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Original Article

Association of single nucleotide polymorphisms in ADAM12 gene with susceptibility to knee osteoarthritis: a case-control study in a Chinese Han population

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Abstract: Objective: Genetic factors play an important role in osteoarthritis (OA) etiology and ADAM12 gene polymorphisms may be involved. This study tried to examine the single-nucleotide polymorphisms (SNPs) of ADAM12 for their association with knee OA susceptibility in a Chinese Han population. Methods: The rs3740199, rs1871054, rs1278279, and rs1044122 SNPs in ADAM12 gene were genotyped in 152 subjects who were diagnosed as knee osteoarthritis and in 179 healthy controls. Results: Rs1871054 was found to be significantly associated with increased risk of OA (C vs. T, OR 1.802 (1.308 to 2.483), $P < 0.0001$) after adjustment of age, gender, and BMI. For other SNPs, no statistically significant associations with OA were found. Conclusion: In conclusion, our data demonstrated the ADAM12 rs1871054 variant was found to be significantly associated with increased OA susceptibility in a Chinese Han population.

Keywords: Single-nucleotide polymorphisms, ADAM12 gene, osteoarthritis, susceptibility

Introduction

Osteoarthritis (OA) is a multifactorial disorder characterized by synovitis, progressive cartilage loss, osteophyte formation, and subchondral sclerosis, which accounts for a large amount of elderly individuals with pain and disability [1, 2]. Arthroplasty surgery acts as the major therapeutic method for OA currently, in despite of the operative invasive, complication, and economic burden. Little is known about the initiating events in OA or the relative importance of bone remodeling compared with that of cartilage degradation. In addition to age, sex, body weight and trauma, in recent years, numerous genetic factors have also been identified in causing OA. Several genome-wide association studies (GWAS) based on large-sample population have demonstrated single nucleotide polymorphisms (SNPs) in various genes were associated with susceptibility to knee osteoarthritis, including vitamin D receptor (VDR); asporin (ASPN), transforming growth factor beta 1 (TGFB1), insulin-like growth factor 1 (IGF1), interleukin-6 (IL6), a disintegrin and metalloproteinase domain 12 (ADAM12), and so on [3-10].

The ADAM family of trans-membrane proteins belongs to the super-family of zinc proteases [11]. There are 19 different ADAM genes, which can secret glycoproteins that are involved in various functions such as: cell-cell interaction, fertilisation, and muscle development [11]. ADAM12 is an active metalloproteinase that possesses cell-binding and cell-signalling properties [12]. Numerous investigations implicate ADAM12 as an important regulator in both normal development of tissues and a variety of pathological states. A previous study demonstrated the ADAM12-S protein could be elevated in some OA patients' sera, and this elevation correlates with grades of the disease [13]. The expression of ADAM12 and variation within the ADAM12 gene have previously been associated with osteoarthritis in various studies [8, 14-17]. While, controversy continues as the results were unable to replicate.

Accordingly, the aims of this study were to determine whether the ADAM12 SNPs (rs3740199, rs1871054, rs1278279, and rs1044122) variants were associated with OA in Chinese Han populations.

Table 1. Summary of the basic characteristics of the groups

Clinical Characteristics	OA Patients	Controls	*P*-Value
No.	152	179	
Age (years)	63.1±5.2	62.2±4.2	n. s
Female/Male	94/58	102/77	n. s
BMI (kg/m^2)	25.6±3.5	24.2±4.0	0.001
KL grade (%)			
2	81 (53.3)	0	
3	47 (30.9)	0	
4	24 (15.8)	0	

Methods

The study was approved by The Ethics Committee of The 117th Hospital of PLA, and informed consent was obtained from patients and control participants.

Study population

A total of 152 patients diagnosed with primary knee OA and 179 age-matched healthy controls who had no symptoms or signs of OA, other types of arthritis, or any joint diseases were recruited in this study. All subjects included in this study were Chinese Han Population. 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 (K/L) grading system (≥ 2 scale). The clinical examination and radiological assessment were performed by two independent examiners who were blinded to the clinical information. Disagreements were resolved through discussion and consensus. The control subjects were consecutively selected among individuals without a personal and family history of OA. Other etiologies causing knee diseases such as inflammatory arthritis (rheumatoid, polyarthritic or autoimmune disease), posttraumatic or postseptic arthritis, skeletal dysplasia or developmental dysplasia were also excluded.

Genotyping

DNA samples were obtained from all the participants from peripheral blood with the Chelex-100 method [18]. The SNPs were then genotyped using Taqman assay (Applied Biosystems 7500, ABI, Foster City, CA) and dual-labeled probes in real-time PCR. The primers and probes were designed and synthesized by Sigma (Sigma-Proligo, The Woodlands, TX). Genotyping was performed by independent laboratory personnel who were blinded to the study, and three authors independently reviewed the genotyping results, data entry, and statistical analyses. In addition, we randomly selected 5% samples of case and control subjects for reproducibility tests at least twice in different days and yielded a 100% concordant.

Statistical analysis

The Statistical Package for Social Sciences software (SPSS, Inc., Chicago, IL, USA), version 16.0 for Windows and the HaploView software were used for statistical analysis in this study. The demographic and clinical data were presented as Mean ± SD and compared between groups by the Student's t-tests. The genotype and allelic frequencies were evaluated by Hardy-Weinberg equilibrium and compared by the Chi-square test. Multivariate logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (CI) after adjustment for age, gender, and BMI. The linkage disequilibrium (LD) mapping and the associations between haplotypes of selected SNPs and risk of OA were estimated by the HaploView software. $P < 0.05$ was considered to indicate a statistically significant difference.

As no previous studies about ADAM12 polymorphism have been performed based on Chinese Han Population, it is hard to define the sample size. To assess the power of our study, we conducted power calculations using a statistical programme (Quanto).

Results

Patient characteristics

Demographic data of the population studied and the number of individuals in each group were shown in **Table 1**. There were no significant differences between groups in terms of age and gender. The mean body mass index (BMI) of the case group (25.6±3.5) was significantly higher than the control group (24.2±4.0) (P=0.001), similar with the previous studies that reported high BMI increased the risk of

Table 2. Genotype and allele distributions of the 4 SNPs for the cases and controls

Group	Genotype (frequency) rs3740199				Allele (%)		H-WE
	GG	GC	CC	GC+CC	G	C	
Case	42	78	32	110	53.3	46.7	/
Control	44	93	42	135	50.6	49.4	0.6
OR (95% CI)[a]	/	0.926 (0.543 to 1.579)	0.787 (0.413 to 1.501)	0.882 (0.532 to 1.461)	/	0.894 (0.653 to 1.224)	
P[a]	/	n. s	n. s	n. s	/	n. s	
Group	Genotype (frequency) rs1044122				Allele (%)		H-WE
	TT	TC	CC	TC+CC	T	C	
Case	47	81	24	105	57.6	42.4	/
Control	56	92	31	123	67.1	32.9	0.517
OR (95% CI)[a]	/	0.995 (0.601 to 1.649)	0.825 (0.417 to 1.632)	0.952 (0.588 to 1.542)	/	0.928 (0.675 to 1.276)	
P[a]	/	n. s	n. s	n. s	/	n. s	
Group	Genotype (frequency) rs1278279				Allele (%)		H-WE
	GG	AG	AA	GA+AA	G	A	
Case	84	59	9	68	74.7	25.3	/
Control	106	60	13	73	76.0	24.0	0.274
OR (95% CI)[a]	/	1.184 (0.737 to 1.901)	0.864 (0.348 to 2.149)	1.125 (0.718 to 1.763)	/	1.043 (0.725 to 1.500)	
P[a]	/	n. s	n. s	n. s	/	n. s	
Group	Genotype (frequency) rs1871054				Allele (%)		H-WE
	TT	TC	CC	TC+CC	T	C	
Case	26	57	69	126	35.9	64.1	/
Control	47	88	44	132	60.0	40.0	0.825
OR (95% CI)[a]	/	1.145 (0.630 to 2.084)	2.705 (1.449 to 5.051)	1.673 (1.108 to 2.903)	/	1.802 (1.308 to 2.483)	
P[a]	/	n. s	0.002	0.037	/	< 0.0001	

[a]ORs and 95% CIs were estimated using multiple logistic regression analyses and adjusted for age, gender and BMI.

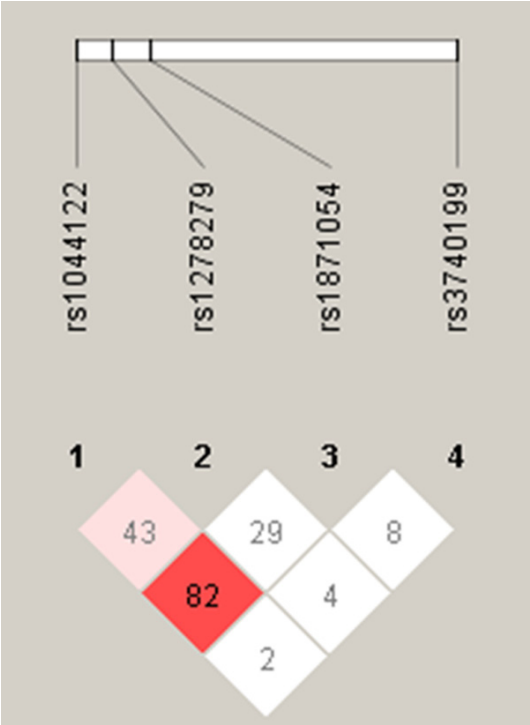

Figure 1. Linkage disequilibrium (LD) across the ADAM12 gene. The results of LD mapping are generated using Haploview software. The values for D' between each SNP are presented in each box. Red/pink boxes (D' < 1, LOD#2), white boxes (D' < 1, LOD < 2), blue boxes (D'=1, LOD < 2), and bright red (D'=1, LOD#2).

Table 3. Genetic association of ADAM12 haplotype (rs3740199, rs1871054, rs1272278, and rs1044122) and OA

Haplotype	*P*
TATC	0.035
TATG	0.025
TACG	0.021
CATG	0.019
CGTC	0.011

developing OA. Approximately 46.7% of the OA patients had a K/L score of 3 or 4.

Association of ADAM12 polymorphisms with OA

As expected, the distribution of the genotypes of SNPs of ADAM12 gene conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. **Table 2** listed the genotyped and allele distributions of the 4 SNPs for the cases and controls. Only rs1871054 was found to be significantly associated with

Table 4. Overview of published studies on the relationship of the ADAM12 polymorphisms with knee osteoarthritis

	Ana M. Valdes (UK 2004)	Ana M. Valdes (UK 2006)	J. Rodriguez-Lopez M. Sc (UK 2009)	K. L. Limer (UK 2009)	I. Kerna (Estonia2009)	Min-Ho Shin (Korea 2012)	I. Kerna (Estonia 2013)
Rs3740199	+	-	-	-	+	-	-
Rs1044122	/	-	/	/	/	/	+
Rs1278279	/	-	/	/	/	/	-
Rs1871054	/	+	/	/	-	/	+

increased risk of OA (C vs. T, OR 1.802 (1.308 to 2.483), $P < 0.0001$) after adjustment of age, gender, and BMI. For other SNPs, no statistically significant associations with OA were found. The linkage disequilibrium (LD) within ADAM12 gene was only found between rs1044122 and rs1872054 showing that these two polymorphisms belong to one haploblock (**Figure 1**).

Association of ADAM12 haplotypes with OA

Haplotype analysis revealed that five haplotypes (TATC, TATG, TACG, CATG, CGTC) associated with the increased risk of OA (**Table 3**).

Discussion

The most important finding of this study was that the ADAM12 rs1871054 variant was found to be significantly associated with increased OA susceptibility in Chinese Han Population.

The genetic background is important determinants of OA. The association of ADAM12 (rs3740199, rs1044122, rs1278279, rs1871054) polymorphisms with OA has been investigated but no consensus have been achieved. Ana M. Valdes et al. firstly demonstrated that OA severity and progression have a multigenic and feature-specific nature with ADAM12 polymorphism (rs3740199) in 2004, UK [8]. In 2006, Ana M. Valdes et al reported another polymorphism (rs1871054) additionally [15]. However, the result could not be replicated during the following years no matter in UK or Korea [16, 19]. Recently, studies from Estonia found the ADAM12 polymorphism (rs3740199, rs1044122, and rs1871054) were associated with increased risk of OA [14, 20]. The results (**Table 4**) changed a lot from each other which may be due to various reasons like the differences in case ascertainment, genotyping difference, and differences in ethnicity. As a result, this study was performed to investigate the relationship between ADAM12 polymorphism and OA based on Chinese Han population.

OA is a complex disorder that involves the whole joint, encompassing, in addition, the cartilage and the synovial membrane, as well as the underlying bone, ligaments and muscles [21]. The matrix metalloproteinases, A disintegrin and metalloproteinase (ADAMs) is one of the main proteolytic enzymes that regulate extracellular matrix turnover in the cartilage [22]. Studies also showed the ADAM12 was up regulated in human OA cartilage [8, 23] which suggested the potential role of ADAM12 in cartilage injury. On the other hand, ADAM12 is reported to be related with synovial inflammation, which is regarded as a major factor associated with the risk of both progression of cartilage degradation and symptoms of diseases [24], as it is upregulated in the synovial tissue in OA patients both on mRNA and protein level [25]. Additionally, meltrin alpha (ADAM12) protein was shown to induce osteoclast formation, and this could be a possible link to bone remodeling in OA development [26]. At the same time, available data suggest that the ADAM12 protein is potentially associated with degradation of the insulin-like growth factor-binding protein 5 (IGFBP-5) in the cartilage [23]. Accordingly, the ADAM12 could be associated with both the chondrocyte proliferation and bone growth, through the release of bioavailable IGF I, which is an important growth factor.

The LD analysis of ADAM12 SNPs in this study demonstrated that two of them (rs1044122 and rs1871054) belong to the same haploblock. Rather weak LD among the other SNP indicates that in genetic risk assessment rs1278279, rs3740199, and one of variants belonging to haploblock must be evaluated separately.

Power analysis is one of the important steps in a genetic association study to identify candidate genes for disease susceptibility. To assess

the power of our study, we conducted power calculations using Quanto software with the following options: an unmatched case-control study design, a significance level of 0.05, a population risk of 30%, a C-allele frequency of 46.7% for rs3740199, a C-allele frequency of 42.4% for rs1044122, a G-allele frequency of 25.3% for rs1278279, a C-allele frequency of 64.1% for rs1871054, and an inheritance dominant mode. The power of our study from 4 SNPs varied from 80 to 95% as the minimum OR's increased from 1.3 to 1.35. Our study was adequately powered to detect an association. Nevertheless, a total of 331 subjects seem to be a relatively small number for detecting weak genetic associations. A larger population or a meta-analysis of published data may provide a better understanding of the potential contributing of this gene to OA risk.

In conclusion, our data demonstrated the ADAM12 rs1871054 variant was found to be significantly associated with increased OA susceptibility in Chinese Han Population.

Disclosure of conflict of interest

None.

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