

A Study of MMP-9 rs3918242 Gene Polymorphism in Egyptian Ischemic Stroke Patients

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ABSTRACT

Background: Stroke is a complex condition influenced by various factors, with ischemic stroke (IS) having diverse etiologies and pathogenic mechanisms. Emerging evidence suggests that its development involves an interplay between genetic and environmental components. One notable genetic factor is the single-nucleotide polymorphism (SNP) rs3918242 in the MMP-9 gene, which may heighten an individual's susceptibility to IS.

Objective: To elucidate the potential association between the MMP-9 rs3918242 polymorphism and the risk of IS, while also exploring the various clinical manifestations observed among the Egyptian IS patient cohort.

Materials and methods: The study recruited 70 IS patients from Benha University Hospitals, Egypt, along with 30 healthy controls. The MMP-9 gene variant (rs3918242) was analyzed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP) employing the *SphI* restriction enzyme.

Results: A significant association of CT genotype (OR =3.22, 95% CI =1.68–6.18, P<0.05) with the risk to develop IS and T allele was also linked to an elevated risk for IS in comparison to the C allele (OR =3.35, 95% CI =2.21–5.08, P<0.05).

Conclusions: The MMP-9 rs3918242 gene polymorphism is a potential risk factor for IS in Egyptians.

Keywords: MMP-9; Egyptian; Ischemic stroke; polymorphism; rs3918242; RFLP.

INTRODUCTION

The World Health Organization (WHO) defines a stroke as a clinical syndrome with a sudden onset of symptoms indicative of focal or diffuse brain function impairment that lasts for more than 24 hours or results in death, with a vascular origin being the sole discernible cause ^[1].

Stroke risk factors are categorized into those that cannot be changed, such as gender, age, ethnicity, family history, and racial background, and those that can be altered, such as high blood pressure, high cholesterol, diabetes, irregular heartbeat, smoking, substance misuse, and excessive alcohol consumption ^[2]. Hypertension remains the most significant modifiable risk factor for stroke, with nearly half of stroke patients having a history of high blood pressure. The higher the blood pressure, the greater is the likelihood of experiencing a stroke ^[3]. There is a clear correlation between blood pressure and stroke prevalence among both hypertensive and normotensive individuals. Research shows that reducing blood pressure by 5–6 mm Hg can decrease the relative risk of stroke by 42% ^[4].

Annually, the world sees more than 12.2 million new cases of stroke, with ischemic strokes making up over 62%, amounting to more than 7.6 million cases. Statistically, one in every four individuals over the age of 25 will experience a stroke at some point in their lives ^[5]. In Egypt, the overall crude prevalence rate of stroke is 963 per 100,000 inhabitants, while the annual incidence ranges from 150,000 to 210,000 cases. Stroke accounts for 6.4% of all deaths in Egypt, ranking third among the leading causes of death following cardiovascular and gastrointestinal diseases ^[6].

Matrix metalloproteinases (MMPs) constitute a group of enzymes that depend on calcium and zinc to function and are implicated in both normal and abnormal human body processes ^[7]. Produced by neurons, microglia, endothelial cells, and astrocytes, MMPs are crucial for the normal breakdown of the extracellular matrix, aiding in angiogenesis, tissue repair, and development ^[8]. They contribute to the development of atherosclerosis through the encouragement of smooth muscle cell migration and growth, as well as the formation and weakening of atherosclerotic plaques ^[9].

MMPs significantly affect the degradation of basement membranes and myelin, play roles in synaptic plasticity, learning, memory, and are implicated in diseases such as Alzheimer's, Parkinson's, and multiple sclerosis, highlighting their importance in neuroinflammation and neurodegeneration ^[10]. The MMP-9 gene, found on chromosome 20q13.12, consists of 13 exons and 12 introns, and it encodes for gelatinase B. This enzyme is pivotal in breaking down gelatin, collagen, and elastin, facilitating the remodeling of the extracellular matrix, and operates by cleaving polypeptides following its release into the extracellular environment ^[11].

MMP-9 is the enzyme most extensively studied in acute ischemic stroke, with its expression significantly increasing following cerebral ischemia. Dysregulated MMP-9 activity can compromise the integrity of the plaque fibrous cap and contribute substantially to proteolytic degradation of the blood-brain barrier. Elevated circulating levels of MMP-9 have shown a notable correlation with disease severity and infarct size in the hyperacute phase ^[12].

Given the multifactorial nature of stroke, emphasizing personalized medicine is crucial for surpassing the current treatment limitations, making it a key focus area for improved diagnosis, management, and prognosis of stroke, allowing for the development of patient-specific care strategies^[13].

Emerging research supports the potential of stem cell therapy and transcranial direct current stimulation (tDCS) in stroke treatment, with considerations for patient age and time since stroke onset for mesenchymal stem cell therapy, and genetic markers like catechol-O-methyltransferase polymorphisms to identify suitable tDCS candidates for treating post-stroke swallowing difficulties^[14,15].

We aimed to explore the association between the MMP-9 rs3918242 gene polymorphism and ischemic stroke (IS) in Egyptian individuals and examining its correlation with clinical manifestations and laboratory findings.

MATERIALS AND METHODS

Design and subjects:

A case-control study was conducted between October 2022 to April 2023 in the Clinical and Chemical Pathology Department at Benha University Hospitals, Egypt.

This study included 70 individuals with IS, the diagnosis of stroke was made by the clinical findings after neurologic examination and the imaging data, which was either by cerebral MRI or computed tomography imaging (CT) and 30 age-matching and sex-matching controls who were apparently healthy and came from the same geographic background as the IS cases. The IS cases were diagnosed at the Neuropsychiatry Department at Benha University Hospitals in Egypt. All participants underwent clinical examinations and their medical history had been taken.

The study included patients aged 50 to 65 years with ischemic stroke, identified through sudden onset focal neurological deficits lasting over 24 hours, regardless of cranial CT or MRI evidence. Participants of any gender were eligible.

Exclusion criteria disqualified cases with hereditary thrombotic diseases, diagnosed brain tumors, heart failure, recent myocardial infarction or unstable angina, aortic dissection, atrial fibrillation, or those in deep coma.

Sample collection:

For each participant, a sterile blood collection was performed from the peripheral veins after fasting for 10 hours. The collected blood (7 ml) was then separated into three parts.

Two millilitres of whole blood were taken in an EDTA tube with a concentration of 1.2 mg/ml. then divided into 2 aliquots; one was used for complete blood count (CBC) and the other was stored at -80°C for later analysis by the RFLP-PCR to detect the MMP-9 (rs 3918242) polymorphism. To perform the coagulation

tests, a sample of 2 ml of blood was mixed with 3.2 mg/ml of sodium citrate in a tube. Three ml of blood was allowed to coagulate via leaving the sample for 30 min in a plain tube at room temperature and then the sample spun at a high speed in a centrifuge to extract the serum. The sample was tested for various tests including: renal function tests (Serum creatinine, serum urea), liver function tests (Aspartateaminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, direct bilirubin), lipid profile tests [Triglyceride (TG), total cholesterol (TC), high density lipoprotein (HDL), and low density lipoprotein (LDL)], and random blood sugar (RBS).

Lab tests:

The complete blood counting was conducted using an automatic cell counter by using (sysmex XN-550, Japan). Coagulation tests including prothrombin time, international normalized ratio and activated partial thromboplastin time, were performed using CS Sysmex, (Japan). liver function tests and renal function tests had been measured by (Abbott) chemical auto analyzer. While lipid profile tests and random blood sugar (RBS) were done using (Dialab auto-analyzer, Austria).

Molecular determination of MMP-9 (rs3918242) SNP by RFLP-PCR method:

The analysis of the MMP-9 (rs3918242) SNP was performed using the RFLP-PCR technique. High-quality DNA was extracted from blood samples treated with EDTA using the gSYNTM Whole Blood Genomic DNA Extraction Kit (Catalog No. GS100, Lot No. FI06207). PCR amplification was carried out on a Thermal Cycling Block (S/N:271003648, Applied Biosystem) using specific primers: the sequence of the forward primer was 5'-GCC TGG CAC ATAGTA GGC CC-3', while the sequence of the reverse primer was 5'-CTT CCT AGC CAG CCG GCA TC-3'. The reaction mixture, totaling 25 µl, included 3.0 µl of DNA, 1.5 µl of each primer, and was supplemented with a genotyping mix and DNase/RNase-free water. The PCR procedure began with an initial denaturation phase at 95°C lasting 2 minutes, followed by 35 cycles consisting of a denaturation step at 92°C for 15 seconds, and an annealing/extension step at 58°C for 30 seconds. PCR products were evaluated via 2% agarose gel electrophoresis. For genotyping, the PCR-RFLP method was applied using the *SphI* HF restriction enzyme (New England BIOLAB). The presence of the MMP-9 rs3918242 polymorphism was confirmed by observing the DNA bands under ethidium bromide staining on an Alpha Innotech Corporation trans-illuminator. Genotypes were identified based on band patterns: a single 435 bp band indicated the homozygote wild type CC genotype, three bands at 188, 247, and 435 bp indicated the heterozygote CT genotype, and two bands at 247 and 188 bp were observed for the mutant TT genotype.

Ethical considerations:

The study was done after being accepted by the Research Ethics Committee, Benha University (Approval number: Ms 36-9-2022). All patients provided written informed consents prior to their enrolment. The consent form explicitly outlined their agreement to participate in the study and for the publication of data, ensuring protection of their confidentiality and privacy. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis

The statistical analysis assessed the impact of the MMP-9 rs3918242 SNP on IS risk by calculating odds ratios (ORs) and 95% confidence intervals (CIs). A P value of less than 0.05 was considered statistically significant within a 95% CI. For quantitative data, descriptive statistics included the mean, standard deviation (SD), and standard error (SE). All statistical analyses were performed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The demographic profiles of both study groups are shown in **Table 1**. Data revealed 70 individuals with IS, most of them were males (57.1%). The average age of the patients was 60.80 ± 4.69 years, most of them were rural (57.1%).

Table 1: The demographic profiles of both study groups

	Ischemic stroke N = 70		Control N = 30		P
Age(years)					
Mean ± SD	60.80 ± 4.69		61.37±4.49		0.576
Sex	No	%	No	%	
Male	40	57.1	20	66.7	0.373
Female	30	42.9	10	33.3	
Residence	No	%	No	%	
Rural	40	57.1	20	66.7	0.373
Urban	30	42.9	10	33.3	

N: number, SD: Standard deviation

The rs3918242 TT genotype was significantly more common in patients than controls. The rs3918242 CC genotype, which is the minor allele frequency, was significantly more common in control than patients. Additionally, the TT genotype increased the possibility of developing IS, while the homozygous CC genotype offered protection against IS. Both groups demonstrated a significantly different allelic distribution. Compared to the C allele, the T allele was associated with a greater IS risk as shown in **Table 2**.

Table 2: The genotypic and allelic frequencies of MMP-9 rs 3918242 compared between IS patients and the control group.

MMP-9 rs3918242	Ischemic stroke N = 70		Control N = 30		P-value	OR (95 % CI)	
	No.	%	No.	%			
Genotypes	CC	14	20.0	22	73.3	Reference	
	CT	26	37.1	6	20.0	<0.001*	3.22(1.68–6.18)
	TT	30	42.9	2	6.7	<0.001*	6.15(2.77–13.67)
Dominant model	CC	14	20.0	22	73.3		Reference
	CT+TT	56	80.0	8	26.7	<0.001*	4.19(2.36–7.42)
Recessive model	CC+CT	40	57.1	28	93.3		Reference
	TT	30	42.9	2	6.7	0.001*	3.71(1.76–7.82)
Alleles	C	54	38.6	50	83.3		Reference
	T	86	61.4	10	16.7	<0.001*	3.35(2.21–5.08)

N: number, OR: odds ratio, CI: confidence interval, according to NCBI database, C: Cysteine, T: thymine, *: Significant, OR>1 has been considered risky.

Table 3 shows lipid profile among patients with ischemic stroke. There were significant differences in TC levels, TG levels, LDL and HDL between the MMP-9 rs3918242 genotype groups.

Table 3: lipid profile in IS patients with CT, TT and CC genotypes.

	MMP-9 rs3918242			P1	Pairwise
	CC N = 14	CT N = 26	TT N = 30		
TC (mg/dL)					
Mean ± SD.	165.4±14.10	195.9±13.20	264.7±56.87	<0.001*	P2=0.053 P3<0.001* P4<0.001*
TG (mg/dL)					
Mean ± SE.	108.6±11.46	145.9±4.40	203.3±17.42	<0.001*	P2=0.019* P3<0.001* P4=0.002*
LDL (mg/dL)					
Mean ± SE.	100.1 ± 3.84	128.9 ± 3.92	179.9 ± 9.52	<0.001*	P2=0.003* P3<0.001* P4<0.001*
HDL (mg/dL)					
Mean ± SD.	42.29±2.76	34.96±1.75	24.97±7.97	<0.001*	P2<0.001* P3<0.001* P4<0.001*

SD: Standard deviation, SE: Standard error, P1: Comparing the different MMP-9 rs3918242 genotypes, P2: Comparing CC and CT, P3: Comparing CC and TT, P4: Comparing CT and TT, *: Significant. TC: Total cholesterol, TG: Triglyceride, LDL: Low density lipoprotein, HDL: High density lipoprotein.

The most common subtype of TOAST (Trial of ORG 10172 in Acute Stroke Treatment) classification in our study was small vessel occlusion, which accounted for 94.3% of the cases (**Figure 1**).

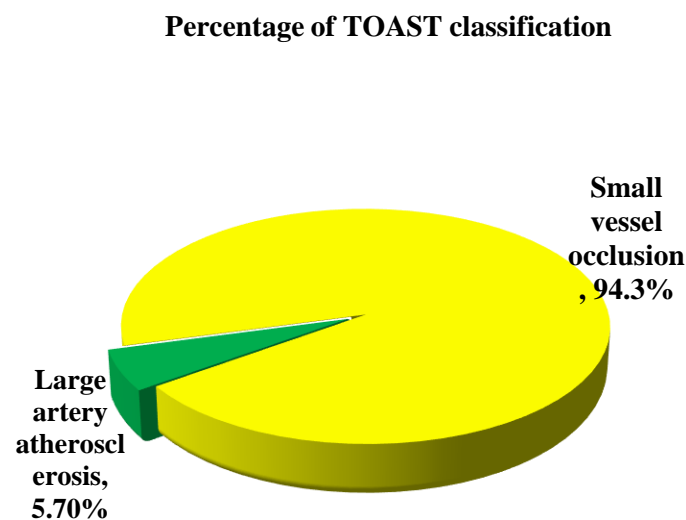


Figure 1. Pie chart for TOAST classification among patients with ischemic stroke.

DISCUSSION

Ischemic stroke (IS), a primary form of stroke, results from the blockage of a blood vessel in the brain. It stands as a leading neurovascular contributor to mortality and disability, influenced by numerous risk factors including high blood pressure, smoking, and obesity^[16].

Matrix metalloproteinases (MMPs) play a pivotal role in atherosclerosis by influencing cellular behaviors and signaling pathways that lead to the growth and rupture of atherosclerotic plaques. Genetic variations in MMPs can alter their expression levels within atherosclerotic lesions and in the bloodstream^[17].

Our research on a group of Egyptian patients showed a higher risk to develop IS among people having the TT genotype (OR=6.15), CT genotype (OR=3.22) compared with those with the CC genotype. Additionally, we found that the T allele was more common in IS patients than the healthy controls.

Research by **Li et al.** on a Chinese cohort found a notably higher occurrence of the MMP-9 rs3918242 TT and CT genotypes in individuals with IS than in healthy controls, showing statistical significance (P < 0.05). Additionally, the frequency of the T allele at this polymorphism was significantly elevated in the group with IS compared to the control participants, indicating a statistical significance (P < 0.05)^[18].

In a similar vein, **Jiang et al.** reported a notable difference in the frequencies of both genotype and allele of the MMP-9 rs3918242 polymorphism between patients with IS and control subjects, suggesting a genetic susceptibility to cerebral infarction^[19].

Additionally, our study revealed significant lipid profile variations among different MMP-9 rs3918242 genotypes, with the TT genotype showing elevated levels of triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and total cholesterol (TC) and reduced levels of high-density lipoprotein cholesterol (HDL-C), all significant at $P < 0.05$.

Our results align with the conclusions drawn by **Wu *et al.***, who, in their meta-analysis, identified a significant correlation between the rs3918242 polymorphism and an elevated risk of large artery atherosclerosis as well as ischemic stroke^[20].

In terms of the TOAST classification of stroke types, our study aligns with findings from **Meneci *et al.***, who reported small vessel strokes as the most prevalent, constituting 51.1% of their cases^[1]. However, this contrasts with **Lv *et al.***, who identified large artery atherosclerosis as the leading subtype, accounting for 60% of cases^[21].

CONCLUSION

In conclusion, the MMP-9 rs3918242 polymorphism emerges as a potential indicator for assessing the risk of ischemic stroke (IS). The TT and CT genetic variants are linked with an increased likelihood of acute ischemic stroke risk factors compared to the CC variants. These insights could aid in evaluating individual risks for IS and in the crafting of targeted strategies for its management and prevention.

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