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# Association Analysis Between HLA-DQA1 Loci and Neuromyelitis Optica Spectrum Disorder in a Han Chinese Population

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**Background:** Genome-wide association studies for neuromyelitis optica spectrum disorder (NMOSD) have established an association between *HLA-DQ* alpha 1 (DQA1) and risk for NMOSD. Though ethnicity is generally considered a major influencing factor in genetic analyses, little is known regarding the association of *HLA-DQA1* polymorphisms with NMOSD in the Han population, especially the single-nucleotide polymorphisms (SNPs) at *HLA-DQA1*.

**Methods:** We genotyped SNP at loci rs28383224 in a case-control study consisting of 137 subjects (51 patients with NMOSD and 86 unrelated controls were recruited) of Han ethnicity. Logistic regression was used to test the association of SNP with NMOSD susceptibility, the sex and age were adjusted, odds ratios and 95% confidence intervals were estimated.

**Results:** The rs28383224 polymorphism and susceptibility to NMOSD were not statistically associated ($P > 0.05$) in the Han population in the current study. No significant difference was found in allelic frequencies or genotypic distributions among different subsets of NMOSD patients ($P > 0.05$).

**Conclusion:** In the current study, there is no evidence that polymorphism of rs28383224 in the *HLA-DQA1* gene is associated with the risk of NMOSD in the Han Chinese population.

**Key Words:** *HLA-DQA1* locus, gene polymorphism, neuromyelitis optica spectrum disorder, association, Chinese

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## BACKGROUND

Neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory demyelinating disease of the central nervous system (CNS) that results primarily in optic neuritis and myelitis.[1] The discovery of aquaporin-4 (AQP4)-immunoglobulin G, an antibody (Ab) against the astrocyte water channel in the CNS, clearly identified NMOSD as a separate disease from multiple sclerosis.[2] The detection of AQP4 antibodies has been validated as a diagnostic criterion for NMOSD. The presence of AQP4 antibody (Ab) also has high specificity for a range of clinical presentations, now referred to as NMOSD, without requiring all the clinical features that were previously essential to make a clinical diagnosis.[3] The etiology of NMOSD arises from complex interactions between autoimmune and genetic variations. Both rare variants and common single-nucleotide polymorphisms (SNPs) are thought to confer risk for NMOSD. Although the majority of NMOSD is sporadic, the discovery of pathogenic genetic loci has provided new insights into the genetic architecture of the disease.

Recent reports have confirmed genes such as AQP4, HLA-DPB, *CD40*, interleukin-17, and *TNFSF4* as susceptibility genes for NMOSD with population-specific heterogeneity.[4–7] The *HLA* region also contributes to the genetic architecture of NMOSD in different populations.[7] Variations in *HLA-DQA* have also been suggested to have associations with NMOSD in various populations. A recent genome-wide association study identified rs28383224, which is located in HLA-DQA1, have a strong association with NMOSD susceptibility in Europeans [odds ratio (OR) = 2.66, $P = 8\times10^{-8}$]. Nevertheless, after Bonferroni correction based on the number of alleles tested, no significant association was observed in a Japanese population ($P > 0.05$).[7,8] Research results vary from different populations, given that ethnicity is an important factor in genetic analysis. To date, the SNP mentioned above have not been reported in the Han population regarding their associations with risk for NMOSD. In this study, we aimed to explore the strength of association between the SNP rs28383224 in HLA-DQA1 and Han Chinese NMOSD patients.

## METHODS

### Subjects

A total of 51 NMOSD patients (8 males and 43 females) and 86 age-matched and sex-matched controls (16 males and 70 females) were enrolled in this study. The ages (mean ± SD) were 46.31 ± 17.27 years for NMOSD and 48.60 ± 16.05 years for controls. There was no significant difference in sex or age between patients and controls (both $P > 0.05$, Table 1). The inclusion criteria were as follows: (1) the idiopathic NMOSD patients were diagnosed according to the 2015 International Consensus Diagnostic Criteria for NMOSD[3] by 2 specialists; (2) Han Chinese populations; and (3) sex-matched and age-matched controls. The exclusion criteria were as follows: (1) coexistence of other CNS disorders; (2) incomplete data on clinical information; and (3) patients had other demyelinating diseases at the first onset. Demographic data and clinical characteristics were recorded for each subject, including sex,

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**TABLE 1.** Demographics and Clinical Characteristics of Subjects

| | NMOSD (N = 51) | Control (N = 86) | *P* |
|---|---|---|---|
| Female:male (female %) | 43:8 (84.31) | 70:16 (81.40) | 0.664 |
| Age (mean ± SD) (y) | 46.31 ± 17.27 | 48.60 ± 16.05 | 0.187 |
| Age at onset (mean ± SD) (y) | 42.96 ± 18.06 | NA | NA |
| Disease duration (mean ± SD) (y) | 3.35 ± 4.417 | NA | NA |
| AQP4-Ab (positive:negative) (positive %) | 43:8 (84.31) | NA | NA |
| EDSS score (mean ± SD) | 2.66 ± 2.12 | NA | NA |
| Core clinical syndromes [n/N (%)] | | NA | NA |
| Acute myelitis | 19/51 (37.25) | NA | NA |
| Optic neuritis | 25/51 (49.02) | NA | NA |
| Acute myelitis+optic neuritis | 5/51 (9.80) | | |
| Area postrema syndrome | 2/51 (3.92) | NA | NA |
| Complicate with autoimmune diseases | 8/51 (15.69) | NA | NA |
| MRI lesions [n/N (%)] | | NA | NA |
| Longitudinally extensive | 29/51 (56.86) | NA | NA |
| Focal | 6/51 (11.76) | NA | NA |
| Brainstem | 3/51 (5.88) | NA | NA |
| Cerebrum | 9/51 (17.65) | NA | NA |
| Optic nerve* | 19/30 (63.33) | NA | NA |

*Optic nerve MRI data availability was 30.

AQP4-Ab indicates aquaporin-4 antibody; EDSS, Expanded Disability Status Scale; MRI, magnetic resonance imaging; NA, not applicable; NMOSD, neuromyelitis optica spectrum disorder.

age, age at onset, disease duration, Expanded Disability Status Scale score,[9] AQP4-Ab status, autoantibodies, core clinical syndromes, and magnetic resonance imaging (MRI) lesions (Table 1). All control subjects were free of neurological disorders determined by history, physical, and laboratory examinations. All subjects were from the mainland Han Chinese and provided written informed consents. The study protocols were approved by the hospital internal Ethics and Scientific Boards.

## MRI Scanning Parameters

Subjects were scanned on a 3 T GE-Discovery 750 scanner at Wenzhou Medical University, Zhejiang, China. For brain MRI, sagittal T1 weighted image (WI), axial fast spin-echo T2WI, axial/sagittal fast spin-echo FLAIR, axial diffusion, and apparent diffusion coefficient mapped images followed by postcontrast axial and coronal T1WI were analyzed. Small field of view axial and coronal T2WI with fat saturation and fat, saturated postcontrasted axial and coronal images were obtained for orbital evaluation. Sagittal T1, T2, STIR, and axial T1, T2WIs were obtained through the spine without contrast, then sagittal and axial T1WIs were obtained after gadolinium administration. All patients received intravenous gadolinium-based contrast media.

## Genotyping

Five milliliters of peripheral blood was drawn from each subject into an EDTA anticoagulant tube. The genomic DNA was extracted and purified using QIAamp DNA Blood Kit (Qiagen, Hilden, Germany) then stored at −20°C till use. The *HLA-DQA1* SNP was amplified by polymerase chain reaction; the sequences of primers used for the amplification of HLA-DQA1 with appropriate annealing temperatures were shown in Supplementary Table 1 (Supplemental Digital Content 1, http://links.lww.com/NRL/A67). Polymerase chain reaction products were examined by direct sequencing using an ABI3730XL genetic analyzer (Applied Biosystems, Life Technologies Co., Carlsbad, CA).

## Data Analysis

Statistical analysis was performed using SPSS 24.0. Allelic associations were calculated by the Pearson $\chi^2$ test. Three additional models, additive, dominant, and recessive, were used to assess the relationships between HLA-DQA1 polymorphism and susceptibility to NMOSD. The age-adjusted and sex-adjusted logistic regression analyses were applied. ORs and 95% confidence intervals (CIs) were calculated. The Bonferroni correction method was applied for multiple comparisons. Hardy-Weinberg equilibrium tests among subjects were performed by Pearson $\chi^2$ test. Age and sex between cases and controls were compared by the Student *t* test and Pearson $\chi^2$ test, respectively. A logistic regression model was applied to analyze the influences of age or sex on the association between each SNP and NMOSD. *P*-values $< 0.05$ were considered statistically significant.

# RESULTS

## Clinical Characteristics

A total of 51 patients with NMOSD (84% females) and 86 controls (81% females) were recruited, ages were 46.31 ± 17.27 years

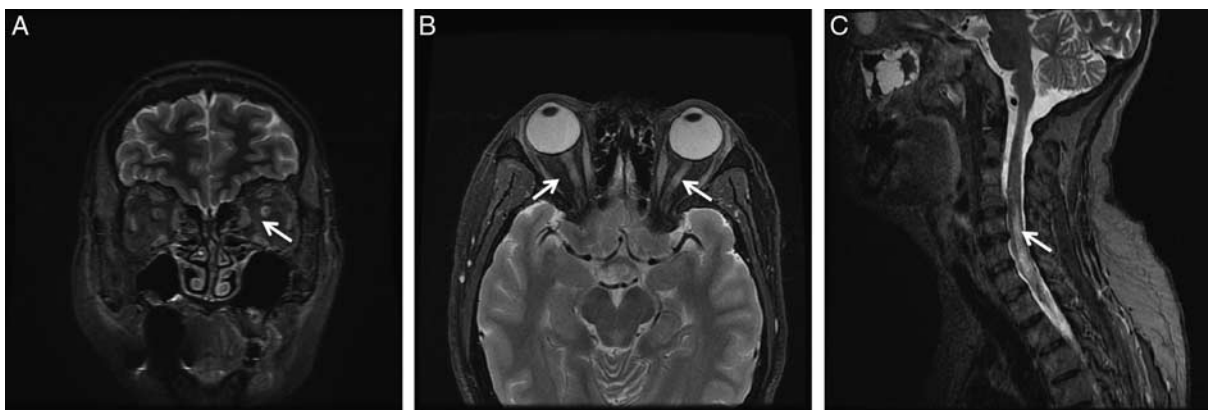


**FIGURE 1.** A 56-year-old woman with aquaporin-4–positive neuromyelitis optica spectrum disorder had left eye vision loss 1 month ago, a sudden visual field defect in the right eye, and numbness of the left lower limb appeared 3 days ago. Hyperintensity of the left optic nerve and swelling of the right optic nerve are visualized in coronal (arrow in A) and axial (arrow in B); a longitudinal extensive hyperintense lesion involving the cervical spinal cord (5 to 7) is seen, as well as swelling of the spinal cord on T2 STIR images (arrow in C).

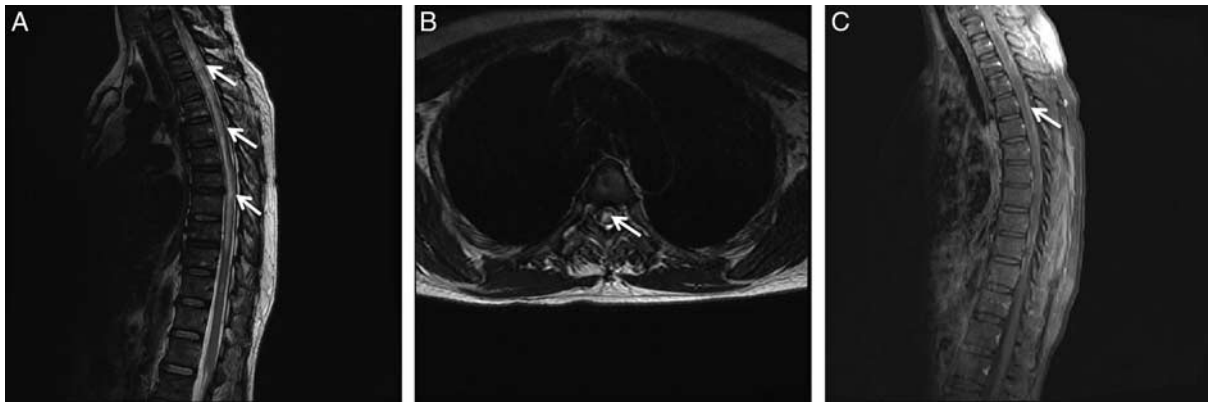


**FIGURE 2.** A 54-year-old woman with aquaporin-4–positive myelitis for 2 months. Spinal magnetic resonance imaging with sagittal T2-weighted image (A), axial (B), and sagittal T2 with gadolinium image (C). A longitudinal extensive T2-hyperintense lesion (arrow in A, B) involving the C4-T10 is seen. After gadolinium administration, a patchy, cloud-like contrast enhancement (arrow in C) on the axial section is observed.

(for NMOSD) and 48.60 ± 16.05 years (for HCs). There was no significant difference in sex or age between patients and controls. We evaluated 51 NMOSD patients and found that 43 patients were seropositive for AQP4-Ab, 8 patients with AQP4-Ab negative. In our study, among 51 NMOSD patients, 7 (~13.7%) had autoimmune disorders, including Sjogren syndrome (n = 4), myasthenia gravis (n = 1), Hashimoto thyroiditis (n = 1), and autoimmune hepatitis (n = 1). Furthermore, the positive concomitant autoantibodies were found, including antinuclear antibody (n = 17), anti-SSA/SSB (n = 7), double-stranded DNA antibodies (n = 3), anti-Ro52 antibody (n = 1), ribonucleoprotein (n = 1), and proliferating cell nuclear antibody (n = 1). Other clinical characteristics, including sex, age, age at onset, disease duration, Expanded Disability Status Scale score, and core clinical syndromes, are shown in Table 1.

## MRI Scanning

All 51 NMOSD patients underwent an MRI scan. Several abnormal lesions were found in brain (n = 9), medulla oblongata (n = 3), spinal cord (n = 35), and optic nerve (n = 19). Typical lesions are shown in Figures 1–3.

## HLA-DQA1 SNP Genotype Associations With Susceptibility to NMOSD

The selected SNP fulfilled the Hardy-Weinberg equilibrium ($P > 0.05$) in both cases and controls. Allelic and genotypic frequencies are summarized in Table 2. No evidence of association with NMOSD was found in the allelic and genotypic frequencies of rs28383224 located in HLA-DQA1. Logistic regression analysis showed no association of rs28383224 with NMOSD (AG vs. AA: OR = 1.028, 95% CI: 0.371-2.848, $P = 0.957$; GG vs. AA: OR = 0.672, 95% CI: 0.283-1.599, $P = 0.369$) (Table 3).

## DISCUSSION

Many factors, including external and internal factors, may be involved in the incidence of NMOSD. In the present study, we analyzed the influence factors of NMOSD at the genetic level. To the best of our knowledge, this is the first study to explore the association between rs28383224 of HLA-DQA1 and sporadic NMOSD in the Han Chinese population. Results demonstrated that SNP rs28383224 was not a risk factor for NMOSD in this population.

The HLA-DQA1, one of the major histocompatibility complex class II family members that locates on chromosome 6p21, may be a potential prognostic biomarker for NMOSD.[5,10,11] Aberrant expression of *HLA-II* may result in insufficient immune response or autoimmunity reaction, leading to lots of diseases, including NMOSD.[12,13] More importantly, numerous studies have shown that *HLA-II* members are

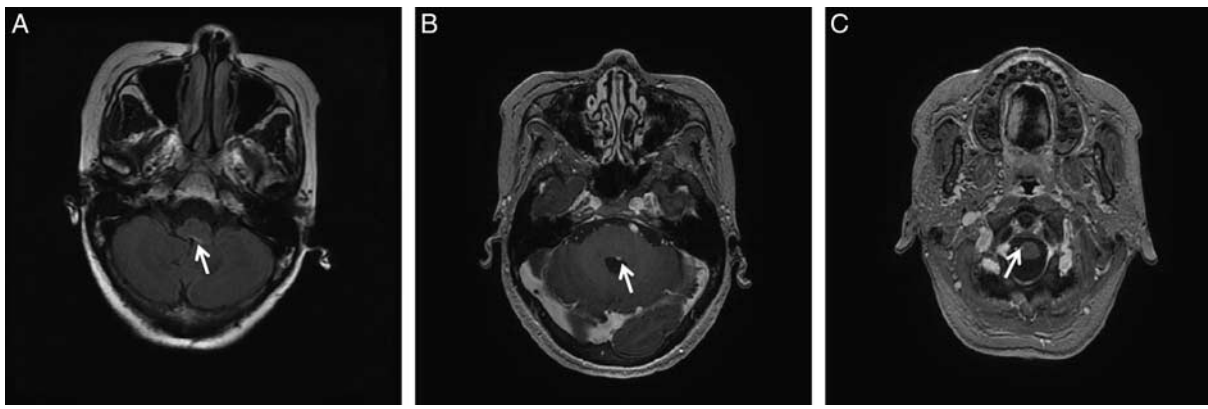


**FIGURE 3.** A case of a 62-year-old woman with neuromyelitis optica spectrum disorder with aquaporin-4 immunoglobulin G seropositivity. Rotating vertigo and vomiting for 15 days followed by 1 week of faintness. Transverse magnetic resonance imaging T2-FLAIR image shows dorsal brainstem (arrow in A) and patchy enhancement (arrows in B, C) after gadolinium administration.

**TABLE 2.** Comparisons of Allelic Frequencies Between Cases and Controls*

| | n (%) | | | |
|---|---|---|---|---|
| **Allele** | **NMOSD** | **Control** | $\chi^2$ | ***P*** |
| rs28383224 | | | | |
| A | 52 (50.98) | 86 (50.00) | 0.025 | 0.875 |
| G | 50 (49.02) | 86 (50.00) | | |

*2×2 $\chi^2$ test was performed to compare the differences between the categorical variables.
NMO indicates neuromyelitis optica.

involved in autoimmune diseases like NMOSD.[14,15] Given the influences of ethnicities and regions, the relationships of the SNPs located in *HLA-II* and NMOSD among different population groups are complex. In Europeans, a recent genome-wide association study identified rs28383224, located in *HLA-DQA1*, which had a strong association with NMOSD susceptibility.[8] A previous study in Japan reported that *HLA-DRBA1*05:03* presented a significantly increased risk of NMOSD, while no significant association of *HLA-DRA1*01:01* or *HLA-DQA1*03:02* with NMOSD was observed in the Japanese population.[16] Our case-control study suggests that rs28383224 is not the risk factor for sporadic NMOSD in a Han Chinese population, which may be attributable in part to the difference in ethnicity. A relatively small number of patients was included in the present study, therefore, we will increase the sample sizes for further investigation. And the potential molecular mechanisms underlying roles of the altered expression of HLA-DQA1 in NMOSD need to be further addressed.

Genetic alterations might lead to changes in protein conformation, and these changes may affect antigenicity. Thus, exploring the pathogenesis of NMOSD from the gene level was a promising research direction. Multiple genes[17–21] such as AQP4, *CD40*, *HLA-DPB*, *TNFSF4*, and *GTF2I* have been known as candidate genes for NMOSD, but inconsistent results were found in different ethnicity or regional research settings. Due to the limited number of patients, the information on the SNP site was relatively limited. The scope of research can be expanded, and the haplotype correlation analysis can be explored with a larger sample size. The continuous understanding and exploration of the genetic mechanism of NMOSD should be investigated, which will provide new clues for clinical solutions.

**TABLE 3.** Associations Between Neuromyelitis Optica Spectrum Disorder and Genotypes of *HLA-DQA1*

| **Locus/Gene (SNP)** | **Genotype** | **N** | **Case [n (%)]** | **OR (95% CI)*** | ***P*** |
|---|---|---|---|---|---|
| *HLA-DQA1* | AA | 18 | 14 (77.78) | 1.00 (1.000-1.000) | Reference |
| rs28383224 | AG | 50 | 24 (48.00) | 1.028 (0.371-2.848) | 0.957 |
| | GG | 18 | 13 (72.22) | 0.672 (0.28-1.599) | 0.369 |

*Age and sex were adjusted.
CI indicates confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

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