Genetic determinants of the humoral immune response in MS

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Abstract

Objective
In this observational study, we investigated the impact of genetic factors at the immunoglobulin heavy chain constant locus on chromosome 14 and the major histocompatibility complex region on intrathecal immunoglobulin G, A, and M levels as well as on B cells and plasmablasts in the CSF and blood of patients with multiple sclerosis (MS).

Methods
Using regression analyses, we tested genetic variants on chromosome 14 and imputed human leukocyte antigen (HLA) alleles for associations with intrathecal immunoglobulins in 1,279 patients with MS or clinically isolated syndrome and with blood and CSF B cells and plasmablasts in 301 and 348 patients, respectively.

Results
The minor alleles of variants on chromosome 14 were associated with higher intrathecal immunoglobulin G levels (β = 0.58 [0.47 to 0.68], lowest adjusted p = 2.32×10^{-23}), and lower intrathecal immunoglobulin M (β = −0.56 [−0.67 to −0.46], p = 2.06×10^{-24}) and A (β = −0.42 [−0.54 to −0.31], p = 7.48×10^{-11}) levels. Alleles from the HLA-B*07:02-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype were associated with higher (lowest p = 2.14×10^{-7}) and HLA-B*44:02 with lower (β = −0.35 [−0.54 to −0.17], p = 1.38×10^{-2}) immunoglobulin G levels. Of interest, different HLA alleles were associated with lower intrathecal immunoglobulin M (HLA-C*02:02, β = −0.45 [−0.61 to −0.28], p = 1.01×10^{-5}) and higher immunoglobulin A levels (HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype, β = 0.40 [0.21 to 0.60], p = 4.46×10^{-3}). The impact of HLA alleles on intrathecal immunoglobulin G and M levels could mostly be explained by associations with CSF B cells and plasmablasts.

Conclusion
Although some HLA alleles seem to primarily drive the extent of humoral immune responses in the CNS by increasing CSF B cells and plasmablasts, genetic variants at the immunoglobulin heavy chain constant locus might regulate intrathecal immunoglobulins levels via different mechanisms.

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Glossary

CIS = clinically isolated syndrome; **GWAS** = genome-wide association study; **HLA** = human leukocyte antigen; **IgA** = immunoglobulin A; **IgG** = immunoglobulin G; **IGHC** = immunoglobulin heavy chain constant; **IGHG** = immunoglobulin heavy constant gamma; **IgM** = immunoglobulin M; **LD** = linkage disequilibrium; **MHC** = major histocompatibility complex; **SNP** = single nucleotide polymorphism.

An elevated immunoglobulin G (IgG) index is seen in 70% of patients with multiple sclerosis (MS), whereas intrathecal synthesis of immunoglobulin M (IgM) and immunoglobulin A (IgA) occurs less frequently (20% and 9%, respectively).[1] Although the amount of intrathecal production of Igs varies strongly between patients, it remains relatively stable over the disease course—even under disease-modifying treatment.[2–4] A genetic contribution to intrathecal Ig synthesis, therefore, likely exists.

We could previously demonstrate in a genome-wide association study (GWAS) that genetic variants located at the immunoglobulin heavy chain constant (IGHC) locus on chromosome 14 are associated with the IgG index in patients with MS or clinically isolated syndrome (CIS).[5] This finding was replicated in a large multicenter GWAS.[6]

In addition, the GWAS by Goris et al.[6] showed that the haplotype rs9271640*A-rs6457617*G that correlates with the human leukocyte antigen (HLA) allele HLA-DRB1*15:01—the strongest known MS risk allele[7,8]—was also associated with higher IgG indices.

The aim of the present study was to further investigate the influence of genetic variants and HLA alleles on intrathecal immunoglobulin synthesis in a large cohort of patients with MS or CIS. Based on our previous findings, we aimed at a more detailed characterization of the association of the IGHC locus and not only intrathecal IgG but also IgM and IgA levels. To further elucidate the mechanisms by which the genetic variants alter the intrathecal immune response, we analyzed possible associations with serum concentrations of IgG, IgA, and IgM, as well as with the proportion of B cells and plasmablasts in CSF and blood.

Methods

Cohorts
We analyzed DNA samples of 1,279 patients with MS or CIS including all patients with available DNA samples and CSF data at the Klinikum rechts der Isar of the Technical University of Munich as well as patients recruited by the German MS competence network.[9] Diagnosis was based on standard diagnostic criteria.[10–13] Of 2,559 patients with available DNA, we excluded all patients with missing data on sex, age, or date of lumbar puncture. In all patients, lumbar puncture had been performed as part of the diagnostic workup. We performed quality control on available genetic data as described below and excluded patients without available genome-wide chip data.

Standard protocol approvals, registrations, and patient consents
We obtained written informed consent from all patients according to the Declaration of Helsinki and collected samples with ethical approval at the recruitment sites. The ethic committee at the Technical University of Munich approved the study.

CSF protein analysis
CSF analysis was performed at each center independently. If CSF data from more than 1 time point were available, we only considered the first CSF sampling data. CSF and serum concentrations for albumin and the 3 Ig classes IgG, IgM, and IgA were measured in parallel by standard turbidimetric or nephelometric assays, depending on the center. We calculated CSF/serum quotients (QIgG, QIgM, QIgA, and Qalb) as well as IgG, IgM, and IgA indices as QIgG/Qalb, QIgM/Qalb, and QIgA/Qalb, respectively.

Flow cytometric analysis
We performed flow cytometric analysis of CSF and blood immune cells for 348 and 301 treatment-naive patients, respectively, as described previously.[14] Flow cytometry data were available only for patients treated at the Klinikum rechts der Isar. We used the following antibodies for staining of B cells and plasmablasts: CD45 (clone HI30, BD Biosciences), CD19 (clone J3.119, Beckman Coulter), and CD138 (clone B-A38, Beckman Coulter) and analyzed the stained cells using a flow cytometer (CyAn ADP, Beckman Coulter). We then gated the cells on CD45 to select all leukocytes and subsequently on CD19 (CD45$^+$ CD19$^+$ B cells) and CD138 (CD45$^+$ CD19$^+$ CD138$^+$ plasmablasts). We determined cell numbers using FlowJo v10 (FlowJo LLC) and calculated percentages of B cells and plasmablasts of all CD45$^+$ cells.

Genotyping and quality control
The previously described variants at the IGHC locus associated with the IgG index are not well represented on most of the available whole-genome genotyping microarrays. We therefore genotyped 16 single nucleotide polymorphisms (SNPs) at the IGHC locus on a MassARRAY system using MALDI-TOF mass spectrometry with iPLEX Gold chemistry (Agena) and called genotypes with Typer Analyzer 4.0.22.67. For genotyping, we collected DNA samples from venous blood and stored the samples at −80°C. During quality control, we excluded variants with a minor allele frequency <1%, a Hardy-Weinberg equilibrium test p value <0.0001, or a call rate <98%, leaving 13 variants for further analysis. Genome-wide genotyping SNP data had been acquired using 2 different microarrays (Illumina

OmniExpress v1.0, v1.1, and v1.2 and Illumina 660-Quad) in different batches at the Max Planck Institute of Psychiatry in Munich, Germany, the Helmholtz Zentrum Munich in Neuherberg, Germany, and the Wellcome Trust Sanger Institute in Cambridge, United Kingdom. Genotype calling had been performed with GenomeStudio Genotyping Module v2.0 or with Illuminus.[15] We conducted quality control of the genotype data using PLINK v1.90b6.9[16,17] as described previously.[8] We excluded individuals with a genotyping rate <98%, cryptic relatedness >1/8, and any genetic outliers with a distance in the first 2 multidimensional scaling ancestry components of the identity-by-state matrix of >5 SDs. We further excluded individuals with deviation of autosomal heterozygosity >4 SDs from the mean and individuals with heterozygosity on the X chromosome of < −0.2.

HLA imputation

We performed HLA allele imputation using SNP2HLA v1.0.3 (Beagle v3.04) and the Type 1 Diabetes Genetics Consortium imputation panel, as previously described.[18–20] After quality control, we selected 98 HLA alleles with 4-digit resolution, an allele frequency of ≥1%, and a Beagle imputation $r^2 \geq 0.3$ for further analysis. We also analyzed the following haplotypes determined using Beagle phasing results: HLA-A*03:01-C*07:02-B*07:02-DRB1*15:01-DQA1*01:02-DQB1*06:02, HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01, HLA-A*02:01-B*44:02-C*05:01-DRB1*04:01, and HLA-A*02:01-B*27:02-C*02:02-DRB1*16:01.

Linkage disequilibrium of the variants on chromosome 14 and HLA alleles

Of 16 genotyped variants, 13 variants at the IGHC locus passed quality control. Using a linkage disequilibrium (LD) threshold of $r^2 > 0.7$, we defined 4 LD groups: A: rs10136766, rs1071803, rs111608686, rs1134590, rs11621145, rs12884389, rs12897751, rs2725142, rs2753571, and rs34398108; B: rs1059216; C: rs61984162; and D: rs8009156. For the 4-digit HLA alleles, 7 LD groups with 2 members each and 5 LD groups with 3 alleles could be identified. We calculated LD using PLINK v1.90b6.9.[16,17]

Statistical analyses

As the primary analysis, we investigated associations of the genotyped variants on chromosome 14 and the imputed HLA alleles with Ig indices (transformed by inverse rank normalization) by linear regression. For the IGHC variants, we either chose a dominant or an additive allelic model for the regression analyses, following visual inspection of the data, and included sex, age at lumbar puncture, and the sequencing plate as covariates. For the imputed HLA alleles, we analyzed dosage data (2× the probability for being homozygous for the allele + 1× the probability for being heterozygous) using the same covariates except the sequencing plate. To correct for population stratification, we added up to 5 first multidimensional scaling components as covariates if they were associated either with the dependent variable or the investigated variant or HLA allele. The DNA samples had been genotyped

using 2 different microarray types. As visual inspection of the multidimensional scaling components showed no distinction between these data sets, we did not include the genotyping chip as a covariate. We determined homoscedasticity of regression residuals using the Breusch-Pagan test; for models showing evidence for heteroscedasticity, we used robust sandwich error estimators (R package sandwich). We tested the normality of residuals by a Shapiro-Wilk test and corrected p values for multiple testing using the Bonferroni procedure for the number of LD groups with $r^2 > 0.7$ (n = 85 independent tests, 4 LD groups of the chromosome 14 variants, and 81 HLA allele LD groups). To determine the phenotypic variance explained by the analyzed genetic variants (R^2), we conducted linear regression analyses using the residuals from a null model including all covariates as the dependent variable and the respective genetic variant as the independent variable.

We carried all variants and HLA alleles significantly associated with any of the Ig indices forward to secondary exploratory analyses on their associations with rank-transformed Ig serum concentrations and proportions of CSF and blood B cells (CD45+ CD19+ cells) and plasmablasts (CD45+ CD19+ CD138+ cells). IgM indices and serum IgM concentrations did not follow a normal distribution after inverse rank transformation. We therefore validated associations with these traits using permutation analyses (100,000 permutations). We performed causal mediation analyses including nonparametric bootstrap for estimation of CIs and p values using the R package mediation[21] with the same covariates as described above with 10,000 simulations. We performed all statistical analyses using R v3.5.1.[22]

Data availability

The data that support the findings of this study are available from the corresponding author on reasonable request.

Results

Study cohort, CSF, and flow cytometry data

Table e-1, links.lww.com/NXI/A277, shows the demographic data and aggregated CSF and flow cytometry parameters for all 1,279 patients. Four hundred twenty of these samples were included in one or both of the mentioned previous genetic studies on IgG indices.[5,6] We observed significant correlations between all 3 Ig indices (Spearman ρ = 0.26, 95% CI [0.20–0.31] for IgG and IgM indices; ρ = 0.18 [0.12–0.24] for IgG and IgA indices; and ρ = 0.47 [0.42–0.52] for IgM and IgA indices).

### Genetic factors at the IGHC locus associated with Ig indices

The minor alleles of all 10 variants from LD group A on chromosome 14 were associated with higher IgG indices (table 1 and figure 1A). All 10 variants were also significantly associated with IgA and IgM indices. However, these associations had a reversed sign, i.e., the minor alleles were

Table 1 Association of variants on chromosome 14 with the Ig indices

Variant (LD group)	EA	AF	Regression on IgG indices			Regression on IgM indices			Regression on IgA indices		
			β (95% CI)	Adjusted p	N	β (95% CI)	Adjusted p	N	β (95% CI)	Adjusted p	N
rs10136766 (A)	A	25.5	0.57 (0.46 to 0.68)	1.02×10^{-21}	1,229	−0.55 (−0.65 to −0.44)	6.35×10^{-22}	1,151	−0.41 (−0.53 to −0.30)	3.57×10^{-10}	1,143
rs1071803 (A)	T	25.8	0.57 (0.46 to 0.68)	1.34×10^{-21}	1,227	−0.57 (−0.67 to −0.46)	1.51×10^{-23}	1,150	−0.41 (−0.52 to −0.29)	7.79×10^{-10}	1,142
rs11160868 (A)	T	25.7	0.58 (0.47 to 0.68)	2.32×10^{-23}	1,262	−0.56 (−0.66 to −0.45)	2.61×10^{-23}	1,189	−0.39 (−0.50 to −0.27)	2.69×10^{-09}	1,181
rs1134590 (A)	C	21.8	0.55 (0.43 to 0.66)	2.89×10^{-19}	1,262	−0.47 (−0.58 to −0.36)	1.37×10^{-15}	1,185	−0.37 (−0.49 to −0.26)	3.34×10^{-08}	1,177
rs11621145 (A)	G	27.1	0.53 (0.42 to 0.64)	8.39×10^{-19}	1,227	−0.53 (−0.64 to −0.42)	2.22×10^{-20}	1,149	−0.40 (−0.52 to −0.29)	1.05×10^{-09}	1,141
rs12884389 (A)	C	28.7	0.51 (0.40 to 0.62)	1.72×10^{-17}	1,229	−0.48 (−0.59 to −0.37)	2.51×10^{-16}	1,152	−0.42 (−0.54 to −0.31)	7.48×10^{-11}	1,144
rs12897751 (A)	G	25.6	0.57 (0.46 to 0.68)	4.92×10^{-23}	1,278	−0.56 (−0.67 to −0.46)	2.06×10^{-24}	1,200	−0.40 (−0.51 to −0.29)	4.05×10^{-10}	1,192
rs2725142 (A)	G	29.0	0.51 (0.40 to 0.62)	1.13×10^{-17}	1,233	−0.48 (−0.59 to −0.37)	1.46×10^{-16}	1,156	−0.40 (−0.51 to −0.28)	1.07×10^{-09}	1,148
rs2753571 (A)	A	29.1	0.51 (0.40 to 0.62)	3.65×10^{-18}	1,231	−0.46 (−0.57 to −0.36)	3.11×10^{-15}	1,154	−0.39 (−0.50 to −0.27)	4.50×10^{-09}	1,146
rs34398108 (A)	A	27.9	0.52 (0.42 to 0.63)	2.40×10^{-19}	1,229	−0.54 (−0.64 to −0.43)	2.71×10^{-21}	1,153	−0.40 (−0.51 to −0.28)	1.19×10^{-09}	1,145
rs1059216 (B)	C	6.2	−0.10 (−0.27 to 0.07)	1.00×10^{-00}	1,270	−0.17 (−0.33 to 0.00)	1.00×10^{-00}	1,193	−0.12 (−0.30 to 0.05)	1.00×10^{-00}	1,185
rs61984162 (C)	A	2.8	0.20 (−0.04 to 0.45)	1.00×10^{-00}	1,276	−0.20 (−0.44 to 0.04)	1.00×10^{-00}	1,198	−0.15 (−0.40 to 0.11)	1.00×10^{-00}	1,190
rs8009156 (D)	T	44.8	−0.21 (−0.29 to −0.13)	5.80×10^{-06}	1,256	0.19 (0.11 to 0.26)	1.07×10^{-04}	1,178	0.14 (0.06 to 0.22)	5.18×10^{-02}	1,170

Abbreviations: AF = allele frequency; EA = effect allele; Ig = immunoglobulin; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; LD = linkage disequilibrium; N = number of patients.
Linear regression on Ig indices transformed by inverse rank normalization and variants on chromosome 14. p Values were adjusted by Bonferroni correction for multiple testing for n = 85 tests.

Figure 1 Ig indices in carriers and noncarriers of the effect alleles of variants on chromosome 14
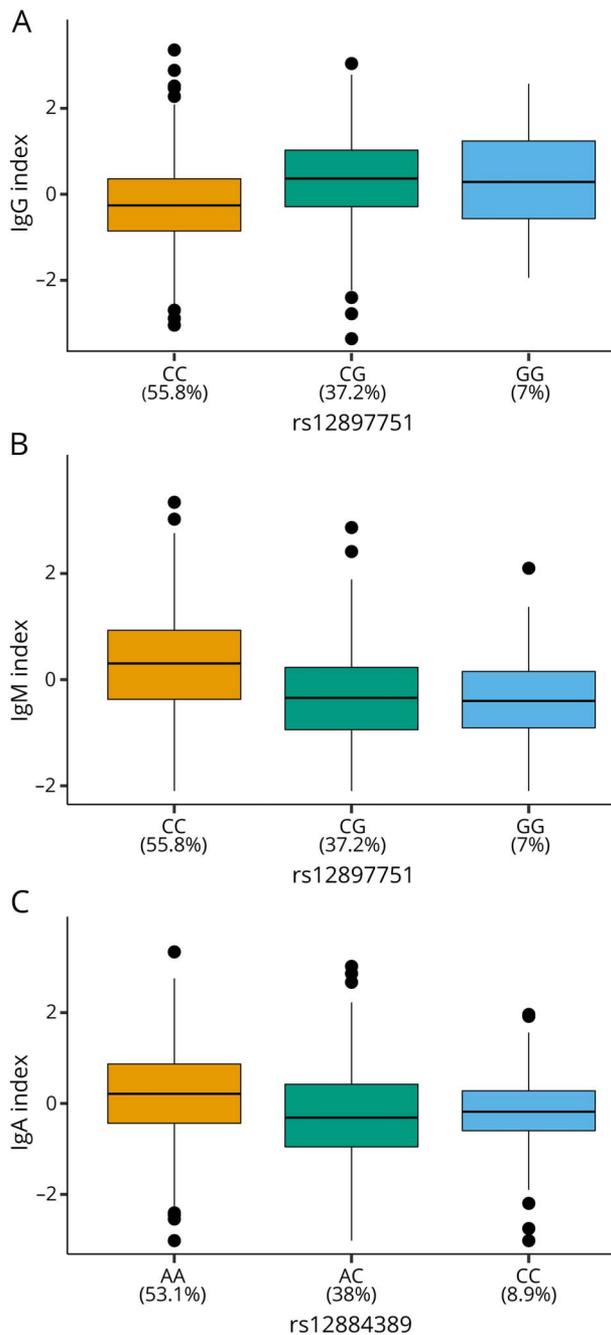
Box plots showing IgG (A), IgM (B), and IgA (C) indices transformed by inverse rank normalization for noncarriers, heterozygotes, and homozygotes of the effect allele of the most significantly associated variants on chromosome 14. Bars represent the median, boxes the interquartile ranges, the vertical lines the data range without outliers, and dots the outliers.

associated with lower IgM (figure 1B) and IgA (figure 1C) indices (table 1). Visual evaluation of the rank-transformed Ig indices by genotype for these variants was consistent with a dominant model of inheritance. The minor allele rs8009156*T was associated with lower IgG indices and higher IgM and IgA indices, but the association with IgA indices was not significant after correction for multiple testing. Associations for the

IgM index were validated using permutation analyses (data not shown).

To fine map the association of variants at the IGHC locus with Ig indices, we performed stepwise conditional regression analyses, adjusting, in each step, for the variants with the most robust support for association. For IgG indices, we identified rs12897751 as the top-associated variant (explaining 7.6% of IgG index variance) and observed no evidence for a second causal effect at this locus. SNP rs12897751 also showed the most robust association with IgM indices (explaining 8.2% of the variance), and we observed weak evidence for a possible second causal effect: SNP rs34398109 was associated at nominal significance when conditioning for rs12897751 ($\beta = -0.24$ [-0.47 to -0.01], $p = 0.037$). For IgA indices, rs12884389 was the top-associated SNP, explaining 4.2% of the variance, and there was weak evidence for a second effect because rs11621145 was associated at nominal significance in the conditional analysis ($\beta = -0.19$ [-0.37 to -0.01], $p = 0.035$).

Association of HLA alleles with Ig indices

Of 98 analyzed 4-digit HLA alleles, 9 showed an association with at least 1 of the 3 Ig indices after correction for multiple testing (table 2). HLA-DRB1*15:01, HLA-DQB1*06:02, HLA-DQA1*01:02, and HLA-B*07:02 were all significantly associated with higher IgG indices (figure 2A for HLA-DQB1*06:02). We performed haplotype level analyses on the extended HLA-A*03:01-C*07:02-B*07:02-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype with stepwise addition of the single HLA alleles of this haplotype. The most robust support for association could be observed for the HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype that explained 2.6% of the variance of IgG indices. When analyzed separately, HLA-DQB1*06:02 showed the most robust association. In addition, HLA-B*44:02 showed an association with lower IgG indices, explaining 1.0% of the IgG index variance. This association was independent of the HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype in a conditional analysis ($\beta = -0.34$ [-0.52 to -0.16], unadjusted $p = 2.64 \times 10^{-04}$).

Only HLA-C*02:02 was associated with IgM indices after correction for multiple testing. This allele was associated with lower IgM indices, explaining 1.8% of the variance. All HLA alleles significantly associated with IgG indices were also associated with IgM indices at a nominal significance level (table 2 and figure 2B), but these associations were not significant after correction for multiple testing.

HLA-DRB1*13:01, HLA-DQB1*06:03, and HLA-DQA1*01:03 were associated with higher IgA indices. All 3 HLA alleles are part of the HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype, and the association of the haplotype was stronger than the association of the single alleles, explaining 1.6% of the IgA index variance (table 2).

Table 2 Associations of HLA alleles with the Ig indices

HLA allele	AF	Regression on IgG indices (no. of patients = 1,279)		Regression on IgM indices (no. of patients = 1,200)		Regression on IgA indices (no. of patients = 1,192)	
		β (95% CI)	Adjusted p	β (95% CI)	Adjusted p	β (95% CI)	Adjusted p
B*07:02	17.8	0.18 (0.08 to 0.28)	2.92×10^{-02}	0.12 (0.02 to 0.22)	1.00×10^{-00}	0.09 (−0.02 to 0.19)	1.00×10^{-00}
DQA1*01:02	33.0	0.17 (0.09 to 0.25)	7.61×10^{-03}	0.11 (0.02 to 0.19)	1.00×10^{-00}	0.05 (−0.04 to 0.13)	1.00×10^{-00}
DQB1*06:02	26.5	0.27 (0.18 to 0.36)	2.41×10^{-07}	0.15 (0.06 to 0.24)	1.05×10^{-01}	0.09 (0.00 to 0.19)	1.00×10^{-00}
DRB1*15:01	27.3	0.24 (0.16 to 0.33)	5.92×10^{-06}	0.13 (0.05 to 0.22)	2.57×10^{-01}	0.09 (0.00 to 0.18)	1.00×10^{-00}
DQA1*01:03	6.0	0.10 (−0.07 to 0.26)	1.00×10^{-00}	0.15 (−0.02 to 0.31)	1.00×10^{-00}	0.37 (0.19 to 0.56)	6.71×10^{-03}
DQB1*06:03	5.5	0.06 (−0.10 to 0.22)	1.00×10^{-00}	0.17 (0.00 to 0.33)	1.00×10^{-00}	0.34 (0.15 to 0.53)	3.40×10^{-02}
DRB1*13:01	5.4	0.10 (−0.07 to 0.27)	1.00×10^{-00}	0.18 (0.01 to 0.35)	1.00×10^{-00}	0.39 (0.19 to 0.59)	8.59×10^{-03}
B*44:02	4.5	−0.35 (−0.54 to −0.17)	1.38×10^{-02}	−0.25 (−0.45 to −0.05)	7.44×10^{-01}	−0.03 (−0.25 to 0.18)	1.00×10^{-00}
C*02:02	4.3	−0.19 (−0.38 to −0.01)	1.00×10^{-00}	−0.45 (−0.61 to −0.28)	1.01×10^{-05}	−0.22 (−0.41 to −0.03)	1.00×10^{-00}
Haplotype1	26.0	0.27 (0.18 to 0.36)	2.14×10^{-07}	0.15 (0.06 to 0.24)	9.07×10^{-02}	0.10 (0.01 to 0.19)	1.00×10^{-00}
Haplotype2	5.5	0.11 (−0.06 to 0.28)	1.00×10^{-00}	0.18 (0.01 to 0.35)	1.00×10^{-00}	0.40 (0.21 to 0.60)	4.46×10^{-03}

Abbreviations: AF = allele frequency; Haplotype1 = HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02; Haplotype2 = HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01; HLA = human leukocyte antigen; Ig = immunoglobulin; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; LD = linkage disequilibrium.
Linear regression on Ig indices transformed by inverse rank normalization and 4-digit imputed HLA alleles. p Values were adjusted by Bonferroni correction for multiple testing for n = 85 tests.

Association of genetic variants and HLA alleles with serum Ig concentrations

To further investigate the mechanisms by which the genetic factors might have an effect on Ig indices, we performed secondary regression analyses on blood Ig concentrations for variants and HLA alleles associated with Ig indices. Except for rs8009156, the minor alleles of all other variants on chromosome 14 associated with IgG indices were also associated with higher IgG serum levels, but the effect sizes of these associations were smaller compared with the analyses on IgG indices (β = 0.15 - 0.28, data not shown). There was no association of any of the analyzed variants at the IGHC locus with serum IgM concentrations, and only rs12897751 was associated with lower serum IgA concentrations. There was no association of any HLA allele with serum Ig levels after correction for multiple testing (data not shown).

Association of genetic variants and HLA alleles with blood and CSF B cells and plasmablasts

Of the analyzed variants on chromosome 14, only rs2725142 and rs2753571 showed an association with proportions of CSF B cells (β = −0.29 [−0.52 to −0.07], adjusted p = 0.022 for both variants), but were associated with a lower proportion of CSF B cells. None of the other analyzed variants on chromosome 14 were significantly associated with the proportions of B cells and plasmablasts in the CSF or peripheral blood (data not shown). HLA-DRB1*15:01 was associated with higher CSF B cell and plasmablast proportions and HLA-DQB1*06:02 and HLA-DQA1*01:02 with higher CSF plasmablast proportions (table 3 and figure 2 shown for HLA-DQB*06:02 as

this was the HLA allele with the strongest association with the IgG indices). HLA-C*02:02 was associated with lower CSF B cell and plasmablast proportions (table 3). HLA-DRB1*13:01, HLA-DQA1*01:03, HLA-DQB1*06:03, and HLA-B*44:02 were not associated with CSF B cells or plasmablasts. There was no significant association of any HLA allele associated with the Ig indices with percentages of peripheral blood B cells or plasmablasts (data not shown).

To investigate whether the association of the HLA alleles from the HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype and HLA-C*02:02 with CSF B cell and plasmablast proportions fully explains the associations with the Ig indices, we performed causal mediation analyses. These analyses could only be performed in a smaller proportion of the patients with available flow cytometry data and showed nominally significant results. We observed full mediation of the effect of the HLA alleles from the HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype on IgG indices by increased CSF B cell or plasmablast proportions (shown for HLA-DQB1*06:02 in figure e-1, links.lww.com/NXI/A277) and full mediation of the effect of HLA-C*02:02 on IgM indices by decreased CSF plasmablast proportions (figure e-1, links.lww.com/NXI/A277).

Epistasis between genetic factors at the IGHC locus and HLA alleles on Ig indices

We tested for epistatic interactions between the variants at the IGHC locus and HLA alleles. No significant interactions were found between the top-associated IGHC variant, rs12897751,
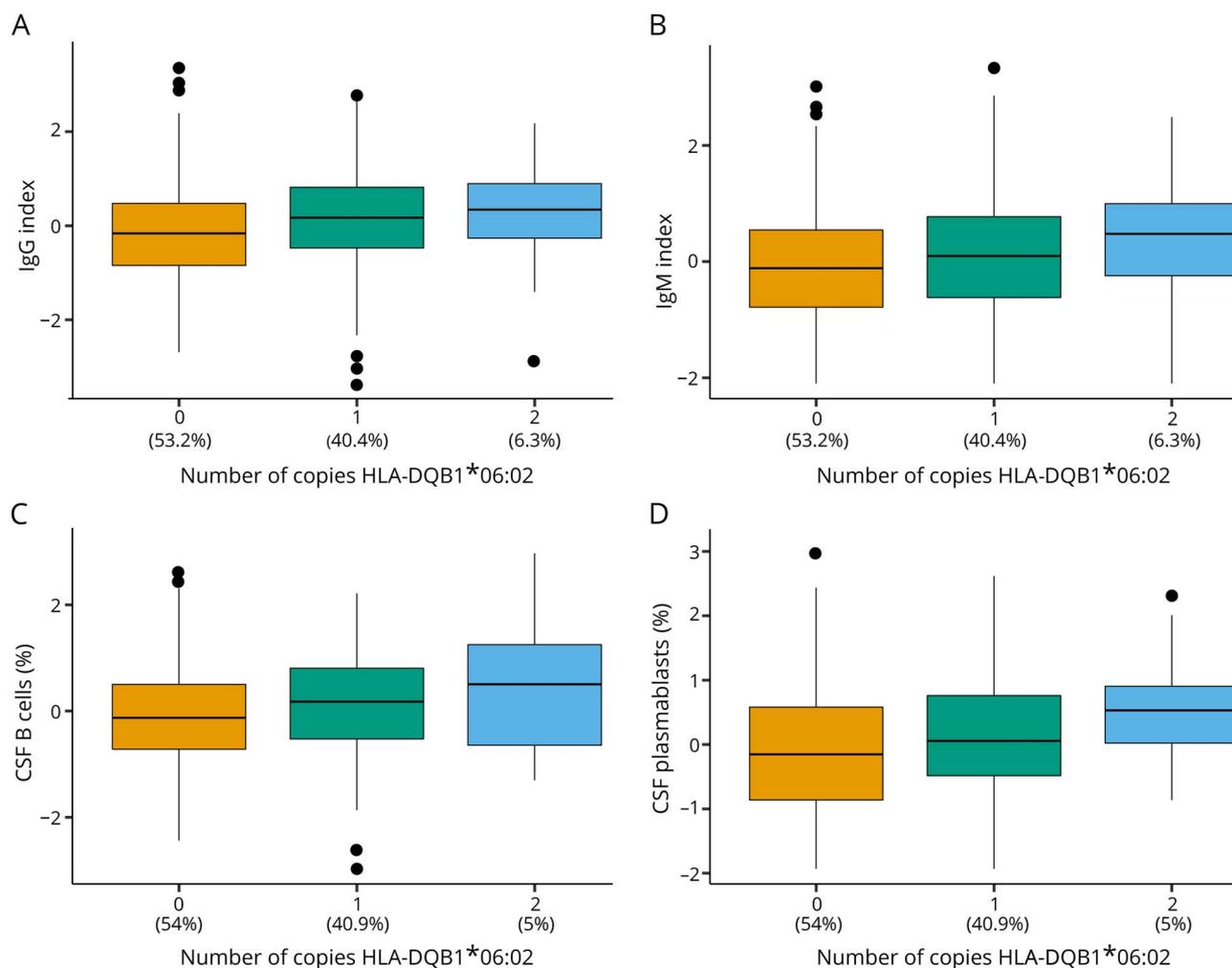
Box plots showing the rank-transformed IgG indices (A), IgM indices (B), CSF B cell (C), and plasmablast (D) proportions (all transformed by inverse rank normalization) for noncarriers, heterozygotes, and homozygotes of HLA-DQB1*06:02. Bars represent the median, boxes the interquartile ranges, the vertical lines the data range without outliers, and dots the outliers.

and alleles from the HLA-B*07:02-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype on IgG indices or between rs12897751 and HLA-C*02:02 on IgM levels. However, we observed evidence for epistatic interaction between rs12897751 and HLA-B*44:02 on IgG indices (interaction term $\beta = -0.58$ [-0.94 to -0.22], $p = 6.12 \times 10^{-03}$, adjusted for 4 independent test, figure e-1C, links.lww.com/NXI/A277) and between rs12884389 and HLA alleles from the HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype on IgA indices (interaction term for the haplotype and rs12884389 $\beta = -0.61$ [-0.97 to -0.26], adjusted $p = 3.22 \times 10^{-03}$, figure e-1D, links.lww.com/NXI/A277).

Discussion

In this study, we report associations of genetic factors in 2 regions—the IGHC locus on chromosome 14 and the major histocompatibility complex (MHC) region on chromosome 6—with IgG, IgA, and IgM indices in patients with MS or CIS.

We confirmed and fine mapped a previously reported association between genetic variants at the IGHC locus and intrathecal IgG indices.[5,6] The effect alleles of a highly correlated group of 10 SNPs were significantly associated with higher IgG indices. SNP rs12897751—an intronic variant in the immunoglobulin heavy constant gamma 3 (*IGHG3*) gene—showed the most robust support for association. Another variant strongly associated with IgG indices, but not independently of rs12897751, was the missense variant rs1071803. Variant rs1071803 (in high LD with rs12897751, $r^2 = 0.95$) defines the IgG1 allotype G1m17 by altering the amino acid sequence of the CH1 domain of IgG1. The G1m17 allotype is part of the Gm21*;17,1;.. and the Gm21*;17,1,2;.. haplotypes, which are prevalent haplotypes in Caucasian and Mongoloid populations.[23] The functional consequences of allotypes are poorly understood. Allotypes have been shown to correlate with IgG plasma concentrations[24,25]; they alter the IgG half-life[26] and might influence the distribution of antibodies to specific tissues, affect class switching, or alter secondary messenger RNA

Table 3 Association of HLA alleles with CSF B cells and plasmablasts

HLA allele	AF	Regression on CSF B cell proportions (no. of patients = 348)		Regression on CSF plasmablast proportions (no. of patients = 348)	
		β (95% CI)	Adjusted *p*	β (95% CI)	Adjusted *p*
B*07:02	17.5	0.13 (−0.06 to 0.31)	8.90×10^{-01}	0.15 (−0.03 to 0.33)	5.35×10^{-01}
DQA1*01:02	32.3	0.19 (0.03 to 0.36)	1.17×10^{-01}	0.23 (0.07 to 0.39)	3.11×10^{-02}
DQB1*06:02	25.5	0.23 (0.05 to 0.40)	5.50×10^{-02}	0.23 (0.06 to 0.41)	3.74×10^{-02}
DRB1*15:01	26.7	0.23 (0.06 to 0.40)	4.45×10^{-02}	0.25 (0.08 to 0.42)	1.91×10^{-02}
DQA1*01:03	6.0	−0.12 (−0.43 to 0.20)	1.00×10^{-00}	−0.09 (−0.40 to 0.22)	1.00×10^{-00}
DQB1*06:03	5.5	−0.12 (−0.43 to 0.19)	1.00×10^{-00}	−0.04 (−0.34 to 0.27)	1.00×10^{-00}
DRB1*13:01	5.8	−0.13 (−0.45 to 0.19)	1.00×10^{-00}	−0.07 (−0.39 to 0.25)	1.00×10^{-00}
B*44:02	3.2	0.07 (−0.35 to 0.49)	1.00×10^{-00}	0.25 (−0.16 to 0.66)	1.00×10^{-00}
C*02:02	4.3	−0.57 (−0.99 to −0.14)	4.52×10^{-02}	−0.57 (−0.91 to −0.23)	5.35×10^{-03}

Abbreviations: AF = allele frequency; HLA = human leukocyte antigen; Ig = immunoglobulin; IgA = immunoglobulin A; IgG = immunoglobulin G; IgM = immunoglobulin M; LD = linkage disequilibrium.
Linear regression on CSF B cell and plasmablast proportions transformed by inverse rank normalization and 4-digit imputed HLA alleles. *p* Values were adjusted by Bonferroni correction for multiple testing for n = 5 tests (only HLA alleles associated with at least one of the Ig indices were tested).

structures and affect the transcription rate.[27,28] Although based on conditional analyses, rs1071803 does not appear to be the causal variant for the association of the IGHC locus and the IgG index, it is however possible that other genetic variants causing Ig allotypy, not genotyped in the present study but in LD with the investigated variants, are causal for this relationship. Alternatively, as rs12897751 has been shown to be associated with higher protein expression levels of IGHG1, IGHG2, and IGHG3 (GTEx v8[29]), an influence of this or another correlated variant at this locus on gene expression might affect IgG levels.

Most of the genetic variants on chromosome 14 associated with Ig indices were also associated with serum IgG levels. These associations were, however, much weaker than the associations with Ig indices. Variants at the IGHC locus might thus not only affect the amount of intrathecal Igs but also have some effect on serum IgG concentrations. Analysis of flow cytometry data from CSF cells showed an association of 2 SNPs on chromosome 14 with CSF B cells. As the effect alleles of these variants were, however, associated with lower CSF B cell proportions, it is unlikely that this explains their association with higher IgG indices.

Of interest, the minor alleles of all variants on chromosome 14 that were associated with higher IgG indices were, at the same time, associated with lower IgM and IgA indices. This is especially striking, as higher IgG indices were correlated with higher IgA and IgM indices. How the variants at the IGHC locus influence intrathecal IgM and IgA is, therefore, unclear; possible scenarios are an increased class switching to IgG-producing cells with resulting lower concentrations of IgA and IgM or feedback mechanisms resulting in reduced synthesis of IgM and IgA.

Eight HLA alleles were associated with Ig indices in this study. Alleles that are part of the extended HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype were significantly associated with higher IgG indices and nominally associated with higher IgM indices. The HLA-DRB1*15:01-DQA1*01:02-DQB1*06:02 haplotype showed the most robust support of association and HLA-DQB1*06:02 was the most significantly associated single allele. Causal mediation analysis showed that the effect of these HLA alleles on the IgG index is completely explained by their association with higher CSF B cell and plasmablast proportions. The same is true for the association of HLA-C*02:02 with lower IgM indices and lower CSF B lymphocyte proportions. HLA-B*44:02 was independently associated with lower IgG indices, and we observed some evidence for epistasis between HLA-B*44:02 and the top-associated IGHC variant rs12897751. The HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype and the individual HLA alleles that are part of this haplotype, on the other hand, were associated with higher IgA indices, but did not show any association with CSF B cells or plasmablasts. We did however observe evidence for epistatic interactions between the HLA-DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype and IGHC variants. Different HLA alleles thus appear to have a differential effect on the intrathecal production of the Ig classes IgG, IgM, and IgA, probably due to different underlying mechanisms. Because of the design of this study, we cannot conclude whether the observed effects are specific for MS or possibly shared by other inflammatory neurologic diseases.

We describe 2 genetic regions—the IGHC locus on chromosome 14 and the MHC region on chromosome 6—that were associated with the amount of intrathecal IgG, IgM,

and/or IgA in patients with MS or CIS. Our findings suggest differential mechanisms by which the 2 genetic regions influence intrathecal Ig synthesis or concentration (figure e-2, links.lww.com/NXI/A277). HLA alleles in LD with a known MS risk allele, HLA-DRB1*15:01, appeared to influence the proportion of intrathecal B cells and plasmablasts and thereby increase the intrathecal synthesis of immunoglobulins, especially of IgG. The same was true for HLA-C*02:02 associated with lower CSF B lymphocyte proportions and IgM indices. The DQA1*01:03-DQB1*06:03-DRB1*13:01 haplotype was associated with higher IgA indices, but not with higher CSF B lymphocyte proportions. We cannot conclude from the present study how these alleles influence CSF IgA levels.

Genetic variants at the IGHC locus on chromosome 14 were associated with higher IgG and lower IgA and IgM indices. These variants did not influence the composition of B lymphocytes in the CSF. We therefore believe that they might influence the amount of intrathecal Ig via other mechanisms, such as an altered CSF immunoglobulin homeostasis—probably as a result of changes of the protein structure caused by the variation in the amino acid sequence associated with the Gm21* haplotypes. Understanding the mechanisms by which the IGHC locus influences intrathecal Ig levels may have implications for the design of future therapeutic antibodies to ensure a better enrichment and persistence in the CNS compartment.

Publication history

Appendix Authors

Name	Location	Contribution
Christiane Gasperi, MD	Technical University Munich, Munich, Germany	Designed and conceptualized the study; performed statistical analyses; and drafted the manuscript
Till F.M. Andlauer, PhD	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Ana Keating, MSc	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Benjamin Knier, MD	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Ana Klein, MD	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Verena Pernpeintner, MD	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content

Name	Location	Contribution
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Ralf Gold, MD	Ruhr-University Bochum, Bochum, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Frauke Zipp, MD	Johannes Gutenberg University Mainz, Mainz, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
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Martin Stangel, MD	Hannover Medical School, Hannover, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Hayrettin Tumani, MD	Clinic of Neurology Dietenbronn, Schwendi, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Brigitte Wildemann, MD	University Hospital Heidelberg, Heidelberg, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Heinz Wiendl, MD	University of Münster, Münster, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
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Uwe K. Zettl, MD	University of Rostock, Rostock, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Ralf A. Linker, MD	University of Regensburg, Regensburg, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Ulf Ziemann, MD	Eberhard-Karls-Universität Tübingen, Tübingen, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content

Appendix *(continued)*

Name	Location	Contribution
Matthias Knop, MD	Max Planck Institute of Psychiatry, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Clemens Warnke, MD	Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
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Friedemann Paul, MD	Charité—Universitätsmedizin Berlin, Berlin, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
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Achim Berthele, MD	Technical University Munich, Munich, Germany	Acquired and analyzed the data and revised the manuscript for intellectual content
Bernhard Hemmer, MD	Technical University Munich, Munich, Germany	Designed and conceptualized the study; supervised the research; and revised the manuscript for intellectual content

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