# Relationship between MMP-9 Gene Polymorphism and Intracranial Aneurysm

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**ARTICLE INFO**

**ABSTRACT**

Matrix metalloproteinase-9 (MMP-9) is a gelatinase, which is a member of the MMPs family. We know that MMP-9 is not only an important gelatinase to regulate the extracellular matrix balance, but also one of the most closely related proteases to the pathogenesis of intracranial aneurysm. The purpose of this study is to investigate the relationship between MMP-9 gene polymorphism and intracranial aneurysm. In this paper, 98 patients who were admitted to a hospital from 2018 to 2019 were selected as the experimental group and the control group according to the relevant standards of intracranial aneurysms. The MMP-9 positive and MMP-9 absorbance values between the two groups were compared, so as to determine the concentration of MMP-9 between the two groups. In addition, the gene distribution and gene frequency analysis of the C-1562T promoter region of MMP-9 were carried out. The results showed that in the control group, the gene distribution frequency of CC type was 67% that of CT type was 31% that of TT type was 2%, that of the experimental group was 52%, and that of CT type was 44%. The results showed that there was a correlation between MMP-9 gene polymorphism and intracranial aneurysm.

## Introduction

MMP-9 has been confirmed that MMP-9 is directly related to the occurrence, development and rupture of intracranial aneurysm. Some studies have shown that the C-1562T in the MMP-9 promoter region is related to the occurrence of coronary artery and other diseases (1-2).

At present, there are little data about amplification frequency and prediction factors. Duan obtained this information from a group of patients and then performed continuous MR angiography (MR a) (1-2). He used the retrospective analysis method to study the MRA performance of 165 cases of 191 UIA patients (3-4). Duan's results show that patients with multiple aneurysms have an increasing trend (5-6). In addition, during a median follow-up period of 47 months, he found that 10% of the increase in UIA significantly increased the risk of larger aneurysms. Aneurysms larger than or equal to 8 mm in diameter were most likely to increase (7-8). This study provides a certain reference value for the clinical application of intracranial aneurysms, but the data are accidental and not feasible (9-10).

Jasińska studied the role of MMPs in cardiovascular disease, especially in myocardial infarction and subsequent heart failure progression (11-12). MMPs are present in the myocardium, which can degrade all matrix components of the myocardium and is the driving force of myocardial matrix remodeling (13-14). Jasińska found that the acute drug inhibition of MMPs or the lack of MMP-9 can reduce the left ventricular dilation of the heart of infarcted mice, so MMP inhibitor can be used as a potential method to treat patients with heart failure risk after myocardial infarction (15-16). Based on the results of this study, he speculated that MMP inhibitors might be a treatment for heart failure (17-18). Although this study has a certain clinical value, the lack of data in this study shows that the reliability of its results remains to be studied (19-20).

## Intracranial aneurysm

Intracranial aneurysm ranks the third in so many cerebrovascular accident diseases, next to cerebral thrombosis and hypertensive cerebral hemorrhage, which is a health killer. An intracranial aneurysm is a protuberance on the wall of the intracranial artery, which is also the main cause of spontaneous subarachnoid hemorrhage. This disease has high mortality and serious disability rate. On the other

hand, the degradation of the extracellular matrix is the key step in the development of ECM. Aneurysms usually come from the dilation of the intracranial artery, because the blood vessels here are complex and easy to form sudden changes in hemodynamics. Most aneurysms are round or wrapped local protruding cystic lesions, sometimes they can be directly generated from the side wall of the artery without branches. The typical histologic feature of aneurysms is that they contain a thin collapsed basal layer, protruding outwards through limited defects in the inner elastic layer and the middle layer (5,6).

At present, we have not fully understood the pathogenesis of the intracranial aneurysm. There are two views on the pathogenesis of the intracranial aneurysm. One view is that aneurysms occur because of the congenital mesothelial defect at the bifurcation of the artery, which can be developed from the remains of primitive embryonic blood vessels. Another view is that the internal elastic membrane is the main factor to maintain the strength of the vascular wall, but the degeneration of the vascular wall and the internal elastic membrane is the basis of the occurrence of the aneurysm. Generally speaking, intracranial aneurysm has not only external environmental risk factors but also internal genetic factors (5,6).

## Matrix metalloproteinase-9

Matrix metalloproteinase (MMPs) is a kind of proteolytic enzyme, which can destroy the zinc and calcium of ECM. Generally speaking, the content of MMPs in the human body is very low under normal circumstances, but it plays a great role in the development of the body and wound healing. On the other hand, under pathological conditions, if there is a high expression, it will participate in the pathophysiological process of some diseases, such as ischemic stroke, cerebral aneurysm, invasion and metastasis of intracranial malignant tumor and brain injury (11-13).

MMP-9 belongs to gelatinase and is a member of the MMPs family. Its molecular weight is 92kd. It is closely related to angiogenesis, atherosclerosis and ischemic brain injury. MMP-9 is a zinc and calcium-dependent protein enzyme produced by some stromal cells. MMP-9 is mainly controlled by a plasminogen activator. Its substrates include gelatin, collagen IV,

collagen V and elastin, and collagen I and III products hydrolyzed by collagenase. Almost all components of extracellular matrix and basement membrane, such as proteoglycan, glycoprotein and collagen protein, the main matrix is collagen IV, which is the main skeleton component of basement membrane and the invasion of tumor Growth, aneurysm formation (13-15). The genetic variation of different types of MMP-9 gene promoters results in a different expression of MMP-9 at the gene transcription level, which leads to different changes of MMP-9 activity in cerebrovascular. When MMP-9 is activated, it can degrade the type IV collagen in the extracellular matrix, destroy the original intact extracellular matrix, cause the abnormal degradation of collagen and elastin in the extracellular matrix of the vascular wall, cause the structural change of the vascular wall, and finally lead to the formation, development and even rupture of intracranial aneurysm.

Generally speaking, in the normal blood circulation of the human body, transcription, excretion, activation and degradation of MMP-9 will be strictly controlled and kept in a state of dynamic balance. Once any of these links are destroyed, this dynamic balance will be destroyed, which will lead to disease (17-20).

## Extracellular matrix

Extracellular matrix (ECM) has a great relationship with the structural integrity of cells and tissues, which can be said to be an important structure to maintain the integrity and function of the vascular wall (21). ECM in the arterial wall provides the necessary support for the integrity of the vascular wall and the normal function of vascular wall cells (22). So, the existence of ECM not only ensures the flexibility and anti-adhesion of the vascular wall but also regulates the physiological function of cells and smooth muscle through the interaction with vascular wall cells. Collagen fiber, elastic fiber, reticular fiber and non-collagen glycoprotein all belong to ECM. They maintain certain flexibility and resistance to the expansion of the arterial wall, so as to ensure the flexibility of cerebrovascular under the influence of long-term large flow, maintain the stability of blood flow, and ensure the normal blood supply of the brain. Collagen and elastin are the most important components of ECM (23). So far, 19 kinds of collagen have been found. Among them, type I and type III

collagen accounted for 90% of the total collagen. Type IV collagen is mainly distributed on the basement membrane. On the other hand, about 90% of the elastic fibers are elastin. In addition, although the content of other components of ECM is relatively small, they are all useful (24-26).

MMP-9 polymorphism may be related to intracranial aneurysms. So, we study the relationship between MMP-9 gene polymorphism and intracranial aneurysm, which is of great value for the clinical prevention and treatment of an intracranial aneurysm. In order to study the relationship between MMP-9 gene polymorphism and intracranial aneurysm, a control group experiment was carried out. The positive rate of MMP-9 in the experimental group was 80%. In the control group, the positive rate of MMP-9 was 2%, $P < 0.05$ between the two groups. In addition, in the study of gene distribution and gene frequency, the results show that MMP-9 gene polymorphism is related to intracranial aneurysms.

## Materials and methods
### Research object

In terms of research objects, this paper selects 50 patients with intracranial aneurysms who went to a hospital for surgical resection between December 2018 and December 2019 as the experimental research objects. In addition, this paper sets up a control experiment and selects 48 patients who went to the hospital for intracerebral hematoma removal and internal decompression in the same period as the control group. These 48 patients include normal arteries including superficial temporal artery, cerebral cortex artery and other diseases. In the experimental group, there were 32 males and 18 females. In the control group, there were 28 males and 20 females.

### Detection of MMP-9 content

For the detection of MMP-9 content, we choose to use the human MMP-9 enzyme-linked immune response kit to measure in all samples, detect the content of MMP-9, and then read the absorbance value of each sample through the enzyme reader, so as to compare the concentration of MMP-9.

On the other hand, we also need to detect the positive rate of MMP-9, which shows that there is brownish yellow granular precipitation in the cytoplasm. In this experiment, we used hpias-100 medical image analysis system for image analysis. Under the same light intensity and exposure time, we put each slice under $10 \times 40$ optical microscope, selected five fields of vision, and tested the positive rate of MMP-9.

### Genomic DNA extraction

We collected 2ml of venous blood from two groups of people. After anticoagulation with EDTA, genomic DNA was extracted by using the Wizard Genomic DNA purification kit. First of all, we add 800 μl of cell lysate to the venous blood sample, and after it is fully mixed, we centrifuge it for 30 s at 10000 R / min, and discard the supernatant, then add 300 μl of cell lysate, after repeated blow, add 150 μl of protein precipitation agent, and centrifuge it for 3 min at 10000 R / min, After that, transfer the supernatant to the EP tube containing 200 μl isopropanol, mix it upside down, and then centrifugate it for 1 min at 10000r / min. after centrifugation, add 1ml of anhydrous ethanol, and centrifugate it again at 10000r / min. this centrifugation lasts for 5 min, and then we can get DNA.

### PCR product analysis

In the PCR product analysis, we first put the rubber plate into the electrophoresis tank, and then add reagents into the sterilized 1.5ml Eppendorf tube. The reaction system is 10 μL, and the added reagents should be in a certain order. The added reagents are 2 μl PCR products, restriction endonuclease *Bbu*l (Promega) 0.5 μL, 10 × Kbuffer buffer solution is 7.5 μL, which is then digested overnight in a 37 ℃ constant temperature water bath. After this procedure was completed, we placed it in 1.8% agarose gel for electrophoresis and electrophoresis. We chose 100bp DNA Marker level electrophoresis, and the electrophoresis lasted 30 minutes. When the electrophoresis is finished, the gel will be taken out and observed under ultraviolet light.

## Results and discussion
### Expression of MMP-9

MMP-9 positive results between the two groups were compared, and the results are shown in Table 1 and Figure 1.

It can be seen from Table 1 and Figure 1 that the total number of people in the experimental group is 50

and that in the control group is 48. Among them, the number of MMP-9 positive people in the experimental group is 40, the number of negative people is 10, and the positive rate is 80%. In the control group, the number of positive MMP-9 was 1, the number of negative MMP-9 was 47, the positive rate was 2%, P < 0.05 between the two groups, that is to say.

**Table 1.** MMP-9 positive results between the two groups

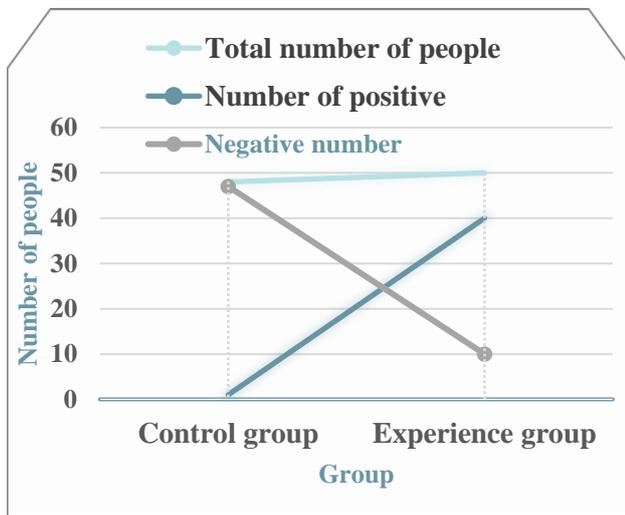| Group | Total number | Positive number | Negative number | Positive rate |
|---|---|---|---|---|
| Control group | 48 | 1 | 47 | 0.02 |
| Experience group | 50 | 40 | 10 | 0.8 |



**Figure 1.** Comparative analysis of MMP-9 positive results between the two groups

**Comparison of absorbance values of MMP-9**

Compare the absorbance value of MMP-9, and the results are shown in Figure 2.
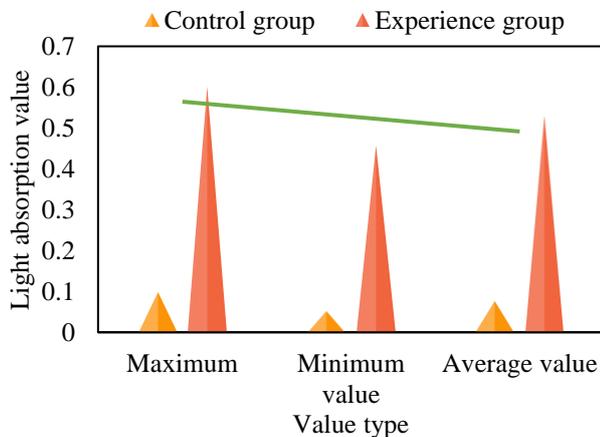


**Figure 2.** Comparison of the absorbance values of MMP-9 between the two groups

It can be seen from Figure 2 that in the control group, the maximum value of MMP-9 is 0.097, the minimum value is 0.051, and the average value is 0.074, while in the experimental group, the maximum value of MMP-9 is 0.601, the minimum value is 0.455, and the average value is 0.528. On the other hand, P < 0.05 between the two groups, that is to say, the data between the two groups have obvious differences, which is statistically significant.

**Gene distribution frequency**

The gene distribution and frequency of site 1562 of the MMP-9 gene between the two groups were analyzed, and the results are shown in Figure 3.
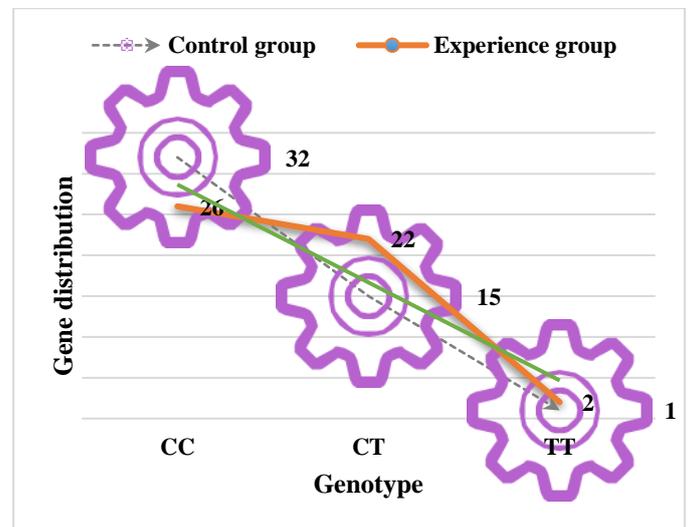


**Figure 3.** Gene distribution analysis of MMP-9-1562

It can be seen from Figure 3 that in the control group, the number of genotype CC is 32, the number of genotype CT is 15, and the number of genotype TT is 1, so the gene distribution frequency of CC is 67%, that of CT is 31%, and that of TT is 2%. In the experimental group, the number of genotype CC was 26, the number of genotype CT was 22, the number of genotype TT was 2, so the gene distribution frequency of CC was 52%, that of CT was 44%, and that of TT was 4%.

Regression analysis of genotype and gene frequency is shown in Figure 4.

C alleles were more common in the two groups. C alleles were found to be equivalent in the two groups. It can be seen from Figure 4 that there is a significant correlation between MMP-9 gene 1562 and the occurrence of intracranial aneurysm, that is to say,

there is a correlation between MMP-9 gene polymorphism and intracranial aneurysm.
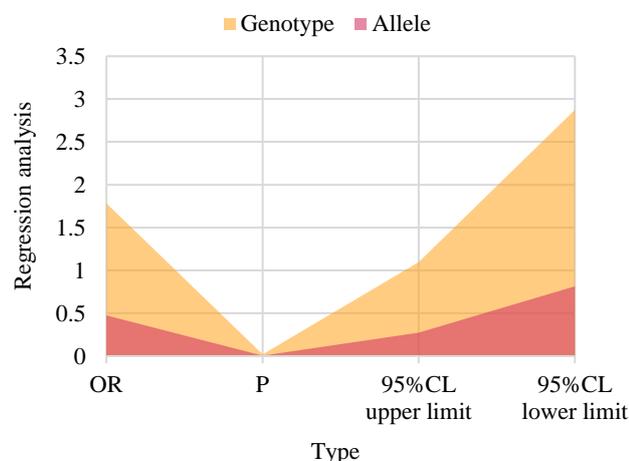


**Figure 4.** Regression analysis results of genotype and gene frequency

We know that intracranial aneurysms are the primary cause of SAH. Except for some relatively large aneurysms which may cause corresponding clinical manifestations due to occupying effect, almost all intracranial aneurysms are found after rupture and SAH. The polymorphism of the MMP-9 gene may be related to intracranial aneurysms.

In this paper, 98 patients in a hospital from December 2018 to December 2019 were selected for a control experiment. During the experiment, the positive rate of MMP-9 and the absorbance value of MMP-9 between the two groups were compared. The results showed that the data between the two groups were $p < 0.05$, that is to say, there were significant differences in the positive rate of MMP-9 and the absorbance value of MMP-9 between the two groups, representing the differences between them The value is statistically significant. This study shows that the expression of MMP-9 affects the occurrence of intracranial aneurysms.

In addition, in order to study the correlation between MMP-9 gene polymorphism and intracranial aneurysms, the gene distribution and gene frequency were analyzed, and the genotype and gene frequency were analyzed by regression. The results of this study showed that the allele frequency of CT genotype in the experimental group was significantly higher than that in the control group, and there was a significant correlation between MMP-9 gene 1562 and the

occurrence of intracranial aneurysm, that is to say, there was a correlation between MMP-9 gene polymorphism and intracranial aneurysm.

## Acknowledgments
None.

## Conflict interest
The authors declare no conflict of interest.

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