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HLA Associations Differ by Ethnicity and Aquaporin-4 Antibody Status in Patients With Neuromyelitis Optica Spectrum Disorders

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Abstract

Background and Objectives

Neuromyelitis optica spectrum disorders (NMOSDs) comprise inflammatory processes of the CNS. Most patients with NMOSD have serum immunoglobulin (Ig) G autoantibodies directed against the astrocytic water channel aquaporin-4 (AQP4-IgG). In this study, we analyzed HLA allelic frequencies in a large cohort of patients with NMOSD, stratified by ethnicity and AQP4-IgG status, compared with healthy controls.

Methods

Next-generation sequencing–based HLA class I and II genotyping was performed in 174 White, 45 Black, and 41 Hispanic AQP4-IgG–positive (AQP4-IgG+) patients with NMOSD; 49 White patients with AQP4-IgG–negative (AQP4-IgG−) NMOSD; and 2,427 White, 244 Black, and 155 Hispanic controls. Correction for multiple testing was performed using the Bonferroni method.

Results

In White AQP4-IgG+ patients with NMOSD, the most significantly associated alleles were *HLA-DQA1*05:01:01* (30.1% vs 11.1%, odds ratio 3.43 [95% CI 2.65–4.42], corrected p = 8.95E-17), *HLA-DQB1*02:01:01* (29.9% vs 11.3%, 3.34 [2.58–4.31], corrected p = 4.33E-16), and *HLA-DRB1*03:01:01* (29.2% vs 11.6%, 3.15 [2.43–4.06], corrected p = 3.66E-14), followed by *HLA-B*08:01:01* (26% vs 10.4%, 3.02 [2.3–3.94] corrected p = 8.79E-12), *HLA-C*07:01:01* (27.8% vs 14%, 2.36 [1.81–3.04], corrected p = 2.44E-07), and *HLA-DRB3*01:01:02* (28% vs 14.7%, 2.27 [1.73–2.95], corrected p = 2.19E-06). The frequency of *HLA-DRB1*08:04:01* was higher in Black AQP4-IgG+ patients with NMOSD than in Black controls but did not achieve statistical significance (19.3% vs 5.7%, 3.92 [1.91–7.86], corrected p = 0.08). Nevertheless, when compared with a larger cohort of Black controls (n = 16,178), the frequency of *HLA-DRB1*08:04* (19.3% vs 5.1%, 4.46 [2.45–7.66], corrected p = 1.88E-03) was significantly higher in Black AQP4-IgG+ patients with NMOSD. No significant HLA associations were detected in AQP4-IgG+ Hispanic patients or White AQP4-IgG− patients with NMOSD.

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Supplementary Material

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Coinvestigators are listed in the appendix at the end of the article.

Glossary

AFND = Allele Frequency Net Database; **C4** = complement component C4; **CIRCLES** = Collaborative International Research in Clinical and Longitudinal Experience Study; **IgG** = immunoglobulin G; **IPND-2025** = International Panel for NMO Diagnosis; **LETM** = longitudinally extensive transverse myelitis; **MOGAD** = MOG antibody–associated disease; **MOG-IgG** = myelin oligodendrocyte glycoprotein-IgG; **NMDP** = National Marrow Donor Program; **NMOSD** = neuromyelitis optica spectrum disorders; **ON** = optic neuritis; **OR** = odds ratio; **RPE** = relative predispositional effect.

Discussion

This study confirms the previously recognized association of *HLA-DRB1*03:01* with AQP4-IgG+ NMOSD in White patients and extends this association to the *HLA-DRB1*03:01:01~HLA-DQA1*05:01:01~HLA-DQB1*02:01:01* haplotype. Furthermore, it identifies an association of *HLA-DRB1*08:04* with AQP4-IgG+ NMOSD in Black patients. However, no HLA associations were detected in White AQP4-IgG– patients with NMOSD. The immunogenetic differences between AQP4-IgG+ and AQP4-IgG– NMOSD support pathophysiologic distinctions between these entities.

Introduction

Neuromyelitis optica spectrum disorders (NMOSDs) are severe inflammatory processes of the CNS, predominantly affecting optic nerves, the spinal cord, and the brainstem. They, respectively, manifest as acute attacks of optic neuritis (ON), myelitis, and brainstem encephalitis.[1] Most patients meeting the current International Panel for NMO Diagnosis (IPND-2015) diagnostic criteria for NMOSD[2] have detectable serum immunoglobulin G (IgG) antibodies to aquaporin-4 (AQP4),[3] an astrocytic water channel.[4] AQP4-IgG has been shown in a variety of experimental models to be pathogenically relevant, and AQP4-IgG-positive (AQP4-IgG+) NMOSD is thus regarded as an autoantibody-mediated astrocytopathy.[5]

The peak age at disease onset of AQP4-IgG+ NMOSD is approximately 40 years, with a strong female predominance.[6] The risk of developing NMOSD is higher in Black and Asian individuals than in White individuals,[7] and familial aggregation of NMOSD has been recognized in approximately 3% of patients,[8] suggesting a possible contribution of genetic risk factors to the development of NMOSD.

The HLA complex is a group of highly polymorphic genes located on the short arm of chromosome 6.[9] Nucleotide variants in HLA class I and II genes (i.e., HLA class I and HLA class II alleles) have been associated with risk or protection to many human diseases, including autoimmune diseases.[10]

The most consistent association between HLA and NMOSD risk thus far identified is with *HLA-DRB1*03:01* in predominantly Western patient populations, as reviewed in a meta-analysis of HLA/NMOSD association studies.[11] However, limitations of previous studies of HLA/NMOSD associations include small sample size, lack of ethnic diversity, and absence of stratification for AQP4-IgG serostatus, as well as use of low-resolution and incomplete HLA genotyping.[11]

In this study, we report the results of a large HLA/NMOSD association study designed to overcome prior limitations. This study included a total of 317 North American patients, for whom comprehensive high-resolution HLA class I and II genotyping data were available. These patients were stratified for ethnicity and anti-AQP4-IgG status and compared with healthy control cohorts.

Methods

Patients With NMOSD

Patients with NMOSD (n = 317) analyzed in this study were participants of the Collaborative International Research in Clinical and Longitudinal Experience Study (CIRCLES), a prospective, multicenter, cross-sectional, and longitudinal observational study that collected longitudinal clinical data and biospecimens of patients with NMOSD.[12] Study enrollment began in November 2013. All patients included in this study had a diagnosis of NMOSD according to the IPND-2006[13] or IPND-2015 criteria.[2]

Primary participant ethnicity of CIRCLES participants derived from self-identification as White/Caucasian, Black/African American, or Hispanic/Latino.[12]

AQP4-IgG serostatus of CIRCLES participants had been determined by the study sites based on reference laboratory assays or by medical record review. AQP4-IgG seropositivity was defined as having detectable AQP4-IgG at any time during the participant's history.[12] Information regarding initial disease presentation phenotype (ON, longitudinally extensive transverse myelitis [LETM, defined as myelitis expanding over 3 or more vertebral segments], or brain/brainstem involvement) was collected at CIRCLES enrollment.[12]

Mobility was assessed using a questionnaire and categorized as "independent" (never requires assistance), "partially dependent" (requires assistance part of the time), "completely dependent" (always requires assistance), and "bedbound" (unable to use any assisted devices).

HLA Genotyping, Sequence Data Analysis, and Genotype Assignment

Details of genotyping, sequence data analysis, and genotype assignment are provided in eMethods.

Control Populations

Data from the University of California San Francisco (UCSF) INDIGO consortium were used as healthy control cohorts for White, Hispanic, and Black populations. This included self-identified 2,427 White (non-Hispanic), 155 Hispanic, and 244 Black participants, respectively. All participants were typed for alleles at *HLA-A*, *HLA-B*, *HLA-C*, *HLA-DRB1*, *HLA-DQA1*, *HLA-DQB1*, *HLA-DPA1*, and *HLA-DPB1* loci. The few cases of patients or controls with missing values were excluded from the analysis of the respective locus. A total of 1,343 (55.3%) White (non-Hispanic) participants and all Hispanic and Black INDIGO participants were also typed for the alleles of the low-expression loci *HLA-DRB3*, *HLA-DRB4*, and *HLA-DRB5*. Comparisons of HLA allele frequencies of patients with NMOSD and participants of INDIGO were performed at the 3-field allele resolution level.

HLA allele frequencies of participants of the USA National Marrow Donor Program (NMDP) were obtained from the Allele Frequency Net Database (AFND)[14] maintained by the Royal Liverpool and Broadgreen University Hospital. Participant ethnicities of NMDP participants were determined using questionnaires.[15] As control population for White patients, we used the NMDP European Caucasian cohort (n = 1,242,890). All participants of this cohort were typed for alleles at *HLA-A*, *HLA-B*, and *HLA-DRB1* loci. In addition, 395,676 participants (31.8%) were typed for *HLA-C* locus.[15] As control population for Hispanic patients, we used the NMDP Hispanic South or Central American cohort (n = 146,714). All participants of this cohort were typed for *HLA-A*, *HLA-B*, and *HLA-DRB1*. In addition, 31,446 participants (21.4%) were typed for *HLA-C* and 5,764 (3.9%) for *HLA-DQB1*.[15] As control population for Black patients, we used the NMDP USA African American pop 2 cohort (n = 416,581). All participants of this cohort were typed for *HLA-A* and *HLA-B*, 99,946 (24%) for *HLA-C*, and 16,178 (4%) for *HLA-DRB1*.[15] The comparison of HLA alleles of patients with NMOSD and participants of the NMDP cohort were performed at 2-field resolution, i.e., the highest level of resolution available for this data set.

As a further control population for White patients, we also used publicly available data from 1,194 participants (n = 537, [45%] female, mean age at blood collection 54.1 years) of the PopGen control cohort, a population-based cohort of randomly selected individuals from the general population of Kiel, Northern Germany.[16] In a questionnaire, 96% of participants of the PopGen cohort declared that they were born in Germany.[17] Details of imputation and genotyping of the PopGen control cohort are provided in eMethods.

Statistical Analysis

For descriptive statistics, data are presented as median (interquartile range), absolute range (minimum—maximum), mean (SD), and absolute and relative frequencies in case of categorical data.

Frequencies of HLA alleles in patients with NMOSD compared with controls were calculated using R (version 2022.07.0 Build 548). Each HLA allele was tested for association with NMOSD case-control status using the Fisher exact contingency table test. We report p values and effect size as odds ratios (ORs) and 95% CIs. A total of 775 HLA alleles with a frequency of ≥1% in the patient as well as in the control cohorts were included in the analyses. Correction for multiple testing was performed by the Bonferroni method (for 888 independent tests). HLA associations with corrected p values < 0.05 were considered significant.

Relative predispositional effect (RPE) analysis was performed to identify HLA alleles disproportionately represented in AQP4-IgG+ NMOSD compared with ethnically matched controls as previously described.[18] A frequency filter (≥1% in patients and controls) was applied before testing to exclude rare alleles. At each iteration, a χ^2 test was applied to the $2 \times k$ contingency table to determine the allele showing the greatest deviation from the expected distribution. The OR and 95% CI for the selected allele were subsequently calculated using Fisher exact test. Only alleles with p values < 0.05 were listed. We assessed allele dosage by comparing heterozygous vs homozygous carriers of *HLA-DRB1*03:01:01* and *HLA-B*08:01:01*, with expected homozygosity calculated under Hardy-Weinberg equilibrium. Because phasing was unavailable, multilocus analyses used unphased co-occurrence: individuals were considered carriers if they had ≥1 copy of each allele specified across loci. This captures complete allele combinations but does not establish phased haplotypes.

Statistical significance of the association of the number of *HLA-DRB1*03:01* alleles with the type of first clinical disease manifestation (ON, LETM, brain/brainstem) and mobility (independent, partially dependent, completely dependent, and bedbound) was assessed by the 2-tailed Fisher exact test, performing 2-group comparisons of patient groups with either 0 or 1 allele and patient groups with either 0 or 2 alleles. Statistical significance of the association of the number of HLA alleles with age at disease onset, and the annualized relapse rate was assessed by Mann-Whitney U test, performing 2-group comparisons of patient groups with either 0 or 1 allele and patient groups with either 0 or 2 alleles. All statistical analyses were performed using R.

Standard Protocol Approvals, Registrations, and Patient Consents

The Collaborative International Research in Clinical and Longitudinal Experience Study (CIRCLES)[12] includes 15 sites across North America and was approved by the institutional review boards of each site and the Data Coordinating Center at the University of Utah. Written informed consent was obtained from all study participants before sample collection.

Data Availability

On reasonable request, approval will be obtained and anonymized data will be shared with any qualified investigator. The R codes used for the analysis, including allele counting and statistical evaluation of HLA associations, are publicly available at github.com/mniederschw/NMOSD-HLA.

Web Resources

Resources are available in AFND (allelefrequencies.net/).[14]

Results

Participants

Demographic and clinical data of patients with NMOSD (n = 317) are summarized in Table 1. Patients with NMOSD were stratified into White AQP4-IgG+ (n = 174), White AQP4-IgG− (n = 49), Hispanic AQP4-IgG+ (n = 41), or Black AQP4-IgG+ (n = 45) groups. Owing to their low number, Hispanic (n = 4) and Black AQP4-IgG− (n = 2) patients were not included in the analyses. Similarly, patients with no data on AQP4-IgG serostatus (n = 2) were also excluded.

HLA Associations in White AQP4-IgG+ Patients With NMOSD

In a comparison of 3-field resolution HLA allele frequencies between White AQP4-IgG+ patients with NMOSD and the INDIGO White healthy control population, *HLA-DQA1*05:01:01*, *HLA-DQB1*02:01:01*, and *HLA-DRB1*03:01:01* were the most significantly associated alleles (ORs 3.43, 3.34, and 3.15), followed by *HLA-B*08:01:01* and *HLA-C*07:01:01* (ORs 3.02 and 2.36), and *HLA-DRB3*01:01:02* (OR 2.27, Table 2). While all of these HLA alleles are part of a conserved combination of alleles referred to as the 8.1 ancestral haplotype,[19] the stronger associations with *HLA-DQA1*05:01:01*, *HLA-DQB1*02:01:01*, *and HLA-DRB1*03:01:01* than with *HLA-B*08:01:01*, *HLA-C*07:01:01*, and *HLA-DRB3*01:01:02* suggested that the primary association is with the *HLA-DRB1*03:01:01*~*HLA-DQA1*05:01:01*~*HLA-DQB1*02:01:01* core haplotype.

By contrast, *HLA-DQB1*02:02:01*, which is most commonly found within the HLA-DR7 haplotype, was less frequent in White AQP4-IgG+ patients with NMOSD than in the INDIGO White control population (Table 2).

Analysis of 2-field resolution HLA allele frequencies between White AQP4-IgG+ patients with NMOSD and NMDP European Caucasian healthy controls confirmed the association of *HLA-DRB1*03:01*, *HLA-B*08:01*, and *HLA-C*07:01* with AQP4-IgG+ NMOSD in White patients (eTable 1). Furthermore, an increased frequency of *HLA-DQB1*02:01*, *HLA-DRB1*03:01*, *HLA-B*08:01*, and *HLA-C*07:01* in White patients with AQP4-IgG+ NMOSD was also confirmed when compared with healthy controls in the PopGen cohort (eTable 2). In the comparison of White AQP4-IgG+ patients with NMOSD and PopGen controls, *HLA-DQB1*06:03* and *HLA-B*15:01* were less frequent in AQP4-IgG+ patients with NMOSD than in PopGen controls.

HLA Associations in Hispanic AQP4-IgG+ Patients With NMOSD

Comparisons of 3-field HLA allele frequencies between Hispanic AQP4-IgG+ patients with NMOSD and healthy Hispanic INDIGO controls did not reveal significant differences after correction for multiple testing (Table 3). *HLA-DRB4*01:03:01* was less frequent in Hispanic APQ4-IgG+ patients with NMOSD but did not achieve statistical significance after Bonferroni correction (corrected p = 0.06). However, among the 17 HLA alleles with uncorrected p values < 0.05 were *HLA-DQB1*02:01:01*, *HLA-DQA1*05:01:01*, *HLA-DRB1*03:01:01*, *HLA-B*08:01:01*, and *HLA-C*07:01:01*.

In an additional comparison of 2-field resolution HLA alleles in Hispanic AQP4-IgG+ patients with NMOSD and a larger number of NMDP Hispanic healthy controls, *HLA-B*08:01* and *HLA-DRB1*03:01* were likewise found to be more frequent in Hispanic patients, but without reaching statistical significance after Bonferroni correction (eTable 3).

HLA Associations in Black AQP4-IgG+ Patients With NMOSD

In a comparison of 3-field resolution HLA allele frequencies in Black AQP4-IgG+ patients with NMOSD and Black INDIGO healthy controls, *HLA-DQA1*04:01:02* and *HLA-DRB1*08:04:01* were more frequent in AQP4-IgG+ patients with NMOSD, but marginally missed achieving statistical significance after correction for multiple testing (*HLA-DQA1*04:01:02*, corrected p = 0.06, *HLA-DRB1*08:04:01*, corrected p = 0.08, Table 4). The *HLA-DQB1*03:19:01* allele that is in linkage disequilibrium with *DRB1*08:04:01* in individuals with African ancestry was also more common in Black AQP4-IgG+ patients with NMOSD than in controls but did not reach statistical significance after correction for multiple testing. Among 12 further HLA alleles with uncorrected p values < 0.05 were *HLA-DQA1*05:01:01*, *HLA-DQB1*02:01:01*, *HLA-DRB1*03:01:01*, and *HLA-B*08:01:01*.

In an additional comparison of 2-field resolution HLA allele frequencies between Black AQP4-IgG+ patients with NMOSD and NMDP African American healthy controls, *HLA-DRB1*08:04* was more frequent in patients than controls (corrected p = 1.88E-03). Among 8 further HLA alleles with uncorrected p values < 0.05 were *HLA-DRB1*03:01* and *HLA-B*08:01* (eTable 4).

Table 1 Demographics and Clinical Characteristics of Patients With NMOSD

	Patients with NMOSD (CIRCLES cohort)				
	All	White AQP4-IgG+	White AQP4-IgG−	Hispanic AQP4-IgG+	Black AQP4-IgG+
n	317[a]	174	49	41	45
Female, n (%)	270 (85.2)	148 (85.1)	39 (79.6)	38 (92.3)	38 (84.4)
Age at first episode onset, y, median (range)	39.7 (2.2–79.9)	39.7 (8.0–79.9)	37.6 (8.4–59.4)	39.4 (2.2–70.2)	39.5 (12.0–65.9)
Age at blood collection, y; median (range)	48.6 (3.4–85.1)	53.3 (15.0–85.1)	45.9 (11.3–76.8)	42.6 (3.4–71.7)	44.3 (16.8–71.2)
ON present during first attack, n (%)	136 (42.9)	67 (38.5)	35 (72.3)	25 (61.0)	18 (40)
LETM present during first attack, n (%)	113 (35,6)	60 (34.5)	21 (42.9)	12 (29.3)	16 (35.6)
Brain clinical syndromes/brainstem lesions/cerebral hemispheric lesions present during first attack, n (%)	77 (31.4)	42 (30.7)	12 (31.6)	11 (35.5)	9 (32.1)
Annualized on study relapse rate, mean ± SD	0.20 ± 0.4	0.18 ± 0.37	0.21 ± 0.39	0.21 ± 0.41	0.20 ± 0.38
Level of mobility (%)					
Independent	194 (61.2)	112 (64.4)	29 (59.2)	21 (46.7)	26 (63.4)
Partially dependent	87 (27.4)	47 (14.8)	16 (32.7)	14 (28.6)	9 (22.0)
Completely dependent	35 (11.0)	14 (4.4)	4 (8.2)	10 (20.4)	6 (14.6)
Bedbound	1 (0.3)	1 (0.5)	0	0	0

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; CIRCLES = Collaborative International Research in Clinical and Longitudinal Experience Study; LETM = longitudinally extensive transverse myelitis; n = number; NMOSD = neuromyelitis optica spectrum disorders; ON = optic neuritis.
[a] Four Hispanic and 2 Black AQP4-IgG− patients as well as 2 patients with no data on AQP4-IgG serostatus were not included in further analyses.

Of note, frequencies of the alleles *HLA-DRB1*08:01* and *HLA-DRB1*08:02*, which are common in White and Hispanic populations, respectively, were not elevated in AQP4-IgG+ patients with NMOSD in these populations. These alleles differ from the *HLA-DRB1*08:04* allele enriched in African ancestry by 2 and 1 amino acid substitutions, respectively (eFigure 1).

Likewise, *HLA-DRB1*03:02*, which has a similar frequency as *HLA-DRB1*03:01* in Black populations and differs from *HLA-DRB1*03:01* by 4 amino acids (eFigure 2), showed no significant association with AQP4-IgG+ NMOSD in Black patients.

Forest plots summarizing the ORs for *HLA-DRB1*03:01*, previously recognized to be associated with AQP4-IgG+ NMOSD,[11] in White, Hispanic, and Black patients with AQP4-IgG+ NMOSD compared with the respective NMDP healthy control cohorts are shown in Figure 1.

HLA Associations in White AQP4-IgG–Negative Patients With NMOSD

A comparison of 3-field HLA allele frequencies between White AQP4-IgG− patients with NMOSD and INDIGO White healthy controls (eTable 5) and a comparison of 2-field HLA allele frequencies between White AQP4-IgG− patients with NMOSD and NMDP European Caucasian controls (eTable 6) did not reveal any significant differences after correction for multiple testing.

*HLA-DRB1*03:01:01* Stratification and Relative Predispositional Effects

To study HLA associations that could potentially be independent of *HLA-DRB1*03:01:01*, we conducted separate analyses of *HLA-DRB1*03:01:01* noncarriers. In a comparison of *HLA-DRB1*03:01:01*-negative AQP4-IgG+ White patients with NMOSD and *HLA-DRB1*03:01:01*-negative White controls (eTable 7), none of the alleles identified in the primary analysis (Table 2) remained significant. While White *HLA-DRB1*03:01:01*-negative AQP4-IgG+ patients exhibited higher frequencies of *HLA-DQA1*01:04:01*, *HLA-DQB1*05:03:01*, *HLA-DPB1*104:01*, and *HLA-DRB1*14:54:01* and a lower frequency of *HLA-DQB1*02:02:01* compared with healthy controls (eTable 7), none of these associations remained significant after Bonferroni correction.

Likewise, among Hispanic noncarriers of *HLA-DRB1*03:01:01*, none of the 5 alleles that were decreased and the 3 alleles that were increased in AQP4-IgG+ patients compared with healthy controls remained significant after Bonferroni correction (eTable 8). Among these, *HLA-A*30:02:01* showed the highest effect size (OR 5.22 [95% CI 0.94–28.96]).

An RPE analysis restricted to alleles with frequencies above 1% in patients and controls confirmed that the alleles of the ancestral haplotype 8.1 identified in our initial analysis represent the primary risk factors in White AQP4-IgG+ patients

Table 2 HLA Allele Frequencies in White AQP4-IgG–Positive Patients With NMOSD and White INDIGO Healthy Controls (3-Field Resolution)

	Allele frequency, n/total n (%)		Two-sided Fisher exact test			
HLA allele	White AQP4-IgG+ patients with NMOSD	White healthy controls (INDIGO)	*p* Value	*p* Value after Bonferroni correction	Odds ratio	95% CI
DQA1*05:01:01	104/346 (30.1)	504/4,532 (11.1)	1.01e-19	**8.95E-17**	3.43	2.65–4.42
DQB1*02:01:01	104/348 (29.9)	510/4,512 (11.3)	4.88e-19	**4.33E-16**	3.34	2.58–4.31
DRB1*03:01:01	101/346 (29.2)	531/4,590 (11.6)	4.12e-17	**3.66E-14**	3.15	2.43–4.06
B*08:01:01	89/342 (26)	490/4,700 (10.4)	9.90e-15	**8.79E-12**	3.02	2.3–3.94
C*07:01:01	95/342 (27.8)	655/4,668 (14)	2.75e-10	**2.44E-07**	2.36	1.81–3.04
DRB3*01:01:02	97/346 (28)	394/2,686 (14.7)	2.47e-09	**2.19E-06**	2.27	1.73–2.95
DQB1*02:02:01	13/348 (3.7)	459/4,512 (10.2)	2.00e-05	**0.02**	0.34	0.18–0.6
DQA1*01:04:01	21/346 (6.1)	102/4,532 (2.3)	1.21e-04	0.11	2.81	1.64–4.59
DRB1*13:01:01	5/346 (1.4)	266/4,590 (5.8)	1.25e-04	0.11	0.25	0.08–0.57
DQA1*01:03:01	7/346 (2)	306/4,532 (6.8)	1.45e-04	0.13	0.29	0.11–0.6
DRB1*14:54:01	19/346 (5.5)	89/4,590 (1.9)	1.46e-04	0.13	2.94	1.67–4.93
A*01:01:01	82/344 (23.8)	752/4,758 (15.8)	2.02e-04	0.18	1.67	1.27–2.17
DQB1*05:03:01	21/348 (6)	107/4,512 (2.4)	3.12e-04	0.28	2.64	1.55–4.32
DRB1*07:01:01	25/346 (7.2)	627/4,590 (13.7)	3.72e-04	0.33	0.49	0.31–0.75
DQB1*06:03:01	7/348 (2)	281/4,512 (6.2)	5.44e-04	0.48	0.31	0.12–0.65
DQB1*03:02:01	18/348 (5.2)	449/4,512 (10)	2.41e-03	n.s.	0.49	0.29–0.8
DPA1*02:01:02	26/342 (7.6)	189/4,744 (4)	3.08e-03	n.s.	1.98	1.24–3.05
DQA1*03:01:01	17/346 (4.9)	427/4,532 (9.4)	3.49e-03	n.s.	0.5	0.28–0.82
DRB4*01:03:01	51/346 (14.7)	563/2,686 (21)	6.79e-03	n.s.	0.65	0.47–0.89
B*40:01:02	7/342 (2)	231/4,700 (4.9)	0.01	n.s.	0.4	0.16–0.86
A*11:01:01	12/344 (3.5)	318/4,758 (6.7)	0.02	n.s.	0.5	0.26–0.91
B*44:03:01	8/342 (2.3)	238/4,700 (5.1)	0.02	n.s.	0.45	0.19–0.91
DPB1*05:01:01	14/348 (4)	96/4,730 (2)	0.02	n.s.	2.02	1.05–3.61
DPB1*01:01:01	27/348 (7.8)	229/4,730 (4.8)	0.02	n.s.	1.65	1.05–2.52
DQB1*03:01:01	53/348 (15.2)	905/4,512 (20.1)	0.03	n.s.	0.72	0.52–0.97
DRB1*04:01:01	18/346 (5.2)	389/4,590 (8.5)	0.03	n.s.	0.59	0.34–0.96
B*15:01:01	10/342 (2.9)	266/4,700 (5.7)	0.04	n.s.	0.5	0.24–0.95
DQA1*05:05:01	34/346 (9.8)	615/4,532 (13.6)	0.05	n.s.	0.69	0.47–1

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; INDIGO = The Immunogenetics of Neurological DIseases working GrOup, UCSF Multiple Sclerosis Biorepository; NMOSD = neuromyelitis optica spectrum disorders; n.s. = not significant ($p > 1$).
The table lists all HLA alleles with uncorrected *p* values < 0.05. HLA alleles with *p* values < 0.05 after Bonferroni correction are highlighted in bold.

(eTable 9). Independent of those primary risk alleles, we observed higher frequencies of *HLA-DRB1*14:54:01*, *HLA-DQA1*01:04:01*, *HLA-DQB1*05:03:01*, and *HLA-DPB1*01:01:01*. Notably, *HLA-DRB1*14:54:01* and *HLA-DQB1*05:03:01* are in linkage disequilibrium,[20] showed higher frequencies in the analysis of *HLA-DRB1*03:01:01* noncarriers, and could, therefore, potentially represent an independent risk factor of NMOSD in White populations. By contrast, *HLA-DQB1*02:02:01* and *HLA-DQB1*06:03:01*, as well as *HLA-DQA1*01:03:01*, were decreased in White AQP4-IgG+ patients with NMOSD.

In Hispanic AQP4-IgG+ patients, step 1 of the RPE analysis (eTable 10) identified the alleles *HLA-A*03:01:01*, *HLA-*

*DQA1*05:01:01*, *HLA-DQB1*02:01:01*, *HLA-DRB1*03:01:01*, and *HLA-DRB4*01:03:01*, which were also identified in the primary analysis (Table 3). In addition, there was a decreased frequency of *HLA-DQA1*03:01:01*, *HLA-DQB1*03:02:01*, and *HLA-DRB1*04:07* compared with controls.

The RPE analysis in Black AQP4-IgG+ patients with NMOSD vs Black healthy controls (eTable 11) identified in step 1 the alleles *HLA-B*08:01:01*, *HLA-DPB1*17:01*, *HLA-DQA1*04:01:02*, *HLA-DQB1*03:19:01*, and *HLA-DRB1*08:04:01*, which were identified previously (Table 4). Independent of those alleles, Black AQP4-IgG+ patients had a higher frequency of *HLA-DQA1*05:01:01* and *HLA-DQB1*02:01:01* (eTable 11). These findings suggest that the *HLA-DRB1*03:01*~*DQA1*05:01*~*DQB1*02:01* haplotype could potentially represent a secondary, independent risk factor of NMOSD in Black individuals.

Genotype Associations for *HLA-DRB1*03:01:01*, *HLA-B*08:01:01*, and Unphased Haplotype Co-Occurrences

In White AQP4-IgG+ patients with NMOSD, *HLA-DRB1*03:01:01* homozygosity was associated with an OR of approximately 1.7× that of heterozygosity, suggesting a dose-response effect (eTable 12). In White AQP4-IgG+ patients with NMOSD, *HLA-DRB1*03:01:01* homozygosity also exhibited a higher OR (5.49) compared with *HLA-B*08:01:01* homozygosity (4.97), which served as single-locus representative of the extended ancestral haplotype 8.1. *HLA-B*08:01:01* homozygosity had an OR of approximately 1.5× that of heterozygosity (Figure 2). Both alleles showed lower frequencies of homozygosity in patients as well as in controls compared with expected frequencies under Hardy-Weinberg equilibrium, consistent with heterozygote excess.

Because phasing information was not available, a phased haplotype analysis could not be performed; instead, we evaluated unphased co-occurrence by counting individuals with at least 1 allele at each locus of the respective haplotype. White AQP4-IgG+ patients with NMOSD showed a greater association with co-occurrence of the core class *HLA-DRB1*03:01:01*~*DQA1*05:01:01*~*DQB1*02:01:01* haplotype than with the extended ancestral haplotype 8.1 alleles (Figure 2, eTable 12). Altogether, these findings suggest that the *DRB1*03:01:01*~*DQA1*05:01:01*~*DQB1*02:01:01* haplotype is the primary risk factor of AQP4-IgG+ NMOSD in

Table 3 HLA Allele Frequencies in Hispanic AQP4-IgG–Positive Patients With NMOSD and Hispanic INDIGO Healthy Controls (3-Field Resolution)

	Allele frequency, n/total n (%)		Two-sided Fisher exact test			
HLA allele	Hispanic AQP4-IgG+ patients with NMOSD	Hispanic healthy controls (INDIGO)	*p* Value	*p* Value after Bonferroni correction	Odds ratio	95% CI
DRB4*01:03:01	8/82 (9.8)	96/310 (31)	6.37e-05	5.66e-02	0.24	0.1–0.53
DQB1*02:01:01	14/82 (17.1)	12/310 (3.9)	1.25e-04	0.1	5.08	2.08–12.63
DQA1*05:01:01	14/82 (17.1)	12/310 (3.9)	1.25e-04	0.1	5.08	2.08–12.63
DPB1*04:02:01	15/82 (18.3)	121/310 (39)	3.90e-04	0.3	0.35	0.18–0.65
DQB1*03:02:01	8/82 (9.8)	86/310 (27.7)	4.32e-04	0.4	0.28	0.11–0.62
DRB1*03:01:01	12/82 (14.6)	11/310 (3.5)	6.14e-04	0.5	4.64	1.79–12.13
DQA1*03:01:01	8/82 (9.8)	82/310 (26.5)	1.04e-03	0.9	0.30	0.12–0.66
DRB1*04:07:01	1/82 (1.2)	37/310 (11.9)	1.42e-03	n.s.	0.09	0–0.56
B*08:01:01	9/82 (11)	9/310 (2.9)	4.77e-03	n.s.	4.10	1.39–12.13
A*03:01:01	11/82 (13.4)	13/310 (4.2)	7.09e-03	n.s.	3.52	1.37–8.93
DPA1*01:03:01	56/82 (68.3)	254/310 (81.9)	9.32e-03	n.s.	0.48	0.27–0.86
A*30:02:01	6/82 (7.3)	6/310 (1.9)	0.02	n.s.	3.98	1.03–15.35
C*07:01:01	9/82 (11)	12/310 (3.9)	0.02	n.s.	3.05	1.09–8.23
DRB1*08:02:01	4/82 (4.9)	41/310 (13.2)	0.03	n.s.	0.34	0.09–0.97
DRB5*02:02	9/82 (11)	14/312 (4.5)	0.04	n.s.	2.6	0.95–6.74
A*01:01:01	10/82 (12.2)	16/310 (5.2)	0.04	n.s.	2.54	0.99–6.26
DRB3*02:02:01	15/82 (18.3)	30/312 (9.8)	0.05	n.s.	2.08	0.98–4.3

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; INDIGO = The Immunogenetics of Neurological DIseases working GrOup, UCSF Multiple Sclerosis Biorepository; NMOSD = neuromyelitis optica spectrum disorders; n.s. = not significant ($p > 1$).
The table lists all HLA alleles with uncorrected *p* values < 0.05.

Table 4 HLA Frequencies in Black AQP4-IgG–Positive Patients With NMOSD and Black INDIGO Healthy Controls (3-Field Resolution)

	Allele frequency, n/total n (%)		Two-sided Fisher exact test			
HLA allele	Black AQP4-IgG+ patients with NMOSD	Black healthy controls (INDIGO)	*p* Value	*p* Value after Bonferroni correction	Odds ratio	95% CI
DQA1*04:01:02	14/88 (15.9)	18/488 (3.7)	6.48e-05	0.06	4.92	2.17–10.98
DRB1*08:04:01	17/88 (19.3)	28/488 (5.7)	9.47e-05	0.08	3.92	1.91–7.86
DQA1*05:01:01	18/88 (20.5)	34/488 (7)	3.34e-04	0.30	3.42	1.72–6.64
DQB1*03:19:01	18/90 (20)	34/488 (7)	3.82e-04	0.34	3.33	1.68–6.44
DQB1*02:01:01	17/90 (18.9)	33/488 (6.8)	6.99e-04	0.62	3.2	1.59–6.28
DRB4*01:03:01	2/90 (2.2)	64/488 (13.1)	1.06e-03	0.94	0.15	0.02–0.59
DRB1*03:01:01	16/88 (18.2)	33/488 (6.8)	1.31e-03	n.s.	3.06	1.49–6.06
B*53:01:01	18/88 (20.5)	41/488 (8.4)	1.72e-03	n.s.	2.8	1.43–5.31
B*08:01:01	10/88 (11.4)	15/488 (3.1)	1.93e-03	n.s.	4.03	1.56–9.99
DPB1*17:01	7/90 (7.8)	7/488 (1.4)	2.41e-03	n.s.	5.77	1.68–19.82
C*04:01:01	28/88 (31.8)	97/488 (19.9)	0.02	n.s.	1.88	1.09–3.17
A*33:03:01	10/88 (11.4)	24/488 (4.9)	0.03	n.s.	2.47	1.01–5.62
DRB1*07:01:01	1/88 (1.1)	35/488 (7.2)	0.03	n.s.	0.15	0–0.91
C*03:04:02	6/88 (6.8)	11/488 (2.3)	0.03	n.s.	3.16	0.93–9.65

Abbreviations: AQP4-IgG = aquaporin-4 immunoglobulin G; n = number; NMOSD = neuromyelitis optica spectrum disorders; n.s. = not significant ($p > 1$); INDIGO = The Immunogenetics of Neurological DIseases working GrOup, UCSF Multiple Sclerosis Biorepository.
The table lists all HLA alleles with uncorrected *p* values < 0.05.

White individuals and that associations with other alleles of the 8.1 ancestral haplotype are due to linkage disequilibrium.

Association of *HLA-DRB1*03:01* and Clinical Findings

We next explored a possible association of immunogenetic findings with clinical variables, focusing on the association of the number of *HLA-DRB1*03:01* alleles with clinical variables in White AQP4-IgG+ patients with NMOSD. There was a nonsignificant tendency toward an increased frequency of ON as first clinical NMOSD manifestation with an increasing number of *HLA-DRB1*03:01* alleles (0 alleles: 41% (n = 75), 1 allele: 41.8% (n = 67), 2 alleles: 54.5% (n = 11); $p = 0.4$ for 0 vs 2 alleles) (Figure 3A). Conversely, there was a nonsignificant tendency toward a decrease in the frequency of LETM as first clinical NMOSD manifestation with an increasing number of *HLA-DRB1*03:01* alleles (0 alleles: 47.1% (n = 70), 1 allele: 38.7% (n = 62), 2 alleles: 22.2% (n = 9), $p = 0.17$ for 0 vs 2 alleles) (Figure 3B). Likewise, brain/brainstem manifestations decreased with an increasing number of *HLA-DRB1*03:01* alleles (0 alleles: 34.3% (n = 70), 1 allele: 31% (n = 58), 2 alleles: 0%, n = 10), with the comparison between 0 and 2 alleles reaching significance ($p = 0.03$) (Figure 3C).

In White AQP4-IgG+ patients, the median age at clinical disease onset tended to increase with the number of *HLA-DRB1*03:01* alleles (0 alleles: 40.1 years (n = 82); 1 allele: 46 years (n = 79); 2 alleles: 57.3 years (n = 11)) without reaching significance ($p = 0.12$ for 0 vs 2 alleles) (Figure 3D).

Neither did we observe an association of the number of *HLA-DRB1*03:01* alleles with the annualized relapse rate nor with mobility in White AQP4-IgG+ patients (data not shown).

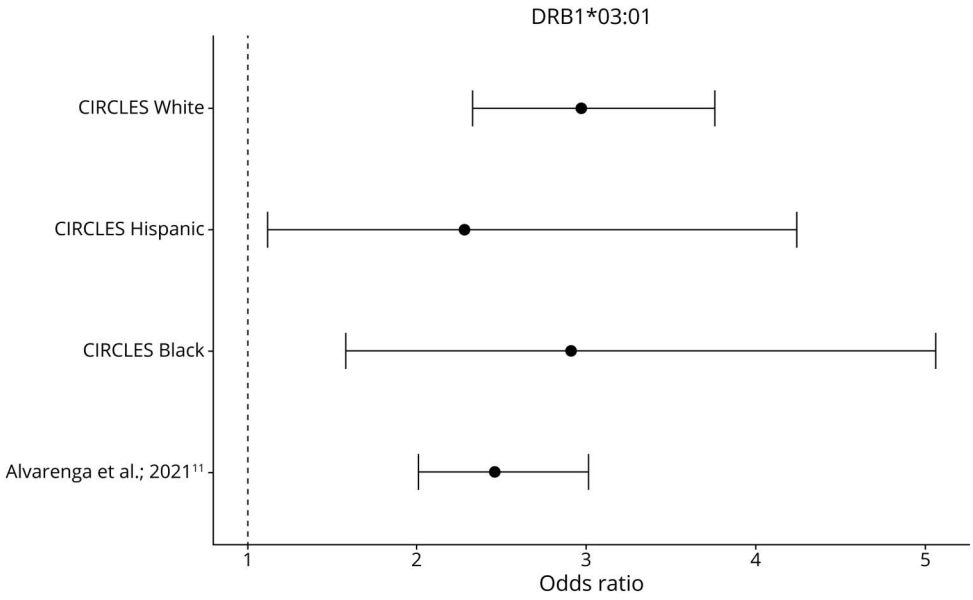
Owing to the small sample size of Black AQP4-IgG+ NMOSD patients with 1 (n = 18) and 2 (n = 1) *HLA-DRB1*08:04* alleles, we did not analyze the association of the number of *HLA-DRB1*08:04* alleles with clinical findings in Black AQP4-IgG+ patients with NMOSD.

Discussion

The key results of this large HLA association study in NMOSD are as follows: (1) the *HLA-DRB1*03:01:01*~*HLA-DQA1*05:01:01*~*HLA-DQB1*02:01:01* haplotype is a genetic risk factor of AQP4-IgG+ NMOSD in White patients; (2) *HLA-DRB1*08:04* is a genetic risk factor of AQP4-IgG+ NMOSD in Black patients; and (3) no association of any HLA allele in White AQP4-IgG− patients with NMOSD was identified.

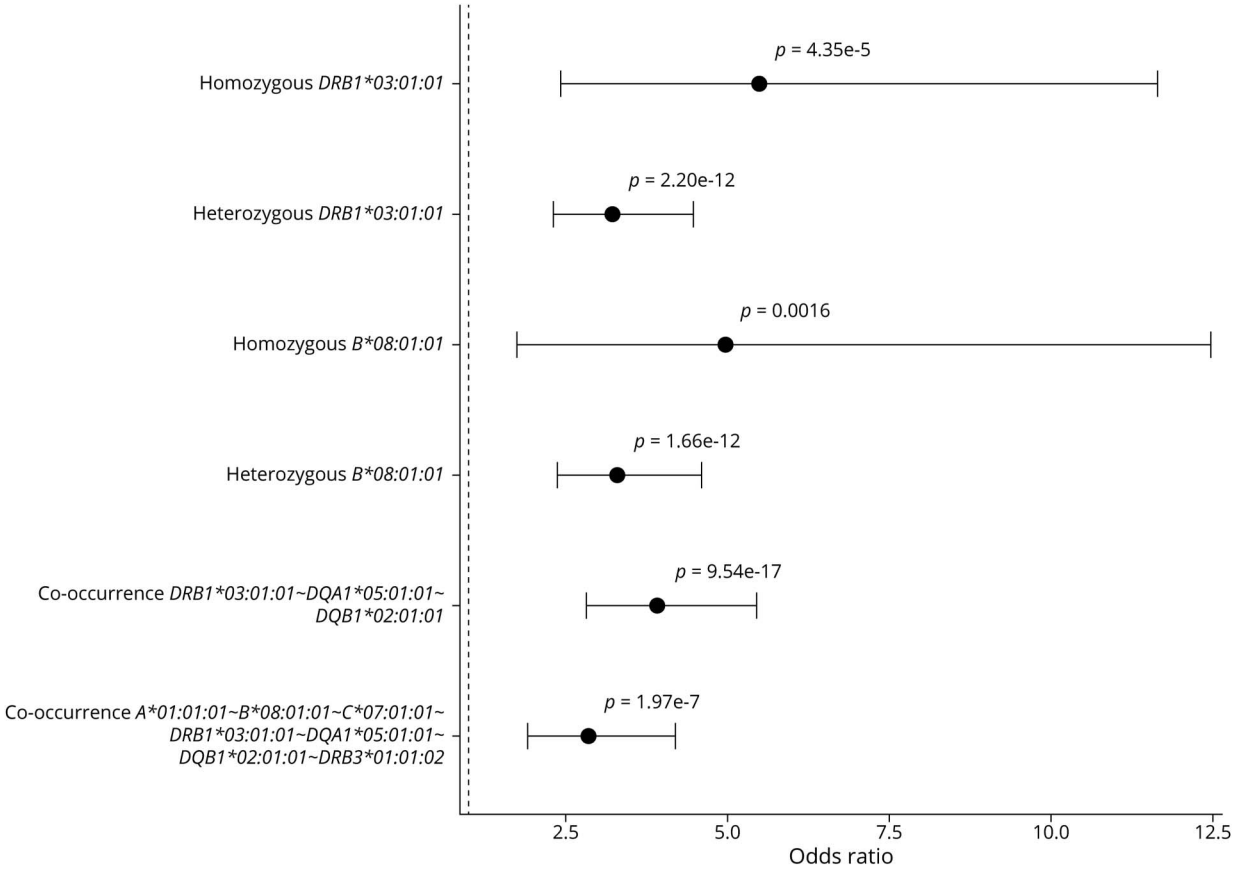
Consistent with these findings, *HLA-DRB1*03:01* has previously been broadly recognized across multiple ethnicities as

Figure 1 Odds Ratios for HLA-DRB1*03:01 in the Different Populations Analyzed in This Study



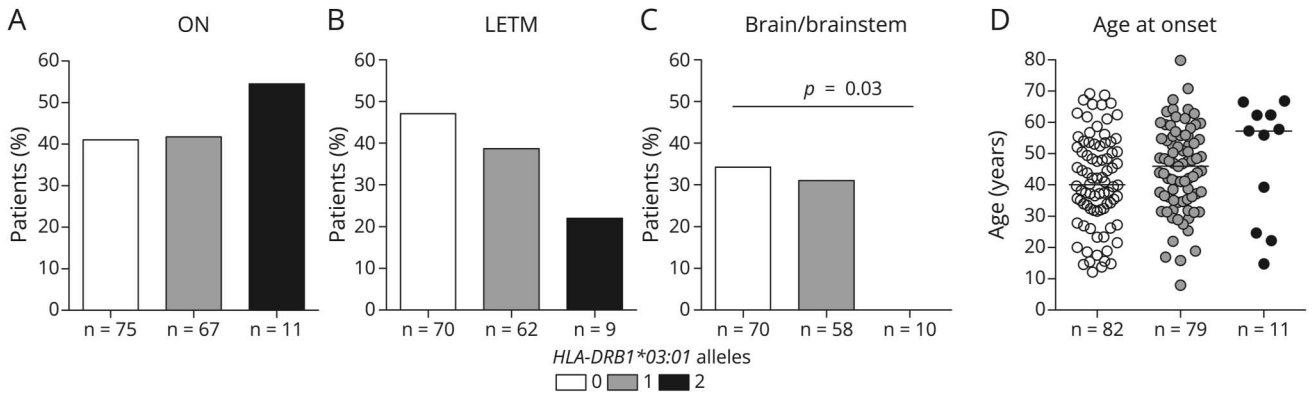
Forest plots showing odds ratios and 95% CIs for *HLA-DRB1*03:01* among White, Hispanic, and Black AQP4-IgG+ patients with NMOSD from the Collaborative International Research in Clinical and Longitudinal Experience Study (CIRCLES) compared with the respective NMDP healthy control cohorts (eTables 1, 3, and 4). For comparison, the odds ratios and 95% CIs for *HLA-DRB1*03* calculated in a meta-analysis of 13 previous HLA/NMOSD association studies by Alvarenga et al.[11] are shown as well. AQP4-IgG = aquaporin-4 immunoglobulin G; NMOSD = neuromyelitis optica spectrum disorders.

Figure 2 Genotype Associations for HLA-DRB1*03:01:01, HLA-B*08:01:01, and Unphased Haplotype Co-Occurrences



Forest plots showing odds ratios with 95% CIs for homozygous and heterozygous genotypes of *HLA-DRB1*03:01:01*, *HLA-B*08:01:01*, the *HLA-DRB1*03~DQA1*05~DQB1*02* core haplotype, and the extended ancestral haplotype 8.1 in White AQP4-IgG+ patients vs White healthy controls from the INDIGO cohort. The respective *p* values are shown and were calculated using the Fisher exact tests. AQP4-IgG = aquaporin-4 immunoglobulin G.

Figure 3 Association of HLA-DRB1*03:01 and Clinical Findings



Bar charts showing the percentage of (A) optic neuritis (ON); (B) longitudinally extensive transverse myelitis (LETM); and (C) brain/brainstem manifestations during first attack among White AQP4-IgG+ patients with NMOSD with 0, 1, or 2 *HLA-DRB1*03:01* alleles. (D) Dot plot showing the age (years) at first clinical manifestation among White AQP4-IgG+ patients with NMOSD with 0, 1, or 2 *HLA-DRB1*03:01* alleles. The number of patients in each group is indicated below the x-axis. The horizontal lines in (D) represent the median. AQP4-IgG = astrocytic water channel aquaporin-4 immunoglobulin G; NMOSD = neuromyelitis optica spectrum disorders.

a primary risk allele for NMOSD.[11,21,22] The OR for *HLA-DRB1*03:01:01* in White AQP4-IgG+ patients with NMOSD (3.15, 95% CI 2.43–4.06) observed herein was somewhat higher than that calculated in a meta-analysis of 13 HLA/NMOSD association studies (2.46, 95% CI 2.01–3.01, Figure 1), which may be due to heterogeneity of the studies from different regions of the world, involving patients with AQP4-IgG+ and AQP4-IgG– NMOSD, included in the meta-analysis.[11]

The NGS-based typing methodology applied in this study allowed to extend and refine the previously described association with *HLA-DRB1*03:01* by identifying the *HLA-DRB1*03:01:01~DQA1*05:01:01~DQB1*02:01:01* haplotype as key genetic risk factor of AQP4-IgG+ NMOSD in White individuals. Indeed, although different alleles of the ancestral haplotype 8.1 (*HLA-A*01:01:01*, *HLA-B*08:01:01*, *HLA-C*07:01:01*, *HLA-DRB1*03:01:01*, *HLA-DQA1*05:01:01*, *HLA-DQB1*02:01:01*, and *HLA-DRB3*01:01:02*) were associated with AQP4+ NMOSD in White patients, the *HLA-DRB1*03:01:01~DQA1*05:01:01~DQB1*02:01:01* core haplotype showed stronger effect sizes than the other associated alleles (Table 2). Additional analyses of *HLA-DRB1*03:01:01* and *HLA-B*08:01:01* homozygosity vs heterozygosity and co-occurrences of alleles (Figure 2 and eTable 12) further support the core *HLA-DRB1*03:01:01~DQA1*05:01:01~DQB1*02:01:01* haplotype as the primary risk factor of AQP4-IgG+ NMOSD in White individuals. By contrast, the observed associations with *HLA-A*01:01:01*, *HLA-B*08:01:01*, *HLA-C*07:01:01*, and *HLA-DRB3*01:01:02* might be due to linkage disequilibrium. Previously reported associations of *HLA-B*08* with AQP4-IgG+ NMOSD in White individuals[11,21,22] might, therefore, be secondary to the association with the *HLA-DRB1*03:01:01~DQA1*05:01:01~DQB1*02:01:01* haplotype. This conclusion is further supported by a recent study identifying an association between *HLA-DRB1*03:01~HLA-DQB1*02:01* and AQP4-IgG+ NMOSD in a Korean population,[23] where *HLA-DRB1*03:01~DQB1*02:01* is not linked to the class I alleles *HLA-B*08:01:01* and *HLA-C*07:01:01*.[24]

The 8.1 ancestral haplotype is a long (>4 megabases) and conserved HLA allele domain, comprising *HLA-A*01:01*, *HLA-C*07:01*, *HLA-B*08:01*, tumor necrosis factor alpha 2 beta 3 and NcoI short allele, *C2*C*, *Bf*s*, *C4A*Q0*, *C4B*1*, *HLA-DRB1*03:01*, *HLA-DRB3*01:01*, *HLA-DQA1*05:01*, and *HLA-DQB1*02:01*.[19] Notably, this extended haplotype also harbors the genes for complement component 4 (C4) within the RCCX (RP-C4-CYP21-TNX) module that lies between the HLA class I and class II regions. Because the RCCX segment can be variably duplicated, individuals may carry 1–3 copies per haplotype of each paralog (C4A and C4B), yielding variable total (diploid) copy numbers.[25] The C4A gene in the 8.1 ancestral haplotype is a null allele, which does not code for a functional C is 4A protein.[26] Of interest, a lower number of C4 gene copies are associated with a higher risk of AQP4-IgG+ NMOSD,[27] and AQP4-IgG+ patients with NMOSD have lower plasma complement C4 levels than healthy controls.[28] Because no data on complement C4 gene copies were available in this study, we could not analyze the association of copy numbers of C4 genes with AQP4-IgG+ NMOSD. Future studies should disentangle the individual contributions of HLA alleles as well as C4A and C4B copy numbers on the risk for AQP4-IgG+ NMOSD.

In addition to the *HLA-DRB1*03:01:01~DQA1*05:01:01~DPB1*02:01:01* haplotype, stratification by *HLA-DRB1*03:01:01* carrier status as well as the RPE analysis revealed *HLA-DRB1*14:54:01~DQB1*05:03:01* as a potential secondary risk haplotype for AQP4-IgG+ NMOSD in White individuals (eTable 7, eTable 9). This haplotype is common in individuals of European and Asian ancestry and has previously been

associated with achalasia, pemphigus vulgaris, and thrombotic thrombocytopenic purpura.[20,29,30]

Although with a lower significance than that of the identified risk alleles, this study identified potentially protective HLA alleles (e.g., *HLA-DQB1*02:02:01*, *HLA-DQB1*06:03:01*, and *HLA-DQA1*01:03:01*) for AQP4-IgG+ NMOSD in White individuals (Table 2, eTable 9). Independent studies will be necessary to confirm these observations.

We found an association of *HLA-DRB1*08:04* with AQP4-IgG+ NMOSD in Black patients. Notably, *HLA-DRB1*08:04* is part of one of the most common haplotypes in Black American individuals.[14,31,32] This haplotype further comprises *HLA-A*33:03*, *HLA-B*53:01*, and *HLA-C*04:01*, all of which were more frequent in Black AQP4-IgG+ patients with NMOSD compared with Black INDIGO and NMDP controls, although not reaching statistical significance after correction for multiple testing (Table 4 and eTable 4).

Although NMOSD is known to be more prevalent[33] and NMOSD attacks might be more severe[34] in Black patients than in White patients with NMOSD, Black patients were underrepresented in previous HLA association studies in NMOSD.[11] Whether and how the association of *HLA-DRB1*08:04* with AQP4-IgG+ NMOSD in Black patients may contribute to differences in the epidemiology and clinical presentation of NMOSD remains to be clarified.[35] An increased frequency of *HLA-DQA1*04:01:02* in Black AQP4-IgG+ patients with NMOSD barely missed significance and re-analysis of this association in larger cohorts, which was not possible in this study because of lack of data on *HLA-DQA1* in the NMDP controls, might be warranted. However, the *HLA-DQA1*04:01* allele is found in 2 distinct common African haplotypes[36] bearing *HLA-DRB1*08:04* and *HLA-DRB1*03:02*. The absence of an association of *HLA-DRB1*03:02* with AQP4-IgG+ NMOSD in Black patients in this study points to *HLA-DRB1*08:04* as the main susceptibility factor, while the association of *HLA-DQA1*04:01* may result from a hitchhiking effect.

Of interest, an RPE analysis revealed increased frequencies of *HLA-DQA1*05:01:01* and *HLA-DQB1*02:01:01* in Black AQP4-IgG+ patients with NMOSD (eTable 11), suggesting that the *HLA-DRB1*03:01∼DQA1*05:01∼DQB1*02:01* haplotype could potentially represent a secondary, independent risk factor for NMOSD in Black individuals.

Likely owing to the limited sample size, we did not identify significant HLA associations in Hispanic AQP4-IgG+ patients with NMOSD after correction for multiple testing (Table 3, eTable 3). However, *HLA-DQA1*05:01:01*, *HLA-DQB1*02:01:01*, and *HLA-DRB1*03:01:01* were among the HLA alleles with uncorrected p values < 0.05 in Hispanic patients, suggesting that this haplotype could possibly be of relevance in this population, too. Analysis of HLA-DRB1*03:01:01 noncarriers revealed the *HLA-A*30:02:01* allele as a potential independent risk factor of NMOSD in Hispanic populations (eTable 8). *HLA-A*30:02:01* is part of the *HLA-A*30:02∼B18:01∼C*05:01* haplotype, which is common in individuals of southern European ancestry and has been associated with susceptibility to myasthenia gravis in an Italian population.[37] Independent studies are needed to clarify whether this haplotype is an independent risk factor of NMOSD in Hispanic individuals.

The lack of any HLA associations in White AQP4-IgG– patients with NMOSD (eTable5, eTable 6) is compatible with the notion that AQP4-IgG– NMOSD represents one or more distinct disease entities whose pathophysiology is different from AQP4-IgG+ NMOSD. Among AQP4-IgG– patients, approximately 40% have IgG against myelin oligodendrocyte glycoprotein (MOG-IgG), a defining feature of MOG antibody–associated disease (MOGAD).[3] AQP4-IgG– patients with NMOSD included in this work were not systematically tested for MOG-IgG. However, 2 studies from the Netherlands and the United Kingdom found no convincing HLA associations with MOGAD, suggesting that AQP4-IgG+ NMOSD and MOGAD are immunogenetically distinct diseases.[21,38] At disease onset, 72.3% of White AQP4-IgG– patients with NMOSD in our cohort presented with ON, a predominant clinical feature of MOGAD,[39] which is compatible with a subset of AQP4-IgG– patients with NMOSD representing undiagnosed MOGAD.

We observed tendencies for an increased age at onset, a higher frequency of ON as first clinical attack, and lower frequencies of LETM and brain/brainstem manifestations as first clinical attack with an increasing number of *HLA-DRB1*03:01* alleles in White AQP4-IgG+ patients with NMOSD (Figure 3). However, owing to the low number of homozygous individuals, these results should be regarded with caution and even larger patient numbers may be required to further address associations of HLA alleles with clinical findings.

Of interest, this study showed differential associations of *HLA-DRB1*03* as well as *HLA-DRB1*08* alleles with AQP4-IgG+ NMOSD. The distinctive amino acid residues present in such susceptibility and nonsusceptibility alleles could thus lead to the identification of critical amino acid residues leading to disease susceptibility. For example, distinct amino acid residues encoded by these alleles could hypothetically influence epitope presentation and underlie autoreactive T-cell polarization in AQP4-IgG+ NMOSD.

One limitation of this study is that despite the overall large number of patients, the lower number of patients in some of the subgroups may have resulted in insufficient power to detect HLA associations. Another limitation is that no genome-wide association study data were available from the patients analyzed in this work. Samples could, therefore, not be controlled for ancestry differences by principal component analysis, and categorization of ethnicity relied on self-identification. Furthermore, although all main comparisons

(Tables 2–4) were conducted in patients and healthy controls from the United States, patients and healthy controls were not from the same study. Thus, a remaining risk of population stratification issues cannot be completely excluded. In addition, no HLA genotyping data from Asian participants in the CIRCLES were available, which precluded analysis of HLA associations in this population. Finally, potential HLA associations in patients with MOGAD with an NMOSD phenotype were not evaluated in this study.

In summary, this study demonstrates an association of the *HLA-DRB1*03:01:01∼DQA1*05:01:01∼DQB1*02:01:01* haplotype with AQP4-IgG+ NMOSD in White patients as well as an association of *HLA-DRB1*08:04* with AQP4-IgG+ NMOSD in Black patients, contributing to the clarification of the immunogenetic background of NMOSD. The absence of HLA associations in AQP4-IgG− NMOSD suggests both genetic and pathophysiologic differences between AQP4-IgG+ and AQP4-IgG− NMOSD.

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Author Contributions

M. Niederschweiberer: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. G. Panagiotaropoulou: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. M.A. Fernandez-Viña: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. G. Montero-Martin: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. K. Osoegawa: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. M. Behne: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. A. Franke: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. W. Lieb: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. S. Jarius: drafting/revision of the manuscript for content, including medical writing for content. B. Wildemann: drafting/revision of the manuscript for content, including medical writing for content. P. Schindler: drafting/revision of the manuscript for content, including medical writing for content. C. Otto: drafting/revision of the manuscript for content, including medical writing for content. F. Paul: drafting/revision of the manuscript for content, including medical writing for content. E.P. Flanagan: drafting/revision of the manuscript for content, including medical writing for content. L.J. Cook: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data. J. Oksenberg: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data. M.R. Yeaman: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data. S. Ripke: drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data. K. Ruprecht: drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data.

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