

***ADAMTS14* gene polymorphism associated with knee osteoarthritis in Thai women**

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ABSTRACT. Osteoarthritis (OA) is the most prevalent form of arthritis in the elderly. This disease is characterized by breakdown and loss of articular cartilage due to genetic, mechanical and environmental factors. Although the pathophysiology of OA is not completely known, several candidate genes have been reported to be associated with OA susceptibility. We assessed the association between genetic variation in the *ADAMTS14* region and knee osteoarthritis susceptibility in the Thai population. The rs4747096 SNP was genotyped by PCR-RFLP on genomic DNA extracted from peripheral blood of 108 OA patients and 119 controls. The PCR product (196 bp) was digested with *BspEI*. A sample with the GG genotype showed two band sizes of 158 and 38 bp, while a sample with the AA genotype showed a single band size of 196 bp. Heterozygotes with the AG genotype showed all three corresponding bands. Genotype distributions,

allele frequencies and model of inheritance in patients and controls were compared. In females, the frequency of the AA genotype and the A allele were significantly higher in knee OA patients than in controls [odds ratio (OR) = 2.79, 95% confidence interval (CI) = 1.05-7.59 and OR = 1.58, 95%CI = 1.00-2.45, respectively]. Moreover, genotypic AA and AG were associated with significantly increased risk for knee OA when compared to GG (OR = 2.72, 95%CI = 1.10-6.87). No significant associations were observed in males. In conclusion, the nsSNP rs4747096 in *ADAMTS14* was associated with knee OA in female Thai patients; therefore, the role of *ADAMTS14* in OA seems to be gender-dependent.

Key words: Osteoarthritis; ADAMTS14; rs4747096; PCR-RFLP; Thai population

INTRODUCTION

Osteoarthritis (OA), a degenerative joint disorder, is the most prevalent form of arthritis in the elderly, which causes pain and restricted motion in joints, particularly the knee and hip (Dieppe and Lohmander, 2005). This disease is characterized by the breakdown and loss of the articular cartilage in parallel with subchondral bone sclerosis, joint space narrowing, and bony outgrowths at the joint margins (osteophytes) (Dieppe and Kirwan, 1994). The multifactorial nature of OA is well accepted, with several attributing factors including a variety of non-genetic factors (obesity, female gender, advanced age, and joint injury) and a combinatorial effect of weak genetic factors (Spector and MacGregor, 2004; Teichtahl et al., 2005; Ikegawa, 2007). Although the pathogenesis of this complex disease is not entirely known, various candidate genes and linkage regions have been shown to be associated with OA susceptibility (Valdes and Spector, 2009). A number of genes involved in developmental processes or maintenance of cartilage and bone have been found to be associated with OA occurrence and progression (Tammachote, 2011). OA-associated genes were categorized into five molecular pathways including inflammation, extracellular matrix (ECM) molecules, Wnt signaling, osteocyte modulation or chondrocyte differentiation, and proteases and their inhibitors (Valdes and Spector, 2009).

Osteoarthritic joints have an imbalance between synthesis and degradation of ECM leading to cartilage destruction. Therefore, metalloproteinases have been considered as the major groups of enzymes responsible for this OA hallmark (Kevorkian et al., 2004; Murphy and Nagase, 2008). A disintegrin and metalloproteinases with thrombospondin motifs (ADAMTSs), members of the Zn²⁺-metalloproteinase superfamily (metzincins), are of interest as novel genetic factors of OA (Murphy and Nagase, 2008). The proteinases in the ADAMTS family resemble and share a common domain structure with the proteinases in the ADAM (A disintegrin and metalloproteinase) family in having a prodomain, an adamalysin/reprolysinlike catalytic domain, a disintegrin-like domain and a cysteine-rich domain. However, the ADAMTS proteinases differ from ADAM proteinases in lacking transmembrane domains and are a putative secretory protein. In addition, ADAMTSs possess a thrombospondin type 1 (TSP1) motif, followed by a spacer region and a variable number of TSP1 repeats, which appear to be involved in binding to ECM components, such as aggrecan and procollagen (Kuno et al., 1997; Hurskainen et al., 1999; Apte, 2004). *ADAMTS14*, which is located on

chromosome 10q22.1, has been recently reported to be a novel OA candidate gene (Rodriguez-Lopez et al., 2009). A non-synonymous single nucleotide polymorphism (nsSNP) of likely deleterious effect in exon 21 of *ADAMTS14* (rs4747096) was demonstrated to be associated with knee OA. Rodriguez-Lopez et al. found that the G allele in rs4747096 was associated with women requiring total knee replacement because of OA and with symptomatic hand OA in the Caucasian population (Rodriguez-Lopez et al., 2009).

To our knowledge, there has been no further published report about the role of this SNP in other populations. Since several studies have indicated the site-specific, sex-specific and ethnic-specific nature of genetic effects on osteoarthritis, this research aimed to analyze the association of rs4747096 nsSNP in *ADAMTS14* with knee OA susceptibility in a Thai population in a case-control study manner.

MATERIAL AND METHODS

Study population

This study has been approved by the Institutional Review Board of the Faculty of Medicine, Thammasat University. Written informed consent was received from all subjects prior to their participation in the study. The 108 cases (88 women and 20 men; mean age 70 years, range 51-91 years) were patients with severe primary knee OA undergoing total knee replacement (TKR) surgery. The indications of TKR were the absence of femoral joint space, no responses to conservative treatment longer than six months, and grades 3-4 of radiographic signs of OA according to the Kellgren-Lawrence grading system (Kellgren and Lawrence, 1957). The control population consisted of 119 healthy individuals (93 women and 26 men; mean age 55 years, range 50-60 years). All were diagnosed by physicians to have no symptoms or signs of OA, other arthritis or any joint disorders (chronic pain, inflammation, tenderness, swelling or limitation of movement), and to have no family history of those diseases. The patients with any systemic inflammatory or autoimmune disorders, or any type of malignant or chronic illness were not included in this study.

Sample collection and DNA extraction

Peripheral blood samples were taken from each participant by standard venipuncture. All blood samples were kept in ethylenediaminetetraacetic acid (EDTA)-containing tubes at 4°C until use. Genomic DNA was extracted from whole blood with innuPREP Blood DNA Master kit (Analytikjena, Seerasen, Einfeld, Germany). The DNA concentration was estimated by measuring the absorbance at 260 nm. Afterwards, DNA was diluted to 50 ng/μL and used as the template in SNP genotyping.

SNP genotyping

The rs4747096 polymorphism was genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The mutated forward primer (5'-GGAACCTTGGGACGCCAGAGGGGCAGTGGGTGCCACAATCCG-3') and the reverse primer (5'-AGGGAGGTGAAGGTCACACA-3') were used for amplification of the area of interest. PCR was conducted in a 20-μL volume containing 2 μL 10X *Taq* buffer, 1.5 mmol

MgCl₂, 0.5 mmol dNTPs, 0.2 μmol of each primer, 100 ng genomic DNA template, and 0.4 U *Taq* DNA polymerase (Fermentas Inc., Hanover, NH, USA). The PCR program consisted of the following steps: initial denaturation of 5 min at 94°C, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 1 min, and a final extension of 72°C for 5 min. The PCR product (196 bp) was incubated overnight at 37°C with the *Bsp*EI restriction enzyme (New England BioLabs, Beverly, MA, USA), which can cut PCR products with the G allele. After incubation, the mixtures were electrophoresed on 3% agarose and visualized by ethidium bromide staining and ultraviolet light transillumination. The expected fragment was 196 bp in AA, 158 and 38 bp in GG and 196, 158 and 38 bp in AG genotypes, respectively. For accuracy testing, several samples with different genotypes were sequenced.

Statistical analysis

Comparison of demographic data was conducted by Excel 2007 (Microsoft® Excel®). The Chi-square test was applied for categorical data, and the unpaired Student *t*-test was applied for continuous data. Genotype and allele proportions in patient and control groups were evaluated for Hardy-Weinberg equilibrium (HWE) as described at the following website: <http://www.oege.org/software/hardy-weinberg.html> (Rodriguez et al., 2009). Genotype distributions, allele frequencies in OA patients and controls, and model of inheritance were determined by the StatCalc program (AcaStat Software, Leesburg, VA, USA). *P* < 0.05 was considered to be statistically significant. The risk associated with individuals' genotypes and alleles was evaluated as the odds ratio (OR) with 95% confidence intervals (95%CI). For gender stratification analysis, female OA patients were compared with female controls and male OA patients with male controls.

RESULTS

Demographic characteristics and the number of individuals in each group of the population studied are illustrated in Table 1. In this study, there were no significant differences between OA patients and healthy controls in terms of gender, weight, height, and body mass index (BMI). However, the average age of the knee OA group was significantly greater than that of controls.

Table 1. Characteristics of osteoarthritis (OA) patients and control individuals.

Variables	Knee OA patients (N = 108)	Controls (N = 119)	P
Female (%)	88 (81.5)	93 (78.2)	0.53
Age [years mean (SD)]	69.94 (8.41)	54.50 (2.72)	<0.001*
Weight [kg mean (SD)]	63.57 (10.92)	63.48 (12.11)	0.95
Height [cm mean (SD)]	155.59 (7.05)	157.18 (6.25)	0.06
BMI [kg/m ² mean (SD)]	26.29 (4.45)	25.65 (4.25)	0.27

*Statistically significant.

The rs4747096 polymorphism was genotyped by PCR-RFLP. PCR product size was 196 bp. *Bsp*EI cleaved the PCR product with G allele into 158- and 38-bp fragments. An example of an electrophoretic gel displaying PCR products after digestion with *Bsp*EI is shown in Figure 1. As the smallest fragment (38 bp) was difficult to be visualized, the presence of a 196-bp fragment corresponded to the AA genotype, the 158-bp fragment corresponded to the GG genotype, and the 196- and 158-bp fragments corresponded to the AG genotype.

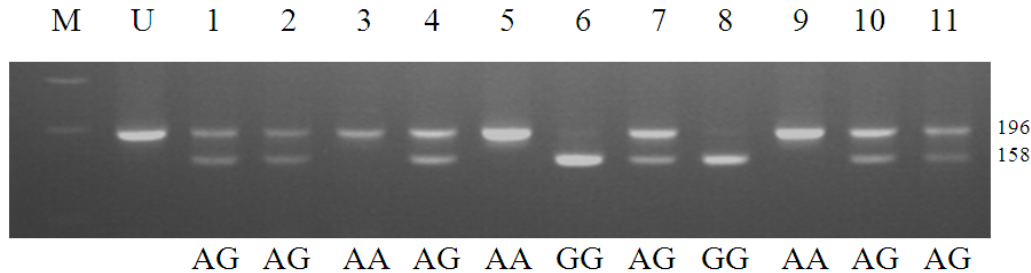


Figure 1. *ADAMTS14* rs4747096 genotyping by PCR-RFLP. The PCR product was digested with *BspEI*, which cleaved the PCR products with G alleles, and subjected to electrophoresis on a 3% agarose gel. Lane M = 100-bp DNA standard marker; lane U = uncut PCR product; lanes 1 to 11 = sample 1-11. Genotypes of each sample are shown in the bottom.

The genotype and allelic frequencies of rs4747096 are summarized in Table 2. Interestingly, the genotype distribution among the controls was not in Hardy-Weinberg equilibrium (HWE) due to the overrepresentation of the rare homozygous genotype (GG), whereas the genotypes of patients were found to be in HWE ($P > 0.05$). AA was the most common genotype in OA patients and normal controls (46.3 and 40.3%, respectively). We found a significant association between the AA genotype and knee OA in female patients ($P = 0.023$). This genotype was linked to a higher risk of knee OA in females (OR = 2.79, 95%CI = 1.05-7.59). In addition, the A allele was also significantly associated with female knee OA patients (OR = 1.58; 95% CI = 1.00-2.45; $P = 0.038$). Moreover, a nearly significant association was found between the AG genotype and knee OA in female patients (OR = 2.65; 95% CI = 0.99-7.19; $P = 0.031$). In contrast, the rs4747096 polymorphism was not associated with OA susceptibility in males in this study.

Table 2. Genotype distribution and allelic frequency of *ADAMTS14* polymorphism between knee osteoarthritis (OA) patients and normal controls.

rs4747096	OA	Control	OR (95%CI)	P
Genotype (%)				
AA				
Whole group	50 (46.3%)	48 (40.3%)	2.17 (0.92-5.19)	0.054
Female	40 (45.5%)	35 (37.6%)	2.79 (1.05-7.59)	0.023
Male	10 (50.0%)	13 (50.0%)	0.77 (0.09-6.35)	0.56
AG				
Whole group	46 (42.6%)	46 (38.7%)	2.08 (0.87-5.02)	0.07
Female	39 (44.3%)	36 (38.7%)	2.65 (0.99-7.19)	0.031
Male	7 (35.0%)	10 (38.5%)	0.70 (0.07-6.39)	0.54
GG				
Whole group	12 (11.1%)	25 (21.0%)	1*	
Female	9 (10.2%)	22 (23.7%)	1*	
Male	3 (15.0%)	3 (11.5%)	1*	
Allele (%)				
A				
Whole group	146 (67.6%)	142 (59.7%)	1.41 (0.94-2.11)	0.08
Female	119 (67.6%)	106 (57.0%)	1.58 (1.00-2.45)	0.038
Male	27 (67.5%)	36 (69.2%)	0.92 (0.35-2.46)	0.86
G				
Whole group	70 (32.4%)	96 (40.3%)	1*	
Female	57 (32.4%)	80 (43.0%)	1*	
Male	13 (32.5%)	16 (30.8%)	1*	

*Reference.

As the SNP showed significant association only with females, the model of inheritance was not analyzed in the whole group and men group. The result from females showed that this polymorphism is inherited by an autosomal dominance aspect, since the AA and AG genotypes significantly increased risk of the disease (OR = 2.72, 95%CI = 1.10-6.87, P = 0.017), whereas the AA genotype showed no significant difference (Table 3).

Table 3. Risk of female knee osteoarthritis (OA) patients associated with *ADAMTS14* -3146A/G genotype according to different models of inheritance.

Model of inheritance	Female			
	OA	Control	OR (95%CI)	P
A dominance, G wild type				
AA or AG	79 (89.8%)	71 (76.3%)	2.72 (1.10-6.87)	0.017
GG	9 (10.2%)	22 (23.7%)	1*	
A recessive, G wild type				
AA	40 (45.5%)	35 (37.6%)	1.38 (0.73-2.61)	0.29
GG or AG	48 (54.5%)	58 (62.4%)	1*	

*Reference.

DISCUSSION

ADAMTS is a family of zinc-dependent proteases that are implicated in a variety of normal and pathological conditions, including arthritis. ADAMTS14 belongs to the ADAMTS family. There are 19 published vertebrate family members of ADAMTSs, numbered 1-10 and 12-20 to date (Cal et al., 2002; Somerville et al., 2003). Although little is still known about its function, ADAMTS14 has high similarities in sequence and domain structure to ADAMTS-2 (56 %) and ADAMTS-3 (63 %) (Colige et al., 2002), which play a major role in the biosynthesis of collagen precursors. There is evidence that these three enzymes have the ability to cleave the N-propeptides of procollagen type I (pNPI activity) and the homotrimeric procollagen type II before they are incorporated into collagen fibers (Lapierre et al., 1971; Tuderman et al., 1978; Hojima et al., 1989, 1994). Moreover, ADAMTS14 has been shown to have pNPI activity *in vitro*, and has been suggested as a possible source of residual pNPI activity observable in the bone, tendon, cartilage, skin, and other tissues of Ehlers-Danlos syndrome type VIIC patients, dermatosparaxic cattle, and *Adams2*-null mice (Fernandes et al., 2001; Colige et al., 2002; Le Goff et al., 2006).

In the present study, we evaluated the association between rs4747096 nsSNP in *ADAMTS14*, a recently discovered OA candidate gene, and presence of knee OA in a Thai population. Originally, this nsSNP designates a change of glutamic acid (GAA) to glycine (GGA) in the COOH-terminal domain of ADAMTS14 protein. The allele G is the ancestral allele, as it exists in *ADAMTS14* orthologues in nonhuman primates such as chimpanzee, whereas the A allele is more common in humans than the G allele (Sherry et al., 2001). Recently, there was evidence of the T allele, which determines the amino acid valine (GTA) in the rs4747096 database. The existence of the new allele seems to complicate the analysis of rs4747096 role in OA. However, this new variation was discovered in genomic DNA from cancer patients (Futreal et al., 2004), and was not apparent in any population in The 1000 Genome Project (2010), including the Asian population even though the whole genome sequencing method was used as genotyping technique. Thus, the T allele was neglected in this study.

Genotype distributions and allele frequencies in a Thai population showed an opposite trend compared to a previous study (Rodriguez-Lopez et al., 2009). The result from this study demonstrated that rs4747096 nsSNP was associated with susceptibility to knee OA in female patients undergoing total knee replacement surgery. The AA genotype carried the highest risk in comparison with the other genotypes. Moreover, the analysis for model of inheritance indicated that the A allele increased the risk of knee OA in women. In contrast, Rodriguez-Lopez et al. study (2009) reported that the G allele was overrepresented and associated with knee OA in Caucasian women. The reason for these contradictory results may be due to differences in ethnicity or shared environmental factors. However, the non-associated results from male population are consistent. Several studies have also revealed contradictory findings of genetic contribution to OA. For example, a polymorphism in *ILIRN* was reported to be associated with disease severity in knee OA in a Caucasian population (Attur et al., 2010), while a study in a Turkish population found no association (Sezgin et al., 2007).

It was believed that the substitution of glutamic acid (acidic) to glycine (nonpolar) in *ADAMTS14* (E1049G) may result in abnormal ability of the protein in processing collagen precursor and finally leading to OA. The positive results of both A and G alleles obtained from the present and previous studies may indicate that the amino acid substitution determined by rs4747096 in *ADAMTS14* is not the direct damaging factor contributing to OA. However, this polymorphic locus may be linked to the OA causative gene on the same chromosome and the SNP is inherited jointly with the disease.

The number of the ancestral G-allele in Thais was higher than in Caucasians, especially in women (38 vs 20%). These figures are consistent with HapMap reports showing that the G allele frequency of this SNP in the East Asian population and European population was approximately 37 and 19%, respectively (The International HapMap Consortium, 2003). The genotype distribution of the control group in this study did not fit HWE, which indicated that specific disturbing influences were introduced. One possible influence is a selective pressure for the G allele, since the GG genotype seemed to be the protective genotype. As Thais are a mixed racial population, gene flow or migration can also be influences that alter the existing gene frequency. However, it is obvious that bias of sample collection can be diminished as the genotype frequency of the other genes studied in the same population was in HWE. The major limitation of the recent finding is the small study population size, especially in the male group. However, in the study of gender-dependent disease like OA this is quite expected.

CONCLUSIONS

In conclusion, we found that the nsSNP rs4747096 in *ADAMTS14* was associated with female OA patients requiring total knee replacement in a Thai population. Therefore, the role of *ADAMTS14* in OA seems to be remarkably gender-dependent. Additional investigations conducted with a larger sample size or different ethnicities are required to validate the present findings.

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