype, GELA cohort.

	Whole cohort				EBV+				EBV-			
	N (%)	Median	Range	P	N (%)	Median	Range	P	N (%)	Median	Range	P
<i>IL10</i>-3575T>A												
rs1800890	TT 196 (44)	12	0-2952	0.29	20 (43)	17	0-326	0.13	85 (47)	12	0-2952	0.42
n = 442	TA 202 (46)	11	0-24		23 (49)	9	0-96		79 (45)	7	0-194	
	AA 44 (10)	6	0-137		4 (8)	0	0-17		15 (8)	12	0-86	
<i>IL10</i>-1082A>G												
rs1800896	AA 151 (33)	11	0-2952	0.76	16 (33)	25.5	0-316	0.08	68 (37)	8	0-2952	0.87
n = 452	AG 229 (51)	11	0-932		26 (53)	16.1	0-96		89 (49)	10	0-194	
	GG 72 (16)	9	0-166		7 (14)	0	0-32		26 (14)	11.3	0-108	
<i>IL10</i>-819C>T												
rs1800871	CC 249 (57)	11	0-931	0.80	25 (53)	17	0-147	0.39	98 (56)	11	0-113	0.43
n = 440	CT 161 (36)	9	0-2952		19 (40)	6	0-316		65 (37)	7	0-2952	
	TT 30 (7)	14	0-183		3 (7)	15	0-41		16 (7)	24	0-183	
<i>IL10</i>-592C>A												
rs1800872	CC 249 (56)	11	0-931	0.80	25 (53)	17	0-147	0.39	98 (56)	11	0-113	0.45
n = 440	AC 162 (37)	9	0-2952		19 (40)	6	0-316		65 (37)	7	0-2952	
	AA 30 (7)	14	0-183		3 (7)	15		0-41	16 (7)	24	0-183	
<i>TNFA</i>-308G>A												
rs1800629	GG 357 (77)	27	6-248	0.17	38 (76)	37	14-143	0.48	144 (78)	28	6-248	0.73
n = 464	AG 98 (21)	26	8-236		12 (24)	33	12-147		37 (20)	27	13-133	
	AA 9 (2)	21	11-36		0 (0)	-	-		3 (2)	21	16-36	
<i>IL6</i>-174G>C												
rs1800795	GG 84 (43)	15	0-390	0.33	9 (43)	24	0-75	0.21	35 (42)	15	0-390	0.68
n = 197	CG 84 (43)	15		0-99	11 (52)	16	4-99		35 (42)	12	0-66	
	CC 29 (14)	22		2-300	1 (5)	300	-		13 (16)	20	3-300	
<i>IL1RN</i>+2018T>C												
rs419598	TT 108 (55)	500	20-5000	0.85	12 (55)	389	82-4614	0.70	45 (55)	559	141-3500	0.59
n = 197	TC 77 (39)	540	26-4000		9 (41)	700	168-1650		31 (38)	531	189-1400	

Whole cohort				EBV ⁺			EBV ⁻		
	N (%)	Median	Range	P	N (%)	Median	Range	P	P
CC	12 (6)	523	242–1300		1 (4)	423	–	6 (7)	367–1300

Table 4

Serum cytokine levels by SNP genotype, SPORE cohort.

	Whole cohort					EBV+			EBV-				
	N (%)	Median	Range	P	N (%)	Median	Range	P	N (%)	Median	Range	P	
<i>IL10</i> -3575T>A													
rs1800890	TT	59 (46)	23	3-1352	0.64	7 (50)	22	10-59	0.12	19 (42)	18	3-1106	0.22
<i>n</i> = 129	TA	55 (43)	27	3-167		7 (50)	35	11-167		20 (45)	26	2-80	
	AA	15 (11)	28	6-89		4 (0)	-	-		6 (13)	30	22-36	
<i>IL10</i> -1082A>G													
rs1800896	AA	42 (33)	22	3-1106	0.40	6 (43)	22	10-126	0.47	12 (27)	19	3-1106	0.31
<i>n</i> = 129	AG	58 (45)	27	3-1352		7 (50)	27	11-167		25 (55)	26	3-80	
	GG	29 (22)	28	3-89		1 (7)	65	-		8 (18)	30	15-37	
<i>IL10</i> -819C>T													
rs1800871	CC	71 (55)	27	3-1352	0.57	5 (36)	27	16-65	0.39	24 (53)	27	3-517	0.67
<i>n</i> = 129	CT	51 (40)	24	3-1106		7 (50)	29	10-107		18 (40)	19	3-1106	
	TT	7 (5)	24	8-61		2 (14)	35	10-59		3 (7)	23	8-37	
<i>IL10</i> -592C>A													
rs1800872	CC	71 (55)	27	3-1352	0.57	5 (36)	27	16-65	0.39	24 (53)	27	3-517	0.67
<i>n</i> = 129	AC	51 (40)	24	3-1106		7 (50)	29	10-107		18 (40)	19	3-1106	
	AA	7 (5)	24	8-61		2 (14)	35	10-59		3 (7)	23	8-37	
<i>TNFA</i> -308G>A													
rs1800629	GG	81 (63)	6	3-333	0.32	10 (71)	3	3-333	0.48	28 (62)	3	3-242	0.59
<i>n</i> = 129	AG	44 (34)	3	3-44		4 (29)	3	3-34		16 (36)	4	3-10	
	AA	4 (3)	5	3-231		0 (0)	-	-		1 (2)	3	-	
<i>IL6</i> -174G>C													
rs1800795	GG	45 (35)	12	2-409	0.87	7 (50)	39	6-130	0.21	15 (34)	10	2-409	0.94
<i>n</i> = 129	CG	68 (53)	13	2-1430		6 (43)	6	1-63		24 (53)	12	1-1430	
	CC	16 (12)	7	2-252		1 (7)	81	-		6 (13)	10	2-70	
<i>IL1RN</i> +2018T>>C													
rs419598	TT	68 (53)	644	15-4459	0.33	10 (71)	1176	15-4459	0.20	22 (49)	587	331-1182	0.07
<i>n</i> = 129	TC	52 (40)	676	64-4762		4 (29)	606	394-1042		19 (42)	734	516-3189	

		Whole cohort			EBV+			EBV-		
		N (%)	Median	Range	P	N (%)	Median	Range	P	P
<i>CCL17</i> –431C>T	CC	9 (7)	550	184–1225		0 (0)	–	–	4 (9)	685 184–1225
	CC	101 (91)	1633	107–4832	0.53	10 (83)	1015	181–4004	37 (95)	2170 239–4832 0.44
	TC	10 (9)	1767	325–3452		2 (17)	2526	1894–3158	2 (5)	1333 511–2155
	TT	0 (0)	–	–		0 (0)	–	–	0 (0)	– –

Table 5
Association of immune SNPs with progression-free survival in classical Hodgkin lymphoma.

Gene	SNP	Model ^a	GELA cohort			SPORC cohort			Combined		
			MAF	HR (95%CI)	P	MAF	HR (95%CI)	P	HR (95%CI)	P	
<i>IL10</i> -3575T>A	rs1800890	TT vs. TA + AA	0.33	1.04 (0.65, 1.64)	0.87	0.33	0.83 (0.49, 1.39)	0.47	0.94 (0.67, 1.33)	0.73	
<i>IL10</i> -1082A>G	rs1800896	AA vs. AG + GG	0.41	1.07 (0.66, 1.73)	0.77	0.43	1.20 (0.68, 2.11)	0.52	1.13 (0.78, 1.62)	0.52	
<i>IL10</i> -819C>T	rs1800871	CC vs. CT + TT	0.25	1.34 (0.85, 2.10)	0.21	0.25	0.82 (0.49, 1.39)	0.47	1.09 (0.77, 1.53)	0.63	
<i>IL10</i> -592C>A	rs1800872	CC vs. AC + AA	0.25	1.33 (0.84, 2.09)	0.22	0.25	0.82 (0.49, 1.39)	0.47	1.08 (0.77, 1.53)	0.64	
<i>TNFA</i> -308G>A	rs1800629	GG vs. AG + AA	0.13	1.15 (0.69, 1.91)	0.58	0.20	1.19 (0.70, 2.03)	0.52	1.17 (0.81, 1.69)	0.40	
<i>IL6</i> -174G>C	rs1800795	GG vs. GC + CC	0.37	0.73 (0.39, 1.37)	0.33	0.41	1.30 (0.74, 2.30)	0.36	1.00 (0.66, 1.53)	0.98	
<i>IL1RN</i> +2018T>C	rs419598	TT vs. TC + CC	0.20	0.80 (0.41, 1.54)	0.51	0.26	0.86 (0.51, 1.45)	0.57	0.81 (0.53, 1.23)	0.32	
<i>IFNG</i> +874A>T	rs2430561	AA vs. TA + TT	0.51	0.81 (0.40, 1.62)	0.55	0.47	0.78 (0.44, 1.38)	0.39	0.79 (0.51, 1.23)	0.30	
<i>CCL17</i> -431C>T	rs223828	CC vs. TC + TT	0.05	1.06 (0.37, 2.96)	0.92	0.06	0.93 (0.41, 2.09)	0.85	0.97 (0.51, 1.84)	0.93	

MAF, minor allele frequency; HR, hazard ratio; CI, confidence interval.

^aDominant model.

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