

## Review Article

# Collagen5A1 and collagen12A1 gene polymorphisms as a risk marker for anterior cruciate ligament ruptures: a meta-analysis

Zhen Lyu<sup>1</sup>, Bing Li<sup>2</sup>, Xiaofei Zhang<sup>2</sup>, Xinglong Ma<sup>2</sup>, Jianxiong Ma<sup>2</sup>, Jun Liu<sup>1</sup>

<sup>1</sup>Department of Joint Surgery, Tianjin Hospital, Tianjin, China; <sup>2</sup>Orthopedic Institute of Tianjin, Tianjin, China

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**Abstract:** Objective: Recent advances have led to the identification of novel genetic polymorphisms that are associated with a risk for anterior cruciate ligament (ACL) ruptures. Currently, 4 loci (rs12722, rs970547, rs240736, and rs139467) in COL5A1 and COL12A1 have been consistently reported. The aim of the present study was to independently replicate 2 recently described ACL rupture susceptibility loci, COL5A1 (rs12722) and COL12A1 (rs970547), in a large case-control cohort and to perform a meta-analysis of all studies published to date to investigate whether genetic susceptibility to ACL rupture risk is gender-related. Methods: A literature search of all relevant studies published before June 30, 2017 was conducted using PubMed, Embase, Web of Science, Cochrane Library, China BioMedicine (CBM), and the China National Knowledge Infrastructure (CNKI) databases. Studies that were related to an association between COL5A1 and COL12A1 (rs12722 and rs970547) polymorphisms with ACL rupture risk susceptibility were identified. A meta-analysis of the pooled and stratified data was performed and assessed using varied genetics with Mantel-Haenszel fixation effects models. Results: Seven case-control studies with a total of 15 comparisons (7 for the COL5A1 rs12722 polymorphism and 8 for the COL12A1 rs970547 polymorphism) met our inclusion criteria. A meta-analysis showed an association between COL5A1 CC genotypes and ACL rupture risk susceptibility. Furthermore, the meta-analysis showed an association between COL12A1 AA genotypes and ACL rupture risk susceptibility. Stratification of ACL rupture patients according to gender groups showed that the COL5A1 CC genotype was significantly associated with ACL rupture risk in male but not in female subjects, whereas the COL12A1 AA genotype was significantly associated with ACL rupture risk in female but not in male subjects. Conclusion: Our results uncovered an association of COL5A1 and COL12A1 with ACL rupture risk and provide highly suggestive evidence for the rs12722/rs970547 loci as risk factors for ACL rupture risk. The present study demonstrates that COL5A1 rs12722 and COL12A1 rs970547 polymorphisms confer susceptibility to ACL ruptures in total subjects and in major gender groups.

**Keywords:** COL5A1, Col12A1, anterior cruciate ligament ruptures, gene polymorphism, meta-analysis

## Introduction

ACL rupture is one of the most severe injuries in sports [1]. The incidence of anterior cruciate ligament (ACL) ruptures is 35/100,000 in the general population [2]. Deacon reported that ACL rupture can increase the risk of osteoarthritis in individuals up to 105 times [3]. It has been suggested that ACL damage is related to movement, especially when the direction of motion is changed and the initial acceleration and deceleration is rapid [4, 5]. However, in 70% of all patients with ACL rupture, the injury was caused by a non-contact mechanism. [6]

Non-contact ACL rupture means that the forces applied to the knee at the time of injury resulted from the athlete's own movements and did not involve contact with another athlete or object [7].

The risk factors for non-contact ACL injury can be divided into external factors and internal factors [8]. Four intrinsic factors have been identified that may increase the risk of ACL damage, including environmental, anatomical, hormonal, and neuromuscular factors [7]. Environmental factors include meteorological conditions [9]. Anatomical factors that can increase the risk of

ACL rupture include the Q angle [10], static and dynamic knee valgus [11], BMI [12] and femoral notch width [13]. Studies have shown that sex hormones play a role in the regulation of collagen synthesis and degradation in human ligament tissue [4]. In addition to these four factors, polymorphisms in collagen genes may be associated with the incidence of ACL ruptures. The COL1A1, COL5A1 and COL12A1 genes encode I, V and type XII collagen alpha 1 chains, respectively, and I, V and type XII collagen constitute the majority of the solid component of the ligament [14]. Other collagens, such as III and VI encoded by the COL3A1 and COL6A1 genes, are also found in the solid component of the ligament [14]. Studies have confirmed that the TT genotype polymorphism of the COL1A1 Sp1-binding site is insufficient in participants with cruciate ligament rupture [15]. The AA genotype of the COL3A1 rs1800255 SNP was associated with increased risk of ACL injuries in a Polish cohort [16]. The TT genotype of the COL6A1 rs35796750 SNP has been associated with increased risk of ossification of the posterior longitudinal ligament [17, 18]. The CC genotype of the COL5A1 rs12722 SNP has been shown to be associated with chronic Achilles tendinopathy [19].

Association between the COL5A1 rs12722 polymorphism and ACL rupture risk has been found in different gender groups, but some studies have yielded conflicting results. Several studies have shown that the COL5A1 rs12722 gene is associated with an increased risk of ACL rupture in female subjects [20], but other studies have shown that the rs12722 SNP is associated with an increased risk of ACL rupture in male subjects [21]. It has been reported that the AA genotype of COL12A1 rs970547 is significantly overexpressed in females but not in males [22], but other reports suggest that there is no association between ACL ruptures and COL12A1 rs970547 in both male and female subjects [23]. These inconsistent results may be caused by small sample sizes, racial or ethnic differences, and clinical or genetic heterogeneity. Therefore, it is important to assess gender-specific associations to determine the nature of genetic associations among different populations. Meta-analysis is a powerful tool that can improve statistical performance by combining the results of multiple studies. Therefore, in this study, we investigated the contribution of COL5A1 rs12722

(T/C) and COL12A1 rs970547 (A/G) polymorphisms to ACL rupture risk in South Africa and Poland populations using a meta-analysis approach.

To achieve the objective, four genetic models, namely, the dominant model, co-dominant model, over-dominant model, and recessive model, were considered. Heterogeneity, sensitivity analysis, and publication bias were also assessed. To the best of our knowledge, this is the first meta-analysis reporting the association of COL5A1 rs12722 (T/C) and COL12A1 rs970547 (A/G) with ACL rupture risk susceptibility in South Africa and Poland populations.

### Materials and methods

#### *Literature search*

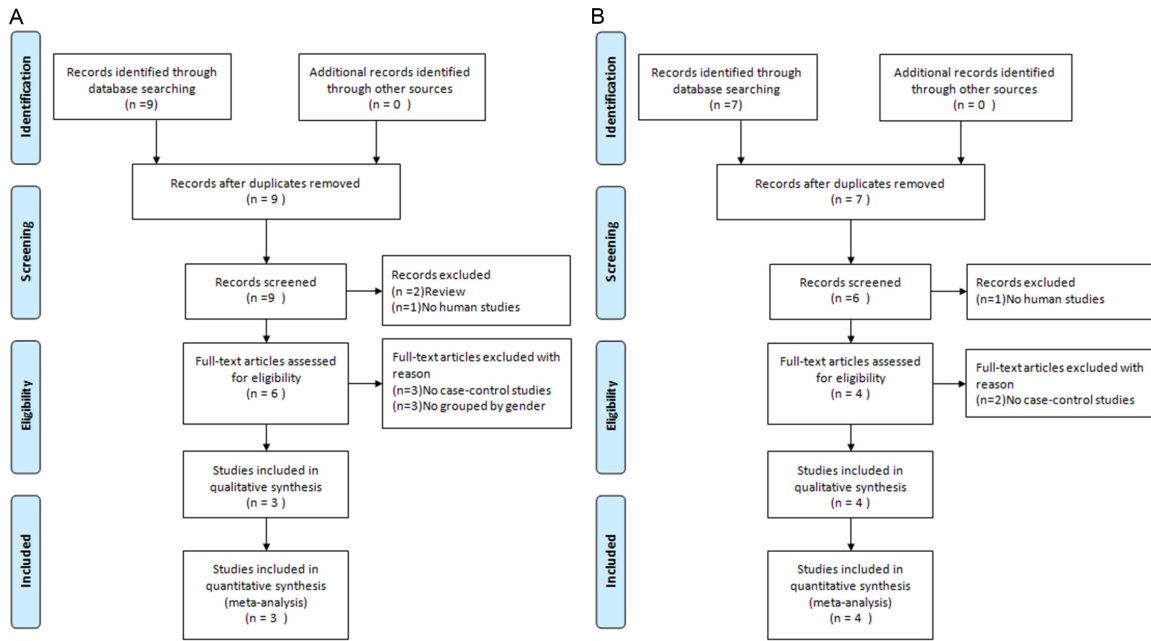
A comprehensive literature search of PubMed, Embase, Web of Science, Cochrane Library, CISCOR, CINAHL, China BioMedicine (CBM), and the China National Knowledge Infrastructure (CNKI), covering all research articles published with a combination of the following key words was carried out: “Anterior Cruciate Ligament Injuries”, “Anterior Cruciate Ligament tear Injuries”, “COL5A1 and ACL rupture”, “COL12A1 and ACL rupture”, and “single nucleotide polymorphism”. When the same population was included in more than one of publications, only the most recent or complete study was included in this meta-analysis. Because this is an analysis of published articles based on the association of COL5A1 and COL12A1 polymorphisms and ACL rupture risk, gender approval was required for this study.

#### *Quality assessment and data collection*

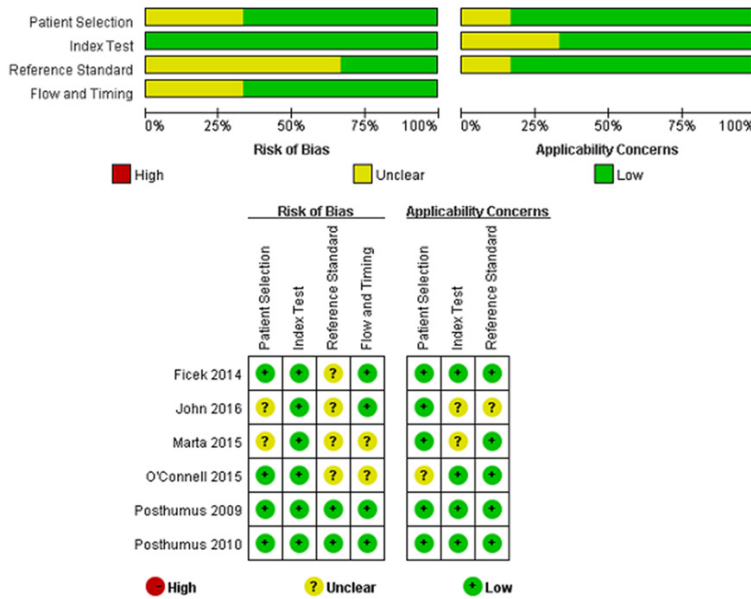
The methodological quality of the included studies was appraised by an adapted version of the QUADAS-2, which consisted of four key domains that discussed patient selection, index test, reference standard, and flow and timing. Risk of bias assessment of the four domains and clinical applicability of the first three domains were assessed with signaling questions. Questions were answered as “yes” for low risk of bias/concerns, “no” for high risk of bias/concerns or “unclear (**Figure 2**).

The literature was evaluated with the Newcastle-Ottawa Scale (NOQAS available at: [http://www.ohri.ca/programs/clinical\\_epidemiolo-](http://www.ohri.ca/programs/clinical_epidemiolo-)

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**Figure 1.** Workflow diagram of the study selection process from initial search to included studies. A. COL5A1 rs12722; B. COL12A1 rs970547. Adapted from PRISMA statement 2009 (<http://www.Prismastatement.org>).



**Figure 2.** Quality assessment of included studies using QUADAS-2 tool criteria. QUADAS-2: The revised Quality Assessment of Diagnostic Accuracy Studies.

HWE (Hardy-Weinberg equilibrium) was used to evaluate the subjects' quality.

Based on the selection criteria, two independent investigators extracted the main characteristics abstracted from the retrieved studies, including the name of the first author, publication year, country of origin, gender of subjects, age of samples, the number of cases and controls, the distribution of genotypes in case and control groups, and the polymorphism detection method. Discrepancies in the data collected were resolved by discussion among the authors and careful re-examination of the full text to avoid conflicting evaluations.

gy/nosgen.doc [24], accessed on 28 February 2016). A NOQAS score uses a "star" rating system to evaluate methodological quality, which is based on three parts: selection, comparability and exposure. Less than 4 stars was considered low quality, 4-6 as moderate quality, and  $\geq 7$  as high quality. Evidence of

### Inclusion and exclusion criteria

To minimize heterogeneity and facilitate appropriate interpretation of the data, the following inclusion criteria were used for the study selection in our meta-analysis: (a) a case-controlled study design based on unrelated individuals,

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(b) sufficient available data (genotype distributions for cases and controls) to estimate an odds ratio (OR) with a 95% confidence interval (CI), (c) articles evaluating the association between COL5A1 rs12722 and COL12A1 rs970547 polymorphisms with ACL rupture risk, (d) studies that recruited surgically diagnosed anterior cruciate ligament rupture patients and healthy controls, and (e) the grouping of case and control participants by gender. Studies were excluded if one of the following existed: (a) studies containing overlapping data, (b) case-only studies, (c) review articles, (d) studies in which the genotype frequencies or numbers could not be ascertained, and (e) studies in which the genotype distribution in controls was not consistent with Hardy-Weinberg equilibrium (HWE) because bias in the control selection or genotyping errors in the control may have led to deviation from HWE. If the same author published a number of articles in the same sample series, our study selected the largest or most recent sample.

### *Statistical analysis*

To examine the strength of association between the COL5A1 rs12722 and COL12A1 rs970547 polymorphisms and ACL rupture risk, pooled odds ratios (ORs), and their corresponding 95% confidential intervals (CIs) were estimated for each study. In this meta-analysis, the association between COL5A1 rs12722 and ACL rupture risk was examined using co-dominant model (CC vs. TC, CC vs. TT), over-dominant model (TT + CC vs. TC), dominant model (TC + CC vs. TT), and recessive model (CC vs. TT + TC). The COL12A1 rs970547 used the co-dominant model (AA vs. AG, AA vs. GG), over-dominant model (AA + GG vs. AG), dominant model (AG + GG vs. AA), and recessive model (GG vs. AA + AG). Subgroup analysis was also performed by gender, defined as male and female, for the rs12722 and rs970547 polymorphisms.

The Odds ratios (ORs) and a 95% confidence interval (95% CIs) were used to determine whether a fixed or random-effects model would be used. For  $p$  values between 0.05 and 50%, the between-study heterogeneity was considered to be significant and the random-effects model was used to calculate the OR. If no significant

heterogeneity was observed, we used a fixed-effects model (DerSimonian-Laird method) [25].  $I^2$  statistics was employed to quantify inter-study variability, where larger values suggested an increasing degree of heterogeneity [26].

The departure of frequencies of the rs12722 and rs970547 polymorphisms from expectation under Hardy-Weinberg equilibrium (HWE) was calculated by Chi-square test in the controls. Forest plots were created to visually assess the major contributors to heterogeneity. Possible publication bias was tested using Begg's [27] funnel plot and Egger's regression test [28] ( $p < 0.5$  was considered a statistically significant publication bias). Sensitivity analysis was performed to examine the impact of individual studies.

All statistical analyses in this study were performed using the statistical software package Stata (Stata Statistical Software: release 11.0 College Station, TX, StataCorp. LP).

### **Results**

#### *Meta-analysis of the association between the COL5A1 rs12722 and ACL rupture: study characteristics*

Nine relevant articles that investigated the association between the COL5A1 rs12722 polymorphism and ACL rupture susceptibility were identified (**Figure 1A**). Six were excluded due to previous review (September AV, et al., 2012; Schwellnus MP et al., 2007) which included the following: studies not done in humans (Baird AE et al., 2014); Not case-control studies (Richard D. Bell et al., 2012, Olimpio Galasso et al., 2012); Not grouped by gender (A V September et al., 2008). Therefore, three studies met the inclusion criteria (Posthumus et al., 2009; O'Connell et al., 2015; Marta et al., 2015;). We treated data from each study as a separate study, however, one of these eligible studies contained data on two different groups (O'Connell et al., 2015), and these groups were analyzed independently. These 3 articles included 4 case-control studies involving 500 ACL rupture patients and 777 controls (**Table 1**). These encompassed various populations, including two from South Africa and two from Poland. However, because the sample populations were derived only from the two countries,

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**Table 1.** Characteristics of included studies in this meta-analysis

First author	Year	Region	Gene (SNP)	Number		Gender (M/F)		Age (years) (M/F)		Genotyping method
				Case	Control	Case	Control	Case	Control	
Posthumus [20]	2009	South Africa	Col5A1 rs12722	129	216	91/38	132/84	28.1±10.5/29.8±12.1	29.0±12.2/28.2±10.0	PCR-RFLP
Posthumus [22]	2010	South Africa	Col12A1 rs970547	129	216	91/38	133/83	28.1±10.5/29.8±12.1	29.0±12.1/28.2±10.0	PCR-RFLP
Ficek [29]	2014	Poland	Col12A1 rs970547	91	143	91/-	143/-	23.0±3.0/-	25.0±2.6/-	Re-Ti-PCR
O'Connell [16]	2015	South Africa	Col5A1 rs12722	242	235	177/65	145/90	26.1±10.4	29.4±11.3	Re-Ti-PCR
O'Connell [16]	2015	Poland	Col12A1 rs970547 Col5A1 rs12722	91	143	71/20	99/44	23.0±3.0	24.0±6.0	Re-Ti-PCR
Marta [21]	2015	Poland	Col5A1 rs12722	138	183	138/-	183/-	27.0±2.0	26.0±3.0	Re-Ti-PCR
John [23]	2016	India	Col12A1 rs970547	50	52	50/-	52/-	-	-	Re-Ti-PCR

**Table 2.** Association of Col5A1 rs12722 and Col12A1 rs970547 with ACL ruptures

SNP	Allele1/Allele2	Gender	First author	Year	Region	Case% (n)			N	Control% (n)			N	P value	HWE	*NOS score
						11	12	22		1163	11	12				
rs12722 (T/C)		Male	Posthumus	2009	South Africa	26	49	15	91	39.0	72.0	21.0	132	0.396	Yes	8
			O'Connell_a	2015	South Africa	27.9 (46)	55.8 (92)	16.4 (27)	165	29.1 (41)	53.2 (75)	17.7 (25)	141	0.899	Yes	7
			O'Connell_c	2015	Poland	33.8 (24)	49.3 (35)	16.9 (12)	71	24.2 (24)	52.5 (52)	23.2 (23)	99	0.328	Yes	7
			Marta	2015	India	35.0(48)	48.0 (66)	17.0 (24)	138	29.0 (53)	50.0 (91)	21.0 (39)	183	0.467	Yes	7
rs12722 (T/C)		Female	Posthumus	2009	South Africa	16.0	19.0	2.0	38	23.0	38.0	23.0	84	0.050	Yes	8
			O'Connell_b	2015	South Africa	44.1 (26)	49.2 (29)	6.8 (4)	59	26.7 (24)	43.3 (39)	30.0 (27)	90	0.001	Yes	7
			O'Connell_d	2015	Poland	40.0 (8)	45.0 (9)	15.0 (3)	20	31.8 (14)	52.3 (23)	15.9 (7)	44	0.926	Yes	7
rs970547 (A/G)		Male	Posthumus	2010	South Africa	57.0	29.0	3.0	91	77.0	52.0	4.0	133	0.359	Yes	8
			Ficek	2014	Poland	67.0 (61)	29.7 (27)	3.3 (3)	91	62.2 (89)	32.9 (47)	4.9 (7)	143	0.487	Yes	8
			O'Connell_a	2015	South Africa	63.1 (101)	30.6 (49)	6.3 (10)	160	59.6 (84)	36.9 (52)	3.5 (5)	141	0.345	Yes	7
			O'Connell_c	2015	Poland	63.4 (45)	32.4 (23)	4.2 (3)	71	67.7 (67)	28.3 (28)	4.0 (4)	99	0.624	Yes	7
		John	2016	India	10	19	15	50	3	26	21	52	0.048	Yes	7	
		Female	Posthumus	2010	South Africa	28.0	9	1	38	44.0	34.0	1.0	83	0.048	Yes	8
			O'Connell_b	2015	South Africa	65.0 (39)	26.7 (16)	8.3 (5)	60	57.6 (49)	41.2 (35)	1.2 (1)	85	0.393	Yes	7
			O'Connell_d	2015	Poland	80.0 (16)	20.0 (4)	0.0 (0)	20	50.0 (22)	43.2 (19)	6.8 (3)	44	0.030	Yes	7

NOS Newcastle-Ottawa Quality Assessment Scale, HWE Hardy-Weinberg equilibrium. \*Study quality was assessed based on the NOQAS. A NOQAS score of < 4 was considered as low quality, 4-6 as moderate quality, and ≥ 7 as high quality. p < 0.05 indicated not consistent with HWE. Gender and population were independent influencing factors, so the articles were classified according to gender. The O'Connell article had two populations, which were divided into four parts considering the gender and population implications, O'Connell\_a means SA male, O'Connell\_b means SA female, O'Connell\_c means PL male, O'Connell\_d means PL female.



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**Table 3.** Meta-analysis of the relationships of Col5A1 rs12722 and Col12A1 rs970547 with ACL ruptures

	Co-dominant model			Dominant model			Over-dominant model			Recessive model		
	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P	OR	95% CI	P
rs970547	AA vs. AG			AG + GG vs. AA			AA + GG vs. AG			GG vs. AA + AG		
Overall	1.41	1.11-1.80	0.227	0.74	0.59-0.93	0.206	1.38	1.09-1.75	0.450	1.15	0.72-1.83	0.408
Male	1.26	0.96-1.65	0.288	0.81	0.62-1.05	0.246	1.22	0.94-1.58	0.741	1.00	0.60-1.67	0.670
Female	2.25	1.29-3.92	0.590	0.52	0.31-0.88	0.314	2.34	1.35-4.07	0.743	2.45	0.74-8.17	0.213
rs12722	CC vs. TC			TC + CC vs. TT			TT + CC vs. TC			CC vs. TT + TC		
Overall	0.66	0.49-0.90	0.396	0.75	0.59-0.95	0.720	0.96	0.77-1.20	0.608	0.64	0.48-0.86	0.245
Male	0.70	0.51-0.98	0.263	0.82	0.63-1.07	0.704	0.92	0.70-1.18	0.310	0.68	0.50-0.93	0.181
Female	0.46	0.20-1.08	0.439	0.54	0.32-0.90	0.872	1.14	0.70-1.86	0.833	0.46	0.20-1.04	0.288

gender specific meta-analyses were implemented in each population.

Characteristics of COL5A1 rs12722 studies included in the meta-analysis are listed in **Tables 1** and **2**. Meta-analysis showed an association between COL5A1 rs12722 and ACL rupture, and there was a positive association in the co-dominant model (CC vs. TC OR=0.66, 95% CI=0.49-0.90, p=0.396) and recessive model (CC vs. TT + TC OR=0.64, 95% CI=0.48-0.86, p=0.245; **Table 3; Figure 3A, 3B**). After stratification by gender, COL5A1 rs12722 CC genotypes were significantly associated with ACL rupture in males (CC vs. TC OR=0.70, 95% CI=0.51-0.98, p=0.263; CC vs. TT + TC OR=0.68, 95% CI=0.50-0.93, p=0.181) but not in females (CC vs. TC OR=0.46, 95% CI=0.20-1.08, p=0.439; CC vs. TT + TC OR=0.46, 95% CI=0.20-1.04, P=0.288; **Table 3; Figure 3C, 3D**).

### *Meta-analysis of the association between the COL12A1 rs970547 and ACL rupture: study characteristics*

Seven relevant articles that investigated the association between the COL12A1 rs970547 polymorphism and ACL rupture susceptibility were identified (**Figure 1A**). Seven were excluded due to the following reasons: not being human studies (Baird AE et al., 2014) and not being case-control studies (Richard D. Bell et al., 2012, Olimpio Galasso et al., 2012). Therefore, four studies met the inclusion criteria (Posthumus et al., 2010; O'Connell et al., 2015; Ficek et al., 2014; John et al., 2016). We treated data from each study as a separate study; however, one of these eligible studies contained data on two different groups (O'Connell et al., 2015), and these groups were

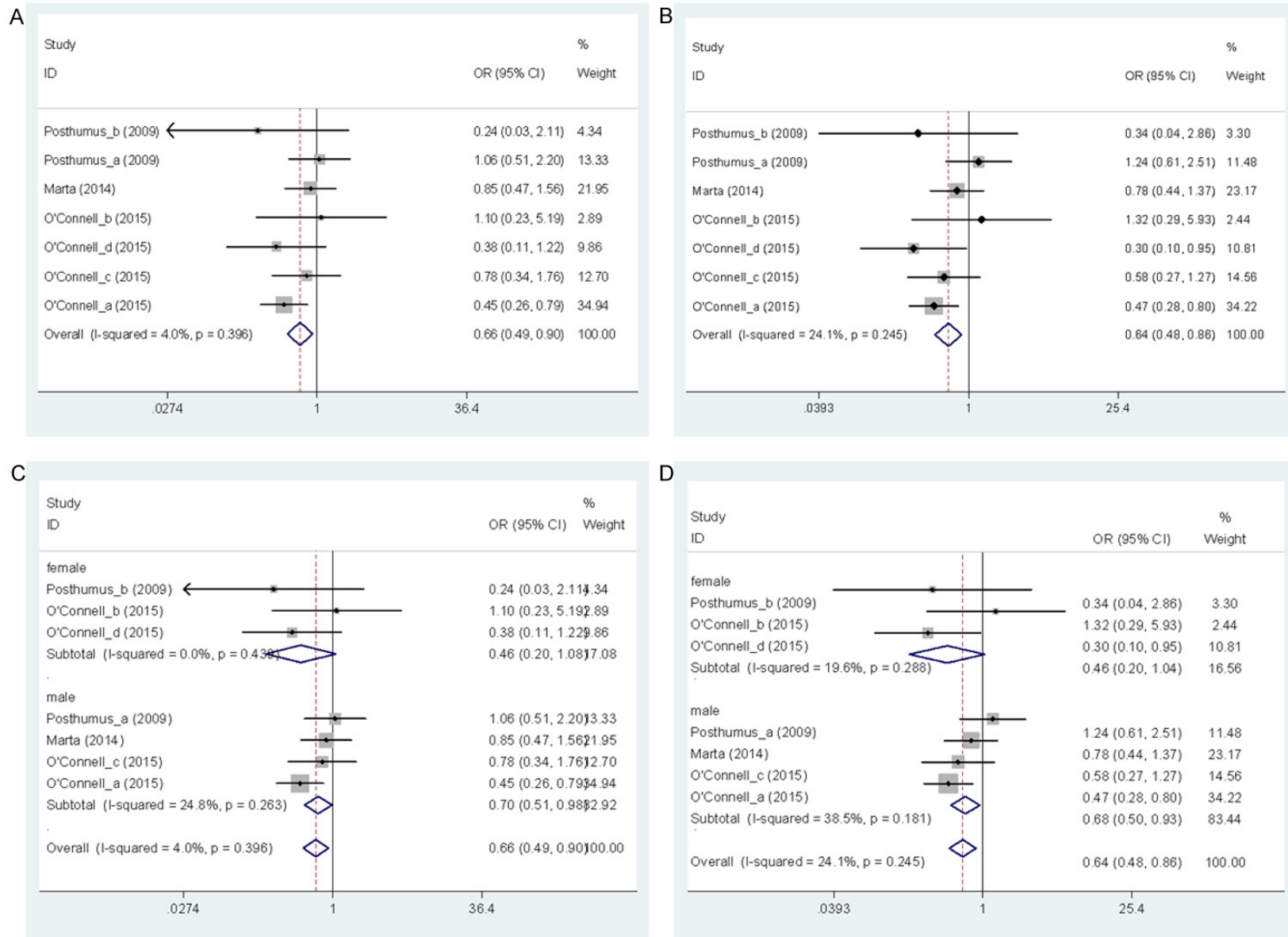
analyzed independently. Overall, there were 4 articles (including 5 case-control studies) for the COL12A1 rs970547 polymorphism, which involved 603 ACL rupture patients and 789 controls in total (**Table 1**). These encompassed two South African populations and two Polish populations. Because the sample population was derived only from the two countries, gender specific meta-analyses are implemented within each population.

Characteristics of COL12A1 rs970547 studies included in the meta-analysis are listed in **Tables 1** and **2**. Meta-analysis showed an association between COL12A1 rs970547 and ACL rupture. Regarding genotypes there was a positive association in the co-dominant model (AA vs. AG OR=1.41, 95% CI=1.11-1.80, p=0.227), and the dominant model (AG + GG vs. AA OR=0.74, 95% CI=0.59-0.93, p=0.296,) (**Table 3, Figure 4A, 4B**). After stratification by gender, COL12A1 rs970547 AA genotypes were significantly associated with ACL rupture in female (AA vs. AG OR=2.25, 95% CI=1.29-3.92, p=0.590; AG + GG OR=0.52, 95% CI=0.31-0.88, P=0.314), but not in male (AA vs. AG OR=1.26, 95% CI=0.96-1.65, p=0.288; AG + GG OR=0.81, 95% CI=0.62-1.05, P=0.246). (**Table 3, Figure 4C, 4D**).

### *Heterogeneity and publication bias*

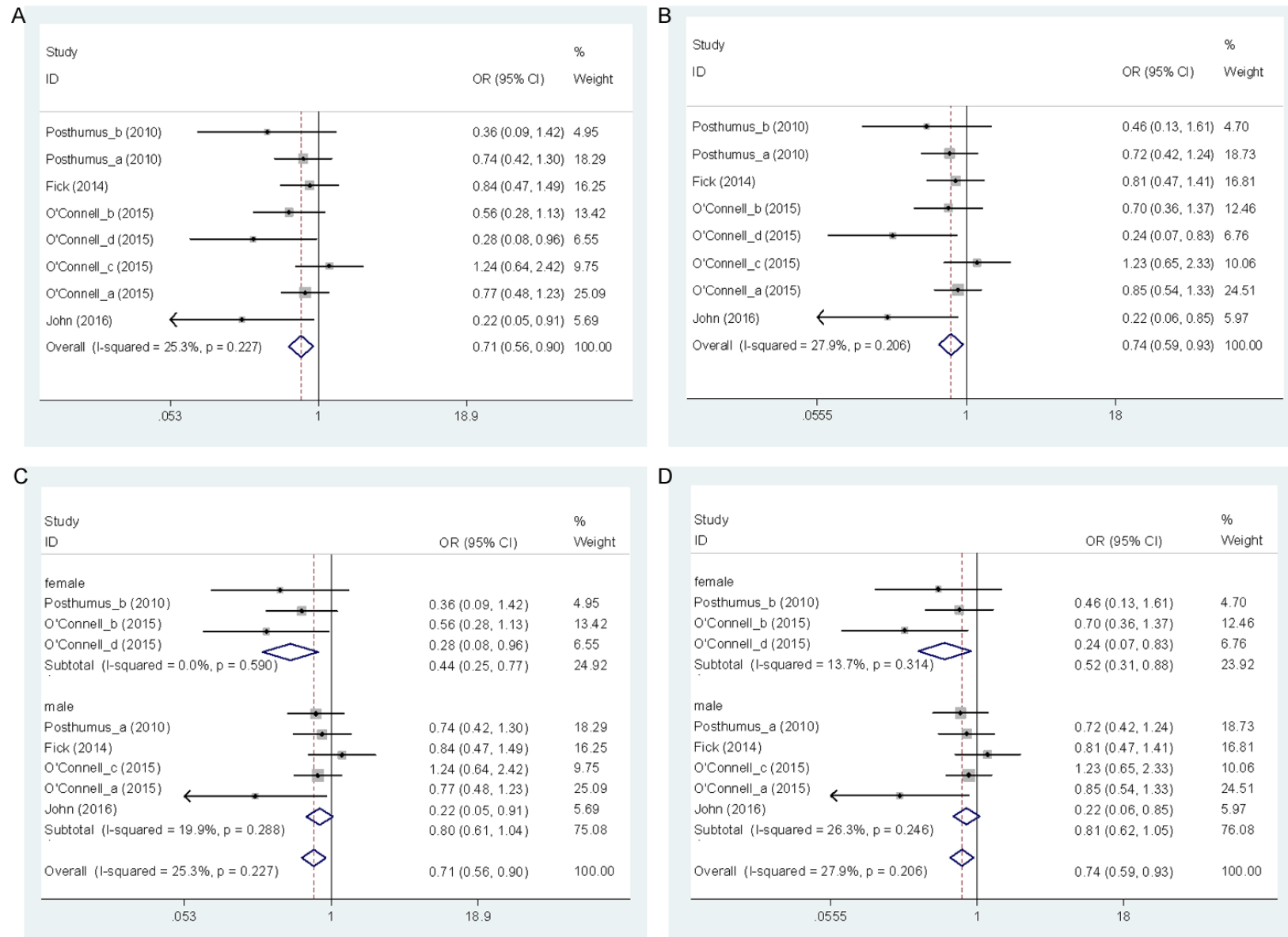
Between-study heterogeneity in terms of the ORs of the COL5A1 rs12722 polymorphism was found in both female and male subjects. Because the *P* values were < 0.5, the fixed effect model was used in the meta-analysis (**Table 3**). Moreover, between-study heterogeneity in terms of the ORs of the COL12A1 rs970547 polymorphism was found among all subjects, both female and male, meta-analysis

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**Figure 3.** Forest plots for the association of Col5A1 rs12722 genetic polymorphisms with ACL ruptures. A. Co-dominant model CC vs. TC; B. Recessive model CC vs. TT + TC; subgroup analysis. C. Co-dominant model CC vs. TC; D. Recessive model CC vs. TT + TC.

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**Figure 4.** Forest plots for the association of Col12A1 rs970547 genetic polymorphisms with ACL ruptures, A. Co-dominant model AG vs. AA; B. Dominant model AG + GG vs. AA; subgroup analysis, C. Co-dominant model AG vs. AA; D. Dominant model AG + GG vs. AA.



of the COL12A1 rs970547 polymorphism was performed using a fixed effect model for these subjects.

Publication bias causes a disproportionate number of studies with positive results, which reduces the accuracy and reliability of meta-analyses. There was no evidence for publication bias found for either COL5A1 rs12722 or COL12A1 rs970547 in our meta-analysis. For COL5A1 rs12722, Egger's test ( $p=0.890$ ,  $>0.05$ ) indicated no publication bias (**Figure 4A**). Meanwhile, Egger's test ( $p$  value =  $0.089$ ,  $>0.05$ ), revealed no evidence of obvious publication bias for COL12A1 rs970547 (**Figure 4B**).

### *Sensitivity analyses for pooled studies*

Sensitivity analysis of the COL5A1 rs12722 and COL12A1 rs970547 polymorphisms was performed to assess the impact of each study on pooled OR. The results suggested that the pooled ORs of these two polymorphisms were not materially altered by the omission of any individual study (**Figure 5**). The summary odds ratio coefficients on the relationships of the COL5A1 rs12722 and COL12A1 rs970547 genetic polymorphisms with susceptibility to osteoarthritis under the dominant mode (**Figure 6**).

### **Discussion**

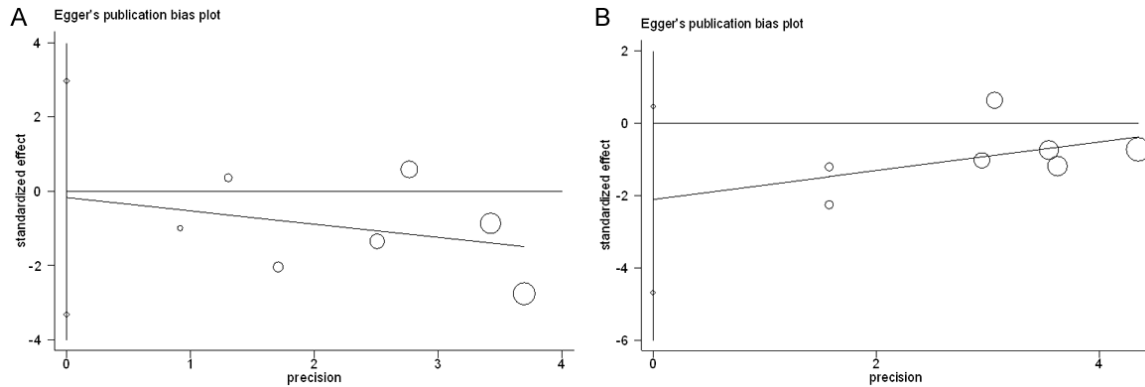
Meta-analysis is a method suitable for detecting the effects of human genetic association studies. We designed this study to examine the association between COL5A1 rs12722 and COL12A1 rs970547 gene polymorphisms and ACL rupture susceptibility. This meta-analysis included 4 studies on the COL5A1 rs12722 [16, 17, 21] and 5 studies on COL12A1 rs970547 [16, 22, 23, 29] polymorphisms and analyzed these gene polymorphisms with respect to their gender status. Our results indicate a significant association between COL5A1 rs12722 and COL12A1 rs970547 polymorphisms and susceptibility to ACL ruptures in all pooled subjects. Stratified analysis showed that the COL5A1 rs12722 CC genotypes are significantly associated with ACL rupture in males but not in females. For COL12A1 rs970547, stratified analysis showed that the AA genotypes were significantly associated with ACL rupture in females but not in males.

The clinical heterogeneity of ACL ruptures may be derived from gender differences, so we did a

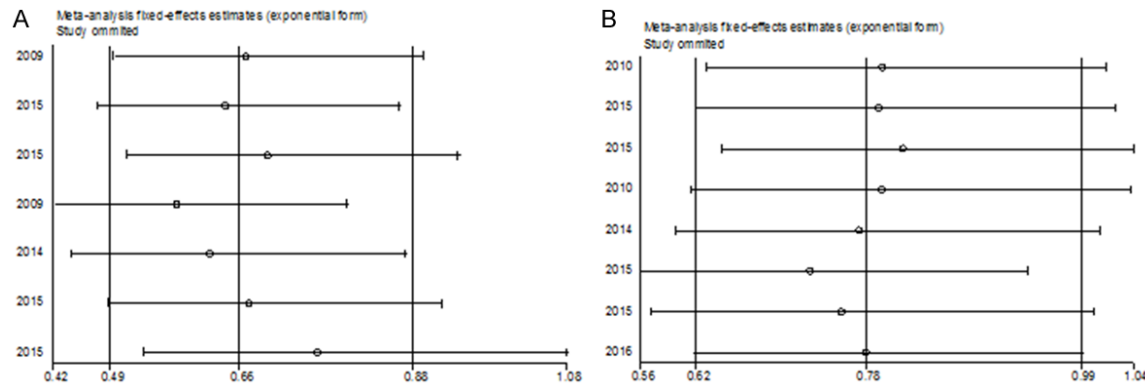
subgroup analysis of gender based on current studies. Some articles [16, 17] have reported over-expression of the CC genotype of COL5A1 rs12722 in ACL rupture patients, as did our study. Interestingly, the same CC genotype was also associated with a reduced risk of chronic Achilles tendon disease [19, 30]. This may be because the COL5A1 gene encodes for the  $\alpha 1$  chain in type V collagen, which is an important structural component of ligaments and tendons [31]. The literature [32] confirms that male patients with ACL ruptures have a significantly higher family history of ligamentous injury, which may be related to family members' exercise habits. However, it is not clear why this relationship between the COL12A1 rs970547 gene polymorphism and ACL ruptures was only present in male participants. Hormones, anatomy, neuromuscular or biomechanical properties may be specific to men, and one hypothesis is that the presence of gene-hormone interactions causes this male-specific genetic association. Sex hormones exert their biologic effects on the ACL by regulating gene expression, such as many of the MMPs [33]. Previous studies have shown that MMP3 and MMP1 gene expression is higher in the ACLs of women compared to men. Raleigh et al. [34] has reported an interaction between a sequence variant within the MMP3 gene and COL5A1 that modifies the risk of Achilles tendinopathy [33]. However, further studies are required to determine whether variants within the MMP3 gene are associated with ACL ruptures, particularly in men.

A number of articles [16, 27] have reported that the AA genotype of COL12A1 rs970547 is over-expressed in patients with ACL rupture, and our study supports this conclusion. Related to the gender association of the COL12A1 rs970547 and ACL ruptures, however, we found that there are no known reports of effects of female sex hormones on COL12A1 regulation or any gene-gene interaction that could explain this observed phenomenon. The gender-specific association in the COL12A1 rs970547 study as well as in the studies on COL5A1 may indicate that genetic variants alter the structural and/or biomechanical properties of the ACL that may compromise this ligament. Therefore, although the biomechanical properties of the ACL are affected by COL12A1 rs970547 in both male and female participants, the polymorphism did not significantly alter the risk of ACL

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**Figure 5.** Eggers funnel plot of the COL5A1 rs12722 (A) and COL12A1 rs970547 (B) polymorphism and ACL rupture in the dominant model. Each circle represents an individual study, while the circle size is proportional to the study weight. Log or natural logarithm of odds ratio.



**Figure 6.** Sensitivity analysis of the summary odds ratio coefficients on the relationships of the COL5A1 rs12722 (A) and COL12A1 rs970547 (B) genetic polymorphisms with susceptibility to osteoarthritis under the dominant mode.

rupture in male participants in this study. Because of this, we need a larger group of homogenous male participants with non-contact ACL ruptures and exposure-matched controls in future investigations.

Although rigorous literature selection criteria were developed to select the literature and we used NOS score and HEW to evaluate the quality of the selected literature so that high-quality literature was chosen for this meta-analysis, there are still many limitations that must be considered. First, because of insufficient raw data, some relevant studies could not be included in this meta-analysis despite the strict search strategy in the study. Second, the literature focuses on populations from South Africa and Poland, so the inclusion of too few studies leads to greater ethnicity heterogeneity in subgroup analyses. Additionally, we used Egger's test, Begg's test, and funnel plots

to evaluate publication bias in the small samples sizes of these studies; therefore, the possibility of bias cannot be ruled out completely.

Despite these limitations, this is the first meta-analysis of the association between COL5A1 and COL12A1 gene polymorphisms and ACL rupture risk, and sensitivity analysis showed that no single study should influence the main conclusion of the present study, confirming its robustness. Current methods were used to assess publication bias, and no other evidence was found to show obvious publication bias, indicating the credibility of our study. Taken together, our findings provide evidence that COL5A1 rs12722 and COL12A1 rs970547 genetic polymorphisms are associated with increased risk of ACL rupture. Thus, COL5A1 rs12722 and COL12A1 rs970547 genetic polymorphisms may be useful for identifying ACL rupture patients at an early stage. More

studies among different ethnicities are required to validate these findings and strengthen our conclusions.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Jun Liu, Department of Joint Surgery, Tianjin Hospital, 406 Jiefang Street, Hexi District, Tianjin, China. Tel: +86 13902170816; E-mail: doctorliutj@163.com

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