ORIGINAL CONTRIBUTION

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

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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*.

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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 studies[1-8] 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

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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 studies[17,18] 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 al[19] 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.

METHODS

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 al[20] 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 Scale[22] 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.blast.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 al[24] 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 mm^2. 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 mm^2. 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 χ^2 test as appropriate, applying the false discovery rate according to Benjamini and Hochberg[27] 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-

Table 1. Patient Demographic, Clinical, and Magnetic Resonance (MR) Imaging Characteristics According to Multiple Sclerosis Subtype

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

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

Table 2. Correlation by Kruskal-Wallis Test of Single-Nucleotide Polymorphisms (SNPs) With Magnetic Resonance Imaging Variables

SNP	Gene	*P* Value		Uncorrected *P* Value for Association With Lesion Volume in the Spinal Cord[a]
		Uncorrected	False Discovery Rate Corrected	
		No. of Focal Lesions in the Spinal Cord		
rs3135388[b]	*MHC2*	<.001	.03	.02
rs2395182[b]	*MHC2*	.001	.03	.003
rs2239802[b]	*MHC2*	.001	.03	.003
rs2227139[b]	*MHC2*	.002	.03	.005
rs2213584[b]	*MHC2*	.003	.05	.009
rs3087456	*C2TA*	.009	.10	.18
		T2-Weighted Lesion Load in the Brain		
rs2107538	*CCL5*	.001	.07	Not applicable

[a] Defined as the total number of segments affected by focal lesions.
[b] Reside on 3 haplotype blocks, with pairwise r^2 values ranging from 0.33 to 0.99.

nificant 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.

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 (*CCL5*) 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

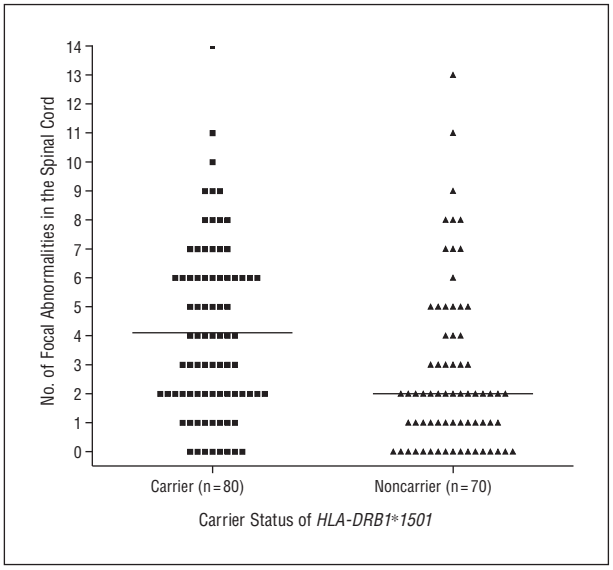

Figure. Carriership of *HLA-DRB1*1501* and focal spinal cord lesions. Scatterplot of focal spinal cord lesions for carriers and noncarriers of *HLA-DRB1*1501* (measured as the presence of the *A* allele of rs3135388). Carriers of *HLA-DRB1*1501* had a median of 4 spinal cord lesions compared with 2 lesions for noncarriers ($P<.001$, Mann-Whitney test). Line reflects the median number of focal abnormalities.

(surrogate marker for *HLA-DRB1*1501*) was associated with significantly more lesions in the spinal cord (**Figure**). The median number of focal abnormalities in carriers of *HLA-DRB1*1501* (n=80) was 4 lesions and in noncarriers (n=70) was 2 lesions ($P<.001$, Mann-Whitney test).

When corrected for multiple testing, 5 SNPs within the MHC class II region (rs3135388, rs2395182, rs2239802, rs2227139, and rs2213584) remained significant, and 1 SNP within the *C2TA* gene (class II transactivator) showed a suggestive result. The 5 HLA SNPs are in strong linkage disequilibrium.

In addition, the aforementioned MHC class II SNPs were also found to be correlated with a higher lesion volume in the spinal cord. Specifically, carriership of *HLA-DRB1*1501* was associated with more segments of the spinal cord affected by focal lesions ($P=.01$, Mann-Whitney test).

No clinical covariate (disease duration, age of patients, or MS subtype) was significantly correlated with the number of focal lesions in the spinal cord. No general linear model that included the 5 significantly associated MHC class II SNPs could be produced because of the high level of collinearity owing to the high linkage disequilibrium (r^2 range, 0.33-0.99). Only rs3087456 (*C2TA*) (OMIM 600005) showed a marginally significant *P* value ($P=.05$) in the general linear model that included rs3135388 ($P=.002$). No association was observed between the presence of diffuse abnormalities and the evaluated SNPs.

COMMENT

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 ($r^2=0.97$).[28]

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[6,29,30] 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.[1,4,6,31] Recent findings show a correlation between carriership of *HLA-DRB1*1501* and Expanded Disability Status Scale scores using an extremes of outcome strategy (comparing patients with severe and mild disability only).[4] In our study, we could not demonstrate a correlation between carriership 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.[32,33] In our study, we also noticed an association between Expanded Disability Status Scale scores and the number of focal abnormalities in the spinal cord ($P=.02$, $\rho=0.185$). 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.[34]

In a recent article, Okuda et al[35] 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][23] 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.[36]

Previous data were sometimes conflicting about the effect of HLA haplotypes on brain MR imaging features. Some studies[31,35] found a relationship between HLA genotypes and brain MR imaging quantitative markers, while other studies[31,37] did not observe this effect of *HLA-DRB1*1501*. In addition, no effect of *HLA-DRB1*1501* was shown on lesion distribution within the brain using T1-weighted and T2-weighted lesion maps.[38] 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

nonself immune recognition.[39] 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.[40]

Moreover, Stromnes et al[41] 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.

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

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eTable. Single-Nucleotide Polymorphisms Included on DNA Chip

Gene	rs-nr	Chromosome	Polymorphism	HWE[a]	MAF
ADAMTS14	rs4747075	10q22	A/G	$P<.01$	0.30
ADAMTS14	rs7081273	10q22	C/G	NS	0.34
ADAMTS14	rs4746060	10q22	C/T	NS	0.08
Apo I/Fas	rs1800682	10q23	C/T	NS	0.47
Apo I/Fas	rs3781202	10q23	C/T	$P<.01$	0.40
Apo I/Fas	rs2234978	10q23	C/T	NS	0.31
BTNL2	rs2076530	6p21.3	A/G	$P<.01$	0.26
CIITA	rs3087456	16p13	A/G	NS	0.26
CACNG4	rs4790896	17q24	A/G	NS	0.41
CCR5	rs333	3p21	-/+	NS	0.11
CD24	rs8734	6q21	C	NA	0.00[b]
CNTF	rs1800169	11q12	A/G	NS	0.12
CRYAB	rs14133	11q21-q23	C/G	NS	0.27
CRYAB	rs762550	11q21-q23	A/G	NS	0.42
CRYAB	rs2234702	11q21-q23	C	NA	0.00[b]
CTLA4	rs231775	2q33	A/G	NS	0.37
CTLA4	rs5742909	2q33	C/T	NS	0.09
EBF1	rs1368297	5q34	A/T	NS	0.38
GABBRA1	rs1805057	6p22	C	NA	0.00[b]
HELZ	rs2363846	17q24	C/T	NS	0.48
HLA	rs2395166	6p21.3	C/T	NS	0.47
HLA	rs2213584	6p21.3	A/G	NS	0.40
HLA	rs2227139	6p21.3	C/T	NS	0.40
HLA	rs3135388	6p21.3	A/G	NS	0.33
HLA	rs9268458	6p21.3	A/C	NS	0.20
HLA	rs6457594	6p21.3	A/G	$P<.01$	0.40
HLA-DRA	rs2395182	6p21.3	G/T	NS	0.38
HLA-DRA	rs2239802	6p21.3	C/G	NS	0.38
IFNAR1	rs2257167	21q22	C/G	NS	0.08
IFNGR2	rs9808753	21q22	A/G	NS	0.14
IKBL	rs3130062	6p21.3	C/T	NS	0.18
IL-10	rs1800896	1q32	A/G	NS	0.46
*IL-1*β	rs1799916	2q14	A	NA	0.00[b]
*IL-1*β	rs1143627	2q14	A/G	NS	0.34
*IL-1*β	rs1143634	2q14	C/T	NS	0.23
IL-1RN	rs419598	2q12-q14	C/T	NS	0.31
IL-1RN	2073 C/T Intron2	2q12-q14	C/T	NS	0.30
IL-2	rs2069763	4q26	G/T	NS	0.36
IL-2	rs2069762	4q26	G/T	NS	0.27
IL-4R	rs1801275	16p12	A/G	NS	0.20
IL-7R	rs11567685	5p13	C/T	NS	0.25
IL-7R	rs7718919	5p13	G/T	NS	0.13
IL-7R	rs11567686	5p13	A/G	NS	0.34
MC1R	rs1805009	16q24	C/G	NA	0.01[b]
MC1R	rs1805006	16q24	A/C	NA	0.00[b]
MEFV	rs28940577	16p13.3	A	NA	0.00[b]
MGC33887	rs987931	17q24	G/T	NS	0.32
MOG	rs3130250	6p22	A/G	NS	0.19
MOG	rs3130253	6p22	A/G	NS	0.12
NDUFA7	rs2288414	19p13.2	C/G	NA	0.03[b]
NDUFA7	rs561	19p13.2	A/G	NS	0.21
NDUFS5	rs2889683	1p34.2	C/T	NS	0.31
NDUFS5	rs6981	1p34.2	A/G	NA	0.04[b]
NDUFS7	rs2074897	19p13.3	A/G	$P<.01$	0.47
NOS2A	rs1137933	17q11.2	A/G	NS	0.25
NOS2A	rs2779248	17q11.2	C/T	NS	0.39
NOTCH4	rs367398	6p21.3	A/G	NS	0.16
PD-1	rs11568821	2q37	G/A	NS	0.11
PITPNC1	rs1318	17q24	A/G	NS	0.21
PITPNC1	rs2365403	17q24	C/G	NS	0.18
PNMT	rs876493	17q11-q23	A/G	NS	0.39
PRKCA	rs7220007	17q24	A/G	NS	0.49
PRKCA	rs887797	17q24	C/T	NS	0.30
PRKCA	rs2078153	17q24	C/G	NS	0.23
PRKCA	rs3890137	17q24	A/G	NS	0.37
PTPN22	rs2476601	1p13	A/G	NS	0.11

Gene	rs-nr	Chromosome	Polymorphism	HWE[a]	MAF
PTPRC	rs17612648	1q31	C/G	NS	0.03[b]
PTPRC	rs4915154	1q31	A/G	NS	0.00[b]
CCL5	rs2280788	17q11.2-q12	C/G	NS	0.02[b]
CCL5	rs2107538	17q11.2-q12	C/T	NS	0.18
Spp1	rs1126616	4q21	C/T	NS	0.23
Spp1	rs1126772	4q21	A/G	NS	0.18
Spp1	rs2853744	4q21	G/T	NS	0.05
Spp1	rs9138	4q21	A/C	NS	0.24
Spp1	rs4754	4q21	C/T	NS	0.24
TNF	rs1800629	6p21.3	A/G	NS	0.17
TRAIL	rs1131568[c]	3q26	C/T	NS	0.32
UCP2	rs659366	11q13	C/T	NS	0.37
VDR	rs1544410	12q13	A/G	NS	0.48
VDR	rs731236	12q13	A/G	NS	0.48

Abbreviations: *ADAMTS14*, a disintegrin and metalloproteinase with thrombospondin motif, type 1 motif 14; *Apo I/Fas*, tumor necrosis factor (TNF) receptor superfamily, member 6; *BTNL2*, butyrophilinlike 2; *CIITA*, class II, major histocompatibility complex, transactivator; *CACNG4*, calcium channel, voltage-dependent, gamma subunit 4; *CCL5*, chemokine (C-C motif) ligand 5; *CCR5*, chemokine (C-C motif) receptor 5; *CNTF*, ciliary neurotrophic factor; *CRYAB*, α-B crystallin; *CTLA4*, cytotoxic T-lymphocyte-associated protein 4; *EBF1*, early B-cell factor 1; *GABBRA1*, gamma-aminobutyric acid (GABA) B receptor 1; *HELZ*, helicase with zinc finger; *HLA*, human leukocyte antigen; *HLA-DRA*, human leukocyte antigen DR alpha; HWE, Hardy-Weinberg Equilibrium in our sample; *IFNAR1*, interferon (alpha, beta, and omega) receptor 1; *IFNGR2*, interferon gamma receptor 2 (interferon gamma transducer 1); *IKBL*, inhibitory κ-B–like gene; *IL-10*, interleukin 10; *IL-1*β, interleukin 1β; *IL-1RN*, interleukin 1 receptor antagonist; *IL-2*, interleukin 2; *IL-4R*, interleukin 4 receptor; *IL-7R*, interleukin 7 receptor; *MAF*, minor allele frequency in our sample; *MC1R*, melanocortin 1 receptor; *MEFV*, Mediterranean fever; *MGC33887*, coiled-coil domain containing 46; *MOG*, myelin oligodendrocyte glycoprotein; NA, not applicable; *NDUFA7*, NADH dehydrogenase (ubiquinone) 1α subcomplex, 7; *NDUFS5*, NADH dehydrogenase (ubiquinone) Fe-S protein 5; *NDUFS7*, NADH dehydrogenase (ubiquinone) Fe-S protein 7; *NOS2A*, nitric oxide synthase 2; *NOTCH4*, Notch homolog 4; NS, not significant; *PD-1*, programmed cell death 1; PITPNC1, phosphatidylinositol transfer protein, cytoplasmic 1; *PNMT*, phenylethanolamine *N*-methyltransferase; *PRKCA*, protein kinase C, alpha; *PTPN22*, protein tyrosine phosphatase, nonreceptor type 22; *PTPRC*, protein tyrosine phosphatase, receptor type C; rs-nr, RefSNP accession identification; *Spp1*, secreted phosphoprotein 1 (osteopontin); *TRAIL*, TNF-related apoptosis inducing ligand; *UCP2*, uncoupling protein 2; *VDR*, vitamin D (1,25-dihydroxyvitamin D3) receptor.

[a] *P* value of HWE significant. $P < .01$ indicates deviation from HWE.

[b] Excluded due to minor allele frequency $P < .05$.

[c] Previous rs-number: rs9880164.