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Association of ADAMTS-5 gene polymorphisms with the susceptibility to knee osteoarthritis in a Chinese Han population

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Abstract

Background Osteoarthritis (OA) is the most prevalent type of arthritis and the main reason for progressive disability in middle-aged and older people. Studies of candidate genes may provide a novel insight and treatment strategy for knee osteoarthritis (KOA). The aim of this study was to investigate the relationship between KOA susceptibility and single-nucleotide polymorphism (SNP) of the ADAMTS-5 gene.

Materials and methods The case group included 188 patients from Luoyang Orthopedic Hospital with clinically and radiographically diagnosed primary KOA, and the control group included 100 age-matched individuals without KOA. Fifteen ADAMTS-5 SNPs were assayed using MALDI-TOF MS. Allelic and haplotypic frequencies were compared between the groups. The relationship between genotype distribution and risk of KOA was analyzed by multivariate logistic regression.

Results The frequency of A allele in rs2249350 site in the KOA group was significantly lower (odds ratio [OR]: 0.761; 95% confidence interval [95% CI]: 0.612–0.947; $P=0.016$), while that of C allele was higher than that in the control group (OR: 1.176; 95% CI: 1.025–1.351; $P=0.016$). AA genotype and gene model, especially recessive gene model at rs2249350 locus, negatively correlated with KOA risk after adjustment for sex, body mass index, age, and occupation (AA vs. CC: OR: 0.288; 95% CI: 0.124–0.669; $P=0.004$; AA vs. CA + CC: OR: 0.348; 95% CI: 0.162–0.749; $P=0.007$). Meanwhile, one protective haplotype, GA (rs229054, rs2249350) (OR: 0.763; 95% CI: 0.614–0.949; $P=0.017$), and one high-risk haplotype, GC (rs229054, rs2249350) (OR: 1.259; 95% CI: 1.032–1.537; $P=0.019$), were found in this study.

Conclusion Despite a limited sample size, our study suggests that the rs2249350 polymorphism in the ADAMTS-5 gene is one of the genetic factors influencing the risk of KOA. The A allele and AA genotype of rs2249350 may protect from KOA, whereas C allele and CC genotype increase the risk of KOA. In addition, the GA haplotype (rs229054, rs2249350) might be associated with a decreased risk of KOA, whereas the GC haplotype (rs229054, rs2249350) may

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be a risk factor for KOA. Additional larger-sized studies in more ethnically diverse populations are needed to confirm these findings.

Keywords ADAMTS-5, Gene, Single nucleotide polymorphism, Association, Aggrecan, Knee osteoarthritis

Background

Osteoarthritis (OA) is the most prevalent type of arthritis and the main reason for the progressive disability in middle-aged and older people [1]. According to the World Health Organization (WHO), approximately 10% of the world's population aged ≥ 60 years suffer from OA, which is characterized by joint pain, stiffness, and limited movement [2–4]. It is generally believed that OA is a result of the interaction of multiple intra-articular pathological changes, including variable degrees of synovitis; softening, ulceration, and fibrillation of articular cartilage; sclerosis of subchondral bone, subchondral cysts, and osteophytes [5, 6]. Although OA can develop in all joints, it most often occurs in the knee joint—the largest and the most complex joint. Knee osteoarthritis (KOA) is one of the most common reasons for total knee joint replacement [7]. KOA is a multifactorial disease and can be caused by a variety of factors, such as aging, obesity, inflammation, joint trauma, and overload physical activity, such as frequent walking up and down stairs, prolonged bending and loading, and sedentary work or driving [8]. In addition to these factors, genetic factors account for 50% of the risk of OA development, and prior research suggests that OA is primarily influenced by population polymorphisms in multiple genes [9–12]. Therefore, studies of candidate genes may provide a novel insight and treatment strategy for KOA [13]. It has been reported that the onset and development of KOA may be influenced by a variety of genes, including interleukin family (IL-1, IL-6, IL-16, IL-17) [14, 15], tumor necrosis factor alpha (TNF-α) [16], collagen (Col2α1, Col9α1, Col11α1) [17], transforming growth factor beta (TFG-β) [18], Smad3 [19], growth/differentiation factor 5 (GDF-5) [20, 21], iodothyronine deiodinase type II (DIO2) [22], secreted frizzled-related protein 3 (Sfrp3) [23], and others.

Aggrecanases are regarded as the most critical enzymes for degradation of cartilage extracellular matrix (ECM). They degrade aggrecan (an extremely important component of the cartilage ECM) [24] by cleaving it at Glu373–Ala374 in the interglobular domain (IGD) [25]. Aggrecanase-1 (a disinterring and metalloproteinase with thrombospondin-like motifs, ADAMTS-4) and aggrecanase-2 (ADAMTS-5) have been suggested as the critical enzymes for the degradation of cartilage and development of OA [24, 26], because they have demonstrated the most effective ability to degrade aggrecan in vitro [27, 28]. More importantly, mice deficient in ADAMTS-5 are protected from early aggrecan loss and cartilage erosion in models of arthritis [29]. In other words, ADAMTS-5 exhibits better biological activity than ADAMTS-4 in vivo, at least in mouse arthritis models [29, 30]. Therefore, the ADAMTS-5 gene was considered as a candidate gene in this study, and we speculated that the onset and development of KOA might be related to the ADAMTS-5 gene.

There have been several studies on the association of ADAMTS-5 single-nucleotide polymorphisms (SNPs) with OA or KOA [31–33]; however, the studies had some limitations and their conclusions were inconsistent. Rodriguez-Lopez et al. collected a sufficient number of cases with OA to analyze the association of SNPs (especially rs229054 and rs2249350) with OA; however, the results were unpromising, that is, none of the SNPs or haplotypes showed a significant association with the susceptibility to OA. Canbek et al. also examined the association of rs229054 and rs2249350 with KOA, but did not find a significant correlation between them. However, the small sample size of that study may have affected the accuracy of the results. In addition, Gu et al. reported that the T allele carrier in rs2830585 could be a protective factor against OA, especially cervical OA [32], while Zhou et al. found that the T allele carrier in rs2830585 was associated with a significantly increased risk of KOA [34]. Hence, the current association studies of ADAMTS5 with OA involve fewer SNP loci, mainly rs229054 and rs2249350, and the conclusions of the studies appear to be inconsistent, or even opposite. Therefore, in this study, we increased the number of SNP loci and explored the association between ADAMTS-5 SNPs and KOA in a Chinese Han population, so as to provide clinical research ideas and data for the association between the susceptibility to KOA and genetic factors.

Materials and methods

Ethical statements

The study was approved by the Ethics Committee of Luoyang Orthopedic Hospital. Written informed consent was obtained from all participants prior to the study, and all of the participants agreed to provide their information and to donate blood samples. Furthermore, they agreed to the publication of the results of this study. All procedures of this study were in accordance with the Declaration of Helsinki [35].

Study population and case selection

A hospital-based case–control design was used in this study. The case group encompassed 188 patients (45–75

years old, 79 males and 109 females) with primary KOA from Luoyang Orthopedic Hospital between January 2016 and December 2017. Primary KOA was diagnosed on the basis of the KOA standards established by the Association of Rheumatology Health Professionals (ARHP) [36], including the presence of knee joint pain, radiographic signs of OA according to the Kellgren–Lawrence (K–L) classification [37], and one or more associated symptoms in the knee joint. The inclusion criteria were age 45 to 75 years, K–L classification ≥ 2, and body mass index (BMI) ≤ 30 kg/m^2. The exclusion criteria were as follows: (1) infectious arthritis, rheumatoid arthritis, ankylosing spondylitis, and other systemic or autoimmune diseases; (2) post-traumatic or post-septic arthritis; (3) developmental dysplasia or skeletal dysplasia; (4) alcohol abuse more than 6 months and smoking more than 12 months.

The control group included 100 individuals (45–75 years old, 54 males and 46 females) free from symptoms or signs of KOA. These individuals were consecutively collected from the subjects who received regular health examinations at Luoyang Orthopedic Hospital within the same time interval. The control group individuals had never experienced any history, signs, or symptoms (joint pain, swelling, tenderness, or restriction of movement) or radiographic evidence of KOA, other arthritis, or joint diseases. The control group individuals were examined by an experienced physician and underwent radiographic examinations. The inclusion criteria were age 45 to 75 years, K–L classification ≤ 1 on radiographic examination, and BMI ≤ 30 kg/m^2. These subjects had no relationship to KOA patients, or family history of KOA disease.

Demographic data and lifestyle and clinical characteristics such as gender, age, BMI classification [38], K–L classification, and occupational category were collected and registered from medical records for all of the participants.

Because this study only examined the hospital population, the Hardy–Weinberg equilibrium (HWE) test was conducted both in the case group and in the control group to ensure the representativeness of the population and to minimize selection bias.

Sample collection

Five-milliliter peripheral blood samples were collected from the participants by venipuncture and placed into ethylene diamine tetraacetic acid (EDTA) tubes for subsequent DNA isolation. The blood samples were stored in a −80° freezer within 1 h after collection. In addition, blood routine and biochemical parameters of the serum were detected.

SNP locus selection

For the ADAMTS-5 gene, the NCBI human genome SNP database (https://www.ncbi.nlm.nih.gov/snp/) and Genome Variation Server 150 website (https://gvs.gs.washington.edu/GVS150/) were used to select tag SNPs. Fifteen tag SNPs with a minimum allele frequency (MAF) ≥ 5% in the Chinese Han population genetic database (CHB) were finally selected for genotyping. All these SNPs can be validated through the NCBI human genome SNP database (https://www.ncbi.nlm.nih.gov/snp/). The 15 SNPs selected in this study are listed in Table 1.

DNA extraction, amplification, and genotyping

DNA was extracted using a QIAamp DNA blood mini kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. The concentration and purity of the extracted DNA samples were measured by a spectrophotometer. The DNA samples showing an optical

Table 1 Information of SNP loci

Gene	SNPs ID	Position	Gene. loc	Variant	Function	MAF (CHB)
ADAMTS-5	rs226794	Chr21:26930036	Extron	G→A	Missense	G = 0.466
ADAMTS-5	rs162497	Chr21:26933989	Intron	G→A	Intron	A = 0.238
ADAMTS-5	rs7510287	Chr21:26937709	Intron	G→A	Intron	A = 0.078
ADAMTS-5	rs233894	Chr21:26953957	Intron	A→G	Intron	G = 0.117
ADAMTS-5	rs2830585	Chr21:26932893	Extron	C→T	Missense	T = 0.078
ADAMTS-5	rs233598	Chr21:26958519	Intron	G→T	Intron	T = 0.073
ADAMTS-5	rs2830586	Chr21:26933158	Intron	T→G	Intron	G = 0.087
ADAMTS-5	rs151065	Chr21:26924005	3′ UTR	G→A	3′ UTR	G = 0.466
ADAMTS-5	rs162496	Chr21:26934256	Intron	A→G	Intron	G = 0.233
ADAMTS-5	rs162509	Chr21:26953456	Intron	G→C	Intron	G = 0.398
ADAMTS-5	rs229054	Chr21:26944497	Intron	G→A	Intron	A = 0.199
ADAMTS-5	rs162499	Chr21:26933328	Intron	A→G	Intron	G = 0.238
ADAMTS-5	rs2249350	Chr21:26950187	Intron	C→A	Intron	A = 0.369
ADAMTS-5	rs233896	Chr21:26952249	Intron	T→G	Intron	G = 0.286
ADAMTS-5	rs151058	Chr21:26939253	Intron	C→T	Intron	T = 0.107

Chr = chromosome; Gene. loc = gene location; MAF (CHB) = minor allele frequency in Chinese Han population (Beijing); missense = missense variant; 3′ UTR = 3′ untranslated regions (within an exon, but not translated)

density (OD) 260/OD 280 ratio in the range between 1.6 and 2.0 were included in the experiment.

Genotyping was performed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using a MassARRAY system (Agena Bioscience®, California, USA). The completed genotyping reactions were spotted onto a 384-well spectroCHIP (Agena Bioscience®, California, USA) using a MassARRAY nanodispenser (Agena Bioscience®, California, USA) and analyzed by MALDI-TOF MS. Genotyping was performed in real time with MassARRAY RT 3.1 (Agena Bioscience®, California, USA) and analyzed on MassARRAYTyper 4.0 (Agena Bioscience®, California, USA). The used primers are indicated in Table 2.

Quality control of the genotyping results was performed using the following methods: (1) the case and control samples were mixed on each plate; (2) genotyping was performed with the clinical staff blinded to the case or control status; (3) 10% of the samples were randomly selected as blinded replicates for genotyping with 100% reproducibility. Genotypes were provided automatically by the software and then determined manually by two different individuals in the laboratory.

Statistical analysis

The Hardy–Weinberg equilibrium (HWE) test was performed using HaploView software for SNP genotypes and alleles in the case and control groups [39, 40]. The HWE was considered satisfied when P was higher than 0.05. Haplotypes were also determined using HaploView software. Applying the SPSS 22.0 software for Windows (SPSS, Chicago, IL), the chi-square test and Fisher's exact test were used to assess the difference in the demographic characteristics (gender, BMI classification, K–L classification, and occupational category) and analyze the relationship between KOA and alleles. Age was analyzed by analysis of variance (ANOVA). The association between KOA and SNP genotypes was assessed by logistic regression analysis, and the odds ratios (ORs) and 95% confidence intervals (95% CIs) were calculated after adjusting for gender, age, BMI classification, and occupational category. The dominant and recessive gene models were selected for gene model analysis. Two-sided P lower than 0.05 was considered statistically significant. Statistical power of this study was analyzed using PASS software (NCSS, LLC, Kaysville, Utah, USA).

Results

General characteristics of the study population

A total of 288 subjects were included in this study, with 188 individuals (65.28%) in the case group and 100 cases (34.72%) in the control group. Demographic characteristics of the study population, such as gender, age, BMI classification, occupational category, and K–L classification, are shown in Table 3. There were statistically significant differences in BMI classification and occupational category between the two groups ($P<0.05$), with more obese patients and physical laborers in the case group. However, there were no significant intergroup differences in other demographic characteristics ($P>0.05$). Nevertheless, because age and gender almost showed statistically significant intergroup differences (P values close to 0.05), we adjusted for age and gender in the following analyses.

HWE test

To ensure the representativeness of the population, the HWE test was conducted both in the case group and in the control group. The results showed that three SNPs, namely, rs233894, rs233896, and rs162509, did not conform to HWE. Therefore, the study was further performed on the 12 SNPs that were consistent with HWE.

Table 2 Primers used for genotyping

SNPs ID	Forward primer sequences (5′–3′)	Reverse primer sequences (5′–3′)	Extended primer sequences (5′–3′)
rs226794	acgttggatgtgattcaggtgaccgatggc	acgttggatgagccagttctcacacacttc	ggcactgaatgtaggc
rs162497	acgttggatgatagtggcagacgtgtttcc	acgttggatgtggtcacttttttcccctttg	ttgttccccggagcagtc
rs7510287	acgttggatgtgtatacagtttggggaagg	acgttggatgtggatgcctctagagctcac	cagggaaggagtaaaaggg
rs233894	acgttggatgctaattccaacatctagtcc	acgttggatggaccagtgtgacataggtat	acatctagtcctaaatgagc
rs2830585	acgttggatgtgctcccagaaacaacggac	acgttggatgttgggagcagcgtaccattg	ttttaagagggccatctacc
rs233598	acgttggatgccttaggtgtatcaagagac	acgttggatggcagagactacattaattgg	ctgttcatcgtaaaggttggc
rs2830586	acgttggatgaagtggaaactttgtcacag	acgttggatggatgaattgcttagcactgg	ggaaactttgtcacagatacag
rs151065	acgttggatgtgcacaaagataactggagg	acgttggatggaagcatgactttctgtgcg	cccctaactggaggattcagcac
rs162496	acgttggatgctgaggtctcaattagacag	acgttggatggggaaaatgcagatactggg	gggttaaaagatgtaaactgcaa
rs162509	acgttggatgccaaggtacagtgtaattgc	acgttggatggtgtcaggaacattaatgcc	aatcgcataattcacaatattgcc
rs229054	acgttggatggcattgtaaatttgttgtgg	acgttggatgcagagcccataattgcattc	tttgttgtggaatatcattttatc
rs162499	acgttggatgacagagatggaccatattag	acgttggatgtcctaagtactgagcacacc	ccatattagaagacataatttacaa
rs2249350	acgttggatgagcacactggagtctttcac	acgttggatgccataatttgcaaacatgccg	gcattttataattaaccactataaag
rs233896	acgttggatggtgcacagaagggcataatc	acgttggatgacaacgtaggagaaaggtgg	ataatcatatatcaaagatttaggttt
rs151058	acgttggatgtctgggtatgaagctgttag	acgttggatgatcctctatcacagggaatc	gtggagctgttagatgcattaggtata

Table 3 General characteristics of the studied Chinese population

	Case group ($n=188$)	Control group ($n=100$)	*P*
Age (mean ± SD, years)	61.3 ± 7.8	59.4 ± 8.3	0.055
Gender, male/female (*n*, %)	79 (42.02)/109 (57.98)	54 (54.00)/46 (46.00)	0.052
BMI classification			
Underweight or normal (*n*, %) (BMI < 23)	44 (23.40)	34 (34.00)	0.002
Overweight (*n*, %) (23 ≤ BMI < 25)	34 (18.09)	29 (29.00)	
Obesity (*n*, %) (BMI ≥ 25)	110 (58.5)	37 (37.00)	
Occupational category (*n*, %)			
Mainly physical labor (*n*, %)	147 (78.19)	66 (66.00)	0.025
Mainly mental labor (*n*, %)	41 (21.81)	34 (34.00)	
K–L classification (*n*, %)			
Grade 0, *n* (%)	0	71 (71.00)	
Grade 1, *n* (%)	0	29 (29.00)	
Grade 2, *n* (%)	49 (26.06)	0	
Grade 3, *n* (%)	86 (45.74)	0	
Grade 4, *n* (%)	53 (28.19)	0	

Table 4 The results of the HWE test for SNPs

SNPs ID	Allele	MAF[#]	Case				Control			
			ObsHET	PredHET	HWpval	MAF	ObsHET	PredHET	HWpval	MAF
rs226794	A: G	G = 0.466	0.554	0.499	0.1814	0.473	0.469	0.5	0.6486	0.5
rs162497	G: A	A = 0.238	0.362	0.373	0.8101	0.248	0.333	0.335	1	0.213
rs7510287	G: A	A = 0.078	0.15	0.148	1	0.08	0.2	0.196	1	0.11
rs233894[§]	A: G	G = 0.117	0.187	0.196	0.7321	0.11	0.12	0.168	0.0492	0.092
rs2830585	C: T	T = 0.078	0.16	0.156	1	0.085	0.17	0.172	1	0.095
rs233598	G: T	T = 0.073	0.181	0.173	0.9585	0.096	0.212	0.221	0.9415	0.126
rs2830586	T: G	G = 0.087	0.17	0.164	1	0.09	0.15	0.188	0.1392	0.105
rs151065	A: G	G = 0.466	0.505	0.497	0.9675	0.462	0.485	0.499	0.8916	0.479
rs162496	A: G	G = 0.233	0.309	0.335	0.3622	0.213	0.27	0.289	0.6893	0.175
rs162509[§]	C: G	G = 0.398	0	0.471	1.2E-27	0.379	0.4	0.466	0.2084	0.37
rs229054	G: A	A = 0.199	0.278	0.294	0.6104	0.179	0.283	0.298	0.8018	0.182
rs162499	A: G	G = 0.238	0.287	0.326	0.1589	0.205	0.271	0.291	0.6603	0.177
rs2249350	C: A	A = 0.369	0.401	0.438	0.3094	0.324	0.41	0.489	0.1441	0.425
rs233896[§]	T: G	G = 0.286	0.372	0.423	0.1369	0.303	0.253	0.34	0.0563	0.217
rs151058	C: T	T = 0.107	0.189	0.197	0.7764	0.111	0.182	0.198	0.664	0.111

MAF[#] = minor allele frequency in the Chinese Han population (Beijing) published in the gene database; MAF = minor allele frequency in the Chinese Han population (Luoyang) in this study; ObsHET = marker's observed heterozygosity; PredHET = marker's predicted heterozygosity (i.e., 2×MAF×(1 − MAF)); HWpval = Hardy–Weinberg equilibrium *P* value, which is the probability that the deviation from HWE could be explained by chance. The table shows that three tag SNPs of the ADAMTS-5 gene do not correspond to HWE (they are marked by "[§]")

The results of the HWE test for the SNPs are shown in Table 4.

It should be noted that all of the SNPs in this study were genotyped successfully. However, few SNPs in some individuals could not be genotyped successfully, and these individuals were excluded from the data statistics.

Association of KOA with SNP alleles

One-way and multifactorial logistic regression analyses were used to elucidate the relationship between the SNPs alleles and the risk of KOA. After logistic regression analysis, we found that the A allele of rs2249350 (A/C) was significantly and negatively associated with the risk of KOA (Table 5). Moreover, the A allele of rs2249350 (A/C) showed a significant association with the risk KOA regardless of whether the effects of confounders such as age, sex, BMI classification, and occupational category were adjusted or not. This suggests that compared with C allele of rs2249350 (A/C), the A allele of rs2249350 (A/C) may be associated with a reduced risk of KOA. In contrast, alleles of other SNPs were not significantly associated with the risk of KOA. Therefore, SNPs alleles other than A allele of rs2249350 (A/C) are probably not associated with the susceptibility to KOA. The results of the unadjusted and adjusted logistic regression analyses are detailed in Table 5.

Association of KOA with SNP genotypes

Statistical analysis of genotypes and different genetic models was performed using logistic regression analysis

Table 5 Results of logistic regression analysis of the relationship between SNPs alleles and KOA risk

SNPs ID	Alleles	Case group, *n* (%)		Control group, *n* (%)		Unadjusted *P* value	Unadjusted OR	Unadjusted 95% CI	Adjusted *P* value	Adjusted OR	Adjusted 95% CI
rs226794	G	174	(47.28)	98	(50.00)	0.539	0.897	0.634–1.269	0.723	0.932	0.632–1.374
	A	194	(52.72)	98	(50.00)	-	1	-	-	1	-
rs162497	A	81	(24.85)	37	(21.26)	0.369	1.224	0.787–1.904	0.226	1.350	0.831–2.195
	G	245	(75.15)	137	(78.74)	-	1	-	-	1	-
rs7510287	A	30	(8.02)	22	(11.00)	0.238	0.706	0.395–1.259	0.067	0.547	0.87–1.044
	G	344	(91.98)	178	(89.00)	-	1	-	-	1	-
rs2830585	T	32	(8.51)	19	(9.50)	0.691	0.886	0.489–1.607	0.502	0.794	0.405–1.556
	C	344	(91.49)	181	(90.50)	-	1	-	-	1	-
rs233598	T	36	(9.57)	25	(12.63)	0.261	0.733	0.426–1.260	0.225	0.683	0.369–1.264
	G	340	(90.43)	173	(87.37)	-	1	-	-	1	-
rs2830586	G	34	(9.04)	21	(10.50)	0.571	0.847	0.478–1.503	0.413	0.763	0.400–1.457
	T	342	(90.96)	179	(89.50)	-	1	-	-	1	-
rs151065	G	172	(46.24)	93	(47.94)	0.700	0.934	0.660–1.322	0.898	0.975	0.661–1.439
	A	200	(53.76)	101	(52.06)	-	1	-	-	1	-
rs162496	G	80	(21.28)	35	(17.50)	0.281	1.274	0.820–1.979	0.130	1.455	0.895–2.367
	A	296	(78.72)	165	(82.50)	-	1	-	-	1	-
rs229054	A	63	(17.90)	36	(18.18)	0.934	0.981	0.624–1.542	0.608	1.145	0.683–1.920
	G	289	(82.10)	162	(81.82)	-	1	-	-	1	-
rs162499	G	73	(20.51)	34	(17.71)	0.431	1.199	0.763–1.882	0.230	1.359	0.824–2.240
	A	283	(79.49)	158	(82.29)	-	1	-	-	1	-
rs2249350	A	121	(32.35)	85	(42.50)	***0.016****	0.647	0.454–0.922	***0.002****	0.522	0.349–0.781
	C	253	(67.65)	115	(57.50)	-	1	-	-	1	-
rs151058	T	41	(11.08)	21	(10.61)	0.991	0.997	0.576–1.727	0.938	1.025	0.551–1.909
	C	329	(88.92)	177	(89.39)	-	1	-	-	1	-

*$P<0.05$, suggesting that this allele corresponding to the P value is associated with the risk of KOA

after adjusting for gender, age, BMI, and occupational category. The results showed that the genotype AA of rs2249350 was associated with a significantly decreased risk of KOA compared with the CC genotype (OR: 0.288; 95% CI: 0.124–0.669; $P=0.004$). In addition, the dominant genetic model ((AA+CA) vs. CC) showed an OR of 0.553 (95% CI: 0.307–0.997; $P=0.049$) and especially the recessive genetic model (AA vs. (CC+CA)) showed an OR of 0.348 (95% CI: 0.162–0.749; $P=0.007$), demonstrating that the A allele and AA genotype of rs2249350 were associated with the reduced risk of KOA. Although homozygous mutant genotypes of rs2830585 (TT), rs233598 (TT), and rs2830586 (GG) showed association with the susceptibility to KOA, the frequency of genotypes rs2830585 (TT), rs233598 (TT), and rs2830586 (GG) in the case and control groups was extremely limited ($n<2\%$), so the statistical results were not very credible and we did not adopt this result in the present study. In future studies, the sample size will be expanded and the association of mutant genotypes (rs2830585 (TT), rs233598 (TT), and rs2830586 (GG)) at these three SNPs with KOA will be further investigated. Other than that, the genotypes and genetic models of other SNPs showed no association with the risk of KOA. The results of logistic regression analysis are shown in Table 6.

Linkage disequilibrium (LD) and haplotype analysis

Additionally, linkage disequilibrium was evaluated and haplotype analysis was conducted. Two blocks were detected in the ADAMTS-5 SNPs by haplotype analysis (Fig. 1). As shown in Table 7, there were no associations between the haplotypes in block 1 and susceptibility to KOA. However, in block 2 (rs229054, rs2249350), the haplotype GC showed a significant association with an increased risk of KOA (OR: 1.259; 95% CI: 1.032–1.537; $P=0.019$), whereas the haplotype GA was associated with a decreased risk of KOA (OR: 0.763; 95% CI: 0.614–0.949; $P=0.017$).

Discussion

As a common chronic condition, KOA is the main cause of pain in the knee joint that affects routine activities such as walking long distances, climbing stairs, sports activities, and even daily household activities, thereby seriously affecting the patients' quality of life. A variety of plausible factors have been confirmed to be involved in this disease. Through years of genome-wide association studies, genetic factors have been considered an important determinant of OA [19]. Therefore, exploring the genetic pathogenesis of OA can help in early diagnosis and improved prevention and treatment of the disease.

Table 6 Association analysis between KOA and SNPs in different genetic models

SNP		Case group, *n* (%)		Control group, *n* (%)		OR	95% CI	Adjusted *P* value
rs226794	Total▲	184	-97.87	98	-98			
	Genotypes							
	AA▼	46	-25	26	-26.53	1	-	-
	AG	102	-55.43	46	-46.94	1.405	0.693–2.846	0.345
	GG	36	-19.57	26	-26.53	0.776	0.337–1.790	0.552
	Dominant							
	AA	46	-25	26	-26.53	1	-	-
	AG + GG	138	-75	72	-73.47	1.18	0.605–2.298	0.627
	Recessive							
	AG + AA	148	-80.43	72	-73.47	1	-	-
	GG	36	-19.57	26	-26.53	0.631	0.310–1.215	0.161
rs162497	Total▲	163	-86.7	87	-87			
	Genotypes							
	GG▼	93	-57.06	54	-62.07	1	-	-
	GA	59	-36.2	59	-67.82	1.248	0.651–2.393	0.505
	AA	11	-6.75	4	-4.6	1.789	0.489–6.542	0.38
	Dominant							
	GG	93	-57.06	54	-62.07	1	-	-
	GA + AA	70	-42.94	33	-37.93	1.318	0.707–2.454	0.385
	Recessive							
	GG + GA	152	-93.25	83	-95.4	1	-	-
	AA	11	-6.75	4	-4.6	1.636	0.459–5.833	0.448
rs7510287	Total▲	187	-99.47	100	-100			
	Genotypes							
	GG▼	158	-84.49	79	-79	1	-	-
	GA	28	-14.97	20	-20	0.518	0.247–1.085	0.081
	AA	1	-0.53	1	-1	0.66	0.018–24.892	0.823
	Dominant							
	GG	158	-84.49	79	-79	1	-	-
	GA + AA	29	-15.51	21	-21	0.523	0.252–1.083	0.081
	Recessive							
	GG + GA	186	-99.47	99	-99	1	-	-
	AA	1	-0.53	1	-1	0.713	0.021–24.159	0.851
rs2830585	Total▲	188	-100	100	-100			
	Genotypes							
	CC▼	157	-83.51	82	-82	1	-	-
	CT	30	-15.96	17	-17	0.856	0.390–1.877	0.697
	TT	1	-0.53	1	-1	0.051	0.003–0.965	**0.047**◆
	Dominant							
	CC	157	-83.51	82	-82	1	-	-
	CT + TT	31	-16.49	18	-18	0.776	0.360–1.673	0.517
	Recessive							
	CC + CT	187	-99.47	99	-99	1	-	-
	TT	1	-0.53	1	-1	0.053	0.003–0.997	**0.050**◆
rs233598	Total▲	188	-100	99	-99			
	Genotypes							
	GG▼	153	-81.38	76	-76.77	1	-	-
	GT	34	-18.09	21	-21.21	0.705	0.338–1.471	0.352
	TT	1	-0.53	2	-2.02	0.046	0.003–0.827	**0.037**◆
	Dominant							
	GG	153	-81.38	76	-76.77	1	-	-
	GT + TT	35	-18.62	23	-23.23	0.641	0.312–1.315	0.225
	Recessive							

Table 6 (continued)

SNP		Case group, *n* (%)		Control group, *n* (%)		OR	95% CI	Adjusted *P* value
	GG + GT	187	-99.47	97	-97.98	1	-	-
	TT	1	-0.53	2	-2.02	0.05	0.003–0.906	**0.043**◆
rs2830586	Total▲	188	-100	100	-100			
	Genotypes							
	TT▼	155	-82.45	82	-82	1	-	-
	TG	32	-17.02	15	-15	1.15	0.518–2.555	0.731
	GG	1	-0.53	3	-3	0.029	0.002–0.377	**0.007**◆
	Dominant							
	TT	155	-82.45	82	-82	1	-	-
	GG + TG	33	-17.55	18	-18	0.897	0.422–1.910	0.779
	Recessive							
	TG + TT	187	-99.47	97	-97	1	-	-
	GG	1	-0.53	3	-3	0.028	0.002–0.365	**0.006**◆
rs151065	Total▲	186	-98.94	97	-97			
	Genotypes							
	AA▼	53	-28.49	27	-27.84	1	-	-
	AG	94	-50.54	47	-48.45	1.093	0.553–2.158	0.789
	GG	39	-20.97	23	-23.71	0.766	0.335–1.750	0.526
	Dominant							
	AA	53	-28.49	27	-27.84	1	-	-
	GG + AG	133	-71.51	70	-72.16	0.987	0.518–1.880	0.968
	Recessive							
	AG + AA	147	-79.03	74	-76.29	1	-	-
	GG	39	-20.97	23	-23.71	0.722	0.361–1.443	0.356
rs162496	Total▲	188	-100	100	-100			
	Genotypes							
	AA▼	119	-63.3	69	-69	1	-	-
	AG	58	-30.85	27	-27	1.374	0.728–2.591	0.327
	GG	11	-5.85	4	-4	1.948	0.539–7.042	0.309
	Dominant							
	AA	119	-63.3	69	-69	1	-	-
	GG + AG	69	-36.7	31	-31	1.452	0.797–2.648	0.223
	Recessive							
	AG + AA	177	-94.15	96	-96	1	-	-
	GG	11	-5.85	4	-4	1.757	0.494–6.244	0.384
rs229054	Total▲	176	-93.62	99	-99			
	Genotypes							
	GG▼	120	-68.18	67	-67.68	1	-	-
	GA	49	-27.84	28	-28.28	1.154	0.596–2.232	0.672
	AA	7	-3.98	4	-4.04	1.081	0.211–5.528	0.926
	Dominant							
	GG	120	-68.18	67	-67.68	1	-	-
	AA + GA	56	-31.82	32	-32.32	1.146	0.606–2.165	0.675
	Recessive							
	GA + GG	169	-96.02	95	-95.96	1	-	-
	AA	7	-3.98	4	-4.04	1.031	0.206–5.175	0.97
rs162499	Total▲	178	-94.68	96	-96			
	Genotypes							
	AA▼	116	-65.17	66	-68.75	1	-	-
	AG	51	-28.65	26	-27.08	1.201	0.627–2.301	0.582
	GG	11	-6.18	4	-4.17	1.899	0.526–6.853	0.328
	Dominant							
	AA	116	-65.17	66	-68.75	1	-	-

Table 6 (continued)

SNP		Case group, *n* (%)		Control group, *n* (%)		OR	95% CI	Adjusted *P* value
	GG + AG	62	-34.83	30	-31.25	1.298	0.703–2.397	0.404
	Recessive							
	AG + AA	167	-93.82	92	-95.83	1	-	-
	GG	11	-6.18	4	-4.17	1.791	0.505–6.360	0.367
rs2249350	Total▲	187	-99.47	100	-100			
	Genotypes							
	CC▼	89	-47.59	37	-37	1	-	-
	CA	75	-40.11	41	-41	0.699	0.371–1.318	0.269
	AA	23	-12.3	22	-22	0.288	0.124–0.669	**0.004**◆
	Dominant							
	CC	89	-47.59	37	-37	1	-	-
	AA + CA	98	-52.41	63	-63	0.553	0.307–0.997	**0.049**◆
	Recessive							
	CA + CC	164	-87.7	78	-78	1	-	-
	AA	23	-12.3	22	-22	0.348	0.162–0.749	**0.007**◆
rs151058	Total▲	185	-98.4	99	-99			
	Genotypes							
	CC▼	147	-79.46	79	-79.8	1	-	-
	CT	35	-18.92	18	-18.18	3.137	0.319–30.812	0.327
	TT	3	-1.62	2	-2.02	3.288	0.315–34.267	0.32
	Dominant							
	CC	147	-79.46	79	-79.8	1	-	-
	CT + TT	38	-20.54	20	-20.2	0.969	0.466–2.013	0.932
	Recessive							
	CC + CT	182	-98.38	97	-97.98	1	-	-
	TT	3	-1.62	2	-2.02	0.315	0.032–3.065	0.32

"Total▲" represents the number and percentage of successful genotyping cases; "▼" represents the wild-type homozygous genotype used as reference; vs. the reference classification, ◆ $P<0.05$

It is well-known that degeneration of cartilage is a crucial pathobiological process in the development and progression of KOA. ECM is mainly composed of collagen, the aggregating proteoglycan aggrecan, and many other macromolecules, while cartilage is composed of a large amount of ECM and a relatively small number of chondrocytes embedded in it [41]. In OA, the degradation of ECM macromolecules surpasses their synthesis, eventually leading to total or partial cartilage erosion [42]. As a critical ECM-degrading enzyme, ADAMTS-5 can cleave the Glu373–Ala374 bond in the IGD of aggrecan [20], thereby mediating the cartilage aggrecan degradation, which is thought to be a significant event in early-stage OA [24].

The ADAMTS-5 gene is located on chromosome 21q21.3 and regulates the synthesis of the ADAMTS-5 protein [24, 43]. Several studies have focused on the association of ADAMTS-5 with the susceptibility to OA; however, their conclusions were inconsistent. Rodriguez-Lopez et al. explored the association between six tag SNPs (rs3746836, rs162495, rs162488, rs9984329, rs233896, and rs233601) and two nonsynonymous SNPs (nsSNP) (rs226794 and rs2830585) of ADAMTS-5 and OA in different parts of the body (e.g., hip OA, knee OA, and hand OA) on samples obtained from four European Caucasian collections. The results of their study did not reveal any significant effects of ADAMTS-5 SNPs on the susceptibility to OA [31]. Nevertheless, as that study is the first and the largest study for the association of OA with ADAMTS5 SNPs, it is worth referring to despite the fact that no positive results were found. Canbek et al. conducted a case–control study in a Turkish population, and their results also showed that the ADAMTS-5 SNPs (rs226794 and rs2830585) variants may not contribute to the susceptibility to KOA [33]. In China, Gu et al. conducted a community-based case–control study and reported that the T allele carrier in rs2830585 could be a protective factor against OA, especially cervical OA [32]. In contrast, Zhou et al. found that the T allele carrier in rs2830585 was associated with a significantly increased risk of KOA [34].

In the previous studies on the association between ADAMTS-5 SNPs and susceptibility to KOA, only a few SNPs have been studied, and the findings have been inconsistent. Thus, the association between ADAMTS-5 polymorphism and OA has not been comprehensively investigated. Therefore, in the present hospital-based case–control study, the associations of 12 tag SNPs

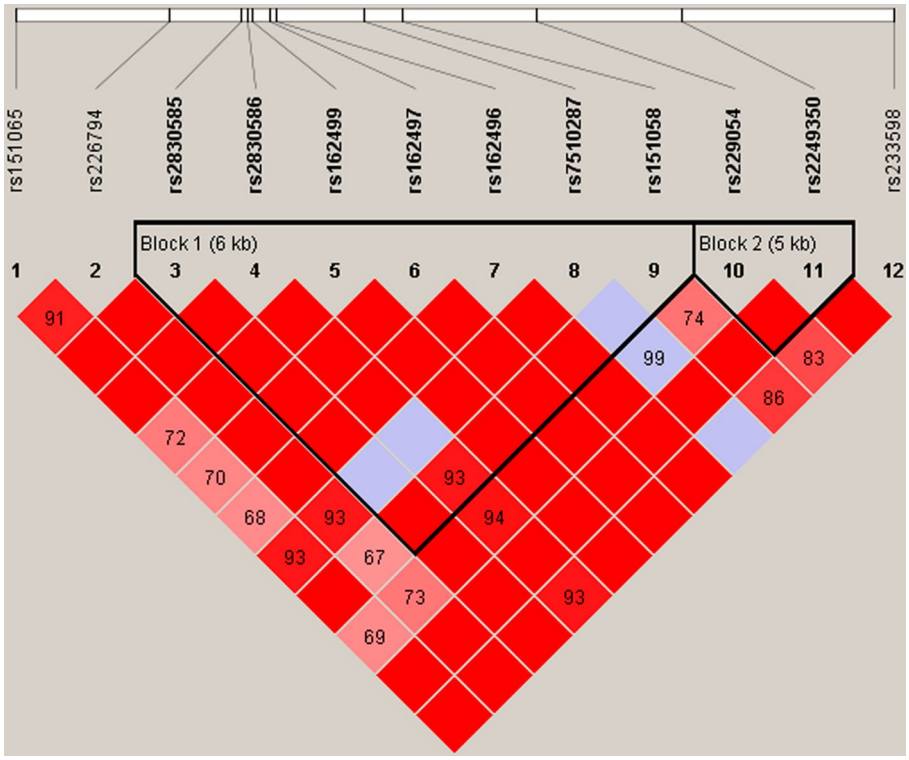

Fig. 1 Linkage disequilibrium (LD) of polymorphic sites in the ADAMTS-5 gene on chromosome 21. The color of each square from white to red represents the D′ value from 0 to 1, indicating a low to high degree of linkage. The numerical value in the squares is D′ multiplied by 100, and the squares with no labeled numbers represent D′ equaling 1. D′ is the ratio of D (coefficient of linkage disequilibrium, a measure of linkage disequilibrium) to its maximum possible absolute value, given the allele frequencies. In addition, the light blue color represents that the LOD (log odds score method) value is less than or equal to 1. LOD is log10 of the ratio of the likelihood data for linked genes to the likelihood data for unlinked genes. LOD > 1 means that there is a linkage, whereas LOD ≤ 1 indicates diminished likelihood of linkage

Table 7 Association of haplotypes and the risk of KOA in the case and control groups

Block ID	SNPs	Haplotype	Haplotype frequencies		*P*	OR	95% CI
			Case group (%)	Control group (%)			
1	rs2830585, rs2830586, rs162499, rs162497, rs162496, rs7510287, rs151058	CTAGAGC	58.38	59.05	0.881	0.989	0.857–1.142
		CTGAGGC	21.09	17.40	0.314	1.201	0.838–1.719
		CTAGAAC	8.01	11.00	0.228	0.725	0.430–1.223
		TGAGAGT	8.51	9.50	0.691	0.896	0.521–1.539
		CTAGAGT	2.77	0.95	0.307	2.660	0.588–12.021
2	rs229054, rs2249350	GC	49.63	39.35	**0.019***	1.259	1.032–1.537
		GA	32.42	42.50	**0.017***	0.763	0.614–0.949
		AC	17.95	18.15	0.991	1.002	0.695–1.445

(rs226794, rs162497, rs7510287, rs2830585, rs233598, rs2830586, rs151065, rs162496, rs229054, rs162499, rs2249350, rs151058) in the ADAMTS-5 gene with the risk of KOA were investigated in a Chinese Han population.

Our results demonstrated a significant difference in the ADAMTS-5 SNPs between the case and control groups, indicating an association between genetic polymorphism and the susceptibility to KOA. We identified the SNP rs2249350 polymorphic AA genotype of ADAMTS-5 and variant A allele as the protective factors against KOA. In contrast, the variant allele, polymorphic genotypes, and genetic models of other ADAMTS-5 SNPs (rs226794, rs162497, rs7510287, rs2830585, rs233598, rs2830586, rs151065, rs162496, rs229054, rs162499, rs151058) showed no associations with the susceptibility to KOA. We also found that the GA haplotype (rs229054, rs2249350) was associated with a reduced risk of KOA, whereas the GC haplotype (rs229054, rs2249350) was associated with an increased risk of KOA.

Our study showed that rs2249350, but not rs2830585 polymorphisms, were associated with the susceptibility to KOA, which conflicts with the conclusions of previous studies that had reported that rs2830585 was associated with the risk of KOA [34]. This discrepancy may be attributed to different sample sizes, ethnicity-dependent effects, and environmental factors. First, the sample size of the case and control groups in this study was much smaller than that in the previous studies. Second, the type of OA, disease severity, and population ethnicity varied among the studies. Other factors may be different BMI, smoking habits, and comorbidities such as diabetes, which may have also contributed to the inconsistent findings. Apart from this, we hypothesize that genetic heterogeneity, different genotyping methods, and random errors may be additional reasons for the different findings between the present and previous studies. Notably, to the best of our knowledge, our study is the first to find a significant association between ADAMTS-5 rs2830585 polymorphism and the susceptibility to KOA in a Chinese Han population.

Due to the crucial role of ADAMTS-5 in cartilage degradation, we hypothesize that the mechanism by which the ADAMTS-5 SNPs affect the susceptibility to KOA may be that the differences in SNPs cause changes in ADAMTS-5 molecular activity or secretion, which in turn could affect the degradation and destruction of cartilage. In a sense, the SNPs in ADAMTS-5 may actually play an important role in molecular biological functions of cartilage metabolism. However, epigenetic aspects such as the effect of SNP on protein expression were not investigated in this study, which should be the focus of future research.

As KOA is a complex disease affected by gene–gene and gene–environmental interactions, a small number of SNP gene mutations is not sufficient to completely represent the genetic susceptibility to the disease, let alone to explain the overall risk of the disease. Therefore, further studies are needed to elucidate the role of other SNPs of ADAMTS-5 and other related genes involved in the pathogenesis of KOA. Meanwhile, the association of gene–gene interactions, gene–environment interactions, and epigenetics with the susceptibility to KOA should also be explored comprehensively.

There were some limitations to this study, including selection bias, small sample size, single ethnicity, and single-center study design. First, there may have been bias in case selection in this study. In order to minimize selection bias, we assessed only primary KOA, and other factors that are strongly associated with secondary KOA or potential confounders were strictly controlled at the time of the recruitment of the study subjects or were controlled by multivariate statistical analysis. Therefore, we believe that the patients in the case group were accurately selected and that the possibility of selection bias in our sample was very low. Second, our study focused on the hospital population, and although we performed the HWT on the case and control groups at the statistical analysis stage to improve the population representativeness of the selected samples and reduce selection bias, we still could not completely eliminate the bias due to population selection. Therefore, in future experiments, we will focus on whole-group, stratified community, population surveys and studies to reduce population selection bias. In addition, due to research funding and time constraints, the sample size of the case and control groups in our study was relatively small. We did our best to ensure that every individual in our study was appropriately selected in order to maximize the accuracy and validity of the sample in the study. Nonetheless, this cannot fully prevent the lack of statistical power of the study to detect subtle differences. Upon calculation, the statistical power of this study was approximately 75%. This suggests that subsequent experiments may necessitate a greater investment of time, funding, and personnel to increase the sample size and manage potential biases. Such measures could greatly enhance statistical power and, in turn, bolster the credibility of our experimental outcomes. Finally, this study only investigated the Chinese Han population and was limited to the Han population in the Luoyang area. However, the results for the Chinese Han population may not be generalizable to other ethnic populations, and the results for a particular ethnic group in a localized area may not be indicative of the entire ethnic group. Therefore, more research on populations from different geographic locations and of different ethnicities is merited.

Conclusion

ADAMTS-5 gene rs2249350 polymorphism is a genetic contributor to the risk of KOA. The A allele and AA genotype of rs2249350 may reduce the risk of KOA, whereas the C allele and CC genotype may increase the susceptibility to KOA. In addition, the GA haplotype (rs229054, rs2249350) might be associated with a decreased risk of KOA, whereas the GC haplotype may protect from KOA. These findings help us to summarize the risk of KOA from a genetic perspective. Nevertheless, in order to confirm these findings, it is necessary to conduct larger studies in more ethnically diverse populations.

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Author contributions
In this study, each author made substantial contributions. Dr. Jingwei Wang and Qiankun Sun collected the blood specimens. Dr. YanXing Guo and Nianhu Li trained and instructed on experimental techniques. Menglong Jia, Longtan Yu, Fangxiu Liu and Lei Wei participated and helped in the collection of experimental data and statistics. Shan Gao was involved in the experiments, statistical analysis of the data, and writing of the paper, and Menglong Jia helped substantially. Shan Gao and Menglong Jia contributed equally to this study and share first authorship; this decision was recognized and supported by all of the authors. All authors reviewed the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The study was approved by the Ethics Committee of Luoyang Orthopedic Hospital. All procedures of this study were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants prior to the study.

Consent for publication
All participants in this study consented the publication of this study.

Competing interests
The authors declare no competing interests.

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