Variants of Interferon Regulatory Factor 5 are Associated with Neither Neuromyelitis Optica Nor Multiple Sclerosis in the Southeastern Han Chinese Population

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Background: Neuromyelitis optica (NMO) and multiple sclerosis (MS) are demyelinating disorders of the central nervous system. Interferon regulatory factor 5 (IRF5) is a common susceptibility gene to different autoimmune disorders. However, the association of IRF5 variants with NMO and MS patients has not been well studied. Therefore, we aimed to evaluate whether IRF5 variants were associated with NMO and MS in the Southeastern Han Chinese population.

Methods: Four single nucleotide polymorphisms (SNPs) were selected and genotyped by matrix-assisted laser desorption/ionization time of flight mass spectrometry in 111 NMO patients, 145 MS patients and 300 controls from Southeastern China.

Results: None of these 4 SNPs was associated with NMO or MS patients.

Conclusions: Our preliminary study indicates that genetic variants in IRF5 may affect neither NMO nor MS in the Southeastern Han Chinese population. Further studies with a large sample size and diverse ancestry populations are needed to clarify this issue.

Key words: Association; Chinese; Interferon Regulatory Factor 5; Multiple Sclerosis; Neuromyelitis Optica

Abstract

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**Methods**

**Subjects**

Between September 2008 to August 2012, 111 NMO patients were recruited according to the 2006 Wingerchuk criteria and 145 MS patients were recruited according to the revised McDonald criteria for MS. All the patients underwent detailed neurological examinations, laboratory tests, and magnetic resonance imaging scans of the brain and/or spinal cord. The patients were followed up at regular intervals. All of the patients were Han Chinese from Southeastern China. In addition, 300 unrelated controls with no history of autoimmune diseases were matched for case ethnicity and region. The study protocol was approved by the local research ethics committees. A signed informed consent was obtained from each participant.

**Detection of anti‑against aquaporin 4 antibodies**

Anti‑AQP4 antibodies were tested with an indirect immunofluorescence assay using HEK293 cells transfected with recombinant human AQP4 gene (Euroimmun, Lubeck, Germany) according to the instruction

Each sample was measured at least twice, with the examiners blind to the origin of the specimens. Samples with twice positive results were deemed to be anti‑AQP4 antibodies positive.

**Genotyping**

Genomic DNA was extracted from peripheral blood using a TIANamp Blood DNA kit (TIANGEN Biotech, Beijing, China). Four selected SNPs were genotyped using the Sequenom MassArray system. We used MassArray Assay Design 3.1 software (Sequenom, San Diego, USA) to design the polymerase chain reaction (PCR) primers used in the genotyping. The PCR and extension primers for these 4 SNPs are shown in Table 1. Alleles were detected using a matrix‑assisted laser desorption/ionization time of flight mass spectrometry platform (MassArray TM, Sequenom Inc., San Diego, CA, USA) according to a previously described method.

**Statistical analyses**

The $\chi^2$ test was used to analyze the Hardy–Weinberg equilibrium. Differences in allele frequencies between controls and cases, odds ratios and 95% confidence intervals were analyzed using the $\chi^2$ test or Fisher’s exact test. All statistical analyses were performed by SPSS 16.0 software (SPSS Inc., USA). The criterion for a significant difference was $P < 0.05$.

**Results**

Overall, we excluded 3 NMO patients, 3 MS patients and 8 controls who had a SNP genotyping success rate <90%, remaining a total of 108 NMO patients, 142 MS patients and 292 controls analyzed in this study. Their demographic and clinical characteristics are listed in Table 2. Anti‑AQP4 antibodies were tested in 68 NMO patients and 35 (51.5%) were positive.

All selected SNPs were in Hardy–Weinberg equilibrium [Table 3]. As showed in Table 4, there was no significant allele or genotype association for rs2280714, rs3807306, rs4728142 and rs729302 identified in NMO patients compared with controls. Similarly, no statistically significant association was observed for any of the SNPs between MS patients and controls. In further analysis, according to the status of anti‑AQP4 antibodies, we found no significance in allele frequencies or genotype distributions among anti‑AQP4 antibodies positive NMO patients, MS patients and controls.

**Discussion**

Interferon regulatory factor 5 is a common susceptibility gene to different autoimmune disorders. Recent findings have revealed the strongest evidence of associations between variants in the human IRF5 locus and a wide range of autoimmune diseases. In a previous association study on rheumatoid arthritis (RA), 4 SNPs in IRF5 including rs375385, rs2004640, rs752637 and rs3807306 were associated with RA.[23] At the same time, they also found that IRF5 polymorphisms were associated with inflammatory bowel diseases and systemic lupus erythematosus.[24,25] In addition, a GWAS of MS patients showed evidence of association between the rs3807306 of IRF5 and the development of MS in the initial screening phase.[5] Subsequently, a study by Kristjansdottir et al. reported that the rs4728142 and rs3807306 were associated with MS in

| Table 1: Primer sequences using for analysis of the IRF5 variants |
|---------------------------------------------------------------|
| Variants | Missing rate % | MS versus controls | NMO versus controls | PCR primers |
|---------|----------------|--------------------|--------------------|-------------|
| rs729302 | 0.01104 | 0.01679 | ACGTTGGATGGGAAATAGACCGAGACCAG | CCGTCCATGGGACAAGGTGAGAC |
| rs4728142 | 0.006623 | 0.007194 | ACGTTGGATGTGGACTCTGGTGTGTAGGTG | CCGTCCATGGGACAAGGTGAGAC |
| rs3807306 | 0.00883 | 0.004796 | ACGTTGGATGTCAGTTCCGCTTTCTGCCC | CCGTCCATGGGACAAGGTGAGAC |
| rs2280714 | 0 | 0 | ACGTTGGATGCCATAAATTCTGACCCTGGC | CCGTCCATGGGACAAGGTGAGAC |

PCR: Polymerase chain reaction; MS: Multiple sclerosis; NMO: Neuromyelitis optica.
Table 2: Characteristics of the participants

| Items                        | MS (n = 142) | NMO (n = 108) | Control (n = 292) |
|------------------------------|--------------|---------------|-------------------|
| Male/female                  | 59/83        | 19/89         | 171/121           |
| Age at analysis, years       | 39.9 ± 13.2  | 43.9 ± 14.5   | 36.9 ± 15.6       |
| Age at onset, years          | 32.3 ± 12.6  | 36.8 ± 14.3   | NA                |
| Relapsing-remitting course, n (%) | 137 (96.5)   | 102 (94.4)    | NA                |
| AQP4-ab positive/total, n (%) | 0/80 (0.0)   | 35/68 (51.5)  | NA                |

MS: Multiple sclerosis; NMO: Neuromyelitis optica; AQP4-ab: Anti-aquaporin-4 antibodies; NA: Not available.

Table 3: Hardy–Weinberg equilibrium test of participants

| Variants | MS (n = 142) | n (expected) | NMO (n = 108) | Controls (n = 292) |
|----------|--------------|--------------|---------------|-------------------|
| rs2280714 |              |              |               |                   |
|          |              | rs729302     | rs4728142     |                   |

Table 4: Allele and genotype distributions of IRF5 variants among MS, NMO and controls

| Variants | Allele/ genotype Controls (n = 292) (%) | MS (n = 142) (%) | NMO (n = 108) (%) | NMO (n = 35) (%) | MS versus controls | NMO versus controls | NMO versus MS | NMO versus controls | NMO versus MS |
|----------|----------------------------------------|------------------|-------------------|------------------|--------------------|---------------------|-----------------|-------------------|-----------------|
| rs2280714| CC                                     | 46 (15.75)       | 26 (18.31)        | 25 (25.93)       | 7 (20.00)          | 0.572               | 0.065           | 0.247             | 0.776           | 0.743           |
|          | CT                                     | 148 (50.69)      | 75 (52.82)        | 47 (43.52)       | 16 (45.71)         | 0.311               | 0.095           | 0.510             | 0.777           | 0.779           |
|          | TT                                     | 98 (33.56)       | 41 (28.87)        | 33 (30.55)       | 12 (34.29)         | 0.344               | 0.550*          | 0.643*            | 0.682*          | 0.286*          |
| rs3807306| GG                                     | 179 (61.30)      | 95 (66.90)        | 73 (67.59)       | 21 (60.00)         | 0.443               | 0.237           | 0.659             | 0.862           | 0.798           |
|          | GT                                     | 104 (35.62)      | 41 (28.87)        | 33 (30.56)       | 14 (40.00)         | 0.372               | 0.207           | 0.677             | 0.952           | 0.659           |
| rs4728142| GG                                     | 211 (72.26)      | 108 (76.06)       | 84 (77.78)       | 25 (71.43)         | 0.656*              | 0.388*          | 0.932             | 0.899*          | 0.612*          |
|          | GA                                     | 77 (26.37)       | 33 (23.24)        | 24 (22.22)       | 10 (28.57)         | 0.372               | 0.207           | 0.677             | 0.952           | 0.659           |
|          | AA                                     | 4 (1.37)         | 1 (0.70)          | 0 (0)            | 0 (0)              | 0.693               | 0.664           | 0.839             | 0.647*          | 0.759*          |
| rs729302 | GG                                     | 29 (9.93)        | 15 (10.56)        | 9 (8.33)         | 2 (5.71)           | 0.464               | 0.819           | 0.720             | 0.920           | 0.626           |
|          | GT                                     | 129 (44.18)      | 68 (47.89)        | 53 (49.08)       | 18 (51.43)         | 0.372               | 0.207           | 0.677             | 0.952           | 0.659           |

*Analyzed by Fisher’s exact test. MS: Multiple sclerosis; NMO: Neuromyelitis optica; NMO*: Total neuromyelitis optica patients; NMO**: Anti-aquaporin-4 antibodies positive neuromyelitis optica patients.
three different population cohorts from Sweden, Spain and Finland.\textsuperscript{[26]}

Here, we constituted a case–control association study in order to investigate the contribution of variants located in \textit{IRF5} in NMO and MS susceptibility. To the best of our knowledge, this has been the first effort, in any population, to address the association between NMO and common variants of \textit{IRF5}. However, no significant difference of genotypes and alleles was detected in NMO patients compared with controls. We also want to determine whether there is an intrinsic association between anti-AQP4 antibodies positive NMO patients and \textit{IRF5} variants. So the NMO group was further divided into two disparate disease entities: Anti-AQP4 antibodies positive patients and anti-AQP4 antibodies negative patients. However, we did not see any association of anti-AQP4 antibodies positive NMO patients with \textit{IRF5} variants. Therefore, \textit{IRF5} variants may not play a major role in genetic predisposition to NMO in the Southeastern Han Chinese population.

In addition, the current study failed to certify a significant association of \textit{IRF5} variants with MS. Similarly, these effects were not replicated by Vandenbroeck \textit{et al.} in North (Bilbao, San Sebastian) Spain.\textsuperscript{[27]} However, the association of \textit{IRF5} variants with MS was observed in the following combined analysis including North, Central and South Spain and Sweden.\textsuperscript{[27]} Although it is difficult to elucidate the reasons for this discrepancy, several explanations should be considered. First and most importantly, \textit{IRF5} polymorphisms are distinct in different ancestral backgrounds. Second, MS is a multifactorial disease caused by the interaction between environmental and inherited factors, different environmental factors such as smoking and lifestyle may affect the results from inherited factors. Third, it could be that the association of these variants has a weak effect size, which this study does not have sufficient power to detect. In addition, there are other SNPs in the same locus associated with MS in the Southeastern Han Chinese population. Hence, further analyses are needed to explore whether other variants associated with MS exist in this locus.

In conclusion, although this is a preliminary study, our results indicate that genetic variants in the \textit{IRF5} gene may affect neither NMO nor MS in the Southeastern Han Chinese population. Further studies with a large sample size and diverse ancestry populations are needed to clarify the associations of \textit{IRF5} variants with NMO and MS.

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