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

Lili Zhou, MD,* Zhiyong He, MD,* Lanbing Zhu, MD,* Juan-juan Zhu, MD,* Jian-Hong Zhu, PhD,**† and Jialin Pan, MD‡

ORIGINAL ARTICLE

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. 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. 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. 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. The HLA region also contributes to the genetic architecture of NMOSD in different populations. Variations in HLA-DQA1 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, as a strong association with NMOSD in the Han Chinese population. The discovery of aquaporin-4 (AQP4) immunoglobulin G, an antibody against the astrocyte water channel in the CNS, clearly identified NMOSD as a separate disease from multiple sclerosis. 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. 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.

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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 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,
age, age at onset, disease duration, Expanded Disability Status Scale score, 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 TIWI were analyzed. Small field of view axial and coronal T2WI with fat saturation and fat, saturated postcontrast 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 TIWIs 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 χ² 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 χ² test. Age and sex between cases and controls were compared by the Student t test and Pearson χ² 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

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) | 43:8 (84.31)   | NA               | NA   |
| EDSS score (mean ± SD)  | 2.66 ± 2.12    | NA               | NA   |
| Core clinical syndromes |               |                  |      |
| Acute myelitis          | 19/51 (37.25)  | NA               | NA   |
| Optic neuritis          | 25/51 (49.02)  | NA               | NA   |
| Acute myelitis+optic neuritis | 5/51 (9.80)  | NA               | NA   |
| Area postrema syndrome  | 2/51 (3.92)    | NA               | NA   |
| Complicate with autoimmune diseases | 8/51 (15.69) | NA               | NA   |
| MRI lesions [n/N (%)]   |               |                  |      |
| 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.

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).
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. Aberrant expression of HLA-II may result in insufficient immune response or autoimmunity reaction, leading to lots of diseases, including NMOSD. More importantly, numerous studies have shown that HLA-II members are
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 \(HLA-DRB1*05:03\) 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.

| Locus/Gene (SNP) | Genotype | Case [n (%)] | OR (95% CI) | \(P\) |
|------------------|----------|--------------|-------------|-----|
| \(HLA-DQA1\)     | AA       | 18 (77.78)   | 1.00 (1.000-1.000) | Reference |
| \(rs28383224\)   | AG       | 50 (48.00)   | 1.028 (0.371-2.848) | 0.957 |
|                  | GG       | 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.

**TABLE 2.** Comparisons of Allelic Frequencies Between Cases and Controls\(^*\)

| 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) | | |

\(*2\times2\) \(\chi^2\) test was performed to compare the differences between the categorical variables.

NMO indicates neuromyelitis optica.

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**REFERENCES**

1. Wingerchuk DM, Hogancamp WF, O’brien PC, et al. The clinical course of neuromyelitis optica (Devic’s syndrome). Neurology. 1999;53:1107–1114.

2. Lennon VA, Wingerchuk DM, Kryzer TJ, et al. A serum autoantibody marker of neuromyelitis optica: distinction from multiple sclerosis. Lancet. 2004;364:2106–2112.

3. Wingerchuk DM, Banwell B, Bennett JL, et al. International consensus diagnostic criteria for neuromyelitis optica spectrum disorders. Neurology. 2015;85:177–189.

4. Ogasawara M, Meguro A, Sakai T, et al. Genetic analysis of the aquaporin-4 gene for anti-AQP4 antibody-positive neuromyelitis optica in a Japanese population. Jpn J Ophthalmol. 2016;60:198–205.

5. Alvarenga MP, Fernandez O, Leyva L, et al. The HLA-DRB1*03:01 allele is associated with NMO regardless of the NMO-IgG status in Brazilian patients from Rio de Janeiro. J Neuroimmunol. 2017;310:1–7.

6. Shi Z, Zhang Q, Chen H, et al. Association of \(CD40\) gene polymorphisms with susceptibility to neuromyelitis optica spectrum disorders. Mol Neurobiol. 2017;54:5236–5242.

7. Liu Z, Liu J, Shi Z, et al. Association of \(TNFSF4\) polymorphisms with neuromyelitis optica spectrum disorders in a Chinese Population. J Mol Med. 2012;79:402–407.

8. Estrada K, Whelan CW, Zhao F, et al. A whole-genome sequence study identifies genetic risk factors for neuromyelitis optica. Nat Commun. 2018;9:1929.

9. Kurtzke JF. Rating neurologic impairment in multiple sclerosis: an Expanded Disability Status Scale (EDSS). Neurology. 1983;33:1444–1452.

10. Yamasaki K. \(HLA-DPB1\)* 0501-associated opticospinal multiple sclerosis: clinical, neuroimaging and immunogenetic studies. Fukuoka Igaku Zasshi. 2000;91:243–245.

11. Brill L, Mandel M, Karussis D, et al. Increased occurrence of anti-AQP4 seropositivity and unique HLA class II associations with neuromyelitis optica (NMO), among Muslim Arabs in Israel. J Neuroimmunol. 2016;293:65–70.

12. Papadopoulos MC, Verkman AS. Aquaporin 4 and neuromyelitis optica. Lancet Neurol. 2012;11:535–544.

13. Prasad S, Chen J, What You Need To Know About AQP4, MOG, and NMOSD. Semin Neurol. 2019;39:718–731.

14. Bruijstens AL, Wong YYM, Van Pelt DE, et al. HLA association in MOG-IgG- and AQP4-IgG-related disorders of the CNS in the Dutch population. Neurol Neuroimmunol Neuroinf. 2020;7:e702.

15. Muñiz-Castrillo S, Vogrig A, Honnorat J. Associations between HLA and autoimmune neurological diseases with autoantibodies. Auto Immun Highlights. 2020;11:2.

16. Ogawa K, Okuno T, Hosomi K, et al. Next-generation sequencing identifies contribution of both class I and II HLA genes on susceptibility of multiple sclerosis in Japanese. J Neuroinflammation. 2019;16:162.

17. Xie JL, Liu J, Lian ZY, et al. Association of \(GTF2IRD1-GTF2I\) polymorphisms with neuromyelitis optica spectrum disorders in Han Chinese patients. Neurol Res. 2019;41:346–353.

18. Wang QS, Xiao HQ, Chen BX, et al. The single nucleotide polymorphism site of aquaporin-4 gene in patients with neuromyelitis optica. Exp Ther Med. 2017;14:6017–6021.

19. Asgari N, Owens T, Freikha J, et al. Neuromyelitis optica (NMO) - an autoimmune disease of the central nervous system (CNS). Acta Neurol Scand. 2011;123:369–384.

20. Romero-Hidalgo S, Flores-Rivera J, Rivas-Alonso V, et al. Native American ancestry significantly contributes to neuromyelitis optica susceptibility in the admixed Mexican population. Sci Rep. 2020;10:13706.

21. Park TJ, Kim JH, Kim HJ, et al. Lack of association between AQP4 polymorphisms and risk of inflammatory demyelinating disease in a Korean population. Gene. 2014;536:302–307.