Multiple Hodgkin lymphoma–associated loci within the HLA region at chromosome 6p21.3

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Introduction

Hodgkin lymphoma (HL) is a common lymph node cancer of germinal center B-cell origin, which is characterized by malignant Hodgkin and Reed-Sternberg (HRS) cells mixed with a dominant background population of reactive lymphocytes and other inflammatory cells.1 Although Epstein-Barr virus (EBV) infection may be causally related to a number of cases, there is little evidence to support the involvement of other environmental risk factors.2

Evidence for inherited genetic influence on susceptibility is provided by the increased familial risk and high concordance between monozygotic twins.3 Since an association between the human leukocyte antigen (HLA) region and Hodgkin lymphoma (HL) was first reported in 1967,4 many studies have reported associations between HL risk and both single nucleotide polymorphism (SNP) and classic HLA allele variation in the major histocompatibility complex. However, population stratification and the extent and complexity of linkage disequilibrium within the major histocompatibility complex have hindered efforts to fine-map causal signals. Using SNP data to impute alleles at classic HLA loci, we have conducted an integrated analysis of HL risk within the HLA region in 582 early-onset HL cases and 4736 controls. We confirm that the strongest signal of association comes from an SNP located in the class II region, rs6903608 (odds ratio [OR] = 1.79, \( P = 6.63 \times 10^{-19} \)), which is unlikely to be driven by association to HLA-DRB, DQA, or DQB alleles. In addition, we identify independent signals at rs2281389 (OR = 1.73, \( P = 6.31 \times 10^{-15} \)), a SNP that maps closely to HLA-DPB1, and the class II HLA allele DQA1*02:01 (OR = 0.56, \( P = 1.51 \times 10^{-7} \)). These data suggest that multiple independent loci within the HLA class II region contribute to the risk of developing early-onset HL. (Blood. 2011; 118(3):670-674)

Methods

Samples and genotyping

We analyzed the constitutional DNA of 615 patients diagnosed with HL (International Classification of Diseases 10 codes C81.0-3) who were ascertained through the Royal Marsden Hospitals National Health Service Trust Family History study, between 2004 and 2008 (mean age at diagnosis 38 years, SD 16 years) and an ongoing national study of cHL in females (mean age at diagnosis 23 years, SD 6 years) being conducted by the Institute of Cancer Research to assess late effects of HL treatment. Both routes of patient ascertainment thus favored the acquisition of early-onset HL cases. All cases were British residents and were self-reported to be of European ancestry.

DNA was extracted from samples using conventional methodologies and quantified using PicoGreen (Invitrogen). Genotyping of cHL cases was conducted using Illumina Infinium HD Human610-Quad BeadChips according to the manufacturer’s protocols (Illumina). DNA samples with GenCall scores < 0.25 at any locus were considered “no calls.” A SNP was deemed to have failed if < 95% of DNA samples generated a genotype at the locus. Cluster plots were manually inspected for all SNPs identified as potentially associated. Publicly available data from the Wellcome Trust Case-Control Consortium 2 study were used as controls. Specifically, this included 2420...
persons from the 1958 Birth Cohort (58BC, also known as the National Child Development Study)\textsuperscript{14} and 2737 from the National Blood Service Collection (NBS), all of which had been genotyped on Illumina 1.2M arrays at the Wellcome Trust Sanger Institute. To ensure quality of genotyping in all assays, at least 2 negative controls and 1% to 2% duplicates (showing a concordance > 99.99%) were genotyped.

Collection of blood samples and clinicopathologic information from subjects was undertaken with informed consent and relevant ethical review board approval from the University of Oxford and the Institute of Cancer Research in accordance with the tenets of the Declaration of Helsinki.

Quality control
For any samples showing a high degree of relatedness, we removed the sample with the lower call rate from the analysis, following previously described protocols.\textsuperscript{13} This obviates the need to adjust for familial correlations and ensuring that unbiased estimates of HL risk associated with genotypes are derived. We excluded SNPs on the basis of deviation from Hardy-Weinberg equilibrium using a threshold of $P < 1.0 	imes 10^{-5}$ in controls. We also removed SNPs with minor allele frequency < 0.05 and call rate < 0.95.\textsuperscript{13} To identify and exclude persons with non-Western European ancestry, case and control data were merged with data on persons of different ethnicities from the International HapMap Project, and dissimilarity measures were used to perform principal component analysis (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). For the control samples, we also followed the guidelines from the Wellcome Trust Case-Control Consortium and removed some additional controls.\textsuperscript{13} After imposing these stringent quality control measures, SNP genotypes were available on 582 cases and 4736 controls (2235 58C and 2501 NBS).

Statistical and bioinformatics analysis
For single SNP analyses, we considered the MHC to be defined by a 4.5-Mb region bordered by the RFP and MLN genes (rs209130 at 28 975 779 bp and rs1547668 at 33 883 424 bp, respectively) at the telomeric and centromeric ends of 6p21, respectively. For the HL imputation, we made use of genome-wide association study SNP data for an extended region in Hardy-Weinberg equilibrium using the algorithms described earlier.\textsuperscript{11,12} Comparison between observed and imputed HLA alleles for a subset of controls, for which we had experimentally determined HLA types, showed a high degree of concordance (> 90% at the 4-digit level; see “Statistical and bioinformatics analysis”). For each person and locus, the imputation methodology returns probabilities associated with the sample having each allelic type; these probabilities have been shown to be reasonably well calibrated.\textsuperscript{13} Thus, we were able to incorporate these probabilities into the general logistic regression framework, to account for any uncertainty associated with imputed types.

We initially considered 1700 SNPs mapping to the 4.5-Mb region that encompasses the classic MHC region at 6p21.3. Figure 1 shows the strength of the single SNP associations across this region. A total of 25 SNPs showed evidence for an association with HL risk at $P < 10^{-7}$, most mapping to HL class II regions. The strongest single-SNP based association was attained for rs6903608 mapping to 32 536 263 bp, about 16 kb centromeric to HLA-DRA ($P = 6.63 	imes 10^{-19}$; Figure 1A; supplemental Table 1).

Five HLA class II alleles showed evidence of association with HL risk at the $1 	imes 10^{-7}$ threshold in the unconditional logistic regression analysis, namely, DQA1*02:01, DRB1*07:01, DQB1*03:03, DRB1*15:01, and DQB1*06:02 (supplemental Table 1). The associations at DQA1*02:01, DRB1*07:01, and DQB1*03:03 confer an increased risk of HL, whereas the associations at DRB1*15:01 and DQB1*06:02 confer a reduced risk. Some of these alleles, such as DRB1*15:01 and DQB1*06:02, are known to be in strong LD in European populations. The strongest association was provided by DQA1*02:01 (odds ratio [OR] = 0.45, $P = 6.17 	imes 10^{-15}$). Of the class I HLA alleles, the strongest association observed was for B*07:02, although it did not reach genome-wide significance (supplemental Table 1; OR = 1.39, $P = 4.40 	imes 10^{-5}$).

To evaluate the independence of associations, we conducted stepwise logistic regression, jointly on SNPs and imputed HLA
alleles, initially including rs6903608 as a covariate in our model. Conditional analysis showed that most, but not all, of the class II variation defined by the SNP genotype could be explained for by rs6903608 (Figure 1B; supplemental Table 2). The strongest secondary signal appeared to be for SNPs rs9277565 (OR = 1.70, $P = 4.57 \times 10^{-13}$) and rs2281389 (OR = 1.73, $P = 6.31 \times 10^{-13}$), which map closely to HLA-DPB1 and one of which, rs9277565, has previously been shown to be in LD ($r^2 = 0.37$) with HLA-DPB1*03:01.20 Note that classic alleles at HLA-DPB1 were not imputed here because of lack of training data. Inclusion of rs2281389 in the logistic model accounted for most of the remaining signal (Figure 1C; supplemental Table 3), except for the protective effect of HLA-DQA1*02:01 (OR = 0.56, $P = 1.51 \times 10^{-7}$). DQA1*02:01 is nearly perfectly correlated with DRB1*07:01, and it is unclear which of the 2 alleles drives the observed signal; here for simplicity, we only refer to DQA1*02:01. When rs6903608, rs2281389, and DQA1*02:01 were all included as covariates to the model, no additional loci showed genome-wide evidence for association (Figure 1D; supplemental Table 4).

Although genetic variation defined by rs6903608 provides the strongest association signal, collectively these data provide evidence for 2 additional independent disease loci, defined by rs2281389 and HLA-DQA1*02:01 (Figure 1; supplemental Tables 2-3). rs2281389 lies in close proximity to HLA-DPB1, variation in which has been associated with HL risk in a number of previously published studies,5,21 with DPB1*03:01 appearing to confer susceptibility (OR = 1.42) and DPB1*02:01 resistance (OR = 0.49) to HL.21

**Discussion**

To examine the relationship between HLA and the risk of developing HL, we have conducted a large case-control study and have systematically examined the relationship between genetic variants and HL risk for the entire 4.5-Mb 6p21.3 region that encodes the MHC.

Using genome-wide association SNP data in principal components analysis has allowed us to minimize the potential problem of population stratification as formal statistical analysis provided no significant evidence that population substructure is a confounding factor in our study. The large dataset and dense set of informative markers in our study have also made possible the detection of independent effects at class II loci. Imputation of HLA alleles from SNP data has allowed us to also analyze systematically the relationship between HLA types and HL risk, at both the 2- and 4-digit levels of significance.

Our study provides unequivocal evidence for an association between the MHC and risk of developing HL. Moreover, and specifically, it provides evidence of a relationship between HLA class II genotypes and HL risk. Although we acknowledge that we did not impute all of the HLA alleles, the analysis we have conducted on a dense set of single SNPs in the region will have been sufficient to recover strong associations at untyped loci. Moreover, our imputations cover the alleles carried by 97.4% of the European population, according to dbMHC (averaged across all loci for which imputations are made). Our analysis found no evidence that class I variation also contributes to disease development, as the signals from the class I region were of moderate significance ($P > 10^{-4}$) and disappeared in the conditional analysis.

Although our study represents the most comprehensive interrogation of the relationship between genetic variation in MHC and HL risk to date, the analysis has limitations. A hallmark of HL epidemiology is a bimodal age-specific incidence, and it has been argued that the disease in young and older adults is etiologically different; in particular, the prevalence of EBV in tumors is higher in older HL patients (~50% age ≥ 40 years compared with...
~ 30% age <40 years). HLA class I polymorphisms have been reported to be associated with development of infectious mononucleosis on primary EBV infection, and HLA-A*02 and HLA-A*01 alleles have been linked to the risk of developing EBV-positive HL. Such observations are in keeping with predominant class I restricted T-cell response to EBV infection. The majority of HL cases we have studied were young (93% age <35 years at diagnosis); hence, our findings are more likely to apply to EBV-negative HL. This suggestion is reinforced by the lack of association between HLA-A*02 or HLA-A*01 alleles and HL in the current study (P > .05). Our results therefore suggest, albeit individually, substantially different genetic contributions to the risk of EBV-positive and EBV-negative HL within the MHC.

The neoplastic multinuclear HRS cells that typify cHL are derived from germinal center B cells that express HLA class II antigens. Several observations suggest that antigen presentation is involved in the pathobiology of HL. HRS cells stimulate T-cell proliferation in mixed lymphocyte reactions and induce cytotoxic activity. Lack of membranous expression of HLA class II by HRS cells of HL is common at primary diagnosis and is associated with extranodal disease, lack of EBV in HRS cells, and absence of HLA class I expression. Expression of HLA class II by HRS cells is retained more frequently in EBV-associated cHL, and in HLA class I–positive HRS cells. Hence, a possible explanation for the association of HLA class II expression status might be that HLA class II directly activates T-helper 1 cells, thereby inducing and maintaining cytotoxic antitumor immune responses. Lack of HLA class II expression results in diminished activation of T-helper 2 and/or Treg cells. Therefore, the class II associations we identified may reflect differential binding affinity of the HLA class II molecule with immunogenetic antigenic peptides modifying class II down-regulation. This postulate is in keeping with class II variation being primarily associated with development of EBV-negative HL, something fully supported by our findings of strong class II associations.

The strongest association identified in our study was attained with rs6903608. Inherited susceptibility to HL is likely to be mediated through the coinheritance of multiple risk alleles. Under a multiplicative model of interaction between risk loci on the basis of the risks associated with heterozygosity and homozygosity for the C-risk allele of rs6903608 (supplemental Table 1), this variant accounts for approximately 6% of the increased risk of HL seen in first-degree relatives of patients. rs6903608 maps to an LD block of 13.8 kb from 32,503,014 to 32,516,820 bp on chromosome 6. The strongest risk allele of rs6903608 (supplemental Table 1), this variant accounts for approximately 6% of the increased risk of HL seen in first-degree relatives of patients.

References


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