

Association of MMP-3 (-1612 5A/6A) polymorphism with knee osteoarthritis in Thai population

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Abstract Osteoarthritis (OA) is a degenerative joint disorder resulting in destruction of articular cartilage, osteophyte formation, and subchondral bone sclerosis. In recent years, numerous genetic factors have been identified and implicated in causing osteoarthritis. One such genetic defect is a single nucleotide polymorphism at position -1612 of matrix metalloproteinase-3 (MMP-3) promoter region, known to lead to three possible genotypes, 5A/5A, 6A/6A, and 5A/6A. The purpose of this study was to investigate the association of MMP-3 -1612 5A/6A gene polymorphism with knee osteoarthritis in Thai population. Genotype distributions and allelic frequencies of MMP-3

-1612 5A/6A polymorphism were investigated in 200 participants (100 patients with knee osteoarthritis and 100 healthy controls). Genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism. There was no statistically significant difference between the groups with respect to genotype distribution ($P > 0.05$). The 5A allele frequency was indicated as 15.5 %, and 6A allele was as 84.5 % in OA patients, whereas it was 10–90 % in the control group. Accordingly, the present study has indicated that the -1612 5A/6A polymorphism genotypes of MMP-3 gene promoter do not play a role in the development of osteoarthritis in the Thai population.

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Introduction

Osteoarthritis (OA) is a degenerative joint disorder resulting in substantial morbidity and disability in the elderly [1]. The genetic etiology of OA is not entirely known. Previously, OA was recognized as a noninflammatory arthropathy. However, recent studies have shown that an inflammatory process plays a part in the pathogenesis of OA [2, 3]. Pro-inflammatory cytokines are implicated as potential mediators in the disease [4]. A number of gene polymorphisms involved in development of knee osteoarthritis have been identified, such as those localized in or adjacent to the encoding sequences for the vitamin D receptor [5], estrogen receptor alpha [6], calcitonin [7], interleukin-6 [8], SMAD3 [9], and matrix metalloproteinase-1 [10].

Matrix metalloproteinases (MMPs), a 28-membered family of zinc-dependent endopeptidases, play essential roles in the processes of tissue remodeling and repair in physiological and pathological conditions [11, 12]. The MMP family is categorized into four main subtypes, consisting of collagenases, gelatinases, stromelysins, and membrane-type matrix metalloproteinases (MT-MMPs) [13]. Increasing evidence has accumulated for their roles in pathogenicity and progression of fibrosis.

MMP-3 is one of the stromelysin expressed in several cell types including human articular chondrocytes and synoviocytes [14]. In addition, MMP-3 is involved in the shedding of protein ectodomains from the cell surface [15] and can degrade a broad spectrum of extracellular matrix substrates, including type III, IV, and V collagens and noncollagenous extracellular matrix components, such as laminins, fibronectin, osteopontin, and proteoglycans [16]. Therefore, MMP-3 can proteolytically activate other MMPs, and pathological conditions may ensue through the overexpression of MMP genes [17]. A common adenosine insertion/deletion polymorphism (5A/6A) at position -1612 upstream of the transcription start site (rs3025039) has been examined within the MMP-3 promoter [18]. The MMP-3 gene possesses a functional promoter polymorphism at position -1612, affecting gene transcription (low-activity 6A allele, high-activity 5A allele) and resulting in low (6A/6A) and high (5A/5A, 5A/6A) promoter activity genotypes [19].

To our knowledge, data on the role of MMP-3 -1612 5A/6A polymorphism in Thai patients with knee OA have as yet not been documented in the literature. In this case-control study, we hypothesized that the -1612 5A/6A single nucleotide polymorphism of the MMP-3 gene promoter would contribute to the susceptibility of knee osteoarthritis. The purpose of this study was to investigate the association between MMP-3 -1612 5A/6A polymorphism and primary knee osteoarthritis in Thai population.

Patients and methods

Study population

This case-control study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University. The present study was conducted in compliance with the guidelines of the Declaration of Helsinki. All subjects gave written informed consent prior to their participation in the study.

A total of 100 patients aged 51–78 years diagnosed with primary knee osteoarthritis (84 women and 16 men; mean age 65.7 ± 1.1 years) and 100 control individuals who had no symptoms or signs of OA, other arthritis, or any joint

diseases (88 women and 12 men; mean age 63.5 ± 2.1 years) were recruited in this study. The diagnosis of knee OA was based on the criteria of the American College of Rheumatology, which included primary OA with any symptoms and radiographic signs of OA according to the Kellgren–Lawrence grading system [20]. Clinical characteristics on disease severity were observed by mean of Kellgren–Lawrence grade and Lequesne's functional index reflecting functional or symptomatic status of patients [21]. Radiographic findings of OA were categorized into Kellgren–Lawrence grade 1, 2, 3, or 4. Functional or symptomatic statuses of patients were classified into mild (Lequesne's functional index $I \leq 10$) or severe (Lequesne's functional index $I \geq 10$). Control subjects were consecutively selected among people without personal and family history of OA. Participants were excluded on the basis of having arthropathy due to gout, pseudogout, rheumatoid arthritis (RA), systemic lupus erythematosus, psoriasis, hemochromatosis, previous knee injury, or previous joint infection. The patients with any systemic inflammatory or autoimmune disorders, or any type of malignant or chronic illness were not included in this study.

Genotyping of MMP-3 gene

Peripheral venous blood samples of 3 ml were drawn from each individual by standard venipuncture. Genomic DNA was isolated from buffy coats by using the commercially available Qiagen Blood Cell and Culture Midi kit. Polymerase chain reaction (PCR) amplifications were performed for the -1612 5A/6A single nucleotide polymorphism (SNP) using the published primer set: 5'-GATT ACAGACAT GGGTCACG-3' (sense primer) and 5'-TTTC AATCAGGACAAGACGAAGTTT-3' (antisense primer). The conditions of the PCR were as previously described with some modifications [22]. Briefly, PCR was performed in a 20- μ l volume containing 5 μ l of genomic DNA template, 2 μ l of 10 \times *Taq* buffer, 2.5 mmol MgCl₂, 2 mmol dNTPs, 200 nmol of forward and reverse primer, and 4 U *Taq* DNA polymerase (Fermentas Inc., USA). The PCR protocol was 95 °C denaturation followed by 35 cycles: denaturation at 95 °C for 45 s, annealing at 53 °C for 45 s and elongation at 72 °C for 30 s, ending with an additional elongation step of 5 min at 72 °C. The amplified sequence was digested by *Xmn*I restriction enzyme (New England BioLabs, Beverly, Massachusetts, USA) at 37 °C overnight. After digestion, the reaction mixtures were separated by 12 % polyacrylamide gel electrophoresis and visualized by ethidium bromide staining. An example of an electrophoretic gel showing PCR product digestion with *Xmn*I is shown in Fig. 1. The 5A/5A genotype corresponded to the presence of 97 bp fragments. The 6A/6A genotype

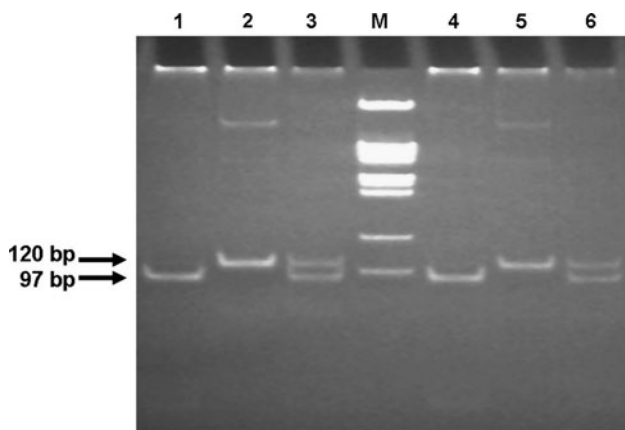


Fig. 1 Genotypes of MMP-3 polymorphism on 12 % polyacrylamide gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Lane 1 and 4: 97 bp represent genotype 5A/5A. Lane 2 and 5: 120 bp represent genotype 6A/6A. Lane 3 and 6: 97 and 120 bp represent genotype 5A/6A. Lane M represents DNA standard marker (100 bp)

Table 1 Characteristics of OA patients and control individuals

| Clinical characteristics | OA patients | Controls |
|---------------------------|-------------|------------|
| Number | 100 | 100 |
| Age (years) | 65.7 ± 1.1 | 63.5 ± 2.1 |
| Age range (years) | 51–78 | 43–71 |
| Female/male | 84/16 | 88/12 |
| BMI (kg/m ²) | 25.7 ± 3.3 | 25.2 ± 3.8 |
| Pain severity mild/severe | 28/72 | – |
| Grade | | |
| 1 | 4 | – |
| 2 | 10 | |
| 3 | 41 | |
| 4 | 45 | |

corresponded to a 120-bp fragment. The 5A/6A genotype corresponded to the presence of 97 and 120 bp fragments.

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software, version 16.0 for Windows. The demographic and clinical data were

compared between groups by the chi-square test and Student’s *t* test. Genotype and allelic frequencies were compared by the chi-square test. Allele and genotype proportions were evaluated for Hardy–Weinberg equilibrium. *P* < 0.05 was considered as indicating statistical significance.

Results

Characteristics of the population studied and the number of individuals in each group are illustrated in Table 1. There were no significant differences between groups in terms of age, gender, and mean body mass index (BMI). In the knee OA patients, the mean age was 65.7 ± 1.1 years. In the healthy controls, the mean age was 63.5 ± 2.1 years (*P* = 0.4). The female/male ratio was 84/16 in the patients with knee OA and 88/12 in controls (*P* = 0.2). Furthermore, average BMI value was not significantly different between groups, 25.7 ± 3.3 kg/m² in the knee OA patients and 25.2 ± 3.8 kg/m² in the controls, respectively (*P* = 0.3).

Table 2 presents the genotype and allelic frequencies of MMP-3 -1612 5A/6A polymorphism. 6A/6A was the most frequent genotype in OA patients, and control groups and genotype frequency fell within Hardy–Weinberg equilibrium. There was no statistically significant difference between the groups with respect to genotype distribution (*P* = 0.19). The 5A allele frequency was indicated as 15.5 %, and 6A allele was as 84.5 % in OA patients, whereas it was 10–90 % in the control group. According to the MMP-3 -1612 5A/6A polymorphism genotypes, there was no association between the genotypes of MMP-3 gene 5A/6A polymorphism and the clinical characteristics of the OA patients and controls.

Discussion

Osteoarthritis (OA) is one of the most common causes of joint disease and limitation of physical activity with advancing age. The knee is the most clinically significant

Table 2 Genotype distribution and allelic frequency of MMP-3 -1612 5A/6A single nucleotide polymorphism between OA patients and controls

| Group | Distribution of genotypes | | | | | | Allelic frequencies | | | |
|-------------|---------------------------|-----|-----------------|------|----------|------|---------------------|------|-----------------|------|
| | 5A5A | | 5A6A | | 6A6A | | 5A allele | | 6A allele | |
| | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % | <i>n</i> | % |
| OA patients | 2 | 2.0 | 27 | 27.0 | 71 | 71.0 | 31 | 15.5 | 169 | 84.5 |
| Controls | 0 | 0.0 | 20 | 20.0 | 80 | 80.0 | 20 | 10.0 | 180 | 90.0 |
| Statistic | $\chi^2 = 1.73$ | | <i>P</i> = 0.19 | | | | $\chi^2 = 2.25$ | | <i>P</i> = 0.13 | |

affected joint with OA characterized by progressive destruction of articular cartilage, which leads to pain and loss of joint function. Although the multifactorial nature of OA is well recognized, genetic factors have been shown to be strong determinants of this disorder. Previous studies have shown that there are a number of candidate genes associated with the knee OA [5–10].

The matrix metalloproteinase family constitutes more than 20 enzymes that are related with degradation of the extracellular matrix [11]. The expression of MMPs has been demonstrated to be affected by the SNP occurring in MMP gene promoters [23]. MMP-3 is a crucial enzyme that directly degrades extracellular matrix and indirectly affects degradation of extracellular matrix by activating other latent MMPs [24]. Serum and synovial MMP-3 expression have been previously reported in patients with OA or with post-traumatic knee injury [25, 26].

An understanding of the factors influencing the production of MMP-3 may allow better identification of patients at risk of developing destructive disease. According to the reports, a common -1612 5A/6A gene polymorphism of MMP-3 promoter has been identified in a wide variety of diseases, including rheumatoid arthritis [27]. Whether the MMP-3 genetic polymorphism at the -1612 region influences the susceptibility or severity in patients with knee osteoarthritis is not fully determined. To address this issue, we analyzed the influence of MMP-3 -1612 5A/6A polymorphism on the risk of knee OA in the Thai population. In this case–control study, our findings demonstrated that the percentage of the MMP-3 -1612 5A/6A polymorphism allele and the distribution of genotypes were not significantly different between the OA patients and controls.

In contrast with our findings, Abd-Allah et al. [28] have shown that there were significant associations between MMP-3 -1171 5A/6A polymorphism and susceptibility with OA in the Egyptian population. Additionally, MMP-3 5A/6A promoter polymorphism was found to be associated with disease outcome in RA [27, 29]. The explanation for these conflicting results remains unclear, but may be attributed to differences in disease advancement, populations, or assays applied.

It should be pointed out, however, that there are several limitations in our study. First, this pilot study was administered as a single-center trial with a relatively small number of subjects. Additional investigation conducted on a random sample of multiple centers with larger sample sizes is essential to validate the present findings. Nonetheless, a large scale prospective study should be launched to confirm the associative effect of this polymorphism with osteoarthritis patients. Secondly, we examined only one polymorphism in most of the genes, which could have missed an association that was to a specific polymorphism.

Haplotype analysis is needed to elucidate the functional variation responsible for MMP-3 expression and may provide further knowledge on the pathways responsible for the association of MMP-3 gene with OA. Another weakness is the lack of data regarding the level and source of MMP-3. More research is needed for future in vitro and in vivo studies of representative cells and tissues to gain insight into MMP-3 production and expression.

In summary, we provide evidence that the -1612 5A/6A polymorphism of the MMP-3 gene promoter is not associated with the susceptibility to knee OA in Thai population. Further studies in different populations and/or in large cohorts of OA patients are warranted to elucidate the definite association between MMP-3 -1612 5A/6A polymorphism and osteoarthritis.

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Conflict of interest The authors declare that they have no conflict of interest.

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