No evidence for shared genetic basis of common variants in multiple sclerosis and amyotrophic lateral sclerosis

An Goris¹,²,†,* , Jessica van Setten³,†, Frank Diekstra⁴,†, Stephan Ripke⁵,⁶,†, Nikolaos A. Patsopoulos⁷,⁸,⁹, Stephen J. Sawcer¹⁰, The International Multiple Sclerosis Genetics Consortium, Michael van Es⁴, The Australia and New Zealand MS Genetics Consortium, Peter M. Andersen¹¹, Judith Melki¹², Vincent Meiningher¹³, Orla Hardiman¹⁴,¹⁵, John E. Landers¹⁶,¹⁷, Robert H. Brown, Jr¹⁶,¹⁷, Aleksey Shatunov¹⁸, Nigel Leigh¹⁸, Ammar Al-Chalabi¹⁸, Christopher E. Shaw¹⁸, Bryan J. Traynor¹⁹, Adriano Chiò²⁰, Gabriella Restagno²¹, Gabriele Mora²², Roel A. Ophoff²³,²⁴, Jorge R. Oksenberg²⁵, Philip Van Damme²,²⁶,²⁷,²⁸, Alastair Compston¹⁰, Wim Robberecht²,²⁶,²⁷,²⁸, Bénédicte Dubois¹,²,²⁶, Leonard H. van den Berg⁴, Philip L. De Jager⁸,⁹,²⁹, Jan H. Veldink¹ and Paul I.W. de Bakker³,⁷,⁸,²⁹,³⁰

¹Laboratory for Neuroimmunology, Experimental Neurology, KU Leuven, Leuven, Belgium, ²Leuven Institute for Neurodegenerative Disorders (LIND), KU Leuven, Leuven, Belgium, ³Department of Medical Genetics, University Medical Center Utrecht, Utrecht, The Netherlands, ⁴Department of Neurology, Rudolf Magnus Institute of Neuroscience, University Medical Center Utrecht, Utrecht, The Netherlands, ⁵Analytic and Translational Genetics Unit, Massachusetts General Hospital, Boston, MA, USA, ⁶Stanley Center for Psychiatric Research, Broad Institute of MIT and Harvard, Cambridge, MA 02142, USA, ⁷Division of Genetics, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, USA, ⁸Program in Medical and Population Genetics, Broad Institute, Cambridge, MA 02142, USA, ⁹Department of Neurology, Center for Neurologic Diseases, Brigham and Women’s Hospital, Boston, MA 02115, USA, ¹⁰Department of Clinical Neurosciences, University of Cambridge, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 0QQ, UK, ¹¹Department of Clinical Neuroscience, Umea University, Umea SE-901 85, Sweden, ¹²Department of Neuropediatrics, University of Paris, Bicetre Hospital, Paris 94275, France, ¹³Department of Neurology, Université Pierre et Marie Curie, Hôpital de la Salpêtrière, Paris 75013, France, ¹⁴Department of Neurology, Beaumont Hospital, Dublin 9, Ireland, ¹⁵Department of Neurology, Trinity College, Dublin 2, Ireland, ¹⁶Department of Neurology, University of Massachusetts School of Medicine, Worcester, MA 01655, USA, ¹⁷Department of Neurology, Massachusetts General Hospital, Charlestown, MA 02129, USA, ¹⁸Department of Clinical Neuroscience, King's College London, Institute of Psychiatry, London SE5 8AF, UK, ¹⁹Neuromuscular Diseases Research Unit, Laboratory of Neurogenetics, National Institute on Aging, National Institutes of Health, Bethesda, USA, ²⁰Department of Neurosciences, University of Turin, Azienda Ospedaliera Città della Salute e della Scienza, Turin 10126, Italy, ²¹Department of Laboratory Medicine, Azienda Ospedaliera Città della Salute e della Scienza, Turin 10126, Italy, ²²Fondazione Salvatore Maugeri, Scientific Institute of Milan, Milan 20138, Italy, ²³Department of Psychiatry, Rudolf Magnus Institute of Neuroscience, UMC, Utrecht, The Netherlands, ²⁴Center for Neurobehavioral Genetics, University of California Los Angeles, Los Angeles, CA 90095, USA, ²⁵Department of Neurology and Institute of Human Genetics, School of Medicine, University of California at San Francisco, San Francisco, CA 94143-0435, USA, ²⁶Department of Neurology, University Hospital, Leuven, Belgium, ²⁷Laboratory for Neurobiology, Experimental Neurology, KU Leuven, Leuven, Belgium, ²⁸Vesalius Research Center (VRC), VIB, Leuven, Belgium, ²⁹Harvard Medical School, Boston, MA 02115, USA and ³⁰Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht 3508 GA, The Netherlands

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†The authors wish it to be known that, in their opinion, the first 4 authors should be regarded as joint First Authors.

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Genome-wide association studies have been successful in identifying common variants that influence the susceptibility to complex diseases. From these studies, it has emerged that there is substantial overlap in susceptibility loci between diseases. In line with those findings, we hypothesized that shared genetic pathways may exist between multiple sclerosis (MS) and amyotrophic lateral sclerosis (ALS). While both diseases may have inflammatory and neurodegenerative features, epidemiological studies have indicated an increased co-occurrence within individuals and families. To this purpose, we combined genome-wide data from 4088 MS patients, 3762 ALS patients and 12,030 healthy control individuals in whom 5,440,446 single-nucleotide polymorphisms (SNPs) were successfully genotyped or imputed. We tested these SNPs for the excess association shared between MS and ALS and also explored whether polygenic models of SNPs below genome-wide significance could explain some of the observed trait variance between diseases. Genome-wide association meta-analysis of SNPs as well as polygenic analyses fails to provide evidence in favor of an overlap in genetic susceptibility between MS and ALS. Hence, our findings do not support a shared genetic background of common risk variants in MS and ALS.

INTRODUCTION

Multiple sclerosis (MS, OMIM: 126200) is a common disease of the central nervous system characterized by inflammation, demyelination and axonal loss (1). Large extended families with the disease are extremely rare (2), but a genetic component in susceptibility to MS has been clearly demonstrated (1). Current knowledge of MS genetics is based on the identification of single nucleotide polymorphisms (SNPs) outside the HLA region (3,4).

Amyotrophic lateral sclerosis (ALS, OMIM: 105400) is a neurodegenerative condition with devastating impact. Multiple cellular events contribute to the pathobiology, including mitochondrial dysfunction, excitotoxicity, protein aggregation in the cytosol, impaired axonal transport, neuroinflammation and dysregulated RNA signaling (5). About 10–20% of cases are familial, and up to 50% of these can be explained by known mutations in 18 genes including SOD1, FUS, TARDBP and C9orf72 (6). The majority of patients are isolated cases, however. Not all results from genome-wide association studies (GWAS) have been replicated, but two regions of association have been confirmed in independent studies: a locus on chromosome 9 and variation in the UNC13A region (7–11).

One of the lessons learned in the GWAS era is the substantial overlap in susceptibility loci between diseases. This has been demonstrated for immune-related (12,13), metabolic (14) and psychiatric (15) disorders and indicates, sometimes unexpectedly, commonalities and differences between diseases. MS indeed shares several susceptibility loci with other immune-related disorders, including type 1 diabetes and Crohn’s disease (3). However, besides the immune component, key features of neurodegeneration, i.e. axonal transection, neuronal cell atrophy and neuronal death, are early pathological events in MS (1). Moreover, the irreversible disability seen in patients correlates stronger with neuronal damage than with inflammatory demyelination (16), although the cause of the neuronal damage remains elusive. On the other hand, for diseases classified as neurodegenerative such as ALS, an inflammatory or immune component has been implicated but is not yet conclusive (17,18). Case reports have described patients affected by both diseases (19–24) and an increased co-occurrence of MS and ALS compared with what is expected has been observed (25,26).

RESULTS

We first investigated previously reported (3,4,7–11) susceptibility loci in one disease for evidence of association in the other. None of the reported ALS susceptibility loci show evidence for association with MS (Table 1). Out of 56 established, independent MS susceptibility loci (3,4), 4 (7.1%) show nominal significance for association with ALS, but none survived multiple testing for the number of SNPs investigated (Table 2). As expected because of the overlap between the datasets used here and those used in the original studies of each disease separately, all previously reported risk factors for either MS or ALS show the same direction of effect for the respective disease in this dataset as in the original studies. Regarding the other disease, 4/5 reported ALS risk SNPs show the same direction of effect in MS as in ALS (sign test $P = 0.38$), and among established MS-associated SNPs, 26/56 (46%) SNPs show the same direction of effect in ALS (sign test $P = 0.69$). Four SNPs were previously highlighted for reaching suggestive $P$-values of $<10^{-5}$ for association with disease course (bout onset versus primary progressive MS) (3). Only one of these shows evidence for association with ALS but in the opposite direction (data not shown).

We next combined summary results from both MS and ALS datasets in a meta-analysis, looking for modest effects in each dataset that strengthen each other in the combined analysis. The combined analysis of both diseases included a total of 5,440,446 SNPs (Fig. 1). The genomic inflation factor ($\lambda_g$) was 1.033 for MS, 0.997 for ALS and 1.005 for the combined MS–ALS meta-analysis. In the meta-analysis, the HLA region reaches genome-wide significance, but this is driven by the MS component ($P$ ALS with same direction of effect $\geq 0.01$). One
Table 1. Association for reported ALS susceptibility loci with MS

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Rsid</th>
<th>Position (hg19)</th>
<th>Gene</th>
<th>Risk allele</th>
<th>P ALS</th>
<th>OR ALS</th>
<th>P MS</th>
<th>OR MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs6700125</td>
<td>59702797</td>
<td>FGGY (9)</td>
<td>T</td>
<td>0.087</td>
<td>1.06</td>
<td>0.085</td>
<td>1.06</td>
</tr>
<tr>
<td>10</td>
<td>rs10260404</td>
<td>15421098</td>
<td>DPPI6 (10)</td>
<td>C</td>
<td>0.0049</td>
<td>1.10</td>
<td>0.55</td>
<td>1.02</td>
</tr>
<tr>
<td>12</td>
<td>rs3499442</td>
<td>27543281</td>
<td>CSF72 (7,8)</td>
<td>T</td>
<td>5.8E-06</td>
<td>1.19</td>
<td>0.26</td>
<td>1.04</td>
</tr>
<tr>
<td>12</td>
<td>rs2366677</td>
<td>26636386</td>
<td>ITPR2 (11)</td>
<td>A</td>
<td>0.080</td>
<td>1.10</td>
<td>0.60</td>
<td>1.03</td>
</tr>
<tr>
<td>19</td>
<td>rs1260932</td>
<td>17752689</td>
<td>UNC13A (7)</td>
<td>C</td>
<td>8.3E-09</td>
<td>1.21</td>
<td>0.39</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Table 2. Association for independent, established MS susceptibility loci with ALS

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Rsid</th>
<th>Position (hg19)</th>
<th>Gene</th>
<th>Risk allele</th>
<th>P MS</th>
<th>OR MS</th>
<th>P ALS</th>
<th>OR ALS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>rs4648356</td>
<td>2709164</td>
<td>MMEL1 (TNFRSF14)</td>
<td>C</td>
<td>0.012</td>
<td>1.09</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>rs11810217</td>
<td>93148377</td>
<td>EVI5</td>
<td>T</td>
<td>0.0032</td>
<td>1.14</td>
<td>0.12</td>
<td>0.94</td>
</tr>
<tr>
<td>1</td>
<td>rs11581062</td>
<td>101407519</td>
<td>SLC30A7</td>
<td>G</td>
<td>0.032</td>
<td>1.08</td>
<td>0.025</td>
<td>1.08</td>
</tr>
<tr>
<td>1</td>
<td>rs1355352</td>
<td>117100957</td>
<td>CD58</td>
<td>A</td>
<td>1.2E-08</td>
<td>1.35</td>
<td>0.97</td>
<td>1.00</td>
</tr>
<tr>
<td>1</td>
<td>rs1322922</td>
<td>192541021</td>
<td>RGS1</td>
<td>A</td>
<td>0.0098</td>
<td>1.11</td>
<td>0.53</td>
<td>1.03</td>
</tr>
<tr>
<td>1</td>
<td>rs7522462</td>
<td>200881595</td>
<td>C9orf72 (7,8)</td>
<td>A</td>
<td>0.00083</td>
<td>1.13</td>
<td>0.023</td>
<td>0.92</td>
</tr>
</tbody>
</table>

Source of variants: (3), except where specified: (4).

\[ r^2 = 0.15 \text{ with adjacent variant rs12466022, } ^a\text{No SNP with } r^2 > 0.6. \]
region, near NPEPPS on chromosome 17 (rs2935183), reaches suggestive association levels of $P < 5 \times 10^{-7}$ but is once again driven by MS [$P_{\text{MS}} = 6.5 \times 10^{-7}; P_{\text{ALS}} = 0.41$].

Lastly, we investigated the possibility of an overlap of small susceptibility effects (polygenic score or ‘en masse’ effect). Therefore, we tested collectively SNPs that reached certain thresholds in the MS or ALS GWASs for association with ALS and MS, respectively. After correction for multiple testing, none of the models were significantly associated with disease (Tables 3 and 4), with the best model for each disease explaining only 0.05% of the phenotypic variance. To test whether the lack of association may have been affected by association results in the HLA region (which is known to be strongly associated with MS, but not with ALS), we repeated the polygenic analysis excluding SNPs in the HLA region (removing all SNPs on chromosome 6 between 29 and 33 Mb). This did not influence the results (Supplementary Material, Table S1).

**DISCUSSION**

In this study, we have applied several statistical approaches to the investigation of shared susceptibility loci between the
neurological diseases MS and ALS, which are both thought to involve inflammatory and neurodegenerative components (1,17,18) and for which case reports and epidemiological studies have reported co-occurrence within individuals or families (19–29). The strength of the study is that different statistical approaches are consistent in demonstrating that the number of regions in the genome with evidence for an overlap in susceptibility between the two diseases is not more than expected by chance. Among 65 loci having previously been implicated in one disease or disease subgroup, only 5 show nominally significant association with the other disease and none survive correction for multiple testing. There was no significant enrichment for the same direction of effect in both diseases. In a combined analysis of both diseases, no region outside of the HLA reaches genome-wide significance and only one reaches suggestive association levels of \( P < 5 \times 10^{-7} \). Moreover, for both these regions with evidence for association in both diseases, results appear driven by strong evidence in MS, despite sample sizes of similar magnitude for both diseases. Furthermore, the polygenic analysis demonstrates that it is unlikely that many common variants with effect sizes that are beyond the detection threshold for association are shared between the two diseases. This contrasts with other diseases where a polygenic risk score calculated for one disease is associated with related diseases, as in the example of schizophrenia and bipolar disorder (15).

MS is a clinically heterogeneous disease, with the majority of patients (~80%) suffering from a bout onset form of the disease with relapses and remissions, possibly followed by secondary progression, and the remaining 20% being characterized by progression from onset (1). It has been speculated that both forms represent a continuous spectrum of disease phenotypes with risk factors driving the balance between inflammation and neurodegeneration (32). Genetic association studies have so far not provided evidence for a different pathogenesis of the two forms (3). On the contrary, \( HLA-DRB1^*1501 \), the strongest risk factor in MS and especially immunological in nature, is shared between both bout onset and primary progressive MS. In this study, there was no evidence for shared loci with the same direction of effect between ALS and primary progressive MS.

A total of >50 common risk variants for MS have now been identified (3,4). There is a highly significant enrichment for immune system genes in this list, with only few variants having a potential neurological function (3). GWAS studies in ALS have seen limited success (8). This discrepancy in the number of common risk variants identified between immunological and other diseases has been suggested to reflect a history of selection and adaptation of variants influencing the immune system (33,34). Mutations in several genes cause familial forms of ALS, and it has been thought that less common (1–5%) or rare (<1%) variants play a role in sporadic forms of the disease as well (35). Similarly, first reports of less common and rare variants in MS are emerging (36,37). This category of variants, which are not well captured by current genome-wide association studies, may explain part of the heritability in MS and ALS that remains unaccounted for by common variants (‘missing heritability’), and potentially the shared neurodegenerative component. Next-generation sequencing offers a technology suited to address this hypothesis.

It has recently been demonstrated that a large proportion of ALS is related to a GGGGCC hexanucleotide repeat expansion in intron 1 of \( C9orf72 \) (38,39), located in a region on chromosome 9p previously highlighted in GWAS studies of ALS (7,8). We did not observe any association of the \( C9orf72 \) region with MS. This is in line with the fact that no repeat expansions were observed in a cohort of 215 MS patients (25). Hence, \( C9orf72 \) variation does not appear to be a risk factor for MS. It has been suggested that MS can act as a modifier that increases the likelihood of \( C9orf72 \) expansions becoming penetrant and causing concurrent ALS (25), although further investigation is required (40).

In summary, the overlap of common variants between MS and other autoimmune disorders is not matched by a similar overlap between MS and other neurological disorders, such as ALS in this study. Whether less common or rare variants explain some of the shared neurodegenerative or neuroinflammatory aspects of both diseases cannot be addressed with the currently available datasets and remains to be examined with emerging technologies.

MATERIALS AND METHODS

We used data from 6 datasets totaling 4088 MS patients and 7144 controls from a recent meta-analysis of MS genome-wide association studies (4). Imputation was performed using Beagle v3.1 and the 1000 Genomes Project (1000G) Phase I (a) reference panel (2010/11 data freeze, 2011/6 haplotypes), and analysis was performed as described previously using the post-imputation probabilities and the first five principal components (PC) as covariates (4), leading to association results for a total of 6 948 682 SNPs with INFO of >0.10 and a minor allele frequency of >0.01 in all 6 datasets.

The ALS study population consists of 3762 patients and 4886 controls over 11 cohorts, for which details have been described previously (7,41). Imputation was performed using Beagle v.3.1.1. software with the 1000G CEU Aug 2010 reference panel. Analysis on dosage data including 3 PC led to association results for 12 249 385 SNPs.

\( A/T \) and \( C/G \) SNPs were removed, and results from both datasets on 5 440 446 overlapping SNPs were combined using an inverse variance fixed-effects model as implemented in the PLINK software package (42). Power was >99% for OR of ≥1.2 and >80% for OR of ≥1.15 at a typical risk allele frequency of 30% and genome-wide significance \( P < 5 \times 10^{-8} \).

Polygenic risk scores were calculated per individual to test the collective impact of SNPs that are associated with ALS on MS and vice versa. For each trait (MS and ALS), we first pruned the association results of the GWAS by linkage disequilibrium \( (r^2 = 0.1) \), preferentially keeping SNPs with lower \( P \)-values. We selected twelve sets of SNPs (models) based on their GWAS \( P \)-values \( (<5 \times 10^{-8}, <5 \times 10^{-7}, <5 \times 10^{-6}, <5 \times 10^{-5}, <5 \times 10^{-4}, <5 \times 10^{-3}, 0.05$, \( <0.1, <0.2, <0.3, <0.4 \) and <0.5). The smallest model contains three SNPs, whereas the models of \( P < 0.5 \) contain >125 000 SNPs (Table 3). Next, we calculated a polygenic risk score in all individuals of the other GWAS by summing up the dosages of the risk alleles in each model, multiplied by the log-odds. We then tested the association between the risk score and the phenotype using logistic regression with the same number of PCs as used in the original analysis of each trait (ALS: PC1-3, MS: PC1-5).
and dummy-coded cohorts as covariates. Nagelkerke $r^2$ was calculated to test the variance explained by each model (43).

**SUPPLEMENTARY MATERIAL**

Supplementary Material is available at HMG online.

Conflict of Interest statement. None declared.

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**REFERENCES**