Role of the HLA System in the Association Between Multiple Sclerosis and Infectious Mononucleosis

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Objective: To determine whether multiple sclerosis (MS) and infectious mononucleosis (IM) share common HLA associations.

Design: A prospective cohort study was conducted from October 1, 1999, through September 30, 2003.


Patients: Participants included 179 individuals who underwent asymptomatic Epstein-Barr virus seroconversion and 175 patients who developed IM.

Intervention: Genotyping for 5 classical HLA loci (HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1).

Main Outcome Measure: Diagnosis of IM and allele frequency.

Results: Allelic analysis showed that HLA-DRB1*01:01 was significantly associated with the development of IM (odds ratio, 3.2; \(P < .001\)). Patients with IM and HLA-DRB1*01:01 had a lower Epstein-Barr virus viral load compared with those without the allele (median, 783 vs 7366 copies/10^6 peripheral blood mononuclear cells; \(P = .03\)).

Conclusion: HLA-DRB1*01:01 is protective against developing MS; thus, a common genetic basis between IM and MS is not supported.

Arch Neurol. 2011;68(4):469-472
manufacturer’s instructions, and stored at −70°C. According to the
described10 on 179 asymptomatic seroconverters and 144 pa-
patients who previously underwent genotyping for EBV serologic
determination. Those with EBV-seronegative results were then monitored for the de-
velopment of IM while at the university (approximately 4 years). In addition, students who developed IM but were not enrolled in the original study were referred by the university health cen-
ter and asked to participate. Upon diagnosis of IM (median, 10
days after onset of symptoms; range, 3–42 days), patients were
examined and asked to provide a blood sample. Students with
seronegative EBV test results on enrollment who did not re-
port symptoms of IM were asked to return for further testing
upon diagnosis. Students with seropositive EBV results were reg-
sarded as asymptomatic seroconverters and formed the EBV-seropositive group. Data on 179 individuals identified as asymptomatic seroconverters (controls) and 175 patients with IM were available for analysis. There was no varia-
tion in ethnicity between groups. Most control participants
were asymptomatic seroconverters (controls) and all those diagnosed as having IM carried the type 1 EBV virus strain; therefore, infection with different EBV types was assumed to be minimal.9

PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood mononuclear cells were separated from whole blood by routine density gradient centrifugation, washed, and counted. DNA was extracted from approximately 5 × 10⁶ cells (Easy-DNA Kit; Invitrogen, Paisley, Scotland), according to the manufacturer’s instructions, and stored at −70°C.

HLA GENOTYPING

High-resolution sequence–based typing of HLA-A, HLA-B, HLA-C, HLA-DRB1, and HLA-DQB1 was performed as de-
scribed10 on 179 asymptomatic seroconverters and 144 patients with IM. Data on an additional 31 patients with IM who previously underwent genotyping for HLA-DRB1 alone were added to the analysis.

VIRUS LOAD QUANTIFICATION

The EBV-DNA load was determined by quantitative competi-
tive polymerase chain reaction as described by Stevens et al.11
The amplification reaction contained 50mM potassium chlo-
ride, 1.5mM magnesium chloride, 10mM TRIS-hydrochloride
buffer (pH, 8.5), 200µM deoxynucleoside triphosphates, 25 pmol
of each primer (5’ primer labeled with biotin), and 1 U of Taq
polymerase. Cycling conditions were 4 minutes at 95°C; 40
cycles at 95°C, 55°C, and 72°C for 1 minute each; and 3 min-
utes at 72°C. Products were captured on a streptavidin-coated plate and probed with digoxigenin-labeled wild-type and in-
ternal standard probes. Optical density was measured and used to
calculate the number of copies.

STATISTICAL ANALYSIS

A Fisher exact test was used to compare allele frequencies be-
tween cohorts at each of the 5 loci. All P values are presented
as uncorrected for multiple testing. Viral copy number was com-
pared across groups using the Mann-Whitney test. A P value
<.05 was considered significant. To correct for multiple test-
ing, permutation tests were performed (10⁶ permutations).

Haplotype analyses were performed (R package haplo.stats,
Version 1.4.4; http://mayoresearch.mayo.edu/schaid_lab/
software.cfm); cohorts were treated as either case or control.
This approach, achieved using the haplo.cc function, adopts an additive-effect linear modeling method to assess associa-
tion between the binary trait and the haplotypes. Various case-
control groupings were assessed, with 2000 control simula-
tions done for each. The minimum haplotype frequency for in-
clusion was set at 0.005, and the most frequent haplotype was
treated as the baseline. Because sample numbers were small, some key haplotypes may not have been modeled.

Initial allele and haplotype frequencies were compared be-
tween the IM cohort (n=144) and EBV-positive group
(n=179) for individuals who underwent genotyping for all 5 HLA loci.
Six different alleles were identified with a frequency differ-
ence yielding a nominal P value of <.05 (Table). HLA-DRB1*01:01 was the most significant allele in the IM vs EBV-positive cohort comparison, being signifi-
cantly more frequent in the IM group (odds ratio,2.49;
P=.003). The fre-
quency of only HLA-DRB1*01:01 remained significant
after permutation testing (corrected P=.048).

In the haplotype analysis, HLA*-03:01–HLA-B*-35:
01–HLA-C*-04:01–HLA-DRB1*01:01–HLA-
DQB1*05:01 was significantly associated with IM (P=.03),
being overrepresented in the IM cohort. All other hap-
lotypes were nonsignificant in all comparisons (P>.05).
When we included the data from 31 patients who un-
derwent genotyping for HLA-DRB1 alone, the associa-
tion of IM with HLA-DRB1*01:01 became stronger (IM

<table>
<thead>
<tr>
<th>Allele</th>
<th>IM Allele Frequency</th>
<th>EBV-Positive Allele Frequency</th>
<th>OR (95% CI)</th>
<th>P Value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-B*15:01</td>
<td>0.04</td>
<td>0.08</td>
<td>0.46 (0.21-0.96)</td>
<td>.04</td>
</tr>
<tr>
<td>HLA-C*02:02</td>
<td>0.01</td>
<td>0.06</td>
<td>0.23 (0.06-0.69)</td>
<td>.004</td>
</tr>
<tr>
<td>HLA-C*04:01</td>
<td>0.14</td>
<td>0.08</td>
<td>1.73 (1.03-2.94)</td>
<td>.03</td>
</tr>
<tr>
<td>HLA-DQB1*03:01</td>
<td>0.12</td>
<td>0.19</td>
<td>0.59 (0.37-0.91)</td>
<td>.01</td>
</tr>
<tr>
<td>HLA-DRB1*01:01</td>
<td>0.10</td>
<td>0.04</td>
<td>2.49 (1.29-5.01)</td>
<td>.003</td>
</tr>
<tr>
<td>HLA-DRB1*04:01</td>
<td>0.06</td>
<td>0.11</td>
<td>0.55 (0.30-0.98)</td>
<td>.03</td>
</tr>
</tbody>
</table>

Abbreviations: CI, confidence interval; EBV, Epstein-Barr virus; IM, infectious mononucleosis; OR, odds ratio.

*Significant at P<.05.
We observed that HLA-DRB1*01:01 is the allele most significantly associated with IM, being overrepresented in individuals who develop this syndrome compared with individuals with asymptomatic EBV infection. The HLA-DRB1*01 allele is protective in MS,12 and we found no association with the strong MS risk allele HLA-DRB1*15. Therefore, our study rules out a common genetic association between MS and IM.

The association of HLA class II with IM is functionally plausible. Epstein-Barr virus preferentially infects B lymphocytes through the binding of the major viral envelope glycoprotein Gp350 to the CD21 receptor on the surface of B cells and through the binding of a second glycoprotein, Gp42, to HLA class II molecules.13 Therefore, it may be that HLA-DRB1*01 is somehow more permissive for EBV entry, leading to enhanced proliferation during the initial stage of infection and effective induction of the immune response. Interestingly, HLA-DRB1*01 has also been shown14-15 to be a potent stimulator of CD8+ and CD4+ T-cell responses after infection with hepatitis B and mumps vaccination—both in terms of magnitude and quality of function. Furthermore, reports16 investigating the role of HLA class II molecules in the control of hepatitis C virus infection show that HLA-DRB1*01 possession correlates with good control and clearance of the virus, suggesting that the immune response is more effective in these individuals. Similarly, in the case of primary EBV infection, it may be that the HLA-DRB1*01 allele strongly binds an immunodominant EBV epitope or a selection of epitopes, leading to a more robust helper T-cell function and the subsequent heightened immune response that characterizes IM. This may in turn control initial virus replication more effectively and minimize the duration of the infection.

Our viral load estimations on samples taken at similar time points (HLA-DRB1*01 positive: median, 11.5 days after onset of symptoms [range, 4-42 days]; HLA-DRB1*01 negative: median, 10 days [range, 3-28]) show a significant reduction in EBV copy number in individuals with HLA-DRB1*01, suggesting that immune control of virus replication is more effective in these individuals. However, the remit of this study did not allow for a detailed analysis of the immune response to confirm this hypothesis. In addition, because of the low allele frequency of HLA-DRB1*01, the attributable risk may be low. It may also be that HLA-DRB1*01 leads to more severe IM rather than susceptibility to IM.

A previous investigation6 highlighted the role of microsatellites in the HLA class I region as being associated with IM; however, the present study suggests that the HLA class II region is the principal association, as HLA-DRB1*01 is the most significantly associated allele in the analysis. Nevertheless, the haplotype analysis highlights the fact that HLA-A*03:01–HLA-C*04:01–HLA-DRB1*01 is the haplotype associated with IM, which may reflect either linkage disequilibrium or epistatic effects to increase the risk of IM.

The cohorts that we studied were perhaps underpowered to fully disentangle the effects of HLA class I and II genes as well as to detect loci exerting a small effect on IM risk, and population stratification could be an issue despite our efforts to match cohorts for ethnicity. Replication of the results obtained is therefore key to understanding in detail the genetic basis of IM. However, a common genetic association between MS and IM would mean that HLA-DRB1*15 would exert effects of similar magnitude in both diseases, which we can exclude. These data suggest that EBV plays a causal role in MS pathogenesis. Further work is warranted to understand the mechanism by which EBV influences MS risk.

Accepted for Publication: December 21, 2010.
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(REPRINTED) ARCH NEUROL / VOL 68 (NO. 4), APR 2011 WWW.ARCHNEUROL.COM

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material support: Conacher, Giovannoni, Crawford, and McAulay. Study supervision: Meier, Ebers, Giovannoni, Crawford, and McAulay.

Financial Disclosure: None reported.

Funding/Support: Funding for this study was provided by the Medical Research Council grant G0801976.

Role of the Sponsor: The study sponsor had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Additional Contributions: The authors thank many colleagues and collaborators at Barts and The London School of Medicine, the University of Oxford, and the Edinburgh University Health Service and its participants. The authors also acknowledge the Histocompatibility and Immunogenetics Laboratory, Royal Infirmary of Edinburgh, for typing of some IM samples.

REFERENCES