Variations of Wnt/β-catenin pathway-related genes in susceptibility to knee osteoarthritis: A three-centre case-control study

Yong Huang | Lifeng Jiang | Haoyu Yang | Lidong Wu | Nanwei Xu | Xindie Zhou | Jin Li

Abstract
Excessive activation of the Wnt signalling pathway in the articular cartilage is demonstrated to be related to the onset and severity of osteoarthritis (OA). However, few studies have investigated the association between variants in Wnt-pathway-related genes and the risk of OA by searching Pubmed and EMBASE. Totally, 471 knee OA patients and 532 controls were recruited from three hospitals to evaluate the associations of five genetic variants (rs61735963, rs2908004, rs10795550, rs1799986 and rs1127379) with the risk of knee OA. These polymorphisms were genotyped through polymerase chain reaction and Sanger sequencing. Genetic risk scores (GRSs) were calculated to evaluate the combined effect of these genetic variants. No significant association was found between OA risk and polymorphisms (rs61735963, rs10795550 or rs1127379). However, WNT16 rs2908004 polymorphism was correlated with a decreased risk of OA, especially among females, smokers, non-drinkers and individuals with age < 60 years or BMI ≥ 25. This SNP was also associated with Kellgren-Lawrence grade and CRP. Similarly, LRP1 rs1799986 polymorphism was correlated with a decreased risk of OA, especially among males, smokers, drinkers and individuals with age < 60 years or BMI ≥ 25. TT genotype was more frequent in the group of VAS ≥ 6 versus VAS < 6. A low GRS was positively correlated with a decreased risk of OA. In addition, rs2908004 or rs1799986 polymorphism reduces the expression of WNT16 or LRP1. In conclusion, two SNPs (rs2908004 and rs1799986) are associated with the decreased risk of OA by regulating the Wnt pathway.

Keywords
bioinformatics analysis, genetic risk score, knee osteoarthritis, polymorphism, Wnt
1 | INTRODUCTION

Osteoarthritis (OA) is the most prevalent chronic joint disease that contributes to cartilage matrix degradation. Major mechanisms of OA is characterized by abnormal joint tissue metabolism and results in cartilage degradation, bone remodelling, joint inflammation, osteophyte formation and loss of normal joint function. About 9.6% of men and 18% of women ≥ 60 years old suffer from symptomatic OA worldwide. Multiple factors, including advanced age, excessive body weight, repeated trauma or surgery to the joint structures, diabetes and hormone-related disorders, contribute to increased risk of OA. Studies provide clear evidence of a heritable component in susceptibility of OA, but the specific genetic factors are largely unknown. It therefore remains a challenge to identify risk alleles or candidate genes that contribute to OA pathogenesis.

The Wnt/β-catenin signalling pathway plays a crucial role in the regulation, proliferation, differentiation and cellular death processes in numerous anomalies of development, growth and homeostasis in animal organisms. We previously found the Wnt/β-catenin pathway plays a key role in OA by inducing the expression of matrix metalloproteinases. Besides, it contributes to cell differentiation and apoptosis and directly regulates chondrocyte phenotype. Once Wnt protein is secreted, it binds to the frizzled receptor and low-density lipoprotein receptor-related protein 5/6 (LRP5/6) in order to stabilize the β-catenin protein and initiate an intricate signalling cascade.

Abnormal activation of the Wnt/β-catenin signalling is involved in the OA pathology, but little is known about the association of the Wnt pathway-related gene polymorphisms with OA susceptibility. Thus, this hospital-based case-control study was conducted to investigate the effects of Wnt pathway-related genes (DVL1, WNT16, ITIH5, LRP1 and SFRP1) polymorphisms on the risk of OA in a Chinese population.

2 | MATERIALS AND METHODS

2.1 | Search strategy

We systematically searched the Pubmed and EMBASE databases up to 12 January 2019 to identify eligible studies using the following keywords: ‘osteoarthritis’, ‘OA’, ‘wnt signalling pathway’, ‘Wnt/β-catenin signalling pathway’, ‘polymorphism’, ‘SNP’ and ‘variant’. We also checked references in eligible studies and reviews for other relevant studies. No restriction was set on language or ethnicity.

2.2 | Protein-protein interaction network and functional annotation

We constructed protein-protein interaction (PPI) network to identify significant interactions among proteins encoded by Wnt pathway-related genes using the search tool STRING (http://string-db.org/). PPI network was constructed by the PPI pairs with protein interaction scores exceeding 0.4. Gene Ontology (GO) is a widely adopted source of gene functional annotation, including biological process, molecular function and cellular component.

2.3 | Study population

We recruited 471 knee OA patients and 532 healthy controls from the Affiliated Changzhou No. 2 People’s Hospital of Nanjing Medical University, the Second Affiliated Hospital of Jiaxing University and the Second Affiliated Hospital, Zhejiang University School of Medicine between November 2014 and July 2018. Knee OA was diagnosed in accordance with the clinical and radiographic criteria of the American College of Rheumatology (1987). Exclusion criteria for OA patients were as follows: (a) autoimmune disorders, malignant tumour, cardiovascular diseases and liver and kidney dysfunction; (b) history of arthritis; and (c) drug abuse more than 3 months. The healthy controls were selected from the outpatients of the above hospitals during the same period. X-ray analysis confirmed no evident symptoms of joint pain and the absence of knee OA. Demographic and clinical information (C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), visual analogue score (VAS) and Kellgren-Lawrence grade) was collected from all participants by using a written questionnaire and reviewing medical records. Individuals who smoked at least one cigarette daily were considered as smokers. Alcohol usage was defined as consumption of at least three alcoholic drinks one week. This study was approved by the Ethics Committees of the above three hospitals and was consistent with the Declaration of Helsinki. Each subject provided written informed consent prior to participation.

2.4 | SNP selection process

Selection process was as follows. First, we identified five OA-related genes (DVL1, WNT16, ITIH5, LRP1 and SFRP1) on the Wnt signalling pathway by reviewing the literature. Second, we found out whether the SNPs on these genes have been studied in the disease. Third, SNPs with minor allele frequency > 0.5, located in the promoter, exon or 3'-UTR were selected. In total, five SNPs in Wnt signalling pathway-related genes were selected, including rs61735963, rs2908004, rs10795550, rs1799986 and rs1127379.

2.5 | Genotyping

Peripheral blood (2 mL) was collected from each subject. Genomic DNA was extracted using a QiAamp DNA blood mini kit (Qiagen, Hilden, Germany) according to the manufacturer’s instruction. DNA samples were tested for purity and concentration with an ultraviolet spectrophotometer and stored at −20°C. PCR primers were designed on Primer Premier 5.0 (Premier, Pala Alto, CA, USA). PCR amplification was conducted in 25-μL volumes each containing 1 μL of the DNA template, 2.5 μL of 3 mmol/L dNTPs, 1.0 μL of each primer (10 mmol/L), 2.5 μL of 10× PCR buffer (added with MgCl2), 1.0 U of Taq DNA polymerase and ddH2O. DNA was denatured at 95°C for 6 minutes, then amplified by 35 PCR cycles (95°C for 30 seconds,
| Author and year | Type of study | Country | Sample size | Genotyping method | Polymorphism | QAS |
|----------------|--------------|---------|-------------|-------------------|--------------|-----|
| Loughlin2004   | Family case-control | UK      | OA/control 936/760 | PCR-RFLP        | TNFAIP6 rs1046668; ITGA6 rs2737085 rs10209072; FRZB rs288326 rs7775 | 8   |
| Urano2007      | Case-only    | Japan   | Spinal OA 304 | TaqMan           | W1S1P1 2364A/G | 7   |
| Kerkhof2008    | Cohort       | Netherlands | Hip/knee/hand OA 2507 | TaqMan       | FRZB rs288326 rs7775; LRP5 Ala1330val; LRP6 Ile1062val | 8   |
| Urano2008      | Case-only    | Japan   | Spinal OA 357 | TaqMan           | LRP5 Q89R | 7   |
| Valdes2009     | Case-control | UK      | Knee/hip OA/control 2170/2849 | PCR         | ANP32A rs7164503 | 7   |
| Lamas2010      | Case-control | Spain   | OA/control 191/283 | N/A             | COL10A1 rs11965969 | 7   |
| Gao2010        | Family cohort | China   | Osteoarthritis 1260 | TaqMan       | FZRB rs643399 rs409238 rs288324 rs4666865 | 7   |
| Leijten2012    | Cohort       | Netherlands | OA 7142 | N/A             | GREM1 rs1881537 rs921510 rs4779584 rs1258726 rs12593365 DKK1 rs11636374 rs12905294 rs16959110 rs16959022 rs12593223 rs11071936 rs10318 | 8   |
| Baker-LePain2012 | Case-control | USA     | Hip OA/controls 451/601 | PCR         | FRZB (rs288326 rs7775) | 9   |
| Garca-Ibarbia2013 | Case-only    | Spain   | Hip fractures/hip OA 500/353 | Sequencing   | WNT4 (rs7526484 rs2235526 rs10917158) WNT10A (rs3806557 rs10177996 rs2385199) WNT16 (rs3779381 rs2908004 rs2707471 rs3803185 rs2707466 rs17143305) SFRP1 (rs3242 rs1127379 rs7820647 rs11786592 rs6651363 rs10109536 rs17652488 rs10958671 rs17574424 rs7832767 rs968427 rs921142) | 9   |

(Continues)
| Author and year | Type of study | Country   | Sample size | Genotyping method | Polymorphism                                                                 | QAS |
|-----------------|---------------|-----------|-------------|-------------------|------------------------------------------------------------------------------|-----|
| Garca-Ibarbia2013 | Case-control  | Spain     | Hip/knee OA/control 680/606 | TaqMan          | WNT1 (rs11610885 rs10878228 rs7397906 rs6581609 rs1900292); WNT10A (rs3006557 rs10177996 rs2385199); WNT16 (rs3779381 rs29008004 rs2707471 rs3801385 rs2707466 rs17143305); DVL2 (rs2074222 rs222837 rs222836 rs2074216); FZD5 (rs3731567 rs35994626); BCL9 (rs666569 rs10793689 rs17160413 rs12081028 rs6659237 rs1546161 rs10793690 rs7523564 rs1015235 rs2353544 rs11240082 rs11240083 rs10900382 rs2353525 rs688325 rs675828 rs3737843 rs11807878 rs3766512); SFRP1 (rs3242 rs1127379 rs7820647 rs11786592 rs6651363 rs10109536 rs17652488 rs10958671 rs17574424 rs7832767 rs968427 rs921142); SFRP4 (rs2598116 rs1052981 rs1802074 rs1802073 rs2598115 rs1450856 rs3807192); TCF7L1 (rs3810822 rs2568217 rs2568225 rs11682047 rs11693369 rs2568197 rs2568203 rs2568210 rs4832148 rs12477587 rs17763853 rs7581133 rs11904127 rs6547606 rs10204252 rs17764504 rs7547856 rs4832156 rs6741339 rs11547160) | 10  |
| Garca-Ibarbia2017 | Case-only     | Spain     | Hip OA/knee OA 173/52 | TaqMan          | WNT16 rs2707466 rs2908004                                                   | 8   |
| Fernandez-Torres2018 | Case-control  | Mexico    | Knee OA/control 141/190 | TaqMan          | LRP6 rs12314259; SOST rs851054; FMN2 rs986690; FRZB rs409328; COL11A1 rs265977 | 8   |
50°C for 1 minutes, 72°C for 30 seconds) and finally extended at 72°C for 10 minutes. PCR products were revealed on 2% agarose gel electrophoresis with ethidium bromide, purified using a DNA purification kit and then genotyped through Sanger sequencing.

2.6 Reverse transcription-polymerase chain reaction

Total RNA was isolated from the whole blood from the OA patients and healthy controls using the Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer’s instructions. Five genes (DVL1, WNT16, ITIH5, LRP1 and SFRP1) expression were detected by RT-PCR with Hifair™ first-strand cDNA synthesis SuperMix and qPCR SYBR Green Master Mix (Yesen, Shanghai, China). Forward and reverse primers used for PCR were as follows: 5’-CGTAAAGTGCTGGGGACCTT-3’, 5’-GATCCACGC ACTCAGGAACT-3’ (SFRP1); 5’-TCCTCACTAACCAGCTCCGT-3’, 5’-GGCGCTCATGTCACTCTTCAC-3’ (DVL1); 5’-CTACAGCTCCCTGC AAACGA-3’, 5’-AGCACCAAGTTATCCCTCGC-3’ (WNT16); 5’-GTC CCTGTGTGTGGGGTC-3’, 5’-ACCCTGAGTCCATCCTGCTC-3’ (ITIH5); 5’-CCGCTCTGATGAGTCTGCTT-3’, 5’-AGTCATTGTCATTGTCGCA TCT-3’ (LRP1); 5’-GATGAGATTGGCATGGCTTT-3’, 5’-GTCACCTTC ACCTTGTCAGT-3’ (β-actin). Statistical analysis used relative quantification, and the 2−ΔΔCT method was used to calculate relative expression.

2.7 Statistical analysis

Continuous variables were expressed as mean ± standard deviation and analysed using Student’s t test or Mann-Whitney U test. Categorical variables were reported as frequency and percentage and analysed using chi-squared test. The genotype frequencies among controls in line with Hardy-Weinberg equilibrium (HWE) were investigated through goodness-of-fit chi-squared test. The risk of gene for OA was evaluated by calculating odds ratios (ORs) and 95% confidence intervals (CIs). Stratification analysis was conducted according to sex, age, smoking, alcohol consumption and body mass index (BMI). The effect size of each SNP was investigated using crude ORs. If the SNPs’ disease risk was independent, the weighted genetic risk score (GRS) was estimated as the sum of (log OR of SNP) × (number of risk alleles carried by the individual) across five SNPs. The GRS was then divided into quartiles according to the normalized value and calculated by binary logistic regression. All statistical analyses were performed on SAS 9.1.3 (SAS Institute, Cary, NC, USA). P < .05 was considered statistically significant.

TABLE 2 Positive sites on the Wnt signalling pathway-related genes

| SNP       | Gene   | Major/minor allele | MAF     | Location       | Results | Country |
|-----------|--------|--------------------|---------|----------------|---------|---------|
| Rs2353525 | BCL9   | T/G                | 0.4293/1250 | Intron         | Risk    | Spain   |
| Rs12341259| LRP6   | C/T                | 0.0094/47    | Intron         | Protect | Mexico   |
| Rs851054  | SOST   | C/A                | 0.4441/2224 | Intron/3’-UTR  | Protect | Mexico   |
| Rs986690  | FMN2   | G/A                | 0.3203/1604 | Intron         | Protect | Mexico   |
| Rs409238  | FRZB   | G/A                | 0.4301/2154 | Intron         | Risk     | Mexico   |
| Rs2615977 | COL11A1| A/C                | 0.2194/1099 | Intron         | Risk     | Mexico   |
| Rs2707466 | WNT16  | C/T                | 0.4970/2489 | Exon           | Risk     | Spain   |
| Rs2929970 | WISP1  | G/A                | 0.4441/2224 | 3’-UTR         | Risk     | Japan    |
| Rs4149434 | LRP5   | A/G                | 0.0200/100  | Exon/promoter  | Protect   | Japan    |
| Rs7164503 | ANP32A | T/C                | 0.3816/1911 | Intron         | Protect   | UK    |
| Rs1259365 | GREM1  | T/C                | 0.2165/1084 | Exon           | Protect   | Netherlands |
| Rs2908004 | WNT16  | G/A                | 0.4896/2452 | Exon           | Protect   | Spain   |

FIGURE 1 The protein interaction network of Wnt/β-catenin pathway-related genes
3 | RESULTS

3.1 | Study characteristics

The systematic database searching returned 25 articles, of which 12 articles were excluded, including three duplicates, and nine reviews, meeting reports or unrelated articles. Finally, 13 articles involving 20 genes and 127 SNPs were included for further analysis. The detailed characteristics of the selected studies are presented in Table 1, including 6 case-control studies, four case-only studies and three cohort studies. Ten articles were conducted among Caucasians and three among Asians. Twelve of the 127 SNPs were associated with...
The OA patients and controls were aged 57.87 and 57.47 years on average respectively (Table 3). About 54% of the participants in the two groups were females. The average BMIs did not differ significantly between groups (P = .632). The majority of OA patients were assigned with Kellgren-Lawrence grade III + IV. Clinical characteristics (eg ESR, CRP, VAS and Lequesnes’ index) of patients were also collected to investigate the pathology of OA.

### 3.4 Wnt pathway-related genes variant analyses

The genotype and allele distributions of five SNPs are shown in Table 4. No significant deviation from HWE was found for any SNP in either group. WNT16 rs2908004 polymorphism was associated with a decreased risk of OA in the co-dominant, recessive, dominant and allelic models. Additionally, the TT genotype versus CC genotype of rs1799986 polymorphism had a 0.49-fold decreased risk of OA (TT vs. CC: OR 0.49; 95%CI 0.29-0.83; P = .008). Furthermore, the CT + TT genotype significantly increased OA risk (CT + TT vs. CC: OR 0.72; 95%CI 0.56-0.93; P = .011). Similar findings were observed in the dominant and allelic models. Notably, these associations were still significant after adjusting sex and age. No meaningfully or statistically significant associations with OA risk were noted in other polymorphisms.

Stratified analyses by sex, age, smoking, drinking and BMI were conducted (Table 5). For rs2908004, the decreased effect was more evident in the subgroups of females, age < 60 and BMI ≥ 25. In addition, the decreased association between LRP1 rs1799986 polymorphism was stronger in the subgroups of males, smokers, non-drinkers, age < 60 and BMI ≥ 25. Subsequently, we investigated the possible association between the two above polymorphisms and clinical characteristics (CRP, ESR, VAS, Lequesnes’ index and K-L grade) of OA patients (Table 6). GG + AA genotype carriers of rs2908004 polymorphism had lower CRP expression and K-L grade (P = .011) compared to the GG genotype. For rs1799986, the TT genotype was more frequent in the group of VAS ≥ 6 versus VAS < 6.

Weighted GRS revealed that individuals in the third/second versus the first quartile had a lower risk of developing OA (Table 7). This relationship remained true after adjusting for sex and age.

The mRNA expression of WNT16 or LRP1 was regulated by the type of rs1799986 polymorphism had a 0.49-fold decreased risk of OA (TT vs. CC: OR 0.49; 95%CI 0.29-0.83; P = .008). Furthermore, the CT + TT genotype significantly increased OA risk (CT + TT vs. CC: OR 0.72; 95%CI 0.56-0.93; P = .011). Similar findings were observed in the dominant and allelic models. Notably, these associations were still significant after adjusting sex and age. No meaningfully or statistically significant associations with OA risk were noted in other polymorphisms.

Stratified analyses by sex, age, smoking, drinking and BMI were conducted (Table 5). For rs2908004, the decreased effect was more evident in the subgroups of females, age < 60 and BMI ≥ 25. In addition, the decreased association between LRP1 rs1799986 polymorphism was stronger in the subgroups of males, smokers, non-drinkers, age < 60 and BMI ≥ 25. Subsequently, we investigated the possible association between the two above polymorphisms and clinical characteristics (CRP, ESR, VAS, Lequesnes’ index and K-L grade) of OA patients (Table 6). GG + AA genotype carriers of rs2908004 polymorphism had lower CRP expression and K-L grade compared to the GG genotype. For rs1799986, the TT genotype was more frequent in the group of VAS ≥ 6 versus VAS < 6.

Weighted GRS revealed that individuals in the third/second versus the first quartile had a lower risk of developing OA (Table 7). This relationship remained true after adjusting for sex and age.

The mRNA expression of WNT16 or LRP1 was regulated by the genotypes of rs2908004 or rs1799986 polymorphism (Figure 3). Our results indicated that WNT16 and LRP1 were upregulated in OA compared to that in healthy controls. There existed a significant difference in the mRNA expression for rs2908004 or rs1799986 polymorphism (P < .05).

4 | DISCUSSION

Two polymorphisms (rs2908004 and rs1799986) correlated with a decreased risk of OA, while no significant association was found between other polymorphisms (rs61735963, rs10795550 or rs1127379) and OA risk. Individuals with lower GRS had a lower risk of developing OA. In addition, rs2908004 or rs1799986 polymorphism reduces the expression of WNT16 or LRP1.

β-catenin-dependent canonical and independent non-canonical Wnt signalling pathways play multiple roles in regulating the cartilage development, growth and maintenance in animal models. Alterations in genes associated with Wnt/β-catenin signalling were
**TABLE 4** Logistic regression analysis of associations between polymorphisms and risk of osteoarthritis

| Genotype      | Cases\(^a\) (n = 471) | Controls\(^a\) (n = 532) | OR (95% CI) | \(P\) | OR (95% CI)\(^b\) | \(P\)\(^b\) |
|---------------|-------------------------|---------------------------|-------------|------|-------------------|------|
| rs2908004 G/A |                         |                           |             |      |                   |      |
| GG           | 158 33.6                | 138 26.0                  | 1.00        | -    | -                 | -    |
| GA           | 228 48.5                | 265 49.9                  | 0.75 (0.57-1.01) | 0.056 | 0.75 (0.57-1.01) | 0.056 |
| AA           | 84 17.9                 | 128 24.1                  | 0.57 (0.40-0.82) | 0.002 | 0.57 (0.40-0.82) | 0.002 |
| GA + AA      | 312 66.4                | 393 74.0                  | 0.70 (0.53-0.91) | 0.009 | 0.69 (0.53-0.91) | 0.009 |
| GG + GA      | 386 82.1                | 403 75.9                  | 1.00        | -    | -                 | -    |
| AA           | 84 17.9                 | 128 24.1                  | 0.68 (0.50-0.93) | 0.016 | 0.68 (0.50-0.93) | 0.015 |
| G allele     | 544 57.9                | 541 50.9                  | 1.00        | -    | -                 | -    |
| A allele     | 396 42.1                | 521 49.1                  | 0.76 (0.63-0.90) | 0.002 | -                 | -    |
| rs10795550 T/C |                        |                           |             |      |                   |      |
| TT           | 285 60.6                | 305 57.4                  | 1.00        | -    | -                 | -    |
| TC           | 162 34.5                | 195 36.7                  | 0.89 (0.69-1.16) | 0.404 | 0.90 (0.69-1.17) | 0.418 |
| CC           | 23 4.9                  | 31 5.8                    | 0.79 (0.45-1.39) | 0.422 | 0.79 (0.45-1.39) | 0.418 |
| TC + CC      | 185 39.4                | 226 42.6                  | 0.88 (0.68-1.13) | 0.322 | 0.88 (0.68-1.14) | 0.328 |
| TT + TC      | 447 95.1                | 500 94.2                  | 1.00        | -    | -                 | -    |
| CC           | 23 4.9                  | 31 5.8                    | 0.83 (0.48-1.44) | 0.505 | 0.83 (0.47-1.44) | 0.498 |
| T allele     | 732 77.9                | 805 75.8                  | 1.00        | -    | -                 | -    |
| C allele     | 208 22.1                | 257 24.2                  | 0.89 (0.72-1.10) | 0.273 | -                 | -    |
| rs1127379 T/C |                        |                           |             |      |                   |      |
| TT           | 120 25.6                | 146 27.5                  | 1.00        | -    | -                 | -    |
| TC           | 234 49.9                | 265 49.9                  | 1.07 (0.80-1.45) | 0.639 | 1.08 (0.80-1.45) | 0.624 |
| CC           | 115 24.5                | 120 22.6                  | 1.18 (0.83-1.67) | 0.368 | 1.18 (0.83-1.68) | 0.358 |
| TC + CC      | 349 74.4                | 385 72.5                  | 1.11 (0.84-1.47) | 0.485 | 1.11 (0.84-1.47) | 0.471 |
| TT + TC      | 354 75.5                | 411 77.4                  | 1.00        | -    | -                 | -    |
| CC           | 115 24.5                | 120 22.6                  | 1.12 (0.84-1.50) | 0.441 | 1.12 (0.84-1.51) | 0.436 |
| T allele     | 474 50.5                | 557 52.4                  | 1.00        | -    | -                 | -    |
| C allele     | 464 49.5                | 505 47.6                  | 1.08 (0.91-1.29) | 0.392 | -                 | -    |
| rs61735963 C/T |                       |                           |             |      |                   |      |
| CC           | 403 85.7                | 444 83.6                  | 1.00        | -    | -                 | -    |
| CT           | 64 13.6                 | 83 15.6                   | 0.85 (0.60-1.21) | 0.359 | 0.85 (0.60-1.21) | 0.358 |
| TT           | 3 0.6                   | 4 0.8                     | 0.82 (0.18-3.71) | 0.801 | 0.82 (0.18-3.68) | 0.793 |
| CT + TT      | 67 14.3                 | 87 16.4                   | 0.85 (0.60-1.20) | 0.345 | 0.85 (0.60-1.20) | 0.348 |
| CC + CT      | 467 99.4                | 527 99.2                  | 1.00        | -    | -                 | -    |
| TT           | 3 0.6                   | 4 0.8                     | 0.85 (0.19-3.80) | 0.827 | 0.84 (0.19-3.77) | 0.819 |
| C allele     | 870 92.6                | 971 91.4                  | 1.00        | -    | -                 | -    |
| T allele     | 70 7.4                  | 91 8.6                    | 0.86 (0.62-1.19) | 0.357 | -                 | -    |
| rs1799986 C/T |                       |                           |             |      |                   |      |
| CC           | 287 61.1                | 282 53.1                  | 1.00        | -    | -                 | -    |
| CT           | 160 34.0                | 203 38.2                  | 0.77 (0.59-1.01) | 0.055 | 0.77 (0.59-1.01) | 0.056 |
| TT           | 23 4.9                  | 46 8.7                    | 0.49 (0.29-0.83) | 0.008 | 0.49 (0.29-0.83) | 0.008 |
| CT + TT      | 183 38.9                | 249 46.9                  | 0.72 (0.56-0.93) | 0.011 | 0.72 (0.56-0.93) | 0.011 |
| CC + CT      | 447 95.1                | 485 91.3                  | 1.00        | -    | -                 | -    |
| TT           | 23 4.9                  | 46 8.7                    | 0.54 (0.32-0.91) | 0.020 | 0.54 (0.32-0.91) | 0.021 |
| C allele     | 734 78.1                | 767 72.2                  | 1.00        | -    | -                 | -    |
| T allele     | 206 21.9                | 295 27.8                  | 0.73 (0.60-0.90) | 0.003 | -                 | -    |

Note: Bold values are statistically significant (\(P < .05\)).

\(^a\)The genotyping was successful in 470 cases and 531 controls for rs2908004, rs10795550, rs61735963 and rs1799986; the genotyping was successful in 469 cases and 531 controls for rs1127379.

\(^b\)Adjust for age and sex.
suggested as susceptibility factors for OA development. Some SNPs of Wnt-related genes influence the genetic predisposition to OA. Therefore, 13 studies changed their focus to the association of polymorphisms in pathway-related genes with risk of OA. Careful reviewing suggests these 13 articles involved 20 genes and 127 SNPs. Twelve of the 127 SNPs were associated with OA among Caucasians. However, no study uncovered any significant association between these SNPs and OA risk among Asians.

As reported, WNT16 supported the homeostasis of progenitor cells in OA, while WNT16‐deficient mice developed more severe OA with lubricin downregulation and increased chondrocyte apoptosis. The expression of inter-alpha inhibitor H5 (ITIH5) differed significantly between OA synovial tissues and healthy tissues. Lipoprotein receptor-related protein 1 (LRP1) could inhibit tumour necrosis factor (TNF)‐α‐induced apoptosis and inflammation in chondrocytes. The reduced expression of secreted frizzled related protein 1 (SFRP1) increased the activation of the Wnt/β‐catenin signalling and rendered the articular cartilage prone to premature.

### TABLE 5

| Variable | Case/control | Recessive model | Homozygous model | Heterozygous model | Dominant model |
|----------|--------------|-----------------|------------------|--------------------|---------------|
|          | GG | GA | AA | GA vs. GG | AA vs. GA | AA vs. GA + GG | AA + GA vs.GG |
| Sex      | Male | 69/71 | 108/121 | 36/56 | 0.93 (0.61‐1.41); 0.721 | 0.66 (0.39‐1.13); 0.129 | 0.69 (0.44‐1.11); 0.124 | 0.84 (0.57‐1.25); 0.397 |
|          | Female | 89/67 | 120/144 | 48/72 | 0.63 (0.42‐0.94); 0.022 | 0.50 (0.31‐0.81); 0.005 | 0.67 (0.45‐1.02); 0.060 | 0.59 (0.40‐0.85); 0.005 |
| Smoking  | Yes | 71/69 | 104/121 | 26/53 | 0.84 (0.55‐1.29); 0.426 | 0.48 (0.27‐0.85); 0.012 | 0.53 (0.32‐0.88); 0.015 | 0.73 (0.49‐1.09); 0.125 |
|          | No | 87/69 | 124/144 | 58/75 | 0.68 (0.46‐1.02); 0.060 | 0.61 (0.39‐0.98); 0.040 | 0.78 (0.53‐1.16); 0.216 | 0.66 (0.45‐0.96); 0.028 |
| Alcohol  | Yes | 76/62 | 111/124 | 45/53 | 0.68 (0.45‐1.04); 0.073 | 0.64 (0.38‐1.08); 0.093 | 0.81 (0.52‐1.27); 0.366 | 0.67 (0.45‐0.99); 0.047 |
|          | No | 82/76 | 117/141 | 39/75 | 0.83 (0.56‐1.24); 0.362 | 0.52 (0.32‐0.86); 0.011 | 0.59 (0.38‐0.90); 0.015 | 0.72 (0.49‐1.06); 0.092 |
| Age (y)  | <60 | 97/80 | 123/157 | 54/72 | 0.65 (0.45‐0.95); 0.026 | 0.62 (0.39‐0.98); 0.041 | 0.80 (0.54‐1.20); 0.284 | 0.64 (0.45‐0.91); 0.014 |
|          | ≥60 | 61/58 | 105/108 | 30/56 | 0.92 (0.59‐1.45); 0.731 | 0.51 (0.29‐0.90); 0.021 | 0.54 (0.33‐0.88); 0.013 | 0.78 (0.51‐1.20); 0.259 |
| BMI      | <25 | 74/66 | 119/139 | 45/61 | 0.77 (0.51‐1.16); 0.212 | 0.66 (0.40‐1.09); 0.107 | 0.78 (0.51‐1.20); 0.260 | 0.74 (0.50‐1.09); 0.123 |
|          | ≥25 | 84/72 | 109/126 | 39/67 | 0.74 (0.49‐1.11); 0.149 | 0.50 (0.30‐0.83); 0.007 | 0.60 (0.38‐0.93); 0.022 | 0.66 (0.45‐0.96); 0.031 |
| Rs1799986 | CC | CT | TT | TT vs. CC | TT vs. CC | TT vs. CT + CC | TT + CT vs.CC |
| Sex      | Male | 123/127 | 79/94 | 11/26 | 0.86 (0.58‐1.27); 0.450 | 0.43 (0.21‐0.92); 0.028 | 0.46 (0.22‐0.96); 0.038 | 0.77 (0.53‐1.11); 0.162 |
|          | Female | 164/155 | 81/109 | 12/20 | 0.70 (0.49‐1.10); 0.056 | 0.57 (0.27‐1.20); 0.138 | 0.65 (0.31‐1.35); 0.246 | 0.68 (0.48‐0.96); 0.030 |
| Smoking  | Yes | 115/134 | 75/86 | 10/23 | 0.63 (0.44‐0.89); 0.010 | 0.49 (0.24‐0.99); 0.048 | 0.50 (0.30‐1.08); 0.078 | 0.90 (0.62‐1.32); 0.592 |
|          | No | 172/148 | 85/117 | 13/23 | 1.01 (0.68‐1.50); 0.966 | 0.50 (0.23‐1.10); 0.086 | 0.58 (0.29‐1.18); 0.132 | 0.60 (0.43‐0.85); 0.003 |
| Alcohol  | Yes | 148/128 | 78/94 | 12/17 | 0.71 (0.49‐1.04); 0.082 | 0.61 (0.28‐1.32); 0.206 | 0.69 (0.32‐1.48); 0.340 | 0.70 (0.48‐1.00); 0.052 |
|          | No | 139/154 | 82/109 | 11/29 | 0.83 (0.58‐1.20); 0.331 | 0.42 (0.20‐0.87); 0.020 | 0.45 (0.22‐0.92); 0.030 | 0.75 (0.53‐1.06); 0.101 |
| Age (y)  | <60 | 174/164 | 88/121 | 13/24 | 0.68 (0.48‐0.97); 0.031 | 0.51 (0.25‐1.03); 0.060 | 0.59 (0.29‐1.18); 0.134 | 0.65 (0.47‐0.91); 0.012 |
|          | ≥60 | 113/118 | 72/82 | 10/22 | 0.92 (0.61‐1.38); 0.677 | 0.48 (0.22‐1.05); 0.065 | 0.49 (0.23‐1.07); 0.072 | 0.82 (0.56‐1.21); 0.326 |
| BMI      | <25 | 142/136 | 84/111 | 12/19 | 0.72 (0.50‐1.04); 0.080 | 0.60 (0.28‐1.28); 0.188 | 0.69 (0.33‐1.45); 0.324 | 0.70 (0.49‐1.00); 0.050 |
|          | ≥25 | 145/146 | 76/92 | 11/27 | 0.83 (0.57‐1.22); 0.343 | 0.41 (0.20‐0.86); 0.018 | 0.44 (0.21‐0.91); 0.026 | 0.74 (0.51‐1.06); 0.095 |

Note: Bold values are statistically significant (P < .05).
bone mineral density (BMD), cortical bone thickness and osteoporotic fracture risk.\textsuperscript{23} Radiographic OA was associated with high BMD and increased rate of bone loss.\textsuperscript{24} WNT16 rs2908004 polymorphism was associated with different OA phenotypes (atrophic or hypertrophic).\textsuperscript{25} Our results indicate WNT16 rs2908004 polymorphism conferred susceptibility to OA by comparing genotype and allele

| TABLE 6 | The associations between rs2908004/rs1799986 polymorphism and clinical characteristics of osteoarthritis |
|---------|---------------------------------------------------------------|
| Characteristics | Genotype distributions | ESR | CRP | K-L grade | VAS | Lequesnes’ index |
| Rs2908004 | | ≤10/≤10 | ≥25/≥25 | III + IV/I + II | ≥6/<6 | ≥12/<12 |
| GG | 61/97 | 15/143 | 133/25 | 151/7 | 153/5 | 111/176 |
| GA | 97/131 | 10/218 | 170/58 | 213/15 | 218/10 | 70/90 |
| AA | 32/52 | 3/81 | 59/25 | 77/7 | 81/3 | 8/15 |
| GA + AA | 129/183 | 13/299 | 229/83 | 290/22 | 299/13 | 78/105 |
| OR (95%CI); P-value | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) | 1.0 (reference) |
| ESR | 1.18 (0.78–1.78); .439 | 0.44 (0.19–1.00); .054 | 0.55 (0.33–0.93); .024 | 0.66 (0.26–1.65); .371 | 0.71 (0.24–2.13); .542 | 1.23 (0.83–1.83); .295 |
| CRP | 0.98 (0.57–1.69); .938 | 0.35 (0.10–1.26); .095 | 0.44 (0.24–0.84); .011 | 0.51 (0.17–1.51); .216 | 0.88 (0.21–3.79); .866 | 0.85 (0.35–2.06); .712 |
| K-L grade | 1.12 (0.76–1.65); .568 | 0.41 (0.19–0.89); .021 | 0.52 (0.32–0.85); .009 | 0.61 (0.26–1.46); .265 | 0.75 (0.26–2.15); .593 |
| VAS | | | | | | |
| ≥6/<6 | 0.66 (0.26–1.65); .371 | 0.51 (0.17–1.51); .216 | 0.88 (0.21–3.79); .866 | 0.61 (0.26–1.46); .265 |
| Lequesnes’ index | | | | | | |
| ≥12/<12 | 0.71 (0.24–2.13); .542 | 0.88 (0.21–3.79); .866 | 0.75 (0.26–2.15); .593 |
| OR (95%CI); P-value | 0.44 (0.19–1.00); .054 | 0.35 (0.10–1.26); .095 | 0.41 (0.19–0.89); .021 |
| ESR | 0.98 (0.57–1.69); .938 | 0.35 (0.10–1.26); .095 | 0.41 (0.19–0.89); .021 |
| CRP | 0.51 (0.17–1.51); .216 | 0.52 (0.32–0.85); .009 |
| K-L grade | 0.61 (0.26–1.46); .265 |
| VAS | | | |
| ≥12/<12 | 0.88 (0.21–3.79); .866 | 0.75 (0.26–2.15); .593 |
| Lequesnes’ index | | | |
| ≥6/<6 | 0.66 (0.26–1.65); .371 | 0.51 (0.17–1.51); .216 |

Note: Bold values are statistically significant (\( P < .05 \)).

| TABLE 7 | The association of genetic risk score of Wnt signalling pathway-related genes with risk of osteoarthritis |
|---------|---------------------------------------------------------------|
| Variable | Case (n = 471) | Control (n = 532) | OR (95% CI) | P value | Adjusted OR (95% CI) | P value |
| Weighted GRS | N (%) | N (%) | | | | |
| First (−0.5,0) | 311 (66.9%) | 287 (53.9%) | 1 | 1 |
| Second (−1.0, −0.5) | 149 (31.6%) | 216 (40.6%) | 0.64 (0.49,0.83) | .001 | 0.63 (0.49,0.82) | .001 |
| Third (−1.5, −1.0) | 5 (1.1%) | 24 (4.5%) | 0.19 (0.07,0.51) | .001 | 0.19 (0.07,0.51) | .001 |

Note: The genotyping was successful in 465 cases and 527 controls. Adjusted for sex and age. Bold values are statistically significant (\( P < .05 \)).
distribution between cases and controls. LRP1 dictates physiological and pathological catabolism of aggrecan by regulating extracellular activity of ADAMTS5. Inhibited shedding of low-density LRP1 reversed the cartilage matrix degradation in OA. The role of LRP1 rs1799986 polymorphism was studied in various diseases, including cardiovascular disease and Alzheimer’s disease (AD). LRP1 rs1799986 polymorphism was associated with lower risk of AD among Asians and could reduce risk of late onset of AD, as revealed by a meta-analysis containing 6455 AD cases and 6304 controls. However, little is known about their association with OA susceptibility. We found TT genotype or T allele significantly increased risk of OA in a Chinese population. Furthermore, the mRNA expression of WNT16 or LRP1 in mutant genotype carriers was significantly lower than that of wild genotype carriers. We hypothesized that rs2908004 or rs1799986 polymorphism conferred susceptibility to OA by influencing its production and function.

Several limitations of this study should be addressed. First, other confounders (eg occupation and diet) were not considered. Second, we recruited cases and controls only from hospitals, which may lead to selection bias during the collection process. Third, we may obtain the false-negative or false-positive results due to limited sample size. Fourth, two positive SNPs in exons may affect the protein expression, leading to OA susceptibility. However, this was not verified by experiments.

This study confirms that WNT16 rs2908004 polymorphism plays a protective role in the pathology of knee OA. Additionally, LRP1 rs1799986 polymorphism is associated with a decreased risk of OA. Nevertheless, larger-size and well-designed case-control studies among other populations are warranted to identify the association between these polymorphisms and knee OA susceptibility.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
YH, XZ and LJ carried out the main experiments and statistical analysis and prepared the manuscript. HY and LW designed the study and prepared the manuscript. YH and XZ wrote the main protocol and prepared the manuscript. YH, JL and NX supervised the study and prepared the manuscript. All authors contributed to and have approved the final manuscript.

ORCID
Yong Huang https://orcid.org/0000-0003-1944-3656
Lidong Wu https://orcid.org/0000-0002-2561-6069
Jin Li https://orcid.org/0000-0002-0144-4688

REFERENCES
1. Xia B, Di C, Zhang J, et al. Osteoarthritis pathogenesis: a review of molecular mechanisms. *Calcif Tissue Int*. 2014;95:495-505.
2. Zhou X, Jiang L, Zhang Y, et al. Genetic variation of aggrecanase-2 (ADAMTS5) in susceptibility to osteoarthritis. *Braz J Med Biol Res*. 2019;52:e8109.
3. De Filippis L, Gulli S, Caliri A, et al. Epidemiology and risk factors in osteoarthritis: literature review data from “OASIS” study. *Reumatismo*. 2004;56:169-184.
4. Brandt KD, Dieppe P, Radin E. Etiopathogenesis of osteoarthritis. *Med Clin North Am*. 2009;93(1):1-24.
5. Chen X, Zhang L, Liang D, et al. Lipid Transporter Activity-Related Genetic Polymorphisms Are Associated With Steroid-Induced Osteonecrosis of the Femoral Head: An Updated Meta-Analysis Based on the GRADE Guidelines, *Front Physiol*. 2018;9:1684.
6. Sun G, Ba CL, Gao R, et al. Association of IL-6, IL-8, MMP-13 gene polymorphisms with knee osteoarthritis susceptibility in the Chinese Han population. *Biosci Rep*. 2019;39(2):BSR20181346.
7. Saito-Diaz K, Chen TW, Wang X, et al. The way Wnt works: components and mechanism. *Growth Factors*. 2013;31:1-31.
8. Zhou X, Lin X, Xiong Y, et al. Chondroprotective effects of palmitine on osteoarthritis in vivo and in vitro: A possible mechanism of inhibiting the Wnt/beta-catenin and Hedgehog signaling pathways. *Int Immunopharmacol*. 2016;34:129-138.
9. Blom AB, Brockbank SM, van Lent PL, et al. Involvement of the Wnt signaling pathway in experimental and human osteoarthritis: prominent role of Wnt-induced signaling protein 1. *Arthritis Rheum*. 2009;60:501-512.
10. Loeser RF. Osteoarthritis year in review 2013: biology. *Osteoarthritis and Cartilage*. 2013;21:1436-1442.
11. Ochoa-Hernandez AB, Juarez-Vazquez CI, Rosales-Reynoso MA, et al. WNT-beta-catenin signaling pathway and its relationship with cancer. Cir Cir. 2012;80:389-398.

12. Skłarczyk D, Morris JH, Cook H, et al. The STRING database in 2017: quality-controlled protein-protein association networks, made broadly accessible. Nucleic Acids Res. 2017;45:D362-D368.

13. Ding J, Zhang Y. Analysis of key GO terms and KEGG pathways associated with carcinogenic chemicals. Comb Chem High Throughput Screen. 2017.

14. Silman AJ. The 1987 revised American Rheumatism Association criteria for rheumatoid arthritis. Br J Rheumatol. 1988;27:341-343.

15. Usami Y, Gunawardena AT, Iwamoto M, et al. Wnt signaling in cartilage development and diseases: lessons from animal studies. Lab Invest. 2016;96:186-196.

16. Zhou Y, Wang T, Hamilton JL, et al. Wnt/beta-catenin signaling in osteoarthritis and in other forms of arthritis. Curr Rheumatol Rep. 2017;19:53.

17. Nalesso G, Thomas BL, Sherwood JC, et al. WNT16 antagonises excessive canonical WNT activation and protects cartilage in osteoarthritis. Ann Rheum Dis. 2017;76:218-226.

18. Conde J, Scotece M, Abella V, et al. Identification of novel adipokines in the joint. Differential expression in healthy and osteoarthritis tissues. PLoS ONE. 2015;10:e0123601.

19. Yang E, Zheng H, Peng H, et al. Lentivirus-induced knockdown of LRP1 induces osteoarthritic-like effects and increases susceptibility to apoptosis in chondrocytes via the nuclear factor-kappaB pathway. Exp Ther Med. 2015;10:97-105.

20. Pasold J, Osterberg A, Peters K, et al. Reduced expression of Sfrp1 during chondrogenesis and in articular chondrocytes correlates with osteoarthritis in STR/ort mice. Exp Cell Res. 2013;319:649-659.

21. Garcia-Ibarbia C, Neila S, Garces C, et al. Non-synonymous WNT16 polymorphisms alleles are associated with different osteoarthritis phenotypes. Rheumatol Int. 2017;37:1667-1672.

22. Wang Y, Liu S, Wang J, et al. Association between LRP1 C766T polymorphism and Alzheimer's disease susceptibility: a meta-analysis. Sci Rep. 2017;7:8435.

23. Zheng HF, Tobias JH, Duncan E, et al. WNT16 influences bone mineral density, cortical bone thickness, bone strength, and osteoporotic fracture risk. PLoS Genet. 2012;8:e1002745.

24. Burger H, van Daele PL, Odding E, et al. Association of radiographically evident osteoarthritis with higher bone mineral density and increased bone loss with age. The Rotterdam Study. Arthritis Rheum. 1996;39:81-86.

25. Yamamoto K, Troeberg L, Scilabra SD, et al. LRP-1-mediated endocytosis regulates extracellular activity of ADAMTS-5 in articular cartilage. FASEB J. 2013;27:511-521.

26. Yamamoto K, Santamaria S, Botkjaer KA, et al. Inhibition of shedding of low-density lipoprotein receptor-related protein 1 reverses cartilage matrix degradation in osteoarthritis. Arthritis Rheumatol. 2017;69:1246-1256.

27. Aledo R, Alonso R, Mata P, et al. LRP1 gene polymorphisms are associated with premature risk of cardiovascular disease in patients with familial hypercholesterolemia. Rev Esp Cardiol (Engl Ed). 2012;65:807-812.

How to cite this article: Huang Y, Jiang L, Yang H, et al. Variations of Wnt/beta-catenin pathway-related genes in susceptibility to knee osteoarthritis: A three-centre case-control study. J Cell Mol Med. 2019;23:8246–8257. https://doi.org/10.1111/jcmm.14696