Original Article

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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Abstract

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

Introduction

Neuromyelitis optica (NMO) is an idiopathic inflammatory demyelinating disorder of the central nervous system characterized by recurrent attacks of severe optic neuritis and myelitis, which is relatively common in Asian populations.[1] Serum immunoglobulin G (NMO), an autoantibody against aquaporin 4 (AQP4), has been shown to be a highly specific biomarker for NMO, distinguishing it from multiple sclerosis (MS).[2] Evidences suggest these diseases are caused by genetic and environmental factors.[3,4]

The genetic component of MS is complex and a number of genes have been implicated in MS susceptibility.[5-8] However, few comprehensive analysis of the association in NMO has been reported. A single nucleotide polymorphism (SNP) within the promoter of *CYP7A1* encoding cytochrome P450 enzyme has been identified as a protective factor of NMO in a genome-wide association study (GWAS),[9] which was replicated in our previous study.[10] Also, the *DPB1**0501 allele was associated with NMO in Japanese and Southern Han Chinese,[11,12] while the *DRB1**03 allele was associated with NMO in Caucasians,[13] suggesting differences in genetic background. In addition, our recent study showed no association between non-MHC MS risk loci and NMO.[14] Thus, the genetic susceptibility of NMO is apparently different from MS.

The interferon regulatory factors (IRFs) play critical roles in cytokine signaling, cell growth and apoptosis, as well as the regulation of the immune response.[15] IRF5 is expressed mainly in dendritic cells, monocytes and B cells and has an important role in interferon production.[16,17] In addition, the B-cell-intrinsic role of *IRF5* is important in promoting the inflammatory process and also in autoimmune pathology by increasing the expression of a kind of antibody isotype.[18]

However, the association of *IRF5* variants with NMO and MS patients has not been well studied. Therefore, the aim of the present study was to evaluate whether *IRF5* variants were associated with NMO and MS in the Southeastern Han Chinese population.

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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.[19,20] 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.[21] 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 the 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.[22]

Statistical analyses

The χ^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 χ^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 %		PCR primers	Mass EXTEND primers
	MS versus controls	NMO versus controls		
rs729302	0.01104	0.01679	ACGTTGGATGGGAAATAGACCAGAGACCAG ACGTTGGATGTGGACTCTGGTGTGTAGGTG	CCCTTCCATGGGACAAGGTGAAGAC
rs4728142	0.006623	0.007194	ACGTTGGATGCCTTCCTCCCCATTTCTTAC ACGTTGGATGTAACAGTGCTGGCTGAATGG	GGCCTCCCCATTTCTTACTAACAC
rs3807306	0.00883	0.004796	ACGTTGGATGTCAGTTCCGCTTTCTGCCC ACGTTGGATGCAGTGAGTCTGGTTTCTGAG	GGGCAACGAAAGTGGCTAGAC
rs2280714	0	0	ACGTTGGATGCCATAAATTCTGACCCTGGC ACGTTGGATGAGGAGGAGTAAGCAAGGAAC	CGACCCTGGCAGGTCC

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	n (expected)					
	MS (n = 142)		NMO (n = 108)		Controls (n = 292)	
rs2280714						
CC	26 (28.40)	χ^2 = 0.66, P = 0.42	28 (24.56)	χ^2 = 1.76, P = 0.18	46 (49.32)	χ^2 = 0.64, P = 0.42
CT	75 (70.21)		47 (53.88)		148 (141.37)	
TT	41 (43.40)		33 (29.56)		98 (101.32)	
rs3807306						
GG	95 (93.95)	χ^2 = 0.34, P = 0.56	73 (74.17)	χ^2 = 0.63, P = 0.43	179 (182.74)	χ^2 = 1.76, P = 0.19
GT	41 (43.11)		33 (30.66)		104 (96.51)	
TT	6 (4.95)		2 (3.17)		9 (12.74)	
rs4728142						
GG	108 (109.16)	χ^2 = 0.81, P = 0.37	84 (85.33)	χ^2 = 1.69, P = 0.19	211 (213.19)	χ^2 = 1.06, P = 0.30
GA	33 (30.69)		24 (21.33)		77 (72.63)	
AA	1 (2.16)		0 (1.33)		4 (6.19)	
rs729302						
CC	15 (16.91)	χ^2 = 0.50, P = 0.48	9 (11.67)	χ^2 = 1.35, P = 0.24	29 (29.94)	χ^2 = 0.06, P = 0.80
CA	68 (64.18)		53 (47.66)		129 (127.12)	
AA	59 (60.91)		46 (48.67)		134 (134.94)	

A P value of 0.05 was considered statistically significant. MS: Multiple sclerosis; NMO: Neuromyelitis optica.

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

Variants	Allele/ genotype	Controls (n = 292) (%)	MS (n = 142) (%)	NMO^T (n = 108) (%)	NMO^P (n = 35) (%)	Chi-square value (P)				
						MS versus controls	NMO^T		NMO^P	
							Versus controls	Versus MS	Versus controls	Versus MS
rs2280714	CC	46 (15.75)	26 (18.31)	28 (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)					
	TT	98 (33.56)	41 (28.87)	33 (30.55)	12 (34.29)					
	C	240 (41.10)	127 (44.72)	103 (47.69)	30 (42.86)	0.311	0.095	0.510	0.777	0.779
	T	344 (58.90)	157 (55.28)	113 (52.31)	40 (57.14)					
rs3807306	GG	179 (61.30)	95 (66.90)	73 (67.59)	21 (60.00)	0.344	0.550*	0.643*	0.682*	0.286*
	GT	104 (35.62)	41 (28.87)	33 (30.56)	14 (40.00)					
	TT	9 (3.08)	6 (4.23)	2 (1.85)	0 (25.93)					
	G	462 (79.11)	231 (81.34)	179 (82.87)	56 (80.00)	0.443	0.237	0.659	0.862	0.798
	T	122 (20.89)	53 (18.66)	37 (17.13)	14 (20.00)					
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)					
	AA	4 (1.37)	1 (0.70)	0 (0)	0 (0)					
	G	499 (85.45)	249 (33.97)	192 (88.89)	60 (85.71)	0.372	0.207	0.677	0.952	0.659
	A	85 (14.55)	35 (66.03)	24 (11.11)	10 (14.29)					
rs729302	CC	29 (9.93)	15 (10.56)	9 (8.33)	2 (5.71)	0.693	0.664	0.839	0.647*	0.759*
	CA	129 (44.18)	68 (47.89)	53 (49.08)	18 (51.43)					
	AA	134 (45.89)	59 (41.55)	46 (42.59)	15 (42.86)					
	C	187 (32.02)	98 (34.51)	71 (32.87)	22 (31.43)	0.464	0.819	0.720	0.920	0.626
	A	397 (67.98)	186 (65.49)	145 (67.13)	48 (68.57)					

*Analyzed by Fisher's exact test. MS: Multiple sclerosis; NMO: Neuromyelitis optica; NMO^T: Total neuromyelitis optica patients; NMO^P: Anti-aquaporin-4 antibodies positive neuromyelitis optica patients.

three different population cohorts from Sweden, Spain and Finland.[26]

Here, we constituted a case–control association study in order to investigate the contribution of variants located in *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 *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 *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 *IRF5* variants. Therefore, *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 *IRF5* variants with MS. Similarly, these effects were not replicated by Vandenbroeck *et al*. in North (Bilbao, San Sebastian) Spain.[27] However, the association of *IRF5* variants with MS was observed in the following combined analysis including North, Central and South Spain and Sweden.[27] Although it is difficult to elucidate the reasons for this discrepancy, several explanations should be considered. First and most importantly, *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 *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 *IRF5* variants with NMO and MS.

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