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Research article

Association between TIMP1 polymorphism and female neuromyelitis optica spectrum disorder in Chinese population

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ABSTRACT

Aims: Earlier studies have indicated an association between the TIMP1 polymorphism and the risk of certain autoimmune diseases, as well as a link between higher TIMP1 levels and blood-brain barrier (BBB) disruption in neuromyelitis optica spectrum disorders (NMOSD). This study aimed to explore the correlation between TIMP1 polymorphism and NMOSD phenotypes.

Methods: Genotyping of three loci (rs4898, rs2070584, rs6609533) in the TIMP1 gene was performed in 126 NMOSD patients and 213 healthy controls (HCs) from North China using the SNaPshot sequencing technique, and a correlation analysis was done between phenotypes and TIMP1 genotype.

Results: The frequency of the rs4898-T, rs2070584-T, and rs6609533-G alleles was significantly higher in NMOSD patients than those in HCs ($p < 0.05$). Accordingly, the rs4898-TT, rs2070584-TT, and rs6609533-GG genotypes were found at a higher frequency in patients than in controls ($p < 0.05$). Haplotype analysis showed TIMP1 T-T-G (rs4898-rs2070584-rs6609533) frequency was higher in female NMOSD patients ($p = 0.019$), and the frequency of T-T-G haplotypes in the BBB disrupted group was higher compared with that in the BBB normal group ($p = 0.04$).

Conclusions: TIMP1 rs4898-T, rs2070584-T, and rs6609533 polymorphism may contribute to the susceptibility of Female NMOSD patients in the Chinese Population. TIMP1 T-T-G (rs4898-rs2070584-rs6609533) haplotype is more common among female NMOSD patients and is linked to heightened disruption of the BBB.

1. Introduction

Neuromyelitis Optica Spectrum Disorder (NMOSD) is an autoimmune demyelinating disorder of the central nervous system that preferentially involves the optic nerves and spinal cord, and to a lesser extent, the brain [1–3]. A substantial body of studies has

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confirmed that NMOSD is associated with aquaporin-4 antibody (AQP4-ab) [4,5]. These antibodies are produced peripherally and, upon crossing the disrupted BBB, target astrocytes, triggering an inflammatory cascade that contributes to disease pathogenesis [6]. The degree of BBB disruption is critically linked to disease development and recurrence in NMOSD [7,8].

Research has shown that in patients with longitudinally extensive transverse myelitis (LETM), a subtype of NMOSD, immunoglobulin G (IgG) significantly induces endothelial cell activation, correlating with clinical markers of BBB disruption and disease activity. Additionally, glucose-regulated protein 78 (GRP78) antibodies are closely associated with the LETM phenotype and disease severity, indicating that the presence of GRP78 antibodies correlates with higher BBB permeability and increased disease severity, as measured by EDSS scores [9]. These findings suggest that other unknown factors may also contribute to NMOSD pathogenesis by disrupting the BBB.

The TIMP1 protein is a natural inhibitor of matrix metalloproteinases (MMPs), mainly MMP-9, inhibiting them in a 1:1 stoichiometric and reversible manner. Imbalanced MMP-9/TIMP1 activities have been observed in many disease conditions [7]. Notably, the serum and cerebrospinal fluid (CSF) concentrations of TIMP1 and MMP-9 were significantly higher in patients with NMOSD than those in controls indicating that TIMP1 participates in the pathogenesis of this disorder possibly through mediating BBB disruption [10–12]. Interestingly, the TIMP1 gene is located on the X chromosome (Xp11.3-p11.23). NMOSD exhibits a strong female predominance, with a female-to-male ratio of up to 9:1[13], similar to many other autoimmune disorders. Previous studies have indicated that genetic polymorphisms on the X chromosome may be associated with NMOSD susceptibility [14]. The TIMP1 gene contains several common polymorphic sites, including rs4898, rs6609533, and rs2070584, which have been implicated in various autoimmune diseases such as asthma, systemic lupus erythematosus [15], and systemic sclerosis [16–18]. The rs4898 polymorphism, located in the protein-coding region of TIMP1, has been shown to alter protein expression and may influence susceptibility to autoimmune diseases [18–20]. Rs6609533 and rs2070584 are reported to belong to the same haploblock as rs4898 [19,21].

However, to the best of our knowledge, the association between TIMP1 polymorphisms and the susceptibility and progression of NMOSD remains unknown. In this study, we attempted to determine whether TIMP1 polymorphisms might confer susceptibility to NMOSD by affecting BBB integrity. Therefore, in this study, we assessed whether TIMP1 polymorphisms are associated with the risk of NMOSD.

2. Methods

2.1. Subjects

All patients with NMOSD included in this study were from the Department of Neurology in our hospital from September 2016 to November 2021. Healthy controls were enrolled at the hospital's medical examination center. Diagnosis of NMOSD was based on the 2015 international consensus diagnostic criteria for neuromyelitis optica spectrum disorders [3]. We excluded patients with (a) hypertension, diabetes, cerebrovascular disease, tumors, and other chronic diseases, and (b) complicated with other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosus, thyroid diseases, etc. The following demographic and clinical data were collected: age, gender, age at onset, AQP4-IgG status (we measured the concentration of AQP4-IgG using the Cell-Based Assay (CBA) method. The test was conducted by the Jiangsu Simcere Biotechnology Co., Ltd.), clinical manifestations, medication history, attack history, and laboratory data including anti-nuclear antibodies (ANA), Oligoclonal bands (OCB), and CSF/blood albumin quotient (Qalb). The CSF/blood Qalb was used as an indicator of BBB disruption [22,23]. The BBB index was calculated as follows: CSF albumin/serum albumin. We analyzed CSF albumin using isoelectric focusing and measured serum albumin using latex-enhanced nephelometry. This study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University and written informed consent was obtained from each participant or his/her legal guardian (20200040). This study was conducted according to the principles expressed in the Declaration of Helsinki.

2.2. Selection of SNPs, DNA extraction, and SNP genotyping

The TIMP gene families contain several polymorphic loci. We selected three common sites (rs4898, rs2070584, and rs6609533) in TIMP1 in this study based on previous genome-wide association studies (GWAS) and confirmatory studies.

Five milliliters of peripheral blood were collected in EDTA anticoagulant tubes from each subject. Genomic DNA was extracted using the Blood Genomic DNA Extraction Kit (JieRui, Shanghai, China), and stored at −20 °C storage for future use. DNA was amplified using PCR MIX (Yisheng Biotech Co., Shanghai, China). The Snapshot kit (ABI, USA) was used for genotyping. Primer information is provided in Table 1.

Table 1
Primer information.

Gene	upstream primer	downstream primer	extension primer
rs4898	ATGGACTCTTGCACATCA	CAGTGTAGGTCTTGGTGAA	ctgactgactgaCACATCACTACCTGCAGTTT
rs2070584	CCCAGGGCTTTCTAGTTAT	GGCAAGATGTGTGAATGG	actgactgactgactgaCAGCTGACAAATCACTGCCT
rs6609534	TGATGTTGTCCAGTGAGG	TCAAGTCAGACTCATGGTAC	ctgactAGGATGCCCCATCCAAACAC

*This primer was applied for the cell experiments.

2.2.1. Statistics analysis

The normality of continuous variables was assessed using the Kolmogorov-Smirnov test. Normally distributed variables were expressed as means ± standard deviations (SD) and compared by the Student's t-test. Categorical variables were expressed as percentages (%) and the chi-squared test was used for comparison between groups. Risk factors for the onset of study endpoints after NMOSD were identified with univariable and multivariable binary logistic regression analyses using the forward conditional method. The online software SHEsis (http://analysis2.bio-x.cn/myAnalysis.php) was used for linkage disequilibrium (LD), Hardy-Weinberg balance test, and haplotype analysis [24]. SNPStats (https://www.snpstats.net/start.htm) was used to construct haplotypes and analyze the interactions with related factors. The computed statistical power for this study was 0.99 ($n = 337$, $P = 0.2$,$\alpha = 0.05$, $OR = 2$) using PASS 15.0. All other statistical analyses were performed using SPSS 21.0 software (IBM Corp., Armonk, NY). $P < 0.05$ was considered to be statistically significant.

3. Result

3.1. Characteristics of the study subjects

The mean age was not significantly different between the patients with NMOSD and healthy controls (HCsp > 0.05). Serum anti-AQP4 antibodies were positively detected in 107 (86.3 %) patients with NMOSD. The attack involved optic nerves, spinal cord, brain, and multiple locations in the CNS in 24(19.35 %), 51(41.13 %), 11(8.87 %), and 38 (30.65 %) NMOSD patients, respectively. Data of QAlb and OCB were available in 30 patients. A total of 35.7 % and 36.6 % of them showed increased Qalb and were positive for OCB, respectively. The demographics and clinical characteristics of participants for TIMP1 SNP analysis are shown in Table 2.

3.2. Data quality analysis of the three SNPs

As the TIMP1 gene is on the X chromosome, the results obtained were segregated by sex. The genotype frequencies of all SNPs conformed to Hardy-Weinberg equilibrium (HWP) (all $p > 0.05$), suggesting that the selected population was representative. The results are shown in Table 3.

3.3. Association between the three SNPs and NMOSD risk

Significant differences in genotype and allele distributions among the groups were found for all three SNPs (all $p < 0.05$). Since the number of male NMOSD patients was limited in this study, the association between the three SNPs and NMOSD risk was assessed in the female cohort. The distributions of the C and T alleles of rs4898 were significantly different between the 2 groups; the T allele frequency distribution in the case group was significantly higher than that in the control group (OR = 0.657, 95 % CI = 0.47–0.93, p = 0.02). In addition, in the recessive models, the prevalence of the TT + CT genotype in the case group was significantly higher than in the control group (OR = 0.50, 95 % CI = 0.27–0.93, p = 0.024). Similarly, rs2070584 and rs6609533 also showed significantly

Table 2
Demographics and clinical characteristics of participants for TIMP1 SNP.

Clinical Characteristics	NMOSD	HC	P values
	n = 126	n = 213	
Sex, no. (%) of females	88.09 %	78.0 %	0.03
Age, y (mean ± SD)	45.26 ± 15.30	45.07 ± 10.84	0.89
Age at onset, y (mean ± SD)	43.83 ± 15.09	NA	NA
AQP4-IgG+, no. (%) of patients	86.3 %	NA	NA
Onset symptoms			
no. (%) of patients			
Optic neuritis	24 (19.35 %)	NA	NA
Acute myelitis	52 (41.13 %)	NA	NA
Brain attacks[a]	11 (8.87 %)	NA	NA
Mix attacks[b]	38 (30.65 %)	NA	NA
Qalb			
normal	18 (60.0 %)	NA	NA
increased	12 (40.0 %)	NA	NA
OCB			
negative	19 (63.3 %)	NA	NA
positive	11 (36.7 %)	NA	NA
ANA status			
negative	95 (76.6 %)	NA	NA
positive	29 (23.4 %)	NA	NA

Abbreviations: NMOSD, neuromyelitis optica spectrum disorder; HC, healthy control group; SD, standard deviation; NA, not assessable.QAlb, CSF/blood albumin quotient; OCB, Oligoclonal bands; ANA status, anti-nuclear antibodies.

[a] Brain attacks, including the brainstem and brain attacks.

[b] Mix attacks, including equal to two or more two damaged sites.

Table 3
Selected SNPs of TIMP1 and PHWE in this study.

SNP	Location	Gropes	HWE	MAF (Present Study Data)		MAF (CHB)	Functional region
				Alley	Frequencies		
rs4898	X:47585586 (GRCh38.p13)	NMOSD	0.28	C	0.42	0.47	Missense Variant
		HC	0.37	C	0.53		F (Phe) > L (Leu)
rs2070584	X:47587120 (GRCh38)	NMOSD	0.28	G	0.43	0.44	Downstream Transcript Variant
		HC	0.37	G	0.53		
rs6609534	X:47587254 (GRCh38)	NMOSD	0.30	A	0.41	0.31	Downstream Transcript Variant
		HC	0.15	A	0.52		

Abbreviations: CHB, Han Chinese in Beijing; MAF, minor allele frequency; NMOSD, neuromyelitis optica spectrum disorder; HC, healthy control group.

different alleles and genotype distributions among female patients with NMOSD and healthy controls. The results indicated that the T allele at rs4898, T allele at rs2070584, and G allele at rs6609533 located on the TIMP1 gene significantly increased the relative risk of NMOSD in females. The results are shown in Table 4.

3.4. Linkage disequilibrium and haplotype analysis

Fig. 1 shows that rs4898, rs2070584, and rs6609533 were in complete linkage disequilibrium (D'>0.98). Haplotype analysis identified three common haplotypes with a frequency higher than 0.03 : haplotype1 = T-T-G (rs4898-rs2070584-rs6609533) with a frequency of 57.4 % in the NMOSD case group and 46.4 % in the healthy control group, haplotype2 = C-G-A with a frequency of 41.2 % in the NMOSD case group and 51.5 % in the healthy control group. There was a statistically significant difference in haplotype distribution between NMOSD cases and healthy controls (OR = 0.61, 95%CI = 0.42–0.89, p = 0.01). The results are shown in Table 5.

Table 4
Association analysis between SNPs and the female NMOSD risk under different models.

SNPs	Model	Genotype	HC N (%)	NMOSD N (%)	OR (95 % CI)	P-value
rs4898	allele	T	157 (47 %)	128 (58 %)	0.66 (0.47–0.93)	0.02
		C	177 (53 %)	94 (42 %)	1	
	Codominant	T/T	34 (20.4 %)	34 (30.6 %)	1	0.03
		C/T	89 (53.3 %)	60 (54 %)	0.66 (0.37–1.18)	
		C/C	44 (26.4 %)	17 (15.3 %)	0.38 (0.18–0.79)	
	Dominant	T/T	34 (20.4 %)	34 (30.6 %)	1	0.046
		C/T-C/C	133 (79.6 %)	77 (69.4 %)	0.57 (0.33–0.99)	
	Recessive	T/T-C/T	123 (73.7 %)	94 (84.7 %)	1	0.024
		C/C	44 (26.4 %)	17 (15.3 %)	0.50 (0.27–0.93)	
	Overdominant	T/T-C/C	78 (46.7 %)	51 (46 %)	1	0.91
		C/T	89 (53.3 %)	60 (54 %)	1.03 (0.64–1.67)	
rs2070584	allele	T	156 (47 %)	124 (57 %)	0.658 (0.466–0.929)	0.02
		G	176 (53 %)	92 (43 %)	1	
	Codominant	T/T	34 (20.4 %)	34 (30.6 %)	1	0.03
		G/T	89 (53.3 %)	60 (54 %)	0.66 (0.37–1.18)	
		G/G	44 (26.4 %)	17 (15.3 %)	0.38 (0.18–0.79)	
	Dominant	T/T	34 (20.4 %)	34 (30.6 %)	1	0.046
		G/T-G/G	133 (79.6 %)	77 (69.4 %)	0.57 (0.33–0.99)	
	Recessive	T/T-G/T	123 (73.7 %)	94 (84.7 %)	1	0.024
		G/G	44 (26.4 %)	17 (15.3 %)	0.50 (0.27–0.93)	
	Overdominant	T/T-G/G	78 (46.7 %)	51 (46 %)	1	0.91
		G/T	89 (53.3 %)	60 (54 %)	1.03 (0.63–1.66)	
rs6609534	allele	G	159 (48 %)	127 (59 %)	1	
		A	173 (52.1 %)	89 (41 %)	0.644 (0.456–0.910)	0.01
	Codominant	G/G	35 (21 %)	35 (31.5 %)	1	0.021
		A/G	90 (53.9 %)	61 (55 %)	0.67 (0.38–1.19)	
		A/A	42 (25.1 %)	15 (13.5 %)	0.35 (0.17–0.75)	
	Dominant	G/G	35 (21 %)	35 (31.5 %)	1	0.045
		A/G-A/A	132 (79 %)	76 (68.5 %)	0.57 (0.33–0.99)	
	Recessive	G/G-A/G	125 (74.8 %)	96 (86.5 %)	1	0.015
		A/A	42 (25.1 %)	15 (13.5 %)	0.46 (0.24–0.88)	
	Overdominant	G/G-A/A	77 (46.1 %)	50 (45 %)	1	0.86
		A/G	90 (53.9 %)	61 (55 %)	1.04 (0.64–1.69)	

Abbreviations: HC: healthy control; NMOSD: neuromyelitis optica spectrum disorder; OR: Odd Ratios.

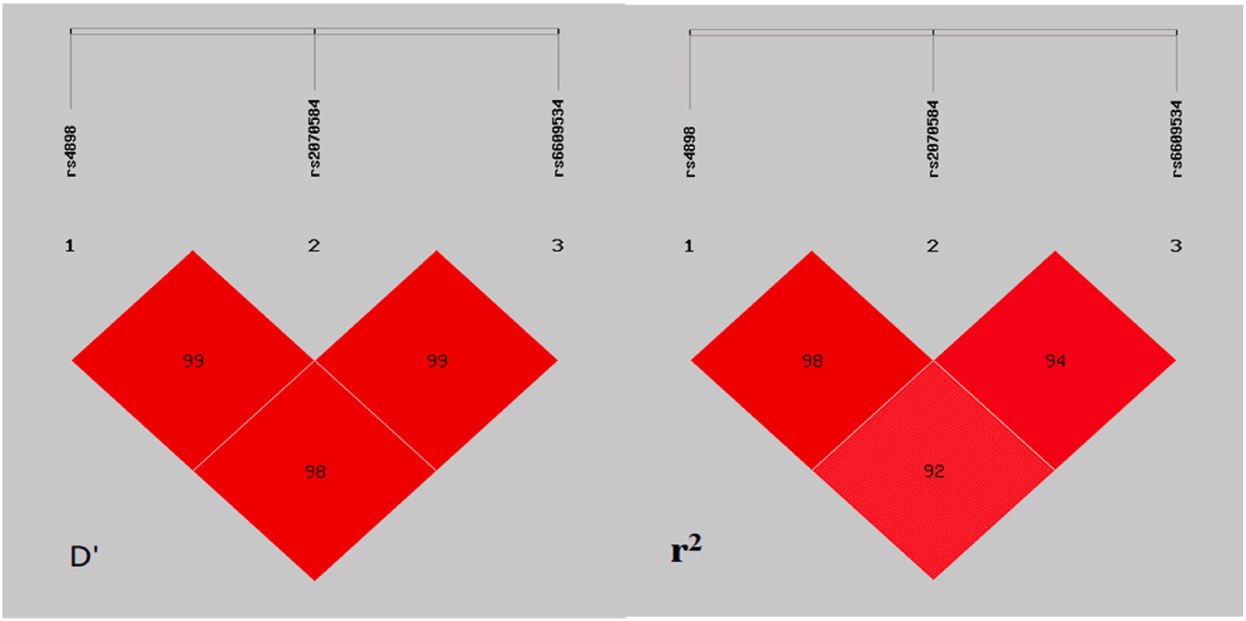

Fig. 1. Linkage disequilibrium (LD) plot of TIMP1 SNPs. Each block represents the linkage disequilibrium relationship between two SNPs. Rrs4898, rs2070584, and rs6609533 were in complete linkage disequilibrium (D'>0.98).

3.5. Stratification analysis of TIMP1-SNPs based on clinical features of female NMOSD patients

Patients with NMOSD were further stratified according to onset symptoms, age at onset, AQP4-IgG status, QAlb status, OCB status, and ANA status to evaluate the haplotype T-T-G frequency in different conditions. No significant differences in haplotype T-T-G distribution were observed when stratified patients by onset symptoms, age at onset, AQP4-IgG status, OCB status, and ANA status ($p > 0.05$).

Table 6 compares the demographic and clinical characteristics of the entire NMOSD cohort (n = 126) with those who had QAlb measurements (n = 30). The mean age was 45.26 ± 15.30 years for the total cohort and 41.31 ± 14.54 years for the QAlb subset (p = 0.179). The percentage of females was similar (88.09 % vs. 89.70 %, p = 0.738), and age at onset was comparable (43.83 ± 15.09 years vs. 40.89 ± 14.71 years, p = 0.352). The QAlb subset had a slightly higher percentage of anti-AQP4 antibody-positive patients (89.7 % vs. 86.3 %, p = 0.738). CSF cell count data were unavailable for both groups. The mean CSF glucose level in the QAlb subset was 4.06 ± 1.08 mmol/L. CSF protein level was 0.25 ± 0.15 mg/dL, and CSF chloride level was 125.05 ± 3.55 mmol/L. Additionally, 63.3 % of the QAlb subset had normal OCB status. The haplotype T-T-G frequency was significantly higher in patients with increased Qalb than those with normal Qalb (OR = 0.204,95%CI = 0.041–1.008, p = 0.044), indicating that the TIMP1 haplotype may be associated with the breakdown of BBB in NMOSD. The results are shown in Table 7.

4. Discussion

Previous studies have demonstrated that genetic polymorphisms are associated with susceptibility to NMOSD, and TIMP1 gene polymorphisms have been implicated in the pathogenesis of various diseases. Our study supports these findings by showing that TIMP1 gene polymorphisms, particularly the T-T-G haplotype, are significantly associated with an increased risk of NMOSD in females and are linked to BBB disruption.

To the best of our knowledge, no previous studies have investigated the role of the TIMP1 polymorphism in NMOSD in any population. Our study found that the T allele at rs4898, T allele at rs2070584, and G allele at rs6609533 located on the TIMP1 gene significantly increased the relative risk of NMOSD in females. Loci in strong linkage disequilibrium (LD) were combined into

Table 5
Associations between TIMP1 haplotypes and risk of NMOSD.

Haplotype	rs4898	rs2070584	rs6609534	HC	NMOSD	OR (95 % CI)	*P* value
1	C	G	A	46.4	57.4	1	
2	T	T	G	51.5	41.2	1.547 (1.092–2.192)	**0.01**
3	C	G	G	1.2	1.4	0.93 (0.20–4.29)	0.92

Abbreviations: NMOSD, neuromyelitis optica spectrum disorder; HC, healthy control; Rare, haplotypes with frequencies<0.01.Significant *P* value in bold.

Table 6
Demographic characteristics of patients with QAlb values compared to the overall patient population.

Items	Total Patients (n = 126)	Patients with QAlb Measurements (n = 30)	p-value
Age (years)	45.26 ± 15.30	41.31 ± 14.54	0.179
Sex (Female)	88.09 %	89.70 %	0.738
Age at onset, y (mean ± SD)	43.83 ± 15.09	40.89 ± 14.71	0.352
Anti-AQP4 Antibodies Positive	86.3 (%)	89.7 (%)	0.738
CSF Cell Count (cells × 10^6/L)	NA	22.4 ± 35	NA
CSF Glucose (mmol/L)	NA	4.06 ± 1.08	NA
CSF Protein (mg/dL)	NA	0.25 ± 0.15	NA
CSF chloride (mmol/L)	NA	125.05 ± 3.55	NA
OCB (normal)	NA	19 (63.3 %)	NA

Abbreviations: SD, standard deviation; NA, not assessable.QAlb, CSF/blood albumin quotient; OCB, Oligoclonal bands; CSF: Cerebrospinal fluid.

Table 7
Stratified analysis of TIMP1-SNPs in different clinical features of patients with NMOSD.

Clinical character	Haplotype	Phenotype N (%)	Phenotype N (%)		OR (95 % CI)	*P*-value
AQP4 status		AQP4IgG-	AQP4IgG+			
	TTG	7 (41.1 %)	34 (31.8 %)		0.66 (0.23–1.89)	0.444
	Others	10 (58.8 %)	73 (68.2 %)		1	
QAlb status		**normal**	**increased**			
	TTG	4 (22.2 %)	7 (58.3 %)		2.41 (1.01–5.80)	**0.04**
	Others	14 (77.8 %)	5 (41.7 %)		1	
OCB status		**negative**	**positive**			
	TTG	7 (36.8 %)	4 (36.4 %)		1.02 (0.37–2.62)	0.98
	Others	12 (63.2 %)	7 (63.6 %)		1	
Onset Symptoms		**optic neuritis**	**myelitis**	**mix attacks**		
	TTG	6 (25 %)	19 (36.5 %)	16 (33.3 %)	NA	0.609
	Others	18 (75 %)	33 (63.5 %)	32 (66.7 %)	NA	
Age at onset		**>43**	**≤43**			
	TTG	19 (27.5 %)	23 (40.4 %)		1.46 (0.89–2.41)	0.129
	Others	50 (72.5)	34 (59.6 %)		1	
ANA status[a]		**positive**	**negative**			
	TTG	33 (34.7 %)	8 (27.6 %)		1.40 (0.56–3.50)	0.47
	Others	62 (65.3 %)	21 (72.4 %)		1	

Abbreviations:AQP4IgG+, AQP4IgG positive; AQP4IgG-, AQP4IgG negative; Age at onset Mean ± SD, years = 43.63 ± 13.32 years.OR: Odd Ratios.
[a] ANA refers to antinuclear antibody. For stratified according to Onset symptoms set 0 = optic neuritis,1 = myelitis,2 = Mix attacks. Mix attacks include patients with more than two locals including the optic nerve, brain, spinal cord, and brain stem , The bold terms indicate statistically significant values.

haplotypes, and complete LD among rs4898, rs6609533, and rs2070584 was observed. Haplotype analysis identified T-T-G (rs4898-rs2070584-rs6609533) with a higher frequency in the NMOSD case group than in the healthy control group. Furthermore, when stratified according to clinical characteristics, the T-T-G haplotype was closely associated with increased permeability of Qalb in NMOSD patients suggesting this haplotype was related to the BBB disruption in this disorder. This alignswith previous studies that reported linkage disequilibrium between rs4898 and rs6609533 [25]. However, the rs4898 locus has also been implicated in susceptibility to other autoimmune diseases such as Crohn's disease [26], sepsis [27], female systemic sclerosis patients [18], and aseptic loosening after total hip arthroplasty [19]. However, different results have been obtained in other studies. For instance, research by Lai et al. indicated that the TIMP1 rs4898 CC genotype increased the risk of lung cancer in Taiwanese populations [21], while Hemant Vira's study showed that the TIMP1 rs4898C allele increased the risk of systemic lupus erythematosus [15]. These discrepancies may be due to distinct genetic or etiologic contributions to different diseases, as well as genetic heterogeneity, and ethnic, and geographic variations that might alter the frequency of specific polymorphisms. This suggests that the heterogeneity of diseases could be a contributing factor.

The TIMP1 rs4898 polymorphism is a functional missense mutation located in the coding region of TIMP1, which may lead to functional changes in the encoded protein. Previous studies have shown that rs4898 regulates TIMP1 protein expression. Works from Meijer MJ et al. found that patients with Crohn's disease-carrying the T allele in the rs4898 genetic polymorphism of TIMP1 presented lower levels of TIMP1 in surgically resected macroscopically inflamed tissue [26]. However, work by Domchek et al. acquired the opposite result in patients with severe sepsis [20]. We obtained results similar to those of Meijer MJ et al. This may be because both Crohn's disease and NMOSD are autoimmune diseases. In this study, our findings suggest that TIMP1 polymorphisms, particularly the rs4898-T allele, may contribute to disease susceptibility and BBB disruption. This indicates a potential disease-specific impact of TIMP1 on NMOSD pathogenesis, which may differ from its role in other conditions. The association with BBB disruption is particularly noteworthy, as it highlights a possible mechanism by which TIMP1 polymorphisms could exacerbate NMOSD.

TIMP1 is elevated in the serum of multiple sclerosis (MS) patients and is involved in the disease's pathogenesis. A study by Zoltán

Krabóth et al. found that MMP-9 and TIMP-1 levels in MS patients were significantly higher during both relapse and remission periods compared to healthy controls [28]. Additionally, the number of relapses correlated with higher levels of MMP-9 and TIMP-1, indicating these enzymes play a crucial role in the early stages of MS and may serve as potential therapeutic targets [28]. Fatemeh Ghasemi Sakha et al. found similar results, showing that MMP-9 and TIMP-1 levels reflect inflammatory activity and inhibition, respectively Another study demonstrated that natalizumab significantly reduced MMP expression and increased TIMP expression in the spinal cord of MS mice, thereby inhibiting BBB damage and inflammation [29]. These studies collectively suggest that TIMP1 plays a significant role in MS and that changes in TIMP1 expression may alter BBB damage and inflammation.

NMOSD and MS share similar pathogenesis mechanisms and were considered the same disease until the discovery of AQP4 antibodies in 2004 [30]. Changes in TIMP1 expression in NMOSD may contribute to BBB disruption in patients. Results from many studies have shown that TIMP1 exerts various functions in autoimmune diseases. Work from Uchida T.et al. showed that NMO patients exhibited significantly elevated TIMP1 levels in CSF than the other groups [12,31]. NMOSD patients showed numerous TIMP1-positive hypertrophic astrocytes at the lesion edge and surrounding areas of the lesions [31]. Regarding MMP9, Hosokawa et al. reported that increased serum MMP9 plays a crucial role in the pathogenesis of NMOSD by modulating the BBB disruption [10,11]. In addition, MMP-9, TIMP-1, and MMP-9/TIMP-1 ratios may act as biomarkers for susceptibility to other autoimmune diseases, such as SLE, sepsis, autoimmune encephalitis, and multiple sclerosis [15,32–34]. Collectively, these results suggested that TIMP1 and MMP-9 are involved in the pathogenesis of NMOSD.

These findings suggest that TIMP1 genetic polymorphisms and haplotypes may play a crucial role in the susceptibility and pathogenesis of NMOSD, particularly in BBB integrity. The study underscores the potential of TIMP1 as a therapeutic target for addressing BBB disruption in NMOSD. Future research should focus on elucidating the precise molecular mechanisms by which TIMP1 polymorphisms influence NMOSD pathogenesis and BBB disruption, as well as exploring targeted therapeutic strategies to improve patient outcomes.

This study had several limitations. Firstly, the limited sample size led to relatively low statistical power. A key limitation of our study is that QAlb measurements were available for only 23.8 % of patients. Despite no significant differences in age, gender, onset age, or AQP4-IgG antibody positivity between those with and without QAlb data, selection bias remains a possibility. Future research should aim to gather a more extensive QAlb dataset to validate our findings and better understand the link between TIMP1 polymorphism and Neuromyelitis. Secondly, the detailed mechanisms by which TIMP1 gene polymorphisms regulate TIMP1 expression and their involvement in the pathogenesis of NMOSD have not been fully elucidated. Additionally, we did not examine the changes in TIMP1 and MMP9 protein levels at different stages of NMOSD or their relationship with gene polymorphisms.

5. Conclusion

TIMP1 rs4898-T, rs2070584-T, and rs6609533 polymorphism may contribute to the susceptibility of Female NMOSD patients in the Chinese Population. TIMP1 T-T-G (rs4898-rs2070584-rs6609533) haplotype is more common among female NMOSD patients and is linked with heightened disruption of the BBB.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Second Hospital of Hebei Medical University and written informed consent was obtained from each participant or his/her legal guardian. This study was conducted according to the principles expressed in the Declaration of Helsinki.

Consent for publication

All authors have given their consent for publication.

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Data availability

The datasets generated and/or analyzed during the current study are available in the [EVA] repository, [Project: PRJEB60368 , Analyses: ERZ16273343].

CRediT authorship contribution statement

Hongjing Yan: Writing – original draft. **Yining Wang:** Data curation. **Ruoyi Guo:** Methodology. **Zhen Jia:** Data curation. **Jia Liu:** Writing – review & editing. **Bin Li:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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List of abbreviations

NMOSD	Neuromyelitis Optica spectrum disorders
BBB	blood-brain barrier
TIMP1	Tissue inhibitor of metalloproteinase-1
HCs	healthy controls
AQP4	anti-aquaporin4
CNS	central nervous system
MMP9	Matrix metalloproteinase-9
ECM	extracellular matrix
CSF	cerebrospinal fluid
ANA	antinuclear antibodies
OCB	Oligoclonal bands
Qalb	CSF/blood albumin quotient
PBMC	Peripheral blood mononuclear cells
ROC	receiver operating characteristic
LD	Linkage disequilibrium
SNP	single-nucleotide polymorphisms

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