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Obesity, osteoarthritis and genetic risk

THE RS182052 POLYMORPHISM IN THE ADIPOQ GENE IS POTENTIALLY ASSOCIATED WITH RISK OF KNEE OSTEOARTHRITIS

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Objectives
Given the function of adiponectin (ADIPOQ) on the inflammatory condition of obesity and osteoarthritis (OA), we hypothesized that the ADIPOQ gene might be a candidate gene for a marker of susceptibility to OA.

Methods
We systematically screened three tagging polymorphisms (rs182052, rs2082940 and rs6773957) in the ADIPOQ gene, and evaluated the association between the genetic variants and OA risk in a case-controlled study that included 196 OA patients and 442 controls in a northern Chinese population. Genotyping was performed using the Sequenom MassARRAY iPLEX platform.

Results
The single nucleotide polymorphism (SNP) rs182052 was found to be potentially associated with knee OA risk (additive model: odds ratio = 1.38; 95% confidence interval 1.07 to 1.76; p = 0.012). Furthermore, a non-significant association was observed for rs182052 and body mass index with regard to OA risk in interaction analyses (p = 0.063). Similarly, no significant interaction was detected for rs182052 and age with regard to OA risk (p = 0.614).

Conclusion
These findings suggest that the SNP rs182052 in the ADIPOQ gene may potentially modify individual susceptibility to knee OA in the Chinese population. Further studies are warranted to investigate our findings in more depth.

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Keywords: Osteoarthritis, Susceptibility, ADIPOQ, rs182052

Article focus
- There are no previous studies investigating the possibility that the single nucleotide polymorphism (SNP) in the adiponectin (ADIPOQ) gene may predispose patients to osteoarthritis (OA) development in an Asian population.
- We investigated the association between the SNP rs182052 in the ADIPOQ gene and OA in the Chinese population.

Key messages
- The SNP rs182052 in the ADIPOQ gene may potentially modify individual susceptibility to knee OA in the Chinese population.
- Further studies are warranted to investigate our findings.

Strengths and limitations
- This is the first report of several polymorphisms in the ADIPOQ gene and knee OA disease in a Chinese population.
- Our findings showed that rs182052 is potentially associated with knee OA.
- Analysis has also shown a borderline association regarding rs182052 SNP, body mass index and risk of OA in our study population.
- More studies need to be conducted with larger sample sizes and using different ethnic groups to validate and further investigate our findings.

Introduction
Osteoarthritis (OA) is the most common chronic joint disease in the elderly, and it is

predicted that it will be the single greatest cause of disability in the general population by 2030.[1] Similar increasing trends in the prevalence of OA are found in China. With ageing populations and a worldwide obesity epidemic, OA is regarded as a global public health issue. Studies show that OA is a multifactorial disease that is influenced by ageing, the environment, genetic predisposition and the interactions between them.[2-6]

There is substantial evidence highlighting the relationship between obesity and OA.[7-9] However, mechanical loading cannot explain the incidence and progression of OA in non-weight-bearing joints such as fingers and wrists.[4,10-12] This suggests probable complex mechanisms linking obesity and OA, including biomechanical, physiological and inflammatory.[13,14] Adipose tissue is well recognized as an active endocrine organ, releasing various adipokines such as adiponectin (ADIPOQ), leptin and visfatin, which are involved in complex biological interactions between fat and other tissues, and play a significant role in bone formation and bone absorption.[15,16] Obesity is one of the strongest predictive and prognostic factors for OA, particularly in knee joints, and, to a lesser extent, the hip.[17] Adiponectin, encoded by the ADIPOQ gene, is a specific protein secreted by adipose tissue.[18] First described in 1995, it has a modular structure consisting of a collagen-like N-terminal domain and a C-terminal globular domain that is similar to tumour necrosis factor-alpha (TNF-α).[19,20] Adiponectin is a complex molecule that plays an important role in the regulation of insulin sensitivity and glucose homeostasis, as well as lipid and fatty acid oxidation.[21] ADIPOQ gene expression has been shown to reduce the production of pro-inflammatory cytokines such as interleukin-6, interleukin-8 and TNF-α, and induce the release of anti-inflammatory cytokines. This suggests a contribution to the low-grade inflammatory state that exists in obesity.[22-24] Recently, ADIPOQ has been found to participate in the inflammatory process, and may trigger articular cartilage injury through the upregulation of cytokines, matrix-degrading enzymes and chemokines in both chondrocytes and synovial fibroblasts.[25-27] A recent case-controlled study reported that the ADIPOQ level positively correlated with disease severity in patients with knee OA.[28] ADIPOQ might be considered as a potentially effective biomarker for joint damage in OA.[29]

The human ADIPOQ gene is located on chromosome 3q27 and spans approximately 17 kb, consisting of three exons and two introns.[30] Previous studies have indicated that the ADIPOQ gene may be identified as a genetic region for phenotypes associated with various diseases, such as obesity, rheumatoid arthritis, diabetes and coronary heart disease.[31-34] However, the study of the relationship between ADIPOQ polymorphisms and OA has been limited.[35]

Obesity may have a shared genetic background with OA based on the well-established epidemiological link. Given the role of ADIPOQ in the inflammatory pathophysiology of obesity and its link to OA, we hypothesized that the ADIPOQ gene expression could modulate the level of ADIPOQ and might potentially be a candidate gene, as a marker for the susceptibility to OA. To the best of our knowledge, no studies on the north east Chinese population about the relationship between ADIPOQ gene variants and the risk of OA have been conducted. The objective of this study was to explore several ADIPOQ polymorphisms and investigate their genetic association with the susceptibility to OA among a Chinese population.

Materials and Methods

Study design and participants. A population-based case-controlled study was carried out to evaluate whether the polymorphism of ADIPOQ was associated with knee OA in Harbin City in Heilongjiang Province, northern China. With the assistance of the local community councils, residents who lived at their registered address during the study period, having permanent living records with Chinese Han nationality, were recruited. The participants were 40 years and over, and residents of the Hongqi community in the Xiangfan metropolitan district, and were recruited using stage-stratified sampling methods, and representing the middle standard of life in Harbin city. A total of 1636 participants completed a questionnaire and underwent clinical and radiological examination. Of these, 110 participants were excluded for the following reasons: no blood sample available (n = 36); no physical examination available (n = 29); and no radiological images available (n = 45). The remaining 1526 participants were classified into four groups based on their clinical and radiological knee status: Group 1) knee pain with radiologically defined OA (n = 196); Group 2) the control group having neither knee pain nor radiological evidence of knee OA (n = 442); Group 3) knee pain without OA, i.e. participants with knee pain but without radiologically defined knee OA (n = 195); and Group 4) radiologically defined knee OA but without knee pain (n = 693). The OA patients in Group 1 had definite symptomatic OA and radiological evidence in at least one knee joint.

The questionnaire was designed to collect data from all participants regarding basic demographics, occupation and sporting activities, previous knee injury, family history of OA and rheumatological manifestations.

Clinical symptoms were defined as significant when signs and symptoms of knee pain were present for at least one month's duration during the previous 12 months. The radiological assessment of OA was made using the Kellgren-Lawrence grading system.[36] Knee radiological images were evaluated independently by two experienced radiologists (JR and SW) who were blinded to patient presentation. Consensus was reached whenever results were divergent. Cases where there was uncertainty were recalled and re-examined by a specialist to ensure the validity. The control group had no signs or

symptoms of arthritis or joint disease. Patients with a previous knee injury and secondary OA, and patients with inflammatory disease or rheumatoid arthritis and developmental dysplasia, were excluded. All participants underwent a physical examination, and body mass index (BMI) (weight/height^2) was recorded. Height was measured to the nearest 0.1 cm using a wall-mounted stadiometer. Body weight was measured with a digital scale to the nearest 0.1 kg. The classification of obesity status was defined according to the criteria of the Working Group on Obesity in China, based on the analysis of data collected from 239 972 Chinese adults in the 1990s:[37] underweight and normal (BMI $\leqslant$ 24.0); overweight (BMI 24.0 to 28.0); and obese (BMI $\geqslant$ 28.0).

The project protocol was reviewed and approved by the Ethics Committee of Harbin Medical University, and written informed consent was obtained from each participant.

Single nucleotide polymorphism (SNP) selection. Public databases of the National Center for Biotechnology Information (NCBI) were used to collect information about SNPs and genes.[38] Based on the HapMap SNP database (phase II + III Feb 09, on NCBI B36 assembly, dbSNP b126) and Haploview 4.2 software,[39] common SNPs (minor allele frequency (MAF) $\geqslant$ 5% in the Chinese Han population) were screened in ADIPOQ gene regions. The context sequences of SNPs with low linkage disequilibrium (LD) analysis ($r^2 < 0.8$) were retained. As a result, three targeting SNPs (rs182052, rs2082940, rs6773957) were finally selected and further determined to perform genotyping assays.

Genotyping assays. Peripheral blood was collected from each subject following informed consent, and genomic DNA from cases and controls was isolated from peripheral blood lymphocytes. Genomic DNA was extracted from the samples with a DNA extraction kit (Qiagen Inc, Valencia, California). Further DNA concentration measures were obtained using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts). Polymerase chain reaction (PCR) was performed in a total reaction volume of 4 μl containing about 10 ng of genomic DNA. The PCR conditions depended on the requirements of each probe according to the manufacturer's indications. The genotype of each sample at this stage was performed using the iPLEX Sequenom MassARRAY platform (Sequenom Inc, San Diego, California).

The following series of methods were used to control the quality of genotyping: 1) case and control samples were mixed on each plate; 2) genotyping was performed blinded to case or control status for the clinical personnel; 3) 5% of the samples were randomly selected for repeat genotyping as blind duplicates and the reproducibility was 100%. As a quality control measure, we included one sample with no template, and one sample duplicate per 96-well plate (a total of four per 384-well plate used). SDS 2.3 Allelic Discrimination Software (Applied Biosystems, Foster City, California) was employed to determine genotypes. Genotypes were provided automatically by the software, and then were determined manually by two different people in the laboratory.

Statistical analysis. The chi-squared test for categorical variables and Student's *t*-test for continuous variables were used to analyze for differences in demographic characteristics, the selected variables and genotypes between cases and controls. The Hardy-Weinberg equilibrium (HWE) for the distribution of each SNP was evaluated using the goodness-of-fit χ^2 test, by comparing the observed genotype frequencies with the expected ones. Genotype-related odds ratios (ORs), their corresponding 95% confidence intervals (CIs) and associated p-values were estimated via unconditional logistic regression. This was calculated for an additive model (minor allele homozygotes *versus* heterozygotes *versus* major allele homozygotes) with adjustment for age, gender, BMI, occupation and physical activity. An association study is designed specifically to reveal associations that depend additively upon the minor allele. That is, the genotype of the individual that has two minor alleles (variant homozygote), rather than having no minor alleles (wild-type homozygote), is twice as likely to affect the outcome in a certain direction as just one minor allele (heterozygote) rather than no minor alleles (wild-type homozygote). The Bonferroni correction was used for multiple comparisons, which was a safeguard against multiple tests of statistical significance on the same data. For this study, three tagging SNPs on the ADIPOQ gene were genotyped, thus the associations between ADIPOQ SNPs and OA risk with p-values < 0.017 (0.05/3) were considered significant after correction for multiple testing. To examine the differences between subgroups, the chi-squared (χ^2)-based Q-test was used to test the heterogeneity of effect sizes (ORs and 95% CIs) derived from corresponding subgroups. All p-values were two-sided, and those less than 0.05 were considered statistically significant. STATA (version 13.1; StataCorp, College Station, Texas) was used for all the statistical analyses.

Results

The basic demographic data of the 196 cases in group 1 with symptoms and radiological changes of OA and the 442 controls in group 2 are summarized in Table I. There was no significant difference detected in gender between OA cases and controls ($\chi^2 = 3.173$, $p = 0.090$). The mean age of cases was significantly higher than that of controls ($t = 6.486$, $p < 0.001$). The distribution of BMI among cases and control is significantly different ($\chi^2 = 8.465$, $p = 0.015$), and OA cases possessed a higher frequency of being overweight (39.29%) and obese (26.02%) compared with the control group (34.84% overweight and 18.78% obese). In terms of smoking and drinking status,

Table I. Distribution of selected variables in group 1 osteoarthritis cases and group 2 controls

Variables	Cases (n = 196)	Control (n = 442)	Test	p-value
Mean age, yrs (SD)	62.19 (8.76)	57.17 (9.19)	t = 6.486	< 0.001
< 57, n (%)*	56 (*28.57*)	213 (*48.19*)	χ^2 = 21.432	< 0.001
⩾ 57, n (%)*	140 (*71.43*)	229 (*51.81*)		
Gender, n (%)			χ^2 = 3.173	0.090
Male	48 (*24.49*)	139 (*31.45*)		
Female	148 (*75.51*)	303 (*68.55*)		
Body mass index (BMI), n (%)			χ^2 = 8.465	0.015
< 24 kg/m^2	68 (*34.69*)	205 (*46.38*)		
24 kg/m^2 ⩽ BMI < 28 kg/m^2	77 (*39.29*)	154 (*34.84*)		
⩾ 28 kg/m^2	51 (*26.02*)	83 (*18.78*)		
Smoking status, n (%)			χ^2 = 0.994	0.330
Ever	46 (*23.47*)	119 (*27.23*)		
Never	150 (*76.53*)	318 (*72.77*)		
Drinking status, n (%)			χ^2 = 1.379	0.254
Ever	52 (*27.81*)	134 (*32.60*)		
Never	135 (*72.19*)	277 (*67.40*)		
Occupation, n (%)†			χ^2 = 1.479	0.224
Managerial	85 (*44.50*)	218 (*49.77*)		
Non-managerial	106 (*55.50*)	220 (*50.22*)		
Physical activity, n (%)‡			χ^2 = 3.286	0.090
Inactive	68 (*34.69*)	177 (*40.04*)		
Less active	78 (*39.80*)	185 (*41.86*)		
More active	50 (*25.51*)	80 (*18.10*)		

*Median age in control group
†The non-managerial occupations: Service; Farming, Forestry and Fishing; Precision Production, Craft and Repair; Operators, Fabricators and Labourers. The managerial occupations: Managerial and Professional; Technical, Sales and Administrative Support
‡Inactive, no reported activity per week; less active, one to four times per week; more active, five or more times per week
χ^2, chi-squared

no significant difference was detected between the cases group and the control group (χ^2 = 0.994, p = 0.330 for smoking; χ^2 = 0.1.379, p = 0.254 for drinking). Also, no significant difference was checked for occupation or for physical activity between the cases group and the control group (χ^2 = 1.479, p = 0.224 for occupation; χ^2 = 3.286, p = 0.090 for physical activity).

The results for the three SNPs are shown in Supplementary table i. Genotyping success rates for all polymorphisms were greater than 98%, and the observed genotype frequencies for these SNPs in the control group were all in agreement with HWE (p > 0.05). Logistic regression analyses were performed for the three SNPs (Table II). It showed that the variant ADIPOQ rs182052 was potentially associated with OA risk. Those individuals with the genotype GA *versus* GG and those with genotype AA compared with GA in rs182052 tended to present a higher risk of OA, and the allele A could increase the OA risk (additive model: OR = 1.38; 95% CI 1.07 to 1.76; p = 0.012). The associations of rs182052 and risk of OA remained significant after correction for multiple testing (n = 3) with p < 0.017. We found no evidence of any significant association between the remaining two SNPs and OA. That is, no statistical difference was found between the association of SNP rs2082940 and OA (additive model: OR = 1.16; 95% CI 0.87 to 1.55; p = 0.303), nor SNP rs6773957 and OA (additive model: OR = 1.03; 95% CI 0.80 to 1.33; p = 0.819) (Supplementary table i).

Further evaluation of the association between rs182052 and knee OA was performed using stratification of age and BMI (Table III). Significant associations between rs182052 and knee OA were found in subjects aged ⩾ 57 years (OR = 1.44; 95% CI 1.07 to 1.93; p = 0.015). Significant associations between rs182052 and knee OA were also detected in subjects with a BMI < 24 (OR = 1.69; 95% CI 1.14 to 2.50; p = 0.009). In addition, no significant heterogeneity was observed among the stratified subgroups of age and BMI (p = 0.666 and 0.321, respectively).

As well as the above stratification, we further explored gene-factor interaction in the identified SNP rs182052. Considering the significant differences in distribution of age and BMI between case and control groups, we further investigated whether the effect of rs182052 on OA risk was modified by age and BMI. Collectively, interaction analyses failed to detect any significant association between rs182052 and BMI on OA risk (multiple interaction p = 0.063) (Table IV). That is, among those individuals with BMI < 24, a combination with GA/AA genotype had a higher chance of developing OA (OR = 2.69; 95% CI 1.33 5.42; p = 0.006). Whereas, among those individuals with BMI ⩾ 24, a combination with the GG genotype presented a higher chance of developing OA (OR = 3.01; 95% CI 1.43 6.34; p = 0.004), and a combination with GA/AA genotype also tended to be more likely to develop OA (OR = 3.59; 95% CI 1.82 7.07; p < 0.001). As for the interaction of identified SNP

Table II. Associations between rs182052 in the adiponectin gene and knee osteoarthritis risk

SNPs	Genotypes	Case	Control	Crude OR (95% CI)	p-value	Adjusted OR (95% CI)*	p-value*
rs182052	GG	49	150	1		1	
	GA	95	204	1.43 (0.95 to 2.14)	0.085	1.46 (0.95 to 2.23)	0.081
	AA	50	84	1.82 (1.13 to 2.93)	0.013	1.88 (1.14 to 3.10)	0.013
	GA/AA	145	288	1.54 (1.05 to 2.25)	0.025	1.58 (1.06 to 2.36)	0.023
	Additive			1.35 (1.07 to 1.71)	0.012	1.38 (1.07 to 1.76)	0.012
rs2082940	CC	95	236	1		1	
	CT	85	168	1.26 (0.88 to 1.79)	0.205	1.31 (0.90 to 1.90)	0.157
	TT	12	33	0.90 (0.45 to 1.82)	0.777	1.09 (0.52 to 2.27)	0.824
	CT/TT	97	201	1.20 (0.85 to 1.68)	0.295	1.27 (0.89 to 1.82)	0.184
	Additive			1.09 (0.83 to 1.42)	0.552	1.16 (0.87 to 1.55)	0.303
rs6773957	AA	50	128	1		1	
	AG	104	216	1.23 (0.82 to 1.84)	0.308	1.09 (0.71 to 1.66)	0.691
	GG	40	90	1.14 (0.69 to 1.87)	0.610	1.05 (0.63 to 1.77)	0.843
	AG/GG	144	306	1.20 (0.82 to 1.77)	0.340	1.08 (0.72 to 1.61)	0.710
	Additive			1.08 (0.84 to 1.37)	0.549	1.03 (0.80 to 1.33)	0.819

*Logistic regression with adjustment for age, gender, body mass index, occupation and physical activity in additive model
SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval

Table III. Stratified analysis on the associations of ADIPOQ rs182052 in osteoarthritis risk

Characteristics	Case*	Control*	OR (95% CI)†	p-value†	p-value het‡
Age, yrs					
< 57	13/28/13	65/106/40	1.28 (0.82 to 2.00)	0.275	0.666
⩾ 57	36/67/37	85/98/44	1.44 (1.07 to 1.93)	0.015	
Body mass index (BMI), kg/m²					
< 24	12/36/20	72/89/43	1.69 (1.14 to 2.50)	0.009	0.321
24 ⩽ BMI < 28	21/36/18	50/70/31	1.09 (0.72 to 1.65)	0.675	
⩾ 28	16/23/12	28/45/10	1.40 (0.82 to 2.39)	0.214	

*Wild-type homozygote/heterozygote/variant homozygote
†Adjusted for age, gender, BMI, occupation and physical activity where appropriate in additive model
‡p for heterogeneity
OR, odds ratio; CI, confidence interval

Table IV. The interaction between rs182052 genotypes and body mass index (BMI) on knee osteoarthritis (OA) risk

BMI (kg/m²)	Genotype	Case	Control	OR (95% CI)	p-value*
< 24	GG	12	72	1	
< 24	GA/AA	56	132	2.69 (1.33 to 5.42)	0.006
⩾ 24	GG	37	78	3.01 (1.43 to 6.34)	0.004
⩾ 24	GA/AA	89	156	3.59 (1.82 to 7.07)	< 0.001
p for multiplicative interaction					0.063

*p-value of interaction analysis between rs182052 and BMI on knee OA risk with adjustment for age, gender, occupation and physical activity
OR, odds ratio; CI, confidence interval

rs182052 and age on OA risk, the similar pattern of non-significant interaction was also detected (multiple interaction p = 0.614) (Supplementary table ii).

Discussion

Osteoarthritis is a complex disease, arising from the interaction of multiple factors including individual genetic factors and environmental factors. Epidemiological studies have shown a strong genetic component to the susceptibility to OA.[2] Growing evidence indicates that the release of additional adipokines may be responsible for the increase of OA observed among obese people.[27,28] Obesity might play a critical role in the development and progression of OA.[40] From a pathophysiological perspective, there is increasing evidence to suggest that ADIPOQ plays an important role in the onset and progression of OA.[29] In our study, we systematically evaluated the association of three tagging polymorphisms in the ADIPOQ gene with OA risk in a case-controlled study of 196 OA cases and 442 controls in a northern Chinese population. The SNP rs182052 was identified to be significantly associated with knee OA susceptibility. However, we found no evidence of significant association between SNP rs2082940, rs6773957 and knee OA risk.

To the best of our knowledge, this is the first study to evaluate the association of several polymorphisms in the ADIPOQ gene and the risk of symptomatic knee OA in an Asian population. For rs182052, the A allele appeared to be one of the risk factors for knee OA in our study. Zhan et al[35] found no statistically significant difference between

ADIPOQ rs1501299 expression and knee OA. In a further study, the same authors showed that rs1501299 and rs2241766 of the ADIPOQ gene were not responsible for OA susceptibility among a Thai population.[41] The different results between the Thai studies and our own study population may be due to genetic backgrounds, gender ratios, population substructure and environmental effects. The sample size in our study was larger than in those previous studies. Furthermore, other factors such as age, gender, BMI, occupation and physical activity were adjusted for in our study, in order to detect the association between ADIPOQ and knee OA.

Our study has several strengths. First, we recruited knee OA cases and selected controls from a community which might better represent the whole population and reduce potential selection bias. Second, the focus on well-defined radiological and clinical features allows for a stringent definition of OA in the study group and may allow differences in gene expression to be observed with more certainty. Osteoarthritis phenotype definitions, reflecting different subsets of OA, have been shown to influence the ability to detect genetic associations.[42] Third, with our detailed investigation into whether the ADIPOQ gene is associated with OA, we systematically evaluated three different tagging polymorphisms in both knee OA cases and controls; the SNP 182052 polymorphism was identified as being associated with knee OA.

However, our study does have limitations. First, OA is a multifactorial disease with a strong genetic component, with various different estimates of influence of genetic factors depending on the joint involved. We only evaluated the SNP rs182052, rs2082940 and rs6773957 in the ADIPOQ gene and the risk of knee OA. Our results cannot be generalized to OA affecting other joints. Second, we only demonstrated an association. We are unable to show causation in terms of how this gene expression influences the process of OA. Third, due to the small sample size (a total of 196 OA cases and 442 control subjects), the statistical power is about 46.5% when detecting an effect size of 1.50 with an α level of 0.05 in relation to the association of the identified SNPs with OA risk, making our study underpowered. Well-conducted larger sample studies are needed to evaluate our findings. Given the modest sample size, especially of younger individuals ($<$ 57 years), and the low study power, our results should be interpreted with caution.

In conclusion, we have investigated the role of genetic variants in the ADIPOQ gene in knee OA within a north east Chinese population. Our results suggest that the variant A allele of rs182052 is associated with an increased risk of knee OA. Further studies are required to validate our findings, and investigate the possible pathophysiological processes that this gene expression may influence. Further studies could focus on gender-specific mechanisms and the aetiology of knee OA and elucidate whether the ADIPOQ gene could be targeted for future therapeutic strategies.

Supplementary material

Tables showing a summary of the three single nucleotide polymorphisms and interaction between rs182052 genotypes and age on knee osteoarthritis risk.

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- L. Jiang: Contribution to the research design and critical revisions, Review of the final version and approval for publication.
- X. Zhu: Writing the final paper, Review of the final version and approval for publication.
- J. Rong: In charge of evaluating knee radiological images, Diagnosing cases, Review of the final version and approval for publication.
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Conflict of Interest Statement

- None declared.