A Novel Risk Locus at 6p21.3 for Epstein-Barr Virus-Positive Hodgkin Lymphoma

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Abstract

Background: A proportion of the genetic variants involved in susceptibility to Hodgkin lymphoma differ by the tumor’s Epstein–Barr virus (EBV) status, particularly within the MHC region.

Methods: We have conducted an SNP imputation study of the MHC region, considering tumor EBV status in 1,200 classical Hodgkin lymphoma (cHL) cases and 5,726 control subjects of European origin. Notable findings were genotyped in an independent study population of 468 cHL cases and 551 controls.

Results: We identified and subsequently replicated a novel association between a common genetic variant rs6457715 and cHL. Although strongly associated with EBV-positive cHL (OR, 2.33; 95% confidence interval [CI], 1.83–2.97; P = 7 × 10−12), there was little evidence for association between rs6457715 and the EBV-negative subgroup of cHL (OR, 1.06; 95% CI, 0.92–1.21), indicating that this association was specific to the EBV-positive subgroup (Phet < P = 10−8). Furthermore, the association was limited to EBV-positive cHL subgroups within mixed cell (MCHL) and nodular sclerosis subtypes (NSHL), suggesting that the association is independent of histologic subtype of cHL.

Conclusions: rs6457715, located near the HLA-DPB1 gene, is associated with EBV-positive cHL and suggests this region as a novel susceptibility locus for cHL.

Impact: This expands the number of genetic variants that are associated with cHL and provides additional evidence for a critical and specific role of EBV in the etiology of this disease.

Introduction

Hodgkin lymphoma is a cancer of the lymphatic system characterized by the presence of B-cell derived Hodgkin Reed–Sternberg (HRS) tumor cells (1). Hodgkin lymphoma is relatively rare, but contributes substantially to worldwide disease burden, totaling 66,000 new cases in 2012 and 25,000 deaths (2). It affects mainly young adults ages 15 to 35 years and older adults ages 55 years and over. Classical Hodgkin lymphoma (cHL) is the major form and comprises four histologic subtypes of which the nodular sclerosis Hodgkin lymphoma (NSHL) subtype is most common followed by mixed cellularity Hodgkin lymphoma (MCHL).
In approximately one third of cHL cases in industrialized countries, the HRS cells are a clonally expanded population of Epstein–Barr virus (EBV)-infected cells. Young adult and NSHL cases are less likely to be EBV-positive than older adult and MCHL cases (3). EBV-positive cases are more likely to be male in contrast with more equal gender representation among EBV-negative cases. EBV-positive cases are associated with recent EBV infection, as evidenced by an association with infectious mononucleosis, and also with weakened immunity, for example, HIV infection or iatrogenic immunosuppression after organ transplantation (4–6). The human leukocyte antigen (HLA) genes, located within the MHC region, have been implicated in the etiology of cHL (7–11). Some associations show heterogeneity by tumor EBV status (9, 11–14) reinforcing the importance of acknowledging EBV status in examining the etiology of cHL.

Here, we expanded our investigation of the MHC region based on data from a previous cHL genome-wide association study (GWAS) and performed EBV status–specific analyses using imputation procedures for both SNPs and HLA alleles.

Materials and Methods

Imputation of 791,716 SNPs in human chromosome 6 was conducted using MACH v1.0 (15) and Minimac (version 2010.12.13; ref. 16). The August 2010 release of the 1000 Genomes Project European (CEU) dataset was used as the reference panel to impute genotypes for 1,200 cHL cases (of which 265 were EBV-positive) from the EPILYMPH study, the Scotland and Newcastle Lymphoma Group (SNLG) studies, the Young Adult Hodgkin’s Disease Case–Control Study (YHHCCS), the Scandinavian Lymphoma Etiology Study, and the Northern Dutch HL study, and 5,726 controls (a large subset that were part of a GWAS of cHL reported previously; ref. 11; Supplementary Table S1). IRB: the study was approved the Northern Dutch HL study, and 5,726 controls (a large (YHHCCS), the Scandinavian Lymphoma Etiology Study, and the Scotland and Newcastle Lymphoma Group (SNLG) studies, the Genomes Project European (CEU) dataset was used as the evidence by an association with infectious mononucleosis, and also with weakened immunity, for example, HIV infection or iatrogenic immunosuppression after organ transplantation (4–6). The human leukocyte antigen (HLA) genes, located within the MHC region, have been implicated in the etiology of cHL (7–11). Some associations show heterogeneity by tumor EBV status (9, 11–14) reinforcing the importance of acknowledging EBV status in examining the etiology of cHL.

Here, we expanded our investigation of the MHC region based on data from a previous cHL genome-wide association study (GWAS) and performed EBV status–specific analyses using imputation procedures for both SNPs and HLA alleles.

Results

We performed SNP imputation of 1,200 cHL cases and 5,726 control subjects of European origin to undertake a comprehensive evaluation of the MHC region in total cHL and by EBV tumor status of cHL, while controlling for the effects of previously described susceptibility variants (11). We did not identify any novel signal (P < 10−8) in total cHL or the EBV-negative subgroup, in addition to previously described associations in MHC class I and class II regions (Supplementary Fig. S1A–S1B). However, three imputed genetic variants (rs6457715, rs6457714, and rs6457711) were associated with the EBV-positive subgroup at genome-wide significance levels (P < 10−7; Fig. 1). The rs6457715, rs6457714, and rs6457711 variants showed evidence of linkage disequilibrium (LD) and conditioning on rs6457715 (A/C) was consistent with a single association signal (risk allele frequencies: EBV-positive cHL = 0.87; controls = 0.79). We directly genotyped rs6457715 within a subset (N = 562; see Materials and Methods) of the sample in which we undertook imputation, and the concordance rate for rs6457715 between categorized dosages and direct genotypes was 99.47%, confirming the high accuracy of the imputation process. Within this discovery set, the rs6457715 major allele (A) was strongly associated with an increased risk of EBV-positive cHL [OR, 2.39; 95% confidence interval (CI), 1.36–3.49; P = 0.0013], but not EBV-negative cHL (P = 0.791) or overall cHL (P = 0.622; Supplementary Table S2). The association was more pronounced in the model adjusted for known MHC cHL susceptibility variants in both the validation and replication cohorts (Supplementary Table S2). Combining the imputation-
We have identified a novel group of highly correlated genetic variants, located near the MHC class-II HLA-DRB1 gene, which are associated with genetic susceptibility to cHL. Statistically robust observations were made in both discovery and validation cohorts, and cross validation of the genotyping methods confirmed their technical fidelity.

The association was restricted to the subgroup of cHL in which EBV was detected within the tumor cells by EBV EBER in situ hybridization and/or LMP-1 immunohistochemistry. This implies that these alleles are only relevant to the subset of cHL that are "EBV-positive cHL," that is, where the HRS cells appear to originate from a clonally expanded population of EBV-infected cell. Most MCHL cases are EBV-positive, however, the heterogeneity by tumor EBV status was also observed following stratification by NSHL and MCHL subtypes of cHL. This strong heterogeneity implies a biologic interaction between EBV and the genetic alleles leading to susceptibility to HL irrespective of the histologic subtype.

The association with the genetic variants was statistically independent of the previously described cHL susceptibility alleles found within the MHC region. A proportion of these other susceptibility alleles also demonstrate heterogeneity in their effects when considering the EBV status of the tumor (9, 11, 12). As noted above for rs6457715, heterogeneity in EBV-stratified cHL risk was observed even following stratification for the NSHL and MCHL subtypes, for the rs2734986 and rs6904029 and EBV-positive cHL and rs6903608 and EBV-negative cHL. All of these observations taken together imply a remarkable relationship between tumor EBV status and the risk conferred by genetic variants within the MHC region and reinforces the importance of acknowledging EBV status when examining the etiology of Hodgkin lymphoma.

We were unable to establish a link between rs6457715 and a particular HLA allele (Supplementary Table S4), albeit within...
the limitation inferring HLA genotypes by imputation. rs6457715 resides within an intronic region of the pseudogene HLA–DPB2, and is suggested to be an HLA–DPB1 expression quantitative trait locus (eQTL; ref. 21). However, multiple additional independent genetic variants at this locus, not associated with EBV-positive cHL, are also suggested to be HLA–DPB1 eQTLs (Supplementary Table S6), implying that differences in HLA–DPB1 expression levels are unlikely to clearly explain the association. Furthermore, we were unable to replicate this eQTL association with rs6457715 and HLA–DPB1. As with the rs6903608 effect on EBV-negative cHL (9), the functional mechanism through which the association with rs6457715 is mediated remains ambiguous.

rs6457715 represents the third independent genetic loci within the MHC region (represented by rs2734986, rs2248462, rs2395185, rs6903608, and rs3823355), sex and eight principal components analysis eigenvectors (or country in the replication analysis). The risk allele is the major allele (A) of rs6457715. Combined GWAS and replication results were generated using inverse variance weighting meta-analysis.

<table>
<thead>
<tr>
<th>Chr 6: rs6457715</th>
<th>Cases</th>
<th>Controls</th>
<th>OR</th>
<th>(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cHL</td>
<td>1,668</td>
<td>6,277</td>
<td>1.22</td>
<td>(1.10–1.35)</td>
</tr>
<tr>
<td>By histology (P_{het} = 0.955)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSHL</td>
<td>1,195</td>
<td>6,277</td>
<td>1.20</td>
<td>(1.06–1.36)</td>
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<tr>
<td>MCHL</td>
<td>309</td>
<td>6,277</td>
<td>1.38</td>
<td>(1.10–1.73)</td>
</tr>
<tr>
<td>By EBV status (P_{het} &lt; 0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV^− cHL</td>
<td>910</td>
<td>6,203</td>
<td>1.06</td>
<td>(0.92–1.22)</td>
</tr>
<tr>
<td>EBV^+ cHL</td>
<td>371</td>
<td>6,203</td>
<td>2.33</td>
<td>(1.83–2.97)</td>
</tr>
<tr>
<td>By histology and EBV (P_{het} &lt; 0.001)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCHL/EBV^−</td>
<td>119</td>
<td>6,203</td>
<td>2.95</td>
<td>(1.89–4.60)</td>
</tr>
<tr>
<td>MCHL/EBV^+</td>
<td>94</td>
<td>6,203</td>
<td>1.06</td>
<td>(0.73–1.54)</td>
</tr>
<tr>
<td>NSHL/EBV^−</td>
<td>214</td>
<td>6,203</td>
<td>1.99</td>
<td>(1.48–2.68)</td>
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<td>NSHL/EBV^+</td>
<td>741</td>
<td>6,203</td>
<td>1.08</td>
<td>(0.93–1.25)</td>
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<td>By age of diagnosis (P_{het} = 0.700)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15–35 years</td>
<td>921</td>
<td>6,277</td>
<td>1.20</td>
<td>(1.04–1.38)</td>
</tr>
<tr>
<td>36–85 years</td>
<td>746</td>
<td>6,277</td>
<td>1.25</td>
<td>(1.07–1.46)</td>
</tr>
<tr>
<td>EBV^+ cHL by study (P_{het} = 0.130)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>EPILYMPH</td>
<td>13</td>
<td>147</td>
<td>1.35</td>
<td>(0.42–4.34)</td>
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<tr>
<td>UK studies</td>
<td>207</td>
<td>3,236</td>
<td>1.98</td>
<td>(1.44–2.72)</td>
</tr>
<tr>
<td>SCALE</td>
<td>83</td>
<td>189</td>
<td>1.70</td>
<td>(0.98–2.95)</td>
</tr>
<tr>
<td>Netherlands</td>
<td>68</td>
<td>1,804</td>
<td>4.33</td>
<td>(2.20–8.52)</td>
</tr>
</tbody>
</table>

Figure 2.
Summary of results for rs6457715 and risk of HL by cHL subgroups. Results of analyses combining the GWAS and replication stages are shown for total cHL and by histology, tumor cell EBV status, age of onset, and study. EBV-positive cHL results are also shown stratified by study, ORs and 95% CIs were derived using multiple logistic regression assuming a log-additive genetic model and adjusting for the five known MHC loci (i.e., rs2734986, rs2248462, rs2395185, rs6903608, and rs3823355), sex and eight principal components analysis eigenvectors (or country in the replication analysis). The risk allele is the major allele (A) of rs6457715. Combined GWAS and replication results were generated using inverse variance weighting meta-analysis.

*Study-specific results for EPILYMPH and UK studies included GWAS and replication data, whereas results for SCALE and the Netherlands studies are those from the GWAS.
conferred by common genetic variants. When considered as a genetic risk score, in a multivariate model, there was an approximately 7-fold difference between the top and bottom 25% of allele carriers. Such a model makes a number of important assumptions, for example, the absence of LD, which is a particularly complex in this region of the genome. Nevertheless, the magnitude of this association implies some potential utility for risk prediction of EBV-positive CHL, particularly in context of other described Hodgkin lymphoma EBV positive risk factors, such as infectious mononucleosis.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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