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Examining the association of MMP-1 gene −1607 (2G/1G) and −519 (A/G) polymorphisms with the risk of osteomyelitis

A case-control study

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Abstract

To investigate the effects of matrix metalloproteinase-1 (*MMP-1*) gene polymorphisms on the onset of osteomyelitis in Chinese Han population.

In all, 80 osteomyelitis patients (case group) and 81 healthy people (control group) were recruited into this case-control study. Polymerase chain reaction-restriction fragment length polymorphism method was utilized to examine the genotypes of *MMP-1* polymorphisms (−1607 2G/1G and −519A/G) in the 2 groups. Genotype and allele differences between the case and control groups were analyzed by chi-square test. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated to present the association strength between *MMP-1* gene polymorphisms and osteomyelitis.

Frequencies of −1607 2G/2G genotype between the case and control groups were statistically significant ($P = .025$). Compared with 1G/1G genotype carriers, the 2G/2G genotype carriers had 1.605 times risk of developing osteomyelitis (OR 2.605, 95% CI 1.116–6.082). Meanwhile, the 2G allele significantly associated with the risk of osteomyelitis (OR 1.735, 95% CI 1.115–2.701). In addition, frequency of −519GG genotype was obviously higher in case group than that in control group ($P = .024$), and GG genotype related to an increased risk of osteomyelitis (OR 2.792, 95% CI 1.127–6.917). Whereas, the −519G allele may be a susceptible factor for osteomyelitis (OR 1.622, 95% CI 1.038–2.536).

The *MMP-1* −1607 (2G/1G) and −519 (A/G) polymorphisms may contribute to the onset of osteomyelitis.

Abbreviations: AGE = agarose gel electrophoresis, CHD = coronary heart disease, CI = confidence interval, ECM = extracellular matrix, EDTA-2Na = ethylene diamine tetraacetic acid disodium salt dihydrate, HWE = Hardy–Weinberg equilibrium, MMPs = matrix metalloproteinases, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SNPs = single nucleotide polymorphisms.

Keywords: matrix metalloproteinase-1, osteomyelitis, polymorphism

1. Introduction

Osteomyelitis is an inflammatory reaction process accompanied with osteoclasia, which is caused by microbial infections.[1] Osteomyelitis may occurs in a single type bone tissue, or spreads to the bone marrow, sclerotin, periosteum, and the surrounding soft tissues at the same time.[2–4] According to different etiologies, osteomyelitis can be commonly divided into the following 3 kinds[5,6]: hematogenous osteomyelitis, caused by the bacterium getting into the bone via blood circulation from lesions; traumatic osteomyelitis, caused by open fractures or skeleton surgeries and its secondary infections; and the osteomyelitis caused by infections spreading from the adjacent tissues to the skeleton. Morbidity rate of the disease is higher among men than among women, and the average onset age of the disease is 21.9 years and most patients are below 50 years old.[7] Osteomyelitis has high healthcare burden.[8] Osteomyelitis is a multifactorial disease affected by both environmental and genetic factors.[9,10] Genetic structure and expression abnormalities including gene mutations, shiftings, insertions, and deletions, and also abnormal regulations may be the fundamental causes of osteomyelitis.[11] In recent years, the potential impact of genetic polymorphisms and mutations on osteomyelitis has become a new research direction.

As a kind of zinc-dependent proteolytic enzyme, the matrix metalloproteinases (MMPs) are the important mediator of the degradation and reconstruction of extracellular matrix (ECM).[12] It plays an important role in both normal physiological process and pathological state. MMP-1, an important member of the MMPs family, is of great importance in the degradation and destruction of articular cartilage and bone, and is closely related to rheumatoid arthritis, osteoarthritis, periodontal disease, and the infiltration of tumors.[13–16] There are many single-nucleotide polymorphisms (SNPs) in the promoter region of the *MMP-1* gene.[17] These polymorphisms

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might affect the expression and function of MMP-1 protein, and then lead to the alteration of physiological process.

Therefore, we selected the −1607 (2G/1G) and −519 (A/G) polymorphisms in the promoter region of the *MMP-1* gene to investigate their relationship with the onset of osteomyelitis, and then explored the osteomyelitis pathogenesis from the angle of molecular genetics.

2. Materials and methods

2.1. Study objects

Osteomyelitis cases were diagnosed by histopathology in Orthopedics Department of Southern District of Affiliated Hospital of Chengde Medical College during May 2013 to May 2015. In all, 80 cases (41 men and 39 women) were with the mean age 35.68 ± 7.16 years. The controls (37 males and 44 females), with an average age 37.12 ± 5.48 years, were healthy people from the physical examination center of the same hospital during the same period. The included subjects had no diabetes, coronary heart disease (CHD), and arthritis, and also liver and kidney diseases. There were no significant differences in sex and age between the 2 groups ($P > .05$). All of the subjects were Chinese Han population and provided the informed consent. Ethic committee of Southern District of Affiliated Hospital of Chengde Medical College approved this study. The experimental process followed the ethical guidelines of the local hospital.

2.2. Genome DNA extraction

First, 5 mL peripheral venous blood of the participants was collected and anticoagulated with ethylene diamine tetra-acetic acid disodium salt dihydrate (EDTA-2Na). Genomic DNA was isolated using a DNA isolation kit (Gentra, China), and the DNA samples were saved at −20°C.

2.3. MMP-1 polymorphisms examination

Primer sequences of *MMP-1* polymorphisms were designed by Primer Premier 5.0 and synthesized by Shanghai Sangon Biotech Co., Ltd. Primer sequences were as follows: −1607 (2G/1G) F: 5′-CCT CTG ATG CCT CTG AGA AGA-3′, −1607 (2G/1G) R: 5′-GTC TTG GGT ACT GGT GAC CG-3′; −519 (A/G) F: 5′-AGG ACT ACA GCT GCA TGA CT-3′, −519 (A/G) R: 5′-CAC AGG TCT AAG AGT ACT CC-3′.

The *MMP-1* polymorphisms were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR reaction used a 25 μL system, including 13.5 μL ddH_2O, 4 μL genome DNA, 2.5 μL 10 × PCR buffer solution, 2 μL dNTP (200 mmol/L), 1 μL TaqDNA polymerase (5 U/L, Promega company), 1 μL forward primer, and 1 μL reverse primer. PCR amplification progression was as follows: initial denaturation at 94°C for 2 minutes, followed by 35 cycles of denaturation at 94°C for 60 seconds, annealing at 54°C for 45 seconds, extension at 72°C for 45 seconds; and finally extension at 72°C for 10 minutes. The PCR products were tested with 3% agarose gel electrophoresis (AGE). Thereafter, 10 U restriction enzyme ALU I (AGCT) and 1.2 μL corresponding 10× buffer were added into 10 μL PCR products. The mixture was mixed well and then incubated at 37°C water bath overnight.

2.4. Results evaluation

The length of the PCR amplification fragment of *MMP-1* −1607 (2G/1G) polymorphism PCR products was 445 bp. Digested products had 3 types: 2G/2G homozygote (445 bp), 1G/1G homozygote (324 and 121 bp), and 2G/1G heterozygote (445, 324, and 121 bp). *MMP-1* −519 (A/G) polymorphism had a 469 bp PCR amplification fragment, and there were also 3 different digested products: GG homozygote (469 bp), AA homozygote (238 and 231 bp), and AG heterozygote (469, 238, and 231 bp) (Fig. 1).

2.5. Statistical methods

Hardy–Weinberg equilibrium (HWE) examination was performed to evaluate the representativeness of the subjects. Chi-square test was utilized to compare the differences between the 2 groups. Relationship between *MMP-1* polymorphisms and osteomyelitis susceptibility was represented by odds ratios (ORs) and 95% confidence intervals (95% CIs). All the data were calculated by SPSS18.0 software. $P < .05$ stood for the statistical significance level.

3. Results

3.1. HWE test

Genotype distributions of the case and control groups were in accordance with HWE ($P > .05$). This signified that the subjects were from the same group, and the data of the control group exhibited good representativeness.

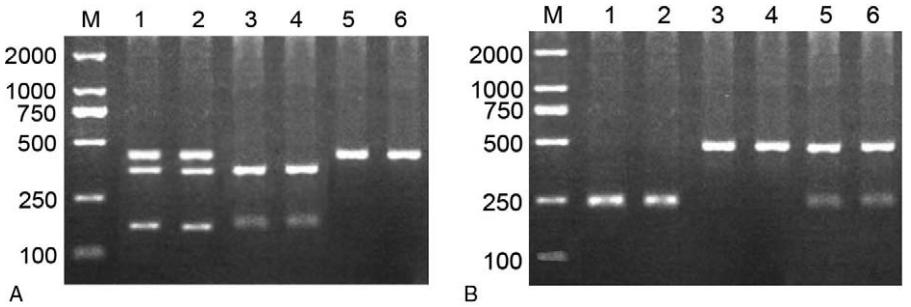

Figure 1. Genotyping results for MMP-1 polymorphisms. A, PCR-RFLP results for-1067 (2G/1G). M refers to marker; 1 and 2 lines refer to 1G2G genotype (455, 324, and 121 bp); 3 and 4 lines refer to 1G1G genotype (324 and 121 bp); 5 and 6 lines refer to 2G2G genotype (455 bp). B, RFLP results for −519 (A/G) polymorphism. M refers to marker; 1 and 2 lines refer to AA genotype (238 and 231 bp); 3 and 4 lines refer to GG genotype (469 bp); 5 and 6 lines refer to AG genotype (469, 238, and 231 bp). MMP-1 = matrix metalloproteinase-1, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.

Table 1

Genotypes and alleles distributions of *MMP-1* −1607 (2G/1G) polymorphism.

		Genotype				Allele		
Group	Number	1G/1G	1G/2G	2G/2G	Number	1G	2G	P_{HWE}
Case	80	19	37	24	160	75	85	.523
Control	81	33	32	16	162	98	64	.118
P		—	0.062	0.025		—	0.014	
OR (95% CI)		—	—	2.605 (1.116–6.082)		—	1.735 (1.115–2.701)	

CI = confidence interval, OR = odds ratios, P_{HWE} = *P* value for Hardy–Weinberg equilibrium.

3.2. Correlations between −1607 (2G/1G) polymorphism and osteomyelitis

Distribution differences of 2G/2G homozygote in 2 groups were statistically significant ($P = .025$; Table 1). People with the 2G/2G homozygote of −1607 (2G/1G) polymorphism had a risk of suffering from osteomyelitis 1.605 times higher than people carrying the 1G/1G genotype (OR 2.605, 95% CI 1.116–6.082). Frequency of 2G allele between the osteomyelitis cases and the healthy controls were also apparently different ($P = .014$), and the osteomyelitis risk of 2G allele carriers was 0.735 times higher than that of 1G allele carriers (OR 1.735, 95% CI 1.115–2.701), so the 2G allele may be a susceptible allele for osteomyelitis.

3.3. Correlation analysis of −519 (A/G) polymorphism and osteomyelitis

Case group had remarkably higher frequency of the GG genotype of −519 (A/G) polymorphism than the control group ($P = .024$; Table 2), and the osteomyelitis risk of GG genotype carriers was 2.792 times as high as that of AA genotype carriers (OR 2.792, 95% CI 1.127–6.917). Compared with A allele, G allele had a significant higher frequency in cases than that in controls, which indicated that the G allele might be related to the enhanced risk of osteomyelitis (OR 1.622, 95% CI 1.038–2.536).

4. Discussion

At present, there are many researches on the *MMP-1* polymorphisms, but most of them are focused on the relationship of the *MMP-1* polymorphisms with cancers.[17–21] But rare of them are about the association with osteomyelitis. The −1607 (2G/1G) polymorphism is located at −1607 bp of the promoter region in the *MMP-1* gene. Cao et al[22] have found that cells with 2G allele could promote changes of MMP-1 protein levels and transcription levels. It is also believed that the SNP was closely related with the development of chronic inflammatory diseases.[23–25] Besides, studies on the pathogenic mechanism of *MMP-1* −519 (A/G) polymorphism are also rare. Pearce et al[26] believe that the −519 (A/G) is located in the most conservative region of *MMP-1* promoter, and SNPs in this region can affect the transcription of *MMP-1* by influencing the combination of transcription factors with the promoter region, thus obviously affect the expression level of the *MMP-1* gene.

Osteomyelitis is a troublesome problem in orthopedic clinic, and its morbidity is still very high in China. Onset of disease is affected by many factors including inherited factors, and infectious and environmental factors.[27–29] It is only in recent years that the risk factors and gene therapies of osteomyelitis have gradually attracted the attention of the scholars, and they gradually become a hotspot in research of osteomyelitis treatment.[30] MMP-1, an important protease in the physiological process of ECM, is mainly expressed in the physiological and pathological conditions, such as the growth and refactoring of the tissues. It also involves in the bone formation and resorption. Additionally, a great many researches showed that bone morphogenetic proteins have important influences on the development and formation process of skeleton, but these domestic and international researches are only limited to animal experimentations and are rarely applied in clinical practices.[31] Therefore, we chose the *MMP-1* gene to discuss its correlation with osteomyelitis.

The present study showed that the *MMP-1* −1607 (2G/1G) and −519 (A/G) polymorphisms had certain influences on the onset of osteomyelitis. For *MMP-1* −1607 (2G/1G) polymorphism, 2G/2G genotype and the 2G allele may facilitate the occurrence of osteomyelitis, respectively, 2.605 and 1.735 times. These results conformed to previous study in Spanish which indicated that −1607 2G allele lead to an increased osteoblast *MMP-1* mRNA and MMP-1 serum levels, and both the 2G/2G genotype and the 2G allele were risk factors for the occurrence of osteomyelitis.[32] Abd-Allah et al[33] showed that 2G allele also contribute to osteoarthritis. Additionally, Lepetsos et al[34] suggested that compared with 1G/1G+2G/2G genotypes, 1G/2G genotype might reduce the risk of knee osteoarthritis. Our result is consistent with previous works. However, the role of −1607 (2G/1G) polymorphism in osteomyelitis and osteoarthritis was different from the results in other diseases.[35,36] For −519 (A/G) polymorphism, the GG genotype was much more frequently observed in cases than in controls, which suggested that GG genotype carriers might be susceptible populations of osteomyelitis. The −519G allele may increases 1.622 times of the

Table 2

Distributions of genotypes and alleles of *MMP-1* −519 (A/G) polymorphism.

		Genotype				Allele		
Group	Number	AA	AG	GG	Number	A	G	P_{HWE}
Case	80	26	32	22	160	84	76	.077
Control	81	33	38	10	162	104	58	.853
P		—	0.851	0.024		—	0.033	
OR (95% CI)		—		2.792 (1.127–6.917)		—	1.622 (1.038–2.536)	

CI = confidence interval, OR = odds ratios, P_{HWE} = *P* value for Hardy–Weinberg equilibrium.

susceptibility to osteomyelitis. Our results conformed to the results in a previous study. Baroneza et al[37] showed that −519G allele increased the susceptibility of posterior tibial tendon. But −519A/G polymorphism had no significant association with the chronic periodontitis.[38] These inconsistent results may be caused by many factors. Different diseases have various pathogenesis, although they may all be cased by inflammation, and the genotype distributions of genetic polymorphisms vary in different ethnics; these can all affect the genetic role of *MMP-1* gene polymorphisms in the disease onset. Thus, replication study should be performed to confirm the genetic association.

In a nutshell, *MMP-1* −1607 (2G/1G) and −519 (A/G) polymorphisms were susceptible factors for the development of osteomyelitis. The results of this study were limited by race, geographical groups, sample size, and experimental methods. Studies on susceptibility genes for osteomyelitis are rare, and researches about the pathogenesis and treatment options of osteomyelitis are still going on. Therefore, it is urgent to explore the osteomyelitis mechanism in deepened medical researches, so as to reduce the incidence and enhance the cure rate of it.

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