Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2012, Article ID 597637, 5 pages
doi:10.1155/2012/597637

Research Article

The Effect of Bradykinin B2 Receptor Polymorphisms on the Susceptibility and Severity of Osteoarthritis in a Chinese Cohort

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Received 13 April 2012; Accepted 12 June 2012

Academic Editor: Thomas Liehr

Background. The B2-bradykinin receptor (BDKRB2) has been reported to associate with onset and development of Osteoarthritis (OA); however, the role of BDKRB2 genetic polymorphisms in OA remains unknown. *Method*. A total of 245 patients with primary knee OA and 264 healthy volunteer were recruited. BDKRB2 gene polymorphisms, −58T/C and +9/−9 bp polymorphisms, were genotyped. *Results*. The genotype distributions and allele frequencies of +9/−9 bp polymorphisms significantly differed between OA and control subjects. Logistic regression analysis showed carriers with −9/−9 genotype had a significantly increased risk for knee OA compared with the +9/+9 genotype (adjusted OR = 2.356, $P < 0.001$). The OR for −9 allele carriage was significantly higher than +9 allele carriage (adjusted OR = 1.52, $P < 0.001$). The +9/−9 bp polymorphisms also determined the OA radiographic severity. The presence of −9 bp was associated with severer OA. The −58T/C polymorphisms did not affect OA risk and severity. *Conclusion*. The +9/−9 bp polymorphisms of BDKRB2 gene may be used as a genetic marker for the susceptibility and severity of OA.

1. Introduction

Osteoarthritis (OA) is a degenerative joint disease that progressively causes loss of joint function and is a major source of physical disability and impaired quality of life in many countries [1–3]. The pathological changes which occur during OA involve all the joint structures, that is, synovium, cartilage, and bone tissues, but the main hallmark of this disease is the degradation of cartilage [4, 5]. The etiology of OA is largely unknown. Aging, trauma, hormonal, and mechanical factors are reported to contribute to the onset and progression of OA [6–8]. In addition, several studies have demonstrated the polymorphisms of some genes may be related to the pathogenesis of OA as well [9–11].

It is now accepted that the excessive and spontaneous inflammation plays a significant role in the molecular pathogenesis of OA, contributing to a highly catabolic state, chondrocyte apoptosis, and the resultant progressive degeneration of articular cartilage [12–14]. Bradykinins, a family of oligopeptides derived from the enzymatic action of kallikreins on kininogens, can promote all the major signs of inflammation, including hyperemia, leakage of plasma proteins, and pain [15–17]. The presence of BK was previously reported in the synovial fluid from patients affected by arthritis of different etiologies, including OA [18, 19]. B_2-bradykinin receptor (BDKRB2) mediates most of the inflammatory actions of bradykinin [20]. B_2-bradykinin receptor is widely present in most tissues, including joint tissues. BDKRB2 has been detected on the synovial lining cells, fibroblasts, and endothelial lining cells of blood vessels from OA patients [18, 21]. Clinically, the administration of B_2 receptor antagonists effectively reduced the inflammatory articular pain and knee OA progression, suggesting the BDKRB2 is involved in the development of OA [18, 22].

The genetic variants of BDKRB2 may lead to altered biological activities of the functional protein. The gene polymorphisms of BDKRB2 have been shown to be related with ACEI-induced cough in hypertensive patients, left ventricular hypertrophy, and insulin resistance [23–26]. However, its relation with OA remains unknown. In this study, we enrolled knee OA patients to explore the role of BDKRB2 in OA.

Table 1: The demographic and clinical characteristics of all subjects.

Variables	Cases ($n = 245$)	Control ($n = 264$)	P
Age (years)	58.6 ± 7.1	58.7 ± 6.4	NS
Sex			
Female	148 (60.4%)	155 (58.7%)	NS
Obesity	132 (53.9%)	116 (43.9%)	0.013
Smoker (%)	99 (40.4%)	98 (37.1%)	0.054
History of heavy labor work (%)	65 (26.5%)	61 (23.1%)	0.051

2. Methods

2.1. Patients. A total of 245 patients with primary knee OA were recruited from Dec 2008 to Feb 2009. The diagnosis of knee OA was based on the American College of Rheumatology criteria [27]. The severity of OA was evaluated according to the Kellgren-Lawrence (KL) grade classification, and only patients with K/L grades of 2 or higher were included. 264 healthy volunteers were enrolled as controls. Both OA and control groups were interviewed to obtain demographic data and all of established risk factors. In the study, other etiologies causing knee diseases such as inflammatory arthritis (rheumatoid, polyarthritic, or autoimmune disease), posttraumatic or postseptic arthritis, skeletal dysplasia, or developmental dysplasia were excluded from OA group. All the control never had any signs or symptoms of arthritis or joint diseases (pain, swelling, tenderness, or restriction of movement). The clinical characteristics of all enrolled subjects, including age, sex, body mass index (BMI), smoke status, bone fracture history, knee activity, and regular excise, were recorded. The study was approved by the ethics review committee of our hospital, and written informed consent was obtained from all participants.

2.2. BDKRB2 Genotyping. Reaction conditions for genotyping the two polymorphic loci (+9/−9 and C −58T) were as follows: DNA (100 ng) was amplified in a 25 μL reaction buffer containing 0.2 mmol/L deoxynucleotide triphosphates, 1.0 mmol/L $MgCl_2$, 20 mmol/L Tris/Cl (pH = 8.4), 50 mmol/L KCl, 0.015 nmol of each primer, and 0.5 U *Taq* polymerase (Invitrogen Corporation, Carlsbad, CA, USA) for 40 cycles of one minute at 94°C, 30 s at 60°C (+9/−9) or 57°C (C −58T), and 10 s at 72°C, followed by a five-minute soak at 72°C in a G-Storm Thermal Cycler (AlphaMetrix Biotech GmbH, Rödermark, Germany). The primers for the *BDKRB2* +9/−9 polymorphism were as follows: forward, 5′-TCCAGCTCTGGCTTCTGG-3′, and reverse, 5′-AGTCGCTCCCTGGTACTGC-3′, and the amplification products were 80 bp (−9) versus 89 bp (+9) [26]. The *BDKRB2* C −58T polymorphism was assayed using a pair of degenerate primers which were as follows: forward, 5′-AAGGTGGCCGCAGCCTTCC-3′, and reverse, 5′-CTCATCTTTCAAGGGCTGGCTA-3′. The reverse primer contained a G-C transversion (underlined) that generated a recognition site (5′-CTAG-3′) for the restriction endonuclease *Bfa*I (New England Biolabs, Ipswich, MA, USA) in the presence of the C allele. If the C allele was present, the 133 bp PCR product digested to 112 + 21 bp. All PCR or digestion products were size-separated by electrophoresis in 8% polyacrylamide gel electrophoresis gels run in 1 × trisborate-EDTA buffer and visualized with ethidium bromide and ultraviolet light. Allele and genotype frequencies were compared by χ^2 analysis. When observed or expected values included a cell with a value less than 5, Fisher's exact test was used. In all cases, significance was accepted at $P < 0.05$ [28].

2.3. Statistical Analyses. χ^2 or Fisher tests were used to compare genotype frequency and demographic distributions between cases and controls. Multiple logistic regression analyses were used to evaluate if each SNP was independently associated with OA when adjusted for the potential confounding effects of important clinical variables. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated. All analyses were performed by using SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA).

3. Results

Table 1 shows demographic and clinical characteristics of all subjects in the study. There were no significant differences in sex, age, smoke status, and history of heavy labor work between knee OA cases and controls. Obesity prevalence was significantly higher in the OA patient group than in controls ($P = 0.013$).

Table 2 described the genotype distributions and allele frequencies of BDKRB2 polymorphisms in knee OA and control subjects. The genotype frequencies for all polymorphisms did not differ significantly from those expected under Hardy-Weinberg equilibrium (both $P > 0.05$). The genotype frequencies and allele frequencies at BDKRB2 −58T/C were similar between OA and control subjects. The −9/−9 genotype was significantly higher in knee OA subjects than in controls (33.46% versus 20.07%). Accordingly, the −9 allele frequency was higher in OA patients than controls (57.754% versus 47.34%, $P < 0.001$). Logistic regression analysis showed a significantly increased risk for knee OA for the −9/−9 genotype compared with the +9/+9 genotype (OR = 2.35, 95% CI: 1.409–3.937; $P < 0.001$) after adjustment with sex, age, BMI, smoke status, history of labor work, regular exercise, and knee activity. The adjusted OR for −9 allele carriage was significantly higher than −9 allele carriage (OR = 1.52, 95% CI: 1.186–1.947, $P < 0.001$).

Table 2: The genotype distributions and allele frequencies of BDKRB2 polymorphisms in knee OA and control subjects.

		OA	%	Control	%	OR	95% CI		χ^2	P
+9/−9	+9/+9	44	17.96%	67	25.38%	1				
	+9/−9	119	48.57%	144	54.55%	1.258	0.801	1.976	0.998	0.318
	−9/−9	82	33.47%	53	20.08%	2.356	1.409	3.938	10.856	<0.001
	+9	207	42.24%	278	52.65%	1.000				
	−9	283	57.76%	250	47.35%	1.520	1.187	1.947	11.034	<0.001
−58T/C	TT	80	32.65%	64	24.24%	1.000				
	TC	102	41.63%	121	45.83%	0.674	0.443	1.028	3.372	0.066
	CC	63	25.71%	79	29.92%	0.638	0.400	1.017	3.581	0.058
	T	262	53.47%	249	47.16%	1.000				
	C	228	46.53%	279	52.84%	0.777	0.607	0.994	4.048	0.044

Table 3: The BDKRB2 polymorphisms and the radiographic severity of OA patients.

		OA (KL ≤ 3)	%	OA (KL > 3)	%	OR	95% CI		χ^2	P
+9/−9	+9/+9	35	23.18%	45	47.87%	1				
	+9/−9	72	47.68%	30	31.91%	3.09	1.67	5.7	13.33	<0.001
	−9/−9	44	29.14%	19	20.21%	2.98	1.48	5.97	9.7	0.002
	+9	142	47.02%	120	63.83%	1				
	−9	160	52.98%	68	36.17%	1.99	1.37	2.89	13.16	<0.001
−58T/C	TT	29	19.21%	15	15.96%	1				
	TC	67	44.37%	52	55.32%	0.67	0.32	1.37	1.22	0.268
	CC	55	36.42%	27	28.72%	1.05	0.49	2.29	0.02	0.895
	T	125	41.39%	82	43.62%	1				
	C	177	58.61%	106	56.38%	1.1	0.76	1.58	0.24	0.628

We further analyzed the genotype and the radiographic severity of OA patients. All the OA patients were grouped into two subgroups: subjects with KL ≤ 3 and those with KL score > 3 (Table 3). We found that the +9/+9 genotype was higher in those with KL score > 3 than in those with KL score < 3. The +9/−9 and −9/−9 genotypes represented higher risks of being severer OA (OR = 3.09, $P < 0.001$, and OR = 2.98, $P = 0.002$, resp.). The −9 carriage showed higher risk for severer OA (OA = 1.99. $P < 0.001$).

4. Discussion

Osteoarthritis (OA) is a painful and degenerating progressive disease of the joints which affects millions of patients worldwide. In this study, we investigated whether BDKRB2 gene polymorphisms influence the susceptibility of OA in a Chinese cohort. Our results showed that the −9/−9 carriers had markedly higher risk for OA compared with +9/+9 carriers. Besides, the +9/−9 and −9/−9 genotypes represented higher risks of being severer OA than +9/+9 carriers. To the best of our knowledge, this is the first study regarding the role of BDKRB2 gene polymorphisms in OA.

BDKRB2 is a vasodilator and inflammatory nonapeptide which is generated in OA synovium. It contributes to the initiation and maintenance of inflammation, to exciting and sensitizing sensory nerve fibres, thus producing pain, and to activating synoviocytes and chondrocytes which are the main cells involved in the homeostasis of synovial fluid and cartilage, respectively [29, 30]. Moreover, BDKRB2 synergistically potentiates the effects produced by proinflammatory cytokines. The BDKRB2 is constitutively expressed in most tissues and is considered a stronger mediator of vasodilation and inflammation through increased production and release of nitric oxide [31, 32].

In humans the BDKRB2 gene has been mapped to chromosome 14q32 [33]. The BDKRB2 gene contains a number of polymorphic loci, including a nine-base insertion/deletion in the first exon of the gene (+9/−9, rs5810761) and C to T transition in the promoter region (C −58T, rs1799722) [34]. The 9 bp deletion (−9) in the gene encoding the BDKRB2 is associated with expression of higher concentrations of receptor mRNA, suggesting its strong functional relevance [35]. The +9/−9 genetic polymorphisms have been reported to be associated with a series of pathological conditions including coronary artery disease, systemic hypertension, and increased left ventricular mass associated with hypertension and pulmonary artery pressure [36–38]. To our surprise, although the role of BDKRB2 in inflammation has been documented, we did not find any reports with regard to the genetic polymorphisms of BDKRB2 gene and inflammation. In this study, we firstly reported the role of genetic polymorphisms of BDKRB2 in OA. We found that the ±9 p polymorphisms, rather than the −58T/C polymorphisms, are not only associated with the OA risk but also the OA severity. This finding suggests that the BDKRB2 +9/−9 polymorphisms may be used as a genetic marker for

the onset and development of OA. However, it should be pointed out that our study is preliminary, and the results need to be further confirmed in larger-scale study, ideally, in different ethnic populations.

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