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Polymorphisms in immune function genes and non-Hodgkin lymphoma survival

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Abstract

Introduction—Cytokines play a critical role in regulating the immune system. In the tumor microenvironment, they influence survival, proliferation, differentiation, and movement of both tumor and stromal cells, and regulate tumor interactions with the extracellular matrix. Given these

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biologic properties, there is reason to hypothesize that cytokine activity influences the pathogenesis of non-Hodgkin lymphoma (NHL).

Methods—We investigated the effect of genetic variation in cytokine genes on NHL prognosis and survival by evaluating genetic variation in individual SNPs as well as the combined effect of multiple deleterious genotypes. Survival information from 496 female incident NHL cases diagnosed during 1996–2000 in Connecticut were abstracted from Connecticut Tumor Registry in 2008. Survival analyses were conducted by comparing Kaplan-Meier curves and hazard ratios (HR) were computed using Cox proportional hazard models adjusting for demographic and tumor characteristics for genes that were suggested by previous studies to be associated with NHL survival.

Results—We found that the variant *IL6* genotype is significantly associated (HR=0.42; 95%CI: 0.23–0.77) with a decreased risk of death, as well as relapse and secondary cancer occurrence, among those with NHL. We also found that risk of death, relapse, and secondary cancers varied by specific SNPs for the follicular, DLBCL, and CLL/SLL histologic types. We identified combinations of polymorphisms whose combined deleterious effect significantly alter overall NHL survival and disease-free survival.

Conclusion—Our study provides evidence that the identification of genetic polymorphisms in cytokine genes may help improve the prediction of NHL survival and prognosis.

Keywords

Non-Hodgkin lymphoma; Cytokines; Single nucleotide polymorphisms; Survival

Introduction

Although the median survival for non-Hodgkin lymphoma (NHL) is approximately 10 years, the course of the disease is highly variable, progressing slowly for indolent tumors and very rapidly for aggressive tumors [1]. Studies have shown that NHL survival patterns vary by subtype [2,3], suggesting different prognostic risk factors for NHL histological subtypes. Established adverse prognostic factors for NHL include older age at diagnosis, higher tumor stage, poor performance score, extranodal involvement and above-normal lactate dehydrogenase [4]. The investigation of the impact of genetic variation in cytokines on NHL pathogenesis and prognosis is of interest as cytokines are secreted proteins that play a critical role in regulating the immune system; they control lymphoid cell development and differentiation, and regulate the balance between the T-helper immune responses, as well as proliferation, differentiation, and the movement and communication between tumor and stromal cells [5]. Given these biologic properties, investigation of the association between variation in cytokine gene expression and the progression of NHL is important in light of the increasing incidence of NHL observed in recent decades [6].

Since the expression of T-helper cytokines can be altered by germ-line genetic variants, we studied the association between common polymorphisms in *Th1* and *Th2* cytokine genes and the risk of NHL in this study population previously [7]. We found that SNPs in critical genes, *IL4*, *IL5*, *IL6*, and *IL10*, were associated with risk of NHL. Subsequent analyses by NHL subtype showed that variants in *IL10* and *IL5* were significantly associated with an increased risk of B-cell lymphoma, and variants in *IL4*, *IL4R*, and *IL6* were significantly associated with an altered risk of T-cell lymphoma. The results raised the possibility that a shift in the balance of the Th1/Th2 response caused by genetic variants in key cytokine genes could have important consequences for the pathogenesis of NHL. In addition to investigation of NHL risk, it is possible that cytokine gene expression plays a role in the progression of NHL.

To more broadly test the hypothesis that inherited variability in cytokine and related immune genes affects NHL survival and disease free survival, we evaluated the association of 82 single nucleotide polymorphisms (SNPs) from 40 candidate immune genes (Table 1) in study participants that have been followed post disease diagnosis. We chose candidate SNPs from genes involved in key immune pathways, particularly those related to cytokine regulation and function in a population-based case-control study among women in Connecticut. We hypothesized that the genetic variation in the cytokines and the combined effect of multiple deleterious genotypes affect NHL prognosis and survival, and we examined the associations by NHL subtype.

Materials and methods

Study population

The study population has been described elsewhere [8,9]. In brief, a total of 1,122 potential female NHL cases aged between 21 and 84 years were identified between 1996 and 2000 through the Yale Comprehensive Cancer Center's Rapid Case Ascertainment Shared Resource (RCA), a component of the Connecticut Tumor Registry (CTR). Among those cases, 167 died before they could be interviewed and 123 were excluded because of doctor refusal, previous diagnosis of cancer (excluding non-melanoma skin cancer), or inability to speak English. Of 832 remaining eligible cases, 601 completed an in-person interview. Pathology slides or tissue blocks were obtained from the hospitals where the cases had been diagnosed. The specimens were reviewed by two independent study pathologists. All NHL cases were classified according to the World Health Organization (WHO) classification system [10,11]. Vital status for these NHL cases was abstracted at the CTR in May-October 2008. Other follow-up information was also abstracted, including date of death, most recent follow-up date, type and date of treatments, dates of relapse and/or secondary cancer, B-symptoms, and tumor stage. There were 250 deaths from any cause and 140 deaths from NHL in the study population. Of the 601 cases, 13 were not able to be identified in the CTR system, 13 were found to have a cancer history prior to diagnosis of NHL, and 79 had genotyping data missing, yielding 496 NHL patients in the final analyses. Of these, 155 had diffuse large B-cell lymphoma (DLBCL); 117 had follicular lymphoma (FL); 57 had chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL); 34 had marginal zone B-cell lymphoma (MZBL); and 37 had T/NK-cell lymphoma (T-cell).

Genotyping

DNA was extracted from blood or buccal cell samples using phenol–chloroform extraction. Genotyping was conducted at the National Cancer Institute Core Genotyping Facility (Advanced Technology Center, Gaithersburg, Maryland, USA) using validated assays on the Taqman (Applied Biosystems, Foster City, California, USA) or MGB Eclipse (Epoch Biosciences, Bothell, Washington, USA) platforms. Sequence data and assay conditions available at: http://snp500cancer.nci.nih.gov [12]. We selected 82 single nucleotide polymorphisms (SNPs) in 40 immune function genes from the designable set of common SNPs (minor allele frequency >5%) genotyped in the Caucasian (CEU) population sample of the HapMap Project (Data Release 20/Phase II, NCBI Build 35 assembly, dpSNPb125) using the software Tagzilla [http://tagzilla.nci.nih.gov/], which implements a tagging algorithm based on the pairwise binning method of Carlson et al. (Table 1) [13]. All 82 SNPs were genotyped in blood-based DNA samples because DNA samples from buccal cell were insufficient for this analysis. When there were multiple transcripts available for genes, the primary transcript was assessed. Duplicate samples from 100 study subjects and 40 replicate samples from each of two blood donors were interspersed throughout the plates used for genotype analysis. The concordance rates for quality control samples were 99–

100% for all assays. We observed no significant departure from Hardy-Weinberg equilibrium in the control population for any of the SNPs analyzed ($P>0.05$).

Statistical analysis

Survival analyses were conducted for both overall survival (OS) and disease-free survival (DFS). In OS analysis, deaths were events and being alive was censoring. In DFS analysis, deaths, relapses and occurrences of secondary cancer were events and otherwise were censorings.

We assumed the Cox proportional hazards (PH) model and estimated hazard ratios (HR) and 95% confidence intervals (95% CI) for the association of each individual genotype with OS and DFS. The homozygote of the most common allele was used as the reference group and coded as 0, and the heterozygote and homozygote variant genotypes were grouped together and coded as 1. The reference groups with a homozygote wild-type genotype were coded as 0, and the heterozygote and homozygote variant genotypes were grouped together to increase power and coded as 1. For most genotypes, the frequency of homozygote variants does not permit analysis. As such, we used the combined approach. Age (continuous), education (high school or less, some college, and college graduate or more), stage (I, II, III, IV, and unknown), B-symptom (yes, no) and initial treatment (none, radiation only, chemotherapy-based regimen, and other) were adjusted as *a priori* confounder variables. Adjustment for race did not result in material changes of the associations, and thus race was not included in the final model.

A multi-SNP model was fitted for NHL and subtypes respectively. First, the SNPs with p-values <0.15 from above single-SNP analysis were selected and fit in a Cox model adjusting for age, education, stage, B-symptom and initial treatment. Then we ran stepwise (backwards) selection for the Cox model to retain a parsimonious number of SNPs while keeping *a priori* confounders in the model. We specified a number of criteria for variable deletion including the statistic for a variable to be added must be significant (0.20) and the value at which any variable that does not produce an *F* statistic significant should be deleted (0.20). Such selection was done for NHL overall as well as each subtype (for both overall survival and disease free survival). A group of four SNPs that concurrently affect NHL survival was identified and the deleterious genotypes were identified from the single-SNP analysis. Kaplan-Meier survival curves were plotted by the number of deleterious genotypes (0, 1, 2, or ≥3) for NHL overall and subtypes. Log-rank statistics were computed to evaluate the difference in survival. The false discovery rate (FDR) method was applied to adjust for multiple comparisons. The FDR provides the expected ratio of erroneous rejections of the null hypothesis compared to the total number of rejected hypotheses. We set FDR=0.2, then calculated p^2=FDR*rank/n, where n is total number of comparisons and determined the value to be significant if p^2>=p.

Given the parameters of the respective models, we computed the log likelihood of the data for the null and alternative models to determine whether the models including the gene combinations improved predictability. We compared the likelihood ratio to a chi-square test statistic. If, the likelihood ratio was greater than the critical value, we rejected the null hypothesis and concluded that the model including the SNPs made a significant improvement to the model without the SNP data.

Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC). The study was approved by the Human Investigation Committee at Yale University and the Connecticut Department of Public Health.

Results

Demographic and tumor characteristics for the 496 NHL cases are presented in Table 2. During follow-up, 211 deaths, 17 recurrence of NHL, and 63 secondary cancers occurred. The mean overall survival was 6.92 years (SD=3.13, range: 0.38–11.68) and the mean disease free survival was 6.61 years (SD=3.20, range: 0.04–11.68).

Frequencies of SNPs in cytokine genotypes in NHL overall and subtypes are presented in Supplemental Table 1. The non-genotyped rates ranged from 8.5% (*IL8RB, ICAM1, FCGR2A*) to 56.2% (*IL4R*).

As shown in Table 3, there was one SNP in *IL6* significantly associated (HR=0.42; 95%CI: 0.23–0.77) with the risk of death in NHL cases after adjusting for demographic and clinical risk factors (Table 3). When NHL subtypes were examined separately, one SNP in *SELE* (rs5361) was found to be significantly associated (HR=0.42; 95%CI: 0.20–0.89) with the risk of death in DLBCL patients; seven SNPs, including *CCR2* (rs1799864) (HR=0.27; 05%CI: 0.08–0.86), *IFNGR1* (rs3799488) (HR=3.19; 95%CI: 1.09–9.34), *IL4R* (rs1801275) (HR=2.35; 95%CI: 1.07–5.19), *IL8* (rs4073) (HR=2.60; 95%CI: 1.10–6.15), *IL8* (rs2227307) (HR=2.57; 95%CI: 1.07–6.17), *MIF* (rs755622) (HR=2.45; 95%CI: 1.09–5.47) and *SELE* (rs5361) (HR=0.10; 95%CI:0.02–0.48) were significantly associated with the risk of death in FL patients; and three SNPs in *IFNGR2* (rs1059293) (HR=3.02; 95%CI: 1.06–8.16), *IL8RB* (rs1126579) (HR=3.54; 95%CI: 1.38–9.06), and *TGFBR1* (rs868) (HR=0.29; 95%CI: 0.09–0.87) were significantly associated with the risk of death in CLL/SLL patients (Table 3).

One SNP in *IL6* (rs1800796) was found to be significantly associated (HR=0.39; 95%CI: 0.22–0.71) with risk of death, relapse or secondary cancer in overall NHL cases (Table 4). When NHL subtypes were examined separately, two SNPs in *FCGR2A* (rs1801274) (HR=1.78; 95%CI: 1.04–3.04) and in *TGFBR1* (rs868) (HR=1.85; 95%CI: 1.02–3.33) were associated with an increased risk of death, relapse or secondary cancer in DLBCL survivors; three SNPs in *IFNGR1* (rs3799488) (HR=2.63; 95%CI: 01.00–6.91), *IL4R* (rs1801275) (HR=2.53; 95%CI: 1.22–5.21), and *SELE* (rs5361) (HR=0.26; 95%CI: 0.09–0.78) were associated with a risk of death, relapse or secondary cancer in FL survivors; four SNPs in *TGFBR1* (rs868) (HR=0.26; 95% CI: 0.09–0.77), *IL8RB* (rs1126579) (HR=3.33; 95% CI: 1.35–8.20), *CSF2* (rs25882) (HR=2.63; 95%CI: 0.99–6.97), and *CCR5* (rs1800940) (HR=0.39; 95% CI: 0.16–0.95) was associated with risk of death, relapse or secondary cancer in CLL/SLL survivors (Table 4).

With backward selection for OS models, three out of 10 SNPs were identified as risk SNPs for NHL OS: *IL6* (rs1800796), *CX3CR1* (rs3732379), *IL4R* (rs1805011); the four out of 10 SNPs were identified as risk SNPs for DLBCL OS: *IL12A* (rs582054), *SELE* (rs5361), *TGFBR1* (rs868), *CTLA4* (rs231775); four out of 11 risk SNP for FL OS were identified as *SELE* (rs5361), *IFNGR1* (rs3799488), *IL6* (rs1800796), *CCR2* (rs1799864); and four out of 8 SNPs were identified as risk SNPs for CLL/SLL OS: *CSF2* (rs25882), *IL8RB* (rs1126579), *IFNGR2* (rs1059293), *IL4* (rs2243250). The homogeneity of OS curves by number of deleterious genotypes and p-values for log-rank tests for NHL overall and subtype cases are shown in Fig. 1. We identified significantly different effects of increasing deleterious genotypes for NHL overall (p=0.0155), for the follicular (p=0.0052) and CLL/SLL (p=0.0428) subtypes, but not for DLBCL (p=0.2175).

With backward selection for DFS models, four out of 8 SNPs were identified as risk SNPs for NHL DFS: *IL4R* (rs1805011), *IL6* (rs1800796), *CX3CR1* (rs3732379), *CCR5* (rs179907); *IL4R* (rs1805011), *TGFBR1* (rs868), *CTLA4* (rs231775), *IL8RA* (rs2234671) were identified as the four risk SNPs for DLBCL DFS; *CARD15* (rs2066844), *IFNGR1*

(rs3799488), *IL4R* (rs1801275), and *IL10* (rs3024509) were identified as risk SNPs for FL DFS; and *CSF2* (rs25882), *IL8RB* (rs1126579), *IFNGR2* (rs1059293), *CCR5* were identified as the four risk SNPs for CLL/SLL DFS. The homogeneity of DFS curves by number of deleterious genotypes and p-values for log-rank tests for NHL overall and subtype cases are shown in Fig. 2. We identified significantly different effects of increasing deleterious genotypes for NHL overall (p=0.0016), for follicular (p=0.0012), and CLL/SLL (p=0.0390) subtypes, but not for DBLCL (p=0.1373).

Discussion

Using a population-based sample of NHL cases diagnosed from 1996 to 2000 and followed through 2008, we identified 11 SNPs from 9 cytokine and related immune regulation genes that were associated with overall survival for NHL and NHL subtypes, and 10 SNPs from 9 cytokine and related immune regulation genes that were associated with overall survival, relapse, or secondary cancer for NHL and NHL subtypes. From these SNPs, we then identified the strongest predictors of survival and combined them into a common carrier model that summed the number of deleterious genotypes. Combining the SNPs with clinical and demographic factors significantly increased the predictive ability of the model over a model restricted to clinical and demographic factors. We were able to identify models that were significantly different based on the number of deleterious genotypes for NHL and the investigated NHL subtypes.

For NHL overall, the clearest signal for both overall survival as well as disease free survival was due to a polymorphism in *IL6*. IL6 is a pleiotropic pro- and anti-inflammatory cytokine and tumor growth factor implicated in the pathogenesis of AIDS-related non-Hodgkin's lymphoma and Kaposi's sarcoma [KS]. This SNP has been shown to affect both the transcription and secretion of IL6 [14, 15]. Several studies have suggested the involvement of a disordered production of IL6 in lymphomas [15– 19] and elevated levels of IL6 have been observed in serum from patients with newly diagnosed lymphomas [20]. Patients with elevated serum levels of IL6 has also been shown to have a poorer overall survival rate as compared with patients with normal levels [21]. The significance of IL6 as a prognostic marker in our study is consistent with such reports. In addition to *IL6*, we also found a signal from *CCR5* for NHL survival. The *CCR5* gene encodes a chemokine receptor protein that plays an important role in many immune-related processes [14] and the variant alleles have been shown to increase the risk of developing AIDs-related NHL though the role in NHL survival has not been evaluated previously.

For the follicular subtype, we found that SNPs in *IFNGR1*, *IL4R*, *IL8*, *MIF*, and *SELE* were significant prognostic factors. In a similar analysis by Cerhan et al [22] of immune function genes and follicular lymphoma survival, they found that SNP markers from *IL8*, *IL2*, *IL12B*, and *IL1RN* were the most robust predictors of survival individually. We also found that *IL8* is a strong predictor of overall survival as well as relapse and secondary cancers for follicular lymphoma cases. Interleukin-8 is produced by a wide variety of cells at inflammatory sites and acts on neutrophils stimulating degranulation and chemotaxis. The identified polymorphism [rs4073] has been associated with higher production of IL8, and IL8 production has been linked with tumor vascularization, metastatic phenotype, and poor prognosis [23–25] including progression in patients with early-stage chronic lymphocytic leukemia [26]. Our finding that follicular lymphoma patients with the homozygous variant genotype [and therefore presumed lower IL8 production] had a poorer survival, is consistent with the Cerhan et al [22] finding and suggests that higher IL8 levels and concomitant greater inflammation may play a protective role in follicular lymphoma. Our finding that *IFNGR1*, *IL4R*, *SELE*, and *MIF* may be other strong predictors of survival for follicular lymphoma in our study population should be replicated in future work.

We found that for DLBCL, a SNP in *SELE* [responsible for the accumulation of blood leukocytes at sites of inflammation] was significant for OS whereas SNPs in *FCGR2A* [involved in the process of phagocytosis and clearing of immune complexes] and *TGFBR1* [transduces the TGF-beta signal from the cell surface to the cytoplasm] were significant for disease free survival. We also found that polymorphisms in *IL8RB* [mediates neutrophil migration to sites of inflammation], *CCR5*, and *TGFBR1* were significant prognostic factors for CLL/SLL survival.

We previously published the association of most of these SNPs with risk of developing NHL from our population-based case-control study [7]. In evaluating the risk of NHL development, we found that SNPs in each of 3 Th2-related cytokines, *IL4*, *IL5*, and *IL10*, were significantly associated with an increased risk for NHL overall which was consistent with results from a pooled analysis. Subsequent analyses by NHL subtype showed that variant SNPs in *IL10* were significantly associated with increased risk for DLBCL and follicular lymphoma. In that analysis, SNPs in *IL6* and *IL8* were not associated with risk of all NHL or follicular lymphoma, respectively. These findings suggest that although genetic variability in immune genes seems to be associated with lymphoma-genesis in general, the specific SNPs and genes involved in NHL etiology may differ from genes involved in prognosis, although more data will be needed to fully evaluate this hypothesis.

The findings from the combined deleterious models for NHL overall and NHL subtypes indicated that improvements could be made to prognostic models by including information on deleterious genotypes. However, when we included the same SNPs identified by another group in our models [22], we did not get significant results. Specifically, when we ran the model for follicular lymphoma including *IL1RN*, *IL12RB*, *IL2*, and *IL8* in our prognostic model for follicular lymphoma survival, we achieved a *p*-value of 0.85 for the test of homogeneity for the likelihood ratio. This may be due to the heterogeneity of patients in the population study. The biological mechanism is a complex system and different subtypes of cancer may have different genetic markers. Another potential explanation for this difference is that we had more detailed data on prognostic clinical factors. Our inability to replicate previous results suggests that although we have significantly improved the predictors of survival in this study population, our results need to be replicated in larger study populations such as pooled cohorts. If our models are found to significantly predict survival in other study populations, this could indicate that the inclusion of genotyping data in the clinical assessment of NHL and NHL subtypes may provide some benefit to clinicians and patients.

An important strength of this study was the population-based ascertainment of newly diagnosed cases. This study is the largest and most comprehensive study of immune candidate SNPs in relation to NHL survival conducted to date. The value of the study also lies in the fact that the choice of SNPs was based on either functional data or prior associations with cancer or other immune-related diseases. Our statistical analyses were comprehensive, and we have been cautious to evaluate the robustness of our results to both false positives and false negatives. We have not, however, reached median survival for the cohort, and further follow-up could lead to other SNP associations. The treatment information for chemotherapy collected by CTR is not detailed and comprehensive. Moreover the information on relapse and secondary cancer might be incomplete especially among patients who were no longer Connecticut residents, which could cause our measure of DFS to be longer than an accurate measure of time to outcome. However, the information bias is unlikely to be associated with their genetic background, thus our observed associations on DFS may be biased towards the null due to this non-differential misclassification. In the DFS analysis, we also considered of deaths in addition to relapses and occurrences of secondary cancer as events instead of censorings, potentially making the OS and DFS analyses more similar than if deaths were censored. Finally, there could be

confounding by comorbid conditions that are associated with these cytokine genotypes and that impact survival.

In order to investigate the generalizability of the study results, we compared our overall survival curve with that of 13,899 female NHL patients aged 21–84 diagnosed during 1996–2000 at 17 Surveillance, Epidemiology and End Results (SEER) registries [27]. The two survival curves were parallel with around 15% difference in survival rates throughout the follow-up period except during the first half year (median time between diagnosis and interview 4.5 months, and mean 7 months), during which 15.8% of SEER patients died, while none of the patients in our study died. Considering that 167 out of 1,122 [14.9%] identified cases were not able to be enrolled in the CT study because they died before interview [8], the overall survival of our case series is comparable to the survival observed by SEER. Our results might not apply to the most aggressive NHL cases with short-term survivals since there were limited cases in our study who died within a half year.

Our results support the hypothesis that inherited genetic variation in cytokine and related regulatory immune genes could influence survival in NHL, as well as the DLBCL, follicular, and CLL/SLL subtypes, and provide further evidence for the contribution of host factors, including the tumor microenvironment, in the prognosis of NHL and NHL subtype survival [28]. Our findings are also consistent with the emerging significance of the complex host genetic background in progression of cancer in general [29]. In the future, the clinical evaluation of host genetics in cancer patients could become a useful tool in tailoring individual patient management and therapy, and complement gene expression profiling as a prognostic tool [29]. Ultimately, the simultaneous evaluation of host and tumor factors would be expected to increase prognostic ability.

In summary, host genetic variability in immune genes, individually and in particularly in combination, appear to be associated with overall survival and disease free survival in NHL and NHL subtypes after accounting for clinical and demographic factors. In this regard, host immunogenetics represents a promising class of prognostic factors that warrant further evaluation. Furthermore, our analysis suggests that such prognostic factors may differ by subtype and should be evaluated accordingly.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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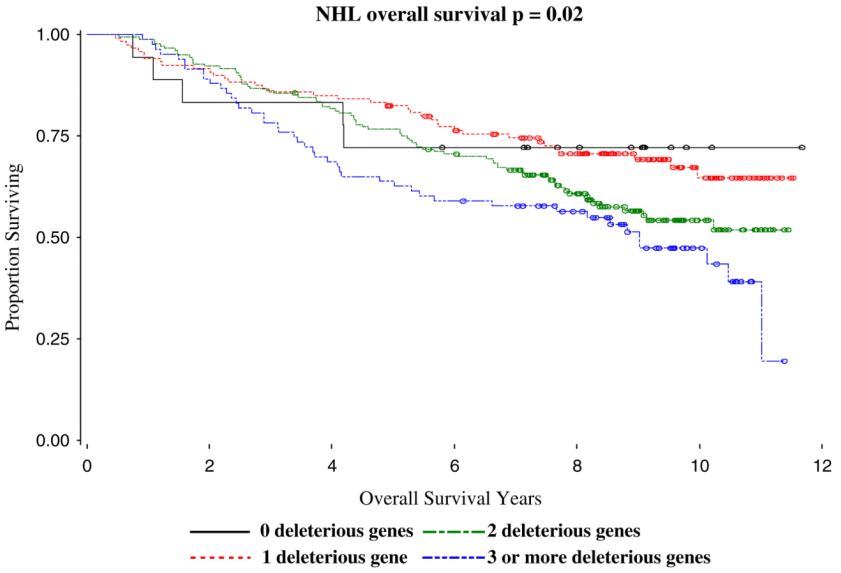
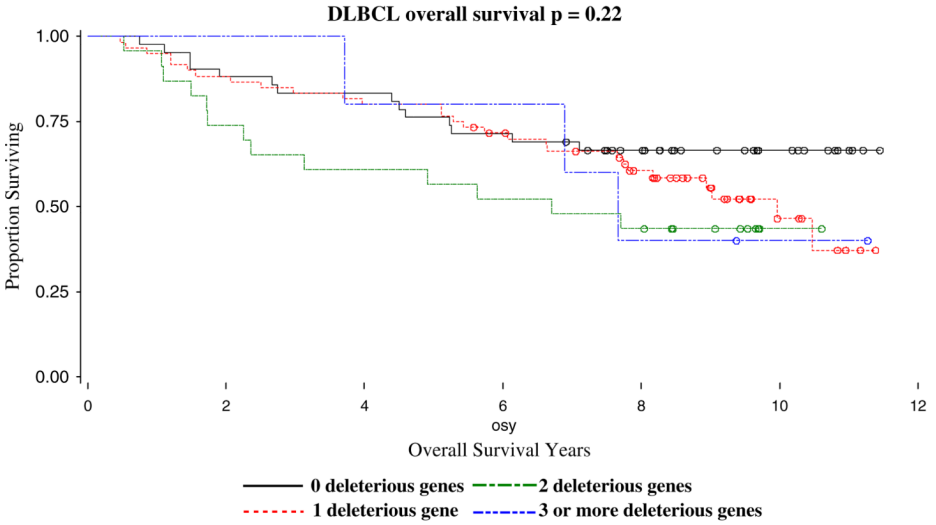
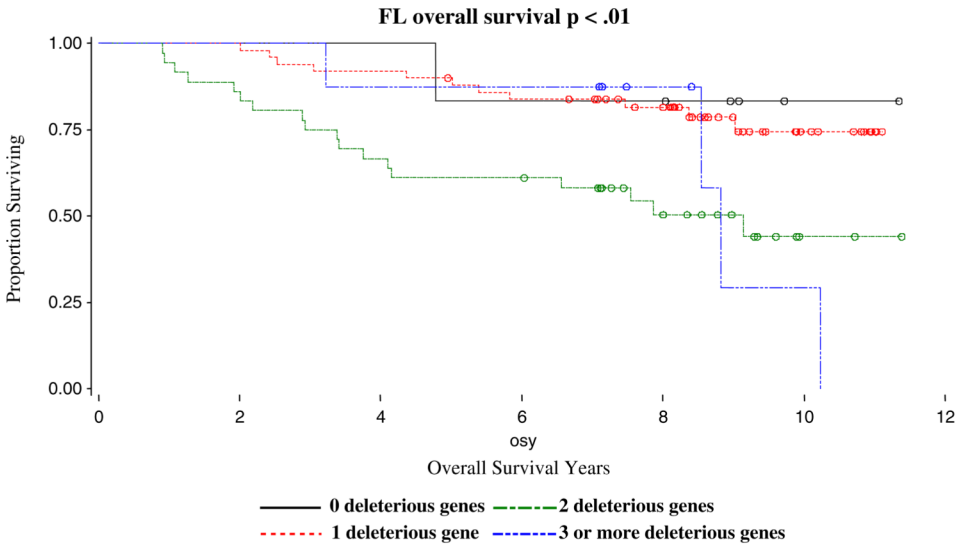


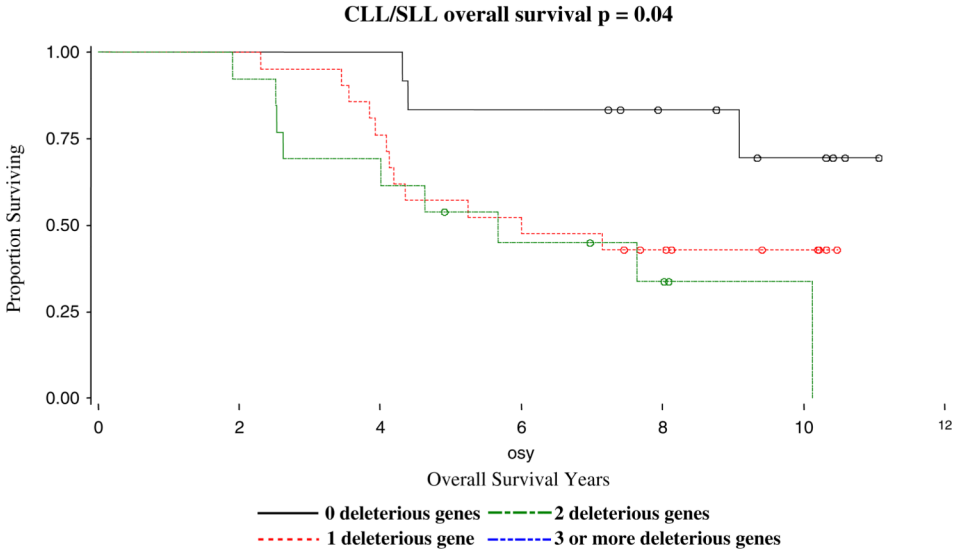
*Included genes: *IL6* [rs1800796], *CX3CR1* [rs3732379], *IL4R* [rs1805011]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].



* Included genes: *IL12A* [rs582054], *SELE* [rs5361], *TGFBR1* [rs868], *CTLA4* [rs231775]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].

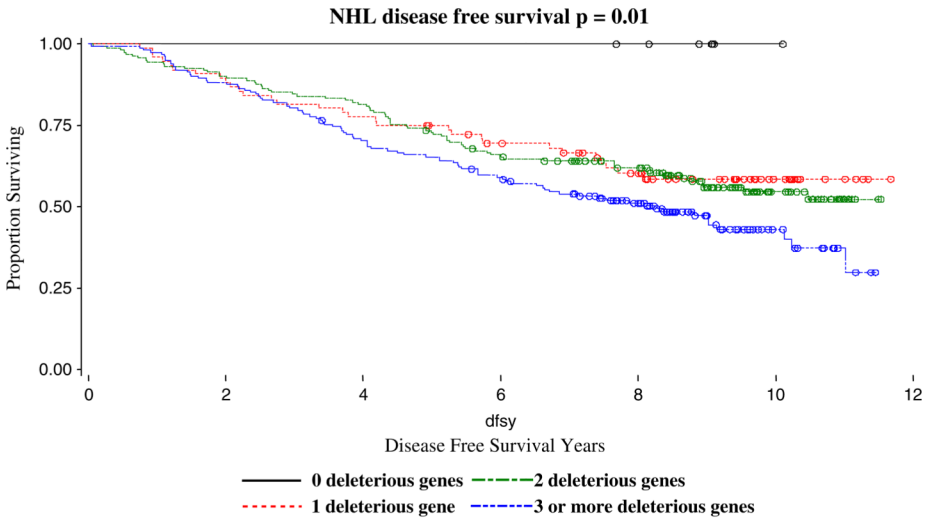


* Included genes: *SELE* [rs5361], *IFNGR1* [rs3799488], *IL6* [rs1800796], *CCR2* [rs1799864]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].

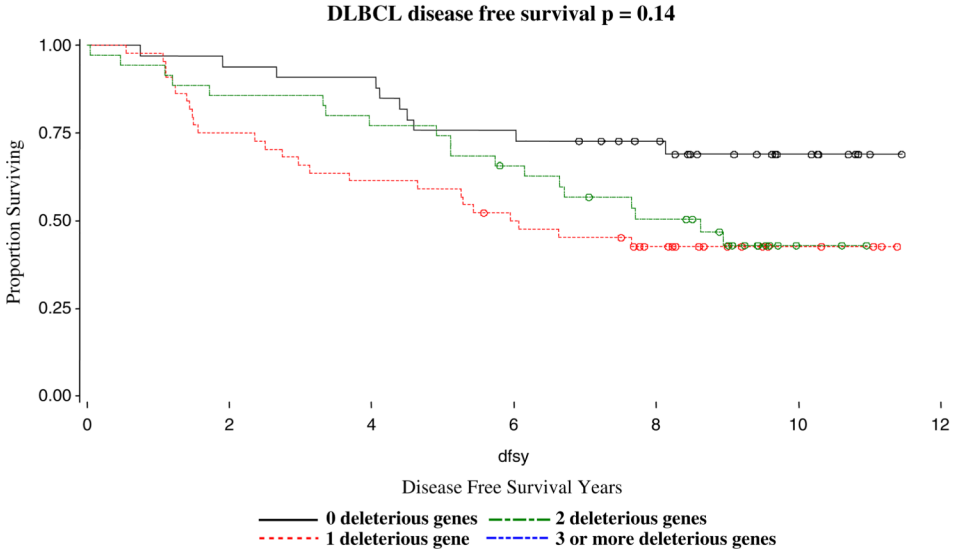


*Included genes: *CSF2* [rs25882], *IL8RB* [rs1126579], *IFNGR2* [rs1059293], *IL4* [rs2243250]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].

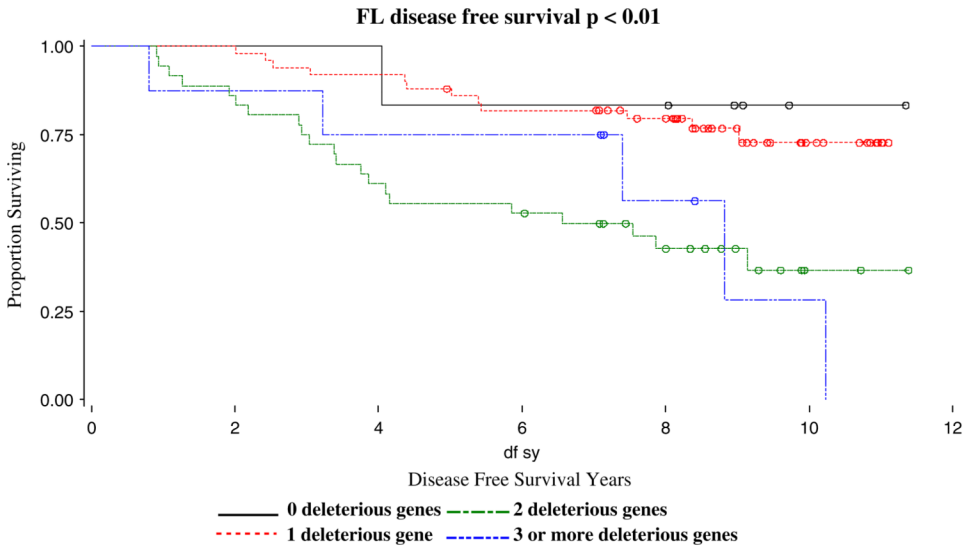
Fig. 1.
Kaplan-Meier overall survival curves by the number of deleterious genotypes for NHL cases, Connecticut, 1995–2001



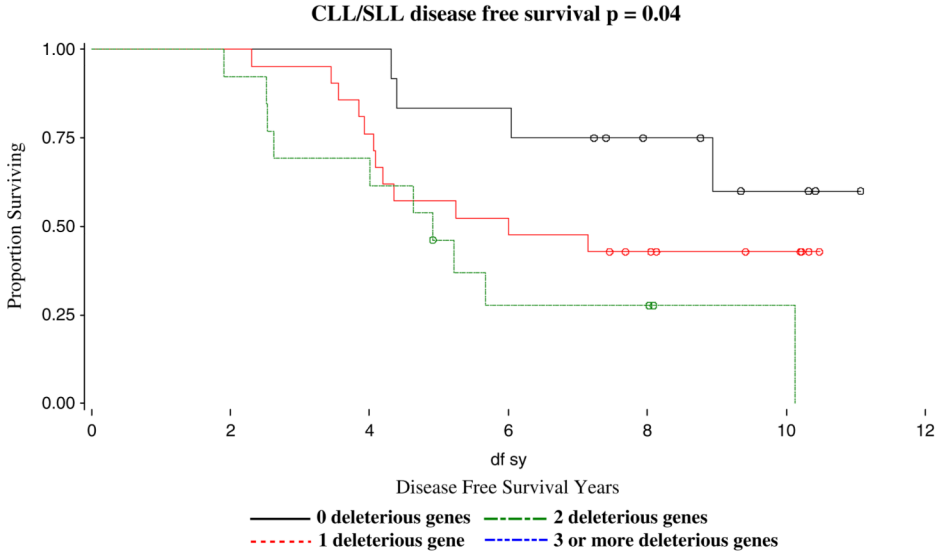
*Included genes: *IL4R* [rs1805011], *IL6* [rs1800796], *CX3CR1* [rs3732379], *CCR5* [rs179987]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].



*Included genes: *IL4R* [rs1805011], *TGFBR1* [rs868], *CTLA4* [rs231775], *IL8RA* [rs2234671]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].



*Included genes: *CARD15* [rs2066844], *IFNGR1* [rs3799488], *IL4R* [rs1801275], and *IL10* [rs3024509]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].



*Included genes: *CSF2* [rs25882], *IL8RB* [rs1126579], *IFNGR2* [rs1059293], *CCR5* [rs179987]
** Models adjusted for age [continuous], education [high school or less, some colleges, and college graduate or more], stage [I, II, III, IV and unknown], B-symptom presence [yes, no] and Initial treatment [none, surgery, radiation, chemotherapy, and other].

Fig. 2.
Kaplan-Meier disease-free survival curves by the number of deleterious genotypes for NHL cases, Connecticut, 1995–2001

Table 1

Candidate genes and SNPs

Gene	Name	Location	SNP rs number
CARD15	Caspase recruitment domain family, member 15	16q12	rs2066847, rs2066844
CCR2	Chemokine, CC motif, receptor 2	3p21	rs1799864
CCR5	Chemokine, CC motif, receptor 5	3p21	rs1800940, rs1799987, rs1800560
CSF2	Colony-stimulating factor 2 (granulocyte)	5q31.1	rs1469149, rs25882
CTLA4	Cytotoxic T lymphocyte-associated 4	2q33	rs231775
CX3CR1	Chemokine, CXC motif	3p21	rs3732379
CXCL12	Chemokine, CXC motif, ligand 12	10q11.1	rs1801157
FCGR2A	Receptor for Fc fragment of IgG, low-affinity IIa (CD32)	1q21-q23	rs1801274
ICAM1	Intercellular adhesion molecule 1 (CD54)	19p13.3-p13.2	rs5491
IFNGR1	Interferon, gamma, receptor 1	6q23-q24	rs3799488
IFNGR2	Interferon, gamma, receptor 2	21q22.11	rs9808753, rs1059293, rs4986958
IFNG	Interferon	12q14	rs1861494, rs2069705
IL10RA	Interleukin 10 receptor, alpha	11q23.3	rs9610
IL10	Interleukin 10	1q31-q32	rs1800871, rs1800872, rs1800896, rs3024509, rs3024496, rs3024491, rs1800890
IL12A	Interleukin 12, alpha	3q25.33	rs568408, rs582054
IL12B	Interleukin 12B	5q33.3	rs3212227
IL13	cytotoxic lymphocyte maturation factor 2	5q23.3	rs20541, rs1800925, rs1295686
IL15RA	Interleukin 15 receptor, alpha	10p15.1	rs2296135
IL16	Interleukin 16	15q25.1	rs859, rs11325
IL1A	Interleukin 1-alpha	2q13	rs17561, rs1800587, rs2856841
IL1B	Interleukin 1-beta	2q14	rs16944, rs1143634, rs1143627
IL1RN	Interleukin 1 receptor antagonist	2q14.2	rs454078
IL2	Interleukin 2	4q26-q27	rs2069762
IL4R	Interleukin 4 receptor	16p12.1-p11.2	rs1805011, rs1801275, rs1049631, rs2107356
IL4	Interleukin 4	5q31.1	rs2243250, rs2243248, rs2070874, rs2243290, rs2243268
IL5	Interleukin 5	5q31.1	rs2069812, rs2069822, rs2069818, rs2069807
IL6	Interleukin 6	7p15.3	rs1800795, rs1800797, rs1800796
IL7R	Interleukin 7 receptor (CD127)	5p13	rs1494555, rs2228141
IL8RA	Interleukin 8 receptor, alpha	2q35	rs2234671
IL8RB	Interleukin 8 receptor, beta	2q35	rs1126579, rs2230054, rs1126580
IL8	Interleukin 8	4q12-q13	rs4073, rs2227307, rs2227306, rs2227538
LEPR	Leptin receptor	1p31	rs1805096, rs7602
MIF	Macrophage migration inhibitory factor	22q11.23	rs755622, rs9282783
SELE	Selectin E	1q22-q25	rs5361
STK11	Serine/threonine kinase 11	19p13.3	rs9282859, rs741764
TGFB1	Transforming growth factor, beta 1	19q13.2	rs1800470
TGFBR1	Transforming growth factor, beta receptor 1	9q22	rs868
VCAM1	Vascular cell adhesion molecule 1	1p32-p31	rs1041163

Table 2

Demographic and clinical characteristics of NHL cases, Connecticut, 1996–2001

	Number	%
Age at diagnosis		
<=45	61	12.30
46–55	100	20.16
56–65	113	22.78
66–75	140	28.23
>=76	82	16.53
Race		
White	475	95.77
Black	16	3.23
Other	5	0.80
Education		
High School or Less	206	41.53
Some College	168	33.87
College graduate or more	122	24.60
Family History		
None	107	21.57
Any other cancer	381	76.82
NHL	8	1.61
Stage		
I	238	47.98
II	61	12.30
III	28	5.65
IV	158	31.85
UK	11	2.22
B-symptom		
Yes	71	14.31
No	425	85.69
Initial Treatment		
None	99	19.96
Surgery	180	36.29
Radiation	39	7.86
Chemotherapy	121	24.40
Other	57	11.49

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Table 3

Hazard ratios for risk of death associated with SNPs (p<0.15) in cytokine genes for NHL overall and subtypes (n for NHL=496, DLBCL=155, FL=117, CLL/SLL=34)

Gene	SNP	HR (95% CI) NHL Overall	HR (95% CI) DLBCL	HR (95% CI) FL	HR (95% CI) CLL/SLL
CCR2	rs1799864			0.27 (0.08–0.86)	
CCR5	rs1799987	0.76 (0.56–1.03)			
CCR5	rs1800940	0.73 (0.53–1.00)			0.46 (0.19–1.15)
CSF2	rs25882				2.73 (0.98–7.65)
CTLA4	rs231775		0.66 (0.37–1.17)	1.31 (0.61–2.83)	
CX3CR1	rs3732379	1.28 (0.94–1.72)			
FCGR2A	rs1801274	1.27 (0.94–1.71)	1.79 (1.01–3.17)		
IFNGR2	rs1059293				3.02 (1.06–8.16)
IFNGR1	rs3799488			3.19 (1.09–9.34)	
IL16	rs11325		0.56 (0.28–1.13)		
IL4R	rs1805011	1.32 (0.91–1.89)	1.82 (0.88–3.77)		
IL4R	rs1801275	1.28 (0.90–1.84)		2.35 (1.07–5.19)	
IL4	rs2243250				0.49 (0.19–1.27)
IL4	rs2243248	0.74 (0.50–1.10)			
IL5	rs2069812	1.25 (0.93–1.69)			
IL6	rs1800796	0.42 (0.23–0.77)[a]	0.25 (0.06–1.06)	0.25 (0.06–1.10)	
IL7R	rs2228141			1.98 (0.82–4.79)	
IL8	rs4073			2.60 (1.10–6.15)	
IL8	rs2227307			2.57 (1.07–6.17)	
IL8	rs2227306			2.23 (0.98–5.11)	
IL8RA	rs2234671		1.74 (0.81–3.84)		
IL8RB	rs1126579				3.54 (1.38–9.06)[a]
IL12A	rs582054		1.51 (0.83–2.76)		
IL10	rs3024509				0.22 (0.04–1.24)
MIF	rs755622			2.45 (1.09–5.47)	
SELE	rs5361	0.68 (0.45–1.03)	0.42 (0.20–0.89)[a]	0.10 (0.02–0.48)[a]	
STK11	rs741764		1.52 (0.87–2.64)		

Gene	SNP	HR (95% CI) NHL Overall	HR (95% CI) DLBCL	HR (95% CI) FL	HR (95% CI) CLL/SLL
TGFBR1	rs868		1.70 (0.91–3.17)		0.29 (0.09–0.87)[a]
VCAM1	rs1041163				2.92 (0.88–9.71)

Reference groups were homozygote wild-type carriers. Models adjusted for age (continuous), education (high school or less, some colleges, and college graduate or more), stage (I, II, III, IV and unknown), B-symptom presence (yes, no, and missing) and initial treatment (none, radiation only, chemotherapy-based regimen, and other)

[a]Remained significant at the p=0.05 level after FDR adjustment

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Table 4

Hazard ratios for risk of death, relapse or secondary cancer occurence associated with SNPs (p<0.15) in cytokine genes for NHL overall and subtypes (n for NHL=496, DLBCL=155, FL=117, CLL/SLL=34)

Gene	SNP	HR (95% CI) NHL Overall	HR (95% CI) DLBCL	HR (95% CI) FL	HR (95% CI) CLL/SLL
CARD15	rs2066844			0.31 (0.07–1.38)	
CCR2	rs1799864			0.39 (0.15–1.05)	
CCR5	rs1800940	0.79 (0.60–1.05)			0.39 (0.16–0.95)
CSF2	rs25882				2.63 (0.99–6.97)
CTLA4	rs231775		0.66 (0.39–1.13)		0.49 (0.21–1.15)
CX3CR1	rs3732379	1.26 (0.95–1.67)			
FCGR2A	rs1801274		1.78 (1.04–3.04)		
IFNGR2	rs1059293				2.51 (0.94–6.74)
IFNGR1	rs3799488	1.33 (0.97–1.83)		2.63 (1.00–6.91)	0.42 (0.14–1.28)
IL4R	rs1805011	1.37 (0.98–1.92)	1.75 (0.88–3.50)	2.14 (0.99–4.64)	
IL4R	rs1801275	1.33 (0.99–1.77)		2.53 (1.22–5.21)[a]	
IL5	rs2069812		0.69 (0.42–1.14)		
IL5	rs2069807			82.5 (7.15–953.0)	
IL6	rs1800797		1.61 (0.91–2.83)		
IL6	rs1800796	0.39 (0.22–0.71)[a]	0.20 (0.05–0.83)[a]	0.32 (0.09–1.10)	
IL7R	rs2228141			1.81 (0.84–3.88)	
IL8	rs4073			2.23 (1.03–4.80)	
IL8	rs2227307			2.15 (0.99–4.65)	
IL8	rs2227306			1.96 (0.93–4.13)	
IL8	rs2227538			108.6 (9.0–1313.2)	
IL8RA	rs2234671		1.81 (0.90–3.65)		
IL8RB	rs1126579	1.25 (0.92–1.68)			3.33(1.35–8.20)
IL12A	rs582054		1.56 (0.88–2.96)		
IL12B	rs3212227			1.70 (0.87–3.32)	
IL10	rs3024509			2.31 (0.90–5.88)	0.20 (0.04–1.08)
IL16	rs859			1.75 (0.87–3.50)	
IL16	rs11325		0.62 (0.33–1.09)		

Gene	SNP	HR (95% CI) NHL Overall	HR (95% CI) DLBCL	HR (95% CI) FL	HR (95% CI) CLL/SLL
MIF	rs755622			1.86 (0.89–3.89)	
SELE	rs5361		0.57 (0.30–1.11)	0.26 (0.09–0.78)[a]	
TGFBR1	rs868		1.85 (1.02–3.33)		0.26 (0.09–0.77)[a]
VCAM1	rs1041163				2.48 (0.78–7.92)

Reference groups were homozygote wild-type carriers. Models adjusted for age (continuous), education (high school or less, some colleges, and college graduate or more), stage (I, II, III, IV and unknown), B-symptom presence (yes, no, and missing) and initial treatment (none, radiation only, chemotherapy-based regimen, and other)

[a]Remains significant at p=0.05 level following FDR adjustment