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Association of adiponectin +276G/T polymorphism with knee osteoarthritis

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Abstract. Osteoarthritis (OA) is a prevalent, degenerative joint disorder leading to the destruction of articular cartilage, osteophyte formation and subchondral bone sclerosis. Genetic and environmental factors are involved in the development of OA. The role of adiponectin gene polymorphisms in OA has not yet been established. The aim of this study was to investigate the association of adiponectin +276G/T (rs1501299) gene polymorphism with knee OA. Genotype distributions and allelic frequencies of adiponectin gene, +276G/T polymorphism were determined in a total of 200 subjects (100 knee OA patients and 100 healthy controls). Single-nucleotide polymorphism (SNP) of the adiponectin +276G/T gene was genotyped by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. The genotype distribution of +276G/T SNP was observed in the Hardy-Weinberg equilibrium for OA patients and controls. No statistically significant difference was identified between the two groups with respect to genotype distributions and allelic frequencies (P>0.05). The T- and G-allele frequencies were indicated as 24.5 and 75.5%, respectively, in OA patients, whereas the frequency was 23-70% in the control group. Findings of this study therefore suggest that the +276G/T SNP was not associated with susceptibility to knee OA.

Introduction

Osteoarthritis (OA) is a degenerative joint disorder leading to stiffness, reduced motion, swelling, crepitus, substantial morbidity and disability (1). It is characterized by synovitis, osteophyte formation, subchondral sclerosis and progressive destruction of articular cartilage, which results in pain and loss of joint mobilization. In recent years, numerous genetic factors have been identified and implicated in causing OA. Previously, OA was recognized as a non-inflammatory arthropathy. Nevertheless, results of previous studies have demonstrated that an inflammatory process plays a vital role in the pathogenesis of OA (2,3). Pro-inflammatory cytokines are considered to play a role as key mediators in the disease (4). A number of gene polymorphisms associated with the development of knee OA have been recently studied, such as those localized in or adjacent to the encoding sequences for growth differentiation factor 5 (5), estrogen receptor α (6), calcitonin (7), interleukin (IL)-6 (8), SMAD3 (9) and matrix metalloproteinase-3 (10).

Adiponectin is a 30-kDa protein encoded by the ADIPOQ gene located on chromosome 3q27 consisting of three exons and two introns. It assembles into complexes of different size, known as trimers (low molecular weight), hexamers (middle molecular weight) and higher order oligomeric complexes (high molecular weight) prior to being secreted (11). Adiponectin has been found to be associated with type of lifestyle and plays a substantial role in the development of metabolic diseases, such as diabetes mellitus and coronary heart disease (12). Although adiponectin may act as an anti-inflammatory mediator in many conditions, its role in joint diseases remains controversial.

To the best of our knowledge, there are currently no published studies regarding the role of adiponectin +276G/T polymorphism in OA patients. In this case-control study, we hypothesized that the +276G/T single-nucleotide polymorphism (SNP) of the adiponectin in intron 2 would contribute to the susceptibility of knee OA. The aim of this study was to examine the association between adiponectin +276G/T polymorphism and primary knee OA in the Thai population.

Materials and methods

Study population. A total of 100 patients diagnosed with primary knee OA (75 females and 25 males; mean age, 68.2±0.9 years) and 100 control individuals who had no symptoms or signs of OA, other types of arthritis, or any joint diseases (80 females and 20 males; mean age, 67.0±1.1 years)

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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 (KL) grading system (13). Radiographic findings of OA were classified as KL grade 1, 2, 3, or 4. The control subjects were consecutively selected among individuals without a personal and family history of OA. Subjects 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. Patients with any systemic inflammatory or autoimmune disorders, or any type of malignant or chronic illness were not included in this study.

This case-control study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University, Thailand. The present study was conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all the subjects prior to their participation in the study.

Genotyping of adiponectin gene. Peripheral venous blood samples were obtained from each subject by standard venipuncture. Genomic DNA was isolated from buffy coats by using illustra blood genomicPrep Midi Flow kit (GE Healthcare, Little Chalfont, UK) and the samples were stored at -20˚C for subsequent analysis. The adiponectin +276G/T (rs1501299) genotypes were determined by polymerase chain reaction (PCR)-restriction fragment length polymorphism analysis. The +276G/T SNP in the adiponectin gene was genotyped by the amplification of genomic DNA using the primers (14): forward, 5'-ACACTGATATAAACGCCATGAA-3' and reverse, 5'-GCAGCAAAGCCAAAGTCTTG-3'. The amplification conditions were as follows: 95˚C for 10 min, followed by 40 amplification cycles at 95˚C for 30 sec, 50˚C for 30 sec, 72˚C for 60 sec and a final extension at 72˚C for 7 min. The amplified PCR product was 168 bp in length. The polymorphism was typed using the enzyme *Bgl*1 (New England Biolabs, Beverly, MA, USA), which yielded 147- and 21-bp fragments (G allele of +276G/T).

In the genotyping experiments, the digestion fragments were subjected to electrophoresis on 12% polyacrylamide gel containing ethidium bromide and visualized on an ultraviolet transilluminator. An example of an electrophoretic gel showing PCR product digestion with *Bgl*1 is shown in Fig. 1. Homozygous GG and TT corresponded to the presence of 147- and 168-bp fragments, respectively, whereas the heterozygous GT corresponded to the presence of both 147- and 168-bp fragments.

Statistical analysis. The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows was used for statistical analysis. The demographic and clinical data were compared between groups by the Chi-square and Student's t-tests. 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 to indicate a statistically significant difference.

Table I. Demographic data of OA patients and control individuals.

Clinical characteristics	Controls	OA patients
No.	100	100
Age (years)	67.0±1.1	68.2±0.9
Female/male	80/20	75/25
BMI (kg/m^2)	24.5±3.7	27.3±3.9
KL grade		
1	-	0
2	-	31
3	-	39
4	-	30

OA, osteoarthritis; BMI, body mass index; KL, Kellgren-Lawrence.

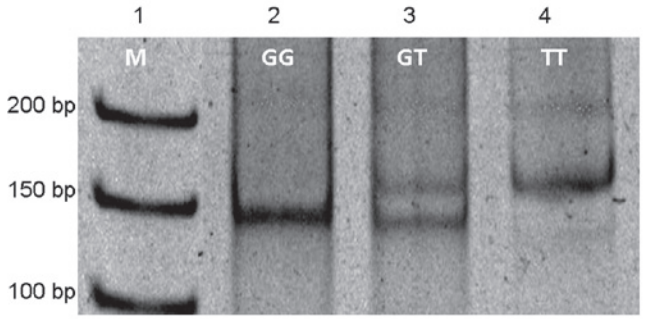

Figure 1. Genotypes of adiponectin polymorphism on 12% polyacrylamide gel electrophoresis with ethidium bromide staining and ultraviolet light transillumination. Lane 1, molecular weight DNA standard marker; lane 2, 147 bp represents homozygous GG; lane 3, 147 and 168 bp represent heterozygous GT; lane 4, 168 bp represents homozygous TT.

Results

Patient characteristics. Demographic data of the population studied and the number of individuals in each group are shown in Table I. 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 68.2±0.9 years. In the healthy controls, the mean age was 67.0±1.1 years (P=0.2). The female/male ratio was 75/25 in the knee OA patients and 80/20 in the controls (P=0.4). Furthermore, the mean BMI value was not significantly different between groups, 27.3±3.9 in the knee OA patients and 24.5±3.7 kg/m^2 in the controls, respectively (P=0.5).

Genotype and allelic frequencies of adiponectin +276G/T polymorphism. GG was the most frequent genotype in the OA patients and control groups and the genotype frequency was within the Hardy-Weinberg equilibrium. There was no statistically significant difference between the groups with respect to genotype distribution (P=0.84) (Table II). The T- and G- frequencies were indicated as 24.5 and 75.5%, respectively, in OA patients, whereas the frequency was 23-70% in the control group. According to the adiponectin

Table II. Genotype distribution and allelic frequency of adiponectin +276G/T single-nucleotide polymorphism between OA patients and controls.

	Genotype distribution				Allelic frequencies		
+276G/T SNP	GG	GT	TT	P-value	G allele (%)	T allele (%)	P-value
Controls	59	36	5	0.84	154 (77.0)	46 (23.0)	0.72
OA patients	58	35	7		151 (75.5)	49 (24.5)	

P-value for difference in distribution of genotypes and allelic frequencies between controls and OA patients. OA, osteoarthritis; SNP, single-nucleotide polymorphism.

+276G/T polymorphism genotypes, there was no association between the genotypes of adiponectin +276G/T polymorphism and the clinical characteristics of the OA patients and controls.

Discussion

OA is a common cause of degenerative joint disease and functional limitation and disability in the elderly. The knee is the most clinically significant site of primary OA involvement. Although great efforts have been made to elucidate the pathophysiology of OA, the genetic factors underlying the development of OA remain unclear. Results of recent studies have shown that there are several candidate genes associated with knee OA (5-10).

Adiponectin, a 244-amino acid polypeptide, represents the highest proportion of all adipokines in the circulation. Adiponectin is structurally homologue to complement factor C1q and tumor necrosis factor-α (TNF-α) (15). It has been shown that adiponectin exerts an anti-inflammatory effect by reducing the release of pro-inflammatory cytokines, e.g., TNF-α and IL-6, and inducing the expression of anti-inflammatory cytokines (16-18). Moreover, numerous studies have shown that adiponectin is capable of counteracting insulin resistance, artherosclerosis and inflammatory processes (15,16,19-21). However, whether adiponectin plays pro- or anti-inflammatory roles in joint disease pathogenesis remains the subject of debate. Recent data have revealed that adiponectin may be secreted by synovial fibroblasts, chondrocytes and infrapatellar fat pad in patients with OA and RA, which led to the increased production of IL-6, IL-8, matrix metalloproteinase and nitric oxide (22,23). These mediators promoted inflammation and joint destruction (16,19). By contrast, Chen *et al* (24) suggested that adiponectin might play a protective role in OA by inducing tissue inhibitor of metalloproteinase-2 expression and suppressing IL-1β-induced matrix metalloproteinase-13 production.

In the present study, we aimed to screen for a susceptibility gene that could facilitate the early diagnosis of OA. Such a genetic screen would enable the identification of individuals who are at a high risk for developing OA. Whether the adiponectin genetic polymorphism at +276G/T influences the susceptibility or severity in patients with knee OA is not fully examined. To address this issue, we investigated the effect of adiponectin +276G/T polymorphism on the risk of knee OA in the Thai population. To the best of our knowledge, this is the first report to evaluate the association between adiponectin +276G/T polymorphism and OA. Our findings demonstrate that the percentage of the adiponectin +276G/T polymorphism allele and the distribution of genotypes were not significantly different between the OA patients and controls.

Limitations of this study involved the relatively small number of enrolled subjects. Further studies conducted on a random sample of multiple centers with larger sample sizes are required to determine whether these findings can be extrapolated to other populations. In addition, this study investigated only one polymorphism in most of the genes, potentially missing any association to a specific polymorphism. Haplotype analysis is necessary for understanding the functional variation responsible for the adiponectin expression and may provide further knowledge on the pathways responsible for the relationship of adiponectin gene with OA. Another limitation is the lack of information regarding the level and source of adiponectin. More studies are in progress to gain insight into adiponectin production and expression.

In conclusion, the present study has suggested that the +276G/T polymorphism genotypes of adiponectin gene do not confer increased susceptibility to knee OA in the Thai population. Additional studies in different and large populations of OA patients are required to elucidate the precise role of adiponectin +276G/T polymorphism and OA.

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