# Review Article Association between asporin gene aspartic acid repeat polymorphism and osteoarthritis: an updated meta-analysis

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**Abstract:** Objective: Osteoarthritis (OA) is a common disease characterized by progressive degeneration of articular cartilage. Several studies have been performed to evaluate the association between asporin gene aspartic acid repeat (ASPN D-Repeat) polymorphism and osteoarthritis susceptibility, but produced conflicting results. Our aim is to determine whether ASPN D-repeat polymorphism is associated with susceptibility to OA. Methods: A comprehensive literature search for all relevant studies was performed. The association between ASPN D-Repeat polymorphism and OA in each ethnic group was investigated. Sensitivity analysis and publication bias test were conducted. Results: Eleven studies with 8463 participants (4842 OA patients and 3621 controls) were enrolled in this study. The combined results revealed no significant association between D13 allele and the risk of OA (D13 allele vs. other alleles combined: OR 0.939, 95% CI 0.844-1.045). Subgroup analysis stratified by ethnicity and OA site further confirmed the irrelevance. The pooled results on the associations between other alleles (D14, D15, D16 and D17, respectively) and the risk of OA were similar to those of D13 allele, no significant association was identified in the overall population or in each subgroup population. Conclusion: This meta-analysis suggested that ASPN D-repeat polymorphism is not associated with OA susceptibility. Since potential confounders could not be ruled out completely, further well-designed studies are required.

Keywords: Asporin, meta-analysis, osteoarthritis, polymorphism

#### Introduction

Osteoarthritis (OA) is one of the most common causes of musculoskeletal disability that primarily affects the knees, hips, hands, and spine [1]. The hallmark of OA is joint pain and immobility, as well as progressive degeneration of articular cartilage involving remodeling of all joint tissues with subsequent joint space narrowing [2]. It was estimated by the World Health Organization (WHO) that 10% of the world's population aged  $\geq$  60 years suffered from symptomatic OA [3], and costs for knee OA treatment were estimated to be over 180 billion dollars per year [4]. Although its etiology remains unclear, several risk factors have been correlated with OA, including age, gender, obesity, mechanical forces, environmental factors and genetic factors [3, 5]. What's more, some candidate genes, such as GDF5 and VDR, have been reported to be associated with OA risk, suggesting that genetic factors play a key role in the pathogenesis of OA [6].

Osteoarthritis is characterized pathologically by progressive degeneration of articular cartilage, which results from an imbalance between synthesis and degradation of the extracellular cartilage matrix [7, 8]. Asporin belongs to the small leucine-rich proteoglycan family and is a biologically active protein of the extracellular cartilage matrix [9]. Asporin protein has been found to be expressed in many tissues, including articular cartilage [10]. TGF-ß signaling is involved in extracellular cartilage matrix synthesis and cartilage repair, and is thus essential for maintaining articular cartilage and preventing osteoarthritis. Asporin directly binds TGF-B, acting as a negative regulator of chondrogenesis by suppressing TGF- $\beta$  function [11, 12]. The

asporin gene (ASPN) lies within human chromosome 9q22-9q21.3 and contains a triplet repeat within exon 2, which codes for a polymorphic stretch of aspartic acid residues (D-repeat) in the N-terminal region of the protein [13]. The number of D-repeat varies from 8 (D8) to 20 (D20), and each allele with a different number of D-repeat may play a different role in OA development [10, 14]. Kizawa observed an association between the ASPN D14 allele and OA susceptibility, and the D14 allele resulted in greater inhibition of TNF-B activity than other alleles [15]. Besides, Kaliakatsos reported that the D15 allele is also a risk allele while the D13 allele may be protective [16].

Several studies have been performed to investigate the association between ASPN D-repeat polymorphism and OA susceptibility. However, the findings reported remain inconsistent [15-21]. The controversial results may be partly attributable to the different populations, limited sample sizes, environmental factors, and failure to take into account other possible confounding factors. Despite positive association found in some study populations, previous meta-analysis published in 2013 and 2014 failed to demonstrate a relationship between ASPN and OA [22, 23]. Since then, several new relevant researches reporting significant association between ASPN and OA risk were published [24-26]. In this study we performed an updated meta-analysis to investigate whether ASPN D-repeat polymorphism is associated with OA susceptibility.

#### Materials and methods

#### Literature search strategy

This meta-analysis was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) statement (<u>Table S1</u>) [27]. A comprehensive literature search for all relevant studies published in English or Chinese was performed in PubMed, Embase, and CNKI (China National Knowledge Infrastructure) databases. The combination of the following key words was used to identify all possible studies: ("asporin" OR "ASPN") AND ("polymorphism" OR "variant") AND "osteoarthritis". References of retrieved studies and review articles were also screened for other relevant studies. The literature search was updated on August 12, 2016.

#### Inclusion and exclusion criteria

The included studies must fulfill the following inclusion criteria: (1) case-control or cohort design; (2) OA was diagnosed according to American College of Radiology (ACR) clinical criteria or radiographic findings (Kellgren-Lawrence grade system), or ascertained by total joint replacement due to primary OA; (3) evaluating the association between ASPN D-repeat polymorphism and OA susceptibility; (4) providing sufficient genotype or allele data for calculation of odds ratio (OR) and 95% confidence interval (CI). The exclusion criteria were as follows: (1) case reports, animal model researches, or reviews; (2) containing invalid or incomplete data; (3) repeated or overlapped publications. If several publications reported findings for the same patients, only the most recent or most informative study was selected. Eligible studies were determined independently by two investigators.

#### Data extraction

For each eligible study, the following data were extracted by two independent reviewers: (1) name of the first author; (2) publication year; (3) study country/region; (4) ethnicity of participants; (5) study design; (6) OA sites; (7) source of controls; (8) numbers of cases and controls; (9) demographics of enrolled subjects; (10) allele counts of the D-repeat polymorphism in cases and controls. A third reviewer was introduced to resolve all the discrepancies during data extraction.

#### Quality assessment

Two independent investigators assessed the quality of the included studies using Newcastle-Ottawa Quality Assessment Scale (NOS) [28]. The studies scored from 0 point (worst) to 9 points (best), based on 8 assessment items. Studies scored 6 or less were classified as low quality, whereas studies with a score of 7 or higher were considered as high quality.

#### Statistical analysis

The association of ASPN D-repeat polymorphism with OA susceptibility was assessed by



calculating pooled ORs and corresponding 95% Cls. Z test was conducted to determine the statistical significance of pooled effect size. Only allele model was analyzed to assess the association, because genotype distribution data was not reported in the original articles. Heterogeneity between different studies was assessed using Q and  $l^2$  statistics [29]. If  $l^2 > l^2$ 50% or *P*-value of Q statistic < 0.10, we use the DerSimonian-Laird random effect model to calculate pooled ORs and 95% Cls [30]. Otherwise, the Mantel-Haenszel fixed effect model was used as the pooling method [31]. We also conducted subgroup analysis stratified by ethnicity and OA site. Sensitivity analysis was performed by removing an individual study each time to evaluate the stability of results. Publication bias was evaluated using funnel plot and Egger's regression test. P-value < 0.05 was considered significant except for the l<sup>2</sup> statistic. All statistical analyses were conducted using STATA version 12.0 (STATA Corporation, College Station, TX, USA).

#### Results

#### Study selection

A flow diagram for the study selection procedure and specific reasons for exclusion is shown in **Figure 1**. A total of 67 articles were

retrieved from a systematic literature search. 44 articles were excluded after title and abstract review, and 23 articles remained for further screening. Afterwards, 9 articles were excluded due to insufficient data or overlapped sample, and 4 articles concerning age at onset of OA or severity of OA were also removed. Finally, 10 articles met the inclusion criteria [15-21, 24-26]. One of these articles provided detailed data on two different groups [15], a case-control group and a cohort group. These two groups were analyzed independently. Therefore, 11 independent studies from 10 articles were included in our metaanalysis.

#### Characteristics of included studies

The primary characteristics of included studies are present in Table 1. A total of 8463 participants with 4842 OA patients and 3621 controls were enrolled in this study, which involved 4 Caucasian, 5 Asian and 2 Latin American populations. All but one comparison were casecontrol designs. OA patients were recruited according to clinical or radiographic findings of OA, or ascertained by total joint replacement. In all the included studies, blood samples were used for DNA extraction and PCR was applied as the genotyping method. As for the sites of OA, 11 studies examined knee OA, 3 studies examined hip OA and only 1 study examined hand OA. Regarding the NOS scale, the quality of all the included studies was fairly high (see Table 1 and Table S2). Because genotype distribution data was not reported in the original articles, only allele model was analyzed to assess the association, and allele counts for the D-repeat polymorphism in ASPN were shown in Table S3.

Association between ASPN D-repeat polymorphism and OA susceptibility

Table 2 and Figure 2 summarized the meta-<br/>analysis results on the association between<br/>ASPN D-repeat polymorphism and risk of OA.Overall, the combined results revealed no sig-

Study.	Voor	Country	Ethnioity	Study	Genotyping	Sampl	e size		A	ge		Association
Study	rear	Country	Etrifficity	design	method	OA (F/M)	Control (F/M)	UA SILE	OA	Control	1005	findings
Kizawa-1	2005	Japan	Asian	Cohort	PCR	137 (99/38)	234 (143/91)	Knee	75.3±5.1	73.6±5.3	9	D13↓, D14↑
Kizawa-2	2005	Japan	Asian	CC	PCR	986 (881/105)	374 (209/165)	Knee, hip	58.3±10.1	28.8±11.9	7	D14†, D17†
Mustafa	2005	UK	Caucasian	CC	PCR	1247 (737/510)	748 (392/356)	Knee, hip	65 (56-85)	69 (55-89)	8	D13↓ (M), D14↑ (M)
Jiang	2006	China	Asian	CC	PCR	218 (151/67)	454 (289/165)	Knee	58.1±18.9	56.3±12.1	9	D14↑
Kaliakatsos	2006	Greece	Caucasian	CC	PCR	158 (138/20)	193 (137/56)	Knee	68.7±8.1	68.2±10.4	7	D13↓, D15↑
Rodriguez-Lopez	2006	Spain	Caucasian	CC	PCR	723 (541/182)	294 (115/179)	Knee, hip, hand	> 55	> 55	7	NS
Atif	2008	USA	Caucasian	CC	PCR	775 (630/145)	511 (341/170)	Knee/hand	F: 70.8±8.6 M: 70.9±7.6	F: 67.5±7.1 M: 69.6±7.0	8	NS
Song	2008	Korea	Asian	CC	PCR	190 (152/38)	376 (154/222)	Knee	60	47.7	7	D13† (F)
Arelano	2013	Mexico	Latin American	CC	PCR	218 (130/88)	222 (130/92)	Knee	58.0±13.2	52.7±11.8	8	D16†, D17†
Jazayeri	2013	Iran	Asian	CC	PCR	100 (72/28)	100 (72/28)	Knee	50-75	50-75	8	D14↓ (F), D15↑ (F)
Gonzalez-Huerta	2015	Mexico	Latin American	CC	PCR	93 (75/18)	118 (98/20)	Knee	56.4±8.8	51.8±8.9	7	D14↑

Table 1. Characteristics of the studies included in the meta-analysis

CC: case-control; F: female; M: male;  $\uparrow/\downarrow$ : increase/decrease the risk of OA; NS: not significant.

Comparison	Sub-group		Test of associatio	on	Test of heterogenecity				
		OR	95% CI	P value	Statistical model	P value	l² (%)		
D13 vs. others	Overall	0.939	0.844-1.045	0.250	Random	0.015	54.6		
	Ethnicity								
	Asian	0.949	0.777-1.159	0.609	Random	0.022	65.0		
	Caucasian	0.902	0.769-1.057	0.202	Random	0.028	66.9		
	Latin American	1.065	0.823-1.378	0.633	Fixed	0.534	0.00		
	OA site								
	Knee OA	0.919	0.815-1.036	0.167	Random	0.012	55.9		
	Hip OA	0.923	0.835-1.020	0.116	Fixed	0.501	0.00		
D14 vs. others	Overall	1.150	0.945-1.400	0.162	Random	0.000	71.1		
	Ethnicity								
	Asian	1.338	0.815-2.198	0.250	Random	0.000	81.9		
	Caucasian	1.036	0.914-1.174	0.581	Fixed	0.598	0.00		
	Latin American	1.097	0.621-1.938	0.750	Random	0.025	80.1		
	OA site								
	Knee OA	1.174	0.954-1.445	0.129	Random	0.000	68.9		
	Hip OA	1.128	0.765-1.662	0.543	Random	0.011	78.0		
D15 vs. others	Overall	1.033	0.944-1.131	0.478	Fixed	0.317	13.3		
	Ethnicity								
	Asian	0.945	0.740-1.206	0.649	Fixed	0.675	0.00		
	Caucasian	1.068	0.963-1.184	0.214	Fixed	0.191	36.8		
	Latin American	0.849	0.500-1.442	0.544	Random	0.097	63.7		
	OA site								
	Knee OA	1.060	0.954-1.178	0.280	Fixed	0.444	0.00		
	Hip OA	1.000	0.871-1.147	0.995	Fixed	0.238	30.3		
D16 vs. others	Overall	1.040	0.860-1.258	0.687	Random	0.079	41.8		
	Ethnicity								
	Asian	0.983	0.668-1.446	0.929	Random	0.042	59.6		
	Caucasian	1.030	0.861-1.232	0.747	Fixed	0.683	0.00		
	Latin American	1.229	0.553-2.729	0.613	Random	0.046	74.9		
	OA site								
	Knee OA	1.000	0.790-1.267	0.998	Random	0.031	51.0		
	Hip OA	1.122	0.942-1.338	0.197	Fixed	0.792	0.00		
D17 vs. others	Overall	1.202	0.871-1.661	0.263	Random	0.053	46.3		
	Ethnicity								
	Asian	1.228	0.679-2.222	0.497	Random	0.054	56.9		
	Caucasian	1.262	0.901-1.768	0.176	Fixed	0.525	0.00		
	Latin American	1.318	0.381-4.567	0.663	Random	0.036	77.2		
	OA site								
	Knee OA	1.324	0.943-1.858	0.105	Random	0.075	42.5		
	Hip OA	0.903	0.551-1.480	0.686	Random	0.108	55.2		

 Table 2. Summary of pooled results on the association between ASPN D-repeat polymorphism and OA risk

nificant association between D13 allele and the risk of OA (D13 allele vs. other alleles combined: OR 0.939, 95% CI 0.844-1.045) (**Table 2**; **Figure 2A**). Furthermore, subgroup analysis stratified by ethnicity suggested that no associations existed in Asians (OR 0.949, 95% CI 0.777-1.159), Caucasians (OR 0.902, 95% CI 0.769-1.057) or Latin American (OR 1.065,





B Funnel plot with pseudo 95% confidence limits P(Egger)=0.431 P(Egger)=0.431 P(Egger)=0.431 P(Egger)=0.431 P(Egger)=0.431 P(Egger)=0.431 P(Egger)=0.599 P(Egger)=0.599P(Egger)=0.599

**Figure 3.** Funnel plots with the Egger's test for publication bias of ASPN D-repeat polymorphism and OA risk. A. D13 vs. others alleles combined; B. D14 vs. others alleles combined. C. D15 vs. others alleles combined; D. D16 vs. others alleles combined; E. D17 vs. others alleles combined. The shapes of the funnel plots appeared to be symmetrical, and no significant publication bias was found by Egger's test either.

95% CI 0.823-1.378). Stratification by OA site also showed no significant association between D13 allele and knee OA risk (OR 0.919, 95% CI 0.815-1.036) or hip OA risk (OR 0.923, 95% CI 0.835-1.020), further confirming the irrelevance between the D13 allele and OA susceptibility. The pooled results on the associations between other alleles (D14, D15, D16 and D17, respectively) and the risks of OA were similar to those of D13 allele and OA risk, no significant association was identified in the overall population or in each subgroup population (**Table 2**; **Figure 2B-E**).

#### Sensitivity analysis and publication bias

Sensitivity analysis was conducted to evaluate the stability of results by subsequently removing individual studies, and no single study qualitatively changed the pooled ORs. Publication bias was examined visually via funnel plot and statistically by using Egger's regression test. The shapes of the funnel plots appeared to be symmetrical and no evidence of significant publication bias was found by Egger's test (**Figure 3**). Thus, the robustness of the present metaanalysis was confirmed.

# Discussion

Osteoarthritis (OA), also known as degenerative arthritis, is the most common cause of physical disability worldwide [1]. Although OA is recognized as a complex, multifactorial disorder, genetic factors are thought to be strong determinants in the pathogenesis of OA [5, 10]. The association between genetic polymorphisms and OA risk has recently attracted increasing attention, and ASPN D-repeat polymorphism was intensively studied [32]. An association between D-repeat polymorphism and risk of OA was firstly observed by Kizawa [15] and confirmed by Jiang [18], but divergent results ranging from strong links to no association were obtained from other replication studies [16, 17, 19-21, 24-26]. Because of the above-mentioned inconclusive results from relatively small, underpowered studies, we conducted this meta-analysis to draw a more definitive conclusion.

In the current meta-analysis, we explored the association between ASPN D-repeat polymorphism and OA susceptibility. The pooled results demonstrated no association between OA and the ASPN D13, D14, D15, D16 and D17 alleles in the overall population. Subgroup analysis stratified by ethnicity and OA site also revealed no association. Sensitivity analysis and publication bias assessment indicated that the results of our meta-analysis were stable. The results of our research are in good agreement with two previous meta-analyses performed by Xing [22] and Song [23]. Compared with the previous studies, our meta-analysis included 3 additional studies. Besides, previous metaanalyses merely compared the differences of D13, D14 and D15 allele frequency between OA patients and controls, while we performed additional meta-analyses to examine potential association of the D16 and D17 alleles with risk of OA. Several genome-wide association studies (GWAS) have been performed and a number of genes, such as CLO11A1, VEGF, IGFBP3 and GDF5, have been identified as associated with OA [33-35]. However, ASPN has not been reported in these GWAS.

Functional studies speculated that the D-repeat polymorphism in asporin may mediate conformational changes that consequently result in diverse ability to suppress TGF- $\beta$  signaling during chondrogenesis [11, 15]. Our negative

results do not coincide with functional studies, one possible reason for the discrepancy is that OA is a complex disease with contributions from multiple genes and non-genetic factors [20]. However, we were unable to perform subgroup analysis by every confounding factor, including gender and body mass index, because few relevant data can be extracted from the original studies. Furthermore, ASPN D-repeat polymorphism have been reported to be significantly associated with age of onset and severity of OA [36-38]. Thus, the association between ASPN D-repeat polymorphism and OA susceptibility could not be excluded.

The present meta-analysis has some limitations that should be taken into account. First, because genotype distribution data was not reported in the original articles, only allele model was analyzed to assess the association. Second, OA is considered as a multifactorial disease, but the effects of gene-environment and gene-gene interactions, which may mask the potential role of ASPN D-repeat polymorphism, were not addressed in this meta-analysis. Third, since most included studies did not provide ORs and 95% CIs adjusted for potential confounding factors such as age, gender and body mass index, our results were primarily based on unadjusted estimates, resulting in relatively inaccurate pooled results. Last, although the funnel plot and Egger's test revealed no publication bias, selection bias may still exist because only studies published in English or Chinese were included, and we are likely to miss some important studies published in other languages.

In conclusion, our meta-analysis demonstrated no association between ASPN D-repeat polymorphism and OA susceptibility. Since potential confounders could not be ruled out completely, further large-volume, well-designed studies are required to confirm these results.

#### Disclosure of conflict of interest

#### None.

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#### Table S1. PRISMA checklist

Section/topic	#	Checklist item	Reported on page#
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibil- ity criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3, 4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	4
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years consid- ered, language, publication status) used as criteria for eligibility, giving rationale.	4, 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to iden- tify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	4
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if ap- plicable, included in the meta-analysis).	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	5
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	5
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I <sup>2</sup> ) for each meta-analysis.	6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selec- tive reporting within studies).	5, 6
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	6
Results			

Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	6, 7
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	7
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	7
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	7,8
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	8
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	8
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	8, 9
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete re- trieval of identified research, reporting bias).	10
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	10
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

#: number of checklist items. From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi: 10.1371/journal.pmed1000097.

Study		Sele	ection	n     Comparability     Exposure/Outcome     Sc $3$ Q4     Q5°     Q6     Q7     Q8 $3$ $4$ $25°$ $26$ $Q7$ $Q8$ $3$ $4$ $25°$ $26$ $Q7$ $Q8$ $3$ $4$	Scores				
	Q1	Q2	Q3	Q4	Q5ª	Q6	Q7	Q8	_
Cohort studies									
Kizawa-1 2005	\$	$\overset{\wedge}{\simeq}$	$\sum_{i=1}^{n}$	$\overset{\wedge}{\sim}$	**			$\stackrel{\wedge}{\simeq}$	9
Case-control studies									
Kizawa-2 2005	${\sim}$	\$	$\overset{\wedge}{\sim}$	\$	-	$\overset{\wedge}{\sim}$	$\stackrel{\wedge}{\sim}$		7
Mustafa 2005	$\overset{\sim}{\sim}$	☆	$\overset{\wedge}{\bowtie}$	$\overset{\wedge}{\sim}$	\$	${\sim}$	$\overset{\sim}{\sim}$		8
Jiang 2006	${\sim}$	\$	$\overset{\wedge}{\sim}$	\$	**	$\overset{\wedge}{\sim}$	$\stackrel{\wedge}{\sim}$		9
Kaliakatsos 2006	${\sim}$	\$	-	\$	$\stackrel{\sim}{\sim}$	${\sim}$	$\stackrel{\wedge}{\sim}$		7
Rodriguez-Lopez 2006	${\leftrightarrow}$	☆	-	\$	$\overleftrightarrow$			☆	7
Atif 2008	${\sim}$	\$	$\overset{\wedge}{\sim}$	\$	$\stackrel{\sim}{\sim}$	${\sim}$	$\stackrel{\wedge}{\sim}$		8
Song 2008	${\leftrightarrow}$	☆	${\bigtriangledown}$	\$	-			☆	7
Arelano 2013	${\leftrightarrow}$	\$	${\swarrow}$	☆	${\leftrightarrow}$	☆	☆	☆	8
Jazayeri 2013		$\overset{\wedge}{\backsim}$	$\overset{\wedge}{\sim}$	$\overset{\wedge}{\sim}$	$\overleftrightarrow$		$\stackrel{\frown}{\simeq}$	☆	8
Gonzalez-Huerta 2015	545	54	-	545	54	545	545	545	7

Table S2. Results of quality assessment for the included studies using the Newcastle-Ottawa Scale

<sup>a</sup>A maximum of 2 stars can be allotted in this category, one for age, the other for other controlled factors.

Table S3. Allele count	s for the D-repeat	polymorphism in	n ASPN in the	included studies
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Study	Year	OA Site				OA							Conti	ol		
			D13	D14	D15	D16	D17	Others	Total	D13	D14	D15	D16	D17	Others	Total
Kizawa-1	2005	Knee	163	30	14	15	15	37	274	314	22	22	31	16	63	468
Kizawa-2	2005	Knee	459	61	34	84	26	122	786	479	36	34	57	35	107	748
		Hip	731	94	37	104	34	186	1186							
		Overall	1190	155	71	188	60	308	1972							
Mustafa	2005	Knee	258	76	116	43	18	45	556	752	190	289	124	26	115	1496
		Hip	858	258	362	158	39	133	1808							
		Knee & hip	67	18	20	15	3	7	130							
		Overall	1183	352	498	216	60	185	2494							
Jiang	2006	Knee	300	41	11	15	5	64	436	604	44	29	39	3	189	908
Kaliakatsos	2006	Knee	118	47	84	20	11	30	310	189	53	74	30	16	18	380
Rodriguez-Lopez	2006	Knee	156	56	93	30	14	27	376	248	74	150	55	12	49	588
		Hip	262	59	156	68	13	48	606							
		Hand	207	57	113	46	13	28	464							
		Overall	625	172	362	144	40	103	1446							
Atif	2008	Knee/hand	749	206	338	NA	NA	257	1550	496	142	212	NA	NA	172	1022
Song	2008	Knee	265	22	13	15	5	60	380	483	65	28	51	7	118	752
Arelano	2013	Knee	205	91	69	38	19	14	436	204	107	66	22	8	37	444
Jazayeri	2013	Knee	82	32	52	22	7	5	200	91	40	45	12	6	6	200
Gonzalez-Huerta	2015	Knee	7	123	25	21	9	1	186	6	134	47	32	16	1	236

# Newcastle-ottawa quality assessment scale for case control studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability

### Selection

Q1) Is the case definition adequate?

- a) yes, with independent validation \*
- b) yes, eg record linkage or based on self reports
- c) no description
- Q2) Representativeness of the cases
  - a) consecutive or obviously representative series of cases \*
  - b) potential for selection biases or not stated
- Q3) Selection of Controls
  - a) community controls \*
  - b) hospital controls
  - c) no description
- Q4) Definition of Controls
  - a) no history of disease (endpoint) \*
  - b) no description of source

#### Comparability

Q5) Comparability of cases and controls on the basis of the design or analysis

- a) study controls for the most important factor.  $\ensuremath{\mathfrak{R}}$
- b) study controls for any additional factor. \*

# Exposure

- Q6) Ascertainment of exposure
  - a) secure record (eg surgical records) \*
  - b) structured interview where blind to case/control status \*
  - c) interview not blinded to case/control status
  - d) written self report or medical record only
  - e) no description
- Q7) Same method of ascertainment for cases and controls
  - a) yes 🕸
  - b) no
- Q8) Non-Response rate
  - a) same rate for both groups \*
  - b) non respondents described
  - c) rate different and no designation

# Newcastle-ottawa quality assessment scale for cohort studies

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

# Selection

- Q1) Representativeness of the exposed cohort
  - a) truly representative of the average population in the community st
  - b) somewhat representative of the average population in the community
  - c) selected group of users
  - d) no description of the derivation of the cohort
- Q2) Selection of the non exposed cohort
  - a) drawn from the same community as the exposed cohort st
  - b) drawn from a different source
  - c) no description of the derivation of the non-exposed cohort
- Q3) Ascertainment of exposure
  - a) secure record ∗
  - b) structured interview \*
  - c) written self-report
  - d) no description
- Q4) Demonstration that outcome of interest was not present at start of study
  - a) yes \*
  - b) no

# Comparability

- Q5) Comparability of cohorts on the basis of the design or analysis
  - a) study controls for the most important factor. \*
  - b) study controls for any additional factor. \*

# Outcome

- Q6) Assessment of outcome
  - a) independent blind assessment \*
  - b) record linkage ∗
  - c) self-report
  - d) no description
- Q7) Was follow-up long enough for outcomes to occur
  - a) yes ≉
  - b) no
- Q8) Adequacy of follow up of cohorts
  - a) complete follow up-all subjects accounted for \*
- b) subjects lost to follow up unlikely to introduce bias small number lost > 70 % follow up, or description provided of those lost
  - c) follow up rate < 70% and no description of those lost
  - d) no statement