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Association of rs2234693 and rs9340799 polymorphisms of estrogen Receptor-1 gene with radiographic defined knee osteoarthritis: A meta-analysis

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ABSTRACT

Objective: To evaluate the association of ESR1 rs2234693 and rs9340799 polymorphisms with radiographic defined knee osteoarthritis (OA), a case-control and meta-analysis was performed.
Methods: A total of 25 case-control studies with 7,144 cases and 8,468 controls with were included.
Results: There was a significant association between rs2234693 polymorphism and radiographic knee OA under heterozygote model (CT vs. TT: OR = 1.164, 95% CI 1.053–1.286, p = 0.003). However, there was no association between rs9340799 and radiographic knee OA. In subgroup analysis by ethnicity, risk estimates were not augmented.
Conclusions: Our results showed that the ESR1 rs2234693 polymorphism might be associated with radiographic defined knee OA, but not rs9340799.

1. Introduction

Osteoarthritis (OA) is one of the main causes of functional limitation and reduced quality of life particularly in elderly worldwide.[1–3] Approximately 19% of American adults aged ≤45 years of age experience some symptoms of OA.[4] According to the epidemiological studies Asian populations had the highest of knee OA than Caucasians.[5] Although the mechanism of OA remains poorly understood, recent findings from molecular epidemiological studies suggest that genetic factors play a strong role in the pathophysiology of OA.[6] Moreover, age, ethnicity, gender are systemic risk factors for knee OA.[7]

Radiography is the most frequently used method for monitoring progression of OA, and useful if surgical intervention is planned or a fracture is suspected. It is allows for an estimation of the space between joints and large bony changes.[8] The Kellgren and Lawrence (K/L) system and Osteoarthritis Research Society International (OARSI) atlas are widely used to define the radiographic knee OA. The literature showed a weak correlation between radiographic OA and joint symptoms. Therefore, radiography cannot be used alone to evaluate OA patients.[9] Despite limitations, radiography is still remains the main method in epidemiology and research to define and measure OA. In addition, radiography has several advantages including widely available, has better defined outcome measures, takes less time and has a much lower cost.[10]

A modest but significant genetic effect in modulating the risk of radiographic knee OA has been reported in several studies.[6,11] In research settings, several case-control studies on the correlation between rs2234693 and rs9340799 polymorphisms of Estrogen Receptor 1 gene (ESR1) and radiographic defined knee OA had been performed. However, the results remain inconclusive and conflicting. A meta-analysis is a proper method to overcome the problem of small sample sizes and inadequate statistical power in individual studies. Therefore, we conducted this meta-analysis on all previous case-control studies to make a comprehensive and accurate assessment of the associations.

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2. Materials and methods

2.1. Case-control study

2.1.1. Subjects

A total of 90 patients with knee OA fulfilled radiographic criteria of OA and American College of Rheumatology (ACR) was recruited to this study between December 2016 and March 2018. Moreover, the control group was composed of 90 matched healthy individuals with no symptoms or signs on clinical examination or radiographic. The protocol for research work was approved by the Human Ethics Committee of Science and Art University and Islamic Azad University, Ashkezar branch, Yazd. Informed written consent was obtained from all the participants before they were enrolled in the study.

2.1.2. Genotyping

DNA was extracted from peripheral blood leukocytes using a commercial DNA Isolation kit following the manufacturer's protocol. The region containing ESR1 rs2234693 and rs9340799 polymorphisms was genotyping by RFLP-PCR assay with the following primers: forward 5′-CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3′ and 5′-TCTTTCTC TGCCACCCTGGCGTCGATTATCTGA-3'. These polymorphisms have been described at http://www.ncbi.nlm.nih.gov/SNP under identification numbers rs2234693 (c.454-397T > C) and rs9340799 (c.454-351A > G). The intron from the *ESR1* gene was amplified by PCR using 50 ng of the genomic DNA in a final reaction volume of 50 µl containing 5 µl of buffer, 0.2 mM dNTPs, 0.2 µM of each primer pair that spanned the polymorphic regions, 2 mM MgSO4, and Taq DNA Polymerase. PCR was carried out in 35 cycles by the following steps: denaturation at 94C for 30 s, annealing at 61C for 40 s, and extension at 37C for 90 s. The PCR products were digested by the PvuII and XbaI restriction endonucleases, respectively. Fragment analysis was performed in 1% agarose gel stained with ethidium bromide, and the bands were separated and photographed under ultraviolet light.

2.2. Meta-analysis

2.2.1. Study identification and selection

PubMed, Google Scholar, Web of Science, Chinese National Knowledge Infrastructure (CNKI), Chinese Wanfang Database, and the Chinese VIP databases for eligible studies examined the association between rs2234693 and rs9340799 polymorphisms of ESR1 gene and radiographic knee OA up to August 15, 2018. The following keywords and terms in various combinations were used: (“knee osteoarthritis” OR “radiographic knee‘) AND (“estrogen receptor 1″ or “estrogen receptor-α” OR “ESR-alpha” OR ‘’ nuclear receptor subfamily 3″ OR “NR3A1″) AND (“rs2234693″ OR “rs9340799″ OR “PvuII T > C″ OR “XbaI A > G “) AND (“intron variant” OR “polymorphism”, OR “mutation” OR “variant” OR “gene” OR “genotype” OR “SNP” OR “allele”). In addition, hand searching of the references of eligible studies, reviews and related meta-analyses, and the abstracts presented at relevant conferences were performed to identify potentially relevant studies. If there were multiple reports of the same study or overlapping data only the study with the largest sample sizes or the most recent one should be in the final analysis.

2.2.2. Inclusion and exclusion criteria

The inclusion criteria were as follows: (1) published case-control or cohort studies; (2) evaluated the association between rs2234693 and rs9340799 polymorphisms of ESR1 gene and radiographic knee OA; (3) detailed numbers of genotypes must have been provided to calculate the odds ratio (OR) and the corresponding 95% confidence intervals (95% CIs). The exclusion criteria were as follows: (1) studies on clinical or biochemical defined knee OA or total knee replacement (TKA); (2) studies with insufficient information to calculated pooled ORs; (3) studies without healthy control group; (4) sibling pairs, family and linkage studies; and (5) reviews, abstracts, posters, case reports, comments and animal studies. For studies with overlapping case series, only the most recent one or the largest sample sizes were considered. If studies did not report detailed data, we would get in touch with authors to obtain the relevant information.

2.2.3. Data extraction

Two authors carefully performed the data extraction independently from all the eligible studies. In the case of disagreement, a joint review of the study was completed by discussion. The following details were retrieved from the included studies: ESR1 gene polymorphisms, author's name, publication year, country where the study was conducted, ethnicity (categorized as Asian, Caucasian, or mixed), number of patients and control subjects, genotyping methods, source of controls (hospital based or population based), numbers of case and control patients in each genotype, minor allele frequencies (MAFs) in control subjects and Hardy–Weinberg equilibrium (HWE) of genotype distribution.

2.2.4. Statistical analysis

Pooled odds ratio (OR) with corresponding 95% confidence interval (CI) were calculated to estimate the strength of association between rs2234693 and rs9340799 polymorphisms of ESR1 gene and radiographic knee OA risk. The significance of the pooled OR was determined using the Z-test and $P < 0.05$ was considered statistically significant. We have estimated the pooled ORs for ESR1 rs2234693 and rs9340799 polymorphisms under five genetic models i.e., allele (B vs. A), homozygote (BB vs. AA), heterozygote (AB vs. AA), dominant (BB + AB vs. AA), and recessive (BB vs. AA + AB), which the A represents the wild (major allele) and the B represents the mutant (minor allele). Heterogeneity between studies was assessed using Cochran's chi-square-based Q-test, which the p-value < 0.05 for the Q-test indicated a lack of heterogeneity across studies. In addition, the I^2 value was used to quantify the percentage of variation between studies, in which 0–25% indicated no observed heterogeneity and larger values showed increasing heterogeneity, with 25–50% regarded as low, 50–75% as moderate, and 75–100% as high. Thus, the fixed effects model was used to pool ORs and 95% confidential interval (CI) when there was no significant heterogeneity. Otherwise, the random effects model (the DerSimonian and Laird method) was utilized. A Hardy-Weinberg equilibrium (HWE) test of rs2234693 and rs9340799 polymorphisms in control subjects was tested by the Chi-square test. If P value > 0.05, the genotype distribution of the control group conformed to HWE. Furthermore, to explore the sources of heterogeneity, we conducted subgroup analysis based on ethnicity and HWE status. To validate the reliability and stability of the pooled ORs, sensitivity analysis was utilized by sequentially omitting individual studies each time, as well as by excluding those studies which were not in agreement with HWE. Publication bias was evaluated by visual inspection of symmetry of Begg's funnel plot of log against its standard error (SE) and the degree of asymmetry was tested using Egger's test, in which $P < 0.05$ was regarded as representative of statistical significance. All the statistical analyses were performed by comprehensive meta-analysis (CMA) version 2.0 software (Biostat, USA). All p values were two sides and less than 0.05 were considered significant.

3. Results

3.1. Case-control study

The genotypes and alleles distribution by cases and healthy subjects are shown in http://cebp.aacrjournals.org/content/13/5/709.long Table 1. The genotype distributions among healthy subjects for both rs2234693 and rs9340799 polymorphisms were consistent with Hardy-Weinberg equilibrium ($p > 0.05$). However, the ORs and 95% CIs did not showed significant differences in genotypes and alleles frequency distributions between radiographic defined knee OA patients and

Table 1
The genotype and the allele frequencies of ESR1 polymorphisms in patients and controls.

Polymorphism	Radiographic knee OA (%)	Control (%)	OR (95% CI)	P-value
rs2234693				
Genotypes				
TT	30(33.3)	24(26.7)	Ref.	
TC	42(46.7)	37(41.1)	1.253(0.695–2.260)	0.453
CC	18(20.0)	29(32.2)	0.526(0.266–1.038)	0.064
Allele				
T	102(53.7)	85(44.7)	1.462(0.965–2.214)	0.073
C	78(46.3)	95(55.3)	0.684(0.452–1.037)	0.073
rs9340799				
Genotypes				
AA	51(56.7)	45(50.0)	Ref.	
AG	28(31.1)	32(35.6)	0.819(0.440–1.523)	0.527
GG	11(12.2)	13(14.4)	0.825(0.348–1.953)	0.661
Allele				
A	130(72.2)	122(67.8)	1.236(0.787–1.942)	0.358
G	50(27.8)	58(32.2)	0.809(0.515–1.271)	0.358

OR: Odds Ratio; CI: Confidence Interval.

healthy subjects for both rs2234693 and rs9340799 polymorphisms ($p < 0.05$)

3.2. Meta-analysis

3.2.1. Study characteristics

Fig. 1 displayed the selection process of this study. A total of 139 articles were identified after an initial search of databases and manual searching. Of these studies, the first screening excluded 55 studies due to duplicates, leaving 84 articles for further selection. After reading titles and abstracts of the remaining studies, 28 articles were excluded for not relevant to ESR1 polymorphisms and radiographic knee OA. Among the remaining 56 studies, 31 articles also were excluded because they were review articles, case reports, explored other diseases instead of radiographic knee OA, did not have sufficient genotype frequencies, and evaluated other polymorphisms of ESR1 gene. Finally, a total of 25 studies in 12 publications with 7,144 cases and 8,468 controls were included in our meta-analysis. The detailed characteristics of the included studies are listed in Table 2. Among these studies, there were 14 studies[12–22] on rs2234693 polymorphism with 4,084 cases and 4,993 controls, and eleven studies[12–17,19–22] on rs9340799 polymorphism with 3,060 cases and 3,475 controls. Four publications were written in Chinese and rest was in English. Of 25 case-control studies, 14 were conducted in Asian populations, 14 were conducted in Caucasian populations, and two were in mixed populations. By source of controls, all studies were population-based (PB) except for one study which not applicable. Two genotyping methods were applied including PCR-RFLP and TaqMan Genotyping Assay. In addition, Genotype distributions in the controls of all the eligible studies were in agreement with HWE, except one study on rs9340799 (Table 2).

3.2.2. Quantitative synthesis

Table 3 listed the main results of the meta-analysis of rs2234693 and rs9340799 polymorphisms of ESR1 gene and radiographic knee OA risk. No association was observed in the rs2234693 polymorphism under the dominant model, but there was a significant difference under the heterozygote model (CT vs. TT: OR = 1.164, 95% CI 1.053–1.286, p = 0.003, Fig. 2). By subgroup analysis, we did not find that the rs2234693 polymorphism increased the risk of radiographic knee OA in Asian and Caucasian populations (Table 3). Moreover, when all eleven eligible case-control studies were pooled into the meta-analysis of rs9340799 polymorphism, we not found a significant association between rs9340799 polymorphism and knee OA under all five genetic models, even in the subgroup analyses by ethnicity (Table 3).

3.2.3. Heterogeneity analysis

For both rs2234693 and rs9340799 polymorphisms of ESR1 gene, significant heterogeneity was found under all five genetic models (Table 3). Therefore, we tested the source of heterogeneity by subgroup analysis based on ethnicity, genotyping methods and HWE status. As shown in Table 3, the I^2 decreased obviously and p-value exceeded 0.05 among Caucasian populations in the three genetic models, indicating that ethnicity might be contributed to substantial heterogeneity for rs2234693 in this meta-analysis (Table 3). However, the subgroup analysis for rs9340799 indicated that ethnicity, genotyping methods and HWE status did not responsible for heterogeneity (Table 3).

3.2.4. Sensitivity analysis

Sensitivity analysis was performed to test the influence of individual studies on the pooled ORs of ESR1 rs2234693 and rs9340799 polymorphisms by omitting each study. However, elimination of each study made no qualitative difference on the pooled ORs. In addition, to testify the impact of HWE-violating studies on pooled ORs, sensitivity analysis was performed by excluding those studies. However, the statistical significance of the results did not change, which indicated that our results were stable.

3.2.5. Publication bias

The publication bias of the included studies was assessed by the Funnel plot and Egger's test. The publication bias analysis was shown in Table 3. For rs2234693, shapes of Funnel plot were symmetrical and also the Egger's test provided statistical evidence for the funnel plot symmetry under all five genetic models, suggesting no evidence of publication bias. However, the asymmetric funnel plot and the Egger's test suggested the presence of publication bias for rs9340799 under all five genetic models, i.e., allele model ($P_{Beggs} = 0.096$ and $P_{Eggers} = 0.002$, Fig. 3A), homozygote ($P_{Beggs} = 0.061$ and $P_{Eggers} = 0.007$), heterozygote ($P_{Beggs} = 0.008$ and $P_{Eggers} \leq 0.001$), dominant ($P_{Beggs} = 0.005$ and $P_{Eggers} \leq 0.001$), and recessive ($P_{Beggs} = 0.005$ and $P_{Eggers} = 0.018$, Fig. 3B) for rs9340799. The existence of publication bias might be a limitation for this meta-analysis because only studies on radiographic knee OA were included. Thus, we applied the Duval and Tweedie non-parametric "trim and fill" method to adjust publication bias results for rs2234693 polymorphism. However, we not observed different results with or without "trim and fill" test, indicating that the current meta-analysis results were statistically robust.

4. Discussion

ESR1 (also known as NR3A1) belong to the nuclear receptor (NR) superfamily of ligand-regulated transcription factors.[23,24] Human ER1 gene is located on chromosome 6q25.1, comprises 7 introns and spanning 140 kb.[23] Two restriction enzymes defined variants in the first intron of the ESR1 gene have been studied in numerous studies including rs2234693 (PvuII C > T) and rs9340799 (XbaI A > G). The PvuII polymorphism site is located on intron 1, 1400 bps upstream of exon 2, and the XbaI site is approximately 50 bps apart from the PvuII site.[25,26]

The present meta-analysis was carried out by critically reviewing previous 24 individual case-control studies and the present case-control study on ESR1 rs2234693 and rs9340799 polymorphisms and radiographic knee OA. Our pooled results based on all studies showed that rs2234693 polymorphism was significantly associated with radiographic knee OA, whereas rs9340799 polymorphism was not significantly associated with radiographic knee OA. There was no evidence for the association between rs2234693/rs9340799 polymorphisms and radiographic knee OA in subgroup analyses based on ethnicity. However, considering the limited studies of both rs2234693 and rs9340799 polymorphisms predominately to the Asian populations, our results for subgroup analyses should be interpreted in a conservative

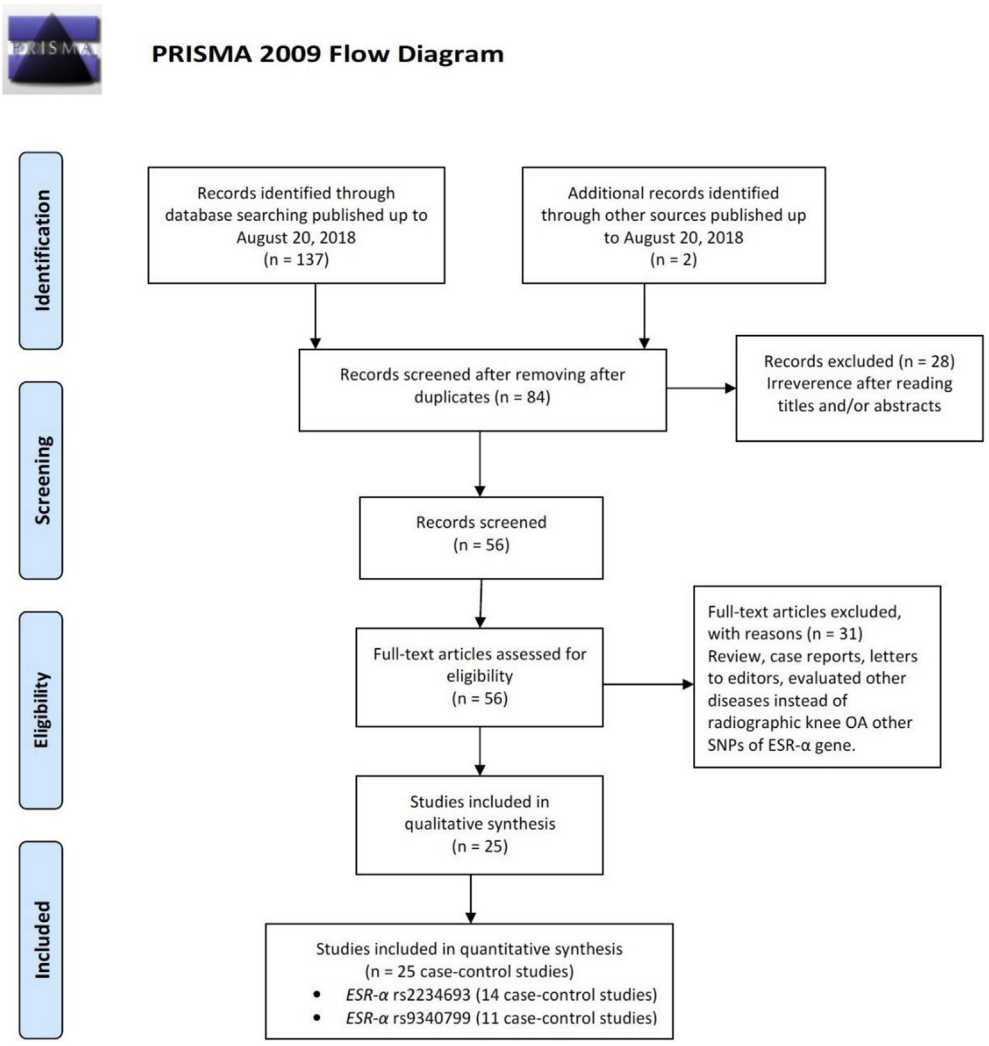

Fig. 1. Flow diagram of the study selection process.

manner and need further validation in larger well-designed studies in different ethnicities. For rs2234693, our results are inconsistence with previous meta-analysis. In 2015, Wang et al., performed a meta-analysis of 15 studies to evaluate the possible association between the ESR1 polymorphism and knee OA. Their pooled results suggest that the rs2234693 polymorphism was associated with an increased KOA risk. However, they have not found significant associations between rs2234693 and rs9340799 polymorphisms of ESR1 gene and susceptibility of knee OA by subgroup analyses based on radiographic diagnostic method.[27] However, the previous meta-analyses did not cover all eligible studies which could lead to publication bias. Therefore, compared to Wang et al., meta-analysis, the present meta-analysis involved the missed studies, recent published studies and also we performed a case-control study, thus markedly minimizing the selection bias. In addition, we performed subgroup analyses based ethnicity and genotyping methods on radiographic knee OA. Also, these studies showed inconsistent results probably due to different definition of knee OA.

Heterogeneity and publication bias may influence the results of a meta-analysis.[28–30] The heterogeneity might result from age, gender, ethnicity, genotyping methods, sample size, source of controls, life style, and environmental factors.[31–33] In this meta-analysis obvious heterogeneity existed in for both rs2234693 and rs9340799 polymorphisms under all five genetic models. By subgroup analysis, we found the ethnicity was a possible source of heterogeneity for rs2234693. However, the ethnicity did not explain heterogeneity for rs9340799 in this meta-analysis. It is possible that selection of studies limited to radiographic defined knee OA may partially contribute to the high heterogeneity in this meta-analysis.

There were some advantages in the current meta-analysis. First, our study is the most comprehensive and statistically more powerful meta-analysis to investigate the association of ESR1 polymorphisms with the radiographic knee OA than the previous meta-analysis. Second, the patient group was uniformly defined radiographic knee OA and control group was only population-based. Third, the literature search process and data extraction in the meta-analysis was well designed and performed in English and Chinese. Although comprehensive analysis was conducted to show the association of rs2234693 and rs9340799 polymorphisms with radiographic knee OA, there are still some limitations should be pointed out. First, only published articles in English and Chines were included, the unpublished and there may still be some unpublished studies could convert the current meta-analysis pooled ORs. Second, almost all of the included studies are from Asia and a few from Caucasians, and thus, the results were underpowered to address an association by ethnicity. Third, Significant heterogeneity existed in the analysis among two polymorphisms under all five genetic models. However, we have performed subgroup analyses by ethnicity and genotyping methods to clarify sources; it was hard to find all potential sources. Forth, publication bias tests and plots only relevant if > 10 studies are included otherwise underpowered to detect much and tend to lead to conclusions that are not justified, suggesting the reliability of

Table 2
Main characteristics of studies included in this meta-analysis.

First Author	Country (Ethnicity)	Genotyping Method	SOC	Case/Control	Cases					Controls					MAFs	HWE
					Genotypes			Allele		Genotypes			Allele			
rs2234693					**TT**	**CT**	**CC**	**T**	**C**	**TT**	**CT**	**CC**	**T**	**C**		
Bergink 2003[12]	Netherlands(Caucasian)	PCR-RFLP	PB	1483/687	434	737	312	1605	1361	225	333	129	783	591	0.430	0.767
Xue 2004[13]	China(Asian)	PCR-RFLP	PB	55/176	17	23	15	57	53	57	87	32	201	151	0.429	0.905
Jin 2004[14]	Korea(Asian)	PCR-RFLP	PB	151/397	61	68	22	190	112	152	183	62	487	307	0.386	0.575
Yan 2004[15]	China(Asian)	PCR-RFLP	NA	53/176	10	24	21	44	66	54	82	40	190	162	0.460	0.408
Yang 2009[16]	China(Asian)	PCR-RFLP	PB	41/40	14	17	10	45	37	12	23	5	47	33	0.412	0.238
Tian 2009[17]	China(Asian)	PCR-RFLP	PB	38/40	16	15	7	47	29	15	16	9	46	34	0.425	0.250
Rianch 2010[18]	UK (Caucasian)	TaqMan	PB	445/862	77	245	123	399	491	167	442	253	776	948	0.549	0.292
	Spain(Caucasian)			272/802	53	140	79	246	298	179	394	229	752	852	0.531	0.699
	Spain(Caucasian)			254/473	46	143	65	235	273	80	217	176	377	569	0.601	0.349
Borgonio-Cuadra 2012[19]	Mexico(Mixed)	PCR-RFLP	PB	115/117	52	49	14	153	77	51	50	16	152	82	0.350	0.507
Liu 2014[20]	China(Asian)	PCR-RFLP	PB	98/196	30	41	27	101	95	63	97	36	223	169	0.431	0.900
Dai 2014[21]	China(Asian)	TaqMan	PB	469/514	167	217	85	551	387	198	242	74	638	390	0.379	0.996
Feng 2017[22]	China(Asian)	PCR-RFLP	PB	520/1042	260	209	51	729	311	665	327	50	1657	427	0.204	0.234
Present 2018	Iran(Asian)	PCR-RFLP	PB	90/90	30	42	18	102	78	24	37	29	85	95	0.527	0.096
rs9340799					**AA**	**GA**	**GG**	**A**	**G**	**AA**	**GA**	**GG**	**A**	**G**		
Bergink 2003[12]	Netherlands(Caucasian)	PCR-RFLP	PB	1483/687	643	682	158	1968	998	372	263	52	1007	367	0.267	0.560
Xue 2004[13]	China(Asian)	PCR-RFLP	PB	55/176	21	24	10	66	44	40	82	54	162	190	0.539	0.408
Jin 2004[14]	Korea(Asian)	PCR-RFLP	PB	151/397	98	49	4	245	57	256	126	15	638	156	0.196	0.917
Yan 2004[15]	China(Asian)	PCR-RFLP	NA	55/176	15	23	17	53	57	32	87	57	151	201	0.571	0.905
Yang 2009[16]	China(Asian)	PCR-RFLP	PB	41/40	28	11	2	67	15	24	13	3	61	19	0.237	0.516
Tian 2009[17]	China(Asian)	PCR-RFLP	PB	38/40	18	16	4	52	24	6	21	13	33	47	0.587	0.598
Borgonio-Cuadra 2012[19]	Mexico(Mixed)	PCR-RFLP	PB	115/117	70	41	4	181	49	62	47	8	171	63	0.269	0.821
Liu 2014[20]	China(Asian)	PCR-RFLP	PB	98/196	36	43	19	115	81	49	92	55	190	202	0.515	0.398
Dai 2014[21]	China(Asian)	TaqMan	PB	469/514	288	152	29	728	210	348	155	19	851	193	0.184	0.736
Feng 2017[22]	China(Asian)	PCR-RFLP	PB	520/1042	276	201	43	796	818	618	366	58	1660	1388	0.666	≤0.001
Present 2018	Iran(Asian)	PCR-RFLP	PB	90/90	51	28	11	130	50	45	32	13	122	58	0.322	0.077

PCR–RFLP: polymerase chain reaction restriction fragment length polymorphism; PB: population based; NA: not applicable; SOC: source of controls; MAFs: minor allele frequencies; HWE: Hardy-Weinberg Equilibrium.

Table 3
Results of the association of ESR1 polymorphisms with radiographic knee OA risk.

Subgroup	Genetic Model	Type of Model	Heterogeneity		Odds Ratio				Publication Bias	
			I^2 (%)	P_H	OR	95% CI	Z_{test}	P_{OR}	P_{Beggs}	P_{Eggers}
rs2234693 (n = 14)	C vs. T	Random	77.97	≤0.001	1.067	0.921–1.236	0.866	0.386	0.912	0.521
	CC vs. TT	Random	69.44	≤0.001	1.158	0.892–1.504	1.100	0.271	0.912	0.638
	CT vs. TT	Fixed	26.70	0.168	1.164	1.053–1.286	2.961	0.003	0.100	0.060
	CC + CT vs. TT	Random	56.64	0.005	1.113	0.945–1.310	1.279	0.201	0.324	0.105
	CC vs. CT + TT	Random	73.70	≤0.001	1.110	0.876–1.406	0.861	0.389	0.661	0.683
Ethnicity										
Asian (n = 9)	C vs. T	Random	80.53	≤0.001	1.126	0.881–1.439	0.951	0.342	0.602	0.306
	CC vs. TT	Random	74.38	≤0.001	1.282	0.821–2.000	1.093	0.274	0.916	0.424
	CT vs. TT	Random	51.84	0.034	1.050	0.821–1.343	0.386	0.699	0.916	0.056
	CC + CT vs. TT	Random	69.93	0.001	1.095	0.818–1.466	0.611	0.541	0.916	0.163
	CC vs. CT + TT	Random	70.98	0.001	1.307	0.901–1.895	1.409	0.916	0.916	0.632
Caucasian (n = 4)	C vs. T	Random	65.99	0.032	0.997	0.860–1.156	−0.039	0.969	0.308	0.204
	CC vs. TT	Fixed	52.37	0.098	1.081	0.911–1.283	0.888	0.374	0.308	0.190
	CT vs. TT	Fixed	0.00	0.913	1.150	0.993–1.330	1.868	0.062	0.734	0.680
	CC + CT vs. TT	Fixed	0.00	0.739	1.139	0.992–1.307	1.841	0.066	0.308	0.338
	CC vs. CT + TT	Random	73.23	0.011	0.906	0.693–1.185	−0.720	0.472	0.308	0.192
rs9340799 (n = 11)										
Asian (n = 9)	G vs. A	Random	87.08	≤0.001	0.915	0.705–1.187	−0.671	0.502	0.096	0.002
	GG vs. AA	Random	76.01	≤0.001	0.792	0.497–1.260	−0.985	0.325	0.061	0.007
	GA vs. AA	Random	66.35	0.001	0.942	0.749–1.185	−0.512	0.609	0.008	≤0.001
	GG + GA vs. AA	Random	77.09	≤0.001	0.882	0.680–1.143	−0.949	0.343	0.005	≤0.001
	GG vs. GA + AA	Random	58.36	0.008	0.944	0.928–1.195	−0.437	0.662	0.042	0.018
	G vs. A	Random	88.28	≤0.001	0.870	0.619–1.223	−0.802	0.423	0.173	0.005
	GG vs. AA	Random	74.84	≤0.001	0.722	0.416–1.255	−1.155	0.248	0.251	0.052
	GA vs. AA	Random	56.20	0.019	0.885	0.686–1.141	−0.944	0.345	0.028	0.003
	GG + GA vs. AA	Random	72.05	≤0.001	0.817	0.607–1.101	−1.326	0.185	0.028	0.003
	GG vs. GA + AA	Random	58.07	0.014	0.877	0.590–1.302	−0.653	0.514	0.175	0.097

Study name	Odds ratio	Lower limit	Upper limit	Z-Value	p-Value	Relative weight
Bergink 2003	1.147	0.933	1.411	1.305	0.192	23.55
Xue 2004	0.886	0.436	1.804	-0.333	0.739	1.99
Jin 2004	0.926	0.616	1.391	-0.371	0.711	6.06
Yan 2005	1.580	0.700	3.566	1.102	0.270	1.52
Yang 2009	0.634	0.235	1.711	-0.900	0.368	1.02
Tian 2009	0.879	0.325	2.380	-0.254	0.800	1.01
Rianch 2010a	1.202	0.880	1.642	1.157	0.247	10.33
Rianch 2010b	1.200	0.835	1.724	0.987	0.324	7.66
Rianch 2010c	1.003	0.654	1.538	0.013	0.990	5.49
Borgonio-Cuadra 2012	0.961	0.554	1.669	-0.141	0.888	3.30
Liu 2014	0.888	0.503	1.566	-0.412	0.681	3.12
Dai 2014	1.063	0.807	1.401	0.435	0.663	13.22
Feng 2017	1.635	1.305	2.047	4.279	0.000	19.83
Present 2018	0.639	0.309	1.321	-1.209	0.227	1.90
	1.164	1.053	1.286	2.961	0.003	

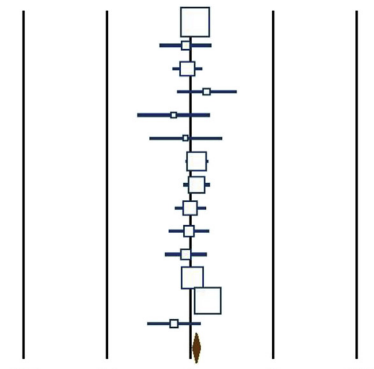

Fig. 2. Forest plots describing the association of ESR1 rs2234693 polymorphism and radiographic knee OA under the heterozygote model (CT vs. TT).

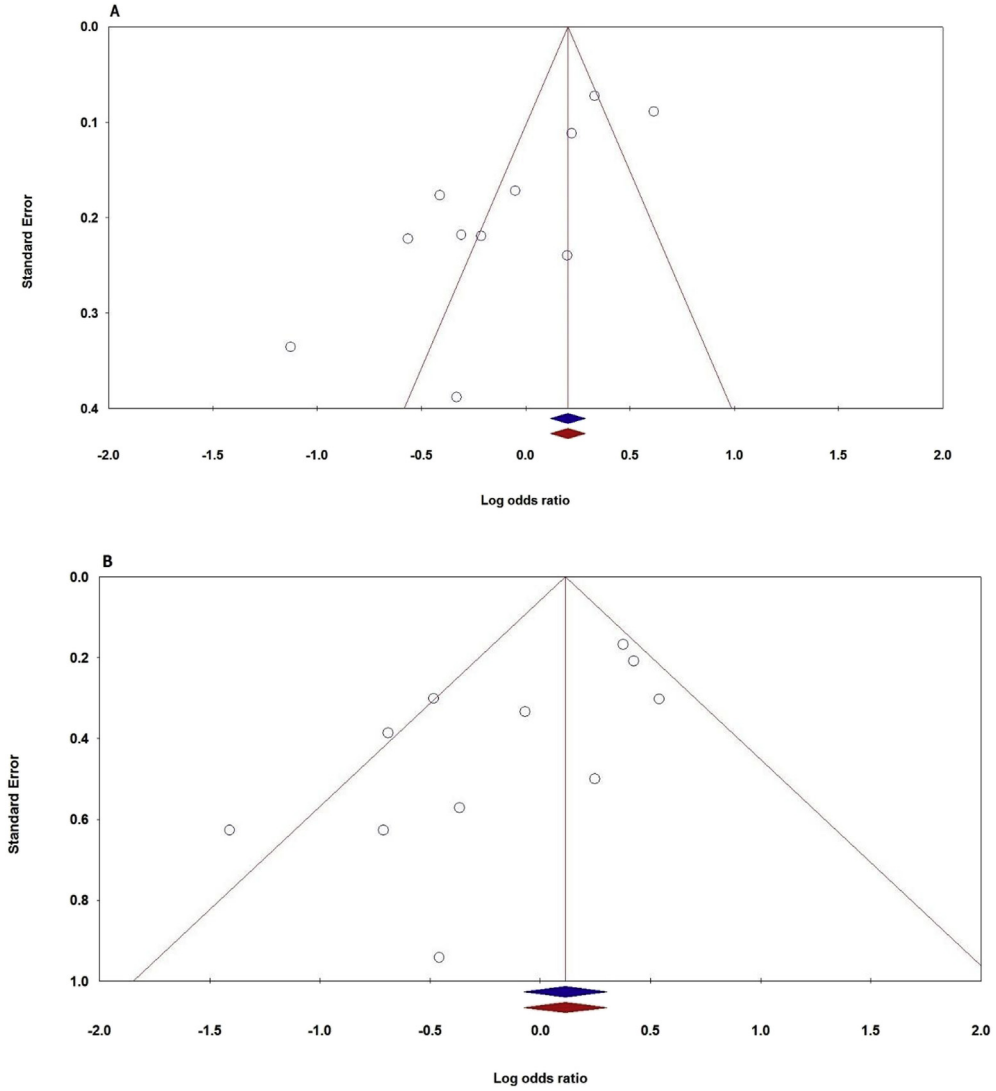

Fig. 3. Begg's funnel plots of the ESR1 rs9340799 polymorphism and radiographic knee OA for publication bias test. **A:** allele model (G vs. A); **B:** recessive model (GG vs. GA + AA). With (blue) and without (red) "trim and fill" test.

our results. Finally, this meta-analysis was based on unadjusted data, as the ORs adjusted for the main confounding variables such as age, gender, BMI, severity of OA, radiographic grading, life style and environmental factor were not available from those studies.

5. Conclusion

In summary, the current meta-analysis suggested that rs2234693 polymorphism might be associated with radiographic defined knee OA, but not rs9340799 polymorphism. Considering the limitations included in the meta-analysis, further large scale, multicenter, and high-quality studies are necessary to confirm our results.

Conflicts of interest

The authors declare that they have no conflict of interest.

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