Association Between Single Nucleotide Polymorphisms of Asporin (ASPN) and BMP5 with the Risk of Knee Osteoarthritis in a Chinese Han Population

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Abstract The aim of this study was to investigate associations between single nucleotide polymorphisms rs13301537 in asporin (ASPN) and rs373444 in the bone morphogenetic protein 5 (BMP5) gene with knee osteoarthritis (OA) susceptibility in a Chinese Han population. ASPN rs13301537 and BMP5 rs373444 polymorphisms were genotyped in patients with knee OA and age- and sex-matched OA-free controls from a Chinese Han population. A total of 510 patients with knee OA and 520 controls were enrolled in the study. CT and CC genotypes of rs13301537, and variant C, were associated with a significantly increased risk of knee OA. On stratification analysis, the association between the risk of OA and rs13301537 CT heterozygotes compared with TT homozygotes was stronger in females and those aged ≥65 years. In contrast, the CT and CC genotypes of rs373444 in BMP5 were not significantly associated with the risk of knee OA, even after further stratification analysis according to age or sex. Our results showed that ASPN rs13301537 T to C change and variant C genotype may contribute to knee OA risk in a Chinese Han population.

Keywords Asporin · Single nucleotide polymorphism · Knee osteoarthritis

Introduction

Osteoarthritis (OA) is a degenerative joint disorder characterized by degradation of cartilage and changes in subchondral bone in synovial joints such as hand, spine, knee, and hip, which leads to structural and functional failure [1, 2]. The knee is the commonly affected joint, and knee OA has an especially high prevalence in Asian population. In China, a survey from Beijing indicated the prevalence of symptomatic and radiographic knee OA in Chinese females aged 60 and over was 15.4 and 42.8 %, and in Chinese males was 5.6 and 21.5 %, respectively [3]. With the aging societies in both developing and developed countries, the trend of OA incidence is on rise worldwide. Therefore, a better understanding of the etiology of OA is much required for a more effective and targeted prevention.

It is generally believed that OA is a multifactorial disease involving both genetic and environmental factors [4–8]. The role of genetic factors influencing OA susceptibility is well documented, and several predisposing genes have been found [9, 10]. Among these genes are bone morphogenetic protein 5 (BMP5) [11, 12] and asporin (ASPN) [13, 14], all involved in TGF-β signaling pathway.

Asporin belongs to a family of small leucine-rich proteoglycans (SLRPs), which compose a major non-collagen component of the extracellular matrix. Functional studies showed that ASPN binds to TGF-β receptor and thereby inhibits TGF-β-induced gene expression [15]. Studies on the SNPs in ASPN have been focused on an aspartic acid (D)-repeat polymorphism in the gene-encoding ASPN; however, the result from different ethnic groups is controversial [16–20]. A recent study revealed that the minor allele of ASPN SNP rs13301537 was associated with hand OA progression [8]. It would be reasonable to postulate ASPN SNP rs13301537 may also related to the knee OA.
BMP5 is a member of the TGF-β superfamily of secreted proteins that involved in synovial joint development and joint tissue homeostasis. SNP rs3734444 was shown to mark allelic imbalanced expression of BMP5 in prostate cancer [21], but no research has been done to study the link between SNP rs3734444 and OA.

As both ASPN and BMP5 are involved in chondrogenesis and chondrocyte proliferation, and there was lack of study about the association between ASPN SNP rs13301537 or BMP5 SNP rs373444 and knee OA risk, we now investigate the possible correlation between SNPs within BMP5 and ASPN and knee OA risk in patients from a Chinese Han population.

**Methods**

**Patients**

In this hospital-based case–control study, 510 consecutive Han Chinese patients diagnosed with knee arthritis between March 2008 and May 2013 at the Department of Orthopaedics, Shaoxing People’s Hospital, Shaoxing, China, and Department of Orthopedics, The 306th Hospital of PLA, Beijing, China, were evaluated for inclusion. Radiographic features were assessed by two examiners blinded to the clinical information to give a global Kellgren–Lawrence (KL) score ranging between 0 and 4, as previously described [22]. Only patients with radiographic OA, defined as a KL score of ≥2, were included in the study. Other aetiologies of knee joint disease such as inflammatory arthritis (rheumatoid, polyarticular, or autoimmune disease), post-traumatic or postseptic arthritis, skeletal dysplasia, or developmental dysplasia were excluded.

Five hundred and twenty age- (±5 years) and sex-matched healthy volunteers were recruited from the same hospital during the same period. Selection criteria for the controls included no history of OA and a KL score <2 on radiographic examination. Age, sex, weight, height, and body mass index (BMI) were recorded for all study participants.

Written informed consent was provided by all participants, and the study protocol was approved by the Ethics Committee of the Shaoxing People’s Hospital, Shaoxing, China.

**Genotyping**

Venous blood samples were obtained from all study participants using ethylene diamine tetra-acetic acid anticoagulant (20 g/l). Genomic DNA was extracted immediately from 200 μl of venous blood using a QIAamp DNA Blood Mini Kit (QIAGEN, Valencia, CA) according to the manufacturer’s instructions and stored at −80 °C until analysis [23]. The SNPs rs13301537 in ASPN and rs3734444 in BMP5 were genotyped using the TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA). The polymerase chain reaction (PCR) was performed using 100 ng of genomic DNA, 0.2 μM of each primer, 0.1 μM of each probe, 200 μM of each deoxyribonucleotide, 3 mM MgCl2, and 1 U Platinum Taq DNA polymerase. PCR amplification was carried out using the 7900HT Fast Real-Time PCR System (Applied Biosystems) according to the manufacturer’s instructions, with an initial denaturation at 95 °C for 2 min, followed by 40 cycles of denaturation at 95 °C for 15 s and annealing at 65 °C for 30 s. Data analyses were performed using ABI PRISM 7900 Sequence Detection System software version 2.3 (Applied Biosystems). Approximately 5 % of the samples were randomly selected for repeated genotyping, for confirmation.

**Statistical Analyses**

The Hardy–Weinberg equilibrium was tested using a goodness-of-fit χ2-test to compare observed and expected genotype frequencies in controls. Differences in the distributions of demographic characteristics and rs13301537 and rs3734444 genotypes between patients with OA and controls were evaluated using the Wilcoxon’s test for analysis of age and the χ2-test for other parameters. Associations between the ASPN or BMP5 variants and OA risk were estimated by calculating the odds ratios (ORs) and 95 % confidence intervals (CIs) using both univariate and multivariate logistic regression analyses with adjustments for age, sex, and BMI. Two-sided tests were used for statistical analyses, and a P value <0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS® version 16.0 (SPSS Inc., Chicago, IL, USA). The statistical power of the study was calculated

| Table 1 Demographic characteristics of Chinese Han patients with osteoarthritis (OA) of the knee and controls |
|---------------------------------------------------|---------------------------------------------------|
| Characteristic                                    | Patients with OA (n = 510)                        |
|                                                  | Controls (n = 520)                                |
| Age (year)                                        | 62.9 ± 7.6                                       | 62.6 ± 8.4 |
| Weight (kg)                                       | 66.6 ± 8.5                                       | 65.2 ± 8.6 |
| Height (cm)                                       | 160.8 ± 7.2                                      | 159.6 ± 7.3|
| Body mass index (kg/cm²)                         | 25.8 ± 2.8                                       | 25.6 ± 3.2 |
| Data presented as mean ± SD                      |                                                   |

No statistically significant between-group differences (P ≥ 0.05, using Wilcoxon’s test for age and χ²-test for other parameters)
using Power and Sample Size Calculation software version 3.0 (Department of Biostatistics, Vanderbilt University, Nashville, TN, USA).

**Results**

A total of 1,030 Chinese Han patients with knee arthritis were evaluated. Of these, 510 had radiographic knee OA and were included in the study. In addition, 520 age- and sex-matched healthy controls were recruited. There were no significant differences in demographic characteristics between the two groups (Table 1).

The observed genotype frequencies for the rs13301537 in ASPN and rs373444 in BMP5 polymorphisms in the controls agreed with that expected according to the Hardy–Weinberg principle (data not shown). The results of repeat genotyping of randomly selected samples were 100% concordant.

The genotype and allele distributions of the rs13301537 and rs373444 polymorphisms in patients with knee OA and healthy controls are shown in Table 2. The genotype distribution for rs13301537 was significantly different between the two groups (P < 0.001). After adjustment for age, sex, and BMI, a significantly increased risk of knee OA was associated with the genotype CT of rs13301537 compared with the TT genotype. In addition, those bearing at least one C allele (CT + CC) had a significantly increased risk of knee OA compared with those without the T allele (TT). However, the CT and CC genotypes of rs373444 were not significantly associated with the risk of knee OA.

Stratification analysis was performed to evaluate the potential association of genetic variants of ASPN rs13301537 with knee OA risk in subgroups based on demographic characteristics. When stratified by age, both young (≤65 years) and old (>65 years) patients showed significant differences in genotype frequencies compared with controls (P < 0.001) (Table 3). When the association between C allele carriers and the risk of OA was evaluated using logistic regression analysis, CT heterozygotes carried a 1.84-fold increased risk of OA compared with TT homozygotes in the young patients group (Table 3). Similarly, in the old patients group, CT heterozygotes carried a 2.43-fold increased risk of OA compared with TT homozygotes (Table 3). When stratified by gender, both male and female patients showed significant differences in genotype frequencies between patients with OA and controls (P < 0.01) (Table 3). In addition, CT heterozygotes carried a 1.55- and 2.82-fold increased risk of knee OA in males and females, respectively, compared with TT homozygotes (Table 3).

In contrast, logistic regression analysis of BMP5 rs373444 genetic variants showed that the CT and CC genotypes were not significantly associated with knee OA susceptibility when stratified by age or sex (Table 4).

**Discussion**

In the present hospital-based case–control study, associations between the potentially functional SNPs rs13301537 in ASPN and rs373444 in BMP5 and the risk of knee OA were investigated in a Chinese Han population. The results showed that the SNP rs13301537 polymorphic CT genotype of ASPN and variant C may contribute to the risk of knee OA and that this risk was increased in older (i.e., >65 years of age) and female patients. To the best of our knowledge, this is the first report linking ASPN SNP rs13301537 with knee OA in a Chinese Han population.
There has been much evidence linking OA pathogenesis and TGF-β signaling pathways [24]. Functional variants in the ASPN gene, which encodes asporin, a negative regulator of TGF-β signaling, have been associated with OA. The SNPs in ASPN have been well documented. It was reported that an aspartic acid (D)-repeat polymorphism in the gene-encoding ASPN was associated with OA of knee and hip joints in Japanese [16]. However, Mustafa et al. [17] assessed the association in UK cases (hip or knee primary OA) and controls, but did not detect significant association. A Greek case–control study [18] indicated that the D13 allele decreased the risk of knee OA, but that the D14 allele did not increase risk. In a Spanish case–control study [19], comprising cases with hand OA or with hip or knee OA ascertained by total joint replacement, no significant associations were detected between the ASPN D-repeat and OA. In a Chinese case–control study [20], comprising cases with symptomatic and radiographically defined knee OA, significant association was detected between the D14 allele and OA ($P = 0.0013, \text{OR} = 2.04$).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with knee OA ($n = 510$)</th>
<th>Controls ($n = 520$)</th>
<th>CC versus TT</th>
<th>CT versus TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 65</td>
<td>250 109 (43.6) 137 (54.8) 4 (1.6)</td>
<td>220 129 (58.6) 88 (40.0) 3 (1.4)</td>
<td>1.58 95 % CI $a$</td>
<td>1.84 95 % CI $a$</td>
</tr>
<tr>
<td>&gt; 65</td>
<td>260 96 (36.9) 161 (61.9) 3 (1.2)</td>
<td>300 175 (58.3) 121 (40.3) 4 (1.4)</td>
<td>1.37 95 % CI $a$</td>
<td>2.43 95 % CI $a$</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>244 110 (45.1) 131 (53.7) 3 (1.2)</td>
<td>240 134 (55.8) 103 (42.9) 3 (1.2)</td>
<td>1.22 95 % CI $a$</td>
<td>1.55 95 % CI $a$</td>
</tr>
<tr>
<td>Female</td>
<td>266 95 (35.7) 167 (62.8) 4 (1.5)</td>
<td>280 170 (60.7) 106 (37.8) 4 (1.4)</td>
<td>1.79 95 % CI $a$</td>
<td>2.82 95 % CI $a$</td>
</tr>
</tbody>
</table>

Data presented as $n$ (%) of patients

$OR$ odds ratio, $CI$ confidence interval

$^a$ Adjusted for the other covariate presented in this table and for body mass index using a logistic regression model for each stratum

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with knee OA ($n = 510$)</th>
<th>Controls ($n = 520$)</th>
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<tr>
<td>Age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 65</td>
<td>250 145 (58.0) 85 (34.0) 20 (8.0)</td>
<td>220 120 (54.5) 88 (40.0) 12 (5.5)</td>
<td>1.38 95 % CI $a$</td>
<td>0.80 95 % CI $a$</td>
</tr>
<tr>
<td>&gt; 65</td>
<td>260 147 (56.5) 95 (36.5) 18 (6.9)</td>
<td>300 163 (54.3) 119 (39.7) 18 (6.0)</td>
<td>1.11 95 % CI $a$</td>
<td>0.89 95 % CI $a$</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>244 136 (55.7) 91 (37.3) 17 (7.0)</td>
<td>240 127 (52.9) 99 (41.2) 14 (5.9)</td>
<td>1.13 95 % CI $a$</td>
<td>0.86 95 % CI $a$</td>
</tr>
<tr>
<td>Female</td>
<td>266 156 (58.6) 89 (33.5) 21 (7.9)</td>
<td>280 156 (55.7) 108 (38.6) 16 (5.7)</td>
<td>1.31 95 % CI $a$</td>
<td>0.82 95 % CI $a$</td>
</tr>
</tbody>
</table>

Data presented as number of patients (%)
The minor allele of ASPN SNP rs13301537 was associated across different races. Only recently, a study revealed that repeat (D) allele of ASPN SNPs and showed variation

These previous studies mostly focused on the aspartic acid-repeat (D) allele of ASPN SNPs and showed variation across different races. Only recently, a study revealed that the minor allele of ASPN SNP rs13301537 was associated with hand OA progression over 6 years [8]. Result from the present study supports the hypothesis that the SNP rs13301537 of ASPN gene might be a risk factor for knee OA. In the present study, individuals carrying ASPN rs13301537 CT + CC or CT genotypes had a higher risk of knee OA than those carrying the TT genotype, suggesting that the rs13301537 C allele might be associated with the development of knee OA.

According to the stratification analysis undertaken in the present study, the association between the risk of knee OA and rs13301537 CT heterozygotes compared with TT homozygotes was stronger in female patients and those >65-year old. This is consistent with the increased incidence of knee OA reported in those >50 years of age and in females [25]. As OA is a complex and multifactorial disease, both gene–gene and gene–environment interactions may occur and a single genetic variant is unlikely to be sufficient to predict overall risk. Further research is therefore needed to elucidate the role of other functional SNPs of ASPN and other related genes involved in similar biological pathways that may be involved in the etiology of OA.

Our study shows that BMP5 rs373444 genetic variants, the CT and CC genotypes, were not significantly associated with knee OA susceptibility when stratified by age or sex. This result is in consistent with a recent study by Bijsterbosch et al. [8] on hand osteoarthritis in a Caucasian population.

The present study had a number of limitations. First, as a hospital-based study, it may be subject to inherent biases; however, the C allele frequency in the control subjects was similar to that in the haplotype map database, and the genotype distributions of the rs13301537 and rs373444 polymorphisms in the controls conformed to the Hardy–Weinberg equilibrium, suggesting that the results did not suffer from selection bias. Secondly, the sample size was relatively modest; therefore, the results should be confirmed in larger-scale studies. Thirdly, only two ASPN and BMP5 SNPs were investigated; SNPs at other loci may also be associated with susceptibility to OA.

In conclusion, the present study was the first to show that the genotype distribution of the ASPN rs13301537 polymorphism was significantly different between patients with knee OA and healthy controls in a Chinese Han population. Larger population-based and in-depth molecular studies are required, to validate these current findings and to elucidate the functional roles of the rs13301537 polymorphism in the etiology of OA.

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Conflict of Interest The authors declare that there are no conflict of interests.

References


