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Association between the aspartic acid D-repeat polymorphisms and osteoarthritis susceptibility

An updated systematic review and meta-analyses

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Abstract

Objectives: Association between the D-repeat of asporin (ASPN) gene and osteoarthritis (OA) was still inconsistent. We performed this meta-analysis to systematically assess the D-repeat polymorphisms in OA susceptibility.

Methods: Relevant studies were enrolled by searching databases. Odd ratios (ORs) with 95% confidence intervals (95% CIs) were used for evaluating the association between ASPN gene and OA. Heterogeneity was calculated using the Q statistic, and three different subgroup analyses were performed on ethnicity, gender, and OA positions respectively. False discovery rate (FDR) was applied to regulate the multiple comparisons.

Results: Twelve qualified articles involving 5190 OA patients and 5167 healthy controls were included. With D13 polymorphism, Caucasian male patients have low OA susceptibility ($P = .008$, $P_{FDR} = .024$, OR [95% CI] = 0.83 [0.73–0.95]). As to D14 polymorphism, all male patients ($P = .0004$, $P_{FDR} = .001$, OR [95% CI] = 1.38 [1.15–1.64]), Asian male patients ($P = .01$, $P_{FDR} = .01$, OR [95% CI] = 1.72 [1.11–2.66]), and Caucasian male patients ($P = .005$, $P_{FDR} = .001$, OR [95% CI] = 1.32 [1.09–1.60]) have high OA susceptibility. In the pooled-population of KOA with D14 polymorphism, overall male patients ($P = .03$, $P_{FDR} = .045$, OR [95% CI] = 1.35 [1.02–1.78]) and Asian male patients ($P = .01$, $P_{FDR} = .03$, OR [95% CI] = 1.72 [1.11–2.66]) have high OA risk. With D16 polymorphism, Latin America patients may have high OA risk ($P = .04$, $P_{FDR} = .15$, OR [95% CI] = 1.43 [1.02–2.01]).

Conclusion: Our results suggest that D-repeat of ASPN gene is mainly associated with male patients. The D13 polymorphism plays a protective role for OA in Caucasians male individuals while D14 plays a risk factor for KOA in male patients.

Abbreviations: ASPN = asporin, CBM = Chinese Biomedical Database, CI = confidence interval, CNKI = Chinese National Knowledge Infrastructure, ECM = extracellular matrix, KOA = knee osteoarthritis, NOS = Newcastle–Ottawa Scale, OA = osteoarthritis, OR = odd ratio, PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-analyses, TGF-β = transforming growth factor-β.

Keywords: asporin, meta-analysis, osteoarthritis, polymorphism

1. Introduction

Osteoarthritis (OA) is a kind of aged-related inflammatory disease of synovial joints that mainly affects knee, hip, hand, and spine,[1] and is characterized by progressive degeneration of articular cartilage and changes in subchondral bone including gradual reduction of extracellular matrix (ECM) and the breakdown of collagen.[2,3] In addition, OA makes a significant economic burden on patients who don't receive effective treatment,[4–7] the pathogenesis of OA is still unknown. Asporin, a novel type of ECM protein, contains a specific polymorphic aspartic acid D-repeat in N-terminal,[8,9] and belongs to a member of leucine-rich proteoglycan protein family, some of which will combine with transforming growth factor-β (TGF-β), and other ECM molecules of cartilage.[10] The high expression level of asporin (ASPN) mRNA in osteoarthritic articular has been reported,[8] and numerous studies suggested that ASPN is a candidate gene in the participation on development and progression of OA, but these results were controversial.

The human ASPN gene locates on chromosome 9q22.13, contains 8 exons, and encodes 382 amino acid proteins with a D repeat that has 10 alleles and the D13 allele is the mostly common.[11] Associations between D13, D14, D15, D16 polymorphism, and OA susceptibility have been widely demonstrated, but results were inconsistent.[12] In 2005, Kizawa et al[13]

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H-LW, XZ and W-TW should be considered co-first author.

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firstly reported that D14 allele was associated with knee OA (KOA) and its frequency increases with disease severity in Japanese population. Furthermore, D14 and D13 were over-represented and under-represented respectively in KOA. These results were duplicated in Chinese population,[14] while the D13 allele instead of D14 demonstrated a tendency of increasing in Korean OA patients.[15] In contrast, compared with health controls, the D14 allele showed a slight increase and the D13 was underrepresented in Caucasian OA patients.[16–18] In the Latin American population, Gonzalez-Huerta et al[19] found a marginal association with the D14 instead of D13, D15, D16 allele in KOA, while Arellano et al[20] suggested that the D16 allele showed a risk factor for KOA after adjusted some covariates. These inconsistent results might be caused by regional and race difference, and their associations need to be evaluated further.

Previous studies showed that the ASPN D14, D13, and D15 alleles were not associated with KOA and hip OA in both Caucasians and Asians.[11,21] However, some issues should be considered. Firstly, the incidence and prevalence of OA were different between male and female in various ethnic populations.[15,22,23] Moreover, in some original articles of meta-analysis conducted, it is that there was a significant difference in male patients while no difference in female patients. Secondly, the sample size of previous studies was not big enough, and the subgroup analysis for Latin America population was not performed. In view of these reasons, we performed an updated meta-analysis which was based on stratified analysis of ethnicity, gender and OA position with a larger sample size conducted to further help understand the role of ASPN gene in OA susceptibility.

2. Materials and methods

2.1. Search strategy and selection criteria

This present paper followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) criteria with all relevant studies searched up to August 2017 from the PubMed, the Web of Science, Chinese National Knowledge Infrastructure (CNKI), and CBM (Chinese Biomedical Database) databases. Key words used for searching were (polymorphism or variation) and (asporin or ASPN) and (osteoarthritis or OA) in the title and abstract with no lingual restrictions. All eligible studies were retrieved, and their bibliographies were checked.

Studies were included in our meta-analysis in case of meeting the following criteria: articles discussing the association between D-repeat polymorphisms of ASPN and OA susceptibility; in accordance with diagnostic criteria of OA in human; the genotype distribution or allele frequencies in case and control groups could be available; research of D-repeat polymorphisms should be executed more than 2 times and their results should be different. Studies were excluded in case of any of following: not meeting the inclusion criteria; irrelevant or duplicated studies; review, meta-analysis, or comment.

2.2. Data extraction

Throughout initial evaluation, relevant data were extracted independently by 2 authors (Honglin Wang and Xu Zhang) according to selection criteria, and a discussion with a third author (Wentao Wu) was engaged in case 2 authors had a different opinion. The following items of each study include: first author's name, publication year, country of origin, ethnicity, sample sizes of patients and controls, the genotype distribution or allele frequencies of each D-repeat polymorphisms in 2 groups, distribution of gender, and the site of OA.

2.3. Assessment of study quality

The Newcastle–Ottawa Scale (NOS) assessment scale was used to assess the quality of studies by 2 authors independently. The scale consists of 9 items which cover three fundamental aspects: selection of cases (4 items); comparability of case and control groups base on the design or analysis (2 items); and assessment of outcome (3 items). A score was awarded for each item which is satisfied by the research. The value ranged from 0 to 9, a score value more than 5 was considered as high quality.

2.4. Ethics

Ethical approval and informed consent were not required for the present meta-analyses.

2.5. Statistical analysis

Associations between D-repeat polymorphisms of ASPN gene and OA risk was assessed as odd ratios (ORs) with 95% confidence interval (95% CIs). Heterogeneity test was performed using χ^2-based Q statistic, and the effects of heterogeneity based on I^2 value of 25%, 50%, and 75% were nominally regarded as low, moderate, and high estimates, respectively. The random-effects model would be analyzed when there was a significant Q statistic ($P < .05$ or $I^2 > 50$), otherwise a fixed-effects model would be used. Subsequently, to explore potential sources of heterogeneity, various subgroups analyses were conducted on ethnicity (Asia vs Caucasian vs Latin America), gender (female vs male) and OA positions (knee vs hip vs hand). In addition, sensitivity analysis was performed by sequential omission of individual study to assess whether one or several studies influence overall results. To estimate the underlying sources of publication bias, Begg rank correlation test and Egger linear regression were used to assess the degree of bias. Then trim and fill method was applied if there was potential publication bias. All statistical analyses were calculated using the STATA 11.0 software (StataCorp, College Station, TX) and Review Manager Software 5.1 (Cochrane Collaboration, Oxford, United Kingdom). Benjamini–Hochberg method of false discovery rate (FDR) was applied to regulate the multiple comparisons, and $P < .05$ was indicated the presence of statistically significant.

3. Results

3.1. Characteristics of eligible studies

The study selection flow was shown in Supplementary Fig 1, http://links.lww.com/MD/C617, and 155 potentially relevant studies have been retrieved from the databases, PubMed (n = 44), Web of Science (n = 89), CNKI (n = 11), and CBM (n = 6). Throughout screening step by step, 12 qualified articles involving 5190 OA patients and 5167 healthy controls were included. Among 12 articles, 5 were from Asians,[13–15,24,25] 4 were Caucasians,[3,16–18] and 3 were Latin Americas;[19,20,26] all articles were associated with KOA while 3 associated with hip OA and one associated with hand OA; stratification analysis by

Table 1

Results of meta-analysis and subgroup analysis in pooled OA patients.

Gene	Subgroup	No.	Sample size		OR (95% CI)	*P*	P_{FDR}	Heterogeneity test	P_{Begg}	P_{Egger}	Model
			Case	Control							
D13	Total	12	5190	5167	0.97 (0.88–1.07)	.52	0.88	P=.008, I^2=56%	1.000	0.445	R
	Asian	5	1849	2366	1.01 (0.84–1.23)	.88	.88	P=.009, I^2=70%	.734	0.266	R
	Caucasian	4	2900	2331	0.91 (0.78–1.066)	.23	.88	P=.01, I^2=71%	1.000	0.431	R
	Latin America	3	441	470	1.04 (0.84–1.29)	.70	.88	P=.79, I^2=0%	1.000	0.371	F
	All female	7	1934	1671	1.04 (0.94–1.14)	.49	1.00	P=.82, I^2=0%	.881	0.658	F
	Asian female	4	526	804	1.09 (0.92–1.29)	.31	1.00	P=.79 I^2=0%	1.000	0.315	F
	Caucasian female	2	1278	737	1.01 (0.89–1.15)	.90	1.00	P=.26, I^2=20%	1.000	NA	F
	Latin America female	1	130	130	1.00 (0.69–1.44)	1.00	1.00	NA	NA	NA	NA
	All male	6	1220	1292	0.89 (0.79–1.01)	.07	.105	P=.32, I^2=15%	.260	.096	F
	Asian male	4	200	580	1.14 (0.89–1.46)	.31	.31	P=.7, I^2=0%	1.000	.376	F
	Caucasian male	2	1020	712	0.83 (0.73–0.95)	**.008**	**.024**	P=1.0, I^2=0%	1.000	NA	F
D14	Total	12	5190	5167	1.14 (0.95–1.38)	.16	.28	P<.001, I^2=75%	1.000	.787	R
	Asian	5	1849	2366	1.33 (0.85–2.07)	.21	.42	P<.001, I^2=83%	.142	.313	R
	Caucasian	4	2900	2331	1.02 (0.91–1.15)	.73	.81	P=.53, I^2=0%	1.000	.996	F
	Latin America	3	441	470	1.04 (0.73–1.49)	.81	.81	P=.08, I^2=61%	1.000	.629	R
	All female	7	1934	1671	1.07 (0.83–1.38)	.6	.82	P=.03, I^2=58%	.652	.974	R
	Asian female	4	526	804	1.11 (0.61–2.02)	.72	.82	P=.004, I^2=77%	.497	.153	R
	Caucasian female	2	1278	737	1.03 (0.85–1.26)	.75	.82	P=.75, I^2=0%	1.000	NA	F
	Latin America female	1	130	130	0.95 (0.62–1.47)	.82	.82	NA	NA	NA	NA
	All male	6	1220	1292	1.38 (1.15–1.64)	**.0004**	**.001**	P=.10, I^2=45%	1.000	.495	F
	Asian male	4	200	580	1.72 (1.11–2.66)	**.01**	**.01**	P=.06, I^2=59%	.497	.474	R
	Caucasian male	2	1020	712	1.32 (1.09–1.60)	**.005**	**.001**	P=1.00, I^2=0%	1.000	NA	F
D15	Total	12	5190	5167	1.02 (0.94–1.10)	.70	.70	P=.30, I^2=14%	.217	.584	F
	Asian	5	1849	2366	0.89 (0.72–1.11)	.30	.40	P=.69, I^2=0%	1.000	.935	F
	Caucasian	4	2900	2331	1.05 (0.96–1.16)	.29	.40	P=.16, I^2=43%	.089	.030	F
	Latin America	3	441	470	0.95 (0.74–1.22	.69	.70	P=.20, I^2=38%	.296	.532	F
	All female	7	1934	1671	0.98 (0.85–1.12)	.76	.90	P=.19, I^2=31%	.176	.530	F
	Asian female	4	526	804	0.98 (0.67–1.43)	.90	.90	P=.05, I^2=63%	.174	.008	R
	Caucasian female	2	1278	737	0.97 (0.83–1.13)	.69	.90	P=.51, I^2=0%	1.000	NA	F
	Latin America female	1	130	130	1.10 (0.67–1.79)	.71	.90	NA	NA	NA	NA
	All male	6	1220	1292	1.04 (0.88–1.25)	.64	.96	P=.60, I^2=0%	.133	.881	F
	Asian male	4	200	580	1.01 (0.62–1.64)	.97	.97	P=.30, I^2=17%	.174	.264	F
	Caucasian male	2	1020	712	1.04 (0.88–1.25)	.63	.96	P=1.00, I^2=0%	1.000	NA	F
D16	Total	11	5190	5167	1.09 (0.98–1.22)	.11	.15	P=.03, I^2=49%	.586	.793	R
	Asian	5	1849	2366	1.09 (0.91–1.30)	.36	.48	P=.03, I^2=62%	1.000	.422	R
	Caucasian	3	2900	2331	1.04 (0.89–1.22)	.64	.64	P=.67, I^2=0%	1.000	.105	F
	Latin America	3	441	470	1.43 (1.02–2.01)	**.04**	.15	P=.06, I^2=64%	1.000	.836	R
	All female	7	1934	1671	1.03 (0.75–1.41)	.86	.86	P=.04, I^2=55%	1.000	.985	R
	Asian female	4	526	804	0.84 (0.48–1.46)	.53	.86	P=.09, I^2=54%	1.000	.158	R
	Caucasian female	2	1278	737	1.05 (0.81–1.38)	.71	.86	P=.23, I^2=30%	1.000	NA	F
	Latin America female	1	130	130	2.10 (1.03–4.30)	**.04**	.16	NA	NA	NA	NA
	All male	6	1220	1292	0.92 (0.74–1.15)	.47	.71	P=.98, I^2=0%	1.000	.585	F
	Asian male	4	200	580	0.96 (0.54–1.70)	.89	.89	P=.88, I^2=0%	.174	.032	F
	Caucasian male	2	1020	712	0.92 (0.73–1.16)	.46	.71	P=1.00, I^2=0%	1.000	NA	F

CI = confidence interval, E = ethnicity, F = fixed, NA = not available NO = number of studies, OR = odds ratios, P_{Begg} = *P* value for Begg test, P_{Egger} = *P* value for Egger test, P_{FDR} = *P* value of false discovery rate, R = random. Significant associations shown in bold.

gender has been performed in 6 articles while it has not been performed in 5 articles. In addition, all articles examined the ASPN D13, D14, D15 and D16 polymorphisms except Atif et al,[3] and high quality with a NOS value more than five in all studies (Supplementary Table 1, http://links.lww.com/MD/C617).

3.2. Results of meta-analysis in pooled OA patients

There were no associations between D13, D14, and D15 polymorphisms of ASPN gene and OA in the pooled population and in the subgroup analysis by ethnicity. Latin America population have high risk of OA (P=.04, OR [95% CI]=1.43 [1.02–2.01]) when they carried with D16 polymorphism, but this statistical difference disappears after adjustment of FDR (P_{FDR}=.15, Table 1 and Fig. 1B). Subgroup analysis revealed that Caucasians male patients with D13 polymorphism have low OA susceptibility (P=.008, P_{FDR}=.024, OR [95% CI]=0.83 [0.73–0.95], Table 1 and Fig. 1A). With the D14 polymorphism, all male patients (P=.0004, P_{FDR}=.001, OR [95% CI]=1.38 [1.15–1.64]), Asian male patients (P=.01, P_{FDR}=.01, OR [95% CI]=1.72 [1.11–2.66]), and Caucasian male patients (P=.005, P_{FDR}=.001, OR [95% CI]=1.32 1.09–1.60]) have high OA susceptibility (Table 1, Fig. 2A). In the stratification of females, there were no significant differences (Table 1).

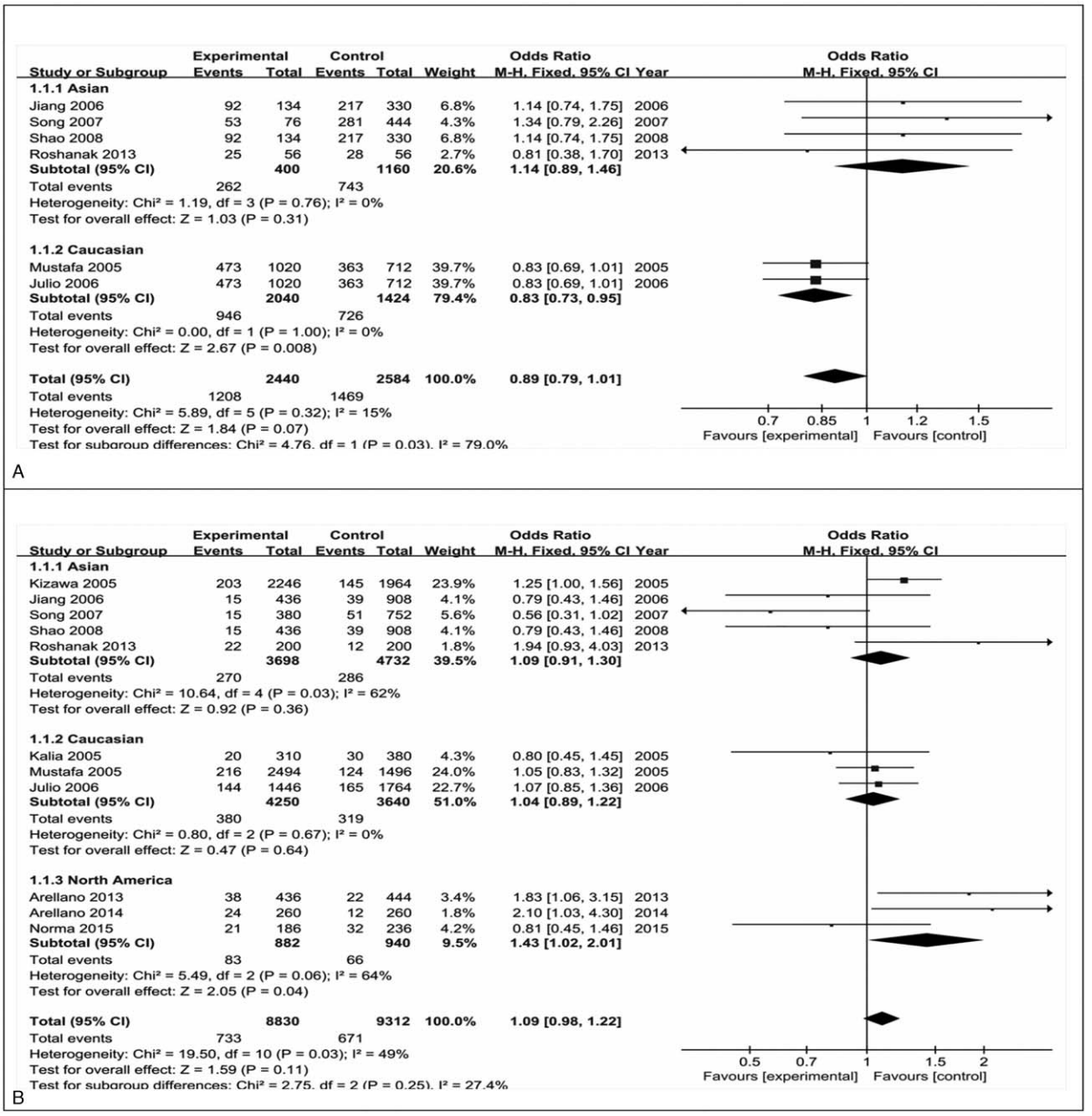

Figure 1. Results of subgroup analysis based on the D13 and D16 polymorphisms (A represents D13 allele is associated with overall-population in male-OA patients, and B represents D16 allele is associated with knee OA in Latin America population).

3.3. Results of meta-analysis in knee and hip OA patients

In the pooled-population of KOA with D14 polymorphism, all male patients ($P=.03$, $P_{FDR}=.045$, OR [95% CI]=1.35 [1.02–1.78]) and Asian male patients ($P=.01$, $P_{FDR}=.03$, OR [95% CI]=1.72 [1.11–2.66]) have high OA risk (Table 2, Fig. 2B). In the meta-analysis of hip OA, there was no statistical significant difference in any subgroup, but an original article found a significant difference in Asian patients ($P=.008$, $P_{FDR}=.024$, OR [95% CI]=1.70 [1.15–2.53]) (Table 3). We recommend that further studies should be extended to other ethnicities.

3.4. Results of heterogeneity test, sensitivity analysis and publication bias

As shown in the Table 2, significant heterogeneity ($P<.05$ or $I^2>50\%$) was found in D14 polymorphism of pooled-population and female individuals as well as in D16 polymorphism of the female patients. In KOA (Table 3), significant heterogeneity was found in D14 polymorphism of pooled-population and male individuals as well as in overall population with D16 polymorphism. In hip OA (Table 3), heterogeneities were also found in D14 polymorphism of pooled-population and male individuals, thus the random effect model was used to calculate results.

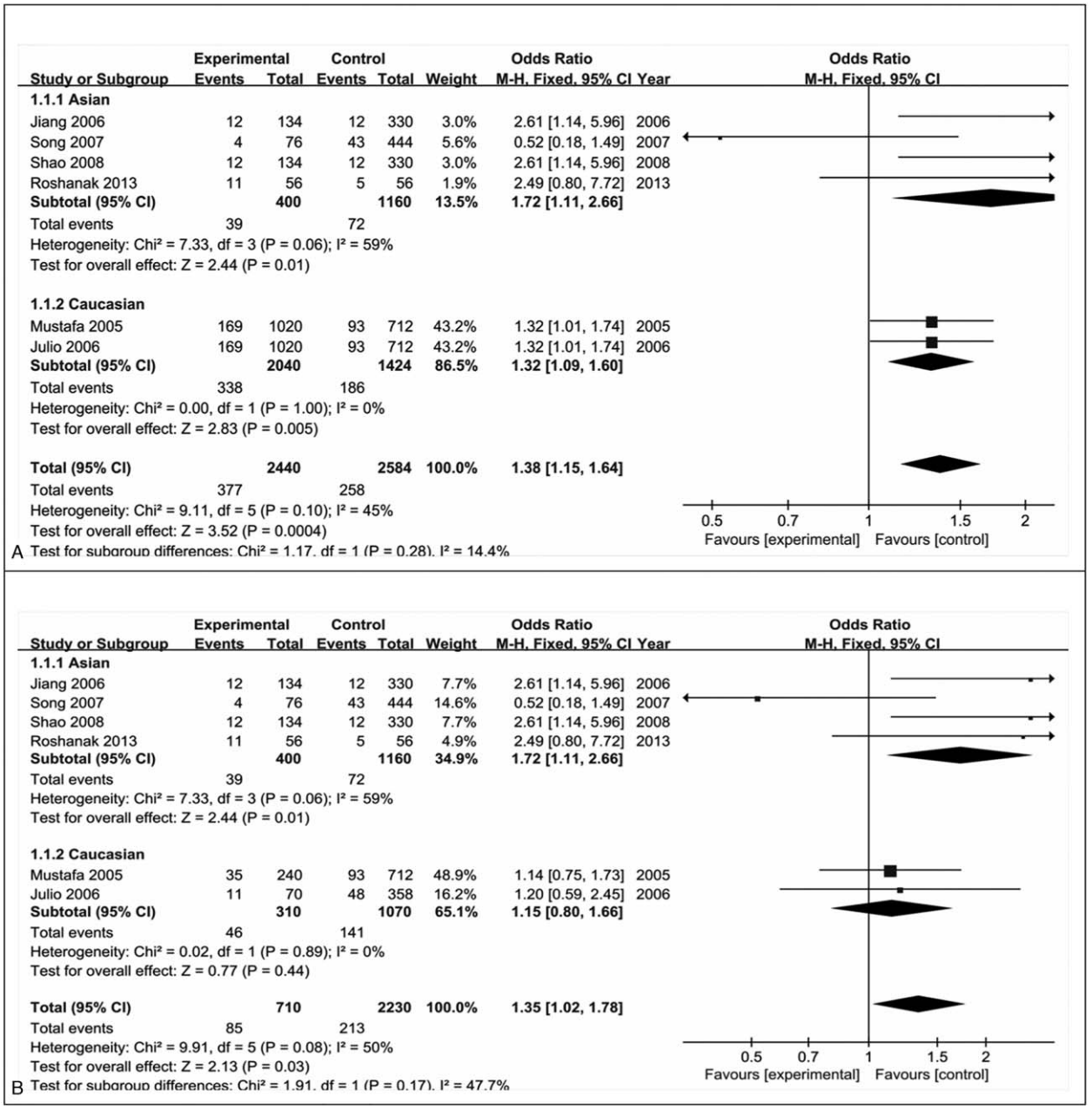

Figure 2. Results of subgroup analysis based on the D14 polymorphism in male patients (A and B represents overall-population patients and knee OA patients in male, respectively).

Sensitivity analysis was conducted to evaluate the effect of each study on the pooled-OR value by omitting one study from merged analysis. The pooled ORs with 95% CIs by sequential omission were not materially altered, suggesting that our results were statistically strong. Begg funnel plot and Egger tests were performed to access the publication bias, and there were no significantly alteration after trim and fill method was performed, indicating that our results were robust in statistics.

4. Discussion

OA is one of most common arthritis in worldwide and is a multifactorial disease in which aging, gene and environmental have been contribute to initiation and progression of disease.[27–29] Many OA associated genes, including ASPN which is an extracellular matrix component and is abundantly expressed in the articular cartilage of OA, have been studied in past years. In 2005, *Kizawa et al*[13] firstly reported the association between the ASPN gene polymorphisms and KOA susceptibility in Japanese. Subsequently, various replicated studies were performed in other populations and other sites of OA.[3,14,16,17,19,20,24] However, these results were inconsistent. We therefore carried out this meta-analysis to systematically evaluate whether ASPN polymorphisms are associated with OA susceptibility by synthesizing relevant studies.

In present study, we performed a meta-analysis approach based on three different subgroups with a lager sample size compared with previous studies.[11,21,30] A total of twelve articles

Table 2

Results of meta-analysis and subgroup analysis in pooled knee OA patients.

Gene	Subgroup	No.	Sample size		OR (95% CI)	*P*	P_{FDR}	Heterogeneity test	P_{Begg}	P_{Egger}	Model
			Case	Control							
D13	Total	14	3158	4953	0.94 (0.85–1.05)	.27	.54	P=.02, I^2=48%	.956	.872	R
	Asian	6	1256	1992	0.96 (0.80–1.16)	.69	.70	P=.01, I^2=66%	.851	.884	R
	Caucasian	5	2491	1348	0.89 (0.76–1.04)	.15	.54	P=.08, I^2=52%	.806	.575	R
	Latin America	3	441	470	1.04 (0.84–1.29)	.70	.70	P=.79, I^2=0%	1.000	.371	F
	All female	7	967	1441	1.00 (0.88–1.13)	1.00	1.00	P=.68, I^2=0%	.881	.826	F
	Asian female	4	526	804	1.09 (0.92–1.29)	.31	.41	P=.79, I^2=0%	1.000	.315	F
	Caucasian female	2	311	507	0.87 (0.71–1.08)	.20	.41	P=.63, I^2=0%	1.000	NA	F
	Latin America female	1	130	130	1.00 (0.69–1.44)	1.00	1.00	NA	NA	NA	NA
	All male	6	355	1115	1.06 (0.89–1.27)	.49	.74	P=.53, I^2=0%	.573	.384	F
	Asian male	4	200	580	1.14 (0.89–1.46)	.31	.74	P=.76, I^2=0%	1.000	.376	F
	Caucasian male	2	155	535	0.99 (0.77–1.28)	.94	.94	P=.13, I^2=0%	1.000	NA	F
D14	Total	14	3158	4953	1.20 (0.99–1.44)	.06	.13	P<.001, I^2=67%	.208	.189	R
	Asian	6	1256	1992	1.43 (0.93–2.20)	.10	.13	P<.001, I^2=79%	.851	.611	R
	Caucasian	5	2491	1348	1.05 (0.91–1.22)	.50	.67	P=.83, I^2=0%	.806	.846	F
	Latin America	3	441	470	1.04 (0.73–1.49)	.81	.81	P=.08, I^2=61%	.296	.532	R
	All female	7	967	1441	1.00 (0.88–1.13)	1.00	1.00	P=.68, I^2=0%	.881	.746	F
	Asian female	4	526	804	1.11 (0.61–2.02)	.72	.96	P=.004, I^2=77%	.497	.153	R
	Caucasian female	2	311	507	1.16 (0.85–1.58)	.36	.94	P=.45, I^2=0%	1.000	NA	F
	Latin America female	1	130	130	0.95 (0.62–1.47)	.47	.94	P=.02, I^2=59%	NA	NA	R
	All male	6	355	1115	1.35 (1.02–1.78)	**.03**	**0.045**	P=.08, I^2=50%	.851	.542	F
	Asian male	4	200	580	1.72 (1.11–2.66)	**.01**	**0.03**	P=.06, I^2=59%	.497	.474	R
	Caucasian male	2	155	535	1.15 (0.80–1.66)	.44	.44	P=.89, I^2=0%	1.000	NA	F
D15	Total	14	3158	4953	1.04 (0.94–1.15)	.45	.90	P=.50, I^2=0%	.043	.170	F
	Asian	6	1256	1992	0.98 (0.77–1.24)	.87	.87	P=.88, I^2=0%	.091	.138	F
	Caucasian	5	2491	1348	1.08 (0.95–1.22)	.23	.90	P=.17, I^2=37%	.734	.772	F
	Latin America	3	441	470	0.95 (0.74–1.22)	.69	.87	P=.50, I^2=0%	.089	.448	F
	All female	7	967	1441	1.05 (0.87–1.27)	.63	.90	P=.21, I^2=28%	.099	.139	F
	Asian female	4	526	804	0.98 (0.67–1.43)	.90	.90	P=.05, I^2=63%	.174	.008	R
	Caucasian female	2	311	507	1.07 (0.84–1.36)	.60	.90	P=.61, I^2=0%	1.000	NA	F
	Latin America female	1	130	130	1.10 (0.67–1.79)	.71	.90	NA	NA	NA	NA
	All male	6	355	1115	0.97 (0.74–1.27)	.82	.97	P=.48, I^2=0%	.189	.860	F
	Asian male	4	200	580	1.01 (0.62–1.64)	.97	.97	P=.30, I^2=17%	.174	.264	F
	Caucasian male	2	155	535	0.95 (0.69–1.31)	.76	.97	P=.37, I^2=0%	1.000	NA	F
D16	Total	13	3158	4953	1.06 (0.85–1.31)	.62	.83	P=.02, I^2=50%	.903	.933	R
	Asian	6	1256	1992	0.97 (0.68–1.40)	.89	.89	P=.03, I^2=60%	.573	.359	R
	Caucasian	4	2491	1348	0.95 (0.75–1.20)	.69	.89	P=.45, I^2=0%	.734	.714	F
	Latin America	3	441	470	1.44 (0.80–2.59)	.22	.83	P=.06, I^2=64%	1.000	.836	R
	All female	7	967	1441	0.98 (0.67–1.42)	.90	.90	P=.04, I^2=54%	.881	.761	R
	Asian female	4	526	804	0.84 (0.48–1.46)	.53	.90	P=.09, I^2=54%	1.000	.158	R
	Caucasian female	2	311	507	0.93 (0.61–1.40)	.71	.90	P=.27, I^2=19%	1.000	NA	F
	Latin America female	1	130	130	2.10 (1.03–4.30)	**.04**	.16	NA	NA	NA	NA
	All male	6	355	1115	0.83 (0.57–1.20)	.32	.48	P=.95, I^2=0%	.039	.267	F
	Asian male	4	200	580	0.96 (0.54–1.70)	.89	.89	P=.88, I^2=0%	.174	.032	F
	Caucasian male	2	155	535	0.75 (0.46–1.22)	.24	.48	P=.86, I^2=0%	1.000	NA	F

CI = confidence interval, E = ethnicity, F = fixed, NA = not available, NO = number of studies, OR = odds ratios, P_{Begg} = *P* value for Begg's test, P_{Egger} = *P* value for Egger's test, P_{FDR} = *P* value of false discovery rate, R = random. Significant associations shown in bold.

with 5190 OA patients and 5167 healthy controls have been enrolled, and all studies have high quality with a NOS value. We found that Latin America population have high risk of OA when they carried with D16 polymorphism (P=.04, OR [95% CI]= 1.43 [1.02–2.01]). This is a novel finding compared with previous studies, Arellano-Pérez-Vertti et al[26] reported that patients with D16 allele had a significant risk for OA. However, the statistical difference was absent after FDR adjustment (P_{FDR}=.15), Gonzalez-Huerta et al[19] suggested that D16 polymorphism was not associated with OA susceptibility but was associated with the OA severity. With the D13 polymorphism, Caucasians male patients have low OA risk (P=.008, P_{FDR}=.024, OR [95% CI]=0.83 [0.73–0.95]), and a protective role of the D13 allele was confirmed with KOA in Greek individuals.[16] These results suggested that the variation of D13 plays a protective role in Caucasians. Meanwhile, male patients with D14 allele have high OA susceptibility, especially in the KOA. These results indicated that D14 polymorphism plays a risk role in male patients and the effect size of it in Asians is more significant than in Caucasians (OR value is 1.72 [1.11–2.66] and 1.32 [1.09–1.60], respectively, Table 2). In addition, previous studies indicated that the association between D14 polymorphism and KOA had global relevance,[30] D14 is a common susceptibility allele for KOA patients in Asians,[13,14] and the association is duplicated in Caucasians, but only in male OA patients.[17] The difference between male patients and female patients may be due to different

Table 3

Results of meta-analysis and subgroup analysis in pooled hip OA patients.

Gene	Subgroup	No.	Sample size		OR (95% CI)	*P*	P_{FDR}	Heterogeneity test	P_{Begg}	P_{Egger}	Model
			Case	Control							
D13	Total	4	1865	2164	0.93 (0.85–1.03)	.15	.30	P=.60, I^2=0%	.308	.169	F
	Asian	1	593	374	0.90 (0.75–1.09)	.29	.30	NA	NA	NA	
	Caucasian	3	1272	1790	0.94 (0.84–1.05)	.30	.30	P=.43, I^2=0%	1.000	.305	F
	Female	2	723	507	0.98 (0.84–1.15)	.83	.83	P=.50, I^2=0%	1.000	NA	F
	Male	2	484	535	0.87 (0.73–1.04)	.13	.26	P=.25, I^2=25%	1.000	NA	F
D14	Total	4	1865	2164	1.12 (0.83–1.52)	.46	.69	P=.03, I^2=67%	1.000	.998	R
	Asian	1	593	374	1.70 (1.15–2.53)	**.008**	**.024**	NA	NA	NA	
	Caucasian	3	1272	1790	1.00 (0.75–1.32)	.98	.98	P=.13, I^2=51%	1.000	.659	F
	Female	2	723	507	0.94 (0.73–1.20)	.61	.84	P=.53, I^2=0%	1.000	NA	F
	Male	2	484	535	1.07 (0.58–1.96)	.84	.84	P=.04, I^2=77%	1.000	NA	R
D15	Total	4	1865	2164	0.98 (0.86–1.12)	.75	.90	P=.26, I^2=24%	.308	.050	F
	Asian	1	593	374	0.68 (0.42–1.09)	.11	.33	NA	NA	NA	
	Caucasian	3	1272	1790	1.01 (0.88–1.16)	.90	.90	P=.49, I^2=0%	.296	.181	F
	Female	2	723	507	1.00 (0.82–1.21)	.98	.98	P=.78, I^2=0%	1.000	NA	F
	Male	2	484	535	1.07 (0.86–1.33)	.56	.98	P=.92, I^2=0%	1.000	NA	F
D16	Total	4	1865	2164	1.14 (0.97–1.35)	.12	.29	P=.76, I^2=0%	.089	.004	F
	Asian	1	593	374	1.17 (0.83–1.63)	.37	.37	NA	NA	NA	
	Caucasian	3	1272	1790	1.14 (0.94–1.38)	.19	.29	P=.56, I^2=0%	.296	.054	F
	Female	2	723	507	1.23 (0.93–1.64)	.15	.30	P=.86, I^2=0%	1.000	NA	F
	Male	2	484	535	0.93 (0.68–1.27)	.65	.65	P=.69, I^2=0%	1.000	NA	F

CI = confidence interval, E = ethnicity, F = fixed, NA = not available, NO = number of studies, OR = odds ratios, P_{Begg} = *P* value for Begg test, P_{Egger} = *P* value for Egger test, P_{FDR} = *P* value of false discovery rate, R = random. Significant associations shown in bold.

allele frequencies, sample size and incidence.[15,24] Generally, the reasons for the discrepancies of D-repeat polymorphism may be as follows: Firstly, inclusion criteria of OA patients in original articles. Some studies enrolled patients according to their clinical manifestations or radiographic evidence,[3,13,14] others used patients tissues.[16–18] Therefore, it was likely that genetic associations were affected by the severity and different stages of OA patients. Secondly, patients from different countries have different genetic background. For example, allele frequency of D13, D14, and D15 in the Japanese is 64%, 4.8%, and 4.5%[13] respectively, while the proportions in the British or Spanish is 50.3%/42.2%, 12.4%/12.6%, and 22.1%/25.5%[17,18] respectively. Finally, lifestyle and environmental factors may make low effect on D14 variant in Caucasians. Furthermore, our results were different from previous studies,[11,21,31] but similar to the first meta-analysis in some ways.[30] Xing et al and Song et al suggested that the D-repeat of ASPN gene may not be associated with OA susceptibility in the Caucasians and Asians and further studies with a large sample size shall be required.[11,21] There was no language and race restrictions in our study, thus more original articles that met the criteria were included.[19,24–26] Shao et al[25] and Jazayeri et al[24] reported that D14 replacing D13 is associated with OA in Chinese and Iran population, respectively. These articles probably provide us with assistance to distinguish our results from previous meta-analysis. In addition, we have performed three different subgroup analyses on ethnicity, gender and the site of OA, so our results may be more accurate and more reliable. Interestingly, the D-repeat polymorphisms were not associated with hip OA, but were associated with KOA. The reason may be contributed to that the pooled sample size of case and control in KOA were 3158 and 4953 respectively and were larger than those in hip OA including 1865 patients and 2164 controls. The prevalence of KOA is higher than hip OA,[32,33] suggesting that it is easier for researchers to enroll and investigate KOA patients than hip OA patients. In addition, the associations of D-repeat polymorphisms with KOA were performed for Caucasians, Asians and Latin Americas,[13,16,19] but the associations of hip OA were not carried out in Latin Americas, which further support the hypothesis that researchers are easier to investigate patients with KOA than hip OA. In our current study, the relationship between hip OA and D-repeat allele was found in Asian population (P=.008, P_{FDR}=.024), and this result should be further validated in other populations with larger sample sizes. However, the heterogeneities could not be completely eliminated after subgroup analysis were carried out, while these heterogeneities observed in the same populations might be related to individuals' difference, genetic heterogeneity, and environmental factor. The exact reason of heterogeneity remains known to us a little and needs to be further investigated. Moreover, when we performed sensitivity analysis by omitting a study from the merged analysis, the results were not materially altered. Due to conflict results emerging between the Begg funnel plot and Egger tests, trim and fill method was performed and results were remained unaltered. These evidences indicate that our results were robust in statistics.

Some limitations in our study were considered. Firstly, potential publication bias might affect our analysis, although our results were robust after Trim and Fill method was applied. Secondly, the heterogeneity and confounding factors might have distorted the results in several subgroups. Therefore, results should be interpreted in modest way. Thirdly, the statistical data for Asian population in overall people (Fig. 2A) is same as that in KOA patients (Fig. 2B) were same. The reason is that the D14 polymorphism associated with male patients are KOA data in Asians and there were few studies to explore other OA categories in Asians, which may broaden our conclusion. Finally, the majority of subgroup analysis was performed in Asian and Caucasian populations, and more original studies should be considered in other ethnicities.

5. Conclusion

In conclusion, our results suggest that D-repeat of ASPN gene is mainly associated with male patients. The D13 polymorphism plays a protective role for OA in Caucasian male patients while D14 is a risk factor to KOA patients. We recommend that further studies should be replicated in other populations to evaluate the effects of ASPN gene on different types of OA.

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