Genetic Variants in the Immunoglobulin Heavy Chain Locus are Associated with the IgG Index in Multiple Sclerosis

Dorothea Buck, MD,[1] Eva Albrecht, DiplStat,[2] Muhammad Aslam, PhD,[1] An Goris, PhD,[3] Natalie Hauenstein,[1] Angela Jochim, MD,[1] International Multiple Sclerosis Genetics Consortium, Wellcome Trust Case Control Consortium[2], Sabine Cepok, PhD,[1] Verena Grummel,[1] Bénédicte Dubois, MD, PhD,[3] Achim Berthele, MD,[1] Peter Lichtner, PhD,[4,5] Christian Gieger, PhD,[2] Juliane Winkelmann, MD,[1,4,5,6] and Bernhard Hemmer, MD[1,6]

Objective: Intrathecal synthesis of immunoglobulin gamma (IgG) synthesis is frequently observed in patients with multiple sclerosis (MS). Whereas the extent of intrathecal IgG synthesis varies largely between patients, it remains rather constant in the individual patient over time. The aim of this study was to identify common genetic variants associated with the IgG index as a marker of intrathecal IgG synthesis in MS.
Methods: We performed a genome-wide association study of the IgG index in a discovery series of 229 patients. For confirmation we performed a replication in 2 independent series comprising 256 and 153 patients, respectively. The impact of associated single nucleotide polymorphisms (SNPs) on MS susceptibility was analyzed in an additional 1,854 cases and 5,175 controls.
Results: Significant association between the IgG index and 5 SNPs was detected in the discovery and confirmed in both replication series reaching combined p values of $p = 6.5 \times 10^{-11}$ to $p = 7.5 \times 10^{-16}$. All identified SNPs are clustered around the immunoglobulin heavy chain (IGHC) locus on chromosome 14q32.33 and are in linkage disequilibrium (r^2 range, 0.71–0.95). The best associated SNP is located in an intronic region of the immunoglobulin gamma3 heavy chain gene. Additional sequencing identified the GM21* haplotype to be associated with a high IgG index. Further evaluation of the IGHC SNPs revealed no association with susceptibility to MS in our data set.
Interpretation: The extent of intrathecal IgG in MS is influenced by the IGHC locus. No association with susceptibility to MS was found. Therefore GM haplotypes might affect intrathecal IgG synthesis independently of the underlying disease

ANN NEUROL 2013;73:86–94

Multiple sclerosis (MS) is considered a chronic inflammatory disease of the central nervous system (CNS) of autoimmune origin involving T and B cells.[1] Both environmental and genetic factors are supposed to contribute to the pathogenesis of MS.[2–7] In a recent genome-wide association study (GWAS), HLA-DRB1*15:01, DRB1*03:01-DQB1*02:01, and DRB1*13:01 were found to be genetic risk factors for MS, whereas the

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.23749

Received Apr 3, 2012, and in revised form Jul 22, 2012. Accepted for publication Aug 31, 2012.

Address correspondence to Dr Hemmer, Department of Neurology, Klinikum rechts der Isar, Technische Universität München, Ismaninger Strasse 22, 81675 Munich, Germany. E-mail: hemmer@lrz.tu-muenchen.de

From the [1]Department of Neurology, Technische Universität München, Munich, Germany; [2]Institute of Genetic Epidemiology, Helmholtz Center Munich–German Research Center for Environmental Health, Neuherberg, Germany; [3]Laboratory for Neuroimmunology, Section of Experimental Neurology, KU Leuven, Leuven, Belgium; [4]Institute of Human Genetics, Technische Universität München, Munich, Germany; and [5]Institute of Human Genetics, Helmholtz Center Munich–German Research Center for Environmental Health, Neuherberg, Germany; [6]Munich Cluster for Systems Neurology (SyNergy), Munich, Germany.

Additional supporting information can be found in the online version of this article.

TABLE 1: Clinical Characteristics and Cerebrospinal Fluid Parameters of Study Series

Characteristic	Discovery Series, n = 229	First Replication Series, n = 256	Second Replication Series, n = 153
Age, mean yr (range)	35 (16–67)	36 (15–71)	41 (14–71)
Gender, F:M	173:56	172:84	106:47
PPMS, No. [%]	11 [4.8]	5 [2.0]	27 [17.6]
IgG index,[a] geometric mean (range)	0.95 (0.29–3.94)	0.85 (0.37–5.20)	0.99 (0.43–5.36)

Clinical characteristics and IgG index of the discovery series, and the first and second replication series. Patients who were excluded after quality control are not shown. Age is given for the time of lumbar puncture.
[a]In this table, the original data of the IgG index are shown to illustrate results; for linear regression analysis, the natural logarithm of the IgG index was used.
F = female; IgG = gamma globulin; M = male; PPMS = primary progressive multiple sclerosis.

classical HLA-A*02:01 allele appeared to be a protective allele.[8] In addition, 57 genes outside the HLA locus have been identified as being associated with increased susceptibility to MS. The majority of these genes are immunologically relevant, thus giving further support to the hypothesis of an autoimmune disease pathogenesis. The involvement of the humoral immune response in MS is suggested by a number of observations; accumulation of B cells and antibody producing plasma cells is observed in MS brain lesions,[9] meninges, and cerebrospinal fluid (CSF),[10] which show signs of clonal expansion and hypermutation.[11] Complement deposition is found in lesions of a subset of MS patients,[12] who favorably respond to plasma exchange for treatment of MS relapse.[13] Treatment with anti-CD20 antibodies, which efficiently deplete B cells, ameliorates the course of MS.[14,15]

Furthermore increased gamma globulin (IgG) in the CSF, which was first described by Kabat et al. in 1942,[16] is considered a marker of a humoral immune response in MS. Over decades, intrathecal synthesis of IgG, mainly consisting of IgG1 and IgG3,[17] or the presence of oligoclonal IgG bands (OCBs) in the CSF, has remained a diagnostic hallmark in MS, although the pathophysiological role and the underlying mechanism of increased intrathecal IgG are still uncertain. Although 90 to 95% of all patients with MS exhibit OCBs in the CSF, a quantitative measurable local synthesis of IgG is only seen in 70% of patients. The extent of IgG production in the CNS compartment can be quantified by the IgG index.[18] In contrast to OCBs, measuring the IgG index allows determination of the extent to which IgG is produced in the local CNS compartment.

Several studies have demonstrated that the extent of intrathecal IgG synthesis is variable among MS patients, but remains stable in the individual patient over time[19–21] and largely unaffected by therapeutic interventions including glucocorticoids, interferon-beta,[22] B-cell depletion,[23] or autologous hematopoietic stem cell transplantation.[24] Therefore, we hypothesized that genetic factors might influence the extent of IgG synthesis in the CSF. To evaluate this hypothesis, we performed a GWAS for single nucleotide polymorphisms (SNPs) associated with the IgG index as a measure of the extent of intrathecal IgG synthesis.

Subjects and Methods

Study Population

In the CSF study, a total of 669 patients with MS or clinically isolated syndrome (CIS) were included. Three independent patient series were analyzed. Patients of the discovery series and the first replication series were recruited in Germany; patients of the second replication series were recruited in Belgium. All patients were of European descent. The discovery series comprised 237 patients, the first replication series 279 patients, and the second replication series 153 patients. After quality control, which is described below, 8 patients of the discovery series and 23 patients of the first replication series were excluded from further analyses.

Association of immunoglobulin heavy chain (IGHC) SNPs with susceptibility to MS was investigated in a data set of 1,854 MS cases and 5,175 controls from the United Kingdom (further details of this data set are described in Sawcer et al[8]). Written informed consent was obtained from all patients. The study protocol was approved by the local ethics committees of the Technische Universität Munich, University Hospitals Leuven, and all institutions in the United Kingdom. Clinical characteristics of the series are displayed in Table 1.

CSF Parameters

CSF parameters were obtained from the CSF reports of routine clinical evaluation. Following national guidelines, CSF examination is being performed regularly in every patient at the time of MS diagnosis at all centers having recruited patients for this study. Therefore, patients included in this study represent an unbiased general population of newly diagnosed MS patients.

In this study, we applied the IgG index, which is a quantitative measure of intrathecal IgG, because metric CSF data are more sensitive to detect differences in intrathecal IgG within a given population of MS patients, being 90 to 95% positive for the qualitative trait OCBs. The IgG index of Delpech and Lichtblau was calculated as follows: IgG index = (IgG CSF/IgG serum)/(albumin CSF/albumin serum).[25] The normal upper limit is 0.66. All laboratories contributing to the study have successfully taken part in intercomparison programs and are certified for routine laboratory testing. Distribution of IgG values was comparable between patient series (see Table 1).

DNA Extraction and Genotyping

DNA was extracted from venous blood by standard methods using either the Gentra Puregene Kit (Qiagen, Hilden, Germany) or illustra Nucleon BACC Genomic DNA Extraction Kit (GE Healthcare, Buckinghamshire, UK).

Samples of the discovery series were genotyped using the Human660-Quad chip (Illumina, San Diego, CA) on the Illumina Infinium platform at the Wellcome Trust Sanger Institute as part of the WTCCC2 project.[8] The Illuminus program was used for genotype calling. Only samples with a call rate >95% were included. At the Sanger Institute, each sample was fingerprinted with a panel of 31 Sequenom markers; 2 samples either failed to genotype on Sequenom or gave data of inadequate quality and were therefore excluded. For additional quality control, a gender check using Sequenom X-linked markers was applied. In 232 samples, gender was confirmed. The remaining 5 samples with misspecified gender or no given gender were excluded. One sample was identified as an ethnic outlier in multidimensional scaling analysis and was therefore excluded. In summary, 8 patients of the discovery series were excluded from further analyses after DNA and genotyping quality control. Only SNPs with a minor allele frequency of $\geq$5% were included. In total, 526,014 SNPs were successfully genotyped and further analyzed in 229 samples of the discovery series.

For replication 17 SNPs were selected, covering all regions that were associated with the IgG index in the discovery series ($p < 1 \times 10^{-5}$). These SNPs showed call rates >95% in the discovery series. Two of the selected SNPS (rs11160868 and rs10136766) were excluded from the IMSGC/WTCCC2 analysis because of problems in the genotyping of the controls, whereas rs11621145, rs2725142, and rs2753571 were not considered in the IMSGC/WTCCC2 GWAS because they were absent from the external control data set.

Genotyping in an independent first replication series was performed using the matrix-assisted laser desorption/ionization time of flight mass spectrometry technology of the Sequenom iPlexGold system (Sequenom, San Diego, CA). Genotype calling was done with SpectroTYPER 3.4 software. Assays were designed using AssayDesign 3.1.2.2 with iPLEX Gold chemistry default parameter. Rs1134590 failed genotyping; rs12884389 could not be called properly. All remaining 12 SNPs had call rates >97% and are shown in Table 2. In 23 samples, personwise call rate was <90%, and therefore these samples were excluded from further analyses.

The second independent replication series comprising 153 cases has also been genotyped on the Human660-Quad chip (Illumina) with the same quality controls as described above. Six SNPs, which had been validated in the first replication series as being significantly associated with the IgG index and/or which reached genome-wide significance in a meta-analysis of the discovery and first replication series, were investigated in this data set for further replication. SNP call rates in this data set were >94%. Genotype clustering of these SNPs has been checked visually by an experienced scientist for all 3 series.

Sequencing and Alignment Analysis

Primer sequences used for sequencing the CH2 and CH3 domain coding exons 6 and 7 of the *IGHG3* gene and their chromosomal locations are described in Supplementary Table 1. To specifically sequence the *IGHG3* exons 6 and 7, we applied a modified protocol described by Dard et al.[26] Briefly, the primer combination F1/R1 was used for initial polymerase chain reaction (PCR) amplification of genomic DNA, which was predigested with the restriction enzyme BspHI (New England BioLabs, Ipswich, MA) to prevent amplification of other immunoglobulin gamma heavy chain (IGHG) genes, namely *IGHG1, IGHG2, IGHG4,* and *IGHGP.* The PCR product corresponding to the size of the *IGHG3* gene was identified on a 1% agarose gel, which was purified and used as a template for Sanger sequencing performed by Eurofins MWG Operon (Munich, Germany) with the primers F2 and F3. Sequencing for *IGHG1* and *IGHG2* was performed with the primers F4 to F7. All sequence reads were tested against the human genome assembly in the National Center for Biotechnology Information (NCBI) blast (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to verify their identity. For sequence alignment analysis and visualization, ClustalW2[27] and Jalview (version2)[28] were used. Sequencing and alignment analysis was performed in 20 samples. Sample selection was based on the rs10136766 genotype to determine those SNPs that are in linkage disequilibrium (LD) with rs10136766 and are known determinants of the GM allotype polymorphism across the IGHG locus. In total 16 GM-allotype defining SNPs were targeted in sequencing and alignment analysis: rs74093865, rs60746425, rs113169458, rs77307099, rs139413052, rs1803797, rs79545032, rs4042056, rs1051112, and 1 SNP at position 106235701 without rs designation for G3m (*IGHG3*); rs1071803, rs1045853, rs11621259, and rs113804727 for G2m (*IGHG2*); rs11627594 and rs8009156 for G1m (*IGHG1*); further details are displayed in Supplementary Table 2. SNP selection and GM allotype polymorphism (and GM haplotype) designation were performed according to the ensemble genome browser (http://www.ensembl.org) and the ImMunoGeneTics information system (www.imgt.org), respectively. All chromosomal locations are provided according to the current NCBI human genome build (GRCh37/hg19).

Statistical Analyses

In the discovery series, the distribution of the IgG index did not follow a Gaussian distribution, but was right-skewed.

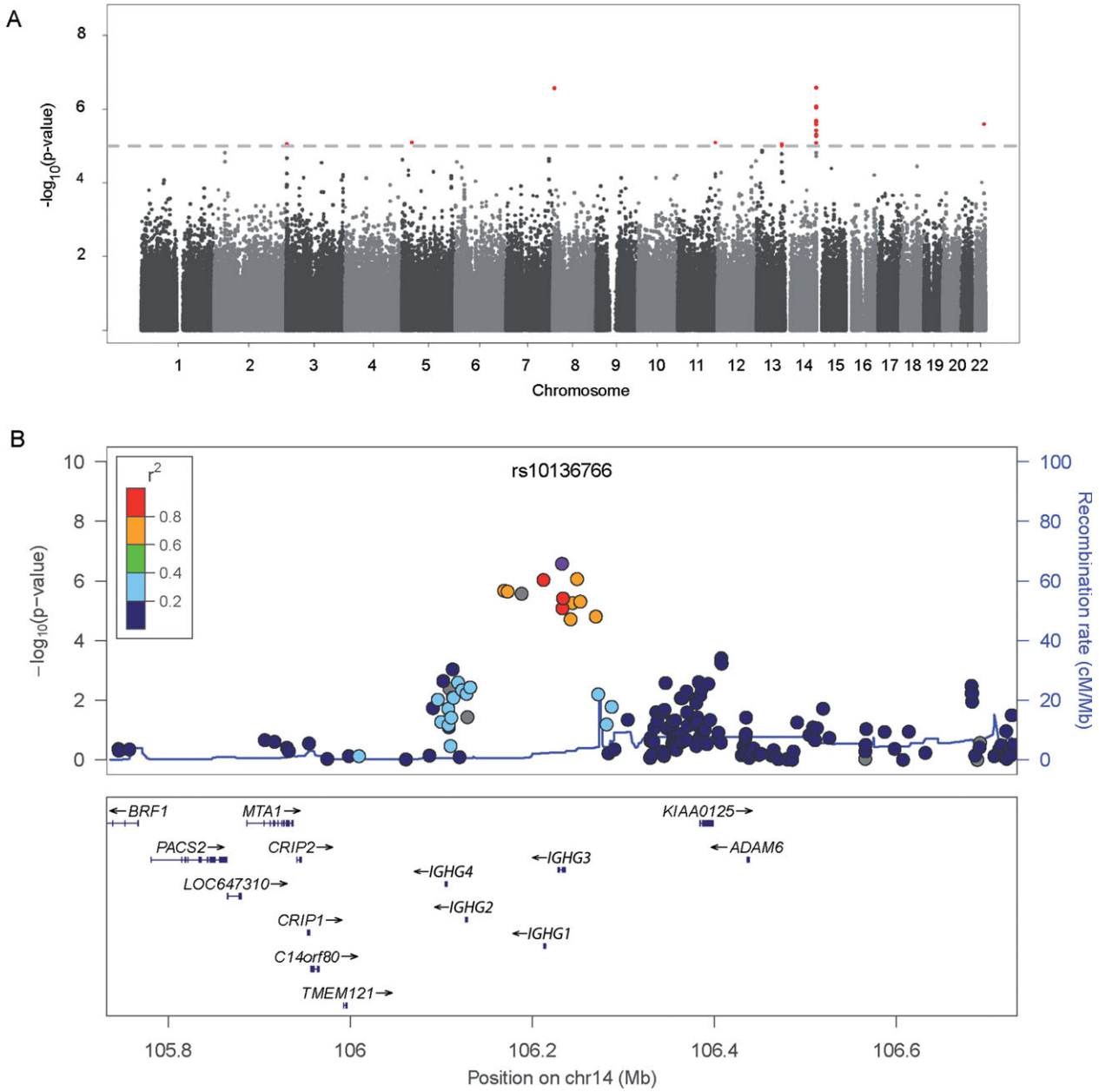

FIGURE 1: (A) Manhattan plot of the gamma globulin (IgG) index, showing $-\log_{10}$ *p* values for all 526,014 analyzed single nucleotide polymorphisms (SNPs) in the discovery genome-wide association study (n = 229), ordered by their chromosomal position. The genomic inflation factor lambda was 1.002. Therefore, no further correction was applied. The horizontal dashed line indicates the *p* value cutoff of 1×10^{-5}. SNPs with $p < 1 \times 10^{-5}$ are highlighted in red. (B) Regional association plot showing −log10 (*p* values) for all SNPs within ±500kb of rs10136766 ordered by their chromosomal position for the IgG index. The index SNP rs10136766 is colored violet. Each other SNP is colored according to its correlation with the index SNP as specified in the color scheme. Correlation structures correspond to hg19/1000g EUR Nov2010 data. Gray color indicates unknown correlation. The annotations of *IGHG1*, *IGHG2*, *IGHG3*, and *IGHG4* were added manually according to the dbSNP database, as they were not indicated by LocusZoom.

Therefore values of the IgG index have been transformed using the natural logarithm in all patient series (Supplementary Fig 1). The association between each single SNP and ln(IgG index) has been tested using linear regression models as implemented in PLINK.[29] The SNP effect has been assumed to be additive. Adjustment has been made for sex. SNPs reaching suggestive significance levels of $p \leq 1 \times 10^{-5}$ in the discovery GWAS were taken for replication in the first replication series. A *p* value of 1×10^{-5} was chosen taking into consideration the rather low sample size. Therefore, results were validated in 2 independent series. In the first replication step, *p* values $<7.1 \times 10^{-3}$, which corresponds to a Bonferroni correction for 7 independent tests (corresponding to 16 SNPs targeting 7 genomic regions), were considered to be significant and tested

TABLE 2: SNPs Associated with the IgG Index

SNP	Chr	Position	Effect Allele	Other Allele	Discovery Series				First Replication Series				Meta-Analysis		Second Replication Series				Meta-Analysis	
					No.[a]	EAF	Beta	*p*	No.[a]	EAF	Beta	*p*	Beta	*p*	No.[a]	EAF	Beta	*p*	Beta	*p*
rs9848578	3	3094310	G	A	229	0.87	0.273	8.8E−06	256	0.85	0.006	9.2E−01	0.142	9.0E−04	—	—	—	—	—	—
rs3816325	5	34682767	G	A	229	0.19	0.230	8.0E−06	255	0.23	−0.046	3.8E−01	0.097	7.2E−03	—	—	—	—	—	—
rs2408007	8	5460838	C	A	229	0.72	0.228	2.7E−07	255	0.77	0.112	3.3E−02	0.181	5.0E−08	153	0.74	−0.117	7.8E−2	0.121	4.7E−5
rs7948482	11	128044966	G	A	229	0.18	0.252	8.0E−06	255	0.19	−0.079	1.6E−01	0.090	2.2E−02	—	—	—	—	—	—
rs9582694	13	104026274	C	A	229	0.14	0.262	8.9E−06	256	0.17	−0.082	1.0E−01	0.068	7.5E−02	—	—	—	—	—	—
rs9582695	13	104036436	G	A	229	0.15	0.259	9.7E−06	256	0.17	−0.085	9.4E−02	0.066	8.2E−02	—	—	—	—	—	—
rs34398108	14	106169056	A	G	227	0.27	0.216	2.1E−06	—	—	—	—	—	—	—	—	—	—	—	—
rs11621145[b]	14	106172880	G	A	218	0.25	0.223	2.2E−06	254	0.29	0.241	2.5E−07	0.232	6.5E−13	144	0.28	0.157	7.9E−03	0.215	3.1E−14
rs1134590	14	106188230	G	A	226	0.21	0.252	2.6E−06	—	—	—	—	—	—	—	—	—	—	—	—
rs11160868[b]	14	106212273	A	G	229	0.24	0.224	9.1E−07	252	0.26	0.247	2.4E−07	0.235	2.5E−13	150	0.26	0.114	5.0E−02	0.206	1.9E−13
rs10136766[b]	14	106232585	A	G	224	0.24	0.250	2.6E−07	256	0.27	0.254	3.4E−08	0.252	6.7E−15	149	0.26	0.152	1.2E−02	0.229	7.5E−16
rs34295723	14	106233106	G	A	198	0.27	0.222	8.2E−06	—	—	—	—	—	—	—	—	—	—	—	—
rs12897751	14	106233748	C	G	211	0.25	0.215	3.8E−06	—	—	—	—	—	—	—	—	—	—	—	—
rs12884389	14	106244123	C	A	229	0.24	0.257	5.4E−06	—	—	—	—	—	—	—	—	—	—	—	—
rs2725142[b]	14	106249173	G	C	227	0.29	0.217	8.5E−07	254	0.31	0.222	1.4E−06	0.220	1.5E−12	151	0.29	0.062	3.0E−01	0.185	1.5E−11
rs2753571[b]	14	106252699	A	C	229	0.29	0.202	4.9E−06	254	0.31	0.227	8.0E−07	0.214	5.8E−12	152	0.29	0.055	3.5E−01	0.180	6.5E−11
rs743931	22	45959540	G	A	229	0.29	0.207	2.6E−06	252	0.28	0.085	1.0E−01	0.157	1.9E−06	—	—	—	—	—	—

SNPs identified in the discovery series as associated with the IgG index ($p < 1 \times 10^{-5}$) and studied in the first independent replication series.
[a]Number of samples.
[b]SNPs confirmed to be associated with the IgG index in the first and the second independent replication series as well as in a meta-analysis of all 3 patient series.
Chr = chromosome; EAF = effect allele frequency; IgG = gamma globulin; SNP = single nucleotide polymorphism.

in a second replication series. In addition, SNPs reaching the genome-wide significance level after meta-analyzing the discovery and first replication series were taken forward for validation in the second replication series. A p value $<9.5 \times 10^{-8}$ was considered genome-wide significant, which corresponds to a Bonferroni correction for 526,014 tests. Meta-analyses were performed using a fixed-effects model applying inverse variance weighting with METAL software (www.sph.umich.edu/csg/abecasis/metal). Figures were plotted using R (www.r-project.org) or Prism (GraphPad Software, La Jolla, CA), as well as LocusZoom[30] for the regional association plots. LD between SNP pairs was calculated using the --ld option in PLINK. For the calculation of explained variances, we subtracted the multiple R^2 value of the covariate model from that of the full model including covariates and SNP in both replication studies separately and assessed the sample size-weighted mean in R.

Power calculations were performed separately for discovery and replication, using Quanto (http://hydra.usc.edu/gxe/). For the discovery analysis among 229 individuals, there was >80% power to detect an SNP explaining 11.4% or more of the phenotypic variance at the suggestive significance level of $p = 1 \times 10^{-5}$. In the first replication series, there was >80% power to validate SNPs explaining >4.8% of the variance with 256 individuals and a significance level of 7.1×10^{-3}. With a sample size of 153 in the second validation series, there was >80% power to validate loci explaining >6% of the variance at a significance level of 0.025 (Bonferroni correction for 2 genomic regions).

Results

GWA Analysis of IgG Index in MS

GWA analysis was conducted for the natural logarithm IgG index and 526,014 SNPs in the discovery series. The genomic inflation factor lambda was 1.002. Therefore, no further correction was applied. The Manhattan plot is displayed in panel A of the Figure. A total of 17 SNPs were identified with p values $<1 \times 10^{-5}$ in the discovery series (see Table 2).

For replication, 14 of the 17 SNPs, covering all 7 identified genetic regions, were studied in an independent series of 256 patients. Three of the 17 SNPs (rs34398108, rs12897751, and rs34295723) were not included in the assay. Rs1134590 gave no adequate signal and rs12884389 was excluded after cluster plot analysis. Five SNPs on chromosome 14 (rs11621145, rs11160868, rs10136766, rs2725142, and rs2753571) were significantly associated with the IgG index (p values 1.4×10^{-6} to 3.4×10^{-8}). In addition, in a first meta-analysis of the discovery and the first replication series, rs2408007 on chromosome 8 reached genome-wide significance ($p = 5.0 \times 10^{-8}$). None of the other 6 SNPs, which were successfully genotyped, replicated in the first replication series or in the first meta-analysis.

For further validation, all 6 SNPs that reached statistical significance in the first replication series and/or first meta-analysis were examined in a second independent replication series of 153 patients. In the second replication series, the p value of rs2408007 was 7.8×10^{-2}, yet the effect is in the opposite direction. Probability values of the 5 SNPs on chromosome 14 range from 3.5×10^{-1} to 7.9×10^{-3}. In a meta-analysis of all 3 patient series, rs2408007 did not reach the genome-wide significance level ($p = 4.7 \times 10^{-5}$), whereas combined p values of the 5 SNPs on chromosome 14 reached from 6.5×10^{-11} to 7.5×10^{-16}.The strongest association was seen for rs10136766. In the replication series, rs10136766 explained 8.9% of the variance of intrathecal IgG synthesis.

All 5 SNPs of chromosome 14 are in LD in our data. R^2 between the index SNP rs10136766 and the other 4 identified SNPs is between 0.93 (rs11160868) and 0.75 (rs11621145) in the discovery series, between 0.95 (rs11160868) and 0.79 (rs2725142) in the first replication series, and between 0.95 (rs11160868) and 0.71 (rs2725142) in the second replication series.

Comparison of the IgG values in carriers who were homozygous for the A or G allele of rs10136766 or who were heterozygous (A/G) confirmed an increased intrathecal IgG production in patients carrying the A allele (Supplementary Fig 2).

Sequencing and Alignment Analysis

The 5 associated SNPs are annotated to the IGHC locus at 14q32.33 (Fig 1B). The best associated SNP, rs10136766, is located in the intronic region between exons 8 and 9 of the IgG3 heavy chain gene *IGHG3* (HGNC: 5527). The *IGHG3* gene exhibits G3m allotype polymorphism, which was originally defined based on serological reagents. Much like the polymorphism in the HLA region, the G3m allotype polymorphism is in close linkage with similar polymorphic determinants of the IgG1 and IgG2 heavy chain genes *IGHG1* (HGNC: 5525) and *IGHG2* (HGNC: 5526), respectively. Therefore, a specific GM haplotype can be determined within the IGHC locus based on SNP genotypes that define the GM allotypes of IgG1, 2, and 3. First we carried out a partial DNA sequencing of the *IGHG3* gene in samples homozygous for the A or G allele of rs10136766 (each n = 10) to assign the identified risk alleles (rs10136766, A) to the specific G3m allotype defining SNPs. Next, partial sequencing of *IGHG1* and *IGHG2* genes was performed in these samples to completely determine the whole spectrum of GM allotype-defining SNPs across the *IGHG1-3* genes (GM haplotype) in LD with rs10136766.

All rs10136766 G/G genotype samples could be designated as homozygous for the GM5*;3;.. haplotype (previously designated as Gm b*;f;..), whereas rs10136766 (A/A) genotype samples were homozygous for either the GM21*;1,17;.. or the GM21*;1,2,17;.. haplotypes (previously designated as Gm g*;z,a;.. and Gm g*;z,a,x;.., respectively). These results suggest that GM21* polymorphisms carrying GM haplotypes are associated with higher intrathecal IgG synthesis. Supplementary Table 2 summarizes alignment and GM allotype designations.

Association with Susceptibility to MS

To evaluate whether the identified SNPs of the IGHC locus are associated with susceptibility to MS, rs11621145, rs2725142, and rs2753571 were analyzed in an additional 1,854 MS cases and 5,175 controls. Rs11160868 and rs10136766 failed quality control in the controls, so no analysis is available for these SNPs. No evidence of association with susceptibility to MS was found in this data set (rs11621145 [G]: odds ratio [OR], 0.94; 95% confidence interval [CI], 0.87–1.02, $p = 0.132$; rs2725142 (G): OR, 0.94; 95% CI, 0.87– 1.02, $p = 0.140$; rs2753571 (C): OR, 1.06; 95% CI, 0.98–1.14, $p = 0.170$).

Discussion

The presence of increased intrathecal IgG is a very characteristic phenomenon in patients with MS. Although it is believed to be of pathogenic relevance and a marker of B-cell involvement in MS, the pathophysiological mechanism and the target of intrathecal IgG has remained unclear. Because the extent of intrathecal IgG differs largely between patients, yet remains stable over time in the individual MS patient (Supplementary Fig 3), a genetic basis has been hypothesized. To evaluate our hypothesis, we performed a genome-wide association analysis for common genetic variants of intrathecal IgG. In a discovery series, 7 regions were identified to be suggestively associated with the IgG index. Six of these regions did not replicate, which might be due to the sample size, and therefore these SNPs need further investigation in a larger sample set.

However, 1 region with 5 genotyped SNPs was confirmed to be significantly associated with intrathecal IgG quantified as IgG index in 2 independent replication series. All associated SNPs are clustered around the IGHC locus on chromosome 14q32.33. Sequencing of the region revealed that high intrathecal IgG is associated with the Gm21* haplotype and low IgG is associated with the GM5*;3;.. haplotype.

Four IGHG genes, *IGHG1, IGHG2, IGHG3,* and *IGHG4*, at 14q32.33, encode the constant region of the IgG subclasses IgG1, IgG2, IgG3, and IgG4, respectively. The constant region of the IgG isotype heavy chain is formed by 3 domains encoded by separate exons. The second (CH2) and third (CH3) domains form the Fc fragment of the IgG3 subtype and are characterized by extensive amino acid polymorphism detectable by serological reagents (G3m allotypes/markers). The Fc fragments of the IgG1 and IgG2 show similar serologically detectable polymorphism, and collectively these variations are defined as GM allotypes. Due to strong linkage between *IGHG1*, *IGHG2*, and *IGHG3* genes, the G1m, G2m, and G3m allotypes are segregated as specific combinations designated as GM haplotypes.[26,31] The human GM allotype variability is likely to influence the structural and functional characteristics of IgGs.[31] $Gm^{a;g}$ and $Gm^{a,x;g}$ phenotypes correspond to the current designation of Gm21*;1,17;.. and Gm21*;1,2,17;.. allotypes.

Polymorphism at the IGHC locus may influence IgG interactions with the neonatal Fc gamma receptor, which is expressed at the blood–brain barrier and is thought to be involved in IgG homeostasis and distribution in the brain.[32–34] In addition, divergent interaction with other Fc gamma receptors may play an active role in determining the extent of antibody-mediated tissue damage by Fc gamma receptor-driven selection (or enrichment) of particular IgG allotype variants in the CNS compartment. Allotype differences may also be relevant for IgG interactions with complement factors. Although the Gm21* haplotype is not associated with higher IgG serum levels,[35] we cannot exclude that it goes along with a higher IgG synthesis of plasma cells and plasmablasts infiltrating the CNS compartment.[10]

Conflicting results have been reported for the correlation of the GM allotype with susceptibility, severity, and course of MS as well as with absolute CSF IgG levels in MS.[35–40] SNPs of the IGHC locus have not been analyzed in the latest MS GWASs,[8] because they have not been adequately genotyped in all controls. In the present study, analysis of the identified candidate SNPs in a subset of cases and controls revealed no evidence of association of the IGHC locus with susceptibility to MS. These findings demonstrate that the IGHC locus might not be of relevance for the development of MS. This would suggest that the locus is rather of general importance for regulating IgG responses independently of disease.

It remains to be determined whether the genetic polymorphism of the IGHC locus impacts on the course of MS. Indirect evidence from different uncontrolled trials and neuropathological studies suggests that the extent of humoral immune response in the CNS may be relevant for the long-term outcome of MS patients.[41–43]

In conclusion, our study demonstrates that the extent of intrathecal IgG antibodies is at least in part genetically determined. Increased intrathecal IgG in MS patients is associated with the IGHC locus. The risk allele of our most closely associated SNP, rs10136766, implicates Gm21* allotype-containing GM haplotypes in higher IgG levels of the CSF. These findings contribute to a better understanding of the mechanisms of increased IgG CSF levels in MS and possibly other inflammatory diseases of the CNS.

Further studies will address how the Gm21* phenotype translates into a higher intrathecal IgG and whether the genetic variations at the IGHC locus play a role in the course of the disease and treatment response.

Acknowledgments

The study was supported by a grant from the German Ministry for Education and Research (German Competence Network Multiple Sclerosis, Control-MS, 01GI0917), and by a Research Grant from the Belgian Charcot Foundation (A.G.) and Research Fund KU Leuven (OT/11/087; A.G. and B.D.). D.B. is supported by the Commission for Clinical Research of the Faculty of Medicine, Technical University Munich. B.D. is a Clinical Investigator of the Fund for Scientific Research (FWO Vlaanderen) and is supported through the Biogen Idec Chair on Translational Research in Multiple Sclerosis and the Bayer Chair on Fundamental Genetic Research regarding the Neuroimmunological Aspects of Multiple Sclerosis. A.G. and B.D. are supported by the Belgian Neurological Society and Scientific Research Multiple Sclerosis–Flanders.

We thank Drs S. Sawcer and G. Hellenthal for helpful discussions and for contributions to the MS susceptibility data in the UK data set. We thank all our colleagues and patients for their contributions to the CSF results.

Potential Conflicts of Interest

D.B.: grants/grants pending, Merck Serono, Biogen Idec; travel expenses, Bayer, Biogen Idec, Merck Serono. B.D.: board membership, Merck Serono, Bayer, Biogen Idec. A.B.: consultancy, Biogen Idec, Merck Serono, Bayer; grants/grants pending, Bayer; speaking fees, Biogen Idec, Bayer, Merck Serono, Teva, Novartis; travel expenses, Biogen Idec, Bayer, Merck Serono, Teva, Novartis; investigator fees, Biogen Idec, Novartis, Merck Serono, Galapagos. J.W.: board membership, consultancy, UCB; grants/grants pending, grants from the German Research Foundation to the Genetics of Iron as well as Genetics of RLS, grants from private foundations such as the Else Kröner Foundation and Fritz Thyssen foundation; speaking fees, UCB, Vifor Pharma, Boeringer Ingelheim; patents, patent filed in relation to the publication Winkelmann et al *Nature Genetics* 2007. B.H.: board membership, Novartis, Biogen Idec; consultancy, Novartis, Biogen Idec, Bayer, Merck Serono, Roche; grants/grants pending, Novartis, Merck Serono, Biogen Idec, Bayer, Teva, Roche.

References

1. Compston A, Coles A. Multiple sclerosis. Lancet 2008;372: 1502–1517.
2. Noseworthy JH, Lucchinetti C, Rodriguez M, Weinshenker BG. Multiple sclerosis. N Engl J Med 2000;343:938–952.
3. Sospedra M, Martin R. Immunology of multiple sclerosis. Annu Rev Immunol 2005;23:683–747.
4. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part I: The role of infection. Ann Neurol 2007;61:288–299.
5. Ascherio A, Munger KL. Environmental risk factors for multiple sclerosis. Part II: Noninfectious factors. Ann Neurol 2007;61:504–513.
6. Hafler DA, Compston A, Sawcer S, et al. Risk alleles for multiple sclerosis identified by a genomewide study. N Engl J Med 2007; 357:851–862.
7. Oksenberg JR, Baranzini SE, Sawcer S, Hauser SL. The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. Nat Rev 2008;9:516–526.
8. Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature 2011;476:214–219.
9. Baranzini SE, Jeong MC, Butunoi C, et al. B cell repertoire diversity and clonal expansion in multiple sclerosis brain lesions. J Immunol 1999;163:5133–5144.
10. Cepok S, Rosche B, Grummel V, et al. Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. Brain 2005;128(pt 7):1667–1676.
11. Owens GP, Kraus H, Burgoon MP, et al. Restricted use of VH4 germline segments in an acute multiple sclerosis brain. Ann Neurol 1998;43:236–243.
12. Storch MK, Piddlesden S, Haltia M, et al. Multiple sclerosis: in situ evidence for antibody- and complement-mediated demyelination. Ann Neurol 1998;43:465–471.
13. Weinshenker BG, O'Brien PC, Petterson TM, et al. A randomized trial of plasma exchange in acute central nervous system inflammatory demyelinating disease. Ann Neurol 1999;46:878–886.
14. Hauser SL, Waubant E, Arnold DL, et al. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. N Engl J Med 2008;358:676–688.
15. Kappos L, Li D, Calabresi PA, et al. Ocrelizumab in relapsing-remitting multiple sclerosis: a phase 2, randomised, placebo-controlled, multicentre trial. Lancet 2011;378:1779–1787.
16. Kabat EA, Moore DH, Landow H. An electrophoretic study of the protein components in cerebrospinal fluid and their relationship to the serum proteins. J Clin Invest 1942;21:571–577.
17. Greve B, Magnusson CG, Melms A, Weissert R. Immunoglobulin isotypes reveal a predominant role of type 1 immunity in multiple sclerosis. J Neuroimmunol 2001;121:120–125.
18. Link H, Huang YM. Oligoclonal bands in multiple sclerosis cerebrospinal fluid: an update on methodology and clinical usefulness. J Neuroimmunol 2006;180:17–28.
19. Walsh MJ, Tourtellotte WW. Temporal invariance and clonal uniformity of brain and cerebrospinal IgG, IgA, and IgM in multiple sclerosis. J Exp Med 1986;163:41–53.

20. Warren KG, Catz I. The relationship between levels of cerebrospinal fluid myelin basic protein and IgG measurements in patients with multiple sclerosis. Ann Neurol 1985;17:475–480.
21. Schipper H. Local cerebrospinal fluid IgG production in multiple sclerosis [in German]. Schriftenr Neurol 1989;30:1–91.
22. Archelos JJ, Storch MK, Hartung HP. The role of B cells and autoantibodies in multiple sclerosis. Ann Neurol 2000;47:694–706.
23. Piccio L, Naismith RT, Trinkaus K, et al. Changes in B- and T-lymphocyte and chemokine levels with rituximab treatment in multiple sclerosis. Arch Neurol 2010;67:707–714.
24. Saiz A, Carreras E, Berenguer J, et al. MRI and CSF oligoclonal bands after autologous hematopoietic stem cell transplantation in MS. Neurology 2001;56:1084–1089.
25. Delpech B, Lichtblau E. Immunochemical estimation of IgG and albumin in cerebrospinal fluid [in French]. Clin Chim Acta 1972;37: 15–23.
26. Dard P, Lefranc MP, Osipova L, Sanchez-Mazas A. DNA sequence variability of IGHG3 alleles associated to the main G3m haplotypes in human populations. Eur J Hum Genet 2001;9:765–772.
27. Larkin MA, Blackshields G, Brown NP, et al. Clustal W and Clustal X version 2.0. Bioinformatics 2007;23:2947–2948.
28. Waterhouse AM, Procter JB, Martin DM, et al. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics 2009;25:1189–1191.
29. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.
30. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. Bioinformatics 2010; 26:2336–2337.
31. Jefferis R, Lefranc MP. Human immunoglobulin allotypes: possible implications for immunogenicity. MAbs 2009;1:332–338.
32. Schlachetzki F, Zhu C, Pardridge WM. Expression of the neonatal Fc receptor (FcRn) at the blood-brain barrier. J Neurochem 2002; 81:203–206.
33. Roopenian DC, Akilesh S. FcRn: the neonatal Fc receptor comes of age. Nat Rev Immunol 2007;7:715–725.
34. Garg A, Balthasar JP. Investigation of the influence of FcRn on the distribution of IgG to the brain. AAPS J 2009;11:553–557.
35. Sandberg-Wollheim M, Baird LG, Schanfield MS, et al. Association of CSF IgG concentration and immunoglobulin allotype in multiple sclerosis and optic neuritis. Clin Immunol Immunopathol 1984; 31:212–221.
36. Pandey JP, Goust JM, Salier JP, Fudenberg HH. Immunoglobulin G heavy chain (Gm) allotypes in multiple sclerosis. J Clin Invest 1981;67:1797–1800.
37. Sesboue R, Daveau M, Degos JD, et al. IgG (Gm) allotypes and multiple sclerosis in a French population: phenotype distribution and quantitative abnormalities in CSF with respect to sex, disease severity, and presence of intrathecal antibodies. Clin Immunol Immunopathol 1985;37:143–153.
38. Haile RW, Goldstein A, Field L, Marazita ML. A linkage analysis of the Gm locus and multiple sclerosis. Genet Epidemiol 1985;2:29–34.
39. Blanc M, Clanet M, Berr C, et al. Immunoglobulin allotypes and susceptibility to multiple sclerosis. An epidemiological and genetic study in the Hautes-Pyrenees county of France. J Neurol Sci 1986;75:1–5.
40. Raknes G, Fernandes Filho JA, Pandey JP, et al. IgG allotypes and subclasses in Norwegian patients with multiple sclerosis. J Neurol Sci 2000;175:111–115.
41. Calabrese M, Federle L, Bernardi V, et al. The association of intrathecal immunoglobulin synthesis and cortical lesions predicts disease activity in clinically isolated syndrome and early relapsing-remitting multiple sclerosis. Mult Scler 2012;18:174–180.
42. Stendahl-Brodin L, Link H. Relation between benign course of multiple sclerosis and low-grade humoral immune response in cerebrospinal fluid. J Neurol Neurosurg Psychiatry 1980;43:102–105.
43. Howell OW, Reeves CA, Nicholas R, et al. Meningeal inflammation is widespread and linked to cortical pathology in multiple sclerosis. Brain 2011;134(pt 9):2755–2771.