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 –519AG 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
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. Ethics 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 CGG-3′, −519 (A/G) F: 5′-AGG ACT ACA GCT GCA TGA CTG-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 ddH2O, 4 μL genome DNA, 2.5 μL 10 × PCR buffer solution, 2 μL dNTP (200mmol/L), 1 μL TaqDNA polymerase (5U/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 (445bp), 1G/1G homozygote (324 and 121bp), and 2G/1G heterozygote (445, 324, and 121bp). MMP-1 −519 (A/G) polymorphism had a 469 bp PCR amplification fragment, and there were also 3 different digested products: GG homozygote (469bp), AA homozygote (238 and 231bp), and AG heterozygote (469, 238, and 231bp) (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 −1607 (2G/1G); M refers to marker; 1 and 2 lines refer to 1G2G genotype (455, 324, and 121bp); 3 and 4 lines refer to 1G1G genotype (324 and 121bp); 5 and 6 lines refer to 2G2G genotype (455bp). B, RFLP results for −519 (A/G) polymorphism. M refers to marker; 1 and 2 lines refer to AA genotype (238 and 231bp); 3 and 4 lines refer to GG genotype (469bp); 5 and 6 lines refer to AG genotype (469, 238, and 231bp). MMP-1 = matrix metalloproteinase-1, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism.
Distribution differences of 2G/2G homozygote in 2 groups were statistically significant \( (P = .025; \text{Table 1}). \) 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 \( (P = .024; \text{Table 2}). \) 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.\[17 \text{–} 21\] 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.\[23 \text{–} 25\] 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.\[27 \text{–} 29\] 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.\[32,33\] Abd-Allah et al.\[34\] 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 \(-519\)G allele may increases 1.622 times of the osteomyelitis risk of GG genotype carriers was 0.735 times higher than that of AA genotype carriers \( (OR\ 1.735, \ 95\%\ CI\ 1.115–2.701), \) so the 2G allele may be a susceptible allele for osteomyelitis.

### Table 1

Distributions of genotypes and alleles of MMP-1 \(-1607\) (2G/1G) polymorphism.

| Group     | Number | 1G/1G | 1G/2G | 2G/2G | Number | 1G | 2G | \( P_{\text{HWE}} \) |
|-----------|--------|-------|-------|-------|--------|----|----|------------------|
| Case      | 80     | 19    | 37    | 24    | 160    | 75 | 85 | .523             |
| Control   | 81     | 33    | 32    | 16    | 162    | 98 | 64 | .118             |
| \( P \)   |        | .025  | .014  |       |        |    |    |                  |
| OR (95% CI)|        | 2.605 | 1.116 | 6.082 |        |    |    |                  |

\( OR = \text{odds ratios}, \ P_{\text{HWE}} = \text{P value for Hardy–Weinberg equilibrium}. \)

### Table 2

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

| Group     | Number | AA | AG | GG | Number | A | G | \( P_{\text{HWE}} \) |
|-----------|--------|----|----|----|--------|---|---|------------------|
| Case      | 86     | 26 | 32 | 22 | 160    | 84 | 76 | .077             |
| Control   | 81     | 33 | 38 | 10 | 162    | 104| 58 | .853             |
| \( P \)   |        | .024 |    |     |        |    |    |                  |
| OR (95% CI)|        | 2.792 (1.127–6.917) |      |     |        |    |    |                  |

\( OR = \text{odds ratios}, \ P_{\text{HWE}} = \text{P value for Hardy–Weinberg equilibrium}. \)
susceptibility to osteomyelitis. Our results conformed to the results in a previous study. Baroneza et al. showed that −519G allele increased the susceptibility of posterior tibial tendon. But −519A/G polymorphism had no significant association with the chronic periodontitis. These inconsistent results may be caused by many factors. Different diseases have various pathogenesis, although they may all be caused by inflammation, and the genotype distributions of genetic polymorphisms vary in different ethnicities, these can all affect the genetic role of MMP-1 gene polymorphisms in the disease onset. Thus, replication studies 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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References

[1] Cornejo P, Mandell GA. Bone scintigraphic findings in MRSA osteomyelitis. Clin Nucl Med 2016;41:153–3.
[2] Murray MR, Schroder GD, Hsu WK. Granulomatous vertebral osteomyelitis: an update. J Am Acad Orthopaed Surg 2015;23:529–38.
[3] Miller JA, Achey RL, Derakhshan A, et al. Neurologic complications, reoperation, and clinical outcomes following surgery for vertebral osteomyelitis. Spine 2015;37.
[4] South EM, Khan MA, Reingold A, et al. Group B streptococcus infections of soft tissue and bone in California adults, 1995-2012. Epidemiol Infect 2015;143:3343–50.
[5] Aggarwal A, Aggarwal AN. Bone and joint infections in children: acute hematogenous osteomyelitis. Ind J Pediatr 2016;83:817–24.
[6] Schmidt HG, Diefenbeck M, Krenn V, et al. [Classification of haematogenous and post-traumatic osteomyelitis]. Zeitschrift fur Orthopadie und Unfallchirurgie 2014;152:334–42.
[7] Agaja SB, Ayorinde RO. Chronic osteomyelitis in Ilorin, Nigeria. South Afr J Surg 2008;46:116–8.
[8] Hackett DJ, Rothenberg AC, Chen AF, et al. The economic significance of orthopaedic infections. J Am Acad Orthopaed Surg 2015;23(suppl: SI–77.
[9] Tande AJ, Patra BJ, Osman DR, et al. Clinical presentation, risk factors, and outcomes of hematogenous prosthetic joint infection in patients with Staphylococcus aureus bacteremia. Am J Med 2016;129:221e1:1–20.
[10] de Souza Mdo S, de Souza CA, Cunha LM, et al. A new look at osteomyelitis development—focus on CCR5delta32. Study in patients from Northeast Brazil. Infect Genet Evol 2015;31:61–3.
[11] Gaviria-Aguadlo C, Aroch C, Tareen N, et al. Genomic heterogeneity of methicillin-resistant Staphylococcus aureus associated with variation in severity of illness among children with acute hematogenous osteomyelitis. PloS One 2015;10:e0150415.
[12] Bae WJ, Shin MR, Kang SK, et al. HIF-2 inhibition suppresses inflammatory responses and osteoclastic differentiation in human periodontal ligament cells. J Cell Biochem 2015;116:1241–55.
[13] Szulcs K, Takaji M, Kombinzen YT, et al. Upregulation of matrix metalloproteinase (MMP)-1 and its activator MMP-3 of human osteoblast via uniaxial cyclic stimulation. J Biomed Mater Res B Appl Biomater 2007;80:491–8.
[14] Liu Q, Zhao J, Tan R, et al. Parthenolide inhibits pro-inflammatory cytokine production and exhibits protective effects on progression of collagen-induced arthritis in a rat model. Scand J Rheumatol 2015;44:182–91.
[15] Luo S, Deng M, Long X, et al. Association between polymorphism of MMP-1 promoter and the susceptibility to anterior disc displacement and temporomandibular joint osteoarthropathy. Arch Oral Biol 2015;60:1675–80.
[16] Almeida RC, Capelli Jr, Teles RP. Levels of gingival crevicular fluid matrix metalloproteinases in periodontally compromised teeth under orthodontic forces. Angle Orthodont 2013;85:1099–14.
[17] Sundar SS, Jayesh SR, Hussain S. Association of matrix metalloproteinase 1 gene promoter mutation and residual ridge resorption in edentulous patients of South Indian origin. J Pharm Bioallied Sci 2015;7(suppl 2):S562–S565.
[18] Dedong H, Bin Z, Peisheng S, et al. The contribution of the genetic variations of the matrix metalloproteinase-1 gene to the genetic susceptibility of gastric cancer. Genet Test Mol Biomark 2014;18:675–82.
[19] Liu L, Wu J, Wu C, et al. A functional polymorphism (-1607 1G→2G) in the matrix metalloproteinase-1 promoter is associated with development and progression of lung cancer. Cancer 2011;117:5172–81.
[20] Wiczerzek E, Reszka E, Gromadzinska J, et al. Genetic polymorphism of matrix metalloproteinases in breast cancer. Neoplasma 2012;59:237–47.
[21] Je W, Kim JW, Park NH, et al. Matrix metalloproteinase-1 promoter polymorphism and epithelial ovarian cancer: does ethnicity matter? J Obstet Gynaecol Res 2007;33:155–60.
[22] Cao Z, Li C, Xiang J. Effect of matrix metalloproteinase-1 promoter genotype on interleukin-beta-induced matrix metalloproteinase-1 production in human periodontal ligament cells. J Periodontol Res 2010;45:109–15.
[23] Lucyszyn SM, de Souza CM, Braosi AP, et al. Analysis of the association of an MMP1 promoter polymorphism and transcript levels with chronic periodontitis and end-stage renal disease in a Brazilian population. Arch Oral Biol 2012;57:954–63.
[24] Morgan AR, Han DY, Lam WJ, et al. Genetic variations in matrix metalloproteinases may be associated with increased risk of ulcerative colitis. Human Immunol 2011;72:1117–27.
[25] Lee A, Ghaname CB, Braun TM, et al. Bacterial and salivary biomarkers predict the gingival inflammatory profile. J Periodontol 2012;83:79–89.
[26] Pearce E, Tregouet DA, Sammgard A, et al. Haplotype effect of the matrix metalloproteinase-1 gene on risk of myocardial infarction. Circ Res 2003;97:1070–6.
[27] Rochford ET, Sabate Bresco M, Zeiter S, et al. Monitoring immune responses in a mouse model of fracture fixation with and without Staphylococcus aureus osteomyelitis. Bone 2013;53:82–92.
[28] Brennan C, Wang VJ. Management of fever and suspected infection in pediatric patients with central venous catheters. Pediatr Emerg Med Pract 2015;12:1–20.
[29] Arens D, Wilke M, Calabro L, et al. A rabbit humerus model of plating and nailing osteosynthesis with and without Staphylococcus aureus osteomyelitis. Eur Cells Mater 2015;30:148–61. [discussion 161–142.]
[30] Taramasso L, Boisson-Dupuis S, Garre ML, et al. Pinel germcella in a child with interferon-gamma receptor 1 deficiency. case report and literature review. J Clin Immunol 2014;34:922–7.
[31] Tso JL, Yang S, Menjivar JC, et al. Bone morphogenetic protein 7 sensitizes O6-methylguanine methyltransferase expressing-glioblastoma stem cells to clinically relevant dose of temozolomide. Mol Cancer Ther 2014;13:545–9.
[32] Montes AH, Valle-Garay E, Alvarez V, et al. A functional polymorphism in MMP1 could influence osteomyelitis development. J Bone Miner Res 2010;25:912–9.
[33] Abd-Allah SH, Shalaby SM, Pasha HF, et al. Variation of matrix metalloproteinase-1 and 3 haplotypes and their serum levels in patients with rheumatoid arthritis and osteoarthritis. Genet Testing Mol Biomark 2012;16:15–20.
[34] Lepetnos P, Pampanos A, Kanavakis E, et al. Association of MMP-1-1607 1G/2G (rs1799750) polymorphism with primary knee osteoarthritis in the Greek population. J Orthopaed Res 2014;32:1155–60.
[35] Huang CD, Lin SM, Chang PJ, et al. Matrix metalloproteinase-1 polymorphism is associated with persistent airway obstruction in asthma in the Taiwanese population. J Asthma 2009;46:41–6.
[36] Lin TH, Yang SF, Chiu CC, et al. Matrix metalloproteinase-1 mirbal expression and -1607 1G/2G gene promoter polymorphism in mitral chordae tendineae rupture. Translat Res 2013;161:406–13.
[37] Baroneza JE, Godoy-Santos A, Ferrera Masa B, et al. MMP-1 promoter genotype and haploype association with posterior tibial tendinopathy. Gene 2014;547:334–7.
[38] Astolfi CM, Shinohora AL, da Silva RA, et al. Genetic polymorphisms in the MMP-1 and MMP-3 gene may contribute to chronic periodontitis in a Brazilian population. J Periodontol 2006;35:699–703.