

Original Article

Association of single nucleotide polymorphisms in IGFBP3 with susceptibility to knee osteoarthritis: a case-control study

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Abstract: Background: The role of IGFBP3 polymorphisms in knee osteoarthritis has not been researched yet; the present study aimed to investigate the association between the polymorphisms of IGFBP3 and the risk of knee OA susceptibility and severity. Method: The rs788748 and rs879966 polymorphisms were genotyped and compared in a population based cohort consisting of 336 OA subjects and 300 age and gender matched controls. Radiographs were obtained before surgery and graded using the Kellgren-Lawrence (KL) system. The association between SNPs and radiographic severity of knee OA was also assessed by linear regression, using K/L grade as outcome variables, and the genotypes as independent variables. Results: For the SNP rs788748, the A allele was found to be associated with a decreased risk of OA in terms of the frequency of allele comparison, extreme genotype comparison, and comparison between GA and GG. Similarly, the G allele of rs879966 was also found to be associated with decreased OA susceptibility in terms of the frequency of allele comparison, extreme genotype comparison, and comparison between TG and TT. Similarly, the decreased severity of OA were also found significantly associated with the A (rs788748, $P = 0.026$) and G (rs879966, $P = 0.006$) allele frequency. Conclusion: The present study demonstrated the SNPs rs788748 and rs879966 of IGFBP3 were associated with a decreased risk of OA development. The SNPs rs788748 and rs879966 of IGFBP3 were also associated with decreased severity of knee OA.

Keywords: IGFBP3, polymorphism, knee OA

Introduction

Osteoarthritis (OA) is one of causes responsible for loss of labor productivity among the mid-aged and the elderly, with the prevalence of 1.3%-11.1% on knee in China, and the prevalence of 19.4% in patients ≥ 60 years old. Despite the increase in molecular knowledge accrued during the past years, the exact pathogenesis of the destructive process remains unknown. Symptomatic OA is now widely accepted as a multifactorial pathology affecting the whole joint complex, including age, body mass index, hormonal and local biomechanical factors, among which the genetic factor plays an important role [1, 2]. The genetics of OA is complex and is not completely understood, genome-wide association scans (GWAS) are

now being employed as powerful, objective tools for mapping susceptibility loci of complex diseases including OA. And as a result, several studies have demonstrated the polymorphism in many genes contributed modestly to the risk for developing OA [3-6]. For example, a large scale Japanese GWAS has identified two single nucleotide polymorphisms (SNPs) mapping to the human leukocyte antigen (HLA) gene region to be strongly associated with knee OA [6]. And variants in three genes have also been reproducibly associated in European-descent populations with knee OA with $P < 5 \times 10^{-8}$, a variant in the promoter region of the growth differentiation factor 5 (GDF5) gene [7], a variant mapping to a gene cluster in chromosome 7 including the component of oligomeric golgi complex 5 (COG5) gene [8], and a variant in the MCF.2

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cell-line-derived transforming sequence-like (MCF2L) gene [9]. Recently, a meta-analysis of GWAS and functional investigation provided suggestive links between polymorphisms of insulin-like growth factor binding protein 3 (IGFBP3) and hip osteoarthritis [10]. As the similar mechanisms in developing hip OA and knee OA, the IGFBP3 polymorphisms may also be associated with knee OA susceptibility. IGFBP3 is one of six insulin-like growth factor binding proteins (IGFBPs) in humans, which is the most predominant binding protein [11, 12]. IGFBPs modulate IGF signaling by binding to the two ligands (IGF-I and IGF-II) that primarily signal through the type I IGF-I receptor (IGF-IR) [12]. IGFBP-3, which is located in the matrix and within chondrocytes in human cartilage, is increased among patients with OA [13, 14]. Taken together, these evidences suggest the possible role of IGFBP3 polymorphisms in knee osteoarthritis, however, no research has been performed upon this till now. The present case control study aimed to investigate the association between the polymorphisms of IGFBP3 and the risk of knee OA susceptibility and severity.

Method

This study included 336 osteoarthritis patients and 300 controls recruited from the Department of Orthopaedics at Xijing Hospital of the Fourth Military Medical University. Informed consent is in accordance with the study protocol, approved by the ethics committee of Xijing Hospital. The controls were age and gender matched healthy Han population selected by excluding from the diagnosis of osteoarthritis. Demographic characteristics (age, gender) and body mass index (BMI) were obtained from all the cases and controls. Individual features of patients with OA and controls were summarized in **Table 1**.

The diagnosis of OA was defined by the symptom, complete clinical evaluation, and radiologic findings, according to the American College of Rheumatology criteria [15]. Radiographs were obtained before surgery and graded using the Kellgren-Lawrence (KL) system according to the following criteria: grade 1 (doubtful narrowing of joint space and possible osteophytes), grade 2 (definite osteophytes and possible narrowing of joint space), grade 3 (moderate multiple osteophytes, definite narrowing of joint

space and some sclerosis and possible deformity of bone ends), grade 4 (large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends).

Inclusion criteria: 1) Any symptom and/or sign of OA. 2) Radiographic abnormalities according to KL grading [16-18] [definition KL > 2]. 3) No evidence for rheumatoid arthritis and other autoimmune diseases as well as chondrodysplasias, infection-induced OA and post-traumatic OA. 4) Informed consent obtained after being informed about the details of the study.

Genotyping

Whole blood samples (5 ml) were obtained from participants and DNA was extracted according to TIAN amp Blood DNA Kit (TIANGEN, Beijing) and stored at -20°C. Analysis of IGFBP-3 SNPs (rs788748 and rs879966) was performed using multiplex polymerase chain reaction (PCR) with an ABI premix (Applied Biosystems, USA). In each 25 µl reaction, 1 µl genomic DNA (100 ng/ml) was amplified by 1.25 U Taq DNA polymerase (Takara, Dalian, China) with 2 µl of 2.5 mM dNTPs and 0.5 µl of each primer. PCR products were analyzed on a 3% ethidium bromide added agarose gel, and photographs were taken under ultraviolet light transilluminator. Subsequently, PCR product was sequenced in an ABI PRISM3100 sequencer using Big Dye Terminator v3.1 Cycle Sequencing method (Applied Biosystems, USA) as recommended by the manufacturer. Candidate SNP regions were detected and typed with the aid of DNA Star Software (DNASTAR, Madison, WI, USA).

Statistical analysis

Statistical calculations were performed using the SPSS Statistics 13.0 (SPSS Inc., Chicago, Ill). Frequency and susceptibility to OA associated with each mutation were compared using the χ^2 test. Two-tailed analyses were performed, and *P* values less than 0.05 were considered statistically significant. Hardy-Weinberg equilibrium (HWE) was checked for IGFBP-3 SNPs in OA and control subjects by Fisher's exact test. The crude and adjusted odds ratio (OR) and the corresponding 95% confidence intervals (CI) were calculated using unconditional multiple logistic regression. The association between SNPs and radiographic severity of knee OA was

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Table 1. The summary of the basic characteristics of the groups

	Case	Control
Number	300	336
Age	60.4 ± 8.2	59.3 ± 7.2
Gender (female/male)	184/116	181/155
BMI (kg/m ²)	26.2 ± 5.3	24.4 ± 6.1
K/L score		336
2	164	
3	95	
4	41	

also assessed by linear regression using as outcome variables, the K/L grade, and as independent variables the genotype.

Result

Characteristics of study subjects

Demographic variables and baseline characteristics of patients are given in **Table 1**. The mean age was (60.4 ± 8.2) years old in study group, and (59.3 ± 7.2) years old in control group, respectively. There were 184 (61.3%) female and 116 (38.7%) male patients, while in controls, there were 181 (53.9%) females and 155 (46.1%) males, respectively. No significant differences between groups in terms of age and gender. Similarly, the mean body mass index (BMI) was also comparable between the cases (26.2 ± 5.3) and the controls (24.4 ± 6.1).

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As expected, the distribution of the genotype of SNP rs788748 and rs879966 conformed to the Hardy-Weinberg equilibrium and the genotyping success rate was 100%. The individual association between the polymorphisms and risk of knee OA after adjusting for age, sex and BMI were analyzed and listed in **Tables 2** and **3**. For the SNP rs788748, the A allele was found associated with a decreased risk of OA in terms of the frequency of allele comparison (A vs. G: adjusted OR = 0.72; 95% CI = 0.55 to 0.88, P = 0.0127), extreme genotype comparison (AA vs. GG, adjusted OR = 0.51, 95% CI = 0.29 to 0.82, P = 0.0195), and comparison between GA and GG (adjusted OR = 0.68; 95% CI = 0.44 to 0.95, P = 0.0489). Similarly, the G allele of rs879966 was also found associated with decreased OA

susceptibility in terms of the frequency of allele comparison (G vs. T: adjusted OR = 0.74; 95% CI = 0.58 to 0.92, P = 0.0395), extreme genotype comparison (GG vs. TT, adjusted OR = 0.50, 95% CI = 0.30 to 0.78, P = 0.0228), and comparison between TG and TT (adjusted OR = 0.55; 95% CI = 0.38 to 0.76, P = 0.0013).

Association between IGFBP3 Polymorphisms and osteoarthritis severity

Also, the genotype frequency of IGFBP3 SNPs rs788748 and rs879966 were analyzed according to the KL grade in the cases. The decreased severity of OA were also found significantly associated with the A (rs788748, P = 0.026) and G (rs879966, P = 0.006) allele frequency.

Discussion

In order to evaluate the role of IGFBP3 gene in osteoarthritis' pathogenesis, we performed a genetic association study using a cohort of 336 OA patients and 300 healthy subjects. We observed that the SNPs rs788748 and rs879966 of IGFBP3 were found associated with a decreased risk of OA development.

Osteoarthritis is the most common form of arthritis affecting 40% of people over the age of 70 years, and is associated with a large health economic burden [19]. There is accumulating evidence linking OA pathogenesis and genetic factors. Genes act through a complex web of mechanisms involving injury and its avoidance; response to injury; body weight; muscle mass; and bone structure and bone turnover or cartilage structure and cartilage turnover or, synergistically, the two together [20, 21]. Although the exact etiology of OA has not been understood yet, the high expression of some biochemical markers, like IGF-1 and insulin-like growth factor binding proteins (IGFBPs) may contribute to OA pathophysiology, diagnosis, and progression [22-24]. The protein normalized IGF-1 and IGFBP-3 were found increased in synovial fluid of OA patients [25]. Higher levels of IGFBPs, in particular IGFBP3, have been observed in OA cartilage, leading to the notion that IGFBPs could decrease IGF-I's bioavailability and could at least be partially responsible for the reduced responsiveness of OA cartilage to IGF-I [26, 27]. IGF-I activates anabolic processes and inhibits catabolism of cartilage, however, IGF-I's anabolic activity is greatly

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Table 2. The individual association between the polymorphism rs788748 and risk of knee OA

Genotype	Controls (n = 336)	Cases (n = 300)	P value	Crude OR (95% CI)	Adjusted OR (95% CI)
rs788748					
GG	110	126			
GA	177	144	0.0489	0.71 (0.51, 1.00)	0.68 (0.44, 0.95)
AA	49	30	0.0195	0.53 (0.32, 0.90)	0.51 (0.29, 0.82)
G allele	397 (59.1%)	396 (66.0%)			
A allele	275 (40.9%)	204 (34.0%)	0.0127	0.74 (0.59, 0.93)	0.72 (0.55, 0.88)

Table 3. The individual association between the polymorphism rs879966 and risk of knee OA

Genotype	Controls (n = 336)	Cases (n = 300)	P value	Crude OR (95% CI)	Adjusted OR (95% CI)
rs879966					
TT	120	149			
TG	173	123	0.0013	0.57 (0.41, 0.80)	0.55 (0.38, 0.76)
GG	43	28	0.0228	0.52 (0.31, 0.89)	0.50 (0.30, 0.78)
T allele	413 (61.5%)	421 (70.2%)			
G allele	227 (38.5%)	179 (29.8%)	0.0395	0.77 (0.61, 0.98)	0.74 (0.58, 0.92)

diminished in OA cartilage, despite the presence of its receptor (IGF-IR) [28]. Serum concentrations of IGF-1 and IGFBP-3 are mainly determined by genetic factors [29]. IGFBPs modulate IGF signalling by binding to the two ligands (IGF-I and IGF-II) that primarily signal through the type I IGF-I receptor (IGF-IR) [12]. IGFBP3 is one of six insulin-like growth factor binding proteins (IGFBPs) in humans, which have higher IGF binding affinity than IGF receptors, regulate the bioavailability of IGF-1 [30].

A recent meta-analysis demonstrated the rs788748 and rs879966 of IGFBP3 were associated with decreased susceptibility of hip OA [10]. The genetic variant allele associated with lower odds of HOA was also associated with lower levels of circulating IGFBP3, and experimental knockdown of IGFBP3 prevented chondrocyte hypertrophic differentiation, a deleterious event in OA pathogenesis that results in cartilage loss. Moreover, IGFBP3 overexpression in articular cartilage from OA patients increased expression of genes associated with cartilage catabolism, and IGFBP3 overexpression in a cellular model of osteogenic differentiation resulted in an increase in matrix mineralisation, consistent with an activation of osteoblastic differentiation [10]. A number of factors that regulate IGFBP3 expression have been identified, but IGFBP3 transcriptional

enhancers that function in chondrocytes are not well characterized. HMGA1 binds to several sites 2 kb upstream of the IGFBP3 promoter and is required for IGFBP3 expression in chondrocytes, but DNA near rs788748 and rs879966 has not been experimentally tested for enhancer activity. HMGA1 binding data was not included in the ENCODE project, but there is evidence that C/EBP β , HMGA1's binding partner, binds DNA near rs788748. Functional studies of this genomic region could help to elucidate the dynamic role IGFBP3 plays in OA development [10].

Conclusion

The present study demonstrated the SNPs rs788748 and rs879966 of IGFBP3 were associated with a decreased risk of OA development. The SNPs rs788748 and rs879966 of IGFBP3 were also associated with decreased severity of knee OA.

Disclosure of conflict of interest

None.

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