No association between identified multiple sclerosis non-MHC risk loci and neuromyelitis optica

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ABSTRACT

Neuromyelitis optica (NMO) and multiple sclerosis (MS) are both autoimmune inflammatory and demyelinating disorders of the central nervous system. Recently, more than 50 MS-susceptibility single-nucleotide polymorphisms (SNPs) have been detected outside the major histocompatibility complex (MHC) region. In this study, we aimed to evaluate the association of these identified non-MHC MS risk loci with Chinese patients with NMO. Thirty-five non-MHC SNPs were selected and genotyped by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in 110 NMO patients and 332 controls from southeastern China. Among the 35 SNPs, only one, rs1800693 in the *TNFRSF1A* locus, was nominally associated with NMO ($P = 0.045$, OR = 1.550, 95% CI = 1.007 – 2.384). However, none of the 35 SNPs was associated with NMO after Bonferroni correction. Our results showed no association between these identified non-MHC MS risk loci and NMO, suggesting there are genetic differences in the etiology of NMO and MS.

Keywords: neuromyelitis optica; major histocompatibility complex; association; Chinese

INTRODUCTION

Neuromyelitis optica (NMO) and multiple sclerosis (MS) are

both autoimmune inflammatory demyelinating disorders of the central nervous system. Although NMO was once considered to be a variant of MS, it is now deemed distinct from MS because of the detection of NMO immunoglobulin G (NMO-IgG), an autoantibody against aquaporin 4 (AQP4)^[1]. The prevalence of NMO is ~1 per 100,000^[2, 3]. Interestingly, NMO is relatively common in Asian populations but rare in Caucasians^[4].

The genetic component of MS is complex and has been studied for decades. For over 30 years, the major histocompatibility complex (MHC) was the only known MS susceptibility locus. However, recent genome-wide association studies have revealed that >50 single nucleotide polymorphisms (SNPs) outside the MHC region are closely associated with susceptibility to MS^[5–12].

Although the genetic susceptibility of NMO remains largely unknown because of the limited number of cases, reports of familial aggregation have provided strong evidence that genetic factors influence the susceptibility to NMO seemingly similar to MS^[13, 14]. However, to date, whether the MS non-MHC risk loci are also associated with NMO has rarely been investigated. In this study, we recruited 110 NMO cases and evaluated whether these non-MHC MS risk SNPs were also associated with Chinese NMO patients.

METHODS

Participants

A total of 110 unrelated NMO patients (21 males, 89

females; mean age 43.75 ± 14.47 years; range 13–73) were included in the study. All patients underwent detailed neurological examinations, laboratory tests, and MRI scans of the brain and/or spinal cord. Patients were diagnosed according to the revised diagnostic criteria for NMO^[15]. In addition, 332 consenting volunteers (191 males, 141 females; mean age 37.60 ± 15.69 years; range 16–70) with no history of autoimmune diseases were recruited as controls matched for case ethnicity and region. All the participants were of Han Chinese descent from southeastern China. They were recruited between 25 October 2007, and 10 March 2012. Most of the NMO patients and controls originated from a previous association study^[16]. Written informed consent was given by participants >18 years of age and guardians on the behalf of those <18 years old prior to inclusion. This study was approved by the Ethics Committee of Huashan Hospital. Anti-AQP4 antibodies were detected with an indirect immunofluorescence assay using HEK293 cells transfected with the recombinant human AQP4 gene (Euroimmun, Lubeck, Germany)^[17]. Each sample was measured at least twice, with the examiners blind to the origin of the specimens. Samples with a positive result twice were considered to be positive for anti-AQP4 antibody.

Genotyping

Genomic DNA was extracted from peripheral EDTA blood using a TIANamp Blood DNA kit (Tiangen Biotech, Beijing). To maximize the statistical power, SNPs with a minor allele frequency <0.10 in Han Chinese in Beijing from the HapMap databases were excluded because of the limited sample size available. Therefore, only 35 non-MHC MS-susceptibility SNPs were selected and genotyped using the Sequenom MassArray system at the Fudan-Van Andel Research Institute Center (School of Life Sciences, Fudan University, China). We used MassArray Assay Design 3.1 software (Sequenom Inc., San Diego, CA) to design the PCR primers used in the genotyping. The PCR and extension primers for these 35 SNPs are shown in Table 1. The resulting products were desalted and transferred to a 384-element SpectroCHIP array (Sequenom). Alleles were detected using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) platform

(MassArray TM, Sequenom) according to a previously described method^[18].

Statistical Analyses

Hardy-Weinberg equilibrium was tested using the χ^2 test. Differences in allele frequencies between cases and controls, odds ratios (ORs), and 95% confidence intervals (95% CIs) were assessed with the χ^2 test or Fisher's exact test. Bonferroni correction was used for multiple comparisons. All statistical analyses were analyzed using SPSS 16.0 software. The criterion for a significant difference was $P < 0.05$.

RESULTS

Overall, 4 NMO patients and 28 controls who had a SNP genotyping success rate <90% were excluded, so a total of 106 NMO patients and 304 controls were analyzed. Their demographic and clinical characteristics are listed in Table 2. Anti-AQP4 antibodies were tested in 77 of 106 NMO patients and 44 (57.14%) were positive, which is lower than that in the previous study^[1]. In addition, complete cerebrospinal fluid analysis at disease onset was available for 67 NMO patients and the oligoclonal-band-positive rate was 10/67 (14.93%).

The average genotyping success rate across the SNPs was 99.5%. All SNPs were in Hardy-Weinberg equilibrium (Table 1). The association testing of 35 SNPs with NMO is shown in Table 3. Among these, only one SNP, rs1800693 in the *TNFRSF1A* locus, was nominally associated with NMO ($P = 0.045$, OR = 1.550, 95% CI = 1.007–2.384). In addition, none of these SNPs was associated with NMO after Bonferroni correction. In further analysis, based on the anti-AQP4 antibody status, there was no significant difference in the allele frequencies in the 35 selected SNPs between the anti-AQP4-positive and negative NMO patients (Table 4).

DISCUSSION

In clinical practice, it is difficult to differentiate NMO from MS due to an overlap of manifestations, such as optic neuritis, spinal syndrome, and female predilection. Since the discovery of NMO-IgG, the discrimination of NMO from

Table 1. List of 35 SNPs analyzed with performance values and primer sequences

Gene	SNP	Missing rate %	NMO HWE <i>p</i> -value	Controls HWE <i>p</i> -value	PCR primers	MassEXTEND primers
EOMES	rs11129295	0.00	0.8125	0.7751	ACGTTGGATGGTGACGTGGCCAGTTTTCTA ACGTTGGATGGCTCATTTAATCTTCACAAC	CCTCGGCCAGTTTTCTAACTTCT
MERTK	rs17174870	0.24	1.0000	1.0000	ACGTTGGATGATATGCCCCACTCCATCCAC ACGTTGGATGCACATATGACCTCTTCCTGC	CCACCCCGGAAAAAGCTTA
BATF	rs2300603	0.24	1.0000	0.4601	ACGTTGGATGTTCTCTCTAAGCAGCCATCC ACGTTGGATGACATAGACTGATGCCGAGAG	CCTCTTCAGTATGAGGCTTTCATTC
No gene	rs669607	1.71	0.3414	1.0000	ACGTTGGATGTCAAAGCTGTTTGGGTGGG ACGTTGGATGTCAGTCCTGATCTTCCCAAC	AGAGCATAATAAAGGAGGAAGAT
HHEX	rs7923837	0.00	0.5548	0.0655	ACGTTGGATGTAGGCAAGAACTTTGTGGC ACGTTGGATGTTGCACGTTGTCAGTTCAGC	TTTGTGGCACTGGTT
TAGAP	rs1738074	0.49	0.4528	0.7166	ACGTTGGATGTCCCAGTGGACTAGAAGGAG ACGTTGGATGCTTTTACATCCGGTGAGCTG	TTTCATAGAAGGAGCAGAGAGTT
GALC	rs2119704	0.73	0.7354	1.0000	ACGTTGGATGGCAGAAGCTTCTGAGACCAC ACGTTGGATGAAGGGAGTAACTGGAGGG	CCCAAGTCAGTATAATTGGTGATCT
NFKB1	rs228614	0.24	0.4553	0.8176	ACGTTGGATGAGTCAGGCTTAAGCAACCAC ACGTTGGATGTGCTTTTACTGTGTTCTTC	GTCCCATTCAGTGCTTTC
MYC	rs4410871	0.00	0.6809	0.1990	ACGTTGGATGGCAGTTACATCTGCAGTGTG ACGTTGGATGTCTGCCGTGAATGAGAAACC	CCTCCCACACTGGAA
MAPK1	rs2283792	0.73	0.5800	0.1514	ACGTTGGATGGGGATCTCAGGTGTTTAAGG ACGTTGGATGTTTCCAGAAGCTGTTGAGGG	CACACTATCAGTAACTACCGT
MYB	rs11154801	1.71	0.6731	0.3709	ACGTTGGATGCTCCTTCAGAAGGTCGAAAC ACGTTGGATGAGCTGTCATGTACCATGCAC	CCTTAAGAAGGTCGAAACCTCAAGT
No gene	rs12466022	1.22	1.0000	0.4165	ACGTTGGATGCCCTTGCTAGAAAGTACC ACGTTGGATGGCTTCTTTATCACCTGACAC	ATATAAGTACCTTGACAAAAC
VCAM1	rs11581062	0.73	1.0000	0.6526	ACGTTGGATGTACGTCGCAGTCAGTTTTT ACGTTGGATGTGTTTCAAAGCCAACCCTCC	TTTCTAAAGAGCCCGAA
ZFP36L1	rs4902647	0.24	0.2542	0.2395	ACGTTGGATGGCTCCTTTGCAGAAAACCTC ACGTTGGATGTAAGCCTATAGCTCCCTTCC	CACCCGTCCCCTCTAAG
ZNF746	rs354033	0.00	0.1585	1.0000	ACGTTGGATGTGGGTGACTGGGTTTCATTG ACGTTGGATGTTCCAGACCCCTCTTACTC	CATTGTGTATGGAGGCTT
IL12B	rs2546890	1.71	0.7083	0.9050	ACGTTGGATGGGCTCAGCAATAGACAAGTG ACGTTGGATGGAATCAATGTGAGGAGCCTG	CGCAATAGACAAGTGATTTCACTG
TNFSF14	rs1077667	0.49	0.3300	0.2977	ACGTTGGATGCAGACAACGTGAATACACCG ACGTTGGATGTCCATATACCCATGTGGACG	AGAGTGTGTGTGGACATGTGGGTACA
IL22RA2	rs17066096	1.95	1.0000	0.3909	ACGTTGGATGTTTCAGCTGAAGGGTGAAGG ACGTTGGATGGAGTGAAACTGATTCCAGGC	CCCGGCCTTCTGCTTAAAC

(To be continued)

(Continued)

Table 1. List of 35 SNPs analyzed with performance values and primer sequences

Gene	SNP	Missing rate %	NMO HWE <i>p</i> -value	Controls HWE <i>p</i> -value	PCR primers	MassEXTEND primers
MALT1	rs7238078	0.24	1.0000	0.5774	ACGTTGGATGATCTGTTACCAATCTCTCC ACGTTGGATGGGCAAGGTGAAAACAAGAG	CTTCACCAATCTCTCCTTCTTTTT
MMEL1	rs4648356	0.24	0.1961	0.6418	ACGTTGGATGATTGGCTGGGTTAACTCC ACGTTGGATGGAAGAAGCAGGCACTGGAAG	GATTGCTTTTTGGTGGACAGAG
CD58	rs1335532	0.00	0.3397	0.4794	ACGTTGGATGGGCATTTTTGCTCCCAAGTG ACGTTGGATGAGAGAAGTGAGAGGGACAAG	GGGTACCAAGTGAGCAGATGG
RGS1	rs2760524	0.24	1.0000	1.0000	ACGTTGGATGCAACACTTTCAGCAACTGGG ACGTTGGATGATAGTGATTGCTCTGCTGTG	CTGGGGAATGAATGCTA
CBLB	rs2028597	0.24	0.0891	0.4048	ACGTTGGATGCTTTTTTCCAGAGATTTCAG ACGTTGGATGCCATGCAACCTATTTTAATC	TAAGATTTCATCCTGGT
TMEM39A	rs2293370	0.00	0.0541	1.0000	ACGTTGGATGGAACAGGCATGTTTGGCATC ACGTTGGATGGAGCCTTGACGATTTAGCAG	CCCCGTTTGGCATCACCCT
IL12A	rs2243123	0.00	1.0000	0.3781	ACGTTGGATGGGTGAATCCAGTGTAAGCAG ACGTTGGATGAGTCTTCTCATGCTGCTCC	GAGGAGCGGGTAGAAGGTC
IL7R	rs6897932	0.00	0.4489	0.8339	ACGTTGGATGCAGAGCGACAGAGAAAAAAC ACGTTGGATGACTGAATGCTCACCACAATC	CAAAAACTCAAAATGCTGATG
IL7	rs1520333	0.24	0.8526	0.5641	ACGTTGGATGAGAGGTGGTATGGGTGTATC ACGTTGGATGTGGGCAAGCAGGTAAGAAAG	CAGCCCACTGGAACCAAAG
IL2RA	rs3118470	0.98	1.0000	0.2763	ACGTTGGATGCTGTGTTTTGGCTCATTGGG ACGTTGGATGGGATGACATGTAAAGGGAGC	TATCTCCCTGGAATCTCA
ZMIZ1	rs1250550	0.00	1.0000	0.0630	ACGTTGGATGAATGATTCCCCAGCCTGAG ACGTTGGATGATGGGAATGATTGGTGTGCG	TCTCTCTCCCATTCTG
TNFRSF1A	rs1800693	0.00	0.7530	1.0000	ACGTTGGATGAAGAAGAGGGAGAGGGCAG ACGTTGGATGGAATGTTAAGGGCACTGAGG	GGATCATGGGCACCAGGTCAC
MPHOSPH9	rs949143	0.49	0.5548	0.8047	ACGTTGGATGTTGCTTCTCCTGAATCGTCTG ACGTTGGATGACCAAGAGGATTGAACAGGG	CCCTTTGTAAATGGAGACA
CLEC16A	rs12708716	0.00	0.5548	0.7489	ACGTTGGATGCACACTTCATCCTCACTGAC ACGTTGGATGGTCTTCAGCTAGTCTCTGG	GTGAAGCGGCTATTACT
IRF8	rs13333054	0.73	0.7095	0.4838	ACGTTGGATGGCTATAACAGCTTGACACAG ACGTTGGATGCATACAAAAGTGAGAAGTGG	ATGCCCAATTAAATTAAGGTAG
STAT3	rs744166	0.73	0.3416	0.1384	ACGTTGGATGTGGCTGTAATGTCTTGAGGG ACGTTGGATGACATTGAGAGGGCAATTGGG	GGGCCTTGAGGGAATCGAGC
TYK2	rs8112449	0.00	0.4614	0.3562	ACGTTGGATGCATGTCTCTGCCTCTCTCG ACGTTGGATGTGTTGCTCAAAGTCTCAAGG	GACCCCTCCAACATC

NMO, neuromyelitis optica; HWE, Hardy-Weinberg equilibrium; SNP, single-nucleotide polymorphism.

Table 2. Demographic and clinical characteristics of patients and controls

	NMO (<i>n</i> =106)	Controls (<i>n</i> =304)
Male/female	20/86	175/129
Age at analysis (years)	43.71 ± 14.26	37.92 ± 15.21
Age at onset (years)	36.70 ± 14.02	NA
Disease duration ^a (years)	7.00 ± 7.33	NA
Relapsing-remitting course, <i>n</i> (%)	96 (90.57%)	NA
NMO-IgG positive/total, <i>n</i> (%)	44/77 (57.14%)	NA
OCB positive/total, <i>n</i> (%)	10/67 (14.93%)	NA

NMO, neuromyelitis optica; NA, not available; OCB, oligoclonal bands. ^aDisease duration (from age at onset to age at analysis).

MS has become more accurate, but there is no single diagnostic criterion for NMO or MS. It is necessary to demonstrate genetic differences between NMO and MS.

Among the genes that have been implicated in MS susceptibility, the greatest individual impact is from the human leukocyte antigen (HLA) locus. The *HLA DRB1*1501* allele is consistently associated with MS in most Europeans, African Americans, and Japanese^[19-21]. In contrast, early studies of Japanese populations suggested some association of optic-spinal MS susceptibility with the *DPB1*0501* allele, which was different from that in MS^[22]. After the discovery of anti-AQP4 antibody, the *DPB1*0501* allele was also reported to be associated with NMO in Japanese and southern Han Chinese, while the *DRB1*03* allele was reported to be a risk factor for NMO in Caucasians, suggesting differences in genetic background^[23-25]. Furthermore, the *DPB1*0501* allele is a risk factor for anti-AQP4-positive NMO, but not for anti-AQP-negative NMO^[26]. However, to date, no specific genetic factors have been found for NMO negative for anti-AQP4 antibody. Taken together, these results suggest that NMO, whether positive or negative for anti-AQP4 antibody, may have an HLA profile different from MS.

Few comprehensive analyses of the association between non-MHC loci and NMO have been reported. A genome-wide association study involving 53 Korean NMO patients and 240 controls found that a promoter SNP in *CYP7A1* that encodes cytochrome P450 has a protective

role in the risk of NMO^[27], which was replicated in our previous study^[16]. Also, the allele of programmed death-1 was found to be associated with NMO^[28]. However, a genetic analysis of *AQP-4* in NMO indicated no association of *AQP-4* variation with susceptibility to NMO^[29]. No genetic variant of *OPAI*, the major causative gene for autosomal dominant optic atrophy, has been associated with NMO^[30]. Thus, the genetics of NMO is still largely unknown and more studies are required to reveal it.

To date, little is known about the genetic differences between NMO and MS apart from the HLA locus. In the current study, we first evaluated the association of 35 non-MHC MS-susceptibility loci with Chinese NMO patients and found that none of them was associated with NMO. Moreover, there was no difference in the allele frequencies of the 35 selected SNPs between the anti-AQP4 positive and negative NMO patients, which indicated that these non-MHC loci are not associated with anti-AQP4 antibody status. Therefore, it is conceivable that NMO differs from MS regarding genetic susceptibility, irrespective of MHC or non-MHC loci, and the development of AQP4 autoimmunity is attributable to a certain genetic background and is mainly mediated by MHC loci.

In summary, our findings provide further evidence for genetic differences between NMO and MS, suggesting two distinct disease entities. However, these data are preliminary and need to be further replicated in a larger cohort.

Table 3. Association of 35 SNPs between NMO patients and controls

Gene	SNP	Risk Allele ^a	NMO RAF %	Control RAF %	NMO vs Controls	
					<i>P</i>	OR (95% CI)
EOMES	rs11129295	A	75.9	71.9	0.251	1.235(0.861-1.773)
MERTK	rs17174870	G	92.9	90.1	0.220	1.443(0.801-2.600)
BATF	rs2300603	A	70.3	73.3	0.402	0.863(0.611-1.219)
No gene	rs669607	C	40.1	36.9	0.412	1.143(0.830-1.576)
HHEX	rs7923837	G	19.0	22.4	0.285	0.807(0.545-1.196)
TAGAP	rs1738074	G	44.4	40.1	0.277	1.192(0.869-1.635)
GALC	rs2119704	C	84.3	80.3	0.201	1.316(0.863-2.007)
NFKB1	rs228614	G	50.9	48.2	0.490	1.116(0.817-1.526)
MYC	rs4410871	G	65.5	66.3	0.849	0.969(0.697-1.347)
MAPK1	rs2283792	C	46.2	41.1	0.190	1.234(0.901-1.691)
MYB	rs11154801	A	33.0	34.8	0.615	0.918(0.658-1.281)
No gene	rs12466022	C	77.0	76.8	0.924	1.018(0.701-1.479)
VCAM1	rs11581062	G	18.6	15.3	0.274	1.258(0.833-1.900)
ZFP36L1	rs4902647	G	28.3	32.9	0.216	0.805(0.571-1.135)
ZNF746	rs354033	G	87.3	89.0	0.499	0.849(0.527-1.367)
IL12B	rs2546890	A	46.2	42.4	0.344	1.165(0.849-1.599)
TNFSF14	rs1077667	G	75.0	73.3	0.637	1.090(0.761-1.562)
IL22RA2	rs17066096	G	7.1	7.6	0.797	0.924(0.504-1.691)
MALT1	rs7238078	A	85.8	80.8	0.096	1.446(0.935-2.235)
MMEL1	rs4648356	C	46.2	45.9	0.930	1.014(0.741-1.388)
CD58	rs1335532	A	41.5	42.4	0.814	0.963(0.701-1.322)
RGS1	rs2760524	G	83.5	79.5	0.211	1.301(0.861-1.966)
CBLB	rs2028597	G	74.5	70.5	0.259	1.227(0.860-1.749)
TMEM39A	rs2293370	G	68.4	65.3	0.412	1.150(0.823-1.607)
IL12A	rs2243123	G	6.1	6.7	0.757	0.903(0.474-1.721)
IL7R	rs6897932	C	86.3	83.9	0.398	1.213(0.775-1.897)
IL7	rs1520333	G	50.0	52.1	0.591	0.918(0.671-1.255)
IL2RA	rs3118470	G	51.9	51.8	0.989	1.002(0.733-1.371)
ZMIZ1	rs1250550	A	41.0	45.6	0.254	0.832(0.606-1.142)
TNFRSF1A	rs1800693	G	17.5	12.0	0.045	1.550(1.007-2.384)
MPHOSPH9	rs949143	G	38.7	37.3	0.712	1.062(0.770-1.466)
CLEC16A	rs12708716	A	82.1	76.3	0.082	1.421(0.955-2.115)
IRF8	rs13333054	A	47.8	46.2	0.714	1.060(0.775-1.451)
STAT3	rs744166	G	42.6	37.9	0.240	1.210(0.880-1.664)
TYK2	rs8112449	G	49.5	51.8	0.567	0.913(0.668-1.248)

CI, confidence interval; NMO, neuromyelitis optica; OR, odds ratio; RAF, risk allele frequency. ^aThe risk alleles are based on the referenced study^[5-12].

Table 4. Association of 35 SNPs between anti-AQP4 antibody positive and negative NMO patients

Gene	SNP	Risk Allele ^a	Positive (n=44) RAF %	Negative (n=33) RAF %	Positive vs Negative <i>P</i>	OR (95% CI)
EOMES	rs11129295	A	73.9	74.2	0.958	0.980(0.473-2.031)
MERTK	rs17174870	G	88.6	93.9	0.257	0.503(0.151-1.682)
BATF	rs2300603	A	73.9	68.2	0.440	1.319(0.653-2.664)
No gene	rs669607	C	38.6	36.4	0.773	1.102(0.569-2.132)
HHEX	rs7923837	G	20.5	18.2	0.725	1.157(0.514-2.607)
TAGAP	rs1738074	G	39.8	40.9	0.887	0.954(0.498-1.828)
GALC	rs2119704	C	80.7	83.3	0.673	0.835(0.362-1.927)
NFKB1	rs228614	G	53.4	48.5	0.545	1.218(0.643-2.308)
MYC	rs4410871	G	61.4	66.7	0.498	0.794(0.407-1.549)
MAPK1	rs2283792	C	39.8	43.9	0.604	0.843(0.441-1.609)
MYB	rs11154801	A	29.5	30.3	0.919	0.965(0.481-1.936)
No gene	rs12466022	C	72.7	80.3	0.276	0.654(0.304-1.408)
VCAM1	rs11581062	G	20.5	15.2	0.398	1.440(0.616-3.366)
ZFP36L1	rs4902647	G	30.7	36.4	0.458	0.775(0.394-1.523)
ZNF746	rs354033	G	92.0	86.4	0.253	1.827(0.643-5.191)
IL12B	rs2546890	A	45.5	37.9	0.346	1.367(0.713-2.620)
TNFSF14	rs1077667	G	70.5	71.2	0.919	0.964(0.477-1.946)
IL22RA2	rs17066096	G	9.1	6.1	0.488	1.550(0.446-5.384)
MALT1	rs7238078	A	81.8	83.3	0.807	0.900(0.387-2.093)
MMEL1	rs4648356	C	48.9	45.5	0.675	1.147(0.605-2.174)
CD58	rs1335532	A	44.3	37.9	0.422	1.305(0.681-2.504)
RGS1	rs2760524	G	79.5	81.8	0.725	0.864(0.384-1.947)
CBLB	rs2028597	G	70.5	75.8	0.465	0.763(0.369-1.577)
TMEM39A	rs2293370	G	67.0	65.2	0.806	1.088(0.555-2.135)
IL12A	rs2243123	G	4.5	10.6	0.148	0.401(0.112-1.433)
IL7R	rs6897932	C	81.8	89.4	0.192	0.534(0.206-1.384)
IL7	rs1520333	G	47.7	48.5	0.926	0.970(0.512-1.837)
IL2RA	rs3118470	G	55.7	53.0	0.744	1.113(0.586-2.112)
ZMIZ1	rs1250550	A	42.0	43.9	0.814	0.926(0.486-1.763)
TNFRSF1A	rs1800693	G	15.9	22.7	0.284	0.643(0.286-1.447)
MPHOSPH9	rs949143	G	35.2	42.4	0.363	0.738(0.383-1.442)
CLEC16A	rs12708716	A	79.5	83.3	0.552	0.778(0.339-1.782)
IRF8	rs13333054	A	44.3	45.5	0.888	0.955(0.503-1.814)
STAT3	rs744166	G	40.9	43.9	0.706	0.883(0.463-1.685)
TYK2	rs8112449	G	45.5	47.0	0.852	0.941(0.496-1.785)

CI, confidence interval; Negative, NMO negative for anti-AQP4 antibody; NMO, neuromyelitis optica; OR, odds ratio; Positive, NMO positive for anti-AQP4 antibody; RAF, risk allele frequency. ^aThe risk alleles are based on the referenced study^[5-12].

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REFERENCES

- [1] Lennon VA, Wingerchuk DM, Kryzer TJ, Pittock SJ, Lucchinetti CF, Fujihara K, *et al.* A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. *Lancet* 2004, 364: 2106–2112.
- [2] Cabrera-Gomez JA, Kurtzke JF, Gonzalez-Quevedo A, Lara-Rodriguez R. An epidemiological study of neuromyelitis optica in Cuba. *J Neurol* 2009, 256: 35–44.
- [3] Cabre P. Environmental changes and epidemiology of multiple sclerosis in the French West Indies. *J Neurol Sci* 2009, 286: 58–61.
- [4] Asgari N, Lillevang ST, Skejoe HP, Falah M, Stenager E, Kyvik KO. A population-based study of neuromyelitis optica in Caucasians. *Neurology* 2011, 76: 1589–1595.
- [5] Hafler DA, Compston A, Sawcer S, Lander ES, Daly MJ, De Jager PL, *et al.* Risk alleles for multiple sclerosis identified by a genomewide study. *N Engl J Med* 2007, 357: 851–862.
- [6] Burton PR, Clayton DG, Cardon LR, Craddock N, Deloukas P, Duncanson A, *et al.* Association scan of 14,500 nonsynonymous SNPs in four diseases identifies autoimmunity variants. *Nat Genet* 2007, 39: 1329–1337.
- [7] Aulchenko YS, Hoppenbrouwers IA, Ramagopalan SV, Broer L, Jafari N, Hillert J, *et al.* Genetic variation in the KIF1B locus influences susceptibility to multiple sclerosis. *Nat Genet* 2008, 40: 1402–1403.
- [8] Australia and New Zealand Multiple Sclerosis Genetics Consortium (ANZgene). Genome-wide association study identifies new multiple sclerosis susceptibility loci on chromosomes 12 and 20. *Nat Genet* 2009, 41: 824–828.
- [9] De Jager PL, Jia X, Wang J, de Bakker PI, Ottoboni L, Aggarwal NT, *et al.* Meta-analysis of genome scans and replication identify CD6, IRF8 and TNFRSF1A as new multiple sclerosis susceptibility loci. *Nat Genet* 2009, 41: 776–782.
- [10] Jakkula E, Leppä V, Sulonen AM, Varilo T, Kallio S, Kempainen A, *et al.* Genome-wide association study in a high-risk isolate for multiple sclerosis reveals associated variants in STAT3 gene. *Am J Hum Genet* 2010, 86: 285–291.
- [11] Sanna S, Pitzalis M, Zoledziewska M, Zara I, Sidore C, Murru R, *et al.* Variants within the immunoregulatory CBLB gene are associated with multiple sclerosis. *Nat Genet* 2010, 42: 495–497.
- [12] Sawcer S, Hellenthal G, Pirinen M, Spencer CC, Patsopoulos NA, Moutsianas L, *et al.* Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011, 476: 214–219.
- [13] Yamakawa K, Kuroda H, Fujihara K, Sato S, Nakashima I, Takeda A, *et al.* Familial neuromyelitis optica (Devic's syndrome) with late onset in Japan. *Neurology* 2000, 55: 318–320.
- [14] Matiello M, Kim HJ, Kim W, Brum DG, Barreira AA, Kingsbury DJ, *et al.* Familial neuromyelitis optica. *Neurology* 2010, 75: 310–315.
- [15] Wingerchuk DM, Lennon VA, Pittock SJ, Lucchinetti CF, Weinshenker BG. Revised diagnostic criteria for neuromyelitis optica. *Neurology* 2006, 66: 1485–1489.
- [16] Zhao GX, Liu Y, Li ZX, Lv CZ, Traboulsee A, Sadovnick AD, *et al.* Variants in promoter region of *CYP7A1* are associated with neuromyelitis optica but not with multiple sclerosis in the Chinese Han population. *Neurosci Bull* 2013, 29: 525–530.
- [17] Matsuoka T, Matsushita T, Kawano Y, Osoegawa M, Ochi H, Ishizu T, *et al.* Heterogeneity of aquaporin-4 autoimmunity and spinal cord lesions in multiple sclerosis in Japanese. *Brain* 2007, 130: 1206–1223.
- [18] Buetow KH, Edmonson M, MacDonald R, Clifford R, Yip P, Kelley J, *et al.* High-throughput development and characterization of a genomewide collection of gene-based single nucleotide polymorphism markers by chip-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Proc Natl Acad Sci U S A* 2001, 98: 581–584.
- [19] Oksenberg JR, Barcellos LF, Cree BA, Baranzini SE, Bugawan TL, Khan O, *et al.* Mapping multiple sclerosis susceptibility to the HLA-DR locus in African Americans. *Am J Hum Genet* 2004, 74: 160–167.
- [20] Brum DG, Barreira AA, Louzada-Junior P, Mendes-Junior CT, Donadi EA. Association of the HLA-DRB1*15 allele group and the DRB1*1501 and DRB1*1503 alleles with multiple sclerosis in White and Mulatto samples from Brazil. *J Neuroimmunol* 2007, 189: 118–124.
- [21] Masterman T, Ligers A, Olsson T, Andersson M, Olerup O, Hillert J. HLA-DR15 is associated with lower age at onset in multiple sclerosis. *Ann Neurol* 2000, 48: 211–219.
- [22] Yamasaki K, Horiuchi I, Minohara M, Kawano Y, Ohayagi Y, Yamada T, *et al.* HLA-DPB1*0501 associated optico-spinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. *Brain* 1999, 122: 1689–1696.
- [23] Matsushita T, Matsuoka T, Isobe N, Kawano Y, Minohara M, Shi N, *et al.* Association of the HLA-DPB1*0501 allele with anti-aquaporin-4 antibody positivity in Japanese patients with idiopathic central nervous system demyelinating disorders.

- Tissue Antigens 2009, 73: 171–176.
- [24] Wang H, Dai Y, Qiu W, Zhong X, Wu A, Wang Y, *et al.* HLA-DPB1*0501 is associated with susceptibility to anti-aquaporin-4 antibodies positive neuromyelitis optica in Southern Han Chinese. *J Neuroimmunol* 2011, 233: 181–184.
- [25] Deschamps R, Paturel L, Jeannin S, Chausson N, Olindo S, Bera O, *et al.* Different HLA class II (DRB1 and DQB1) alleles determine either susceptibility or resistance to NMO and multiple sclerosis among the French Afro-Caribbean population. *Mult Scler* 2011, 17: 24–31.
- [26] Yoshimura S, Isobe N, Matsushita T, Yonekawa T, Masaki K, Sato S, *et al.* Distinct genetic and infectious profiles in Japanese neuromyelitis optica patients according to anti-aquaporin 4 antibody status. *J Neurol Neurosurg Psychiatry* 2013, 84: 29–34.
- [27] Kim HJ, Park HY, Kim E, Lee KS, Kim KK, Choi BO, *et al.* Common CYP7A1 promoter polymorphism associated with risk of neuromyelitis optica. *Neurobiol Dis* 2010, 37: 349–355.
- [28] Asgari N, Nielsen C, Stenager E, Kyvik KO, Lillevang ST. HLA, PTPN22 and PD-1 associations as markers of autoimmunity in neuromyelitis optica. *Mult Scler* 2012, 18: 23–30.
- [29] Matiello M, Schaefer-Klein JL, Hebrink DD, Kingsbury DJ, Atkinson EJ, Weinshenker BG. Genetic analysis of aquaporin-4 in neuromyelitis optica. *Neurology* 2011, 77: 1149–1155.
- [30] Sitarz KS, Yu-Wai-Man P, Hudson G, Jacob A, Boggild M, Horvath R, *et al.* Genetic variations within the OPA1 gene are not associated with neuromyelitis optica. *Mult Scler* 2012, 18: 240–243.