Human Leukocyte Antigen Association Study Reveals DRB1*04:02 Effects Additional to DRB1*07:01 in Anti-LGI1 Encephalitis

Vicente Peris Sempere, MSc,* Sergio Muñiz-Castrillo, MD, PhD,* Aditya Ambati, PhD, Sophie Binks, MD, Anne-Laurie Pinto, MSc, Veronique Rogemond, PhD, Sean J. Pittock, MD, Divyanshu Dubey, MD, Michael D. Geschwind, MD, PhD, Jeffrey Marc Gelfand, MD, Sonam Dilwalli, MD, Soon-Tae Lee, MD, PhD, Julian Knight, PhD, Katherine S. Elliott, PhD, Sarosh Irani, MD, PhD, Jérôme Honnorat, MD, PhD,* and Emmanuel Mignot, MD, PhD†

Neurol Neuroimmunol Neuroinflamm 2022;9:e1140. doi:10.1212/NXI.0000000000001140

Abstract

Background and Objectives
To study human leukocyte antigen (HLA) allele associations in anti-leucine–rich glioma-inactivated 1 (LGI1) encephalitis.

Methods
A multiethnic cohort of 269 patients with anti-LGI1 encephalitis and 1,359 controls was included. Four-digit HLA sequencing and genome wide association single-nucleotide polymorphism typing imputation (0.99 concordance) were used for HLA typing. Significance of primary and secondary associations was tested using χ², Fisher exact tests, or logistic regression with the control of population stratification covariates when applicable.

Results
DRB1*07:01 and DQA1*02:01, 2 alleles in strong linkage disequilibrium, were associated with the disease (90% vs 24%, OR = 27.8, p < 10⁻⁵₀) across ethnicity independent of variation at DRB3 and DQB1, 2 flanking HLA loci. DRB1*07:01 homozygosity was associated with a doubling of risk (OR = 2.1, p = 0.010), suggesting causality. DRB1*07:01 negative subjects were younger (p = 0.003) and more frequently female (p = 0.015). Three patients with malignant thymomas did not carry DRB1*07:01, whereas patients with other tumors had high DRB1*07:01 frequency, suggesting that the presence of tumors other than thymomas may be coincidental and not causal. In both DRB1*07:01 heterozygous individuals and DRB1*07:01 negative subjects, DRB1*04:02 was associated with anti-LGI1 encephalitis, indicating an independent effect of this allele (OR = 6.85, p = 4.57 × 10⁻⁶ and OR = 8.93, p = 2.50 × 10⁻₃, respectively). DRB1*04:02 was also independently associated with younger age at onset (β = −6.68, p = 9.78 × 10⁻³). Major histocompatibility complex peptide-binding predictions using LGI1-derived peptides revealed divergent binding propensities for DRB1*04:02 and DRB1*07:01 alleles, suggesting independent pathogenic mechanisms.

Discussion
In addition to the established primary DRB1*07:01 association in anti-LGI1 encephalitis, we observe a secondary effect of DRB1*04:02 with lower age at onset. Our study provides evidence for secondary effects within HLA locus that correlate with clinical phenotypes in anti-LGI1 encephalitis.

*These authors contributed in equal proportions to the analysis and the interpretation of the data, and design of the study as co-first authors.
†These authors designed, planned, and theorized the study from the beginning and have coordinated the collection and analysis of the data as co-last authors.

From the Stanford University Center for Sleep Sciences (V.P.S., A.A., and E.M.), Stanford University School of Medicine, Palo Alto, CA; French Reference Center for Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis (S.M.-C., A.-L.P., V.R., and J.H.), Hospices Civils de Lyon, Hôpital Neurologique, Synatac Team (S.M.-C., A.-L.P., V.R., and J.H.), NeuroMyoGene Institute, INSERM U1217/CNRS UMR5310, Université Claude Bernard Lyon 1, Université de Lyon, France; Oxford Autoimmune Neurology Group (S.B. and S.I.), Nuffield Department of Clinical Neurosciences, University of Oxford; Department of Neurology (S.B. and S.I.), John Radcliffe Hospital, Oxford, United Kingdom; Department of Laboratory Medicine and Pathology (S.J.P. and D.D.), and Department of Neurology (S.J.P. and D.D.), Mayo Clinic, Rochester, MN; Department of Neurology (M.D.G., J.M.G., and S.D.), University of California, San Francisco; Department of Neurology (S.-T.L.), Seoul National University Hospital, South Korea; and Wellcome Centre for Human Genetics (J.K. and K.S.E.), Nuffield Department of Medicine, University of Oxford, United Kingdom.

Go to Neurology.org/NN for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.
Anti-leucine–rich glioma-inactivated 1 (LG11) encephalitis is one of the most common autoimmune encephalitis with approximate incidence of 0.83/million/year in Europe.1 The classic presentation is in men (66%) of around 65 years. Faciobrachial dystonic seizures (FBDSs) are a characteristic and often presenting clinical feature of the disease, with other focal seizures in addition to amnesia, disorientation, and psychiatry features following.1-4 Despite an initial good response to most immunotherapies,5,6 cognitive sequelae after the disease are common and disabling.1,7

LG11 is a secreted neuronal protein preferentially expressed in the hippocampus, which forms a trans-synaptic complex between Kv1.1 potassium channels and AMPA receptors through its partners ADAM23 and ADAM22.9 Human antibodies against LG11 block these interactions,10,11 although they have different properties depending on the location of their epitopes, for example, if targeting the leucine-rich repeat (LRR) domain, which forms LG11 homodimers, or the epitope (EPTP) domain that binds to ADAM22/23.12,13 No consistent environmental triggers have been described, and anti-LG11 cases are only rarely paraneoplastic, with a few cases associated with thymoma.1,14

Strikingly, anti-LG11 encephalitis is strongly associated with the human leukocyte antigen (HLA) allele DRB1*07:01 in ~90% of Whites15-17 and Koreans,18 although a recent study found an association with DRB1*03:01 in 11 Chinese cases.19 Additional weaker effects have been suggested in HLA-DPB1 and HLA class I genes, although these remain unconfirmed.15 In this article, we extended the analysis of HLA genotypes in approximately 200 patients across 3 ethnic groups, confirming and extending results and their clinical relevance.

Methods

Subjects
A total number of 269 patients (246 Whites and 23 of Asian or African descent) diagnosed with anti-LG11 encephalitis and referred to (1) the Oxford Autoimmune Neurology Group in England with the contribution of the University of California, San Francisco, and Mayo Clinic, Rochester, MN (n = 121), and (2) the French Reference Center for Paraneoplastic Neurological Syndromes and Autoimmune Encephalitis in Lyon, France (n = 148), were included. Detection of anti-LG11 antibodies in serum and/or CSF was performed, as previously reported.2,20 HLA typing data on a subset of 68 patients recruited through Oxford and 72 patients recruited through Lyon have been previously published.8,16 The presence of FBDS and clinical severity measured by the modified Rankin score (mRS) at different stages of the disease (onset, maximum, and last follow-up) and tumoral status were determined from clinical or notes review.

Patients and controls recruited in Lyon were genotyped using the Affymetrix PMRA array, whereas patients recruited in Oxford were genotyped on Illumina GSAMD v1.0 and v2.0. A subset of controls were drawn from a different cohort and were genotyped with Illumina Infinium PsychArray-24 and had high-resolution HLA sequencing. All genotype data operations were performed using PLINK. All cohorts were imputed to the 1000 Genome Phase III21 after haplotype phasing and merged using QCTOOL. High-quality imputed calls ($R^2 \geq 0.9$) were used to extract genetic principal components using PLINK. A Euclidean distance-based measure was used to automatically match patients to the closest controls in a 1:5 ratio.

HLA imputation was performed using HLA Genotype Imputation with Attribute Bagging.22,23 Overall HLA imputation concordance was evaluated within a subset of 111 Oxford-recruited patients16 and 71 patients recruited in Lyon8 who were next-generation HLA-typed and found DRB1 concordance to be 99.4% and 99.6%, respectively. No differences were found between the Oxford and Lyon cohorts; thus, the results were analyzed as a single group after matching control subjects using principal component analysis (PCA). Genotypes with an imputation probability lower than 0.3 were removed.

Standard Protocol Approvals, Registrations, and Patient Consents
This study was approved by local ethics committees, and written informed consent was obtained from all the patients for the storage and use of biological samples and clinical information for research purposes.

Statistical Analyses
Categorical variables are presented as percentages and quantitative variables as means with SD. Although we report uncorrected $p$ values, our primary analyses were Bonferroni or false discovery rate (FDR) corrected per allele over 5% carrier frequency so that only findings corrected for multiple comparisons were discussed and pursued. These primary analyses included (1) comparisons of HLA frequencies in all subjects (HLA phenotype carrier analysis) and (2) effects of the “other alleles” in DRB1*07:01 heterozygous subjects (compared with heterozygous subjects and expected allele counts derived from the control population). After these findings, we subsequently
analyzed the effects of DRB1*07:01 homozygosity, differential effects of DRB3~DRB1*07:01~DQA1*02:01~DQB1 haplotypes, and effects in DRB1*07:01 negative cases, both within the White subgroup using \( \chi^2 \) statistics and across ethnic groups using general logistic regressions, with successive conditioning of the various HLA-associated alleles and using population principal component matching when necessary. These secondary analyses were not Bonferroni-corrected or FDR-corrected because these were considered confirmatory. The analytical plan followed was similar to that reported for narcolepsy by Ollila et al.\(^{24}\) A significance level was set at \( p < 0.05 \). Analyses were performed using R.

**Data Availability**

Raw data are available on request to the corresponding author.

**Results**

**Primary Association of DRB1*07:01 in Anti-LGI1 Encephalitis**

Consistent with previous studies,\(^8,15-17,19\) 88.9% of the cases (239/266, 3 patients removed because of low imputation probability) carried HLA-DRB1*07:01 (Table 1), independent of ethnicity. DRB1*07:01 homozygosity was associated with an odds ratio of 6.85, \( p = 2.16 \times 10^{-3} \), in the DRB1*07:01 heterozygous cases (Table 1). Using logistic regression analysis, we explored whether these haplotypes confer differential predisposition. As given in Table 2, these haplotypes had identical effects on disease predisposition. Because both DQA1 and DQB1 variations contribute to the HLA binding of the DQ molecule, this result suggests a primary effect of DRB1*07:01 on disease predisposition.

**Secondary Association of DPB1*03:01 and DRB1*04:02 in Anti-LGI1 Encephalitis**

We next studied the effects of DRB1 alleles in trans of DRB1*07:01. To do so, we compared non-DRB1*07:01 DRB1 allele counts in trans of DRB1*07:01 positive cases (1) vs non-DRB1*07:01 DRB1 allele counts in trans of DRB1*07:01 positive controls (Table 3, top) and (2) vs all non-DRB1*07:01 allele counts in controls, a comparison which should be identical to that of non-DRB1*07:01 allele counts in controls considering the Hardy-Weinberg equilibrium (Table 3, middle and bottom, this comparison has more statistical power). Any identified allelic association in the Hardy-Weinberg study was sequentially and iteratively removed to explore whether another, yet unidentified allele remained significant until no alleles remained significant. To avoid issues with population structure matching in each stratum, this analysis was performed in Whites only. DRB1*04:02, an allele found in only 1.9% of controls, was significantly associated with the disease (OR = 4.41, \( p = 2.16 \times 10^{-3} \) in DRB1*07:01 positive controls, and OR = 6.85, \( p = 4.57 \times 10^{-6} \) in all controls’ allele counts) in the DR7 heterozygous patients. Other effects included the susceptibility effects of DRB1*09:01 and DRB1*01:01 and protective effects of DRB1*15:01 and DRB1*13:02.

**Table 1** Effect of DRB1*07:01 Zygosity on Anti-LGI1 Encephalitis Risks (Top), Age, and Sex (Bottom)

<table>
<thead>
<tr>
<th>DRB1-DRB1</th>
<th>Cases (n = 266)</th>
<th>Controls (n = 1,330)</th>
<th>( p ) Value</th>
<th>OR (95% CI)</th>
<th>( p ) Value</th>
<th>OR (95%) CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:01-07:01</td>
<td>0.113 (30)</td>
<td>0.017 (22)</td>
<td>8.18E-65</td>
<td>50.50 (25.84-98.68)</td>
<td>0.01</td>
<td>2.01 (1.13-3.58)</td>
</tr>
<tr>
<td>07:01-no 07:01</td>
<td>0.786 (209)</td>
<td>0.232 (308)</td>
<td>7.26E-84</td>
<td>25.97 (16.94-43.81)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>No 07:01-no 07:01</td>
<td>0.102 (27)</td>
<td>0.752 (1,000)</td>
<td>Ref</td>
<td>Ref</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 2** Mean Age and Sex for DRB1*07:01 Zygosity on Anti-LGI1 Encephalitis Risks (Top), Disease Onset and Sex (Bottom)

<table>
<thead>
<tr>
<th>DRB1-DRB1</th>
<th>Mean age ± SD</th>
<th>( t ) Test cis</th>
<th>( t ) Test trans</th>
<th>Male</th>
<th>Female</th>
<th>Homozygous</th>
<th>Heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td>07:01-07:01</td>
<td>65.30 ± 9.24</td>
<td>0.916</td>
<td>N/A</td>
<td>0.122 (22)</td>
<td>0.091 (8)</td>
<td>0.831</td>
<td>0.83 (0.30-2.06)</td>
</tr>
</tbody>
</table>

**Abbreviations:** LGI1 = leucine-rich glioma-inactivated 1; N/A = not applicable; Ref = reference.

**Reference groups were used to compute \( p \) values and ORs. Cases and controls are reported in frequencies and sample size.**

**Footnotes:**

- Three patients were excluded because of low DRB1 imputation probability.
- Bonferroni corrected.
other DRB1 effects. Unlike previously reported,16 we could not confirm any independent effects in HLA-A, B, or C loci, although expected class I alleles associated with DR7 haplotypes were enriched in cases vs controls when not controlled for the presence of DR7 (data not shown).

**Table 2** Differential Effects of Various DR-DQ Haplotypes Carrying DRB1*07:01 on Disease Susceptibility

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Cases (n = 266)</th>
<th>Controls (n = 1,329)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>02:01-03:03-07:01-01:03</td>
<td>0.234 (62)</td>
<td>0.071 (95)</td>
<td>7.31E-39</td>
<td>24.17 (14.68–39.79)</td>
<td>0.195</td>
<td>0.76 (0.51–1.13)</td>
</tr>
<tr>
<td>02:01-02:07-01:01-02:01</td>
<td>0.239 (82)</td>
<td>0.081 (108)</td>
<td>7.31E-39</td>
<td>28.12 (17.43–45.35)</td>
<td>0.573</td>
<td>0.89 (0.61–1.28)</td>
</tr>
<tr>
<td>02:01-02:07-01:01-01:01</td>
<td>0.502 (133)</td>
<td>0.117 (156)</td>
<td>2.41E-72</td>
<td>31.57 (20.19–49.36)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
<tr>
<td>Non-DRB1*07:01</td>
<td>0.102 (27)</td>
<td>0.752 (1,000)</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Abbreviations: N/A = not applicable; Ref = reference. Cases and controls reported in frequencies and sample size. * Three patients have been removed because of low imputation probability. **Bonferroni corrected.

**Association of DRB1*04:02 in Non-DRB1*07:01 Positive Anti-LGI1 Encephalitis Cases**

We next explored the possibility of a residual HLA association in the rare DRB1*07:01 negative patients with European ancestry (Table 4). Strikingly, DRB1*04:02 was also overrepresented in

**Table 3** Heterozygous and Hardy-Weinberg Study on DRB1*07:01 Anti-LGI1 White Patients

<table>
<thead>
<tr>
<th>DRB1*07:01-X</th>
<th>DRB1*07:01-X</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:01</td>
<td>0.068 (13)</td>
<td>0.156 (46)</td>
<td>5.98E-03</td>
</tr>
<tr>
<td>13:02</td>
<td>0.005 (1)</td>
<td>0.061 (18)</td>
<td>4.29E-03</td>
</tr>
<tr>
<td>01:01</td>
<td>0.200 (38)</td>
<td>0.082 (24)</td>
<td>2.47E-04</td>
</tr>
<tr>
<td>04:02</td>
<td>0.084 (16)</td>
<td>0.020 (6)</td>
<td>2.16E-03</td>
</tr>
<tr>
<td>09:01</td>
<td>0.084 (16)</td>
<td>0.014 (4)</td>
<td>3.47E-04</td>
</tr>
<tr>
<td>Other alleles</td>
<td>0.556 (106)</td>
<td>0.667 (196)</td>
<td>Ref</td>
</tr>
</tbody>
</table>

**Hardy-Weinberg DRB1*07:01-X**

<table>
<thead>
<tr>
<th>DRB1*07:01-X</th>
<th>Cases (n = 2,114)</th>
<th>Controls (n = 2,087)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:01</td>
<td>0.148 (313)</td>
<td>0.42 (0.24–0.75)</td>
<td>0.150 (313)</td>
<td>0.49 (0.23–0.74)</td>
</tr>
<tr>
<td>13:02</td>
<td>0.061 (128)</td>
<td>0.08 (0.01–0.59)</td>
<td>0.061 (128)</td>
<td>0.08 (0.01–0.58)</td>
</tr>
<tr>
<td>01:01</td>
<td>0.086 (181)</td>
<td>0.26 (1.81–3.93)</td>
<td>0.087 (181)</td>
<td>0.26 (1.79–3.88)</td>
</tr>
<tr>
<td>04:02</td>
<td>0.013 (28)</td>
<td>6.85 (3.64–12.91)</td>
<td>0.013 (28)</td>
<td>5.38 (3.59–12.74)</td>
</tr>
<tr>
<td>09:01</td>
<td>0.013 (27)</td>
<td>3.22E-06</td>
<td>7.11 (3.76–13.44)</td>
<td>Ref</td>
</tr>
<tr>
<td>Other alleles</td>
<td>0.680 (1,437)</td>
<td>Ref</td>
<td>Ref</td>
<td>0.689 (1,437)</td>
</tr>
</tbody>
</table>

**Hardy-Weinberg DRB1*07:01-X**

<table>
<thead>
<tr>
<th>DRB1*07:01-X</th>
<th>Cases (n = 2,059)</th>
<th>Controls (n = 1,878)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15:01</td>
<td>0.152 (313)</td>
<td>0.41 (0.23–0.73)</td>
<td>0.176 (313)</td>
<td>0.37 (0.21–0.65)</td>
</tr>
<tr>
<td>13:02</td>
<td>0.062 (128)</td>
<td>0.08 (0.01–0.57)</td>
<td>0.068 (128)</td>
<td>0.07 (0.01–0.52)</td>
</tr>
<tr>
<td>01:01</td>
<td>0.088 (181)</td>
<td>2.59 (1.76–3.82)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>04:02</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>09:01</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Other alleles</td>
<td>0.754 (1,437)</td>
<td>Ref</td>
<td>Ref</td>
<td>0.765 (1,437)</td>
</tr>
</tbody>
</table>

Abbreviations: LGI1 = leucine-rich glioma-inactivated 1; N/A = not applicable; Ref = reference. Reference groups were used to compute p values and ORs. Cases and controls reported in frequencies and sample size. * Not corrected because of low sample size. **False discovery rate corrected.
these cases, indicating the effects of this allele both in DR7 positive and negative cases (OR = 8.93, p = 0.003). By contrast, DRB1*09:01 and DRB1*01:01 were not associated with the disease.

Clinical Associations

As reported previously, DRB1*07:01 negative subjects had a younger age at onset (56.7 vs 65.5 years, mean age, p = 0.003) and were more frequently female (53% vs 46%, p = 0.015). Intriguingly, the presence of DRB1*04:02 was also associated with reduced disease onset age (~6.7 years, p < 0.01, eTable 3, links.lww.com/NXI/A694). However, this finding could not be confirmed in DRB1*04:02 positive/DRB1*07:01 negative cases because of the small sample size (n = 4), despite a very low average age at onset (mean age ~40 years old, p = 0.057) in comparison with DRB1*07:01 positive patients (Table 5).

Next, these striking findings led us to examine whether DRB1*04:02 could also associate with a different clinical presentation. To address this, we analyzed the effect of DRB1*07:01 and DRB1*04:02 on the mRS scores at onset, maximal mRS score, mRS score in the last visit, and the presence of FBDS (eTable 4, links.lww.com/NXI/A694). The results showed a moderate association of DRB1*04:02 with an increased mRS score at onset (p = 0.049).

Additionally, we studied the association of DRB1*07:01 and DRB1*04:02 in patients with tumors (n = 32) and matched controls. Of 32 patients, 3 were diagnosed with malignant thymomas, none of them carrying DRB1*07:01 or DRB1*04:02. Conversely, in patients with tumors other than malignant thymomas, we found that the association of DRB1*07:01 with the disease status was also present and of the same magnitude as in patients without tumor (eTable 5, links.lww.com/NXI/A694).

HLA Binding Predictions

As DRB1*07:01 and DRB1*04:02 share some residues (eFigure 1, links.lww.com/NXI/A694), we finally examined if specific LGI-derived peptides could bind uniquely to these subtypes. We parsed the full-length sequences of LGI1 from the UniProt database26 in the FASTA format (accession number O95970). We used NetMHCIIpan4.027 with offset correction for DRB1*07:01 and DRB1*04:02 HLA binding prediction in consecutive overlapping peptides with a length of 15 amino acids. We considered rank values ≤ 1 as strong binders, and >1 but ≤ 3 as medium binders and compared DRB1*07:01 and DRB1*04:02 binding spectrums with that of the remaining DRB1 (eTables 6 and 7, links.lww.com/NXI/A694).

Thirty-three LGI1-derived peptides with a length of 15 amino acids were predicted as medium/strong binders for DRB1*07:01, encompassing 12 core-peptides located in both the LRR and EPTP domains. This binding peptide-spectrum was shared in more than 50% with DRB1*01:01, DRB1*04:01, DRB1*09:01, and DRB1*16:01. However, only ~18% of the peptides predicted as medium/strong binders for DRB1*07:01 (~30% of the binding cores) were predicted as well for DRB1*04:02; thus, overlap with these predisposing subtypes was low. The common medium/strong binders between

Table 4 Carriers Frequencies in European DRB1*07:01 Negative Subjects

<table>
<thead>
<tr>
<th>Allele</th>
<th>Cases (n = 25)</th>
<th>Controls (n = 910)</th>
<th>p Valuea</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*04:02</td>
<td>0.160 (4)</td>
<td>0.021 (19)</td>
<td>2.50E-03</td>
<td>8.93 (2.79–28.54)</td>
</tr>
<tr>
<td>DRB1*09:01</td>
<td>0.000 (0)</td>
<td>0.025 (23)</td>
<td>1.000</td>
<td>0.00 (0.00–6.61)</td>
</tr>
<tr>
<td>DRB1*01:01</td>
<td>0.200 (5)</td>
<td>0.169 (154)</td>
<td>0.598</td>
<td>1.22 (0.35–3.43)</td>
</tr>
</tbody>
</table>

Cases and controls are reported in frequencies and sample size. *p Value not corrected for multiple testing because of low sample size.

Table 5 DRB1*07:01 and DRB1*04:02 Alleles Effect on Age and Sex

<table>
<thead>
<tr>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Cases (n = 269)</th>
<th>Mean age ± SD</th>
<th>p Value</th>
<th>Male (n = 181)</th>
<th>Female (n = 88)</th>
<th>p Value</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*07:01 neg</td>
<td>DRB1*04:02 pos</td>
<td>0.015 (4)</td>
<td>40.00 ± 17.14</td>
<td>0.057a</td>
<td>0.011 (2)</td>
<td>0.023 (2)</td>
<td>0.588</td>
<td>0.43 (0.59–0.40)</td>
</tr>
<tr>
<td>DRB1*07:01 pos</td>
<td>DRB1*04:02 pos</td>
<td>0.059 (16)</td>
<td>63.00 ± 11.33</td>
<td>0.369</td>
<td>0.061 (11)</td>
<td>0.057 (5)</td>
<td>1.000</td>
<td>0.96 (0.31–2.89)</td>
</tr>
<tr>
<td>DRB1*07:01 neg</td>
<td>DRB1*04:02 neg</td>
<td>0.093 (25)</td>
<td>58.00 ± 13.63</td>
<td>0.021</td>
<td>0.061 (11)</td>
<td>0.159 (14)</td>
<td>0.013</td>
<td>0.34 (0.15–0.80)</td>
</tr>
<tr>
<td>DRB1*07:01 pos</td>
<td>DRB1*07:01 pos</td>
<td>0.112 (30)</td>
<td>65.30 ± 9.24</td>
<td>0.828</td>
<td>0.122 (22)</td>
<td>0.061 (8)</td>
<td>0.831</td>
<td>1.20 (0.50–2.85)</td>
</tr>
<tr>
<td>DRB1*07:01 pos</td>
<td>DRB1*04:02 neg</td>
<td>0.721 (195)</td>
<td>65.70 ± 10.49</td>
<td>Ref</td>
<td>0.746 (135)</td>
<td>0.670 (59)</td>
<td>Ref</td>
<td>Ref</td>
</tr>
</tbody>
</table>

Abbreviation: Ref = reference.
Reference groups were used to compute p values and ORs. Cases and controls reported in frequencies and sample size.
* Note that DRB1*04:02 has an association with lower age at onset across all cases with the disease (eTable 3, links.lww.com/NXI/A694).
DRB1*07:01 and DRB1*04:02 were predicted to bind with other DRB1 as well (eTable 6, links.lww.com/NXI/A694).

In the LRR domain, 4 peptides with core FLFTPSLQL were predicted to bind with DRB1*07:01 with ranks ≤1%, 3 of them also binding to DRB1*09:01 and DRB1*16:01 with ranks >1%. On the other hand, in the EPTP 6 domain, 4 peptides with binding core IQRMPSRGS were predicted as strong binders to DRB1*04:01 with ranks ≤1% (Figure 1), only sharing binding prediction with ranks >1%, with DRB1*04:04 (3 peptides), DRB1*08:01 (1 peptide), and DRB1*11:04 (1 peptide).

**Discussion**

This study expands our understanding of the role of HLA in anti-LGI1 encephalitis. First, we confirmed that the disease is strongly associated with DRB1*07:01 across different ethnicities. Second, homozygosity for the main disease association allele was found to be linked with an increased risk of developing the disease with minor or no effects on disease presentation or age at onset, suggesting a causal effect. This effect is similar to that observed in other HLA class II autoimmune diseases associated with single HLA class II heterodimers, such as narcolepsy with DQA1*01:02-DQB1*06:02,24,28,29 celiac disease with DQA1*05-DQB1*02,30,31 and rheumatoid arthritis with DRB1*04.32 An explanation for these “allele dosage” effects may be that the encounter between the HLA-DRB1*07:01, the initial autoantigen trigger, and CD4+ autoreactive T cells is a rare event that can be modeled as a stochastic process proportional to HLA class II molecule amounts. This may contrast with effects on age at onset, which has been shown to involve different HLA associations in many instances, possibly reflecting regulatory CD4+ T cells or subsequent CD8+ T-cell or B-cell effects. For instance, DQB1*06:02/DQB1*03:01 heterozygosity but not DQB1*06:02 homozygosity is strongly associated with an earlier age at onset in narcolepsy.33

Another finding of this study is the observation of a strong secondary predisposing association with a rare DRB1*04 subtype, DRB1*04:02. This association was significant in both DRB1*07:01 heterozygous and DRB1*07:01 negative subjects. DRB1*04:02 carriers appeared with an earlier age at onset and a slightly increased severity. Younger age at onset was also observed in DRB1*07:01 negative subjects, which moreover showed a female predominance, confirming our previous results.8 Of interest, 3 rare cases with malignant thymoma did not carry DRB1*07:01 or DRB1*04:02, suggesting the absence of HLA association. This mirrors our finding in diseases with antibodies against contactin-associated protein-like 2 where only nonparaneoplastic cases were associated with DRB1*11:01.34 Patients with nonthymoma tumors did not differ in DRB1*07:01 carrier frequency compared with nonparaneoplastic cases, suggesting that the presence of these diverse tumors, unlike malignant thymoma, is coincidental.1,7,8 Of note, the association between DRB1*07:01 or DRB1*04:02 and anti-LGI1 encephalitis would increase in significance if these thymoma-associated cases are removed from the analysis.

Possible associations were found with DRB1*09:01, a subtype also associated with DQA1*01:02-DQB1*06:02;24,28,29 because DQB1*03:03 is also present in approximately one-third of DRB1*07:01 haplotypes associated with the disease (Table 2), this could suggest DQB1 effects additional to DRB1*07:01 effects. We, however, believe this to be unlikely because DRB1*07:01 predisposes to anti-LGI1 encephalitis, independently of DQB1*03:03 (Table 1). Furthermore, this association was present in Whites and Asians in trans of...
patients could represent a di
one and a slight increase in severity in both DRB1*07:01 positive
sera, and CSF samples.

In addition to the above, we explored the possibility of residual
effects in other HLA genes after controlling for DRB1 effects. A
notable finding was a strong independent protective effect of
DPA1*01:02→DPB1*03:01, a finding distinct from previously
reported DPA1*02:01→DPB1*11:01 predisposing effects.16 Of
interest, DPB1*03:01 is a high-expression allele35 and has been
involved in susceptibility (not protection) to a number of other
autoimmune diseases, such as multiple sclerosis,36–38 severe
aplastic anemia,39 primary biliary cholangitis,40 and B27 negative
ankylosing spondylitis.41 By contrast, although HLA class I al-
leles known to be associated with DRB1*07:01, such as B*57:01,
B*44:03, C*06:02, and C*16:01, were increased in the sample
uncorrected for DRB1*07:01 (data not shown) as previously
reported,16 all HLA class I association disappeared after con-
trolling for the main DR7 association (data not shown).

To explore whether specific motifs of LGI1 could be involved in
these effects, we finally conducted peptide-binding prediction
analysis for critical DRB1 alleles. In contrast to other DRB1*04
molecules, DRB1*04:02, like DRB1*07:01, shares I and D at
positions 67 and 70 (eFigure 1, links.lww.com/NXI/A694),
positions critical to HLA pocket binding 4 (P4), possibly sug-

In conclusion, our study confirmed a primary effect of DRB1*07:
01 on disease susceptibility and revealed solid novel HLA asso-
ciations with DRB1*04:02 and DPB1*03:01. The DRB1*04:02
association was notable because of a secondary effect on age at
onset and a slight increase of severity in both DRB1*07:01 positive
and negative patients. This suggests that DRB1*04:02 positive
patients could represent a different subgroup or a modulation of
the existing sporadic phenotype that should be further explored.

Acknowledgment
The authors thank NeuroBioTec Hospices Civils de Lyon
BRC (France, AC-2013-1867, NFS96-900) for banking DNA,
sera, and CSF samples.

Study Funding
This study was supported by research grants NIH U01-
AI152590 and Fondation pour la recherche médicale (FRM,
DQ20170336751). It has been developed within the frameworks
of the LABEX CORTEX (reference ANR-11-LABX-0042) and
the BETPSY project (reference ANR-18-RHUS-0012), both
operated by the French National Research Agency (ANR).
SRI was supported by the Wellcome Trust (104079/Z/14/Z),
Medical Research Council (MR/V007173/1), BMA Research
Grants—Vera Down grant (2013), Margaret Temple (2017),
Epilepsy Research UK (P1201), the Fulbright UK-US com-
mision (MS Society research award), and the NIHR Oxford
Biomedical Research Centre. This research was funded in whole,
or in part, by the Wellcome Trust (Grant number 104079/Z/
14/Z). S. Binks has received support from the NIHR and
Wellcome Trust. For the purpose of Open Access, the author has
applied a CC BY public copyright license to any Author Ac-
cepted Manuscript version arising from this submission.

Disclosure
S.R. Israni is a coapplicant and receives royalties on patent
application WO/210/046716 (U.K. patent no., PCT/
GB2009/051441) entitled “Neurological Autoimmune Dis-
orders”; the patent has been licensed for the development of
assays for LGI1 and other VGKC-complex antibodies; has
filed a patent (Diagnostic Strategy to improve specificity
of CASPR2 antibody detection; Ref. JA94536P.GBA); has re-
ceived research support from and/or consultancy with UCB,
Immunovant, MedImmune, ADC therapeutics, CSL Behring
and ONO Pharmaceuticals. The remaining authors report no
disclosures relevant to the manuscript. Go to Neurology.org/NN
for full disclosures.

Publication History
Received by Neurology: Neuroimmunology & Neuroinflammation
References


Human Leukocyte Antigen Association Study Reveals DRB1*04:02 Effects Additional to DRB1*07:01 in Anti-LGI1 Encephalitis
Vicente Peris Sempere, Sergio Muñiz-Castrillo, Aditya Ambati, et al.
Neurol Neuroimmunol Neuroinflamm 2022;9;
DOI 10.1212/NXI.0000000000001140

This information is current as of February 3, 2022

Updated Information & Services
including high resolution figures, can be found at:
http://nn.neurology.org/content/9/2/e1140.full.html

References
This article cites 41 articles, 7 of which you can access for free at:
http://nn.neurology.org/content/9/2/e1140.full.html#ref-list-1

Citations
This article has been cited by 1 HighWire-hosted articles:
http://nn.neurology.org/content/9/2/e1140.full.html#otherarticles

Permissions & Licensing
Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at:
http://nn.neurology.org/misc/about.xhtml#permissions

Reprints
Information about ordering reprints can be found online:
http://nn.neurology.org/misc/addir.xhtml#reprintsus

Neurol Neuroimmunol Neuroinflamm is an official journal of the American Academy of Neurology. Published since April 2014, it is an open-access, online-only, continuous publication journal. Copyright © 2022 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2332-7812.