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Original Article / Özgün Araştırma

Association Between Matrix Metalloproteinase-9 *C-1562T* Gene Polymorphism and Ischemic Stroke

Matriks Metalloproteinaz-9 C-1562T Gen Polimorfizmi ile İskemik İnme Arasındaki İlişki

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Abstract

Objective: Matrix metalloproteinases (MMPs) are calcium-dependent zinc-containing proteases containing more than 28 enzymes that cause the degrading of the extracellular matrix. Although MMPs play key roles in many biological processes, they influence some pathological processes such as ischemic stroke (IS). Among MMPs, the enzyme most associated with IS is the MMP9 enzyme. This study aimed to evaluate the effect of the *C-1562T* (rs3918242) gene polymorphism of *MMP9* on the development of IS in Turkish patients living in the Trakya Region.

Materials and Methods: Our study involved 60 patients with IS and 60 controls. The patients with IS were categorized according to the Trial of ORG 10172 in Acute Stroke Treatment classification of stroke subtypes, and *MMP9 C-1652T* gene polymorphism was identified using polymerase chain reaction, followed by restriction fragment length polymorphism using the Pael (SphI) restriction enzyme.

Results: Genotypes were defined as CC, CT, and TT according to the presence of C and T alleles. No significant differences were identified in the genotype distribution and allele frequency of *MMP9 C-1562T* gene polymorphism between the patients with IS and controls.

Conclusion: Our findings suggest that *MMP9 C-1562T* gene polymorphism is not associated with the risk of IS in Turkish patients living in the Trakya Region.

Keywords: Matrix metalloproteinase, stroke, MMP9, C-1562T, rs3918242

Öz

Amaç: Matriks metaloproteinazlar (MMP), kalsiyum bağımlı çinko içeren proteazlardır. Yirmi sekizden fazla enzim içeren MMP'ler, hücre dışı matriksin bozulmasına neden olur. MMP'ler birçok biyolojik süreçte önemli rol oynamalarına rağmen, iskemik inme (İİ) gibi bazı patolojik süreçlerde de rol oynarlar. MMP'ler arasında İİ ile en çok ilişkili olan enzim MMP9 enzimidir. Bu çalışmanın amacı *MMP9 C-1562T* (rs3918242) gen polimorfizminin Trakya Bölgesi'nde yaşayan Türk hastalarda İİ gelişimine etkisini değerlendirmektir.

Gereç ve Yöntem: Çalışmamız 60 İİ hastası ve 60 kontrol içermektedir. İskemik inme hastaları Akut İnme Tedavisinde ORG 10172 denemesine göre kategorize edildi. *MMP9 C-1652T* gen polimorfizmi, polimeraz zincir reaksiyonu ve ardından kesim enzimi Pael (SphI) ile kesim fragmanı uzunluk polimorfizmi kullanılarak tanımlandı.

Bulgular: Genotipler, C ve T alellerinin varlığına göre CC, CT ve TT olarak tanımlandı. Hastalar ve kontroller arasında *MMP9 C-1562T* gen polimorfizminin genotipik dağılımı ve alelik frekansı açısından anlamlı bir fark bulunamadı.

Sonuç: Sonuç olarak bulgularımız, *MMP9 C-1562T* gen polimorfizminin, Trakya Bölgesi'nde yaşayan Türk hastalardaki İİ riski ile ilişkili olmadığını göstermektedir.

Anahtar Kelimeler: Matriks metalloproteinaz, inme, MMP9, C-1562T, rs3918242

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Introduction

Stroke is the second leading cause of death and the most common cause of disability globally (1). A considerable amount of research has been conducted to clarify the etiological and pathological mechanisms of this multifactorial disease. The etiology and pathogenesis of ischemic stroke (IS) are diverse. Environmental factors (such as diabetes, hypertension, alcohol intake, and smoking) have been confirmed as key risk factors of IS (2). In addition, because not all individuals have been exposed to the aforementioned environmental factors, genetic factors have been correlated with the occurrence and progress of IS (3,4).

The activation and altered expression of matrix metalloproteinases (MMPs) are thought to play a role in the development of IS. The levels and activity of MMP9 (gelatinase B), which has a molecular weight of 92 kDa and is also known as type IV collagenase, increase in the acute phase of stroke and remain high for months after the incident (5). Studies have demonstrated that MMP9 activity is controlled by a functional substitution of the C and T (C/T) alleles at the −1562 position in the promoter region of the human *MMP9* gene, which is located on chromosome 20q12.2-13.1. These studies have revealed that the T-1562 allele has higher promoter activity in driving gene expression than the C-1562 allele (6,7). In particular, relatively high expression levels of MMP9 were identified in the human brain tissue of patients with IS, indicating that MMP9 may contribute to ischemic brain injury (8).

Some meta-analyses have been conducted to assess the association of *MMP9* gene polymorphism with IS susceptibility, but no association has been detected (9,10). In recent years, studies have associated MMP9 variants with the risk of IS. Although studies have reported an association between MMP9 variants and IS susceptibility, the results remain controversial (11). In our study, we aimed to investigate the role of *MMP9 C-1562T* gene polymorphism in the development of IS in Turkish patients living in the Trakya Region.

Materials and Methods

Patients

The patient group consisted of 60 Turkish adult patients with IS, who were categorized according to the Trial of ORG 10172 in Acute Stroke Treatment classifications for stroke subtypes into large and small vessel stroke (12). All the patients with IS were enrolled in the study when they were hospitalized during acute stroke or when they came to the neurology department for further examinations in the chronic phase of stroke. Stroke was diagnosed through clinical findings after neurologic examination and imaging data obtained from either cerebral magnetic resonance imaging or computed tomography. Patients diagnosed with cerebral hemorrhage, transient ischemic attack, cardioembolic stroke, or recent myocardial infarction were excluded from the study. The control group consisted of 60 Turkish adults who were treated at the hospital's department of physical therapy and rehabilitation without any clinical evidence of cerebrovascular disease. Both the controls and patients were recruited from the province of Edirne and its surrounding areas. All participants gave informed consent, and the study was approved by Trakya University Ethics Committee of Scientific Research in Edirne (protocol no: TÜTF-BAEK 2016/176, decision no: 13/20, date: 20.07.2016).

Determination of the Matrix Metalloproteinase-9 Genotype

Peripheral venous blood of both patient and control groups was collected in ethylenediamine tetraacetic acid vials, and DNA was extracted from whole blood using blood DNA kits (Thermo Fisher Purelink® Genomic DNA Mini Kit). The DNA purity and quantity were assessed using a spectrophotometer and checked using 0.8% concentration agarose gel electrophoresis stained with 2 µl of ethidium bromide. Polymerase chain reaction (PCR) followed by restriction fragment length polymorphism using the Pael (SphI) restriction enzyme was used to identify *C-1562T* (rs3918242) gene polymorphism in the *MMP9* gene. Subsequently, C-1562T polymorphism in the *MMP9* gene was analyzed through the amplification of a 436 bp (Figure 1) fragment using PCR (6,13). The following primers were used for the amplification reaction: forward primer 5'-GCC TGG CAC ATA GTA GGC CC-3'; reverse primer 5'-CTT CCT AGC CAG CCG GCA TC-3' (Figure 1). The PCR reaction mixture (25 µl) contained 200 ng of DNA, deoxynucleotide triphosphates (0.1 mM of each), primers (200 pmol of each), 1X Taq buffer with ammonium sulfate [$(NH_4)_2SO_4$; 75 mM of Tris hydrochloride buffer (pH 8.8), 20 mM of $(NH_4)_2SO_4$, 0.01% Tween 20], 2.5 mM of magnesium chloride, and 1.25 U of Taq DNA polymerase (Thermo Fisher Scientific). This mixture was amplified using a Techne DNA Thermal Cycler (TechGene) with 6 min of denaturation at 95 °C, followed by 35 cycles with denaturation for 30 s at 94 °C, annealing for 30 s at 61.5 °C, and extension for 1 min at 72 °C, followed by 7 min of extension at 72 °C. The PCR products were checked using 2% concentration agarose gel electrophoresis stained with 2 µl of ethidium bromide (Figure 2). The Pael (SphI) restriction enzyme then digested 10 µl of the PCR products. Fragments were separated on 2% concentration agarose gel electrophoresis stained with 2 µl of ethidium bromide (Figure 3).

Statistical Analysis

The statistical analysis was performed using TURCOSA (Turcosa Analytics, Kayseri, Türkiye) statistical software. Allele frequencies were calculated from the genotypes of all participants. The Hardy–Weinberg equilibrium was evaluated using a chi-squared test with 1 degree of freedom. Genotype distribution and allele frequencies were compared between the patient and

GCCTGGCACATAGTAGGCCCTTTAAATACAGCTTATTGGGCCGGGCGCCATG
GCTCATGCCCGTAATCCTAGCACTTTGGGAGGCCAGGTGGGCAGATCACTTGA
GTCAGAAGTTCGAAACCAGCCTGGTCAACGTAGTGAAACCCCATCTCTACTAA
AAATACAAAAAATTT|AGCCAGGCGTGGTGGC***GCA*****C****GC***CTATAATACCAGCTAC
TCGGGAGGCTGAGGCAGGAGAATTGCTTGAACCCGGGAGGCAGATGTTGCAGT
GAGCCGAGATCACGCCACTGCACTCCAGCCTGGGTGACAGAGTGATACTACAC
CCCCCAAAAATAAAATAAAATAAATAAATACAACTTTTTGAGTTGTTAGCAGG
TTTTTCCCAAATAGGGCTTTGAAGAAGGTGAATATAGACCCTGCCC**GATGCCG**
GCTGGCTAGGAAG

Figure 1. Sequencing of the 436 bp region containing the C-1562T position of the *MMP9* gene. Underlined and bold letters denote forward and reverse primer sequences; italic and bold letters represent the restriction site for SphI (5'-GCATG↓C-3'); the italic, bold, and underlined letter (C) is the position of the *MMP9* C-1562T
MMP: Matrix metalloproteinases

control groups using Pearson's chi-squared test of independence with 2 × 2 contingency and z-statistics. The relationship between the genotypes of *MMP9 C-1562T* gene polymorphism and development of IS was assessed through logistic linear regression analysis after adjusting for the confounding covariates. Age, hypertension, diabetes mellitus, smoking, and alcohol intake were included as the covariates in the regression model. Statistical significance was set at $P < 0.05$.

Results

In the present study, −1562 C/T (rs3918242) gene polymorphism in the *MMP9* gene was investigated in 60 patients and 60 controls. The demographic and clinical characteristics of all research participants are summarized in Table 1. In the comparison between patients and controls, no significant statistical differences were identified in terms of age ($P = 0.52$), sex ($P = 0.28$), diabetes mellitus ($P = 0.67$), alcohol intake ($P = 0.82$), or smoking ($P = 0.08$), but significant differences were determined for hypertension ($P = 0.01$).

All genotype distributions were in Hardy–Weinberg equilibrium (X^2: 4.69, $P = 0.09$). The genotype distribution of *MMP9 C-1562T* gene polymorphism and allele frequency for the C and T alleles in the patient and control groups are presented in Table 2. No significant statistical differences were identified in either allele frequency or genotype distribution between the two groups. The genotype distribution among patient subgroups is summarized in Table 3. No significant statistical differences were observed in the genotype distribution of patient subtypes.

Discussion

MMPs, produced by endothelial cells, microglia, astrocytes, and neurons, are involved in the progress of atherosclerosis by activating the migration and proliferation of smooth muscle cells and inducing the destabilization of atherosclerotic plaque.

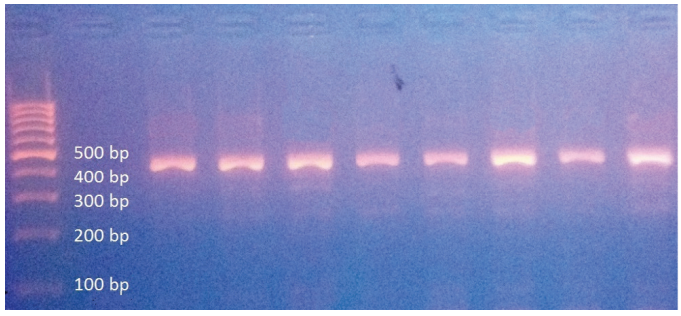

Figure 2. Ethidium bromide-stained gel of the polymerase chain reaction products (436 bp) of the C-1562T region of the *MMP9* gene. GeneRuler 100 bp DNA ladder was used
MMP: Matrix metalloproteinases

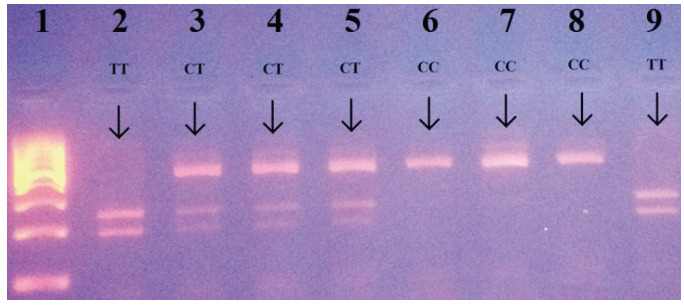

Figure 3. Ethidium bromide-stained gel of Pael (SphI) digested products. Line 1: 100 bp DNA ladder; lines 2 and 9: TT alleles (194 and 242 bp); lines 3–5: CT alleles (194, 242, and 436 bp); and lines 6–8: CC alleles (436 bp)

Table 1. Demographic and clinical characteristics of the research participants

	Patient group (n = 60)	Control group (n = 60)	*P* value
Age (years)	61.48 ± 12.50	59.90 ± 14.50	0.52
Sex (male/female)	40/20	31/29	0.28
Hypertension (%)	61.7	40	0.01
Diabetes mellitus (%)	20.0	25	0.67
Smoking (%)	45.0	26.7	0.08
Alcohol intake (%)	20.0	21.7	0.82

Table 2. Genotype and allele frequencies of matrix *metalloproteinase 9 C-1562T* gene polymorphism in the groups

Genotype frequency	Patient group (%)	Control group (%)
CC genotype frequency*	60.00	68.33
CT genotype frequency*	35.00	31.66
TT genotype frequency*	5.00	0.0
Total genotype frequency*	100	100
C allele frequency*	78.50	84.17
T allele frequency*	22.50	15.83
Total allele frequency*	100	100

*Non-significant between groups

Table 3. Genotypes among patient subgroups					
Genotypes	**Undetermined etiology**	**Atherothrombotic**	**Cardioembolic**	**Atherothrombotic and cardioembolic**	***P* value**
CC	5 (12.50%)	10 (25.00%)	21 (52.50%)	4 (10.00%)	
CT	5 (29.41%)	0 (0%)	7 (41.18%)	5 (29.41%)	0.412
TT	1 (33.33%)	1 (33.33%)	1 (33.33%)	0 (0%)	

An imbalance in MMP activity is associated with cardiovascular and cerebrovascular diseases (14), and some *MMP9* gene polymorphisms regulate MMP9 activity. Studies have identified MMP9 C-1562T polymorphism within the promoter of the *MMP9* gene (15). The transition of the T-1562 allele to the C-1562 allele increases the promoter activity of MMP9 (16). Because MMP9 exhibits relatively high expression levels in the human brain tissue of patients with IS, it may contribute to ischemic brain injury (17).

When the cells of the central nervous system are injured by ischemia, MMP9 is released (18). The *MMP9* gene is located in the chromosome 20q12.2-13.1 region and contains 13 exons and 12 introns (7), and MMP9 is vital for matrix degradation, extracellular matrix remodeling, cellular adhesion, and cell growth (18,19). Additionally, MMP9 is thought to play a role in regulating cell proliferation, differentiation, angiogenesis, apoptosis, and host defense (2,19).

In our study, we did not identify any significant relationship between the C-1562T polymorphism of the *MMP9* gene and the development of IS. This is consistent with the results of numerous other studies. A study performed in a Polish population revealed no correlation between MMP9 C-1562T variants and the risk of IS. In this study, no significant difference was determined between CC, CT, or TT genotype ratios or C and T allele ratios in patients with IS compared with controls (20). Wang et al. (21) conducted a study in a Chinese population in Guangxi Province, revealing results consistent with those of Szczudlik and Borratynska (20);

However, significant associations between MMP9 C-1652T variants and an increased risk of IS have been identified in some case-control studies (12,22,23,24,25).

The study performed in a Polish population in 2015 by Buraczynska et al. (13) revealed that the T allele in −1562 C/T polymorphism, compared with that of a control group, might lead to an approximately 1.9-fold increase in the risk of stroke in patients carrying the T allele and a 2.3-fold increase in T allele carriers with diabetes. This finding is inconsistent with a previous study conducted in a Polish population, which demonstrated no association between MMP9 C-1562T polymorphism and an increased risk of IS (20).

Nie et al. (22) conducted a study in 396 Chinese patients with IS and 400 controls, revealing that MMP9 C-1562T genotype and allele frequencies differed between the two groups and the MMP9 C-1562T allele was associated with a 1.5-fold increase in IS incidence.

Zhao et al. (23) demonstrated that *C-1562T* gene polymorphism and IS pathogenesis are closely related and that this gene polymorphism interacts with body mass index to increase the risk of IS. Moreover, the CC genotype of C-1652T of the *MMP9* gene increases the risk of IS compared with the TT genotype in a Chinese population (23).

A similar result was obtained in a study conducted by Hao et al. (24) involving 317 patients with stroke and 317 healthy participants. They identified that individuals carrying the CC genotype and TC + CC genotype were associated with a significantly increased risk of IS compared with those carrying the TT genotype, thus correlating MMP9 *C-1652T* polymorphism with an elevated risk of IS. The authors also demonstrated that this gene polymorphism has a significant interaction with smoking to increase the risk of IS (24).

Li et al. (25) demonstrated a significant difference in the genotype and allele frequencies of MMP9 *C-1652T* polymorphism between patients with IS and controls, indicating that the MMP9 C-1562T T allele was a genetically susceptible gene for cerebral infarction and cerebral hemorrhagic stroke.

In a meta-analysis of 13 studies conducted by Jiang et al. (26) involving 3,996 patients and 3,815 controls, a correlation was identified between MMP9 C1562T polymorphism and IS in the Chinese population, with the TT+TC genotype determined to increase the risk of IS.

Conclusion

In this study, no relationship was identified between the *C-1562T* gene polymorphism of the *MMP9* gene and the probability of developing IS in the Turkish population living in the Trakya Region. These findings suggest that individuals with genotype CC, CT, or TT or with allele C or T do not have a relatively high risk of IS compared with the whole Turkish population.

In conclusion, although some research suggests an association between single nucleotide polymorphisms such as MMP9 C-1562T and an increased risk of IS, further investigation based on larger sample sizes utilizing more diverse population selections will help us gain better insight into any potential linkages between genetic variations and diseases such as stroke.

Early diagnosis of IS may be possible if the genes that are effective in the development of IS are identified. The identification and understanding of these genetic factors can help identify individuals at risk of developing this condition, allowing for early intervention and treatment. This improved knowledge could also lead to more successful therapies as well as preventive measures such as lifestyle modifications or medications that target those specific genes associated with this disorder.

Ethics

Ethics Committee Approval: Trakya University Ethics Committee of Scientific Research in Edirne (protocol no: TÜTF-BAEK 2016/176, decision no: 13/20, date: 20.07.2016).

Informed Consent: All participants gave informed consent.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: A.S.S., B.G., Concept: İ.K., T.S., Design: İ.K., T.S., Data Collection or Processing: İ.K., A.S.S., Analysis or Interpretation: İ.K., T.S., B.G., M.Y., Literature Search: İ.K., Writing: İ.K.

Conflict of Interest: No conflict of interest was declared by the authors.

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References

1. Ince B, Necioglu D; Turkish Stroke Study Group. Organization of stroke care in Turkey. Int J Stroke 2017;12:105-107.
2. Chaturvedi M, Kaczmarek L. MMP-9 Inhibition: a therapeutic strategy in ischemic stroke. Mol Neurobiol 2014;49:563-573.
3. Guldiken B, Sipahi T, Guldiken S, et al. Glu298Asp polymorphism of the endothelial nitric oxide synthase gene in Turkish patients with ischemic stroke. Mol Biol Rep 2009;36:1539-1543.
4. Sipahi T. Lack of Evidence for Contribution of eNOS, ACE and AT1R gene polymorphisms with development of ischemic stroke in Turkish subjects in Trakya Region. In: Hernandez-Rodriguez P (ed). Polymerase Chain Reaction, Rijeka: University Campus STeP Ri; 2012:455-468.
5. Clark AW, Krekoski CA, Bou SS, Chapman KR, Edwards DR. Increased gelatinase A (MMP-2) and gelatinase B (MMP-9) activities in human brain after focal ischemia. Neurosci Lett 1997;238:53-56.
6. Zhang B, Ye S, Herrmann SM, et al. Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis. Circulation 1999;99:1788-1794.
7. Zhang B, Henney A, Eriksson P, et al. Genetic variation at the matrix metalloproteinase-9 locus on chromosome 20q12.2-13.1. Hum Genet 1999;105:418-423.
8. Rosell A, Ortega-Aznar A, Alvarez-Sabín J, et al. Increased brain expression of matrix metalloproteinase-9 after ischemic and hemorrhagic human stroke. Stroke 2006;37:1399-1406.
9. Fan ZZ, Ge ZM, Cai HB, et al. Association between the MMP-9-1562 C>T polymorphism and the risk of stroke: a meta-analysis. Mol Biol Rep 2014;41:6787-6794.
10. Wen D, Du X, Nie SP, Dong JZ, Ma CS. Association between matrix metalloproteinase family gene polymorphisms and ischemic stroke: a meta-analysis. Mol Neurobiol 20014;50:979-985.
11. He T, Wang J, Wang XL, Deng WS, Sun P. Association between the matrix metalloproteinase-9 rs3918242 polymorphism and ischemic stroke susceptibility: a meta-analysis. J Stroke Cerebrovasc Dis 2017;26:1136-1143.
12. Adams HP Jr, Bendixen BH, Kappelle LJ, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993;24:35-41.
13. Buraczynska K, Kurzepa J, Ksiazek A, Buraczynska M, Rejdak K. Matrix metalloproteinase-9 (MMP-9) gene polymorphism in stroke patients. Neuromolecular Med 2015;17:385-390.
14. Ghilardi G, Biondi ML, DeMonti M, et al. Matrix metalloproteinase-1 and matrix metalloproteinase-3 gene promoter polymorphisms are associated with carotid artery stenosis. Stroke 2002;33:2408-2412.
15. Siefert SA, Sarkar R. Matrix metalloproteinases in vascular physiology and disease. Vascular 2012;20:210-216.
16. Chen Q, Jin M, Yang F, et al. Matrix metalloproteinases: inflammatory regulators of cell behaviors in vascular formation and remodeling. Mediators of Inflamm 2013;2013:928315.
17. Fornage M, Mosley TH, Jack CR, et al. Family-based association study of matrix metalloproteinase-3 and -9 haplotypes with susceptibility to ischemic white matter injury. Hum Genet 2007;120:671-680.
18. Guo MF, Yu JZ, Ma CG. Mechanisms related to neuron injury and death in cerebral hypoxic ischaemia. Folia Neuropathol 2011;49:79-87.
19. Genge AK, Blocki A, Franke RP, Jung F. Vascular endothelial cell biology: an update. Int J Mol Sci 2019;20:4411.
20. Szczudlik P, Borratynska A. Association between the-1562 C/T MMP-9 polymorphism and cerebrovascular disease in a Polish population. Neurol Neurochir Pol 2010;44:350-357.
21. Wang Y, Zhang L, Huang H, et al. Relationship between the matrix metalloproteinase-9 gene polymorphisms and ischemic stroke. Int J Clin Exp Pathol 2019;12:949-956.
22. Nie SW, Wang XF, Tang ZC. Correlations between MMP-2/MMP-9 promoter polymorphisms and ischemic stroke. Int J Clin Exp Med 2014;7:400-404.
23. Zhao JH, Xu YM, Xing HX, et al. Associations between matrix metalloproteinase gene polymorphisms and the development of cerebral infarction. Genet Mol Res 2015;14:19418-19424.
24. Hao YH, Tian SH, Sun M, et al. Association between matrix metalloproteinase gene polymorphisms and development of ischemic stroke. Int J Clin Exp Pathol 2015;8:11647-11652.
25. Li Y, Chen LF, Yao SY, et al. Association of polymorphisms of the matrix metalloproteinase 9 gene with ischaemic stroke in a Southern Chinese Population. Cell Physiol Biochem 2018;49:2188-2199.
26. Jiang Y, Liu HY, Wang Y, et al. Meta-analysis of matrix metalloproteinase (MMP)-9 C1562T polymorphism and susceptibility to ischemic stroke in the Chinese population. J Int Med Res 2020;48:300060520926427.