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ORIGINAL RESEARCH

Association of MTMR3 rs12537 at miR-181a Binding Site with Ischemic Stroke in Southern Chinese Han Population

Linfa Chen[1,2,*], Shan Wei[2,*], Yutian Zhang[3,*], You Li[3], Zishan Li[2], Pengru Huang[1], Chun Xiao[2], Yusheng Zhang[1]

[1]Department of Neurology, The First Affiliated Hospital of Jinan University, Guangzhou, 510630, People's Republic of China; [2]Huizhou Third People's Hospital, Guangzhou Medical University, Huizhou, 516002, People's Republic of China; [3]Guangdong Key Laboratory of Age-Related Cardiac and Cerebral Diseases, Affiliated Hospital of Guangdong Medical University, Zhanjiang, 524001, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yusheng Zhang, Department of Neurology, The First Affiliated Hospital of Jinan University, Guangzhou, 510630, People's Republic of China, Email zhangys@jnu.edu.cn; Chun Xiao, Huizhou Third People's Hospital, Guangzhou Medical University, Huizhou, 516002, People's Republic of China, Email HuizhouXiaoc@163.com

Background: Single nucleotide polymorphisms (SNPs) at microRNA (miRNA)–binding sites influence the development of ischemic stroke (IS) by affecting the expression of specific target genes. Myotubularin-related protein 3 (MTMR3), which is involved in autophagy, is directly targeted by miR-181a. This research examined the potential association between the SNP rs12537 in the miRNA-181a binding location within the 3′ untranslated region (3′-UTR) of MTMR3 and the incidence and prognosis of IS.

Methods: An improved multitemperature ligase detection reaction assay was used to perform genotyping analysis in two independent case-control datasets consisting of 1128 subjects with IS and 1140 healthy controls with matched ages.

Results: The distribution frequencies of the T allele ($p = 5.2\times10^{-4}$) of SNP rs12537 in MTMR3 were elevated significantly in IS patients as compared to healthy controls. Further categorization based on IS subtypes revealed that individuals carrying the variation T allele were linked with a higher risk of suffering large-artery ($p = 1.2\times10^{-3}$) and small-artery ($p = 7.0\times10^{-4}$) atherosclerotic stroke subtypes. The T allele of rs12537 was shown to be linked to both moderate and severe stroke (NIHSS $\geq$ 6) ($p = 0.011$), as well as a poor short-term outcome ($p = 0.016$) of IS. A significant correlation was also found between the rs12537 T allele mutation and a decrease in MTMR3 ($p = 0.019$), as well as an elevated miR-181a ($p = 0.021$) and LC3B ($p = 0.026$) in individuals with IS.

Conclusion: This study identified a novel role for the rs12537 variant in influencing IS susceptibility and prognosis, potentially by modulating MTMR3 expression and leading to increased autophagy.

Keywords: ischemic stroke, MTMR3, polymorphism, risk, prognosis

Introduction

Ischemic stroke (IS) is a severe cerebrovascular disease that has a high death rate and causes significant disability.[1] During the past decade, many genome-wide association studies (GWAS) have shown various genomic regions linked to the susceptibility to IS[2] which have broadly expanded the knowledge of the genetic architecture of this disease. These findings have significantly enhanced the understanding of the genetic makeup of the disease. However, those identified genes that provide a risk do not completely explain the exact biochemical pathways that are responsible for the development of IS. Furthermore, the relationship between environmental risk factors and genetic predisposition adds complexity to the genetic development of IS.

MicroRNAs (miRNA) are essential for controlling several biological processes through the post-transcriptional modification of gene expression.[3] Genetic variations in miRNA-associated genes have the potential to affect various phenotypic outcomes.[4] Studies have reported that circulating levels of miR-181a were significantly elevated in IS, suggesting its

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involvement in the disease's pathogenesis.[5] In vivo experiments demonstrated that silencing miR-181a led to a considerable decrease in infarct size, mitigated neuron loss due to ischemia, and enhanced neurological outcomes.[6] miR-181a has been reported to target genes involved in inflammation and lipid metabolism, which are key factors in atherosclerosis.[5] In addition, miR-181a is found to affect the expression of proteins involved in endothelial cell function and vascular permeability.[6] These pathogenic mechanisms are involved in the causation of IS subtypes, and miR-181a may play diverse roles in different types of IS. Furthermore, a genetic variant, rs322931, identified through a GWAS, has been linked to an elevated risk of IS. Earlier research also pointed to myotubularin-related protein 3 (MTMR3) as a direct target of miR-181a.[5,7] The MTMR3 belongs to the myotubularin family. Neuro-muscular disorders are caused by mutations in certain myotubularin members.[8] The MTMR3 inhibits constitutive autophagy by controlling the amounts of phosphatidylinositol 3-phosphate (PI3P) in the surroundings.[9] The MTMR3 was also shown to regulate the effects of pattern recognition receptors (PRRs) and the release of cytokines in inflammatory bowel disease (IBD).[10] Recent research has shown a significant decrease in the expression of MTMR3 in a model of myocardial ischemia-reperfusion injury (MIRI).[11] More recently, MTMR3 has been identified as a susceptible gene and potential therapeutic target for unruptured intracranial aneurysms.[12] Given that autophagy and inflammation are significant factors in the development of IS, these indications have led to the conclusion that MTMR3 may have a function in the pathological process of IS.

The single nucleotide polymorphism (SNP) rs12537, which is located in the 22q12 region within the MTMR3 gene, resides in the miR-181a binding site in the 3′UTR of MTMR3. This SNP has been linked with a higher risk of gastric cancer (GC).[7] A GWAS conducted in the population of Chinese Han has linked the MTMR3 rs12537 variant to an elevated risk of IgA nephropathy.[13] Senousy et al reported a significant link between the rs12537 TT genotype with the occurrence of systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Egyptian patients.[14] Nevertheless, the influence of rs12537 on the risk and severity of IS has not yet been explored. The objective of this research was to examine if there is an association between the MTMR3 SNP rs12537 with the risk and prognosis of IS in the population of Southern Chinese Han.

Materials and Methods

Subject Recruitment and Sample Collection

This two-stage hospital-based case-control study, conducted in two stages, enrolled a total of 1128 IS subjects and 1140 healthy control participants. The initial cohort included 615 IS patients who were enrolled by the Department of Neurology at the Affiliated Hospital of Guangdong Medical University from 2015 to 2017. To validate the results seen in the first cohort, a replication cohort consisting of 513 IS patients was recruited from the Department of Neurology at Huizhou Third People's Hospital between 2020 and 2023. Both the initial and replication cohorts consisted of unrelated Han Chinese adults from Guangdong province. The IS patients' diagnosis was conducted according to the International Classification of Diseases, using history, physical exams, clinical signs, and cranial CT or MRI results. Following the guidelines set by the Trial of Org 10172 in the Acute Stroke Treatment (TOAST) categorization system,[15] all subjects diagnosed with IS were categorized into subtypes including large-artery atherosclerosis (LAA), small-artery occlusion (SAO), cardioembolic (CE), and unspecified etiology (UE).[15] LAA strokes, which feature narrowing of cerebral arteries and rupture of atherosclerotic plaques, usually manifest with acute, severe symptoms. The risk of complications is elevated, and outcomes tend to be unfavorable because of the unstable nature of the plaques.[16] SAO strokes stem from small-vessel pathologies like lipohyalinosis and microatheroma. They commonly affect the elderly, especially those with hypertension and diabetes.[17] CE strokes happen when emboli originating from the heart, commonly associated with atrial fibrillation or other cardiac disorders, block cerebral vessels, leading to areas of tissue death in the brain.[18] UE strokes encompass cases where, even after thorough diagnostic evaluation, the underlying cause of the ischemic event remains elusive.[19]

Patients who previously had a record of transient ischemic attacks, cerebral hemorrhage, subarachnoid hemorrhage, cardioembolism, coronary artery diseases, haematologic disorders, autoimmune diseases, malignant tumors, or chronic infectious diseases were not allowed to participate in the research. Furthermore, a participant who had previously been diagnosed with a stroke was not included in this research.

The initial control group consisted of 615 subjects (311 male and 304 female) who were matched for age and ethnicity and visited the Health Examination Center of the Affiliated Hospital of Guangdong Medical University at the same time as the IS patients. In the replication study, 525 healthy individuals (323 men and 203 women), matched for both sex and age, were randomly selected from Huizhou within the same timeframe. Individuals with recent cerebrovascular disease or myocardial infarction, and any of the above-mentioned conditions in the exclusion criteria for IS patients were not included in the control group, and the replication cohort adhered to the same inclusion and exclusion criteria as the original cohort.

The National Institute of Health Stroke Scale (NIHSS) score was used to assess the severity of stroke at the time of admission, where scores below 6 indicated a minor stroke, and scores of 6 or above signified a moderate to severe stroke. To evaluate functional outcomes, the modified Rankin Scale (mRS) score was recorded 3 months post-onset, with mRS $\leq$ 2 signifying favorable outcomes and mRS > 2 indicating poor outcomes. At 3 months, follow-up was conducted for 1016 patients either via phone or in-person visits; however, 112 patients (9.9%) were lost to follow-up. All participants in the study voluntarily provided written informed consent before their inclusion. The research was conducted according to the ethical principles outlined in the Declaration of Helsinki, ensuring the protection of human subjects. The study protocol received formal approval from the Ethics Committees of both the Affiliated Hospital of Guangdong Medical University (2015–064KT) and Huizhou Third People's Hospital (2020-MR-96), underscoring the commitment to ethical standards throughout the research process.

Genotyping

Peripheral venous blood samples were used to isolate genomic DNA with the TIANamp Blood DNA Kit (Tiangen Biotech, Beijing, China). The rs12537 polymorphism of the MTMR3 gene, reported in a GWAS study, was analyzed using the iMLDR-TM technique with the following primers, forward:5′- GCAGAGTGGGCTCAGTGT-3′, reverse:5′-TGTCCACGTTTGGTCCTC-3′. This method involves the use of specific primers designed to anneal to the target DNA sequence, followed by a ligase reaction that links the primers to form a product detectable by capillary electrophoresis.

RNA Extraction and Real-Time PCR

The isolation of peripheral blood mononuclear cells (PBMCs) was carried out using LymphoprepTM (Axis-Shield PoCAS, Oslo, Norway) with the help of density gradient centrifugation, as previously outlined.[20] Total RNA extraction from PBMCs was conducted using the RNAprep Pure Blood Kit (TianGen Biotech, Beijing, China), following the protocol provided by the manufacturer. RNA was reverse transcribed into complementary DNA using the RevertAid cDNA Synthesis Kit (Thermo Fisher Scientific) in accordance with the detailed protocol provided by the manufacturer. The levels of expression of MTMR3, GAPDH, and miR-181a were measured by qRT-PCR with SYBR green,[20] as previously outlined. Fold change was calculated by using the $2^{-\Delta\Delta Ct}$ formula. Table S1 lists the primers used in this study. Quantification of the relative expression levels for each sample was conducted using three technical replicates to ensure precision and reliability. To verify the accuracy of the amplification products, melting curve analysis was performed, providing additional confirmation of the specificity and correctness of the results.

Detection of Serum LC3B Levels

Prior to conducting the analysis, serum samples were carefully collected from the participants and immediately stored at a temperature of −80°C to ensure the preservation of the biological material. To measure the levels of LC3B, an ELISA was performed using a kit (Tiangen Biotechnology, Beijing, China). The procedure strictly adhered to the detailed instructions provided by the manufacturer, ensuring accuracy and consistency in the detection process.

Statistical Analysis

Statistical analysis was carried out using GraphPad Prism 5.0 (GraphPad Software, Inc., San Diego, CA, USA) and SPSS version 19.0 (IBM, NY, USA). The chi-square test was employed for the calculation and assessment of differences between categorical data, while the *t*-test was employed for continuous data. The study implemented a multivariate logistic regression model to investigate the associations between the MTMR3 rs12537 variant and functional outcome after a stroke, including the determination of 95% confidence intervals (CIs) and odds ratios (ORs). Levels of MTMR3, miR-181a, and LC3B across various genotypes in both patient and control groups were analyzed. The Student's *t*-test was used for comparing normally distributed data, while the Mann–Whitney *U*-test was employed for data that did not follow a normal distribution. Furthermore, linear regression analyses were conducted to account for potential confounding factors, including age, gender, smoking status, hypertension, diabetes mellitus, and hyperlipidemia. The Bonferroni correction was employed for multiple comparisons and to reduce the occurrence of type 1 errors. Specifically, we performed a total of 12 comparisons (6 for each cohort) to account for the different genotypes and alleles analyzed. The Bonferroni correction was applied by dividing the significance level ($\alpha = 0.05$) by the number of comparisons, resulting in a corrected significance level of 0.0042. In this study, 112 patients (9.9%) were lost to follow-up at 3 months. To address potential biases, sensitivity analyses compared baseline characteristics (age, gender, smoking, hypertension, diabetes, NIHSS scores) between those lost and those who completed follow-up, revealing no significant differences. A p-value < 0.05 was considered to have statistical significance.

Results

Demographic Characteristics

Table 1 lists the demographic details of the 1128 IS patients and 1140 healthy controls that were included in this investigation. A flow chart of the study was shown in Figure 1. Across the two study cohorts, insignificant differences were found in age, total cholesterol, serum uric acid, triglycerides, or HDL-cholesterol between patients and controls. However, significant differences were observed between the control and IS groups in smoking status ($p < 0.001$), diabetes ($p < 0.001$), hypertension ($p < 0.001$), and homocysteine levels ($p < 0.001$). The initial cohort demonstrated significant

Table 1 Characteristics of Ischemic Stroke Cases and Controls

Variables	Initial Cohort			Replication Cohort		
	IS (n=615)	Control (n=615)	*P value*	IS (n=513)	Control (n=525)	*P value*
Mean age (years)	62.47±9.89	61.53±8.91	0.11	62.32±9.52	61.45±8.76	0.13
Male/female	408/207	311/304	**< 0.001**	341/172	322/203	0.093
Smokers, n (%)	167 (27.2)	73 (11.9)	**< 0.001**	158 (30.8)	91 (17.3)	**< 0.001**
Hypertension, n (%)	466 (75.8)	199 (32.4)	**< 0.001**	375 (73.1)	194 (37.0)	**< 0.001**
Diabetes, n (%)	184 (29.9)	51 (8.3)	**< 0.001**	149 (29.0)	65(12.4)	**< 0.001**
Uric acid (mmol/L)	319.8±88.4	322.1±90.9	0.72	346.54±105.27	338.72±96.35	0.13
Total cholesterol (mmol/L)	5.07±1.18	5.06±0.99	0.94	4.84±1.13	4.58±1.04	0.088
Triglycerides (mmol/L)	1.62±1.09	1.42±0.97	**< 0.001**	1.64±1.03	1.65±1.08	0.88
HDL-cholesterol (mmol/L)	1.30±0.64	1.41±0.42	**< 0.001**	1.22±0.75	1.25±0.64	0.49
LDL-cholesterol (mmol/L)	3.07±1.03	3.01±0.97	0.42	3.18±1.05	3.05±0.98	**0.039**
HCY (mmol/L)	11.60±6.06	10.33±3.33	**< 0.001**	13.25±4.85	12.13±3.56	**< 0.001**
Antihypertensive Medication	313(50.9)	NA		242(47.2)	NA	
Antidiabetic Drugs	153(24.9)	NA		127(24.8)	NA	
Antiplatelet Therapy	609(99.0)	NA		508(99.0)	NA	
Statins	603(98.0)	NA		502(95.6)	NA	
Intravenous thrombolysis	22(3.6)	NA		17(3.3)	NA	
Endovascular treatment	89(14.5)	NA		69(13.5)	NA	

Notes: Continuous data are presented as the mean ± SD, median (range) or n (%). P < 0.05 is indicated in bold font.
Abbreviations: IS, Ischemic stroke; HDL-cholesterol, high-density lipoprotein cholesterol; LDL-cholesterol, low-density lipoprotein cholesterol; HCY, Homocysteine; NA, not available.

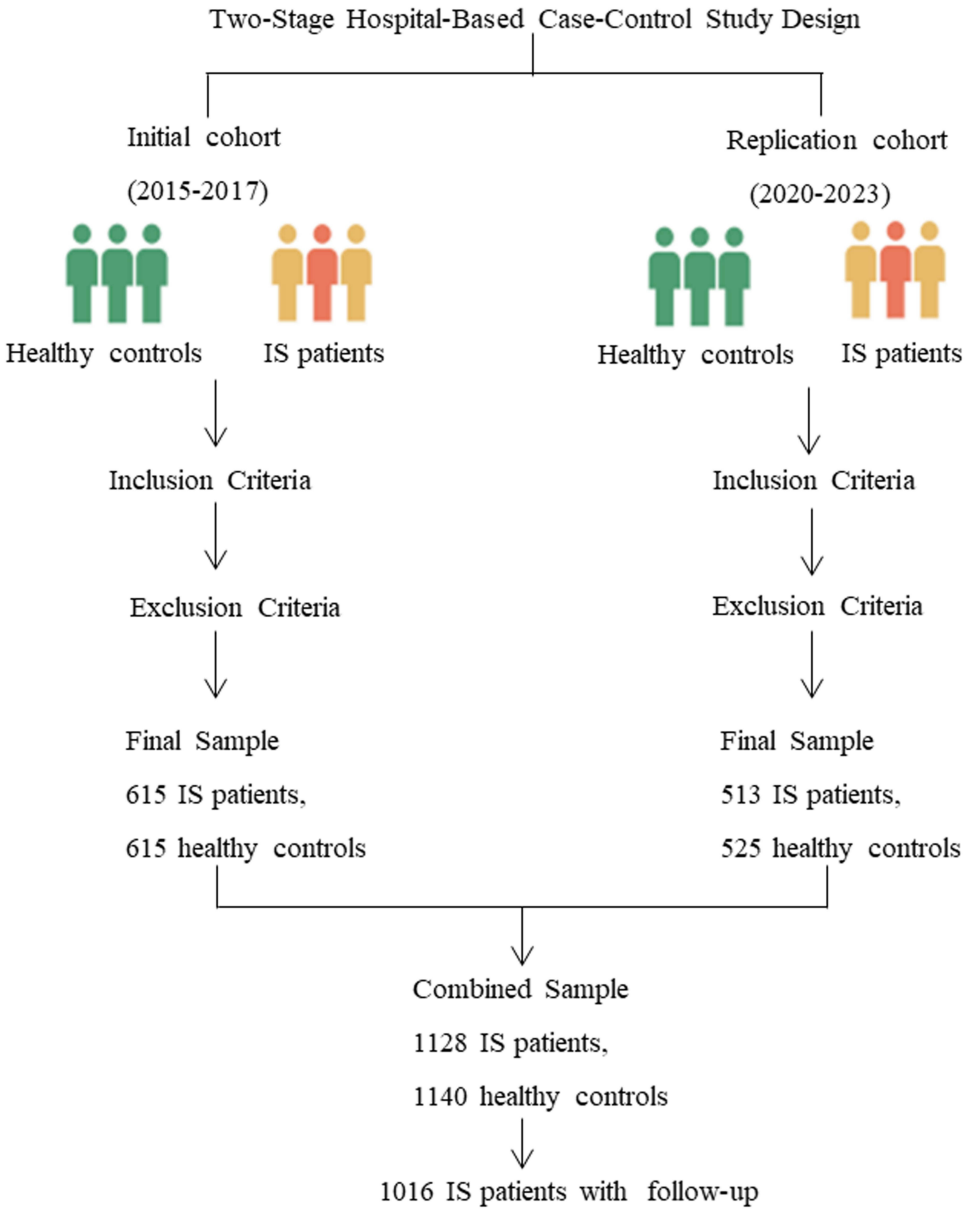

Figure 1 A flow chart of the study.

disparities in gender, triglycerides, and HDL-cholesterol levels between controls and IS patients, whereas the replication cohort did not reveal differences in gender, triglycerides, or HDL-cholesterol levels. LDL-cholesterol levels were elevated significantly in IS patients in the replication cohort, although insignificant variation was observed between the IS case group and controls in the initial cohort.

Relationship between the MTMR3 rs12537 Variants and the Risk of IS

Table 2 summarizes the genotype and allele frequencies for the *MTMR3* rs12537 variation in IS patients and controls. The Hardy-Weinberg equilibrium test displayed no deviation in either group ($p > 0.05$). The initial cohort analysis indicated a significant association between the rs12537 variants and IS risk ($p = 0.040$). This association was validated in the replication cohort with a p-value of 0.017. The frequency of rs12537 was shown to be significantly different in IS patients vs the control group in a dominant model (CC vs CT/TT). The initial cohort had a p-value of 0.027, whereas the replication cohort had a p-value of 7.3×10^{-3}. On the other hand, the recessive model did not show any significant

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Table 2 Genotype and Allele Frequencies of MTMR3 rs12537 Variant Between IS Patients and Controls, and Corresponding ORs for IS

Genotype &Allele	Initial Cohort				Replication Cohort				Combined Cohort	
	IS Patients (n=615)	Controls (n=615)	OR (95% CI)	P[a] value	IS Patients (n=513)	Controls (n=525)	OR (95% CI)	P[a] value	OR (95% CI)	P[a] value
rs12537										
CC	376(61.1)	421(68.5)		**0.017**	308(60.0)	351(66.9)		**0.040**		**1.3×10^{-3}**
CT	208(33.8)	175(28.5)			175(34.1)	156(29.7)				
TT	31(5.1)	19(3.1)			30(5.8)	18(3.4)				
CC vs CT/TT	239(38.9)	194(31.5)	1.38 (1.09–1.74)	**7.3×10^{-3}**	205(40.0)	174(33.1)	1.33(1.03–1.72)	**0.027**	1.36(1.14–1.61)	**7.2×10^{-4}**
CC/CT vs TT	584(95.0)	596(96.9)	0.60 (0.34–1.08)	0.11	483(94.2)	504(96.0)	0.58(0.32–1.05)	0.070	0.59(0.39–0.89)	**0.013**
C allele	960(78.0)	1017(82.7)	1.00(reference)		791(77.1)	852(81.6)	1.00(reference)		1.00(reference)	
T allele	270 (22.0)	213(17.3)	1. 34(1.10–1.64)	**3.9×10^{-3}**	235(22.9)	192(18.4)	1.32(1.06–1.63)	**0.011**	1.33(1.15–1.54)	**5.2×10^{-4}**

Notes: Data are presented as number (%) [a] adjusted for age, gender, smoking, hypertension, diabetes mellitus and hyperlipidaemia. P < 0.05 is indicated in bold font.

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differences between the IS group and the control group for the rs12537 variant when analyzed separately. However, when the data from both groups were combined for analysis, the recessive model revealed a significant difference in the rs12537 variant between IS patients and controls, with a *p*-value of 0.013. The frequencies of the variation T allele at rs12537 were considerably higher in the IS group vs the control group, with a *p*-value of 0.011 in the first cohort and a p-value of 7.3×10^{-3} in the replication cohort.

Associations between the MTMR3 rs12537 Variants and Demographic Characteristics

The general characteristics of subjects according to *MTMR3* rs12537 genotypes and alleles are outlined in Table 3. Upon adjusting for age, sex, hypertension, diabetes, and smoking status, it was observed that an increased risk of IS was correlated with the MTMR3 rs12537 T allele in age ≥ 60 ($p = 2.6\times10^{-3}$), male ($p = 3.6\times10^{-3}$) and non-hypertensive patients ($p = 0.021$) (Table 3).

Association of MTMR3 rs12537 Variants with Stroke Subtypes

To determine whether the MTMR3 rs12537 variations were limited to a specific subgroup, the IS patients were classified into stroke subgroups based on the TOAST categorization. As indicated in Table 4, carriers of the rs12537 T allele had a significantly higher risk of large-artery atherosclerotic (LAA) stroke ($p = 1.2\times10^{-3}$) and small-artery atherosclerotic (SAA) stroke ($p = 7.0\times10^{-4}$) compared to the control group.

Association between MTMR3 rs12537 Variants and the Severity of Stroke

The baseline characteristics of patients were categorized according to their NIHSS scores (Table 5). Multivariate logistic regression analysis found a correlation between the rs12537 variants and a higher risk of severe stroke ($p = 0.011$). In the recessive genetic model, a significant correlation was found between CT+TT genotypes and an NIHSS score of ≥ 6, with a *p*-value of 0.011, suggesting that these genotypes are linked with greater stroke severity. Patients carrying the rs12537 variant T allele also showed a higher risk of NIHSS ≥ 6 ($p = 0.011$).

MTMR3 rs12537 Variants and Short-Term Outcome of IS

The short-term prognosis of IS was evaluated three months after the stroke using the mRS. The baseline characteristics of the two patient groups are described in Table 6. Patients with an mRS > 2 were more likely to have elevated basal NIHSS scores ($p < 0.001$) and to receive intravenous thrombolysis ($p = 0.036$) and endovascular treatment ($p = 0.036$). The

Table 3 A Comparison Between the Baseline Characteristics of the MTMR3 rs12537 Genotypes and Alleles in the IS Patient and Control Groups

Characteristics	IS Patient Group					Control Group					P_G[a] value	P_A[a] value
	Genotype n (%)			Allele n (%)		Genotype n (%)			Allele n (%)			
	CC	CT	TT	C	T	CC	CT	TT	C	T		
Age												
≥60	356(60.4)	199(33.8)	34(5.8)	911(77.3)	267(22.7)	399(69.4)	160(27.8)	16(2.8)	958(83.3)	192(16.7)	**0.012**	**2.6×10^{-3}**
<60	328(60.9)	184(34.1)	27(5.0)	840(77.9)	238(22.1)	373(66.0)	171(30.3)	21(3.7)	917(81.2)	213(18.8)	0.19	0.065
Gender												
Male	451(60.2)	256(34.2)	42(5.6)	1158(77.3)	340(22.7)	431(68.1)	182(28.8)	20(3.1)	1044(82.5)	222(17.5)	**0.015**	**3.6×10^{-3}**
Female	233(61.5)	127(33.5)	19(5.0)	593(78.2)	165(21.8)	341(67.3)	149(29.4)	17(3.4)	831(82.0)	183(18.0)	0.19	0.065
Diabetes												
Yes	199(59.8)	115(34.5)	19(5.7)	513(77.0)	153(23.0)	270(68.7)	112(28.5)	11(2.8)	652(83.0)	134(17.0)	**0.048**	**0.012**
No	485(61.0)	268(33.7)	42(5.3)	1238(77.9)	352(22.1)	502(67.2)	219(29.3)	26(3.5)	1223(81.9)	271(18.1)	**0.048**	**0.012**
Hypertension												
Yes	511(60.8)	285(33.9)	45(5.4)	1307(77.7)	375(22.3)	80(69.0)	33(28.4)	3(2.6)	193(83.2)	39(16.8)	0.19	0.065
No	173(60.3)	98(34.1)	16(5.6)	444(77.4)	130(22.6)	692(67.6)	300(29.3)	34(3.3)	1684(82.1)	368(17.9)	0.059	**0.021**

Notes: P_G: P value of the difference in alleles between the case and control groups; P_A: P value of the difference in genotype between the case and control groups. [a]adjusted for age, gender, smoking, hypertension, diabetes mellitus and hyperlipidaemia. P < 0.05 is indicated in bold font.

Table 4 The Relationship Between MTMR3 rs12537 Variant and IS Stratified by TOAST Classification in IS Patients

	MTMR3 rs12537							
	Genotype			P value[a]	Allele		P value[a]	OR (95% CI)
	CC	CT	TT		C	T		
Control (n=1140)	772(67.7)	331(29.0)	37(3.3)		1875(82.2)	405(17.8)		
Cases								
LAA (n=703)	430(61.2)	235(33.4)	38(5.4)	**5.1×10^{-3}**	1095(77.9)	311(22.1)	**1.2×10^{-3}**	1.31(1.11–1.55)
SAA (n=315)	182(57.8)	116(36.8)	17(5.4)	**2.9×10^{-3}**	480 (76.2)	150(23.8)	**7.0×10^{-4}**	1.45(1.17–1.79)
CE (n=41)	31(75.6)	8(19.5)	2(4.9)	0.31	70(85.4)	12(14.6)	0.47	0.79(0.43–1.48)
UE +OE (n=69)	41(59.4)	24(34.8)	4(5.8)	0.23	106(76.8)	32(23.2)	0.11	1.40(0.93–2.11)

Notes: [a]adjusted for age, gender, smoking, hypertension, diabetes mellitus and hyperlipidaemia. P < 0.05 is indicated in bold font.
Abbreviations: LAA, Large-artery atherosclerosis; SAA, Small-artery atherosclerosis; CE, Cardioembolic; UE, Undetermined aetiology; OE, other aetiology.

Table 5 Association of MTMR3 rs12537 Polymorphism with Stroke Severity of IS

Variables	NIHSS<6	NIHSS≥6	P value[a]	AOR (95% CI)
Age ≥ 60	211(50.1)	378(53.5)	0.29	1.14(0.90–1.46)
Male	271(64.4)	478(67.6)	0.27	1.16(0.90–1.49)
Smoking	117(27.8)	208(29.4)	0.59	1.08(0.83–1.42)
Diabetes	113(26.8)	220(31.1)	0.14	1.23(0.94–1.61)
Hypertension	304(72.2)	537(76.0)	0.18	1.22(0.92–1.60)
rs12537				
CC(684)	278(66.0)	406(57.4)	**0.011**	
CT(383)	123(29.2)	260(36.8)		
TT(61)	20(4.8)	41(5.8)		
CC/CT vs TT	401(95.2)	666(94.2)	0.45	0.81(0.47–1.40)
CC vs CT/TT	143(34.0)	301(42.6)	**0.011**	1.44(1.12–1.85)
C allele	679(80.6)	1072(75.8)	1.00(reference)	
T allele	163(19.4)	342(24.2)	**0.011**	1.33(1.08–1.64)

Notes: [a]adjusted for age, gender, smoking, hypertension, diabetes mellitus and hyperlipidaemia. P < 0.05 is indicated in bold font.

rs12537 CT/TT genotype was significantly associated (p = 0.016) with a 1.34-fold increased risk of poor outcomes compared to the CC genotype when confounding factors were controlled. Patients with IS who carried the rs12537 T allele were more likely to experience unfavorable outcomes with greater disability (p = 0.016).

Correlation between rs12537 Variants and MTMR3 and miR-181a Expression

A comparative determination was performed on the expression levels of *MTMR3* mRNA and *miR-181a* in the PBMCs of 89 IS patients and 94 healthy controls. In the IS patients, the expression of *MTMR3* mRNA was significantly reduced (Figure 2A) and miR-181a was significantly increased (Figure 2B) when compared with the controls (p = 0.010 and p < 0.001, respectively). Furthermore, the correlation between the average *MTMR3* mRNA and miR-181a levels in individuals with IS was examined, along with their *MTMR3* genotype. An elevated *MTMR3* mRNA expression was significantly observed in IS patients who possessed the mutated rs12537 T allele, with a p-value of 0.019 (Figure 2C). A significant drop in miR-181a levels was detected in IS patients with the rs12537 T allele (p = 0.021) (Figure 2D). However, controls with the rs12537 CC or CT+TT genotypes did not show statistically significant differences in *MTMR3* mRNA (p=0.63) or miR-181a (p=0.28) expression.

Table 6 Association of MTMR3 rs12537 Polymorphism with Short-Term Outcome of IS

Variables	mRS (0–2)	mRS (3–6)	P value[a]	AOR (95% CI)
Age ≥60	369(51.2)	161(54.6)	0.33	1.15(0.87–1.50)
Male	471(65.3)	201(68.1)	0.42	1.14(0.85–1.52)
Smoking	205(28.4)	88(29.8)	0.65	1.07(0.79–1.44)
Diabetes	205(28.4)	95(32.2)	0.26	1.20(0.89–1.60)
Hypertension	540(74.9)	210(71.2)	0.24	0.83(0.61–1.12)
Basal NIHSS score, admission	2(1–4)	8(6–14)	**<0.001**	
Antihypertension	348(48.3)	152(51.5)	0.37	1.14(0.87–1.49)
Antidiabetic drugs	175(24.3)	77(26.1)	0.58	1.10(0.81–1.50)
Antiplatelet	713(98.9)	293(99.3)	0.73	1.64(0.35–7.79)
Statin	705(97.8)	290(98.3)	0.81	1.32(0.48–3.63)
Intravenous thrombolysis	19(2.6)	16(5.4)	**0.036**	2.12(1.07–4.18)
Endovascular treatment	90(12.5)	52(17.6)	**0.036**	1.50(1.03–2.18)
rs12537				
CC	449(62.3)	167(56.6)	**0.016**	
CT	243(33.7)	102(34.6)		
TT	29(4.0)	26(8.8)		
CC/CT vs TT	692(96.0)	269(91.2)	**0.011**	2.31(1.33–3.99)
CC vs CT/TT	272(37.7)	128(43.4)	0.11	1.27(0.96–1.67)
C allele	1141(79.1)	436(73.9)		
T allele	301(20.9)	154(26.1)	**0.016**	1.34(1.06–1.67)

Notes: [a]adjusted for age, gender, smoking, hypertension, diabetes mellitus and hyperlipidaemia. P < 0.05 is indicated in bold font.

MTMR3 rs12537 Variants and Serum LC3B Levels According to Various Genotypes

Serum LC3B levels were assessed in 86 healthy controls and 82 IS patients. Patients with IS had significantly elevated blood LC3B levels vs control group ($p < 0.001$; Figure 3A). Serum LC3B levels were increased significantly in patients with the MTMR3 rs12537 CT+TT genotype compared to those with the CC genotypes ($p = 0.026$) (Figure 3B). However, this significance was not detected in the control group when comparing individuals with the rs12537 CT +TT genotype to those with the CC genotype.

Discussion

In the present two-stage hospital-based case-control study, a novel correlation was found between the rs12537 variant of MTMR3 and an increased risk of IS. The data further suggest that the rs12537 T allele may be linked to a higher risk of stroke in patients with LAA and SAA subtypes. Carriers of the rs12537 T allele also displayed decreased MTMR3 levels and elevated expression of miR-181a and LC3B compared to controls. Furthermore, a significant association was identified between the rs12537 T allele and negative functional outcomes in IS patients, indicating that this genetic variant might serve as a predictive biomarker for IS.

MTMR3, a member of the myotubularin-related protein family, contributes to the process of dephosphorylating PI3P. The production of PI3P is crucial for initiating autophagy as well as controlling the size of autophagosomes[21,22]. MTMR3 functions as a suppressor of basal autophagy by modulating the level of PI3P in certain locations.[9] In monocyte-derived macrophages, it decreases the levels of PRR-induced PI3P and autophagy. This, in turn, increases caspase-1 activation, the secretion of autocrine IL-1β, the signaling of NF-κB, and the total release of cytokines in IBD.[10] Macrophages from individuals carrying the MTMR3 rs713875 variant, who are at risk for IBD, also showed elevated MTMR3 expression along with increased cytokine secretion.[10] Furthermore, a recent study employing RNA-seq and RT-qPCR confirmed a significant reduction in MTMR3 expression in samples from an MIRI model compared to healthy controls.[11] Since autophagy and activation of inflammatory factors are key players in the pathogenesis of stroke, and as an important effector of stroke-related miRNA, miR-181a, it is speculated that MTMR3 may be involved in the

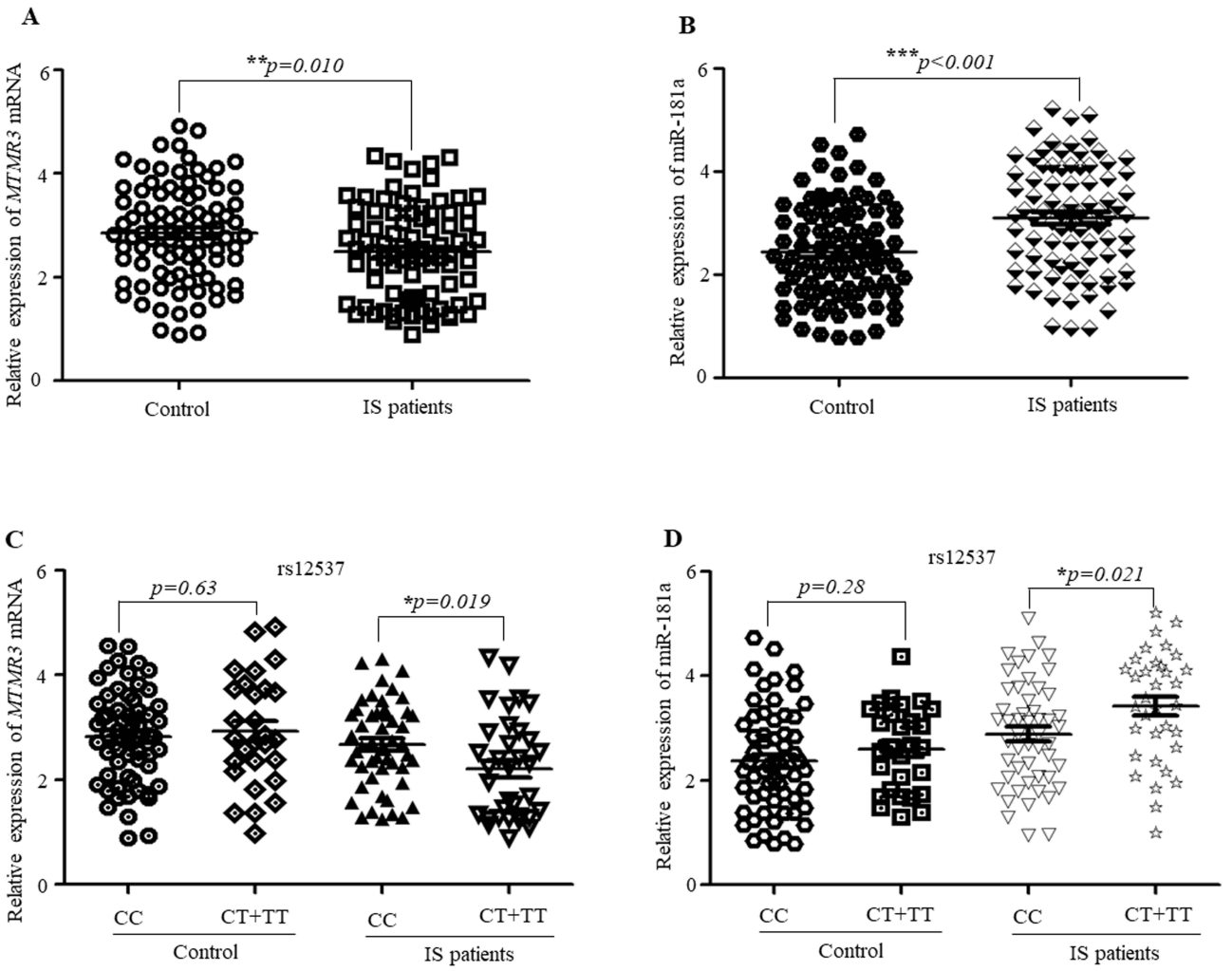

Figure 2 Relative MTMR3 (**A**) and miR-181a (**B**) expression in PBMCs from IS patients (n = 89) and healthy controls (n = 94). (**C**) Relative MTMR3 expression in IS patients and control subjects with the rs12537 CC and CT+TT genotypes. GAPDH served as a normalization control. (**D**) Relative miR-181a expression in IS patients and control subjects with the rs12537 CC and CT+TT genotypes. U6 served as the normalization control. Data are expressed as medians with interquartile ranges. IS: ischemic stroke. *p<0.05, **p<0.01, ***p<0.001.

pathogenesis of IS. This was supported by the present findings that *MTMR3* expression was statistically decreased in the peripheral blood of IS patients.

In this study, we showed that the *MTMR3* rs12537 variants were associated with increased IS risk and poor functional outcomes. MicroRNAs (miRNAs) are essential in regulating numerous biological processes by influencing gene expression at the post-transcriptional level. Genetic polymorphisms in genes related to miRNAs can influence these regulatory functions, potentially leading to a broad spectrum of phenotypic variations.[23] Numerous studies have revealed that SNPs can affect how efficiently miRNAs bind to the 3′ UTR of target genes, thereby impacting an individual's risk and prognosis for various diseases.[10] MTMR3 was selected as a direct target of miR-181a.[7] The SNP rs12537 present in the miR-181a-binding site in the 3′ UTR of the *MTMR3* gene, and Lin et al first reported that MTMR3 rs12537 is associated with susceptibility and prognosis of GC in the Southern Han Chinese population.[7] The rs12537 T/C variant in MTMR3 has been linked to IgA nephropathy in Han Chinese,[13] as well as RA and SLE in Egyptian patients.[14] A previous study recognized rs12537 as a new genetic susceptibility factor for ER-positive breast cancer (BC) specifically in the Han Chinese population, with potential implications for the methylation of MTMR3.[24] This study discovered a link between MTMR3 rs12537 variants and an increased risk of IS, particularly in the LAA and SAA subtypes. This suggests that the genetic predisposition to these subtypes may be influenced by the regulation of MTMR3 expression and autophagy. In contrast, no significant association was found for CE stroke,

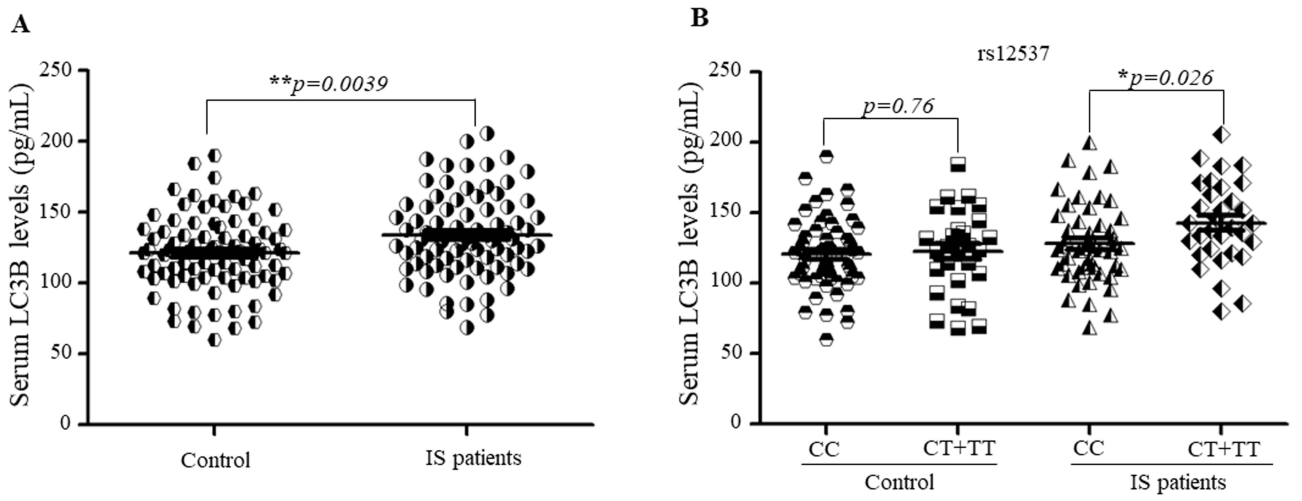

Figure 3 Serum LC3B levels stratified by MTMR3 rs12537 genotypes in IS patients and controls. (**A**) The LC3B levels in serum were measured in IS patients (n=82) and the controls (n=86). (**B**) Serum LC3B levels in controls and IS patients stratified by MTMR3 rs12537 CC and CT+TT genotypes. *p<0.05, **p<0.01 indicates a significant difference in serum LC3B levels between controls and IS patients.

highlighting the distinct pathophysiological mechanisms underlying different stroke subtypes. Furthermore, the study revealed that the risk of IS was statistically elevated in individuals with the rs12537 T allele who were older, male, and not diagnosed with hypertension. This suggests that the rs12537 variant may interact with environmental factors to contribute to the progression of IS. Furthermore, the findings show that the MTMR3 rs12537 T allele is significantly associated with poor functional outcomes in IS, indicating that the rs12537 variant may serve as a prognostic marker for IS susceptibility and prognosis.

Genetic predisposition and environmental factors are widely recognized as contributors to IS susceptibility, though the interplay between these variables remains complex and poorly characterized. Robust epidemiological evidence links age, sex, smoking, diabetes, and hypertension to increased IS risk.[25,26] Aging, the strongest non-modifiable risk factor, elevates IS vulnerability in older populations, who exhibit higher mortality rates and worse prognoses compared to younger individuals.[26] Stroke incidence is also sex-dependent, with men experiencing a 1.3–1.5-fold higher risk than women.[27] Among modifiable risk factors, diabetes and hypertension are particularly significant. Diabetes mellitus doubles the risk of IS, particularly affecting small-vessel disease. Diabetic patients often exhibit delayed recovery and higher rates of post-stroke disability.[28] A meta-analysis further demonstrated that hypertension confers an overall relative risk of 5.43 for stroke.[29] In line with prior findings, subgroup analyses of our cohort revealed that the MTMR3 rs12537 T allele was associated with significantly elevated IS risk in older, male individuals without hypertension. This observation underscores the complex interplay between genetic (eg, rs12537 variant) and environmental factors (age, sex, blood pressure status) in determining IS susceptibility.

We observed that the rs12537 T allele was linked with lower *MTMR3* mRNA expression and higher miR-181a expression in this study. The rs12537 variant is functional due to the substitution of the C allele with the T allele, which creates an additional miR-181a binding site (positions 1653–1673) in the 3′ untranslated region (UTR) of MTMR3. This additional site leads to full complementarity and increased mRNA degradation.[7] Lin et al found that individuals with the rs12537 CT genotype displayed reduced *MTMR3* mRNA expression levels compared to those with the CC genotype in the context of GC.[7] Senousy et al reported that individuals with the rs12537 TT genotype had significantly lower levels of MTMR3 and higher levels of LC3B in patients with RA and SLE.[14] Consistent with these findings, the current study observed that the rs12537 T allele was correlated with decreased MTMR3 mRNA expression and increased miR-181a levels in PBMCs of IS patients. These findings underscore a new role for rs12537 in influencing the severity and progression of IS, potentially by decreasing MTMR3 levels through enhanced miR-181a binding. Xu et al observed that MTMR3 expression was reduced in BC tissues, with an increase in methylation levels.[24] Therefore, it is likely that the rs12537 variant may regulate the methylation of MTMR3, resulting in decreased MTMR3 levels in IS patients. Additionally, targeting the miR-181a/MTMR3 axis may provide a novel therapeutic strategy for IS.

The rs12537 T allele was responsible for lower *MTMR3* expression and higher LC3B expression in the present study. Autophagy has been well-established as a key factor in various neurodegenerative diseases, including IS.[30] Mutations in genes associated with autophagy are now known to be contributing factors in certain Mendelian disorders.[31] Genetic polymorphisms in autophagy-related genes have also been linked to increased susceptibility to specific pathological conditions, highlighting their importance in disease risk and progression.[31] Several researches have shown that when oxidative stress and inflammation increase after IS, the autophagic process can be activated. The outcome of this process depends on the type of stress, how long it lasts, and the cells involved; it can have positive as well as negative effects. A balanced level of autophagy benefits neurons and aids in the recovery of cerebral function by mitigating inflammation and oxidative stress. However, when autophagy becomes excessive, it can result in neuronal cell death and further exacerbate brain damage.[32] As a PI3P phosphatase, MTMR3 plays a role in the inhibition of autophagy initiation.[21] Autophagy pathway dysregulation has been associated with several neurological conditions, especially IS.[30] The present study showed that IS patients carrying the rs12537 T allele had lower MTMR3 levels and higher LC3B levels compared to those with other genotypes, indicating that the rs12537 variant could impact autophagy by reducing MTMR3 expression and thereby increase IS risk and poor prognosis. In IS patients, carrying the rs12537 variant may cause MTMR3 reduction, which disrupts autophagy balance: as a PI3P phosphatase, diminished MTMR3 allows PI3P accumulation, triggering excessive autophagy. While moderate autophagy protects neurons by alleviating inflammation and oxidative stress, rs12537-associated PI3P-driven hyper-autophagy exacerbates neuronal cell death and brain damage, worsening stroke severity.[32]

Limitations

There are some limitations to this study; exercise caution when interpreting the findings. Firstly, as a hospital-based study, selection bias was unavoidable. However, the use of a replicated population could be seen as a strength, as it helps reduce genetic variability. Secondly, MTMR3 and miR-181a levels were compared across different genotypes in limited patient and control cohorts, which may have introduced a type II error. The MTMR3 expression was also not evaluated at the protein level, which may weakens the mechanistic claims. Other functional variants could also influence MTMR3 expression and play a role in IS. For instance, the rs713875 polymorphism has been linked to increased MTMR3 expression and altered PRR-induced outcomes in IBD.[10] This study is limited by the inclusion of a single-ethnicity cohort, which restricts the generalizability of our findings to other populations. Future studies should include diverse ethnic groups to validate our results. The mechanisms by which rs12537 variants affect MTMR3 expression, as well as the role of the miR-181a/MTMR3 axis in IS pathogenesis, are still not fully understood. Therefore, further independent studies are required to confirm and validate these findings and determine their relevance across different ethnic groups before drawing definitive conclusions.

Conclusion

This study is the first to identify MTMR3 rs12537 variants at the miR-181a-binding site as potential genetic markers associated with an increased risk and poorer prognosis of IS. Furthermore, the rs12537 T allele may impact the genetic predisposition of IS by influencing the MTMR3 expression and leading to increased autophagy. However, further investigations are needed to validate these results and to clarify how MTMR3 variants influence the onset and progression of IS.

Future Directions

Our study provides novel insights into the role of the MTMR3 rs12537 variant in ischemic stroke susceptibility and prognosis. However, further research is needed to fully elucidate the underlying mechanisms and validate our findings. First, use CRISPR-based editing in cellular models to validate the causal role of rs12537 in modulating MTMR3 expression and autophagy-related neuronal damage. Second, validate findings in multi-ethnic cohorts to assess generalizability and explore population-specific genetic associations with IS. Three, investigate small molecules or RNA-based therapies targeting the miR-181a/MTMR3 axis through preclinical and clinical studies for potential therapeutic applications. Fourth, conduct longitudinal studies to develop rs12537 as a prognostic biomarker for IS prognosis and personalized treatment strategies.

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Disclosure

The authors have no actual or potential competing interest related to this manuscript.

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