Association between aspartic acid repeat polymorphism of the asporin gene and susceptibility to knee osteoarthritis: a genetic meta-analysis

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Objective: Knee osteoarthritis (KOA) is a common disease that is characterized by the degeneration of joint cartilage in the knee. Genetic factors have been implicated in KOA. Recently, several genetic studies have suggested that susceptibility to KOA is affected by the number of aspartic acid (D) residues in the amino-terminal of the asporin protein, but evidence remains conflicting. Therefore, the objective of the present meta-analysis was to investigate whether or not the D-repeat polymorphism is associated with susceptibility to KOA.

Methods: A systematic search of all relevant studies published through Dec 2012 was conducted using the MEDLINE, EMBASE, OVID, and ScienceDirect. Allelic counts were evaluated for the D14 and D13 alleles respectively. The included studies were only assessed in the analysis of the following allele model: D14 allele vs others alleles combined, D13 allele vs others alleles combined, and D14 allele vs D13 allele.

Results: Seven studies (eight comparisons) with 5515 total participants (2334 KOA patients and 3181 controls), which involved four Caucasian and four Asian populations, were eligible for inclusion. Meta-analysis was conducted for genotype D14 vs others combined, D13 vs others combined, and D14 vs D13. In the stratification based on ethnicity, studies were divided into Caucasian and Asian populations. We did not detect positive association between KOA and the D14 allele in Asian populations (OR = 1.527, 95% CI: 0.879–2.653) and in Caucasian populations (OR = 1.053, 95% CI: 0.905–1.225). There was also no positive association between susceptibility to KOA and D13 allele in Asian populations (OR = 0.950, 95% CI: 0.732–1.233) and in Caucasian populations (OR = 0.866, 95% CI: 0.723–1.037).

Conclusion: The present results suggest that the D-repeat of asporin gene (ASPN) may not be a major susceptibility locus in the Caucasian and Asian populations with KOA. Because of the limitations of the present meta-analysis, accurate conclusions could not be drawn based on the current evidence, and further studies with large sample size are required.

Introduction

Osteoarthritis (OA) is the most common form of skeletal disease and is characterized by the progressive loss of articular cartilage in synovial joints and changes in the adjacent bone. Patients with OA present initially with stiffness and pain. The joint may show swelling caused by an effusion and synovitis. Locking may also occur as the bare bony surfaces grate against each other, causing severe pain. OA is the most common cause of the limitation of daily life after middle age. The knee is the most commonly affected joint with OA. It is estimated that about 15% of Americans (40 million Americans) suffer from some form of arthritis, with approximately six million Americans affected in the knee. The knee osteoarthritis (KOA) commonly affects individuals over 45 years of age but has been identified in all age groups. The following risk factors have been correlated with KOA: age, obesity, female gender, repetitive knee trauma, joint laxity, muscle weakness, mechanical forces, environmental factors, kneeling, squatting and meniscal injuries, and genetic factors

Current viewpoint in KOA is that an imbalance between synthesis and degradation of the extracellular cartilage matrix through mechanisms controlled by chondrocytes leads to KOA. Asporin is a new member of the small leucin-rich proteoglycan family of transforming growth factor-β (TGF-β) binding proteins and an
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protein of the extracellular cartilage matrix9,10. Asporin is expressed in several tissues, including adult articular cartilage11 and connective tissues10. The asporin gene (ASPN) has been mapped to the human chromosome 9q22–9q21.3. It contains a triplet repeat within exon 2, coding for a polymorphic stretch of aspartic acid (D) residues in the N-terminal region of the protein10. This consecutive aspartic acid residue (D-repeat) has 10 alleles encoding 10–19 residues, with the D13 allele being most common. An association between the D-repeat polymorphism and KOA susceptibility was previously demonstrated. However, published results have been inconsistent. A Japanese study12 first reported a D-repeat polymorphism in the ASPN was associated with susceptibility to KOA, and also reported that the D14 allele (an allele containing 14 D-repeats) was over-represented, and the D13 allele was under-represented in KOA. This result was confirmed in the Han Chinese13. However, the association has not been found in studies in Caucasians14–16. In the present study, we therefore performed a meta-analysis to investigate whether or not the D-repeat polymorphism is associated with susceptibility to KOA.

Materials and methods

Search strategy

We performed a systematic research of Medline, Embase, ScienceDirect, and OVID to identify published epidemiological studies through Dec 2012 that were related to D-repeat polymorphisms of ASPN and KOA. The medical subject headings (MeSH; National Library of Medicine, Bethesda, Maryland) “asporin”, “genetic polymorphism”, “osteoarthritis”, “knee”, “ASPN protein, human” and the free-text words “D-repeat” or “aspartic acid” were combined. No language or other restrictions were placed on the search. Furthermore, the reference lists of all the full-text papers were examined to identify any initially omitted studies.

Inclusion and exclusion criteria

To be eligible for inclusion in the present meta-analysis, the following items were established: (1) observational studies that addressed KOA patients and healthy controls; (2) KOA was diagnosed on basis of clinical and radiographic findings or ascertained by total joint replacement; (3) original studies that evaluated the association between D-repeat polymorphism and susceptibility to KOA; (4) studies had sufficient genetic frequency for extraction. Interim analyses, overlapping study populations, and comparisons of laboratory methods were excluded.

Study selection

Two reviewers (DX and JW) independently screened the titles and abstracts for the eligibility criteria. Subsequently, the full text of the studies that potentially met the inclusion criteria were read and the literature was reviewed to determine the final inclusion. We resolved disagreements by reaching a consensus through discussion.

Date extraction

Two of the authors (DX and JW) independently extracted the following data from each full-text report using a standard data extraction form. The data extracted from studies included the title, authors, year of publication, study design, number of cases or controls, ethnicity, gender, allele count, and allele frequency in cases or controls.

Statistical analysis

The data analysis was conducted using STATA 12.0 (Statacorp, College Station, Texas). Allelic counts were evaluated for the D14 and D13 alleles respectively. The included studies were only assessed in the analysis of allele model (D14 allele vs others alleles combined, D13 allele vs others alleles combined, and D14 allele vs D13 allele) because of no specific genotype distribution reported in the original articles.

Between-study heterogeneity was tested using the Q statistics, $P < 0.1$ was considered statistically significant. The Mantel–Haenszel method for fixed effects and the Der-Simmonian and Laird method for random effects were used to estimate pooled effects17. We used fixed-effects methods if the result of the Q test was not significant. Otherwise, we calculated pooled OR and 95% CI assuming a random-effects model. Fixed effects assume that genetic factors have similar effects on autoimmune diseases susceptibility across all studies, and that observed variations between studies are caused by chance alone16. Random-effects model assumes that different studies may have substantial diversity and assesses both within- and between-study variation13. A recently developed measure $I^2$ was used to quantify the inconsistency among the studies’ results with values of 50% or higher, and large heterogeneity for values of 75% or higher20. Data are shown as OR with 95% CI, with two-tailed $P$-values and statistical significance was set at $P < 0.05$ (two-tailed). A sensitivity analysis was performed for the effect size omitting the trial for which data were imputed, which was to assess the changes in overall effect.

Publication bias was conducted both visually by using a funnel plot and statistically via Begg’s funnel plots and Egger’s bias test, which measures the degree of funnel plot asymmetry21,22. The Begg adjusted rank correlation test was used to assess the correlation between test accuracy estimates and their variances. The deviation of Spearman’s rho values from zero provides an estimate of funnel plot asymmetry. Positive values indicate a trend towards higher levels of test accuracy in studies with smaller sample sizes. The Egger’s bias test detects funnel plot asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision. If asymmetrical plots were showed, then we performed the trim and fill analysis to assess the stability in overall effect. This approach removes small studies until symmetry in the funnel plot is achieved by recalculating publication bias, before the removed studies are replaced with their missing mirror-image counterparts. A revised estimate is then conducted using all original studies and hypothetical studies.

Results

Search results

A total of 55 titles and abstracts were preliminarily reviewed, of which seven published literature15–18,21,24 eventually satisfied the eligibility criteria (Fig. 1). All of the included studies investigated the relation between the ASPN D-repeat polymorphism and susceptibility to KOA. Of these, one study15 contained data on two different groups, a cohort group and a case-control group. We analyzed these two comparisons for D-repeat polymorphism independently. Therefore, a total of eight comparisons were included in the present meta-analysis.

Demographic characteristics

In total, eight comparisons with 5515 total participants (2334 KOA patients and 3181 controls), which involved four Caucasian
and four Asian populations, were eligible for inclusion. The comparisons originated from case-control or cohort design, and sample size per comparison varied from 345 to 1286. All but one comparison was case-control designs. KOA patients of three included studies were enrolled according to signs and symptoms of OA, or radiographic evidence of OA. However, the four studies recruited KOA patients who had undergone joint replacement. The control groups of included studies comprised 3181 individuals who had no signs or symptoms of OA or joint disease. Characteristics of included studies in the meta-analysis are presented in Table I.

### Allelic frequency in different ethnic groups

Allelic counts were evaluated for the D14 and D13 alleles respectively. The frequencies of the D14 allele and the D13 allele differed between the Caucasian and Asian studies (Table II). The differences of the allele frequencies between the two ethnic groups were tested by chi-square test. The P-values for the tests were all significant (Table II). The frequency of the D14 allele was higher in KOA cases than in control groups in all studies, except for two studies. The frequency of the D13 allele was lower in KOA cases than in control groups in all studies, except for two studies.

### Results of meta-analysis

A summary of the meta-analysis findings of the association between D-repeat polymorphism and susceptibility to KOA is shown in Table III. The summary OR for the D14 allele vs other alleles combined and its 95% CI included 1 (OR = 1.262, 95% CI: 0.982–1.622), which indicated that no significant association was found between D14 allele and KOA. The same result was also

### Table I

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Study design</th>
<th>Eligible subjects (n)</th>
<th>Age</th>
<th>Gender (M/F)</th>
<th>Genotyping method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jiang et al.</td>
<td>2006</td>
<td>China</td>
<td>CC</td>
<td>218</td>
<td>58.1 ± 18.9</td>
<td>67/151</td>
<td>165/289</td>
</tr>
<tr>
<td>Rodriguez-Lopez et al.</td>
<td>2006</td>
<td>Spain</td>
<td>CC</td>
<td>188</td>
<td>&gt;55</td>
<td>55/336</td>
<td>179/115</td>
</tr>
<tr>
<td>Kaliakatsos et al.</td>
<td>2006</td>
<td>Greece</td>
<td>CC</td>
<td>155</td>
<td>68.1 ± 8.2</td>
<td>20/138</td>
<td>56/137</td>
</tr>
<tr>
<td>Mustafa et al.</td>
<td>2005</td>
<td>UK</td>
<td>CC</td>
<td>278</td>
<td>65 (56–85)</td>
<td>120/158</td>
<td>356/392</td>
</tr>
<tr>
<td>Kizawa et al. (cohort)</td>
<td>2005</td>
<td>Japan</td>
<td>Cohort</td>
<td>137</td>
<td>75.3 ± 5.1</td>
<td>38/99</td>
<td>91/143</td>
</tr>
<tr>
<td>Kizawa et al. (case-control)</td>
<td>2005</td>
<td>Japan</td>
<td>CC</td>
<td>393</td>
<td>72.5 ± 7.4</td>
<td>63/330</td>
<td>165/209</td>
</tr>
<tr>
<td>Song et al.</td>
<td>2008</td>
<td>Korea</td>
<td>CC</td>
<td>190</td>
<td>60</td>
<td>38/152</td>
<td>222/154</td>
</tr>
<tr>
<td>Atif et al.</td>
<td>2008</td>
<td>USA</td>
<td>CC</td>
<td>775</td>
<td>70.8 ± 8.6</td>
<td>145/630</td>
<td>170/341</td>
</tr>
</tbody>
</table>

CC: case-control; M: male; F: female; n: number.
obtained in the analysis of D13 allele vs others alleles combined (OR = 0.904, 95% CI: 0.783–1.043). In the subgroup analysis based on ethnicity, studies were divided into Caucasian and Asian populations. There was also no association between D-repeat polymorphism and susceptibility to KOA in the Caucasian and Asian groups (Figs. 2–4) (Table III). The publication bias test was performed for overall populations. No significant publication bias was showed for overall populations by Begg’s rank correlation method (P = 0.711) and Egger weighted regression method (P = 0.576).

Sensitivity analysis

A sensitivity analysis was performed by omitting Song et al.24 which induced heterogeneity in the subgroup analysis for Asian populations. The results of sensitivity analysis are shown in Table IV. There was significant association between D14 allele and susceptibility to KOA, but was not replicated in association of the D13 allele with KOA. In Spanish population, the D-repeat polymorphism in ASPN was not associated with KOA or not.

Discussion

OA is the most common cause of joint disease and physical disability worldwide1. Molecular messengers, including IL-1, TNF and nitric oxide appear to be responsible for changes in the composition of cartilage joint. The association of genetic polymorphism and KOA has recently attracted growing attention. Most researches were focused on the genes that encode for the proteins responsible for the maintenance of articular cartilage and for susceptibility to OA. ASPN is an extracellular matrix component that belongs to a small leucine-rich proteoglycan family and is abundantly expressed in the articular cartilage of individuals with OA3. ASPN may regulate TGF-β-mediated factors in the development of OA4,5. There is a triple nucleotide repeat, coding for a polymorphic stretch of D residues, in the amino-terminal region of the ASPN. There is a dispute whether ASPN D-repeat polymorphism is associated with KOA or not.

In the present study, we investigated the association of ASPN D-repeat polymorphism with KOA susceptibility in different ethnicities using meta-analysis approach. Seven published studies (eight comparisons) were included with a total of 2334 KOA and 3181 controls in the present study. Kizawa et al.12 firstly reported a significant association between the D-repeat polymorphism and KOA in Japanese population using a cohort sample and a case-control study. However, Mustafa et al.16 suggested that the ASPN polymorphism is not a major influence on KOA etiology in UK population. Kaliakatsos et al.11 indicated that the frequency of the D13 allele was significantly lower in KOA patients, but that the difference was not observed in the frequency of the D14 allele in Greek population. In Spanish population, the D-repeat polymorphism in ASPN was not an important factor in KOA susceptibility4. In a Chinese case-control study, significant association was detected between the D14 allele and KOA, but was not replicated in association of the D13 allele with KOA13. Song et al.24 reported that no significant difference was observed between Korean KOA patients and healthy participants in the allele frequency of the D13/D14 allele compared with other alleles, whereas a significant difference between female KOA patients and their controls in the allele frequency of the D13 allele was found compared to the other alleles. Atif et al.22 assessed the association in US cases and controls, but did not detect significant association between D-repeat polymorphism in ASPN and susceptibility to KOA. Overall, the pooling results demonstrated that no significant correlation was observed between D14 and D13 alleles frequency and KOA susceptibility in Caucasian and Asian individuals, which is consistent with the previous functional study where the inhibitory effect of the D13 allele on TGF-β induction on cartilage

Table II

<p>| Allele counts for the D-repeat polymorphism in ASPN in the included studies |
|-------------------------------------|-----|-----|-----|-----|-----|-----|</p>
<table>
<thead>
<tr>
<th>Group</th>
<th>Study</th>
<th>Ethnicity</th>
<th>Case D14</th>
<th>D13</th>
<th>Others</th>
<th>Control D14</th>
<th>D13</th>
<th>Others</th>
</tr>
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<tbody>
<tr>
<td>Asian</td>
<td>Jiang et al.17</td>
<td>Asian</td>
<td>41</td>
<td>300</td>
<td>95</td>
<td>44</td>
<td>604</td>
<td>260</td>
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<tr>
<td>Kizawa et al. (cohort)12</td>
<td>Asian</td>
<td>30</td>
<td>163</td>
<td>81</td>
<td>0.109</td>
<td>22</td>
<td>314</td>
<td>132</td>
</tr>
<tr>
<td>Song et al.24</td>
<td>Asian</td>
<td>61</td>
<td>459</td>
<td>266</td>
<td>0.078</td>
<td>36</td>
<td>479</td>
<td>233</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>22</td>
<td>265</td>
<td>93</td>
<td>0.058</td>
<td>65</td>
<td>483</td>
<td>204</td>
</tr>
<tr>
<td>Caucasian</td>
<td>Atif et al.23</td>
<td>Caucasian</td>
<td>154</td>
<td>1187</td>
<td>535</td>
<td>167</td>
<td>1880</td>
<td>829</td>
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<tr>
<td>Rodriguez-Lopez et al.14</td>
<td>Caucasian</td>
<td>206</td>
<td>749</td>
<td>595</td>
<td>0.133</td>
<td>142</td>
<td>496</td>
<td>384</td>
</tr>
<tr>
<td>Kaliakatsos et al.11</td>
<td>Caucasian</td>
<td>56</td>
<td>151</td>
<td>164</td>
<td>0.323</td>
<td>74</td>
<td>248</td>
<td>266</td>
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<tr>
<td>Mustafa et al.16</td>
<td>Caucasian</td>
<td>47</td>
<td>119</td>
<td>146</td>
<td>0.151</td>
<td>51</td>
<td>192</td>
<td>141</td>
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<tr>
<td>Total</td>
<td></td>
<td>76</td>
<td>258</td>
<td>222</td>
<td>0.137</td>
<td>190</td>
<td>752</td>
<td>554</td>
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<tr>
<td>Total</td>
<td></td>
<td>385</td>
<td>1282</td>
<td>1127</td>
<td>0.138</td>
<td>457</td>
<td>1688</td>
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Table III

<table>
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<th>Comparison</th>
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<th>No. of studies</th>
<th>Type of model</th>
<th>Test of association</th>
<th>Test of heterogeneity</th>
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<tr>
<td></td>
<td></td>
<td>Case</td>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D14 vs others combined</td>
<td>Overall</td>
<td>2334</td>
<td>3181</td>
<td>8</td>
<td>Random</td>
<td>1.262</td>
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<tr>
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<td></td>
<td>European</td>
<td>1396</td>
<td>1743</td>
<td>4</td>
<td>Random</td>
<td>1.708</td>
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<tr>
<td>D13 vs others combined</td>
<td>Overall</td>
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<td>3181</td>
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<td>Random</td>
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<td>1438</td>
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<td>Random</td>
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<tr>
<td></td>
<td>European</td>
<td>1396</td>
<td>1743</td>
<td>4</td>
<td>Random</td>
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<td>D14 vs D13</td>
<td>Overall</td>
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<td>3181</td>
<td>8</td>
<td>Random</td>
<td>1.313</td>
</tr>
<tr>
<td></td>
<td>Asian</td>
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<td>1438</td>
<td>4</td>
<td>Random</td>
<td>1.571</td>
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<tr>
<td></td>
<td>European</td>
<td>1396</td>
<td>1743</td>
<td>4</td>
<td>Fixed</td>
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<table>
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<th>Comparison</th>
<th>OR</th>
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<th>Q test</th>
<th>P value</th>
<th>I²</th>
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<tr>
<td>D14 vs others combined</td>
<td>1.262</td>
<td>0.982–1.622</td>
<td>0.069</td>
<td>24.19</td>
<td>0.001</td>
<td>71.1%</td>
</tr>
<tr>
<td>D13 vs others combined</td>
<td>0.904</td>
<td>0.783–1.043</td>
<td>0.167</td>
<td>21.12</td>
<td>0.004</td>
<td>66.8%</td>
</tr>
<tr>
<td>D14 vs D13</td>
<td>1.313</td>
<td>1.009–1.708</td>
<td>0.042</td>
<td>24.61</td>
<td>0.001</td>
<td>71.6%</td>
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</table>
marker gene is similar to those of the D16 and D17 alleles. The result of the present meta-analysis differed from the previous meta-analysis. In the previous meta-analysis, Nakamura et al. reported that strong association was found between D-repeat and KOA in Asians. A new study in Korean populations, which reported no difference in the frequency of the different ASPN alleles between the KOA patients and the healthy controls, was included in the present study comparing with the previous one. This study, as a source of heterogeneity, may exert an influence on the stability on the results of the present meta-analysis. We, therefore, performed a sensitivity analysis in Asian populations by omitting the new study to evaluate the stability of the pooling results. However, the results of sensitivity analysis were opposite to the previous pooling results, which indicated that the pooling results were lacking enough stability. Thus, we had no enough confidence to draw the exact conclusion in Asian populations based on the current evidence.

Fig. 2. ORs and 95% CI of individual studies and pooled data for stratification study of the association between the ASPN D-repeat polymorphism and KOA in the comparison of the D14 allele vs other alleles combined.

Fig. 3. ORs and 95% CI of individual studies and pooled data for stratification study of the association between the ASPN D-repeat polymorphism and KOA in the comparison of the D13 allele vs other alleles combined.
Only two included studies\textsuperscript{16,24} conducted stratification according to gender of the participants. Song et al.\textsuperscript{24} observed a statistically significant association of the D13 allele with KOA in Korean female population. Mustafa et al.\textsuperscript{16} reported that there was significant difference for male patients in the frequency of the D14 allele. However, we were unable to perform a subgroup analysis according to different gender, since raw data of individual subject cannot be obtained from each included study. Furthermore, the heterogeneity of included studies may also be due to differences in inclusion criteria. Four studies\textsuperscript{14–16,24} used only OA tissues after joint replacement surgery, but three studies\textsuperscript{12,13,23} recruited KOA patients based on the symptoms of KOA or radiographic evidence. Thus, it could be argued that the included studies are investigating different stages of KOA. Moreover, the heterogeneity of included studies includes the following: quality of included studies, gender proportion, age, diagnostic criteria, racial differences in allele frequency, different genetic background, environmental factors, sampling criteria and cultural difference. The heterogeneity of genetic effects between individual studies may be caused by the existence of gene-environmental or genetic interaction. Although we performed subgroup analyses that were stratified by ethnicities and sensitivity analysis by omitting a study, heterogeneity cannot be completely resolved. Accordingly, although the results of the meta-analysis should be considered appropriate, the above clinical heterogeneity should be considered when interpreting the findings.

As we know, KOA is considered a multi-factorial disorder. It is likely that non-genetic factors could modify the susceptibility genes, including the ASPN. The differences of genetic effects and the effects of D-repeat polymorphism may be influenced by other genes or by environmental factors that vary between or within ethnicities. Therefore, further studies are required to characterize the ASPN according to the D-repeat number and determine how non-genetic factors, such as environmental factors and other interacting proteins, modify genetic risk.

The primary limitations of this meta-analysis include the following: (1) We were unable to conduct subgroup analysis for every confounding factor, including gender and age. The following reasons might be responsible for this limitation: the small number of included studies, few raw data regarding individual patients extracted from the original studies, and a lack of unified criterion for recruitment amongst the different studies; (2) Although clinical heterogeneity was partially addressed by subgroup analysis according to ethnicity, heterogeneity cannot be completely resolved. We could not distinguish any substantial differences in heterogeneity between the studies. Owing to the unavailability of some important variables in the original studies and limited number of the included studies, meta-regression analysis could not be performed to investigate the sources of heterogeneity. The stability of the pooled results may be disturbed by these unresolved confounding factors; (3) The effect of genetic and environmental interactions was not addressed in our meta-analysis; (4) The statistical efficacy may be inadequate because of the finite number of included studies, especially in the subgroup analyses.

In conclusion, the present meta-analysis demonstrated that the D-repeat of ASPN may not be a major susceptibility locus in the...
Caucasian and Asian populations with KOA. However, the heterogeneity of included studies affects the stability of the results, especially in Asian populations. Accurate conclusions could not be drawn based on the current evidence. More studies are required to determine the significance of the asporin polymorphism in a larger population and to define the effect of the size of D-repeat on susceptibility to KOA.

Author contributions

Project conceptualization: D Xing and XL Ma.
Study design: D Xing, J Wang and XL Ma.
Data collection/validation: D Xing, J Wang and JX Ma.
Data analysis: SW Zhu, Y Chen, Y Yang and BY Ma.
Result interpretation: D Xing, BY Ma, J Wang and JX Ma.
Reporting & editing: WG Xu, Y Yang, D Xing and J Wang.
Final approval of the version to be submitted: D Xing, J Wang and XL Ma.
Project guarantor: XL Ma.

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Conflict of interest statement

The authors declare that they have no conflict of interest.

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References