Original Contribution

HLA-DRB1*1501 and Spinal Cord Magnetic Resonance Imaging Lesions in Multiple Sclerosis

Madeleine H. Sombekke, MD; Carsten Lukas, MD; J. Bart A. Crusius, PhD; Diego Tejedor, PhD; Joep Killestein, MD, PhD; David Arteta, PhD; Antonio Martínez, PhD; Bernard M. J. Uitdehaag, MD, PhD; Dirk L. Knol, PhD; A. Salvador Peña, MD, PhD; Jeroen J. G. Geurts, PhD; Philip L. De Jager, MD, PhD; Frederik Barkhof, MD, PhD; Hugo Vrenken, PhD; Chris H. Polman, MD, PhD

Background: Multiple sclerosis (MS) is a heterogeneous neurologic disease with extensive variation with respect to the most affected central nervous system region (brain vs spinal cord).

Objective: To test the hypothesis that this variation in lesion location (brain vs spinal cord) might be (partially) genetically determined.

Design: Candidate gene study.

Setting: Academic research.

Patients: Patients were selected for the availability of DNA material, clinical variables, and brain and spinal cord magnetic resonance images (evaluating T2-weighted lesion load in the brain and the number of spinal cord lesions).

Main Outcome Measures: For genotyping, we used a DNA chip containing a set of genes mentioned in previous publications noting their relation to different phenotypes of MS. We assessed the association between brain and spinal cord abnormalities and the genotypes of the patients.

Results: One hundred fifty patients were included in the analysis. Five single-nucleotide polymorphisms within the major histocompatibility complex region were associated with the number of focal abnormalities in the spinal cord. The most significant was rs3135388 (surrogate marker for the HLA-DRB1*1501 allele). Carriers of HLA-DRB1*1501 had a median of 4 spinal cord lesions compared with 2 lesions for noncarriers (P < .001). No significant association was noted between the single-nucleotide polymorphisms and T2-weighted lesion load in the brain.

Conclusions: Carriership of HLA-DRB1*1501 (via rs3135388) was associated with the extent of focal abnormalities in the spinal cord. Spinal cord lesions might be an explanation for increased MS disease severity in patients carrying HLA-DRB1*1501.

Arch Neurol. 2009;66(12):1531-1536

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system characterized by inflammation, demyelination, and axonal loss in the brain and spinal cord. The current hypothesis is that MS is caused by a complex interplay of genetic and environmental factors. The genetic influence is characterized by the interaction of multiple genes that exert modest effects. The most striking among these is the association of the major histocompatibility complex (MHC) with MS susceptibility, which has consistently been reported over the past decades.1

Clinically, MS is a heterogeneous disease with a diverse spectrum of neurologic deficits and variable outcome. Some studies1-8 have focused on genetic predictors of disease phenotypes (such as disease severity, disease subtypes, magnetic resonance [MR] imaging characteristics, and response to treatment), showing some effect of different genes.

Besides clinical heterogeneity, considerable variation exists between patients regarding type and anatomic location of the lesions.9,10 Varying degrees of involvement of the cerebrum, brainstem, cerebellum, and spinal cord have been described in postmortem and MR imaging studies.11,12 This variability is unexplained, and much effort is being put in unraveling it. Several arguments point toward a genetic role in the regional distribution of lesions in the brain and spinal cord in MS. First, differences in pathologic manifestation of MS have been observed between Asian and Western populations. Asian-type MS is
characterized by predominant involvement of the optic nerve and spinal cord, whereas Western-type MS predominantly involves the brain. Genetic differences within the HLA region between these populations could underlie this variability.3,13-16 Second, recent studies17,18 confirm a tendency of patients to develop relapses in the same locations, including spinal cord lesions. This tendency for localized exacerbations could be genetically predetermined. Third, involvement of a genetic factor has already been demonstrated in lesion distribution in experimental autoimmune encephalomyelitis (an animal model that shows many similarities to MS): Butterfield et al19 showed that in mouse experimental autoimmune encephalomyelitis the lesions in the brain and spinal cord were controlled by different quantitative trait loci.

Many researchers have demonstrated the clinical relevance of spinal cord lesions in diagnosing MS and disability accumulation. However, we are unaware of any studies that included spinal cord MR imaging variables in genetic analyses searching for genotype-phenotype correlations.

In this study, we used a DNA chip containing a set of single-nucleotide polymorphisms (SNPs) in candidate genes to assess the genetic effect on regional lesion distribution in the brain and spinal cord as seen on MR imaging. The SNPs on this chip were selected based on published associations with MS pathogenesis, prognosis, or response to treatment.

STUDY PARTICIPANTS

Unrelated Dutch Caucasian patients were selected retrospectively from natural history studies conducted in the Department of Neurology and Radiology, Vrije Universiteit Medical Center, Amsterdam, the Netherlands. Patients were selected for the availability of DNA material, as well as brain and spinal cord MR images that fulfilled certain standardized requirements (described herein) and were obtained less than 2 years apart. The study was performed with the approval of the medical ethics committee of the VU University Medical Center, and informed consent was obtained from all participants. Patients were diagnosed as having MS as ascertained by Poser et al20 or revised McDonald criteria.21 For patients included in the analyses, clinical data were collected, including age, sex, type of disease, age at onset, disease course, and duration of disease. Disability status was determined for all subjects using Kurtzke’s Expanded Disability Status Scale22 and, whenever available, the Multiple Sclerosis Functional Composite Scale.23

SELECTION OF SNPs

Single-nucleotide polymorphisms were selected based on involvement in MS pathogenesis, prognosis, or response to treatment according to the literature published before July 2007. The polymorphisms were confirmed and associated with an identifier using dbSNP database (http://www.ncbi.nlm.nih.gov/SNP). Nucleotide sequences for the design of allele-specific probes and polymerase chain reaction primers were retrieved in the SNPper database (http://snpper.chip.org/bio). Sequence-specific probes and primers were designed using freely available Primer3 software (http://frodo.wi.mit.edu/).

If a polymorphism was not present in the database, position and sequences were established by performing a BLAST search (http://www.ncbi.nlm.nih.gov/BLAST.cgi) using the data available in the literature.

GENOTYPING

Genomic DNA was isolated from anticoagulated blood using DNAzol reagent (Molecular Research Center, Inc, Cincinnati, Ohio). Genotyping was performed using a newly developed low-density DNA microarray based on allele-specific probes. The design, fabrication, validation, and analysis of the arrays were performed using the procedure described by Tejedor et al24 with minor modifications.

BRAIN MR IMAGING

Scans were acquired on 1.0-T or 1.5-T scanners (Siemens AG, Erlangen, Germany) with standard head coils using 2-dimensional conventional or fast spin-echo proton density-weighted and T2-weighted images (repetition time, 2200-3000 milliseconds; echo times, 20-30 and 80-100 milliseconds) with section thicknesses of 3 to 5 mm, a maximum gap between sections of 0.5 mm, and an in-plane resolution of 1 × 1 mm². Lesions were identified by an expert reader and then outlined on the corresponding proton density-weighted image using home-developed semiautomated seed-growing software based on a local thresholding technique (Show_images).25 Lesion areas were multiplied by the intersection distance to obtain total T2-weighted brain lesion volume for each patient.

SPINAL CORD MR IMAGING

Spinal cord scanning included a cardiac-triggered sagittal proton density-weighted and T2-weighted dual-echo spin-echo sequence with a section thickness of 3 mm covering the whole spinal cord (repetition time, 2500-3000 milliseconds; echo times, 20-30 and 80-100 milliseconds), with a gap between sections of 0.3 mm, and an in-plane resolution of 1 × 1 mm². From this sequence, the number of focal lesions and the presence of diffuse abnormalities were scored by an experienced reader (C.L.). Diffuse abnormalities were defined as poorly delineated areas with increased signal intensity compared with the signal intensity of spinal cerebrospinal fluid best seen on proton density-weighted images.26 Lesion volume was assessed in the spinal cord as the total number of focal pathologic segments involved (total extension of all spinal cord lesions over several corresponding vertebral segments).

STATISTICAL ANALYSIS

First, the associations between the brain variable (T2-weighted lesion load) and the spinal cord variables (the number of focal lesions and the presence of diffuse abnormalities) were tested per SNP and per clinical variable. We used the nonparametric Spearman ρ rank correlation test, Kruskal-Wallis test, Mann-Whitney test, and χ² test as appropriate, applying the false discovery rate according to Benjamini and Hochberg27 to correct for multiple testing. The corrected number represents the expected proportion of false discoveries for a given P value cutoff. We used the cutoff point of P < .05 after false discovery rate correction. Spearman rank correlation coefficient was used to test the correlations between 2 scaled variables.

Second, multivariate analysis by general linear models was performed on the associated SNPs (P < .10 significance after correction for multiple testing). We used log-transformed brain T2-weighted lesion loads and the square root–transformed number of focal abnormalities in the spinal cord to correct for sig-

(Reprinted) Arch Neurol/Vol 66 (No. 12), Dec 2009 www.archneurol.com

©2009 American Medical Association. All rights reserved.
significant clinical variables and the type of MR imaging system (1.0 T vs 1.5 T).

All analyses were performed using commercially available software packages. These included SPSS (version 15; SPSS Inc, Chicago, Illinois), Excel 2003 (Microsoft Corporation, Redmond, Washington), and HelixTree (Golden Helix, Inc, Bozeman, Montana).

**RESULTS**

**PATIENT CHARACTERISTICS**

One hundred fifty patients were included in the analysis. Our patient group reflects a representative population with MS, with 36.7% being male and 20.0% having primary progressive MS (Table 1). Most patients (132 of 150) demonstrated abnormalities (mostly focal lesions) on spinal cord MR imaging, while all patients had abnormalities on brain MR imaging.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (N=150)</th>
<th>Remitting Relapsing (n=88)</th>
<th>Secondary Progressive (n=32)</th>
<th>Primary Progressive (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex, No. (%)</td>
<td>55 (36.7)</td>
<td>26 (29.5)</td>
<td>17 (53.1)</td>
<td>12 (40.0)</td>
</tr>
<tr>
<td>Age at MR imaging, ya</td>
<td>41.4 (11.2)</td>
<td>36.1 (9.2)</td>
<td>46.5 (8.9)</td>
<td>51.2 (9.8)</td>
</tr>
<tr>
<td>Disease duration, ya</td>
<td>7.1 (7.4)</td>
<td>4.4 (6.2)</td>
<td>12.8 (7.0)</td>
<td>9.2 (7.1)</td>
</tr>
<tr>
<td>Expanded Disability Status Scale score, median (interquartile range)</td>
<td>3.5 (2.5)</td>
<td>2.0 (2.0)</td>
<td>5.5 (2.5)</td>
<td>4.0 (3.0)</td>
</tr>
<tr>
<td>Brain T2-weighted lesion load, mL</td>
<td>7.7 (10.3)</td>
<td>4.9 (6.6)</td>
<td>16.2 (14.6)</td>
<td>7.0 (9.2)</td>
</tr>
<tr>
<td>Focal lesions in the spinal cord</td>
<td>3.4 (3.0)</td>
<td>3.3 (2.8)</td>
<td>4.5 (3.9)</td>
<td>2.8 (2.3)</td>
</tr>
<tr>
<td>Patients with diffuse abnormalities, No. (%)</td>
<td>20 (13.3)</td>
<td>9 (10.2)</td>
<td>6 (18.8)</td>
<td>5 (16.7)</td>
</tr>
</tbody>
</table>

Data are given as mean (SD).
Interquartile range is between 25% and 75%.

**GENOTYPING**

In total, 80 SNPs in 44 genes were studied on the DNA chip. Twelve SNPs were excluded from further analysis (5 SNPs were monomorphic, and 7 SNPs had a minor allele frequency <5% [eTable; http://www.archneurol.com]). Hardy-Weinberg equilibrium was calculated for all SNPs.

**CORRELATION BETWEEN LESION LOAD IN THE BRAIN AND GENOTYPES**

In the univariate analyses of T2-weighted lesion load in the brain vs all SNPs on the DNA chip, the only suggestive correlation was rs2107538 (CCL5) (OMIM 187011) (Table 2). Two clinical covariates were significant and were included in the general linear model for brain T2-weighted lesion load, namely, disease duration and MS subtype. The type of MR imaging system (1.0 T vs 1.5 T) was not associated with T2-weighted lesion load in the brain. After inclusion of rs2107538 in the model that contained the clinical covariates, this SNP showed significant association with brain T2-weighted lesion load (P = .03).

**CORRELATION BETWEEN SPINAL CORD ABNORMALITIES AND GENOTYPES**

Several MHC SNPs were found to be related to the number of focal spinal cord abnormalities (Table 2). The most significant is SNP rs3135388. Carrier status of the A allele
In the present candidate gene study, we observed an association between several SNPs within the MHC class II region and the number of focal abnormalities in the spinal cord. The most significant results were found for rs3135388. This SNP is a surrogate marker for the HLA-DRB1*1501 allele ($r^2=0.97$).

After correction for multiple testing, no genes were significantly related to T2-weighted lesion load in the brain. The MHC class II SNPs that were associated with more lesions in the spinal cord and with a higher lesion volume in the spinal cord do not seem to affect the presence of T2-weighted lesions in the brain.

Studies have unambiguously shown that HLA-DRB1*1501 strongly influences MS susceptibility. There is some evidence that this haplotype might also be associated with a more severe disease course. Recent findings show a correlation between carrier-ship of HLA-DRB1*1501 and Expanded Disability Status Scale scores using an extremes of outcome strategy (comparing patients with severe and mild disability only). In our study, we could not demonstrate a correlation between carri-ership of HLA-DRB1*1501 and Expanded Disability Status Scale scores. This might be because of the impossibility of performing the extremes of outcome analysis owing to a small sample size. However, findings from this study suggest that spinal cord lesions might be an additional explanation for the described relationship between HLA-DRB1*1501 and MS disease severity.

Abnormalities in the spinal cord correlate with the degree of disability and with the date of diagnosis. In our study, we also noticed an association between Expanded Disability Status Scale scores and the number of focal abnormalities in the spinal cord ($P=.02$, $p=0.185$). Future studies should include additional MR imaging variables of the spinal cord such as atrophy (cross-sectional area), as this variable might correlate better with clinical disability.

In a recent article, Okuda et al showed in their patients that HLA-DRB1*1501 affects disease severity as measured by clinical variables and by brain MR imaging variables. In our patients, we could not demonstrate this effect on T2-weighted lesion load in the brain or on cognitive function (using PASAT [Paced Auditory Serial Addition Task] scores). Post hoc power analysis detected a power of less than 25% to detect a T2-weighted lesion load difference of 1 mL, suggesting that a type II error cannot be ruled out. This also warrants careful interpretation of the suggestive positive finding of rs2107538 (CCL5) in association with brain T2-weighted lesion load, although previous findings have shown an influence of this gene on other MR imaging variables.

Previous data were sometimes conflicting about the effect of HLA haplotypes on brain MR imaging features. Some studies found a relationship between HLA genotypes and brain MR imaging quantitative markers, while other studies did not observe this effect of HLA-DRB1*1501. In addition, no effect of HLA-DRB1*1501 was observed on lesion distribution within the brain using T1-weighted and T2-weighted lesion maps. To date, we are unaware of any study that specifically relates HLA-DRB1*1501 to spinal cord abnormalities on MR imaging in a Caucasian population.

The molecular basis of the association with greater involvement of the spinal cord in patients carrying the HLA-DRB1*1501 allele remains unknown. Major histocompatibility complex class II genes are involved in self vs
nonspecific immune recognition. These genes encode for polymorphic surface glycoproteins. Variability in this region may determine individual differences in T-cell responses. It is postulated that MHC class II may present variable central nervous system antigens to T cells, possibly producing different lesion distribution in animals and in humans.

Moreover, Stromnes et al reported different lesion distribution in the central nervous system (spinal cord vs brain parenchyma) in 2 mouse experimental autoimmune encephalomyelitis models with different MHC strains. This was found to be mediated by variable preferential MOG (myelin oligodendrocyte glycoprotein) epitope presentation and ultimately by an alternative ratio of T-helper type 17 to T-helper type 1. The present study indicates a different mechanism of lesion formation in the brain vs the spinal cord, with a possible indirect role of the MHC class II genes. The role of the genes on this mechanism is unclear. The translation of these findings from animal models to the human situation warrants further studies.

Because of the complexity of the MHC class II region (epistatic effects and high linkage disequilibrium), future studies using high-density HLA mapping are warranted to unravel the genetic influence on lesion distribution and to determine the responsible HLA allele.

In conclusion, this study demonstrates more focal lesions in the spinal cord among carriers of the HLA-DRB1*1501 allele. If confirmed in independent samples, these observations may provide important insight in MS disease heterogeneity and its underlying mechanisms.

Accepted for Publication: June 12, 2009.

Author Affiliations: Departments of Neurology (Drs Sombekke, Killestein, Uitdehaag, and Polman), Radiology (Drs Lukas, Geurts, Barkhof, and Vrenken), Pathology (Drs Crusius, Peña, and Geurts), Epidemiology and Biostatistics (Drs Uitdehaag and Knol), and Physics and Medical Technology (Dr Vrenken), VU University Medical Center, Vrije Universiteit Amsterdam, Amsterdam, the Netherlands; Department of Diagnostic and Interventional Radiology, St Josef Hospital, Ruhr University, Bochum, Germany (Dr Lukas); Progenika Biopharma, SA, Derio, Spain (Drs Tejedor, Ar eta, and Martínez); and Center for Neurologic Diseases, Department of Neurology, Brigham and Women's Hospital and Harvard Medical School, and Partners Center for Personalized Genetic Medicine, Boston, Massachusetts (Dr De Jager).

Correspondence: Madeleine H. Som bekke, MD, Department of Neurology, VU University Medical Center, Vrije Universiteit Amsterdam, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands (m.sombekke@vumc.nl).

Author Contributions: Study concept and design: Sombekke, Lukas, Crusius, Tejedor, Killestein, Ar eta, Martínez, Uitdehaag, Peña, Vrenken, and Polman. Acquisition of data: Sombekke, Lukas, Crusius, and Barkhof. Analysis and interpretation of data: Sombekke, Lukas, Crusius, Tejedor, Killestein, Martínez, Uitdehaag, Knol, Geurts, De Jager, Barkhof, Vrenken, and Polman. Drafting of the manuscript: Sombekke, Crusius, Tejedor, Ar eta, Martínez, and Vrenken. Critical revision of the manuscript for important intellectual content: Crusius, Tejedor, Killestein, Ar eta, Martínez, Uitdehaag, Knol, Peña, Geurts, De Jager, Barkhof, Vrenken, and Polman. Statistical analysis: Sombekke, Uitde haag, Knol, and De Jager. Obtained funding: Tejedor, Ar eta, Martínez, and Polman. Administrative, technical, and material support: Crusius, Tejedor, Ar eta, Martínez, and Vrenken. Study supervision: Crusius, Uitde haag, Barkhof, Vrenken, and Polman.

Financial Disclosure: Dr Lukas received a research grant from Bayer Schering Germany. Drs Tejedor, Ar eta, and Martínez are employees of the corporate sponsor. Dr Killestein has been involved in clinical trials of companies that market drugs for multiple sclerosis (Bayer Schering Germany, AG, Biogen Idec, Merck Serono, and Teva Neuroscience) and some companies that have development programs for future drugs to treat multiple sclerosis. Dr Uitdehaag received honoraria for consultancy from Novartis and Merck Serono. Dr De Jager received speaking and advisory board fees from Biogen Idec, honoraria for serving on the advisory board of Teva Neuroscience, and research support from Affymetrix Inc and Source MDx Inc. Dr Vrenken received program grant 05-358c and project grants 05-366MS and 08-633MS from the Dutch Multiple Sclerosis Research Foundation and project grant EADNI-06-10004 from the Alzheimer’s Association. Dr Polman received consulting fees or speaking fees from Actelion, Biogen Idec, Bayer Schering Germany, Teva Neuroscience, Merck Serono, Novartis, GlaxoSmithKline, UCB, Roche, and Antisense Therapeutics, as well as grant support from Biogen Idec, Bayer Schering Germany, GlaxoSmithKline, Novartis, UCB, Merck Serono, and Teva Neuroscience.

Funding/Support: This study was supported by Progenika Biopharma, SA. The MS Center Amsterdam is supported by program grant 05-358 from the Dutch Multiple Sclerosis Research Foundation. Dr De Jager is a Harry Weaver Neuroscience Scholar of the National Multiple Sclerosis Society.

Role of the Sponsor: The corporate sponsor participated in the design and conduct of the study (including selection of single-nucleotide polymorphisms for the DNA chip, which is intellectually protected by the corporate sponsor), the genetic analysis, and the review and approval of the final manuscript.

Additional Information: The eTable is available at http://www.archneurol.com.

Additional Contributions: M. van de Wiel, PhD, provided statistical advice. We thank all the patients for their participation.

REFERENCES


cluded to aid in decision making. Twenty of those chapters follow a specific disease approach to epidemiology, diagnosis, differential diagnosis, therapy, and prognosis. They begin with a short vignette and end with a “Pearls and Pitfalls” section. One chapter is dedicated for “Basics of Neuroimaging,” and the last one touches on the “Grades of Recommendations Based on Level of Evidence.” Although the stereotypic outline of the chapters is helpful for junior house officers using this book, this presentation is not always applicable to all chapters.

Frontera has done a phenomenal job in summarizing the available literature and, when relevant, including tables describing the different studies to help answer complex clinical scenarios for which standard of care is lacking (there is no shortage of such clinical dilemma in the NNICU). References cited in the text are listed at the end of each chapter. Different medications used in the NNICU setting and their dosage are listed without ambiguity; however, an appendix including all the drugs mentioned in the book and a few others not mentioned but frequently used in a general ICU would have been very useful for quick consultation. This could have included different enteral feeding formulas that were not discussed in the “Glucose Management and Nutrition” chapter.

The section on “Airway Management and Sedation” is clear and very informative, unlike the one that follows, tackling “Ventilator Management”; it is likely to add some confusion to the understanding of the subject in a young, inexperienced reader. This is in part because of the ambiguity that plagues nomenclature in mechanical ventilation practice in general. The chapters on “Brain Death and Organ Donation” and “Induced Normothermia and Hypothermia” are excellent and logically included in the book.

General ICU matters like sepsis and acute respiratory distress syndrome are discussed; however, even for a young house officer, a higher level of elaboration on these subjects could be sought. Moreover, the book kept out certain subjects like perioperative care of critically ill neurologic and neurosurgical patients, for example, in carotid endarterectomy, craniotomy for large tumor resection (especially at the skull base), and complicated vascular bypass surgery, to name a few.

In concluding, this book is probably the best available NNICU handbook. It is a must for house officers embarking on their NNICU rotations or those working in a general, nondedicated ICU. Future editions will certainly improve on the minimal imperfections.