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THE EFFECT OF MMP-2 (-1306 C/T) GENE VARIATION IN THE DEVELOPMENT OF ISCHEMIC STROKE

MMP-2 (-1306 C/T) GEN VARYASYONUNUN İSKEMİK İNME GELİŞİMİNDEKİ ETKİSİ

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ABSTRACT

The aim of this study is to determine the role of MMP-2 (-1306 C/T) gene variation in the development of ischemic stroke. Our study was carried out with 93 ischemic stroke patients and 94 healthy controls. Polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were applied to determine the genotype distributions of MMP-2 (-1306 C/T) gene variation. Although the significant difference was not determined between ischemic stroke patients and healthy control groups in terms of age and alcohol parameters in our study ($p>0.05$), the significant difference was determined between these groups in terms of smoking, cerebrovascular disease history, hypertension, diabetes mellitus and heart disease parameters. Although CT heterozygous genotype of MMP-2 (-1306 C/T) gene variation was determined higher in ischemic stroke patient group compared to healthy controls, the significant difference was not detected between patient and control groups in terms of MMP-2 (-1306 C/T) gene variation genotype distributions. In addition, TT homozygous genotype of MMP-2 (-1306 C/T) gene variation was not observed in ischemic stroke patient group and healthy control group. In conclusion, MMP-2 (-1306 C/T) gene variation was not identified as a genetic risk factor for the development of ischemic stroke in the Thrace population of Turkey. Comprehensive studies with larger populations are needed to determine the relationship between MMP-2 (-1306 C/T) gene variation and the development of ischemic stroke.

Keywords: Ischemic stroke, MMP-2 gene, MMP-2 (-1306 C/T) gene variation, PCR, RFLP

ÖZET

Bu çalışmanın amacı, MMP-2 (-1306 C/T) gen varyasyonunun iskemik inme gelişimindeki rolünü belirlemektir. Çalışmamız 93 iskemik inme hastası ve 94 sağlıklı kontrol ile gerçekleştirildi. MMP-2 (-1306 C/T) gen varyasyonu genotip dağılımlarını belirlemek için polimeraz zincir reaksiyonu (PZR) ve restriksiyon fragment uzunluk polimorfizmi (RFLP) yöntemleri uygulandı. Çalışmamızda iskemik inmeli hasta ve sağlıklı kontrol grupları arasında yaş ve alkol parametreleri bakımından anlamlı bir farklılık belirlenmemesine rağmen ($p>0.05$), sigara, serebrovasküler hastalık öyküsü hipertansiyon, diyabetes mellitus ve kalp hastalığı parametreleri bakımından anlamlı bir farklılık saptanmıştır ($p<0.05$). İskemik inmeli hasta grubunda MMP-2 (-1306 C/T) gen varyasyonunun CT heterozigot genotipi sağlıklı kontrollere kıyasla anlamlı derecede daha yüksek belirlenmiş olmasına rağmen, hasta ve kontrol grupları arasında MMP-2 (-1306 C/T) gen varyasyonu genotip dağılımları bakımından anlamlı bir farklılık saptanmamıştır ($p>0.05$). Ayrıca iskemik inmeli hasta grubunda ve sağlıklı kontrol grubunda MMP-2 (-1306 C/T) gen varyasyonunun TT homozigot genotipi gözlenmemiştir. Sonuç olarak, Türkiye'nin Trakya popülasyonunda MMP-2 (-1306 C/T) gen varyasyonu iskemik inme gelişimi için genetik bir risk faktörü olarak belirlenmemiştir. MMP-2 (-1306 C/T) gen varyasyonu ve iskemik inme gelişimi arasındaki ilişkiyi net olarak belirlemek için daha geniş popülasyonlar ile gerçekleştirilecek kapsamlı araştırmalara ihtiyaç duyulmaktadır.

Anahtar kelimeler: İskemik inme, MMP-2 geni, MMP-2 (-1306 C/T) gen varyasyonu, PZR, RFLP

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INTRODUCTION

Cerebrovascular diseases (CVD) are characterized by cerebral ischemia (Chang et al., 2016). Stroke is basically classified into two groups as ischemic stroke and hemorrhagic stroke (Zhang et al., 2016). Ischemic stroke is an important cause of morbidity and mortality (Chen et al., 2018). Cerebral ischemia has been associated with various pathological conditions such as disruption of the blood-brain barrier, vasogenic edema, and neuronal death (Chang et al., 2016). Ischemic stroke represents approximately 60-80% of all strokes (Liu et al., 2020; Kolb et al., 2019). Ischemic stroke is a multifactorial disease and genetic and environmental factors play a role together in its pathogenesis (Mukherjee and Patil, 2011). Identification of genetic factors that may be effective in the pathogenesis of ischemic stroke is clinically very important for the development of new treatment strategies (Chen et al., 2018).

The significant relationship between ischemic stroke and inflammatory response has been reported. Inflammatory proteins and mediators of inflammation are effective in the pathogenesis of ischemic stroke (Niu et al., 2018). Matrix metalloproteinases (MMPs) are composed of more than 25 proteins (Chang et al., 2016). MMPs are a zinc-containing endopeptidase and play an important role in extracellular matrix degradation (Chen et al., 2018). MMPs are effective in various physiological processes such as cell growth, differentiation, proliferation and migration. MMPs secreted by inactive proenzymes are degraded activated by extracellular proteinases (Daskalopoulou et al., 2007). MMP family is classified as collagenases (MMP-1, MMP-8, MMP-13), gelatinases (MMP-2, MMP-9), stromelysins (MMP-3, MMP-10), membrane type MMPs and other MMPs according to structural similarities and substrate properties. Especially MMP-1, MMP-2, MMP-3, MMP-9 and MMP-12 play an effective role in the pathophysiology of ischemic stroke (Chang et al., 2016).

MMP-2 is known as gelatinase A and MMP-2 gene has been associated with vascular diseases such as ischemic stroke (Li et al., 2021; Lin et al., 2017). Increased MMP-2 levels have been detected during ischemic stroke (Niu et al., 2018). MMP-2 activity increases in the late phase of ischemic stroke. Thus, in the ischemic nucleus, MMP-2 may play a neuroprotective role (Chang et al., 2016).

Two common genetic variations have been identified in the MMP-2 gene as MMP-2 (-1306 C/T) and MMP-2 (-735 C/T) gene variations (Wagsater et al., 2011). MMP-2 (-1306 C/T) gene variation has been associated with increased oxidative stress (Li et al., 2021). MMP-2 (-1306 C/T) gene variation identified in the promoter region of MMP-2 is characterized by cytosine / thymine (C/T) base substitution and MMP-2 expression and activity are altered as a result of this genetic variation (Chang et al., 2016). The significant relationship has been reported between MMP-2 gene variations and the development of lacunar stroke. In addition, genetic variations identified in the MMP-2 gene have been associated with functional outcome after stroke (Guo et al., 2017).

Therefore, the aim of our study is to determine the role of MMP-2 (-1306 C/T) gene variation in the development of ischemic stroke in the Thrace Region of Turkey.

MATERIALS AND METHODS

Ischemic stroke patient and healthy control groups

Ethics committee approval was obtained for this study from the Trakya University Faculty of Medicine Non-Invasive Ethics Committee with the protocol code of TÜTF-BAEK 2017/199. Signed informed consent forms were collected from each of the individuals in the ischemic stroke patient and healthy control groups. Our study was carried out with peripheral venous blood samples taken for routine examinations from Trakya University Faculty of Medicine Department of Neurology. These blood samples contained ethylenediaminetetraacetic acid (EDTA). Experimental parts of our study were carried out in Trakya University Faculty of Medicine Department of Biophysics. Our study consisted of 93 ischemic stroke patients and 94 healthy controls. The patient group consisted of participants above 19 years of age who were diagnosed with ischemic stroke. The healthy control group consisted of participants above 19 years of age who were not diagnosed with ischemic stroke. Pregnant and breastfeeding women, those with any neurodegenerative disorder, and participants younger than 19 years of age were excluded from our study. The flow diagram for the ischemic stroke patient and healthy control groups is presented in Figure 1.

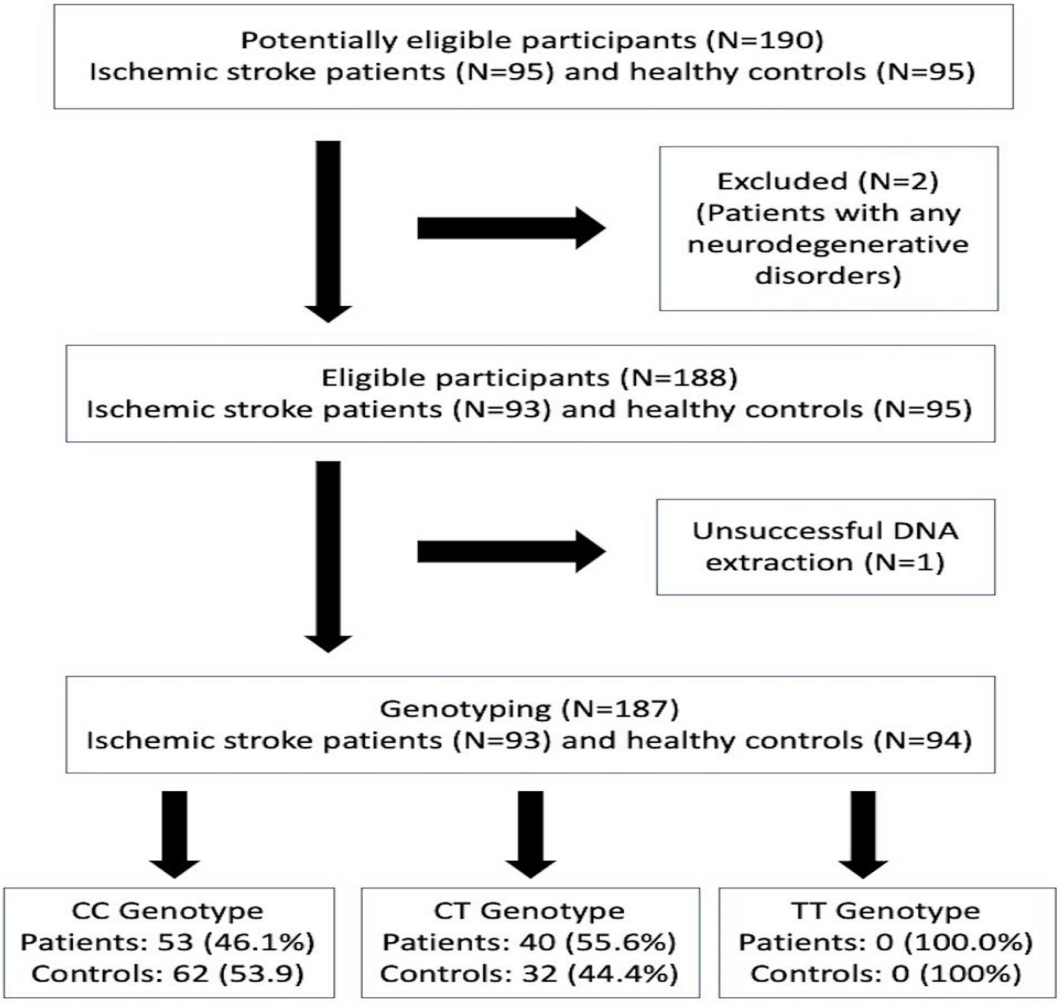

Figure 1. Flow diagram for the ischemic stroke patient and healthy control groups

DNA isolation

DNA isolation was performed from peripheral venous blood samples containing EDTA of ischemic stroke patients and healthy control groups. Invitrogen DNA blood kit was used for DNA isolation.

PCR-RFLP Methods

Genotype distributions of MMP-2 (-1306 C/T) gene variation were determined using PCR and RFLP methods. 188 bp PCR product was observed in 2% agarose gel electrophoresis for MMP-2 (-1306 C/T) gene variation. CC homozygous genotype (188bp, 5bp), CT heterozygous genotype (188bp, 162bp, 26bp, 5bp) and TT homozygous genotype (162bp, 26bp, 5bp) for MMP-2 (-1306 C/T) gene variation were observed and BfaI (XspI) restriction enzyme was used in RFLP method. Primer sequences, PCR conditions and restriction enzyme for MMP-2 (-1306 C/T) gene variation are presented in Table 1. In addition, the product lengths of the genotype distributions were observed in 2.5% gel electrophoresis for MMP-2 (-1306 C/T) gene variation and are presented in Table 2 (Figure 2).

Table 1: Primer series, PCR conditions and restriction enzyme for MMP-2 (-1306C/T) gene variations

Gene Variations	Primer series	PCR Conditions		RE
MMP-2 (-1306C/T)	**FP:**5'-CTTCCTAGGCTGGTCCTTACTGA-3 ' **RP:**5'-CTGAGACCTGAAGACCTAAAGAGCT-3'	5 minutes at 94^0C 1 minute at 95^0C 1 minute at 62^0C 1 minute at 72^0C 7 minutes at 72^0C	35cycle	***BfaI (XspI)***

FP: Forward primer; **RP:** Reverse primer; **PCR:** Polymerase chain reaction; **RE:** Restriction enzyme

Table 2. MMP-2 (-1306 C/T) gene variation genotype distributions product lengths

Gene Variation	PCR Product Length	Genotype Distributions Product Lengths
MMP-2 (-1306C/T)	**188bp**	**CC ⟶ 188bp, 5bp** **CT ⟶ 188bp, 162bp, 26bp, 5bp** **TT ⟶ 162bp, 26bp, 5bp** (26bp and 5bp are not observed; 100bp marker)

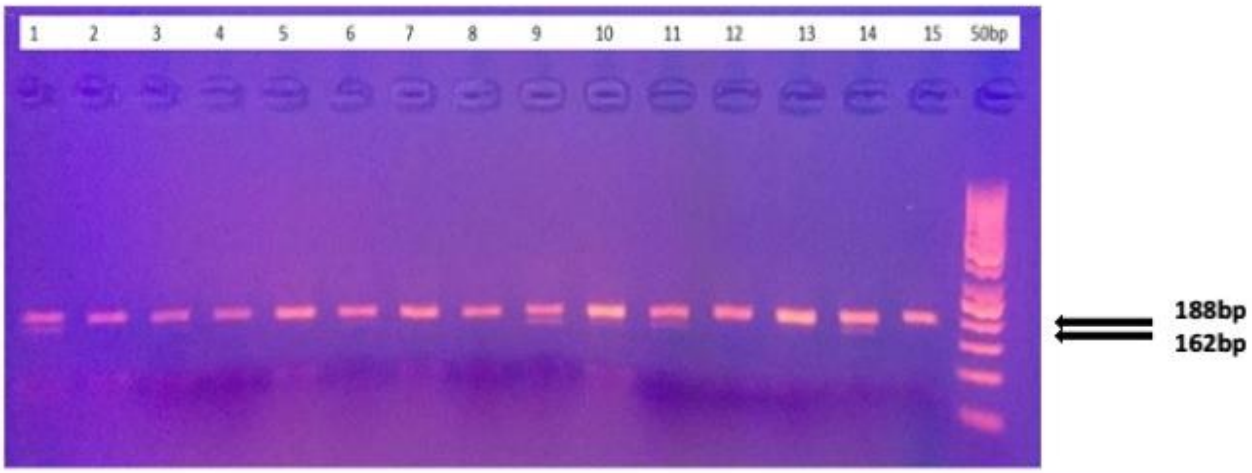

Figure 2. (CC genotype in MMP-2 -1306 C/T gene polymorphism (188bp, 2, 3, 4, 5, 6, 7, 8, 12 and 13 numbered bands), CT genotype (188bp, 162bp, 1, 9, 10, 11 and 14 numbered bands); 5 bp not observed; 50bp marker)

Statistical analysis

Statistical analysis for our study was performed using SPSS (Statistics Package of Social Science) program. Age parameter was compared between ischemic stroke patients and healthy control groups using Independent Samples test. The parameters of alcohol, smoking, CVD history, hypertension, diabetes mellitus and heart disease were compared between the patient and control groups using the Chi-Square test. In addition, genotype distributions of MMP-2 (-1306 C/T) gene variation were compared between patient and control groups with Chi-Square test.

RESULTS

In our study, the significant difference was not determined between ischemic stroke patients and healthy control groups in terms of age and alcohol parameters (p>0.05). However, the significant difference was detected between these groups in terms of smoking, CVD history, hypertension, diabetes mellitus and heart disease (p<0.05) (Table 3).

Table 3. Comparison of Clinical Findings Between Patient and Control Groups

Clinical Findings	Patient Group (n=93)	Control Group (n=94)	p
Age (year)	62,452 ± 12,0339	60,277 ± 12,1871	0,221[a]
Alcohol use, n (-,+)	70/23	72/22	0,832[b]
Smoking, n (-,+)	52/41	67/27	**0,029[b]***
CVD history, n (-,+)	78/15	93/1	**0,001[b*]**
Hypertension, n (-,+)	36/57	64/30	**<0,001[b*]**
Diabetes Mellitus, n (-,+)	60/33	76/18	**0,012[b*]**
Heart Disease, n (-,+)	66/27	92/2	**<0,001[b*]**

[a]Student-t test; [b]Chi-Square test; *: Significance (p<0,05); −/+: Nonexistent/existent; **CVD**: cerebrovascular diseases

CT heterozygous genotype of MMP-2 (-1306 C/T) gene variation was observed more in the ischemic stroke patient group compared to the healthy control group. However, the significant difference was not detected between the patient and control groups in terms of genotype distribution of MMP-2 (-1306 C/T) gene variation (p>0.05). In addition, TT homozygous genotype of MMP-2 (-1306 C/T) gene variation was not observed in the ischemic stroke patient group and healthy control group (Table 4)

Table 4. Comparison of MMP-2 (-1306 C/T) gene variation genotype distributions between patient and control groups

Genotype Distributions		Patient Group (n=93)	Control Group (n=94)	p
MMP-2 -1306 C/T	**CC**	53 (46,1%)	62 (53,9%)	0.208[a]
	CT	40 (55,6%)	32 (44,4%)	
	TT	–	–	

[a]Chi-Square test, **TT:** Thymine-Thymine; **CT:** Cytosine-Thymine; **CC:** Cytosine-Cytosine

DISCUSSION AND CONCLUSION

Ischemic stroke is a vascular neurological disease and one of the leading causes of mortality and morbidity (Niu et al., 2018). Cerebral ischemic stroke represents approximately 80% of all strokes. Genetic and environmental factors play role together in the pathogenesis of ischemic stroke (Zhang et al., 2016). MMPs are structurally composed of signal peptide, prodomain, catalytic domain, hemopexin domain and hinge region. MMPs are zinc-dependent proteolytic enzymes and are effective in various physiological processes such as cell growth, proliferation, differentiation, migration. MMPs, which are secreted as inactive proenzymes, are degraded and activated by extracellular proteinases (Niu et al., 2018). Changes in MMP activities have been associated with CVDs and cardiovascular diseases (Zhao et al., 2016).

MMP-2 has been associated with processes such as cerebral ischemia-reperfusion, hemorrhagic transformation and neuron apoptosis. MMP-2 plays an important role in extracellular matrix degradation (Niu et al., 2018). It may be effective in vascular cellular aggregation by penetrating the basement membrane and blood-brain barriers. The MMP-2 gene contains 13 exons and 12 introns. Multiple variants have been identified in the MMP-2 gene which is located on chromosome 16q13-21. MMP-2 (-1306 C/T) and MMP-2 (-735 C/T) gene variations are characterized by C/T base substitution in the promoter region of the MMP-2 gene. These common genetic variations have been associated with altered transcriptional activity. It is thought that genetic variations identified in the MMP-2 gene may affect the relationship between MMP-2 levels and the development of ischemic stroke (Li et al., 2021). MMP-2 has assumed a dual role in the pathophysiology of ischemic stroke. MMP-2 shows an early pathological effect after focal ischemic injury, and therefore, blood-brain barrier damage may occur

through basal lamina degradation. MMP-2 also plays an important role in endogenous repair, regulation of cerebral blood flow during the recovery phase after ischemic stroke (Lin et al., 2017).

In previous studies, MMP-2 has been associated with angiogenesis and inflammatory response. It has also been reported that the MMP-2 gene is effective in the pathogenesis of ischemic stroke (Han et al., 2017; Jacob-Ferreira et al., 2013). In a study, significantly increased serum MMP-2 levels were detected in the acute phase of ischemic stroke. This condition has been associated with development of vasogenic edema through disruption of the blood-brain barrier and increased vascular permeability (Brouns et al., 2011). In another study, significant changes in serum MMP-2 levels have been associated with neurological impairment after ischemic stroke (Halder et al., 2013; Sternlicht and Werb, 2001). The significant relationship has been reported between MMP-2 (-1306 C/T) gene variation and atherosclerotic cerebral infarction (Xu et al., 2016). In a study performed with Taiwanese population, increased MMP-2 levels have been associated with the pathogenesis of ischemic stroke (Lin et al., 2017). In a South Chinese population study, the C allele of the MMP-2 rs 1132896 gene variant and the T allele of the MMP-2 rs 243849 gene variant have been associated with a reduced risk of ischemic stroke (Niu et al., 2018). In another study performed with the Chinese population, the CC genotype of the MMP-2 (-1306 C/T) gene variation has been associated with increased transcriptional activity (Chang et al., 2016). In a study carried out with a northern Chinese population, the significant association has been reported between MMP-2 (-1306 C/T) gene variation and the development of ischemic stroke (Zhang et al., 2016).

Although CT heterozygous genotype of MMP-2 (-1306 C/T) gene variation was observed more in ischemic stroke patients in this study performed with Thrace population, this gene variation was not determined as a genetic risk factor for the development of ischemic stroke. Comprehensive studies with larger populations are needed to clearly determine the role of MMP-2 (-1306 C/T) gene variation in the development of ischemic stroke. Determining the roles of genetic variations such as the MMP-2 (-1306 C/T) gene variation in the development of ischemic stroke will provide important biomarkers for the diagnosis, progression and treatment of ischemic stroke.

Compliance with Ethical Standards

Ethical approval

For this study, it was applied to Trakya University Faculty of Medicine Non-Invasive Clinical Research Ethics Committee. Ethics committee approval has been obtained for our study with TÜTF-BAEK 2017/199 protocol code.

Consent to participate

Signed informed consent forms have been collected from the participants.

Conflict of interest

The authors declare that they have no conflict of interest

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REFERENCES

Brouns, R., Wauters, A., De Surgeloose, D., Mariën, P., De Deyn, P.P. (2011). Biochemical markers for blood-brain barrier dysfunction in acute ischemic stroke correlate with evolution and outcome. Eur Neurol, 65(1), 23-31.

Chang, J.J., Stanfill, A., Pourmotabbed, T. (2016). The Role of Matrix Metalloproteinase Polymorphisms in Ischemic Stroke. Int J Mol Sci, 17(8), 1323.

Chen, L., Yang, Q., Ding, R., Liu, D., Chen, Z. (2018). Carotid thickness and atherosclerotic plaque stability, serum inflammation, serum MMP-2 and MMP-9 were associated with acute cerebral infarction. Exp Ther Med, 16(6), 5253-5257.

Daskalopoulou, S.S., Daskalopoulos, M.E., Perrea, D., Nicolaides, A.N., Liapis, C.D. (2007). Carotid artery atherosclerosis: what is the evidence for drug action? Curr Pharm Des, 13(11), 1141-59.

Guo, T., Hao, H., Zhou, L., Zhou, F., Yu, D. (2017). Association of SNPs in the TIMP-2 gene and large artery atherosclerotic stroke in southern Chinese Han population. Oncotarget, 9(4), 4698-4706.

Halder, A.K., Saha, A., Jha, T. (2013). Exploring QSAR and pharmacophore mapping of structurally diverse selective matrix metalloproteinase-2 inhibitors. J Pharm Pharmacol, 65(10), 1541-54.

Han, S.W., Kim, S.H., Lee, J.Y., Chu, C.K., Yang, J.H., Shin, H.Y.,…,Heo, J.H. (2007). A new subtype classification of ischemic stroke based on treatment and etiologic mechanism. Eur Neurol, 57(2), 96-102.

Jacob-Ferreira, A.L., Schulz, R. (2013). Activation of intracellular matrix metalloproteinase-2 by reactive oxygen-nitrogen species: Consequences and therapeutic strategies in the heart. Arch Biochem Biophys, 540(1-2), 82-93.

Kolb, B., Saber, H., Fadel, H., Rajah, G. (2019). The endocannabinoid system and stroke: A focused review. Brain Circ, 5(1), 1-7.

Li, Y., Ouyang, Q.R., Li, J., Chen, X.R., Li, L.L., Xu, L., Yu, M. (2021). Correlation between matrix metalloproteinase-2 polymorphisms and first and recurrent atherosclerotic ischemic stroke events: a case-control study. J Int Med Res, 49(6), 3000605211022967.

Lin, H.F., Hsi, E., Huang, L.C., Liao, Y.C., Juo, S.H., Lin, R.T. (2017). Methylation in the matrix metalloproteinase-2 gene is associated with cerebral ischemic stroke. J Investig Med, 65(4), 794-799.

Liu, L., Chen, W., Zhou, H., Duan, W., Li, S., Huo, X.,…,Wang, Y. (2020). Chinese Stroke Association Stroke Council Guideline Writing Committee. Chinese Stroke Association guidelines for clinical management of cerebrovascular disorders: executive summary and 2019 update of clinical management of ischaemic cerebrovascular diseases. Stroke Vasc Neurol, 5(2), 159-176.

Mukherjee, D., Patil, C.G. (2011). Epidemiology and the global burden of stroke. World Neurosurg, 76, S85-S90.

Niu, F., Wei, B., Yan, M., Li, J., Ouyang, Y., Jin, T. (2018). Matrix metalloproteinase-2 gene polymorphisms are associated with ischemic stroke in a Hainan population. Medicine (Baltimore), 97(39), e12302.

Sternlicht, M.D., Werb, Z. (2001). How matrix metalloproteinases regulate cell behavior. Annu Rev Cell Dev Biol, 17, 463-516.

Wågsäter, D., Zhu, C., Björkegren, J., Skogsberg, J., Eriksson, P. (2011). MMP-2 and MMP-9 are prominent matrix metalloproteinases during atherosclerosis development in the Ldlr(-/-)Apob(100/100) mouse. Int J Mol Med, 28(2), 247-53.

Xu, L., Rx, Z., Q, L. (2016). Association between MMP-2 gene polymorphism and atherosclerotic cerebral infarction. Adv Anat Sci, 22, 273-275.

Zhang, L., Ren, R., Mu, S. (2016). The correlation of *MMP-2* polymorphisms and stroke. Int J Clin Exp Med, 9(2), 4460-4466.

Zhao, J.H., Xu, Y.M., Xing, H.X., Su, L.L., Tao, S.B., Tian, X.J.,…,Ji, S.B. (2016). Associations between matrix metalloproteinase gene polymorphisms and the development of cerebral infarction. Genet Mol Res, 14(4), 19418-24.