Influence of COL9A1 and COL19A1 Polymorphisms on Kaschin-Beck Disease Risk

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Abstract

Objective We aimed to determine whether COL9A1 and COL19A1 polymorphisms were associated with Kaschin-Beck disease (KBD) risk.

Methods Five single nucleotide polymorphisms (SNPs) in COL9A1 and COL19A1 were genotyped in 316 KBD patients and 320 healthy controls using the Agena MassARRAY platform. The association between genetic polymorphisms (COL9A1: rs3806093, rs603410 and rs621347; COL19A1: rs9346371 and rs555313) and KBD risk were assessed using logistic regression model by calculating odds ratio (OR) and 95% confidence interval (CI).

Results After adjustment with age and sex, the frequency distributions of genotypes in rs3806093 and rs9346371 were significantly different between cases and controls. COL9A1 rs3806093 significantly increased KBD risk in co-dominant (OR = 14.80, 95%CI = 1.42-154.80, p = 0.024) and recessive (OR = 16.39, 95%CI = 1.60-168.20, p = 0.019) models. Meanwhile, COL9A1 rs555313 was associated with KBD risk in recessive model (OR = 3.80, 95%CI = 1.01-14.27, p = 0.048). In addition, haplotype analysis revealed two blocks (block 1: rs3806093, rs603410 and rs621347; block 2: rs9346371 and rs555313).

Conclusion COL9A1 and COL19A1 polymorphisms were associated with KBD risk in the Chinese Han population, suggesting roles of COL9A1 and COL19A1 in the development of KBD.

Introduction

Kaschin-Beck disease (KBD) is a chronic osteochondropathy, characterized by cartilage degeneration, chondrocyte necrosis and apoptosis(1, 2). KBD mainly
distributed from southeastern Siberia to China. There are approximately 690,000 KBD patients and more than 10 million people are likely to suffer from KBD in China(3). The etiology of KBD remains unclear, studies suggest that KBD is a complex disease made by interactions between environmental factors and genetic factors(4). More than 40% of KBD risk could attribute to genetic components(3). Certain susceptibility genes may have effects on KBD risk.

Collagens are the most abundant proteins, the collagen family comprises 28 members (I-XXVIII)(5, 6). One of the three alpha chains of type IX collagen are coded by COL9A1 gene, which is essential for the functional longevity of joint cartilages and connected with osteochondropathy(7, 8). Studies in knockout mice have shown that lack of type IX collagen is associated with early onset osteoarthritis. Mutations in COL9A1 are associated with osteoarthritis, lumbar disc disease, and multiple epiphyseal dysplasia(9-11). Specially, it is reported that COL9A1 polymorphism (rs6910140) was significantly associated with KBD risk in a northwest Chinese Han population(12). COL19A1 encodes the alpha chain of type XIX collagen, a member of the fibril-associated collagens with interrupted helices (FACIT) collagen family. COL19A1 was localized to 6q12-q14, the same region of COL9A1 gene(13). Type XIX collagen was involved in the initial stages of skeletal muscle cell differentiation(14). Studies reported that Type XIX collagen may contribute to brain disorders(15), but no data on the relationship between COL19A1 polymorphisms and KBD risk.

In this study, we aimed to investigate whether polymorphisms of COL9A1 and COL19A1 affect the risk of KBD. We conducted a case-control study and focused on five polymorphisms (rs3806093, rs603410 and rs621347 of COL9A1; rs934631 and rs555313 of COL19A1) to assess the associations between genetic polymorphisms
and KBD risk.

Methods

Study subjects

A total of 636 Chinese Han individuals (316 KBD patients, 320 healthy controls) were recruited from Affiliated Hospital of Xizang Minzu University, Xianyang, China. According to the national diagnostic criteria of China (WS/T 207–2010), all KBD patients was diagnosed by two KBD experts. The exclusion criteria included people who had clinical symptoms or radiographic changes of other osteochondropathy. The healthy controls were randomly collected from disease-free individuals who had health examination in the Affiliated Hospital of Xizang Minzu University. This study was in the Declaration of Helsinki and approved by the Ethics Committee of Affiliated Hospital of Xizang Minzu University, and we obtained written informed consents from all study subjects before study.

Genotyping

Based on the data of the Han Chinese population in Beijing (CHB) from the 1000 Genomes project and previous studies(4), we selected three SNPs (rs3806093, rs603410 and rs621347) of COL9A1 gene and two SNPs (rs9346371 and rs555313) of COL19A1, with minor allele frequency (MAF) greater than 5%. Genomic DNA was extract from whole blood using blood DNA kit (GoldMag Co. Ltd., Xi’an, China) and was measured by Nanodrop 2000 (Thermo Scientific, Waltham, Massachusetts, USA). We genotyped five SNPs of COL9A1 and COL19A1 using Agena MassARRAY platform (Agena, San Diego, CA, USA). The Agena MassARRAY Assay Design 3.0 Software (San Diego, CA USA) was used to design the primers for each SNP (Table 1). Additionally, we managed and analyzed data by the Agena Typer 4.0 Software
(San Diego, CA, USA)(16).

Statistical analysis
Statistical analysis was conducted with SPSS 21.0 (IBM®, Armonk, New York, USA)(17). P < 0.05 was considered statistically significant. The Hardy-Weinberg equilibrium (HWE) of each SNP was assessed by Fisher’s exact test in the control group. Logistic regression analysis adjusted by age and sex was used to evaluate the association of COL9A1 and COL9A1 polymorphisms with KBD risk through calculating odds ratio (OR) and 95% confidence interval (CI). Allele model and genetic models (co-dominant, dominant, recessive and additive) were assessed by the chi square test and PLINK software. Then, haplotype analysis was performed by Haploview software (version 4.2) and PLINK software.

Results
Study subjects
A total of 316 cases (183 men and 133 women) and 320 controls (239 men and 81 women) were included in this study. The characteristics of study subjects are presented in Table 2. The mean ages of cases and controls were 54.70 ± 17.14 and 19.00 ± 1.60 years old, individually. There are significant differences in the distribution of age and sex between two groups.

Association of COL9A1 and COL9A1 polymorphisms with KBD risk
The loci information of five SNPs in COL9A1 and COL19A1 are shown in Table 3. All SNPs are in HWE and the MAFs in two groups are listed in this table. The association of COL9A1 and COL19A1 polymorphisms with KBD risk are presented in Table 4. The distribution frequencies of genotypes in COL9A1 rs3806093 and COL19A1 rs9346371 are significantly different between the two groups. Rs3806093 is significantly
associated with higher risk of KBD in co-dominant (OR = 14.80, 95%CI = 1.42–154.80, p = 0.024) and recessive (OR = 16.39, 95%CI = 1.60–168.20, p = 0.019) models. Individuals with TT genotype of rs9346371 are associated with increased KBD risk compared with TC-CC genotype (OR = 3.80, 95%CI = 1.01–14.27, p = 0.048). There are no significant associations between other SNPs and KBD risk (p > 0.05).

Haplotype analysis

We performed the haplotype analysis of COL9A1 and COL19A1 polymorphisms with KBD risk (Table 5). There are no significant relationship between COL9A1 and COL19A1 polymorphisms and KBD risk (p > 0.05). As shown in Figure 1 and Figure 2, we observed two blocks (block 1: rs3806093, rs603410 and rs621347; block 2: rs9346371 and rs555313).

Discussion

In this study, three SNPs in COL9A1 (rs3806093, rs603410 and rs621347) and two SNPs in COL19A1 (rs9346371 and rs555313) were included to explore the association of genetic polymorphisms and risk of KBD. The results showed that rs3806093 of COL9A1 and rs9346371 of COL19A1 were significantly associated with increased KBD risk.

KBD is an endemic multiple and deformed osteoarthropathy(18). The electron microscopic analysis have showed that a reduction in the collagen fibril diameter and a loss of the fibril banding patterns in the cartilage matrix among the KBD patients(19). Type IX collagen plays a vital role in the degradation of cartilage and bone(12). Several studies have revealed that genes polymorphisms encoding type IX collagen had relationship with osteoarthropathy risk(8, 20). Previous study
genotyped fifteen SNPs in COL9A1 and found rs6910140 of COL9A1 plays an important role in the risk and severity of KBD in the Chinese Han population(12). Our data confirmed the association between COL9A1 gene and KBD risk. In co-dominant and recessive models, rs3806093 of COL9A1 had a strong association with KBD susceptibility ($p < 0.05$). COL19A1 encodes type XIX collagen, which mainly expressed in central neurons and is necessary for hippocampal synapses formation(21). In this study, we assessed the association between COL19A1 polymorphisms and KBD risk. We observed that rs9346371 of COL19A1 was associated with the risk of KBD in recessive model. Our results suggest that polymorphisms of COL9A1 and COL19A1 may be involved in the development of KBD. However, further studies are needed to explore the exact mechanism of genetic polymorphisms in KBD.

Some limitations should also be considered. First, the relatively small sample size in this study. Second, we cannot do stratification analysis due to the lack of information on subjects. Third, many environmental factors and lifestyle could affect the susceptibility of KBD, we could not eliminate all factors in this study. Hence, larger sample size and well designed studies are required to validate the association of COL9A1 and COL19A1 polymorphisms with KBD risk.

Conclusion

In conclusion, we found polymorphisms of COL9A1 and COL19A1 were associated with the risk of KBD, suggesting the role of COL9A1 and COL19A1 polymorphisms in the development of KBD. Further studies are required to validate the influence of COL9A1 and COL19A1 polymorphisms on KBD risk.
Declarations

Ethics approval and consent to participate

This study was in the Declaration of Helsinki and approved by the Ethics Committee of Affiliated Hospital of Xizang Minzu University, and we obtained written informed consents from all study subjects before study.

Competing interests

The authors declare that they have no competing interests

Consent for publication

Not applicable.

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Authors contribution

XH drafted the manuscript. JWZ and YHW performed the DNA extraction and genotyping; LW and MB performed the data analysis; DYY and MB performed the sample collection and information recording; XH and TBJ conceived and supervised the study.

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Tables

Table 1 Primers used in the study

| Gene  | SNP          | 1st-PCRP | 2nd-PCRP |
|-------|--------------|----------|----------|
| COL9A1| rs3806093    | ACGTTGGATGAAAGCAGTCCAGTCTTTGAACAC | ACGTTGGATGTGAACCTCAGTCTTTGAACAC |
|       | rs603410     | ACGTTGGATGCCTTACTATGCTTTACTCT    | ACGTTGGATGGCAGTTAGCGGAGA          |
|       | rs621347     | ACGTTGGATGCCAGGACAAAGTCTTTGAC    | ACGTTGGATGTCAGTGTCTTTGAC          |
| COL19A1| rs9346371   | ACGTTGGATGCTTTCTATGCTTAATACAG    | ACGTTGGATGAAAGAATGCTTTGAC         |
|       | rs555313     | ACGTTGGATGCTTTATGCTTTATGCTTCAGC | ACGTTGGATGGGGTCTTTGAC             |

SNP, single nucleotide polymorphism; PCRP, polymerase chain reaction primer; UEP, unextended mini sequencing primer; DIR, direction; SEQ, sequence

Table 2 Characteristics of study subjects

| Characteristics | Cases (N = 316) | Controls (N = 320) | P value |
|-----------------|-----------------|--------------------|---------|
| Age             | 54.70 ± 17.14   | 19.00 ± 1.60       | < 0.001 |
| Sex             |                 |                    |         |
| Man             | 183 (57.91%)    | 239 (74.69%)       | < 0.001 |
| Woman           | 133 (42.09%)    | 81 (25.31%)        |         |

Table 3 The loci information of five SNPs in COL9A1 and COL19A1
| Gene     | SNP        | Chromosome position | Alleles | SNP location | MAF (cases) | MAF (controls) | HWE test (P) |
|----------|------------|---------------------|---------|--------------|-------------|----------------|---------------|
| COL9A1   | rs3806093  | 6: 70273226         | A/G     | intron       | 0.163       | 0.166          | 0.550         |
|          | rs603410   | 6: 70274945         | T/G     | intron       | 0.214       | 0.214          | 1.000         |
|          | rs621347   | 6: 70276646         | A/G     | intron       | 0.375       | 0.381          | 1.000         |
| COL19A1  | rs9346371  | 6: 70210157         | T/C     | 3'UTR        | 0.349       | 0.361          | 0.278         |
|          | rs555313   | 6: 70214317         | T/C     | 3'UTR        | 0.465       | 0.441          | 1.000         |

SNP, single nucleotide polymorphism; MAF, minor allele frequency; HWE, Hardy-Weinberg equilibrium

Table 4 Association of COL9A1 and COL19A1 polymorphisms with Kaschin-Beck disease risk

| SNP       | Genotype | Count (case) | Count (control) | Model   | OR (95%CI)   | P    |
|-----------|----------|--------------|-----------------|---------|--------------|------|
| rs3806093 | A/G      | 103/529      | 106/534         | Allele  | 0.98(0.73-1.32) | 0.899|
| AA        |          | 8            | 7               | Co-dominant | 14.80(1.42-154.80) | 0.024|
| AG        |          | 87           | 92              |          | 0.68(0.19-2.38)  | 0.542|
| GG        |          | 221          | 221             |          | 1             |      |
| AA-AG     |          | 95           | 99              | Dominant | 1.04(0.34-3.15) | 0.944|
| GG        |          | 221          | 221             |          | 1             |      |
| AA        |          | 8            | 7               | Recessive | 16.39(1.60-168.20) | 0.019|
| AG-GG     |          | 308          | 313             |          | 1             |      |
|           |          |              |                 | Additive | 1.51(0.59-3.87) | 0.386|
| rs603410  | T/G      | 135/497      | 135/497         | Allele  | 1.00(0.76-1.31) | 1.000|
|   |   |   |   |   |
|---|---|---|---|---|
| TT | 15 | 14 | Co-dominant | 0.41(0.01-11.54) | 0.599 |
| TG | 105 | 107 |   | 0.70(0.24-2.05) | 0.517 |
| GG | 196 | 195 |   | 1 |   |
| TT-TG | 120 | 121 | Dominant | 0.68(0.24-1.94) | 0.469 |
| GG | 196 | 195 |   | 1 |   |
| TT | 15 | 14 | Recessive | 0.47(0.02-12.43) | 0.653 |
| TG-GG | 301 | 302 |   | 1 |   |
| rs621747 | A/G | 237/395 | 244/396 | Allele | 0.97(0.78-1.22) | 0.818 |
| AA | 47 | 46 | Co-dominant | 1.37(0.35-5.35) | 0.649 |
| AG | 143 | 152 |   | 0.49(0.16-1.49) | 0.206 |
| GG | 126 | 122 |   | 1 |   |
| AA-AG | 190 | 198 | Dominant | 0.66(0.25-1.78) | 0.411 |
| GG | 126 | 122 |   | 1 |   |
| AA | 47 | 46 | Recessive | 1.93(0.54-6.88) | 0.313 |
|        | AG-GG  | T/C       | Allele | Additive    | P-value |
|--------|--------|-----------|--------|-------------|---------|
|        | 269    | 219/409   |        | 0.97(0.48-1.99) | 0.941   |
|        | 274    | 231/409   |        | 0.95(0.75-1.19) | 0.650   |
|        |        |           |        |             |         |
| rs9346371 | T/C   | 33        |        | 3.69(0.85-16.00) | 0.081   |
|        |        | 153       |        | 0.95(0.31-2.93) | 0.929   |
|        |        | 128       |        | 1           |         |
|        |        |        |        |             |         |
|        |        |           |        |             |         |
|        |        |           |        |             |         |
|        |        |           |        |             |         |
| rs555313 | T/C   | 74        |        | 0.30(0.06-1.56) | 0.152   |
|        |        | 146       |        | 0.48(0.15-1.50) | 0.205   |
|        |        | 96        |        | 1           |         |

**Allelic Frequencies:**

- **AG-GG:** 269 (33%), 274 (40%)
- **rs9346371 T/C:** 219/409 (33%), 231/409 (40%)
- **rs555313 T/C:** 294/338 (74%), 282/358 (62%)
| Haplotype   | Frequency in cases | Frequency in controls | Dominant   | OR (95% CI) | P   | Recessive | OR (95% CI) | P   | Additive | OR (95% CI) | P   |
|------------|--------------------|-----------------------|------------|-------------|-----|-----------|-------------|-----|----------|-------------|-----|
| TT-TC      | 210                | 220                   | Dominant   | 0.44(0.15-1.31) | 0.139 |           |             |     | 0.50(0.12-2.09) | 0.345 |     |
| CC         | 96                 | 100                   | 1          |             |     |           |             |     | 1        |             |     |
| TT         | 74                 | 62                    | Recessive  | 0.50(0.12-2.09) | 0.345 |           |             |     | 0.53(0.24-1.19) | 0.124 |     |
| TC-CC      | 242                | 258                   | 1          |             |     |           |             |     |          |             |     |

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval

Bold data means significant difference (p < 0.05)

Table 5 Haplotype analysis of COL9A1 and COL19A1 polymorphisms with Kaschin-Beck disease risk

| Gene | SNPs | Haplotype | Frequency in cases | Frequency in controls | Without adjustment | With adjustment |
|------|------|-----------|--------------------|-----------------------|--------------------|-----------------|
|      |      |           |                    |                       | OR (95% CI)        | P               | OR (95% CI) | P   |
| COL9A1 | rs38060 93| GTA | 0.212               | 0.216                 | 0.98(0.75-1.28)    | 0.876           | 0.66(0.26-1.69) | 0.392 |
|        | rs603 410| AGA | 0.163               | 0.166                 | 0.98(0.73-1.32)    | 0.897           | 1.51(0.59-3.87) | 0.386 |
|        | rs62 1347| GGG | 0.377               | 0.381                 | 0.98(0.78-1.23)    | 0.865           | 0.53(0.24-1.19) | 0.941 |
| COL19A1 | rs93463 71| CT  | 0.463               | 0.441                 | 1.09(0.8-1.36)     | 0.424           | 0.53(0.24-1.19) | 0.124 |
|        | rs555 313| TC  | 0.347               | 0.361                 | 0.94(0.74-1.19)    | 0.595           | 1.68(0.73-3.66) | 0.192 |
|        |         | CC  | 0.188               | 0.198                 | 0.94(0.71-1.23)    | 0.640           | 1.15(0.41-3.15) | 0.750 |

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval

Figures
Figure 1

Haplotype block map for the SNPs of COL9A1 Block includes rs3806093, rs603410
Haplotype block map for the SNPs of COL19A1 Block includes rs9346371 and rs555313.
