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Cytokine signaling pathway polymorphisms and AIDS-related non-Hodgkin lymphoma risk in the Multicenter AIDS Cohort Study

Hui-Lee Wong[1], **Elizabeth C. Breen**[2], **Ruth M. Pfeiffer**[1], **Brahim Aissani**[3], **Jeremy J. Martinson**[4], **Joseph B. Margolick**[5], **Richard A. Kaslow**[3], **Lisa P. Jacobson**[5], **Richard F. Ambinder**[5], **Stephen Chanock**[1], **Otoniel Martínez-Maza**[2], and **Charles S. Rabkin**[1]

[1]National Cancer Institute, Rockville, MD

[2]David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, CA

[3]University of Alabama at Birmingham, Birmingham, AL

[4]University of Pittsburgh, Pittsburgh, PA

[5]Johns Hopkins University, Baltimore, MD

Abstract

Cytokine stimulation of B-cell proliferation may be an important etiologic mechanism for acquired immunodeficiency syndrome (AIDS)-related non-Hodgkin lymphoma (NHL). The Epstein-Barr virus may be a co-factor, particularly for primary central nervous system (CNS) tumors, which are uniformly EBV-positive in the setting of AIDS. Thus, we examined associations of genetic variation in *IL10* and related cytokine signaling molecules (*IL10RA, CXCL12, IL13, IL4, IL4R, CCL5* and *BCL6*) with AIDS-related NHL risk and evaluated differences between primary CNS and systemic tumors. We compared 160 Multicenter AIDS Cohort Study (MACS) participants with incident lymphomas, of which 90 followed another AIDS diagnosis, to HIV-1-seropositive controls matched on duration of lymphoma-free survival post-HIV-1 infection (N=160) or post-AIDS diagnosis (N=90). We fit conditional logistic regression models to estimate odds ratios (ORs) and 95 percent confidence intervals (95%CIs). Carriage of at least one copy of the T allele for the *IL10* rs1800871 (as compared to no copies) was associated with decreased AIDS-NHL risk specific to lymphomas arising from the CNS (CC *vs.* CT/TT: OR=0.3; 95%CI: 0.1, 0.7) but not systemically (CC *vs.* CT/TT: OR=1.0; 95%CI: 0.5, 1.9) ($P_{heterogeneity}$=0.03). Carriage of two copies of the “low IL10” haplotype rs1800896_A/

Roles of authors

Hui-Lee Wong: wrote manuscript and performed analyses
Elizabeth C. Breen: participated in study design and data interpretation
Ruth M. Pfeiffer: provided statistical consultancy
Brahim Aissani: participated in data interpretation
Jeremy J. Martinson: involved in on-going cohort
Joseph B. Margolick: involved in on-going cohort
Richard A. Kaslow: participated in study design and data interpretation
Lisa P. Jacobson: participated in study design and management of dataset
Richard F. Ambinder: participated in study design
Stephen Chanock: lead genotyping efforts
Otoniel Martínez-Maza: participated in study design and data interpretation
Charles S. Rabkin: managed study, participated in study design, data analyses, data interpretation and manuscript-writing.

rs1800871_T/rs1800872_A was associated with decreased lymphoma risk that varied by number of copies (P_{trend}=0.02). None of the ORs for the other studied polymorphisms was significantly different from 1.0. Excessive IL10 response to HIV-1 infection may be associated with increased risk of NHL, particularly in the CNS. IL10 dysregulation may be an important etiologic pathway for EBV-related lymphomagenesis.

Keywords

cytokine; SNPs; AIDS-related lymphoma

Introduction

While the incidence of the most common immune system malignancy, non-Hodgkin lymphoma (NHL), has been increasing over the past half century, the etiologic factors underlying this steady increase remain unclear. The strongest risk factor for NHL is immune deficiency. The risk of NHL in human immunodeficiency virus (HIV)-infected persons with acquired immunodeficiency syndrome (AIDS) is elevated at least 50 times above that seen in the general population. Both AIDS-related NHL and NHL in immunocompetent Western populations generally arise from B cells (reviewed in (1). However, the majority (70 to 90%) of AIDS-related lymphomas are high histologic-grade (WHO classification) and clinically aggressive tumors with extranodal involvement at diagnosis. AIDS-related NHL may be classified by site of primary tumor into systemic and primary central nervous system (CNS) lymphoma. Primary CNS lymphoma is particularly associated with immune deficiency as marked by low $CD4^+$ T-lymphocyte cells counts, with an incidence at least 1000 times higher than the general population (2).

AIDS-related NHL is an informative model to investigate specific components of the immune system related to lymphoma development. HIV induces profound defects in cell-mediated immunity leading to dysregulated cytokine signaling. Cytokines are messengers of the immune system and act in a transient fashion. Elevated levels of B-cell stimulatory cytokines in HIV-infected people may drive proliferation of B-lymphocytes and lead to increased risk of NHL. Circulating protein levels of these labile B-cell stimulatory cytokines are often low, and difficult to measure.

While cytokine levels are dynamic, the inter-individual variation in cytokine production is, in part, genetically determined. Common genetic polymorphisms, e.g., single nucleotide polymorphisms (SNPs), with differential allelic effects may be a better indicator of long-term immune function and easier to measure than cytokine level. Thus, we examined the associations between cytokine SNPs in relation to the risk of NHL in HIV-infected men. We also assessed possible differences in these genetic associations between systemic and primary central nervous system (CNS) lymphomas.

Methods and Materials

Study population

The present study is a case-control study nested in the Multicenter AIDS Cohort Study (MACS), an observational study of HIV infection and AIDS (3). We studied AIDS-NHL cases that occurred during an era spanning the introduction of the first treatment for HIV infection and the development of highly active antiretroviral therapy (HAART). 9 (5%) of the cases and 7 (4%) of the controls reported ever using HAART prior to the NHL diagnosis of the index case; 111 (62%) of cases and 91 (51%) of controls reported any HIV treatment prior to the diagnosis of NHL. Cases and controls were identified from among 5622

homosexual men enrolled in 1984–85 and 1987–1991 at four study centers in the U.S. (Baltimore, Chicago, Los Angeles, and Pittsburgh). At semi-annual visits, clinical information and blood samples were obtained from participants, confirmed by medical record review, and blood was stored in a central repository. Data on HIV-1 serostatus, absolute CD4 T cell counts, and plasma HIV viral load were collected with standardized quality-assured protocols (4, 5). The baseline visit for viral load data was defined as either the third or fourth MACS follow-up visit if HIV seropositive at enrollment (1.0 or 1.5 years after enrollment), whichever included both a CD4 cell count and a plasma HIV RNA measurement, or, at least 18 months after the participant became HIV seropositive. Plasma concentrations of HIV-1 RNA were determined by either reverse-transcriptase-polymerase chain reaction assay (Amplicor HIV-1 Monitor assay, Roche Molecular Systems, Branchburg, NJ) or the branched-DNA assay (Chiron Corp, Emeryville, CA), and all values were standardized to their Amplicor equivalents with a lower detection limit of 50 HIV-1 RNA copies/mL (4–6). The protocols and questionnaires utilized in the MACS (available at www.statepi.jhsph.edu/macs/macs.html) were approved by the Institutional Review Board at each center. The current genetic study follows definition of a case-control study of serum cytokines and AIDS-NHL risk nested in MACS (analyses in progress).

Cases—Cases consisted of HIV-infected persons diagnosed with AIDS-NHL prior to April, 2003, for whom at least one serum sample from a time point preceding AIDS-NHL diagnosis was available in the MACS repository. Eligible cases included 58 participants with primary CNS lymphoma and 121 with systemic AIDS-NHL, of which a total of 56 CNS and 104 systemic case-control sets had adequate DNA for genotyping assays. We compared these cases to two sets of controls (matched on post-HIV and post-AIDS periods, respectively) to identify findings specific to AIDS-NHL risk, not confounded by HIV progression.

Controls matched on post-HIV infection lymphoma-free period—One set of controls was selected from cohort members who were HIV-1-infected and lymphoma-free as of April 2003, matched on: 1) year of first HIV-seropositive visit based on known date of HIV seroconversion for seroconverters or date of enrollment as HIV-seropositive, ± 1 year, 2) subsequent follow-up at least as long as lymphoma-free survival duration of the NHL case, and 3) expected sample availability at equivalent time point(s) as the case (± 1 year). One HIV+ control was randomly selected from among 6 to 1526 eligible controls for each case. At the time of AIDS-NHL diagnosis in the case, the median time that cases and HIV+ controls had been followed with HIV-infection was 6.7 and 6.9 years, respectively.

Controls matched on post-AIDS lymphoma-free period—Individuals diagnosed with an AIDS-defining condition may be more immune compromised than other individuals with the same duration of HIV infection. In order to account for this difference, a second set of controls was selected for the subset of cases in which NHL followed another AIDS-defining condition, excluding AIDS cases diagnosed by CD4 T cell count alone (7). These AIDS controls were matched on: 1) year of AIDS diagnosis (± 1 year), 2) a post-AIDS lymphoma-free survival duration at least as long as that of the NHL case, and 3) expected sample availability at equivalent time points as the case (± 1 year). For each case, there were between 2 and 195 eligible post-AIDS controls.

SNP selection—We examined 13 candidate SNPs in cytokine and receptor genes related to suspected pathways in AIDS-NHL development, including B-cell stimulatory cytokines and their activators (*IL10*, *IL10RA*, *BCL6*), allergy-related cytokines (*IL13*, *IL4*, *IL4R*), and chemokines (*CXCL12*, *CCL5)* (Supplementary Table 1).

IL10 is a cytokine that has both immunomodulatory and immunosuppresive effects, with pleiotropic cellular effects that include 1) enhancement of B cell viability, particularly with IL4 co-stimulation and lymphoma growth at the auto/para-crine level, 2) induction of differentiation of CD40-stimulated B cells and apoptosis of *S. aureus*-activated B cells, and 3) inhibition of T cell activation and effector functions (17). Three SNPs in the *IL10* promoter form haplotypes associated with differential IL10 production (Supplementary Table 1). The IL10 receptor (IL10R) mediates the actions of IL10.

B-cell lymphoma 6 protein (*BCL6*) is a proto-oncogene that is frequently translocated in diffuse large B cell lymphomas, including those seen in HIV infection. BCL6 regulates germinal center B cell differentiation and inflammation, and has also been implicated in mast cell production of T-helper type 2 cytokines, which mediate cutaneous and mucosal allergic reactions. IL4, IL13, IL4R and CXCL12 regulate the proliferation and maturation of B cells. IL4 and IL13 influence growth and survival of non-Hodgkin's B-lymphoma cells *in vitro*. Both CXCL12 and CCL5 have roles in both HIV susceptibility and B cell lymphoma development. In addition, IL4, IL13, IL4R, CCL5 and CXCL12 are involved in allergic and atopic phenotypes that have been associated with reduced risk for AIDS-NHL.

Genotyping—Genomic DNA was isolated from EBV-immortalized lymphoblastoid cell lines using Gentra Puregene DNA extraction kits (Qiagen Inc., Valencia, CA) for eligible cases and controls in the MACS repository. Of the 179 eligible cases, six cases had inadequate DNA quantity or quality for genotyping. In addition, 14 of the 179 post-HIV infection controls were excluded because of inadequate DNA quantity or quality. With one set excluded for both case and control DNA, there were 160 complete case-control sets matched for duration of post-HIV infection.

98 of these 160 AIDS-NHL cases occurred after another AIDS-defining illness. However, DNA quantity or quality was inadequate for 8 of their post-AIDS matched controls. Thus, genotypes were analyzed for 90 complete case-control sets matched for post-AIDS survival duration.

TaqMan assays (Applied Biosystems, Foster City, CA) for the SNPs was performed at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute. Protocols for each specific assay are documented at http://snp500cancer.nci.nih.gov. The SNPs were verified in a panel of 102 individuals of self-described Caucasian (n=31), African-American (n=24), Hispanic (n=23) and Pacific Rim (n=24) ethnicity by re-sequencing approximately 300–600 base pairs of DNA on either side of the putatively polymorphic locus. To verify the quality of DNA samples, we typed 15 tetranucleotide short tandem repeat loci and the amelogenin locus (AmpFℓSTR® Identifiler® PCR Amplification Kit, Applied Biosystems, Foster City, CA). Among subjects with adequate DNA, genotyping success ranged from 94.5% to 99.5% for the 13 SNPs, depending on the genotype.

Statistical Methods

Single locus Analyses—Departures from Hardy-Weinberg equilibrium (HWE) were assessed by testing the difference between the observed (sampled) and expected (under HWE) genotype frequencies in both sets of controls using a χ^2 test. To assess strength of association between genotypes and cancer risk, conditional logistic regression models were used to estimate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). Potential confounders included in the models were age at time of NHL diagnosis in the matched case and race (non-Hispanic Whites, non-Hispanic Blacks, others).

We fit separate models that compared heterozygous or variant homozygous genotypes to common homogygous genotypes as a reference category, assuming no specific mode of inheritance. We also tested for linear trends associated with the number of variant alleles, under an additive model of inheritance. For rare SNPs, carriers of at least one variant allele were compared to common homozygotes (rs2280788/CCL5 - 28C>G in Table 2 and rs1800871/IL10-819 and rs1800872/IL-592 in Table 3). *P*-values for linear trends and for dominant genotype coding were derived from Wald statistics. For cases and controls matched on duration of lymphoma-free survival post-HIV infection, we calculated stratum-specific ORs and 95% CIs for anatomic sites of primary disease (i.e., CNS *vs*. systemic). Stratum-specific p-values for additive or dominant models were compared using a one-degree of freedom Wald test. For the smaller group of cases and controls matched on lymphoma-free survival post-AIDS, we did not compare primary CNS *vs*. systemic genetic associations due to sparse data.

Haplotype Analyses for IL10 promoter SNPs—Linkage disequilibrium among the three IL10 SNPs was assessed by r2. Haplotype analyses were performed for cases and controls matched on duration of lymphoma-free survival post-HIV infection and not on the matched sets of lymphoma-free survival post-AIDS due to sparse data. To estimate haplotype frequencies from genotype information, the expectation-maximization (EM) algorithm implemented in the Haploview software (8) was used to resolve phase uncertainties. For the rs1800896_G/rs1800871_C/rs1800872_C(GCC) and rs1800896_A/rs1800871_T/rs1800872_A (ATA) haplotypes respectively, the association of haplotype pairs with NHL were estimated with the haplotype regression dosage method implemented in the HapStats program, assuming an additive model. We did not perform haplotype analyses by site of primary disease (CNS *vs*. systemic) due to limited power.

All *P*-values are two-sided and statistical analyses were performed using STATA 9.0 (Stata Corp, College Station, TX) and SAS Genetics (SAS Institute Inc., Cary, NC).

Results

Compared to the post-HIV controls, NHL cases had lower CD4 counts at multiple time points prior to NHL diagnosis, higher plasma HIV load at baseline and tended to be older and of white race (Table 1). The post-AIDS controls were comparable to post-AIDS NHL cases except for $CD4^+$ T-lymphocyte counts at less than three years prior to NHL diagnosis.

Single Locus Analyses

Genotype distributions (Table 2) did not depart from Hardy-Weinberg equilibrium in the controls. rs1800871(IL10-819) and rs1800872(−592) were in tight LD (r^2=0.98) and their genetic associations were highly correlated. rs1800896(IL10-1082) and rs1800872 were not in high LD (r^2=0.18). Thus, the associations between rs1800896 and rs1800872 and AIDS-related NHL, respectively, are distinct informative genetic associations.

rs1800871, and the strongly correlated rs1800872, were associated with AIDS-NHL case status in comparison to both post-HIV infection and post-AIDS matched controls (Table 2). Compared to post-HIV controls, NHL case status was inversely associated with carriage of the A allele of rs1800872 and in a gene-dosage-dependent manner (P_{trend}=0.01). In similar analyses, rs1800896 showed no association with NHL risk (Table 2).

Table 3 presents SNP associations with primary CNS and systemic lymphoma *vs*. post-HIV controls. Among the primary CNS lymphoma cases, none were homozygotes for the respective minor alleles for either rs1800871 or rs1800872. Therefore ORs could not be calculated for those individual genotypes. However, persons with at least one copy of the T

allele for rs1800871 were at lower risk of primary CNS lymphoma as compared to persons without a copy (OR=0.3; 95%CI: 0.1, 0.7). There was no apparent association with systemic lymphoma (OR=1.0(95%CI: 0.5, 1.9)) (Table 3, $P_{heterogeneity}$=0.03). In contrast, rs1800896 was associated with increased risk of systemic NHL (GG vs. GA/AA: OR=2.4; 95%CI: 1.0, 5.9) but not primary CNS lymphoma (OR=1.0; 95%CI: 0.4, 2.1); however, the ORs for CNS and systemic AIDS-NHL do not significantly differ (Table 3, $P_{heterogeneity}$=0.12). Interestingly, for the individual genotypes of rs1800896, systemic NHL appeared to be associated with either heterozygous or homozygous G, as both comparisons with AA yielded increased odds ratios.

The other examined SNPs in *IL10RA*, *CXCL12*, *IL13*, *IL4*, *IL4R*, *CCL5*, and *BCL6* were not significantly associated with NHL case status overall as compared to either post-HIV infection controls or to post-AIDS /controls (Table 2), nor with primary CNS or systemic lymphoma separately (Table 3).

Haplotype Analyses

The frequencies of the three common *IL10* promoter haplotypes rs1800896_G/ rs1800871_C/rs1800872_C (GCC), rs1800896_A/rs1800871_C/rs1800872_C (ACC) and rs1800896_A/rs1800871_T/rs1800872_A (ATA), were 43.3%, 32.0% and 24.3% respectively. There were only 2 (0.4%) copies (estimated) of all other haplotypes. Among cases and post-HIV infection controls, the risk of AIDS-NHL with the ATA haplotype decreased in a haplotype dose-dependent manner (P_{trend}=0.02; OR=0.3 (95%CI: 0.1, 1.0)), with OR of ATA/other (i.e., GCC or ACC) *vs*. other/other=0.7 (95%CI: 0.5, 1.1) and OR of ATA/ATA vs. other/other=0.4 (95%CI: 0.1, 1.6.). The associations with the number of GCC haplotypes were not significant.

Discussion

NHL that arises in HIV-infected individuals can be distinguished into primary CNS and systemic lymphoma(9). Primary CNS lymphoma is a particularly significant cause of morbidity and mortality among HIV-infected individuals. In our study, individuals with at least one copy of either rs1800871_T or rs1800872_A allele, linked to lower IL10 production (10, 11) and circulating IL10 levels (12), were at decreased risk of primary CNS lymphoma. In contrast, the rs1800896_G allele, linked to higher IL10 production, was not associated with overall AIDS-NHL risk, compared to either the post-HIV or post-AIDS controls. The rs1800896_G allele has a marginally significant association with systemic AIDS-NHL (GG *vs*. GA/AA: 2.4 (1.0, 5.9)). However, the effect of rs1800896 on primary CNS lymphoma and systemic AIDS-NHL did not statistically differ ($P_{heterogeneity}$=0.12). Breen *et al* have previously reported in a smaller study that elevated serum IL10 levels preceded the onset of AIDS-NHL, and that the *IL10* promoter haplotypes associated with higher *IL10* gene expression occur more frequently among the MACS subjects with AIDS-NHL compared to MACS subjects without lymphoma (12). Consistent with these previous data, our current analyses on an expanded number of MACS AIDS-NHL cases and matched MACS HIV seropositive controls showed an association with rs1800872 in a combined case group of CNS and systemic NHL as compared to post-HIV controls. We consider associations observed in both of the matched sets of cases with post-HIV and post-AIDS controls to be specific to AIDS-NHL risk and unlikely due to confounding by AIDS overall. The effect size remained similar when a subset of cases with AIDS was compared to post-AIDS controls; however the association was statistically insignificant, probably due to insufficient power. We also found decreased risk with *IL10* diplotypes that have been associated with low *IL10* gene expression or production in some (13, 14) but not all (15, 16) *in vitro* studies. The genotypes we examined in other immunity pathways did not appear to

be associated with AIDS-NHL risk. To date, these findings from the MACS cohort remain the only reported genetic association study of immunity and AIDS-related NHL risk. We now report that the association of the genetic variants encoding elevated IL10 levels with AIDS-related NHL is specific to primary CNS lymphoma. Interestingly, while the rs1800872_A allele, that correlates to higher IL10 production is associated with decreased risk for AIDS-NHL((12) and current study), the rs1800872_A allele however has been previously associated with faster AIDS progression(11). These observations may reflect the pleiotropic effects of IL10 as both an anti-inflammatory and B-cell-stimulatory cytokine with potential contrasting effects on the course of AIDS progression and development of NHL in HIV seropositive individuals (17).

Our novel finding that rs1800871 associates specifically with a subset of AIDS-NHL, primary CNS NHL, unravels potential new mechanisms in lymphomagenesis and provides a new lead for future studies. One feature distinguishing AIDS-related primary CNS NHL is the presence of the human gammaherpesvirus Epstein-Barr virus (EBV), which can immortalize B cells *in vitro* (18). EBV viral products are found in virtually 100% of CNS lymphomas of HIV-infected individuals (19), but only in 30%–50% of AIDS-related systemic tumors (20). Furthermore, EBV infection of the tumor clone is predictive of CNS involvement along with systemic AIDS-NHL(21). Our findings add to the evidence associating high IL10 production or levels with NHL subgroups in which EBV has been implicated. High IL10 levels are a hallmark of EBV-positive AIDS-Burkitt lymphoma(22, 23), and IL10 expression levels are higher in EBV-positive AIDS-primary effusion lymphomas cell lines than in EBV-negative cell lines (24). EBV also encodes a homologue of human IL10, the EBV *BCRF1* gene product (25, 26). Taken together, the data suggest that IL10 activity contributes to oncogenicity of EBV by inducing latent membrane protein 1 (LMP1), the major viral mediator of lymphocyte transformation (27). Another potential mechanistic role for elevated IL10 production in EBV-positive NHL is the inhibition of functional anti-viral immunity, allowing the outgrowth of these virus-infected cells. While IL10 is a B cell stimulatory cytokine, it also is a potent suppressive cytokine for T cells and macrophages (17). Therefore, relatively high levels of IL10 would be expected to impair the generation of effective cytotoxic T cell responses to EBV. Given that a significant fraction of the total human T cell repertoire is devoted to the response to EBV-infected cells(28), inhibition of T cell anti-viral immunity would allow the expansion of EBV-transformed B cells, especially in those HIV-infected persons who already have significantly impaired T cell immunity.

NHL is a heterogeneous disorder that likely has disparate etiologies, both in the general population a well as in the setting of HIV infection. Our data may specifically implicate the IL10 pathway in the generation of EBV-positive lymphomas. We failed to find associations in other cytokine pathways or for systemic NHL overall. However, we did not have sufficient data to separately analyze the small number of EBV-positive systemic NHLs. Also, we only examined a limited panel of genetic variants in a few pathways. Identifying other cytokine pathways that may differ for specific NHL etiologic groups will require a more comprehensive genetic scan using a combination of tagSNPs as well as rare, putative “functional” SNPs in a larger study.

Nonetheless, the current study represents the first comprehensive examination of a panel of potentially functional variants in B cell stimulatory cytokine genes in relation to risk of AIDS-NHL. The relative concordance of findings in comparisons with a subset of cases and controls matched on post-HIV and post-AIDS lymphoma-free duration strengthens our interpretation that the observed associations of *IL10* polymorphisms are with AIDS-NHL risk rather than with HIV progression more generally.

Specific cytokine pathways may underlie the molecular and pathological mechanisms of lymphomagenesis in various settings. For example, some NHL subtypes in the general population (*e.g.*, diffuse large B-cell lymphoma and marginal zone lymphoma) seem to be more related to immune dysfunction as compared to others (follicular lymphoma and chronic lymphocytic leukemia/small lymphocytic lymphoma) (29). The rs1800896_Gallele for IL10 has been associated with an increased risk of diffuse large B-cell lymphoma in one study of immunocompetent individuals (30), although several other studies failed to find rs1800896, rs1800871 or rs1800872 associations with NHL overall or with either diffuse large B-cell or follicular lymphoma (31–33). In addition, a distal *IL10* promoter SNP, rs1800890 (−3575T/A) with unknown functionality was associated with diffuse large B-cell lymphoma in some(30, 31) but not all (33) of these studies.

NHL in the general population appears to be remarkably heterogeneous. AIDS-related NHL may serve as a model system for the elucidation of cytokine pathways and viral co-factors that may help clarify distinct etiologies of this complex malignancy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

NHL	non-Hodgkin lymphoma
AIDS	Acquired Immune Deficiency Syndrome
SNP	single nucleotide polymorphism
CNS	central nervous system

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Table 1

Characteristics of the AIDS-related non-Hodgkin lymphoma (NHL) study, Multicenter AIDS Cohort.

Characteristics	post-HIV infection[a]			post-AIDS[b]		
	Cases (N= 160)	Controls (N=160)	*P*-value	Cases (N=90)	Controls (N=90)	*P*-value
Age at NHL diagnosis, yr	41 (25–60)	39 (24–60)	0.02	40 (25–58)	39 (28–65)	0.27
Race						
White,non-Hispanic	83%	88%	0.04	81%	89%	0.40
Black	11%	8%		11%	4%	
Others	6%	4%		8%	7%	
CD4+ T-lymphocyte counts/mm^3						
> 3 yr pre-NHL	387(5, 1349)	561(3, 2030)	<0.0001	369(5, 1127)	397(19, 1123)	0.23
1–3 yr pre-NHL	209(6, 1178)	522(37, 1544)	<0.0001	114(6, 1178)	238(9, 1123)	0.001
<1 yr pre-NHL	74(0, 707)	468(4, 1255)	<0.0001	32(0, 691)	98(0, 1159)	0.001
Early plasma HIV-1 RNA levels, copies/mL[c]	5.5 (2.0, 8.4)	4.6 (1.8, 7.4)	<0.0001	5.7 (2.0, 8.4)	5.4 (2.4, 8.5)	0.35

[a] cases and controls matched on post-HIV infection lymphoma-free period

[b] cases and controls matched on post-AIDS lymphoma-free period

[c] HIV viral load at visit closest to 18 months following HIV seroconversion or entry into study

Summary statistics are presented as median (range) or percentages and *P*-values were derived from Kruskal-Wallis test

Table 2

Odds ratios (ORs) and 95% confidence intervals (CIs) for polymorphisms and AIDS-related non-Hodgkin's lymphoma, utilizing cases and controls matched on post-HIV or post-AIDS lymphoma-free duration

Genetic variants	Post-HIV infection		Post-AIDS	
	Cases/ Controls	OR (95% CI)[a]	Cases/ Controls	OR (95% CI)[a]
IL10				
rs1800896 (−1082)				
AA	43/50	Ref	26/22	Ref
AG	72/71	1.4 (0.8, 2.5)	40/45	0.7 (0.3, 1.7)
GG	39/31	1.3 (0.7, 2.7)	20/17	0.8 (0.3, 2.1)
		P_{trend}=0.39		P_{trend}=0.68
rs1800871 (−819)				
CC	109/88	Ref	63/59	Ref
CT	47/57	0.7 (0.4, 1.2)	25/27	0.9 (0.5, 1.7)
TT	2/9	**0.2 (0.03, 0.99)**	1/4	0.1 (0.01, 1.5)
		P_{trend}=0.03		P_{trend}=0.22
rs1800872 (−592)				
CC	107/88	Ref	61/57	Ref
CA	49/57	0.7 (0.4, 1.1)	26/27	0.9 (0.5, 1.7)
AA	11/9	**0.2 (0.03, 0.8)**	1/4	0.1 (0.01, 1.5)
		P_{trend}=0.01		P_{trend}=0.24
IL10R rs9610				
GG	46/47	Ref	27/31	Ref
GA	74/77	1.0 (0.6, 1.8)	40/36	1.3 (0.6, 2.5)
AA	36/30	1.3 (0.6, 2.9)	21/20	1.0 (0.4, 2.4)
		P_{trend}=0.50		P_{trend}=0.87
CXCL12 rs1801157				
TT	94/105	Ref	54/58	Ref
TG	50/42	1.4 (0.8, 2.3)	28/22	1.4 (0.7, 3.1)
GG	5/6	0.6 (0.2, 2.1)	2/7	0.2 (0.02,1.7)
		P_{trend}=0.64		P_{trend}=0.69
IL13 rs1800925				
CC	92/106	Ref	54/59	Ref
CT	59/42	1.5 (0.9, 2.6)	32/28	1.4 (0.7, 2.7)
TT	6/11	0.7 (0.2, 1.9)	3/2	1.7 (0.3,10.9)
		P_{trend}=0.62		P_{trend}=0.34
IL4				
rs2243250				

Genetic variants	Post-HIV infection		Post-AIDS	
	Cases/ Controls	OR (95% CI)[a]	Cases/ Controls	OR (95% CI)[a]
CC	101/105	Ref	55/69	Ref
CT	47/44	1.3 (0.8, 2.2)	28/15	2.0 (0.9, 4.4)
TT	7/8	1.0 (0.3, 3.0)	5/5	1.4 (0.3, 4.4)
		P_{trend}=0.53		P_{trend}=0.29
rs2070874				
CC	109/112	Ref	59/68	Ref
CT	44/42	1.3 (0.7, 2.3)	27/17	1.6 (0.7, 3.8)
TT	5/5	1.2 (0.3, 4.6)	3/4	0.6 (0.1, 3.4)
		P_{trend}=0.44		P_{trend}=0.63
IL4R rs1801275				
AA	96/90	Ref	48/57	Ref
AG	50/50	1.0 (0.6, 1.7)	31/24	1.1 (0.5, 2.13)
GG	11/13	1.1 (0.4, 3.0)	8/1	8.4 (1.0, 69.0)
		P_{trend}=0.92		P_{trend}= 0.06
CCL5				
rs2280788				
CC	154/156	Ref	87/82	Ref
CG/GG	3/5	2.7 (0.5, 15.4)	3/7	0.4 (0.1, 1.9)
		P=0.84		P=0.27
rs2107538				
GG	97/103	Ref	54/51	Ref
GA	49/46	1.2 (0.7, 2.0)	27/30	1.0 (0.5, 2.0)
AA	9/9	1.1 (0.3, 4.0)	6/4	1.1 (0.3, 4.4)
		P_{trend}=0.58		P_{trend}=0.91
BCL6				
rs1056932				
TT	67/57	Ref	39/44	Ref
TC	62/66	0.9 (0.5, 1.5)	29/35	1.0 (0.5, 2.0)
CC	24/35	0.7 (0.3, 1.3)	18/11	1.7 (0.7, 4.3)
		P_{trend}=0.22		P_{trend}=0.29
rs3774306				
AA	62/49	Ref	35/39	Ref
GA	61/68	0.7 (0.4, 1.3)	29/34	1.2 (0.6, 2.7)
GG	28/39	0.7 (0.4, 1.3)	21/16	1.4 (0.6, 3.2)
		P_{trend}=0.22		P_{trend}=0.43

[a]Estimates from conditional logistic regression, adjusted for age at NHL diagnosis (or comparable time in controls) and race (non Hispanic White, Blacks, Others); significant results are highlighted in bold. Totals vary for some SNPs due to failed genotyping.

Table 3

ORs and 95% CIs for polymorphisms and AIDS-NHL in cases and controls matched on post-HIV lymphoma-free duration, stratified by NHL tumor location

	Central Nervous System		Systemic		
Genetic variants	**Cases/ Controls**	**OR (95% CI)[a]**	**Cases/ Controls**	**OR (95% CI)[a]**	***P* heterogeneity**
IL10					
rs1800896 (−1082)					
AA	22/22	Ref	21/28	Ref	
AG	17/14	1.1 (0.4, 2.8)	55/57	2.2 (0.9, 5.6)	
GG	14/14	0.8 (0.3, 2.4)	25/17	**3.0 (1.0, 9.5)**	
GG *vs*. GA/AA		1.0 (0.4, 2.1)		**2.4 (1.0, 5.9)**	
		P_{trend}=0.81		P_{trend}=0.06	0.12
rs1800871 (−819)					
CC	45/30	Ref	64/58	Ref	
CT/TT	10/26	**0.3 (0.1, 0.7)**	39/40	1.0 (0.5, 1.9)	
		P=0.008		P=0.96	**0.03**
rs1800872 (−592)					
CC	45/30	Ref	62/59	Ref	
AC/AA	11/26	**0.3 (0.1, 0.7)**	40/41	0.8 (0.4, 1.5)	
		P=0.008		P=0.50	0.07
IL10R rs9610					
GG	11/11	Ref	35/36	Ref	
GA	29/31	1.1 (0.4, 3.2)	45/46	1.0 (0.5, 1.9)	
AA	13/12	1.0 (0.3, 4.0)	23/18	1.8 (0.7, 5.1)	
		P_{trend}=0.98		P_{trend}=0.36	0.61
CXCL12 rs1801157					
TT	31/37	Ref	63/68	Ref	
TG	20/10	2.7 (1.1, 6.9)	30/32	1.0 (0.5, 1.9)	
GG	2/4	0.6 (0.1, 3.7)	3/2	0.9 (0.1, 6.1)	

	Central Nervous System		Systemic		
Genetic variants	**Cases/ Controls**	**OR (95% CI)[a]**	**Cases/ Controls**	**OR (95% CI)[a]**	***P* heterogeneity**
		P_{trend}=0.28		P_{trend}=0.89	0.36
IL13 rs1800925					
CC	32/38	Ref	60/68	Ref	
CT	22/14	1.8 (0.7, 4.5)	37/28	1.2 (0.6, 2.4)	
TT	2/4	0.8 (0.1, 4.6)	4/7	0.6 (0.1, 2.2)	
		P_{trend}=0.52		P_{trend}=0.88	0.54
IL4 rs2243250					
CC	33/40	Ref	70/69	Ref	
CT	20/16	1.7 (0.6, 4.7)	26/26	1.1 (0.6, 2.2)	
TT	2/0	1.6 (0.2, 12.8)	4/6	0.7 (0.2, 2.7)	
		P_{trend}=0.34		P_{trend}=0.90	0.38
IL4R rs1801275					
AA	28/32	Ref	68/58	Ref	
AG	20/17	1.2 (0.5, 2.7)	30/35	0.9 (0.5, 1.9)	
GG	6/2	6.6 (0.7, 57.0)	5/12	0.4 (0.1, 1.7)	
		P_{trend}=0.12		P_{trend}=0.29	0.07
CCL5 rs2107538					
GG	34/38	Ref	72/67	Ref	
GA	17/14	1.3 (0.6, 2.9)	34/34	1.2 (0.6, 2.5)	
AA	3/3	1.2 (0.1, 19.0)	7/6	1.3 (0.3, 5.7)	
		P_{trend}=0.60		P_{trend}=0.52	0.27
BCL6 rs1056932					
TT	24/19	Ref	38/30	Ref	
TC	19/20	0.7 (0.2, 2.0)	42/48	0.8 (0.4, 1.5)	
CC	10/14	0.7 (0.2, 2.1)	18/25	0.7 (0.3, 1.6)	

	Central Nervous System		Systemic		
Genetic variants	**Cases/ Controls**	**OR (95% CI)[a]**	**Cases/ Controls**	**OR (95% CI)[a]**	***P* heterogeneity**
		P_{trend}=0.44			0.93
rs3774306					
AA	25/21	Ref		Ref	
GA	21/23	0.7 (0.2, 2.0)	42/36	1.0 (0.5, 1.8)	
GG	7/11	0.7 (0.2, 2.1)	41/43	0.6 (0.3, 1.5)	
		P_{trend}=0.48	17/24	P_{trend}=0.36	0.97

[a]Estimates from conditional logistic regression, adjusted for age at NHL diagnosis (or comparable time in controls) and race (non Hispanic White, Blacks, Others); significant results are highlighted in bold.

IL4 rs2070874 and *CCL5* rs2280788 were not included due to sparse strata. Totals vary for some SNPs due to failed genotyping.