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ORIGINAL RESEARCH

Myelin oligodendrocyte glycoprotein-associated disorders are associated with HLA subtypes in a Chinese paediatric-onset cohort

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ABSTRACT

Objective Myelin oligodendrocyte glycoprotein-associated disorders (MOGADs) are a rare new neurological autoimmune disease with unclear pathogenesis. Since a linkage of the disease to the human leucocyte antigen (HLA) has not been shown, we here investigated whether MOGAD is associated with the HLA locus.

Methods HLA genotypes of 95 patients with MOGADs, assessed between 2016 and 2018 from three academic centres, were compared with 481 healthy Chinese Han individuals. Patients with MOGADs included 51 paediatric-onset and 44 adult-onset cases. All patients were seropositive for IgG targeting the myelin oligodendrocyte glycoprotein (MOG).

Results Paediatric-onset MOGAD was associated with the *DQB1*05:02–DRB1*16:02* alleles (OR=2.43; OR=3.28) or haplotype (OR=2.84) of *HLA* class II genes. The prevalence of these genotypes in patients with paediatric-onset MOGAD was significantly higher than healthy controls ($p^{\text{adj}}=0.0154$; $p^{\text{adj}}=0.0221$; $p^{\text{adj}}=0.0331$). By contrast, adult-onset MOGAD was not associated with any HLA genotype. Clinically, patients with the *DQB1*05:02–DRB1*16:02* haplotype exhibited significantly higher expanded disability status scale scores at onset ($p=0.004$) and were more likely to undergo a disease relapse ($p=0.030$). HLA–peptide binding prediction algorithms and computational docking analysis provided supporting evidence for the close relationship between the MOG peptide subunit and *DQB1*05:02* allele. In vitro results indicated that site-specific mutations of the predicted target sequence reduced the antigen–antibody binding, especially in the paediatric-onset group with *DQB1*05:02* allele.

Conclusions This study demonstrates a possible association between specific *HLA* class II alleles and paediatric-onset MOGAD, providing evidence for the conjecture that different aetiology and pathogenesis likely underlie paediatric-onset and adult-onset cases of MOGAD.

INTRODUCTION

Myelin oligodendrocyte glycoprotein-associated disorders (MOGADs) refer to a newly described group of neurological autoimmune diseases, characterised by seropositivity for IgG targeting the MOG (MOG-IgG).^{1 2} Some researchers consider

that MOGAD is a distinct disease entity, immunopathologically different from the conventional multiple sclerosis and aquaporin-4 (AQP4)-IgG-positive neuromyelitis optica spectrum disorders (NMOSDs), and has distinct clinical manifestations, treatment response and prognosis.^{2–4} The pathogenesis of MOGAD is not fully understood, and the potential genetic risk factors have not been studied previously.

Compared with other idiopathic inflammatory demyelination diseases, MOGAD more commonly occurs in children, and exhibits a relapsing course in some patients. Although glucocorticoid therapy is effective for the treatment of MOGADs, some patients showed steroid sensitive and reduction can lead to relapse. Multiple studies have reported age-dependent autoimmunity related to the frequency and titres of MOG-IgG.^{5 6} Young children with MOGADs often present with acute disseminated encephalomyelitis (ADEM), whereas older children and adults often exhibit recurrent optic neuritis, myelitis and brainstem encephalitis.⁷ Clinical phenotypes at onset are more frequently characterised by encephalopathy in paediatric patients with MOGADs, while older patients often exhibit ON.^{8 9} MOG-IgG titres are also significantly higher in paediatric patients than in adults.^{10 11} The differences in multiple features among patients with paediatric-onset and adult-onset MOGADs suggest different underlying pathogenetic mechanisms, which are still unclear.

Among genes that predispose individuals to systemic or neurological autoimmune diseases, the human leucocyte antigen (*HLA*) gene is potentially the most relevant and crucial candidate.^{12 13} Most autoimmune neurological disorders, such as multiple sclerosis, NMOSDs and anti-*N*-methyl-D-aspartate receptor encephalitis, are associated with specific HLA loci, especially the class II loci,^{14–16} suggesting an important role of antigen presentation in the pathogenesis of these diseases. In this study, we aimed to investigate the potential association between HLA locus and MOGADs, and explore the implications of these unique HLA subtypes in the immunopathogenesis of MOGADs. We hypothesised that HLA might be involved in the pathogenesis of MOGADs, and due to the differences in disease characteristics, there may be different associations of HLA between children and adults.

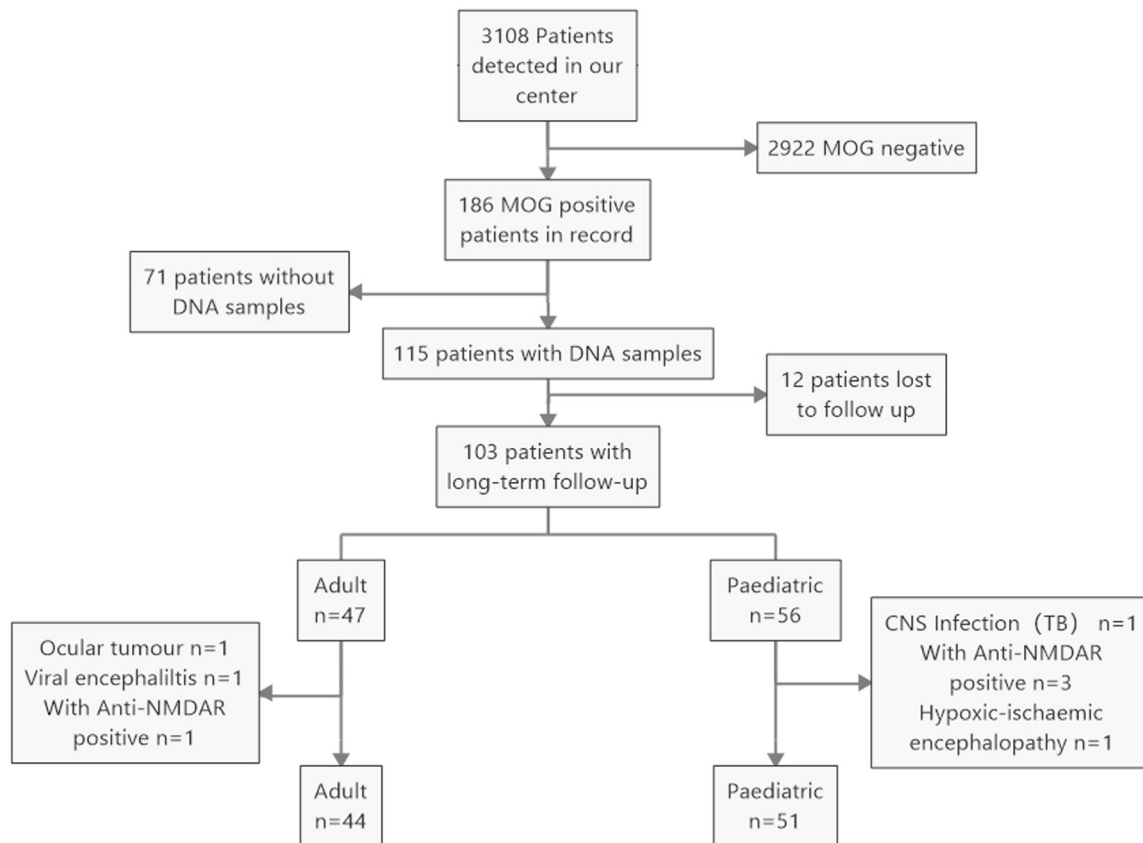


Figure 1 Flowchart of patient cohort enrolment and exclusion. Overall, 3108 patients with IIDDs were screened for MOG-IgG, and only 186 positive cases were identified. Of these, 71 patients did not have enough samples for DNA extraction and 12 patients were lost to follow-up. In several patients, diseases other than IIDDs were identified, and several patients were anti-NMDAR antibody positive. Eventually, 44 patients comprised the adult-onset MOG-encephalomyelitis cohort and 51 patients comprised the paediatric-onset cohort. CNS, central nervous system; IIDDs, idiopathic inflammatory demyelinating diseases; MOG-IgG, myelin oligodendrocyte glycoprotein-immunoglobulin G; NMDAR, *N*-methyl-D-aspartate receptor.

SUBJECTS AND METHODS

Subjects

From March 2016 to December 2018, 3108 patients from three academic centres (the Third Affiliated Hospital of Sun Yat-sen University, Zhongshan Ophthalmic Center of Sun Yat-sen University and Guangzhou Women and Children Medical Centre) in Guangzhou were referred to our laboratory for serum MOG-IgG testing. All patients had clinical manifestations related to central nervous system (CNS) demyelinating diseases and were tested for serum MOG-IgG and AQP4-IgG by a cell-based assay (CBA), as described previously.⁸ In total, 186 patients were seropositive for MOG-IgG, and 71 of them had no peripheral blood specimens stored after the test. A total of 115 MOG-IgG-positive patients were collected and followed up every 2 months in this study. The end time of follow-up was April 2019 and no other follow-up end point was set artificially. Twelve patients were lost to follow-up or the follow-up time was less than 4 months. After follow-up, 8 patients were finally diagnosed with non-CNS inflammatory demyelinating disease. Therefore, 95 patients were finally included in this study as the overall cohort, and they were all Han Chinese (figure 1). Final diagnoses were reported by the referring centres during the last follow-up and were based on the 2015 Wingerchuk criteria for NMOSDs,¹⁷ the 2017 McDonald criteria for multiple sclerosis¹⁸ and the 2012 criteria for ADEM.¹⁹ Relapse was defined as the sudden emergence of new symptoms lasting over 24 hours and separated by at least 1 month since the last episode. Expanded

Disability Status Scale (EDSS) scores were evaluated at the peak of recurrence. Written informed consent was obtained from all patients or their representatives, and in the case of paediatric patients, consent was signed by the legal guardian.

Molecular cloning and CBA

Full-length human MOG (Hu-MOG) was subcloned into the internal ribosome entry site 2-enhanced green fluorescent protein (pIRES2-EGFP) plasmid. We mutated the 42nd amino acid of the Hu-MOG protein from proline (P) to serine (S) (Hu-P42S) and subcloned it into the same expression vector. These constructs comprised a C-terminal EGFP-tag. Using the QuikChange Site-Directed Mutagenesis Kit (Stratagene, Santa Clara, California, USA), point mutations were introduced into Hu-MOG gene. The oligonucleotides used were 5'-GTGG GGTGGTACCGCTCCCCCTTCTCTAGGGTG-3' (P42S) and the corresponding reverse complementary oligonucleotides.

Hu-MOG and Hu-P42S plasmids were transiently transfected into HEK293T cells using Lipofectamine2000 (Thermo Scientific, Carlsbad, CA, USA). HEK293T cells were blocked with goat serum and immunolabelled with AlexaFluor 546-tagged secondary antibody against human IgG (1:1000; Thermo Scientific). Immunofluorescent images were acquired using a Zeiss Axiovert A1 fluorescence microscope (online supplementary figure 1). Each positive sample was independently tested at least twice.

HLA genotyping

Blood samples from all patients were collected and stored at -80°C . Genomic DNA was extracted from the peripheral blood of each patient with MOGADs and HLA genotyping was performed as described previously.²⁰ Briefly, the HLA class I (*HLA-A, B* and *C*) and class II (*DRB1, DOA1, DQB1, DPA1, DPB1*) genotypes for each patient were determined at the four-digit allele level using a PCR sequence-based typing method. The HLA frequencies of 481 geographically and ethnically matched healthy controls were derived from previous reports, in which all samples were from the Southern Han voluntary donors of haematopoietic stem cells from the Shenzhen Branch of China bone marrow bank. The exact beforementioned HLA genotyping method was used for genotyping the control group.²⁰

HLA-peptide binding prediction

The NetMHCIIpan V.3.2 server was utilised for HLA class II peptide-binding prediction.²¹ The MOG protein sequence obtained from the National Centre for Biotechnology Information (NCBI accession number: NP_996532.2) was submitted to the server. Finally, *DQB1*05:02* and *DRB1*16:02* were selected and compared in order to predict the binding epitopes within the protein.

In silico docking

We performed computational docking to describe the binding of the *DQB1*05:02* and the MOG segment (predicted by the above method). Since the crystallographic structure of *DQB1*05:02* has not been experimentally determined, a heterodimer structure including *DQB1*05:02* was created by homology modelling using a Swiss-Model from the *HLA-DQB1*02:01* template structure (Protein Data Bank code 4d8p). Tertiary structure modelling and peptide docking were performed as described previously.¹⁶ AutoDockTools was used to add hydrogens, assign charges and specify rotatable bonds to the proteins.²² MOG segment docking to *DQB1*05:02* was performed using the AutoDockVina software with the docking grid encompassing the entire peptide-binding cleft and the option 'number of torsions' being set to 0.²³

Statistical analyses

Fisher's exact test was used to compare the frequencies of the various *HLA* alleles or haplotypes between the MOGADs and control groups. The degree of association between the *HLA* data and MOGADs was expressed as the OR and 95% CIs. The *p* values determined by Fisher's exact test were adjusted for multiple testing with the false discovery rate criterion proposed by Benjamini and Hochberg. Only the adjusted *p* (p^{adj}) values under 0.05 (two-tailed) were considered to be statistically significant. To assess the significance of clinical variables between groups, Mann-Whitney tests and Fisher's exact test were performed for data lacking a normal distribution. All statistical analyses were conducted using GraphPad Prism V.7.0 and R-studio.

RESULTS

Demographic and clinical status of patients with MOGADs

As described in the Subjects and methods section, all the serum tests were conducted by experienced testing personnel through CBA method and all the MOGADs were diagnosed by two neurologists separately, according to the 2018 MOGADs consensus.^{24, 25} A total of 95 patients with MOGADs were enrolled, including 51 cases of paediatric-onset MOGADs (≤ 14 years old) and 44 cases of adult-onset (> 14 years old, figure 1).

Table 1 Demographic and clinical features of patients with MOGADs

	Total n=95	Paediatrics n=51	Adults n=44	P value
Current age, years, median (range)	16 (3–68)	10 (3–18)	32 (17–68)	–
Age of onset, years, median (range)	13 (2–67)	8 (2–14)	30 (15–67)	–
Follow-up time, months, median (range)	12(4–36)	12(4–36)	12(4–30)	–
Inducement, n (%)				
Previous infection	22 (23)	18 (35)	4 (9)	0.031
Vaccine	3 (3)	2 (4)	1 (2)	>0.999
Others	3 (3)	1 (2)	2 (5)	0.595
Phenotype at onset, n (%)*				
ON	58 (61)	25 (49)	33 (75)	0.012
Myelitis	11 (12)	4 (8)	7 (16)	0.336
Encephalopathy	30 (32)	26 (51)	4 (9)	<0.0001
Brainstem syndrome	6 (6)	4 (8)	2 (5)	0.683
Others	4 (4)	3 (6)	1 (2)	0.621
Initial EDSS, median (range)	3 (1–8)	3 (1–8)	2 (1–5)	0.024
Time to relapse, m median (range)†	8 (1–81)	10 (1–51)	8 (1–81)	0.480
Relapse type, n (%)*†				
ON	56 (54)	19 (40)	37 (66)	0.011
Myelitis	17 (17)	4 (9)	13 (23)	0.062
Encephalopathy	31 (30)	23 (49)	8 (14)	<0.001
Brainstem syndrome	9 (9)	4 (9)	5 (9)	>0.999
Others	1 (1)	0	1 (2)	>0.999
Acute phase treatment, n (%)				
Intravenous MTP	85/95 (90)	47/51 (92)	38/44 (86)	0.506
intravenous IG	13/95 (14)	12/51 (24)	1/44 (2)	0.025
PLEX or IAD	2/95 (2)	1/51 (2)	1/44 (2)	>0.999
None or others	7/95 (7)	3/51 (6)	4/44 (9)	0.701
Chronic therapies, n (%)				
Steroids	67/95 (71)	34/51 (67)	33/44 (75)	0.499
Immunosuppressive therapies	18/95 (19)	7/51 (14)	11/44 (25)	0.195
Immunomodulatory therapies	7/95 (7)	5/51 (10)	2/44 (5)	0.448

Values in bold indicate that there are differences between adults and paediatric individuals ($p < 0.05$).

*Patients may exhibit multiple phenotypes simultaneously.

†Forty-six patients relapsed for a total of 103 relapses; 23 paediatric-onset patients with 47 relapses and 23 adult-onset patients with 56 relapses.

EDSS, Expanded Disability Status Scale; IAD, immunoadsorption; MOGADs, myelin oligodendrocyte glycoprotein-associated disorders; MTP, methylprednisolone; ON, optic neuritis; PLEX, plasma exchange.

The clinical data for all patients are listed in table 1. Eighteen (35%) paediatric patients had a previous infection before onset, whereas only four adults had a previous infection ($p = 0.031$). Adult-onset patients were more likely to have an initial ON-like phenotype than paediatric-onset patients (25 vs 33, $p = 0.012$), while the paediatric patients more often had an initial encephalopathy phenotype (26 vs 4, $p < 0.0001$). Initial EDSS scores were higher among paediatric-onset patients, implying a more severe clinical attack (3 vs 2, $p = 0.024$).

HLA genotypes in patients with MOGADs and healthy controls

First, the frequencies of *HLA* class I and II alleles were compared between the 95 patients and 481 healthy controls. Among patients, the frequency of the *DQB1*05:02* allele was 18.95%, whereas

Table 2 HLA alleles in patients with MOGADs and in healthy controls

HLA allele	Patients (n=95 × 2)			Controls (n=481 × 2)			P value	P ^{adj} value	OR (95% CI)
	Positive	Negative	Freq	Positive	Negative	Freq			
<i>DQB1</i> *05:02	36	154	0.1895	103	859	0.1071	0.0023	0.0316	1.95 (1.25 to 3.00)
<i>DPB1</i> *13:01	7	183	0.0368	82	880	0.0852	0.0246	0.1105	0.41 (0.16 to 0.91)
<i>DRB1</i> *08:03	4	186	0.0211	69	893	0.0717	0.0055	0.1278	0.28 (0.07 to 0.76)
<i>DRB1</i> *16:02	17	173	0.0895	41	921	0.0426	0.0106	0.1278	2.21 (1.15 to 4.08)
<i>DPA1</i> *02:02	124	66	0.6526	550	412	0.5717	0.0438	0.1754	1.41 (1.01 to 1.98)
<i>DQA1</i> *01:02	51	139	0.2684	192	770	0.1996	0.0407	0.2215	1.47 (1.01 to 2.13)
<i>DQA1</i> *01:03	7	183	0.0368	79	883	0.0821	0.0332	0.2215	0.43 (0.16 to 0.94)
<i>DRB1</i> *14:05	8	182	0.0421	16	946	0.0166	0.0446	0.2675	2.60 (0.95 to 6.54)
<i>DRB1</i> *15:02	11	179	0.0579	26	936	0.0270	0.0397	0.2675	2.21 (0.97 to 4.73)
<i>DQB1</i> *05:03	15	175	0.0789	40	922	0.0416	0.0385	0.2697	1.97 (0.99 to 3.75)
<i>C</i> *07:02	22	168	0.1158	171	791	0.1778	0.0429	0.3144	0.61 (0.36 to 0.98)
<i>C</i> *06:02	2	188	0.0105	38	924	0.0395	0.0493	0.3144	0.26 (0.03 to 1.02)
<i>A</i> *02:07	34	156	0.1789	119	843	0.1237	0.0465	0.4683	1.54 (0.98 to 2.37)

Table shows only HLA alleles with an unadjusted p value of <0.05. P values are adjusted for multiple testing via the FDR step-up method. FDR, false discovery rate; freq, frequency; HLA, human leucocyte antigen; MOGADs, myelin oligodendrocyte glycoprotein-associated disorders.

for controls it was only 10.71% (OR=1.95, 95% CI=1.25–3.0, p=0.002, p^{adj}=0.031 table 2, online supplementary table 1). Notably, when a comparison was conducted between the 51 paediatric-onset patients and 481 healthy controls, a more significant association was identified between *DQB1**05:02 (OR=2.43, 95% CI=1.39–4.11, p=0.001, p^{adj}=0.015) and *DRB1**16:02 (OR=3.28, 95% CI=1.55–6.25, p=0.001, p^{adj}=0.022, online supplementary table 2, figure 2) and disease. Furthermore, we found that the frequency of the *DQB1**05:02–*DRB1**16:02 haplotype was higher in paediatric-onset patients than in healthy controls (OR=2.84, 95% CI=1.17–6.36, p=0.0165, p^{adj}=0.0331), though this difference was not statistically significant for all patients after correction (p=0.0478, p^{adj}=0.0956). Interestingly, adult-onset patients yielded no specific association with any HLA alleles (online supplementary table 3, figure 2).

Association between *DQB105:02–*DRB1**16:02 haplotype and clinical data of patients with MOGADs**

We next investigated *DQB1**05:02–*DRB1**16:02 haplotype’s association with the clinical features of the paediatric-onset patients with MOGADs by comparing the *DQB1**05:02–*DRB1**16:02 carriers with the *DQB1**05:02–*DRB1**16:02 non-carriers. As shown in table 3, the two patient subgroups were comparable in age, initial EDSS and relapse frequency.

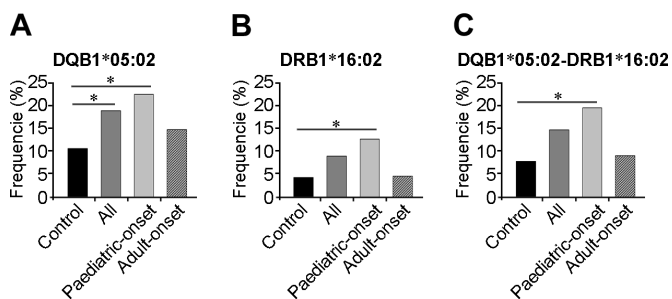


Figure 2 Frequencies of significant alleles and haplotypes in patients with MOGADs and controls. Comparison of the allele frequencies of (A) *DQB1**05:02, (B) *DRB1**16:02 and (C) *DRB1**16:02–*DQB1**05:02 haplotypes among controls, all patients and patients with paediatric-onset and adult-onset MOGADs. *P<0.05. MOGADs, myelin oligodendrocyte glycoprotein-associated disorders.

However, patients carrying the *DQB1**05:02–*DRB1**16:02 haplotype exhibited more severe clinical symptoms, significantly higher initial EDSS scores (4.25 vs 3, p=0.004) and more frequent relapses (8/10 vs 15/41, p=0.030, table 3). The number of *DQB1**05:02–*DRB1**16:02 carriers in adult group was too small to determine any statistical significance.

HLA–MOG binding prediction and computational docking

The potential association between the various HLA subtypes and MOGADs led us to produce the HLA peptide-binding prediction algorithms. Using the NetMHCIIpan V3.2 server as a tool for analysis, we identified an epitope within the MOG that was predicted to be recognised by *DQB1**05:02. This epitope with the target sequence ‘VGWYRPPFS’, is located at the 37th amino acid position in the MOG protein’s N-terminus. Therefore, the sequence ‘VGWYRPPFS’, which is part of the MOG protein’s

Table 3 Association between *DQB1**05:02–*DRB1**16:02 haplotype and clinical features in paediatric-onset patients with MOGADs

	Patients with haplotype n=10	Controls n=41	P value
Age, years, median (range)	11 (3–16)	10 (3–18)	–
Age of onset, years, median (range)	4 (2–14)	8 (2–13)	–
Inducement, n (%)			
Previous infection	3 (30)	15 (38)	0.730
Phenotype at onset, n (%)*			
ON	5 (50)	20 (49)	>0.999
Myelitis	2 (20)	2 (5)	0.168
Encephalopathy	5 (50)	21 (51)	>0.999
Brainstem syndrome	0	4 (10)	0.573
Others	0	3 (7)	>0.999
Initial EDSS, median (range)	4.25 (2–8)	3 (1–6.5)	0.004
Baseline titre, median (range)	320 (100–640)	100 (100–1280)	0.387
Patients with relapse, n (%)	8 (80)	15 (37)	0.030
Relapse type, n (%)*			
ON	9/16 (56)	10/31 (32)	0.131
Myelitis	2/16 (13)	2/31 (7)	0.597
Encephalopathy	6/16 (38)	18/31 (58)	0.227
Brainstem syndrome	0	4/31 (13)	0.284

*Patients may exhibit multiple phenotypes simultaneously. EDSS, Expanded Disability Status Scale; MOGADs, myelin oligodendrocyte glycoprotein-associated disorders; ON, optic neuritis.

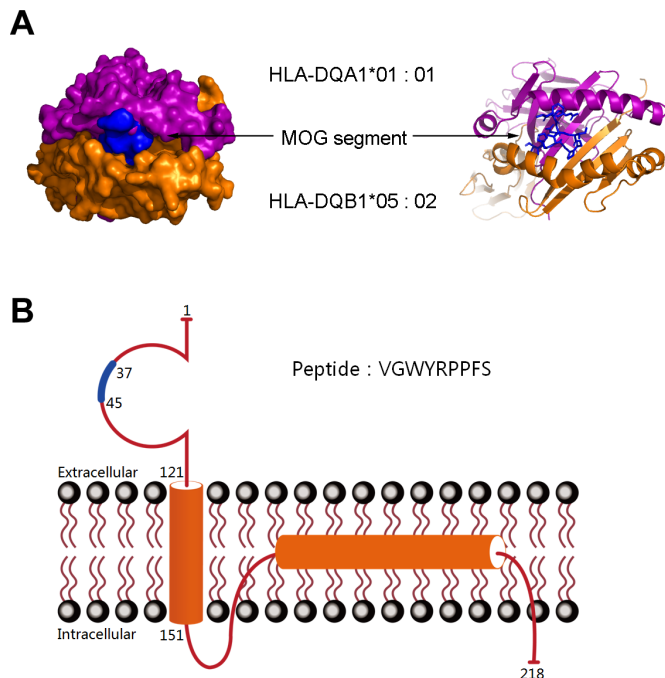


Figure 3 Computational docking of the MOG segment VGWYRPPFS to the HLA-DQB1*05:02 heterodimer and structure of the MOG protein. (A) In silico docking simulation resulted in the placement of the MOG segment within the peptide-binding groove between HLA-DQB1*05:02 and HLA-DQA1 with docking scores (ΔG) of -10.4 kcal/mol. Mesh on the surface of HLA indicates close contact between the atoms and MOG protein segment. The ribbon structure shows the ligand-binding domains. (B) The structure of the MOG protein and the blue sections indicate the location of the peptide segment. HLA, human leucocyte antigen; MOG, myelin oligodendrocyte glycoprotein.

extracellular domain, has a high probability of binding to the DQB1*05:02 protein.

To further evaluate the relationship between the predicted epitope and major histocompatibility complex (MHC) II molecules, we performed an in silico docking of the ligand to the DQB1*05:02 protein. Computational docking determined that the target sequence 'VGWYRPPFS' fit precisely into the peptide-binding groove between DQB1*05:02 and HLA-DQA1, with a docking score (ΔG) of -10.4 kcal/mol (figure 3). This in silico docking result suggests a positive interaction between the predicted epitope and DQB1*05:02.

Mutation of a VGWYRPPFS-specific site reduces the ability of antigen–antibody binding

A homology comparison between the humans, rats and mice protein sequences shows that the target sequence is conserved except for the P locus, which is a S locus in rats and mice. Since it has been reported that this site affects the binding of the MOG antigen and antibodies,²⁶ we chose this site for the mutation. The mutant plasmid Hu-P42S was constructed as described in the Subjects and methods section. The Hu-P42S plasmid was transiently transfected into HEK293T cells and 95 samples, that seropositive for MOG-IgG, were detected. The results showed that mutations of the predicted target sequence reduced the ability of antigen–antibody binding, especially in the paediatric-onset group with the DQB1*05:02 allele. Overall, in 74% of the 19 paediatric-onset patients with the DQB1*05:02 allele, no positive signals were observed, which was statistically significant compared with

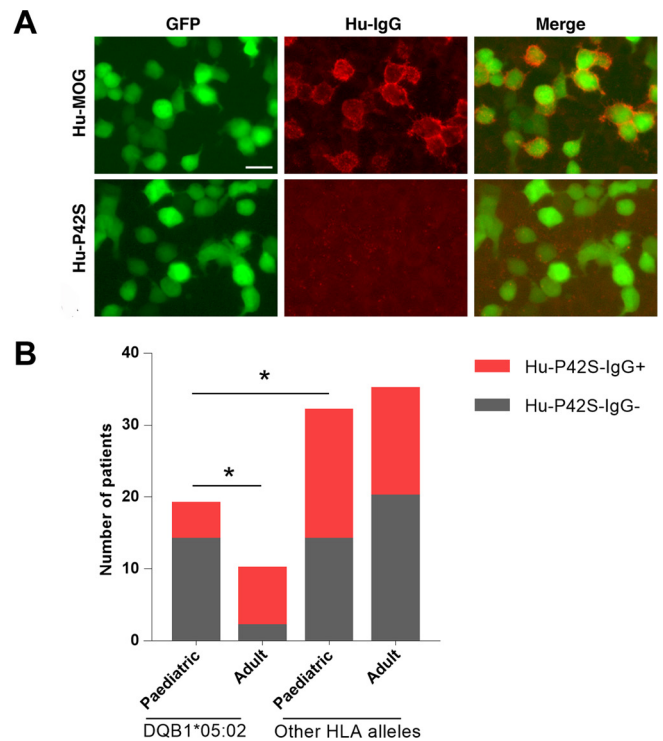


Figure 4 Mutation of the specific site of the target sequence (VGWYRPPFS) reduces the ability of antigen–antibody binding especially in paediatric-onset group with DQB1*05:02. (A) HEK293T cells were transiently transfected with full-length human MOG (Hu-MOG) or an MOG mutation plasmid (Hu-P42S) and patient serum tested by an in-house CBA. Binding of IgG from patient serum using an anti-human IgG antibody (red) to cells transfected with MOG (green), merged images (yellow). Some patients' serum could only be bound to Hu-MOG but not to Hu-P42S. Scale bar=20 μ m. (B) Overall, 95 seropositive samples for MOG-IgG were detected with Hu-P42S, and the percentage of patients with positive or negative signals were compared in four groups, including paediatric-onset patients with a DQB1*05:02 allele, paediatric-onset patients with a DQB1*05:02 allele, paediatric-onset patients with other HLA alleles and adult-onset patients with other HLA alleles. * $P < 0.05$. CBA, cell-based assay; HLA, human leucocyte antigen; MOG, myelin oligodendrocyte glycoprotein; MOG-IgG, myelin oligodendrocyte glycoprotein-immunoglobulin G.

the adult group ($p = 0.016$) and the paediatric-onset group with the other HLA allele ($p = 0.047$) (figure 4). The 42nd amino acid of the Hu-MOG was included in the predicted target peptide VGWYRPPFS, suggesting that this peptide contained important antigen–antibody binding sites, and that the DQB1*05:02 allele was involved only in paediatric-onset MOGADs.

DISCUSSION

As a new disease entity, the pathogenesis of MOGADs is still unclear, and there are currently no reports of its potential association with the HLA loci. MOG-IgG, the biomarker of MOGADs, was first identified in patients with CNS demyelinating diseases.²⁷ While the presence of the DPB1*05:01 allele is a known risk factor for NMOSDs in Japanese and Southern Han Chinese populations,^{28 29} it also leads to an increased incidence of AQP4-IgG positivity in patients with NMOSDs. The DRB1*09:01 allele has also been reported to be a protective factor in both multiple sclerosis and NMOSDs in the Japanese and Chinese patients.^{15 28 30} DRB1*15:01–DQB1*06:02 alleles are the major susceptible HLA

subtypes in multiple sclerosis and are usually inherited as haplotypes due to linkage disequilibrium.^{14,30} In this study, MOGAD was found, for the first time, to be associated with the *DQB1*05:02-DRB1*16:02* alleles or haplotype of *HLA* class II genes in Chinese paediatric-onset patients. These susceptible loci differed from those associated with NMOSDs and multiple sclerosis, indicating a distinct pathogenesis for MOGADs.

The *DQB1*05:02* and *DRB1*16:02* alleles have also been associated with an increased risk for several autoimmune diseases. The *DQB1*05:02* allele is associated with relapsing polychondritis,³¹ the muscle-specific kinase antibody positive in patients with myasthenia gravis (MG, MuSK-MG),^{32,33} and anti-acetylcholine receptor MG.³¹ The *DRB1*16:02* allele is positively correlated with Graves' disease,³⁴ anti-topoisomerase I autoantibody-positive systemic sclerosis³⁵ and AQP4-IgG-positive NMOSDs.¹⁵ In relapsing polychondritis and MG, both *DQB1*05:02-DRB1*16* alleles and haplotypes are involved. Interestingly, these autoimmune diseases are predominantly mediated by autoantibodies, suggesting that *DQB1*05:02* and *DRB1*16:02* may play an important role in autoantibody production.

MOG-IgG can be detected in both paediatric and adult patients and recent studies have focused on the clinical differences between these two groups. MOG-IgG is found more often in children than in adults and the associated disease classification is often associated with a monophasic course.^{10,36} The results of this study demonstrate that only paediatric-onset MOGAD is associated with unique *HLA* subtypes, as adult-onset patients with MOGADs do not exhibit any specific association with *HLA* alleles or haplotypes. The inability to reach a statistical significance may be due to the small adult group size. Besides susceptibility, our study also showed a relationship between the presence of the *DQB1*05:02-DRB1*16:02* haplotype and clinical features. Patients carrying the haplotype had a significantly worse level of disability and disease relapse. These findings provide genetic evidence for multiple pathogenic mechanisms that differ between paediatric-onset and adult-onset MOGADs and suggest that different clinical therapies may be required. The pathogenesis of MOGADs is still unclear, and as an autoimmune disease, multiple contributing genetic and environmental factors may be involved. While *HLA* subtypes could be potential genetic risk factors, it cannot explain all aspects of the MOGADs. As there are many differences between children and adults regarding diet, living habits and vaccination and so on, it may lead to differences in disease susceptibility. The differences in the development of the blood-brain barrier and maturation of the immune system among children and adults may also result in the differential disease phenotype and clinical characteristics of patients with MOG-IgG with different ages at onset. We hypothesise that some characteristics of children make them more susceptible and thereby highlight the variable impact of *HLA*. This, however, requires further research for definite confirmation.

The *HLA* system is known to be high polymorphic, and the distribution and frequencies of *HLA* alleles are highly variable among the different ethnic groups. Due to the different frequencies of the alleles among populations, the association between specific diseases and *HLA* loci tends to be population-specific. As far as we know, similar results may be obtained for ethnic groups with a similar genetic background. For example, in Caucasians, *DRB1*15:01-DQB1*06:02* alleles have been consistently associated with susceptibility to multiple sclerosis,¹⁴ whereas the *DPB1*05:01* allele confers susceptibility to anti-AQP4 antibody-positive NMOSDs in the Japanese and Southern Han Chinese populations.^{28,29} In this study, MOGAD was found to be

associated with the *DQB1*05:02-DRB1*16:02* alleles or haplotype in the Chinese paediatric-onset patients. Although no other report exists on the potential association between *HLA* loci and MOGADs, we believe that this result may be applicable to other cohorts, at least in Asian populations with a similar *HLA* genetic background. It is worth mentioning that the difference in *HLA* association between the paediatric-onset and adult-onset patients with MOGADs in this study may also be applicable to non-Asian populations. While, further studies that include a larger number of patients and various populations are required to confirm these results.

Interestingly, bioinformatic analyses also predicted a strong epitope (target sequence: VGWYRPPFS) for the *DQB1*05:02* protein. This target sequence is located between aa37 and aa45 relative to the N-terminus of the MOG protein, which is part of the N-terminal extracellular domain (aa1–121). The extracellular region of the MOG protein also contains major T cell and B cell epitopes,^{37,38} as well as the epitope for the antibody-antigen interactions responsible for the formation of pathogenic antibodies.^{37,39} Furthermore, MOG peptides (aa35–55) are highly encephalitogenic and can induce experimental autoimmune encephalomyelitis in animals.⁴⁰ In animal models, the anti-MOG immune response can be modified by MHC haplotypes,⁴⁰ but in humans, the mechanisms by which they act are still undefined. Our study shows that mutation of the 42nd amino acid of MOG reduces the ability of antigen-antibody binding, especially in the paediatric-onset group with *DQB1*05:02*. Collectively, these results suggest that *HLA-DQB1*05:02* plays a role in the pathogenesis of MOGADs and may be a possible reason that some carriers differed from others in their responses to the treatment.

This study had several limitations. First, the size of patient samples in this study was relatively small and the delineation of different ages cut-off for paediatric/adult onset of the cohort may affect the results; therefore, further studies with a larger number of patients are required to confirm these results. Second, since the *HLA* gene has a high degree of polymorphism as well as regional and ethnic distribution differences, the association between *HLA* subtypes and MOGADs seems to be population specific. Finally, the carrier frequencies of *DQB1*05:02-DRB1*16:02* alleles or haplotype were rather low ($\leq 30\%$) in patients with MOGADs, suggesting the existence of other potential features in the disease course that may have contributed to the different presentations between paediatric-onset and adult-onset patients with MOGADs.

In summary, to the best of our knowledge, the present study is the first to demonstrate that the *DQB1*05:02-DRB1*16:02* alleles or haplotype are associated with paediatric-onset MOGADs, in terms of both disease susceptibility and clinical severity. Our findings suggest that these *HLA* genotypes are risk factors for paediatric-onset MOGADs and represent a predictive factor for more severe clinical symptoms. This study also provides valuable insight into a novel disease mechanism, although further studies are needed to validate this association and explore the underlying mechanism.

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REFERENCES

- Bradl M, Reindl M, Lassmann H. Mechanisms for lesion localization in neuromyelitis optica spectrum disorders. *Curr Opin Neurol* 2018;31:325–33.
- Hennes E-M, Baumann M, Schanda K, et al. Prognostic relevance of MOG antibodies in children with an acquired demyelinating syndrome. *Neurology* 2017;89:900–8.
- Sato DK, Callegaro D, Lana-Peixoto MA, et al. Distinction between MOG antibody-positive and AQP4 antibody-positive NMO spectrum disorders. *Neurology* 2014;82:474–81.
- Jarius S, Ruprecht K, Kleiter I, et al. MOG-IgG in NMO and related disorders: a multicenter study of 50 patients. Part 2: epidemiology, clinical presentation, radiological and laboratory features, treatment responses, and long-term outcome. *J Neuroinflammation* 2016;13:280.
- Reindl M, Waters P. Myelin oligodendrocyte glycoprotein antibodies in neurological disease. *Nat Rev Neurol* 2019;15:89–102.
- Zhou Y, Jia X, Yang H, et al. Myelin oligodendrocyte glycoprotein antibody-associated demyelination: comparison between onset phenotypes. *Eur J Neurol* 2019;26:175–83.
- Jarius S, Ruprecht K, Stellmann JP, et al. MOG-IgG in primary and secondary chronic progressive multiple sclerosis: a multicenter study of 200 patients and review of the literature. *J Neuroinflammation* 2018;15:88.
- Chen L, Chen C, Zhong X, et al. Different features between pediatric-onset and adult-onset patients who are seropositive for MOG-IgG: a multicenter study in South China. *J Neuroimmunol* 2018;321:83–91.
- Ramanathan S, Mohammad S, Tantsis E, et al. Clinical course, therapeutic responses and outcomes in relapsing MOG antibody-associated demyelination. *J Neurol Neurosurg Psychiatry* 2018;89:127–37.
- Hennes E-M, Baumann M, Schanda K, et al. Prognostic relevance of MOG antibodies in children with an acquired demyelinating syndrome. *Neurology* 2017;89:900–8.
- Hacohen Y, Wong YY, Lechner C, et al. Disease course and treatment responses in children with relapsing myelin oligodendrocyte glycoprotein antibody-associated disease. *JAMA Neurol* 2018;75:478–87.
- Sollid LM, Pos W, Wucherpfennig KW. Molecular mechanisms for contribution of MHC molecules to autoimmune diseases. *Curr Opin Immunol* 2014;31:24–30.
- Gontika MP, Anagnostouli MC. Anti-Myelin oligodendrocyte glycoprotein and human leukocyte antigens as markers in pediatric and adolescent multiple sclerosis: on diagnosis, clinical phenotypes, and therapeutic responses. *Mult Scler Int* 2018;2018:8487471.
- Oksenberg JR, Baranzini SE, Sawcer S, et al. The genetics of multiple sclerosis: SNPs to pathways to pathogenesis. *Nat Rev Genet* 2008;9:16–26.
- Yoshimura S, Isobe N, Matsushita T, 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.
- Shu Y, Qiu W, Zheng J, et al. HLA class II allele *DRB1*16:02* is associated with anti-NMDAR encephalitis. *J Neurol Neurosurg Psychiatry* 2019;90:652–8.
- Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. *Neurology* 2015;85:177–89.
- Thompson AJ, Banwell BL, Barkhof F, et al. Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. *Lancet Neurol* 2018;17:162–73.
- Krupp LB, Tardieu M, Amato MP, et al. International pediatric multiple sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. *Mult Scler* 2013;19:1261–7.
- Jin S, Zou H, Zhen J, et al. [Study of polymorphisms of HLA class I (-A, -B, -C) and class II (DRB1, DQA1, DQB1, DPA1, DPB1) genes among ethnic Hans from Southern China]. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2017;34:110–4.
- Jensen KK, Andreatta M, Marcatili P, et al. Improved methods for predicting peptide binding affinity to MHC class II molecules. *Immunology* 2018;154:394–406.
- Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J Comput Chem* 2009;30:2785–91.
- Trott O, Olson AJ. Autodock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem* 2010;31:455–61.
- Jarius S, Paul F, Aktas O, et al. MOG encephalomyelitis: international recommendations on diagnosis and antibody testing. *J Neuroinflammation* 2018;15:134.
- López-Chiriboga AS, Majed M, Fryer J, et al. Association of MOG-IgG serostatus with relapse after acute disseminated encephalomyelitis and proposed diagnostic criteria for MOG-IgG-Associated disorders. *JAMA Neurol* 2018;75:1355–63.
- Mayer MC, Breithaupt C, Reindl M, et al. Distinction and temporal stability of conformational epitopes on myelin oligodendrocyte glycoprotein recognized by patients with different inflammatory central nervous system diseases. *J Immunol* 2013;191:3594–604.
- Di Pauli F, Mader S, Rostasy K, et al. Temporal dynamics of anti-MOG antibodies in CNS demyelinating diseases. *Clin Immunol* 2011;138:247–54.
- Matsushita T, Matsuoka T, Isobe 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;73:171–6.
- Wang H, Dai Y, Qiu W, et al. HLA-DPB1 0501 is associated with susceptibility to anti-aquaporin-4 antibodies positive neuromyelitis optica in southern Han Chinese. *J Neuroimmunol* 2011;233:181–4.
- Qiu W, James I, Carroll WM, et al. HLA-DR allele polymorphism and multiple sclerosis in Chinese populations: a meta-analysis. *Mult Scler* 2011;17:382–8.
- Terao C, Yoshifuji H, Yamano Y, et al. Genotyping of relapsing polychondritis identified novel susceptibility HLA alleles and distinct genetic characteristics from other rheumatic diseases. *Rheumatology* 2016;55:1686–92.
- Bartocioni E, Scuderi F, Augugliaro A, et al. HLA class II allele analysis in MuSK-positive myasthenia gravis suggests a role for DQ5. *Neurology* 2009;72:195–7.
- Testi M, Terracciano C, Guagnano A, et al. Association of HLA-DQB1*05:02 and DRB1*16 alleles with late-onset, Nonthymomatous, AChR-Ab-Positive myasthenia gravis. *Autoimmune Dis* 2012;2012:541760.
- Chen P-L, Fann CS-J, Chu C-C, et al. Comprehensive genotyping in two homogeneous Graves' disease samples reveals major and novel HLA association alleles. *PLoS One* 2011;6:e16635.
- He D, Wang J, Yi L, et al. Association of the HLA-DRB1 with scleroderma in Chinese population. *PLoS One* 2014;9:e106939.
- Hyun J-W, Woodhall MR, Kim S-H, et al. Longitudinal analysis of myelin oligodendrocyte glycoprotein antibodies in CNS inflammatory diseases. *J Neurol Neurosurg Psychiatry* 2017;88:811–7.
- Adelmann M, Wood J, Benzell I, et al. The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. *J Neuroimmunol* 1995;63:17–27.
- Mesleh MF, Belmar N, Lu CW, et al. Marmoset fine B cell and T cell epitope specificities mapped onto a homology model of the extracellular domain of human myelin oligodendrocyte glycoprotein. *Neurobiol Dis* 2002;9:160–72.
- Molnarfi N, Schulze-Topphoff U, Weber MS, et al. MHC class II-dependent B cell APC function is required for induction of CNS autoimmunity independent of myelin-specific antibodies. *J Exp Med* 2013;210:2921–37.
- Warnecke A, Musunuri S, N'diaye M, et al. Nitration of MOG diminishes its encephalitogenicity depending on MHC haplotype. *J Neuroimmunol* 2017;303:1–12.