Association of *GTF2IRD1–GTF2I* polymorphisms with neuromyelitis optica spectrum disorders in Han Chinese patients
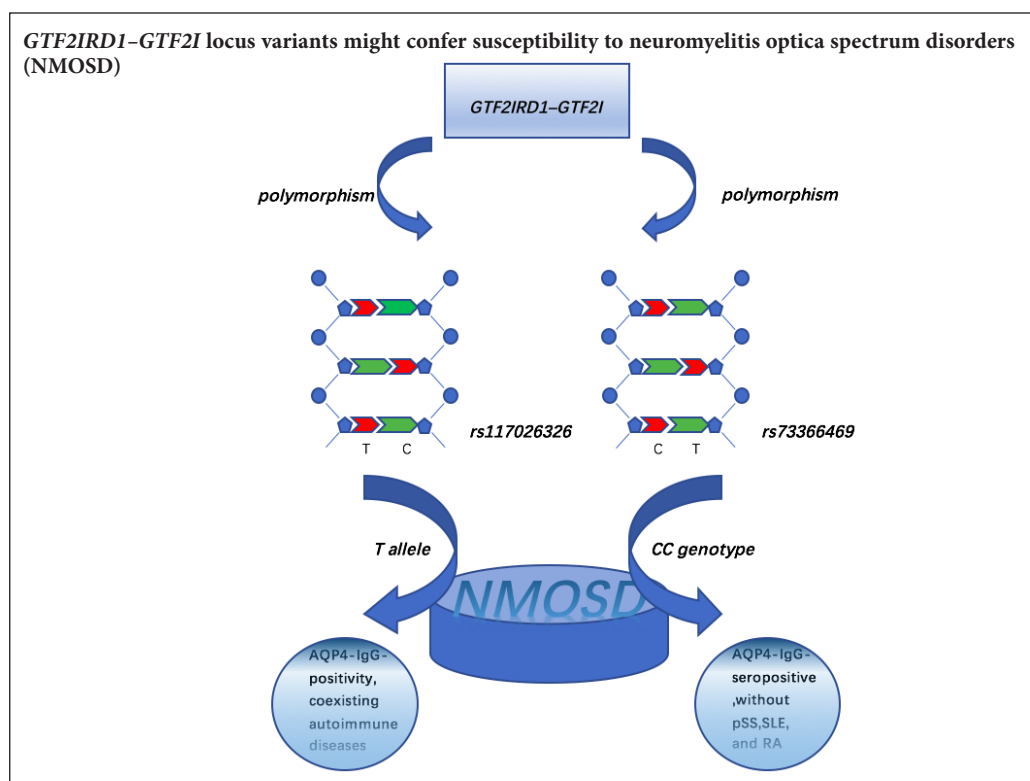
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Graphical Abstract

GTF2IRD1–GTF2I locus variants might confer susceptibility to neuromyelitis optica spectrum disorders (NMOSD)

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Abstract

Variants at the GTF2I repeat domain containing 1 *(GTF2IRD1)–GTF2I* locus are associated with primary Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis. Numerous studies have indicated that this susceptibility locus is shared by multiple autoimmune diseases. However, until now there were no studies of the correlation between *GTF2IRD1–GTF2I* polymorphisms and neuromyelitis optica spectrum disorders (NMOSD). This case control study assessed this association by recruiting 305 participants with neuromyelitis optica spectrum disorders and 487 healthy controls at the Department of Neurology, from September 2014 to April 2017. Peripheral blood was collected, DNA extracted and the genetic association between *GTF2IRD1–GTF2I* polymorphisms and neuromyelitis optica spectrum disorders in the Chinese Han population was analyzed by genotyping. We found that the T allele of rs117026326 was associated with an increased risk of neuromyelitis optica spectrum disorders (odds ratio (*OR*) = 1.364, 95% confidence interval (*CI*) 1.019–1.828; *P* = 0.037). This association persisted after stratification analysis for aquaporin-4 immunoglobulin G antibodies (AQP4-IgG) positivity (*OR* = 1.397, 95% *CI* 1.021–1.912; *P* = 0.036) and stratification according to coexisting autoimmune diseases (*OR* = 1.446, 95% *CI* 1.072–1.952; *P* = 0.015). Furthermore, the CC genotype of rs73366469 was frequent in AQP4-IgG-seropositive patients (*OR* = 3.15, 95% *CI* 1.183–8.393, *P* = 0.022). In conclusion, the T allele of rs117026326 was associated with susceptibility to neuromyelitis optica spectrum disorders, and the CC genotype of rs73366469 conferred susceptibility to AQP4-IgG-seropositivity in Han Chinese patients. The protocol was approved by the Ethics Committee of West China Hospital of Sichuan University, China (approval number: 2016-31) on March 2, 2016.

Chinese Library Classification No. R446; R741

Introduction

Neuromyelitis optica (NMO, Devic's disease) is an autoimmune inflammatory disorder of the central nervous system characterized by severe attacks of optic neuritis and acute transverse myelitis, and is distinct from multiple sclerosis (Lennon et al., 2004; Wingerchuk et al., 2006). The discovery of immunoglobulin G antibodies to aquaporin-4 (AQP4-IgG), which is a specific biomarker of NMO (Lennon et al., 2004), has helped to further define the concept of NMO spectrum disorders (NMOSD) (Wingerchuk et al., 2007), for which the diagnostic criteria were published in 2015 by the International Panel for NMO Diagnosis (Wingerchuk et al., 2015). Notably, Asians are reported to have a higher prevalence of NMOSD than white populations (Kim and Kim, 2016). Although the etiology and pathogenesis of NMOSD have not been fully elucidated, a genetic component to susceptibility to NMOSD has been established over recent years. Is single nucleotide polymorphism, one type of genetic variation, involved in the pathogensis of NMOSD?

Recently, gene modification therapy has attracted increasing attention. If genetic variations can be associated with susceptibility to NMOSD, this would provide a theoretical basis for gene modification therapy.

Genetic association studies in NMOSD have identified several susceptibility loci, including human leukocyte antigen (*HLA*) (Zéphir et al., 2009; Pandit et al., 2015), cluster of differentiation 58 (CD58) (Kim et al., 2014; Liu et al., 2017), interleukin 17 (Wang et al., 2012), 25-hydroxyvitamin D(3)-1alpha-hydroxylase (Zhuang et al., 2015), Fc receptor-like 3 (Wang et al., 2016) and cluster of differentiation 40 (*CD40*) genes (Shi et al., 2017). The most consistently replicated of these associations is with the *HLA-DRB1*03* allele (Zéphir et al., 2009; Brum et al., 2010; Deschamps et al., 2011; Pandit et al., 2015).

GTF2IRD1 and *GTF2I* at 7q11.23 encode multifunctional phosphoproteins that are critical factors involved in general transcription and signal transduction, ultimately contributing to the regulation of T- and B-cell activation (Sacristán et al., 2009; Roy, 2012). TFII-I encoded by *GTF2I* might interact with B-cell specific transcription factors, such as Bright, thereby playing an important role in establishing enhancer–promoter communication and regulating immunoglobulin heavy chain transcription (Rajaiya et al., 2006; Roy et al., 2011). Furthermore, GTF2IRD1 can regulate transcription by mediating chromatin modification (Carmona-Mora et al., 2015). *GTF2IRD1* and *GTF2I* have been reported to be the main genes responsible for the neurocognitive profile of Williams–Beuren syndrome (Antonell et al., 2010; Vandeweyer et al., 2012), and a *GTF2IRD1* mutation in the Williams–Beuren syndrome critical region results in craniofacial abnormalities (Howard et al., 2012). Recently, variants at the *GTF2IRD1–GTF2I* locus have also been found to be associated with primary Sjögren's syndrome (Li et al., 2013; Zheng et al., 2015; Song et al., 2016), systemic lupus erythematosus (Li et al., 2015; Morris et al., 2016; Sun et al., 2016), and rheumatoid arthritis (Kim et al., 2016), indicating that this susceptibility locus is shared by multiple autoimmune diseases. Furthermore, NMOSD probably coexists with these autoimmune diseases (Pittock et al., 2008; Nagaishi et al., 2011), which implies that variants at the *GTF2IRD1–GTF2I* locus might also confer susceptibility to NMOSD.

To the best of our knowledge, there are no available data on the relationship between *GTF2IRD1–GTF2I* polymorphisms and the risk of NMOSD. Therefore, this study examined whether certain single nucleotide polymorphisms (SNPs) at this locus predispose individuals from a Han Chinese population from western China to NMOSD. Our study analyzed the association between *GTF2IRD1–GTF2I* alleles, genotypes, linkage disequilibrium, and haplotypes and NMOSD. Additionally, this study analyzed the AQP4-IgG positive subgroup of NMOSD patients and the subgroup with coexisting other autoimmune diseases, to explore the correlation between *GTF2IRD1–GTF2I* polymorphisms and the expression of autoantibodies.

Participants and Methods

Study participants

This was a case-control study. From September 2014 to April 2017, 305 participants with sporadic NMOSD, who fulfilled the diagnostic criteria of 2015 International Panel for NMO Diagnosis (Wingerchuk et al., 2015), and were receiving outpatient or inpatient treatment at the Department of Neurology were prospectively consecutively enrolled. Over the same time period we also enrolled 487 unrelated healthy controls, matched with the patients according to age and sex.

All the participants were of Han Chinese ethnicity. Clinical data, including age at disease onset, disease duration, annual relapse rate, Expanded Disability Status Scale score, serum AQP4-IgG status (cell-based assay), clinical phenotypes, magnetic resonance imaging lesions, and coexisting autoimmune diseases, were recorded. Peripheral venous bloods (3–5 mL) were also collected from all participants in tubes containing ethylenediaminetetraacetic acid.

Written informed consent was obtained from all participants before their enrollment. This study was carried out according to the requirements of the *Declaration of Helsinki* and the protocol was approved by the Ethics Committee of West China Hospital of Sichuan University, China (approval number: 2016-31) on March 2, 2016.

SNP detection and genotyping

Based on previous studies (Li et al., 2013; Jabbi et al., 2015; Zheng et al., 2015; Kim et al., 2016; Morris et al., 2016; Song et al., 2016; Sun et al., 2016), five SNPs, including GTF2IRD1 rs4717901 and rs11981999, GTF2I rs117026326 and rs2527367, and rs73366469 in the *GTF2IRD1–GTF2I* intergenic region, were selected for genotyping (**Table 1**).

Genomic DNA was isolated from peripheral venous blood leukocytes using the AxyPrep Blood Genomic DNA Midi-prep Kit 25-prep (AxyGen, Shanghai, China) as per the manufacturer's instruction and stored at −20°C. All of the SNPs were determined using a custom-by-design 48-

Table 1 Five SNPs identified at the *GTF2IRD1–GTF2I* locus in this study

SNP	Substitution	Chromosome	Position	Gene name	Region
rs117026326	C > T	7	74126034	*GTF2I*	Intron
rs2527367	T > C	7	74099138	*GTF2I*	Intron
rs73366469	T > C	7	74033600	*GTF2IRD1–GTF2I*	Intergenic
rs4717901	A > C	7	74016979	*GTF2IRD1*	downstream (3'UTR)
rs11981999	T > A	7	74002083	*GTF2IRD1*	Intron

SNPs: Single nucleotide polymorphisms (rs117026326, rs2527367, rs73366469, rs4717901, rs11981999) identified at the *GTF2IRD1–GTF2I* locus in this study. UTR: Untranslated region.

Plex SNPscan Kit (Genesky Biotechnologies Inc., Shanghai, China), as previously described (Chen et al., 2012). The kit was developed based on patented SNP genotyping technology (Genesky Biotechnologies Inc.), which includes double ligation and multiplex fluorescence polymerase chain reaction. A random sample accounting for ~5% (*n* = 40) of the total DNA samples was directly sequenced to confirm the genotyping results using Big Dye-terminator version 3.1 and an ABI3730XL automated sequencer (Applied Biosystems, Carlsbad, CA, USA).

Statistical analysis
To ensure the reliability of our study, all participants' sample size and power were calculated using the uncorrected Chi-squared statistic at α = 0.05, using Power and Sample Size Calculation software v.3.1.2 (Department of Biostatistics, Vanderbilt University, Nashville, TN, USA).

Chi-square tests were applied to compare the allele frequencies and sex distribution between groups and to analyze the Hardy-Weinberg equilibrium in the cases and controls. Age was compared between groups using Student's *t*-test. Logistic regression analysis was applied to assess the association with NMOSD susceptibility after adjusting for age and sex under dominant, recessive, and additive models. The odds ratios (*ORs*), 95% confidence intervals (95% *CIs*), and *P*-values were calculated, and a two-sided *P* < 0.05 was considered statistically significant. Stratification analyses for AQP4-IgG positivity and coexisting autoimmune diseases (primary Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis) were performed. Statistical analysis was carried out using PLINK v1.07 (Shaun Purcell, Mount Sinai School of Medicine and Harvard University, USA) and SPSS Statistics software version 21.0 (IBM SPSS, Chicago, IL, USA). Linkage disequilibrium analysis and haplotype construction were performed using Haploview software v4.2 (Daly Lab at the Broad Institute, Cambridge, MA, USA). The genetic associations of haplotypes were determined with SHEsis software (Bio-X Inc., Shanghai, China) (Shi and He, 2005).

Results

Baseline characteristics of NMOSD patients and controls
The demographic and clinical characteristics of the NMOSD patients and healthy controls are presented in **Table 2**. The average age was 41.99 ± 12.15 years for the NMOSD patients and 40.67 ± 11.56 years for the controls, with a sex distri-

Table 2 Demographic and clinical characteristics of NMOSD patients and controls

	NMOSD patients (*n* = 305)	Controls (*n* = 487)	*P*-value
Female/male (female %)	264/41(86.56%)	407/80(83.6%)	0.256
Age (years)	41.99 ± 12.15	40.67 ± 11.56	0.136
Age at onset (years)	37.45 ± 13.32	NA	NA
Disease duration (years)	5.95 ± 5.99	NA	NA
EDSS score	2.68 ± 2.22	NA	NA
Annual relapse rate	0.69 ± 0.58	NA	NA
AQP4-Ab (positive: negative, positive%)[a]	236/55(81.1%)	NA	NA
Clinical phenotype [*n*(percent)]			
Neuromyelitis optica	184(60.33)	NA	NA
Transverse myelitis only	95(31.15)	NA	NA
Optic neuritis only	22(7.21)	NA	NA
Others	4(1.31)	NA	NA
MRI lesions[b] (*n*(percent))			
Spinal cord	208/241(86.30)	NA	NA
Longitudinally extensive	150/208(72.11)	NA	NA
Focal	58/208(27.89)	NA	NA
Medulla oblongata	40/203(19.70)	NA	NA
Pons	38/203(18.71)	NA	NA
Midbrain	16/203(7.88)	NA	NA
Hypothalamic and thalamic	15/203(7.39)	NA	NA
Cerebellum	10/203(4.93)	NA	NA
Cerebrum	41/203(20.19)	NA	NA
Autoimmune disorders [*n*(percent)]			
Primary Sjögren syndrome	27/305(8.85)	NA	NA
Systemic lupus erythematosus	6/305(1.96)	NA	NA
Rheumatoid arthritis	4/305(1.31)	NA	NA
Autoimmune thyroid disorders	22/305(7.21)	NA	NA
Others	5/305(1.64)	NA	NA

Data are expressed as the mean ± standard deviation or frequency (%). Student's *t*-test for age and the Chi-square test for sex were used to compare cases and controls. [a]AQP4-Ab data were available in 291 patients; [b]MRI data were available in 241 patients. NMOSD: Neuromyelitis optica spectrum disorders; NA: not available; EDSS: expanded disability status scale; AQP4: aquaporin-4; MRI: magnetic resonance imaging.

bution (female/male) of 264/41 and 407/80 respectively. No significant differences in age (*P* = 0.256) or the sex ratio (*P* = 0.136) were observed between cases and controls. AQP4-Ab positivity was found in 236 (81.10%) NMOSD patients,

while AQP4-Ab was negative in 55 patients (18.90%) and the status of 14 patients was unknown. The majority of the NMOSD patients presented with the clinical phenotype of NMO (60.33%), and the remainder presented with transverse myelitis (31.15%), optic only neuritis (7.21%), or other clinical syndromes (1.31%).

SNP genotyping and the association with NMOSD risk

The allele and genotype frequencies of the genotyped SNPs and their associations with the risk of NMOSD are shown in **Table 3**. All of the SNPs were in Hardy-Weinberg equilibrium in the patients and controls ($P > 0.05$).

The minor T allele of rs117026326 was associated with an increased risk of NMOSD ($OR = 1.364$, 95% CI 1.019–1.828; $P = 0.037$). The additive model showed a similar association of rs117026326 with NMOSD ($OR = 1.359$, 95% CI 1.006–1.837; $P = 0.046$). However, the genotype and allele frequencies of other SNPs (rs11981999, rs4717901, rs2527367, and rs73366469) did not exhibit any association with NMOSD susceptibility.

The genotyping results were consistent. Furthermore, the call rate for each SNP was above 99%.

Stratification analysis for AQP4-IgG positivity

The distribution of the allele and genotype frequencies of *GTF2IRD1–GTF2I* SNPs in AQP4-IgG-seropositive NMOSD patients is shown in **Table 4**. After stratification for AQP4-IgG positivity, significant association with an increased risk of NMOSD persisted for rs117026326 (under an allelic model: $OR = 1.397$, 95% CI 1.021–1.912; $P = 0.036$; under a recessive model: $OR = 3.341$, 95% CI 1.022–10.93; $P = 0.046$) (**Table 4**). Furthermore, the CC genotype of rs73366469 was found to be more frequent in AQP4-IgG-seropositive patients than in controls ($OR = 3.15$, 95% CI 1.183–8.393; $P = 0.022$). However, there was still no significant association between the other three SNPs (rs11981999, rs4717901, and rs2527367) and the risk of NMOSD (**Table 4**).

Stratification analysis according to coexisting autoimmune diseases

Because both rs117026326 and rs73366469 confer risk for primary Sjögren's syndrome, systemic lupus erythematosus, and rheumatoid arthritis, patients with these particular autoimmune diseases were excluded from the case group and genetic association analyses were performed for both SNPs. The minor T allele of rs117026326 was still associated with an increased risk of NMOSD (under an allelic model: $OR = 1.446$, 95% CI 1.072–1.952; $P = 0.015$; under an additive model: $OR = 1.448$, 95% CI 1.064–1.969; $P = 0.018$; under a dominant model: $OR = 1.435$, 95% CI 1.022–2.015; $P = 0.037$)

Table 3 Allele and genotype frequencies of five SNPs identified at the GTF2IRD1–GTF2I locus in NMOSD patients and controls, along with the association with risk of NMOSD

SNP	Allele/ Genotype	NMOSD [n(%)]	Control [n(%)]	P^{HWE}	Analysis Model	OR(95% CI)	P-value
rs117026326	T	95(15.6)	116(11.9)	0.52			
	C	515(84.4)	858(88.1)		Allelic[a]	1.364(1.019–1.828)	0.037*
	TT	7(2.3%)	5(1.0)		Additive[b]	1.359(1.006–1.837)	0.046*
	TC	81(26.6)	106(21.8)		Dominant[b]	1.344(0.966–1.872)	0.08
	CC	217(71.1)	376(77.2)		Recessive[b]	2.487(0.771–8.029)	0.128
rs11981999	A	211(34.6)	306(31.5)	0.402			
	T	399(65.4)	666(68.5)		Allelic	1.151(0.929–1.427)	0.2
	AA	33(10.8)	44(9.1)		Additive	1.116(0.892–1.395)	0.337
	AT	145(47.5)	218(44.8)		Dominant	1.150(0.857–1.542)	0.352
	TT	127(41.6)	224(46.1)		Recessive	1.143(0.706–1.851)	0.587
rs2527367	C	72(12.1)	109(11.2)	0.249			
	T	538(87.9)	865(88.8)		Allelic	1.062(0.774–1.457)	0.709
	CC	5(1.6)	3(0.6)		Additive	1.015(0.732–1.406)	0.93
	CT	62(20.3)	103(21.2)		Dominant	0.956(0.675–1.363)	0.818
	TT	238(78.1)	381(78.2)		Recessive	2.611(0.607–11.23)	0.197
rs4717901	C	132(21.6)	198(20.3)	0.889			
	A	478(78.4)	776(79.7)		Allelic	1.082(0.845–1.387)	0.532
	CC	12(3.9)	19(3.9)		Additive	1.074(0.832–1.385)	0.584
	CA	108(35.4)	160(32.9)		Dominant	1.107(0.821–1.493)	0.503
	AA	185(60.7)	308(63.2)		Recessive	0.974(0.462–2.052)	0.945
rs73366469	C	107(17.5)	121(15.5)	0.23			
	T	503(82.5)	823(84.5)		Allelic	1.159(0.884–1.521)	0.285
	CC	10(3.3)	8(1.6)		Additive	1.168(0.882–1.546)	0.279
	CT	87(28.5)	135(27.7)		Dominant	1.112(0.811–1.524)	0.509
	TT	208(68.2)	344(70.7)		Recessive	2.277(0.871–5.956)	0.093

"D" represents wild type and "d" represents mutant type. Allelic: d *vs.* D; Additive: dd *vs.* dD *vs.* DD; Dominant: dd + dD *vs.* DD; Recessive: dd *vs.* dD + DD. [a]Chi-square test; [b]logistic regression analyses adjusted by age and sex. *Indicates significant differences at $P < 0.05$. SNP: Single-nucleotide polymorphism; NMOSD: neuromyelitis optica spectrum disorders; OR: odds ratio; CI: confidence interval.

Table 4 Stratification analysis for AQP4-IgG positivity: allele and genotype frequencies of five SNPs identified at the *GTF2IRD1–GTF2I* locus in AQP4-IgG-seropositive NMOSD patients and controls, along with the association with the risk of NMOSD

SNP	Allele/Genotype	NMOSD [n(%)]	Control [n(%)]	Analysis Model	OR(95% CI)	P-value
rs117026326	T	75(15.9)	116(11.9)			
	C	397(84.1)	858(88.1)	Allelic[a]	1.397(1.021–1.912)	0.036[*]
	TT	7(3.0)	5(1.0)	Additive[b]	1.375(0.995–1.899)	0.054
	TC	61(25.8)	106(21.8)	Dominant[b]	1.323(0.923–1.897)	0.128
	CC	168(71.2)	376(77.2)	Recessive[b]	3.341(1.022–10.93)	0.046[*]
rs11981999	A	165(35.0)	306(31.5)			
	T	307(65.0)	666(68.5)	Allelic	1.17(0.927–1.476)	0.186
	AA	28(11.9)	44(9.1)	Additive	1.11(0.872–1.414)	0.398
	AT	109(46.2)	218(44.8)	Dominant	1.101(0.799–1.518)	0.556
	TT	99(41.9)	224(46.1)	Recessive	1.251(0.751–2.085)	0.39
rs2527367	C	60(12.7)	109(11.2)			
	T	412(87.3)	865(88.8)	Allelic	0.399(0.826–1.618)	0.398
	CC	4(1.7)	3(0.6)	Additive	1.096(0.772–1.556)	0.609
	CT	52(22.0)	103(21.2)	Dominant	1.046(0.718–1.524)	0.815
	TT	180(76.3)	381(78.2)	Recessive	2.732(0.583–12.79)	0.202
rs4717901	C	101(21.4)	198(20.3)			
	A	371(78.6)	776(79.7)	Allelic	1.067(0.815–1.397)	0.638
	CC	11(4.7)	19(3.9)	Additive	1.042(0.791–1.373)	0.771
	CA	79(33.5)	160(32.9)	Dominant	1.036(0.747–1.437)	0.833
	AA	146(61.8)	308(63.2)	Recessive	1.134(0.525–2.454)	0.749
rs73366469	C	84(17.8)	121(15.5)			
	T	388(82.2)	823(84.5)	Allelic	1.18(0.149–0.880)	0.268
	CC	10(4.2)	8(1.6)	Additive	1.189(0.878–1.609)	0.263
	CT	64(27.1)	135(27.7)	Dominant	1.085(0.768–1.533)	0.642
	TT	162(68.7)	344(70.7)	Recessive	3.15(1.183–8.393)	0.022[*]

"D" represents the wild type and "d" represents the mutant type. Allelic: d vs. D; Additive: dd *vs*. dD *vs*. DD; Dominant: dd + dD *vs*. DD; Recessive: dd *vs*. dD + DD. [a]Chi-square test; [b]logistic regression analyses adjusted by age and sex. [*]Indicates significant differences at *P* < 0.05. SNP: Single-nucleotide polymorphism; NMOSD: neuromyelitis optica spectrum disorders; *OR*: odds ratio; *CI*: confidence interval.

Table 5 Stratification analysis according to coexisting autoimmune diseases: allele and genotype frequencies of rs117026326 in NMOSD patients without primary Sjögren's syndrome, systemic lupus erythematosus, or rheumatoid arthritis and the association with the risk of NMOSD

SNP	Allele/genotype	NMOSD [n(%)]	Control [n(%)]	Analyzing model	OR(95% CI)	P-value
rs117026326	T	88(16.4)	116(11.9)			
	C	450(83.6)	858(88.1)	Allelic[a]	1.446(1.072–1.952)	0.015[*]
	TT	7(2.6)	5(1.0)	Additive[b]	1.448(1.064–1.969)	0.018[*]
	TC	74(27.5)	106(21.8)	Dominant[b]	1.435(1.022–2.015)	0.037[*]
	CC	188(69.9)	376(77.2)	Recessive[b]	2.802(0.869–9.031)	0.084

[a]Chi-square test; [b]logistic regression analyses adjusted according to age and sex. [*]Indicates significant differences at *P* < 0.05. SNP: Single-nucleotide polymorphism; NMOSD: neuromyelitis optica spectrum disorders; *OR*: odds ratio; *CI*: confidence interval.

(**Table 5**). Similarly, the frequency of the CC genotype of rs73366469 remained higher in AQP4-IgG-seropositive patients than in controls (*OR* = 3.476, 95% *CI* 1.312–9.212; *P* = 0.012) (**Table 6**).

Linkage disequilibrium analysis of haplotypes and association with the risk of NMOSD
A linkage disequilibrium plot is shown in **Figure 1**. One linkage disequilibrium block consisting of two SNPs (rs11981999 and rs4717901) was constructed using an algorithm designed by Gabriel et al. (2002). Three haplotypes with a frequency > 5% (AA, AC, and TA) were identified in the study subjects. However, we did not find any significant haplotypes in the selected block (**Table 7**).

Discussion
This study investigated the influence of *GTF2IRD1–GTF2I* polymorphisms on the risk of NMOSD in a Han Chinese population for the first time. We found that the rs117026326 polymorphism in *GTF2I* was strongly associated with an increased risk of NMOSD. Because AQP4-IgG-seronegative patients with NMOSD are a heterogeneous clinical subgroup, having different immunopathogenetic mechanisms, it was necessary to conduct stratification analysis accord-

Table 6 Stratification analysis according to coexisting autoimmune diseases: allele and genotype frequencies of rs73366469 in AQP4-IgG-seropositive patients without primary Sjögren's syndrome, systemic lupus erythematosus, or rheumatoid arthritis, and along with the association with the risk of NMOSD

SNP	Allele/genotype	NMOSD [n(%)]	Control [n(%)]	Analyzing model	OR(95% CI)	P-value
rs73366469	C	78(17.8)	121(15.5)			
	T	342(82.2)	823(84.5)	Allelic[a]	1.243(0.920–1.680)	0.156
	CC	10(4.8)	8(1.6)	Additive[b]	1.250(0.917–1.705)	0.157
	CT	58(27.6)	135(27.7)	Dominant[b]	1.137(0.797–1.622)	0.479
	TT	142(67.6)	344(70.7)	Recessive[b]	3.476(1.312–9.212)	0.012*

[a]Chi-square test; [b]Logistic regression analyses adjusted by age and sex; *Indicates significant difference at $P < 0.05$. SNP: Single-nucleotide polymorphism; NMOSD: neuromyelitis optica spectrum disorders; OR: odds ratio; CI: confidence interval.
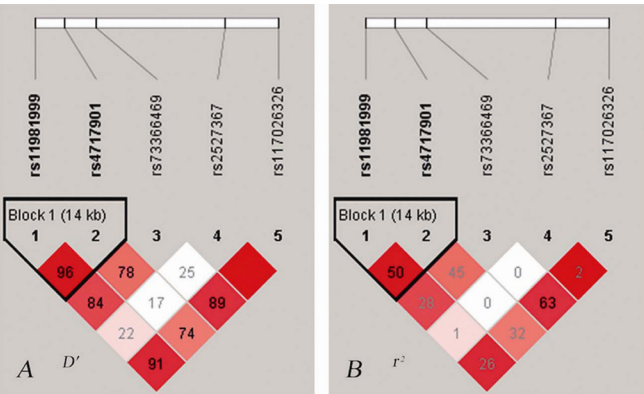
Figure 1 Linkage disequilibrium tests for five SNPs at the GTF2IRD1–GTF2I locus.
(A, B) Linkage disequilibrium coefficients (|D'| (A) and r^2 (B)) of the five SNPs. The relative physical positions of the five SNPs at the GTF2IRD1–GTF2I locus are indicated by the top white line. Each box represents the linkage disequilibrium relationship between two SNPs. SNP: Single-nucleotide polymorphism

Table 7 Results of the analysis of selected haplotypes at the GTF2IRD1–GTF2I locus for the potential association with the risk of NMOSD

Haplotype	NMOSD [n(%)]	Control [n(%)]	OR(95% CI)	P-value
AC	130(21.3)	191(19.7)	1.099(0.855–1.412)	0.461
AA	81(13.3)	115(11.8)	1.148(0.847–1.556)	0.376
TA	397(65)	660(67.9)	0.871(0.703–1.080)	0.209

Loci chosen for haplotype analysis: rs11981999 and rs4717901. All haplotype frequencies < 0.05 were ignored in the analysis. All of the P-values were calculated using logistic regression analyses, and adjusted by sex and age. $P < 0.05$ was considered statistically significant. OR: Odds ratio; CI: confidence interval; NMOSD: neuromyelitis optica spectrum disorders.

ing to AQP4-IgG serostatus. After stratification for AQP4-IgG positivity, a significant association with susceptibility to NMOSD persisted in rs117026326. Surprisingly, the rs73366469 CC genotype was associated with AQP4-IgG-seropositivity, which implies that there is an association with AQP4-IgG but not with NMOSD.

Furthermore, three major haplotypes constructed from two SNPs (rs11981999 and rs4717901) in one linkage disequilibrium block showed no significant difference between the cases and controls. The *GTF2IRD1* polymorphisms rs11981999 and rs4717901 have been reported to confer risk of systemic lupus erythematosus (Li et al., 2015; Sun et al., 2016), while the *GTF2I* rs2527367 polymorphism is associated with complex behavioral traits such as human anxiety (Jabbi et al., 2015). Most notably, the rs4717901 polymorphism, which is located in the 3′ flanking region of the *GTF2IRD1* gene, might regulate mRNA stability and translational efficiency. However, no association was observed between any of these three SNPs and the risk of NMOSD, even after stratification for AQP4-IgG positivity. This may be due in part to the different genetic background between NMOSD and systemic lupus erythematosus, as well as other complex behavioral traits.

Additionally, stratification analysis according to coex-

isting autoimmune diseases was performed. The *GTF2I* rs117026326 polymorphism has been identified as the strongest susceptibility locus for primary Sjögren's syndrome in Han Chinese (Li et al., 2013), but not in Caucasians (Lessard et al., 2013), according to genome-wide association studies. Subsequently, it was reported that the rs117026326 polymorphism is associated with anti-Sjøgren syndrome antibody A-positivity (Zheng et al., 2015) in Han Chinese or primary Sjögren's syndrome in southern Chinese females (Song et al., 2016), in addition to being associated with rheumatoid arthritis (Kim et al., 2016) and systemic lupus erythematosus (Li et al., 2015; Sun et al., 2016). Moreover, rs73366469 has been shown to confer susceptibility to rheumatoid arthritis (Kim et al., 2016), primary Sjögren's syndrome (Li et al., 2013), and systemic lupus erythematosus (Sun et al., 2016; Zhao et al., 2017) in Asian populations. In systemic lupus erythematosus patients, a recent study (Zhao et al., 2017) detected the strongest association signal at rs73366469, which was consistent with another study performed in an Asian population (Sun et al., 2016). In contrast, the association of the rs73366469 polymorphism with systemic lupus erythematosus in European Americans has been confirmed at only a modest significance level, and this association was not found in African Americans (Zhao et al., 2017). Therefore, the coexistence of the abovementioned autoimmune diseases in patients may confound the correlation between *GTF2IRD1–GTF2I* polymorphisms and NMOSD in Han Chinese. Considering this factor, we further excluded

patients with primary Sjögren's syndrome, systemic lupus erythematosus, or rheumatoid arthritis, and the significant associations with the T allele of rs117026326 and the genotype CC of rs73366469 remained. Collectively, our findings demonstrate that polymorphisms in *GTF2IRD1–GTF2I* may be associated with the risk of NMOSD, which can facilitate the understanding of the pathogenesis of NMOSD.

However, the exact molecular mechanisms through which these polymorphisms are implicated in the pathogenesis of NMOSD have yet to be elucidated. It is likely that they influence the expression of *GTF2I*, *GTF2IRD1*, or other neighboring genes and thereby alter the immune responses mediated by T or B lymphocytes, leading to the subsequent breakdown of immune tolerance and production of AQP4-IgG. Recently obtained evidence demonstrated that rs117026326 can regulate the expression of *GTF2IRD2* (downstream of *GTF2I*), but not for *GTF2I*, *GTF2IRD1*, or *NCF1* (Kim et al., 2016). Another study also detected no association between rs117026326 genotypes and the transcript levels of *GTF2I* and *GTF2IRD1* in peripheral blood mononuclear cells from patients with systemic lupus erythematosus and controls (Zhao et al., 2017). As the rs117026326 polymorphism is located within the intron of *GTF2I*, while rs73366469 is located in the intergenic region, it needs to be clarified whether the susceptibility variants exert an effect by regulating their own expression level or that of other neighboring genes. Another possibility is that these variants interact with unidentified variants of other genes or microRNAs. We should also keep in mind that the association of these polymorphisms with the risk of NMOSD may be due to a direct causative effect of these SNPs, or may occur because they are in linkage disequilibrium with other functional variants located in or near the *GTF2IRD1–GTF2I* region. In a recent study published in Nature Genetics, a missense variant in NCF1 was found to be associated with susceptibility to multiple autoimmune diseases, based on the hypothesis that rs117026326 might tag causal variant(s) of *NCF1* that are not present in the 1000 Genomes Project (Zhao et al., 2017).

Several limitations of our study should be noted. First, we focused only on the reported disease-associated SNPs within the *GTF2IRD1–GTF2I* locus, which may have led us to neglect several other susceptibility variants in this region. Second, all participants came from southwest China, and the cohort consisted exclusively of individuals of Han ethnicity. Therefore, our findings require replication in other ethnicities. Thus, the relationship between *GTF2IRD1–GTF2I* polymorphisms and NMOSD needs to be verified in larger cohorts. Nevertheless, our study provides valuable insights and will contribute to future investigations in this field.

In conclusion, the results of our study suggest that the T allele of rs117026326 is associated with susceptibility to NMOSD, and the rs73366469 CC genotype confers susceptibility to AQP4-IgG seropositivity in Han Chinese patients. The strong association between *GTF2IRD1–GTF2I* polymorphisms and the risk of NMOSD may provide new approaches for investigating the pathogenesis of NMOSD and developing alternate treatment options for NMOSD.

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