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Review Article

Tandem repeats of aggrecan gene and susceptibility of disc degeneration diseases: a case-control study and meta-analysis

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Abstract: Several studies have examined the association between aggrecan gene variable number of tandem repeats (VNTR) polymorphism and risk of intervertebral disc degeneration (IDD). However, the results are still controversial. We recruited 98 IDD patients and 94 controls from May 2011 to August 2015. All the subjects were genotyped using the PCR-based invader assay. The differences of allele distributions between IDD patients and controls were investigated in case-control study. A systematic search of all relevant studies was conducted. The observational studies that were related to an association between aggrecan VNTR polymorphism and IDD were identified. The association between aggrecan VNTR polymorphism and risk of IDD was assessed by meta-analysis. The case-control study showed that aggrecan VNTR polymorphism was associated with the susceptibility of IDD in a Chinese population ($P < 0.05$). Furthermore, other eight previously reported studies were included to perform meta-analysis. The meta-analysis showed that aggrecan VNTR polymorphism was associated with risk of IDD in Asian (OR = 1.50, 95% CI = 1.17-1.93) but not in Caucasians (OR = 1.35, 95% CI = 0.84-2.18). The present study suggested that aggrecan VNTR polymorphism with shorter allele may be associated with the increased risk of IDD in Chinese population. The further meta-analysis provides additional evidence supporting the above result in Asian. However, such association may not be statistically significant in Caucasians.

Keywords: Intervertebral disc degeneration, case-control study, meta-analysis, aggrecan, gene polymorphisms

Introduction

Intervertebral disc degeneration (IDD) is a major pathological process implicated in low back pain, and is a prerequisite to disk herniation [1]. Low back disorders have been the most common musculoskeletal problems in the industrialized societies [2]. The disorders are a major source of disability and have a substantial impact on the cost of health care. However, the cause of IDD is still unknown completely.

IDD has been attributed to the accumulation of environmental factors, primarily mechanical insults and injuries, imposed on the "normal" aging changes [3]. However, epidemiological studies on families and twins have suggested that inheritance may be the major determinant of IDD [4-6]. So far, several gene polymorphisms have been demonstrated to be associated with the risks of IDD [7].

Aggrecan, a large aggregating proteoglycan, is one of the major structural components of intervertebral disc and cartilage [8, 9]. The localized high concentration of aggrecan provides the osmotic property that is necessary for common tissue function. This function is related to the structure of aggrecan, especially to the large number of chondroitin sulfate (CS) chains that present on their core protein. The CS chains are presented in 2 adjacent regions of the aggrecan core protein, termed the CS1 and CS2 domains. In humans, the region of the aggrecan gene encoding the CS1 domain exhibits size polymorphism, which can result in variation in the degree of CS substitution of aggrecan in different individuals [10]. Humans

are known to uniquely exhibit variable numbers of tandem repeat (VNTR) polymorphism within the aggrecan CS1 domain [11].

The association between aggrecan VNTR polymorphism and the risk of IDD has been investigated in several recent studies. Kawaguchi et al. firstly reported that subjects with shorter VNTP length of the aggrecan had a risk of having multilevel IDD [12], which was supported by the studies by Solovieva [13], Cong [14], and Mashayekhi [15]. However, the studies by Roughley [16] and Noponen-Hietala [17], showed no such association.

Although several studies have investigated the role of aggrecan VNTR polymorphism in the development of IDD among various populations, results are still conflicting. To confirm the association between aggrecan VNTR polymorphism and IDD, we performed a case-control study for the association of the above gene polymorphisms with risk of IDD in Chinese population, and then conducted a meta-analysis to derive a relatively comprehensive picture of the relationship between aggrecan VNTR polymorphism and the risk of IDD.

Materials and methods

Inclusion and exclusion criteria

The subjects of this study included those with low back pain. All participants had a magnetic resonance imaging (MRI) scan and/or CT. The subjects who visited our outpatient clinic for lower back pain and/or sciatica were included in the study. According to degenerated or no degenerated intervertebral disc findings in lumbar, the participants were divided into two groups. We attempted to exclude any environmental factor that might be related to lumbar disc disease. The subjects withobesity, a smoking history, and heavy physical occupations were excluded. Obesity was defined as a body mass index of 30 or higher. Heavy physical occupations included occupations exposed to handling heavy materials, postural loading, and vehicular vibration.

Subjects

A total of 192 subjects were recruited in the present study. All participants were recruited from Department of orthopedics, The Affiliated Hospital of Qingdao University, between May 2011 and August 2015. The present study was approved by the ethics committee of Nanjing Medical University, and all participants gave written, informed consent.

Of total subjects, 98 patients were already diagnosed with symptomatic IDD in lumbar. All symptomatic IDD patients had radicular pain with signs of positive nerve root tension or neurologic deficit, a confirmatory imaging study (both MRI and CT in 93.8% of the patients; only MRI in 5.1% and only CT in 3.1%) demonstrating lumbar disc herniation (LDH) corresponding to their symptoms and presence of symptoms for at least 6 weeks. All IDD patients had their medical records reviewed or they participated in a medical interview documenting clinical, laboratory, and radiographic data, which were evaluated by a radiologist and 2 orthopedists through physical and radiologic examination (MRI or CT or both). The control group consisted of the 94 healthy blood donors without symptoms of LDH who were not diagnosed with LDH. All participants did not have occupational or lifestyle risk factors, such as heavy manual labor, occupational driving, and heavy smoking. The mean age of the case and control groups was 41.5 years (range, 24-63 years) and 48 years (range, 30-69 years), respectively.

Genotyping

We selected the aggrecan VNTR polymorphism for association analysis. Genomic DNA was extracted from peripheral blood leukocytes using genomic DNA isolation kits (Promega, Madison, WI) according to the manufacturer's instructions. The primers, probes and reaction conditions were available upon request. SNPs were genotyped by the PCR-based invader assay (Third Wave Technologies) using ABI 7900 (Applied Biosystems, Foster City, CA, WI) [18]. Genotyping was done by laboratory personnel blinded to subject status. Of the samples, 10% were tested twice to validate the genotyping results with 100% reproducibility. Two authors independently reviewed the genotyping results, data entry, and statistical analysis.

Meta-analysis

Candidate studies had to meet the following criteria: (1) all patients meeting the diagnostic cri-

Table 1. Distribution of the aggrecan VNTR alleles in case-control study

Observed allele repeat No.	Allele distribution in case group	Allele distribution in control group
32	0	1
31	0	3
30	0	4
29	3	8
28	8	10
27	20	18
26	17	17
25	15	11
24	13	9
23	7	4
22	5	3
21	3	2
20	3	3
19	2	1
18	2	0

Table 2. The aggrecan VNTR polymorphism between the case and control group

Aggrecan VNTR polymorphism	Case group	Control group	OR	95% CI
A13-25 (shorter alleles)	50	33	1.45	0.86-2.45
A26-A27 (common alleles)	37	35	1.01	0.59-1.74
A28-A32 (longer alleles)	11	26	0.41	0.19-0.87

teria for IDD, (2) case-control study focused on the relationship between aggrecan VNTR polymorphism and the risk of IDD, and (3) sufficient original data for calculating odds ratios (ORs) with corresponding 95% confidence intervals (CIs). The major reasons for excluding studies were design other than a case-control study, duplicate publications, and no available data reported.

Two of the authors independently extracted the following data from each full-text report using a standard data extraction form. The data extracted from the studies included the title, authors, year of publication, study design, number of cases or controls, ethnicity, genotyping method, genotype distribution, and numbers of genotypes in cases and controls.

Statistical analysis

Standard χ^2 analysis was used to examine distribution of aggrecan VNTR polymorphism between IDD patients and controls in case-control study. Hardy-Weinberg equilibrium was tested by a goodness-of-fit χ^2 test. All *P*-values presented are 2-tailed, and all analyses were performed with the SPSS for Windows version 11.5 statistical package.

Meta-analysis was performed with STATA 12.0 (Stata corp, college station, Tex). Comparing with single-nucleotide polymorphisms, which are characterized by 2 alleles and 3 genotypes, the aggrecan VNTR polymorphism is characterized by 13 alleles [19]. For each study, we divided alleles into 3 groups: A13-25 (shorter alleles), A26-A27 (common alleles), and A28-A32 (longer alleles), as done in the previous researches [12, 13, 19]. This approach investigated whether an excess of alleles in 1 of the 3 groups altered the risk for IDD.

The ORs and 95% CIs for each study were calculated to evaluate the association between the alleles of aggrecan VNTR polymorphism and IDD. Statistical heterogeneity was assessed using Q statistics. We performed the Laird *Q* test for heterogeneity and also calculated the I^2 statistic for each analysis. If the *P* value was 0.05 or less, indicating obvious heterogeneity between studies, a random effects (the DerSimonian and Laird method) model was used to calculate the pooled OR; otherwise, a fixed-effects (the Mantel-Haenszel method) model was performed. $P < 0.05$ implied statistical heterogeneity, and a random effects model was used in those circumstances.

Publication bias was conducted both visually by using a funnel plot and statistically via Begg funnel plots and Egger's bias test, which measures the degree of funnel plot asymmetry [20, 21]. 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 toward 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

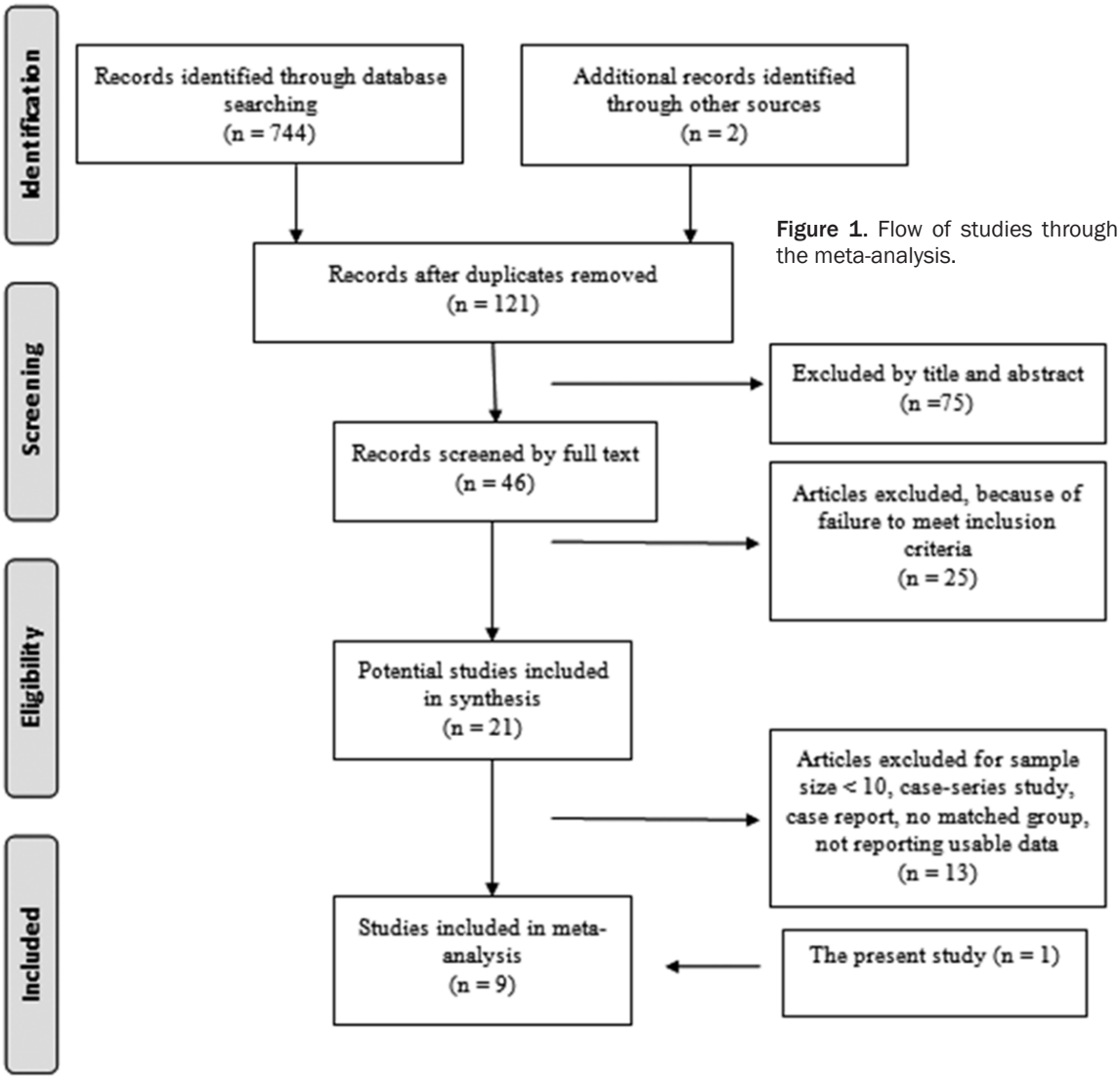

Figure 1. Flow of studies through the meta-analysis.

effect estimates against their precision. Sensitivity analysis was performed by omitting each study in turn to assess the results stability. Two-sided *P* values less than 0.05 were considered statistically significant.

Results

Case-control association study

A total of 192 subjects (98 cases with a mean ± SD age of 33.5±5.6 years and 94 controls with 33.2±4.9 years) were successfully genotyped and subjected to statistical analysis. Sixteen alleles and 42 genotypes were observed in this study in all participants. Of the 94 control group participants, 26 (13.5%) participants were homozygotes and 166 (86.5%) participants were heterozygotes. No deviation from Hardy-Weinberg equilibrium (*P* = 0.415) was observed in the control group [22]. The frequency of allele in the studied subjects is presented in **Table 1**. The number of repeats in these alleles ranged from 18 to 32. There were no participants with 30 or more allele repeats in the disease group. There is a statistically significant difference of aggrecan VNTR polymorphism in shorter and longer allele between case group and control group (**Table 2**). The most frequent among all the alleles were allele 27 (case group 20.4% and control group 19.1%, respectively).

Meta-analysis

A total of 46 titles and abstracts were preliminarily reviewed, of which 8 of the published literature [12-15, 23-26] eventually satisfied the

Table 3. Characteristics of included studies

Author	Year	Country	Ethnicity	Case group		Control group		Diagnostic criteria	Detection method
				Sample size	Age (M ± SD)	Sample size	Age (M ± SD)		
Kawaguchi et al.	1999	Japan	Asian	64	21.3 (20-29)	64	21.3 (20-29)	MRI+CT	PCR
Solovieva et al.	2007	Finland	Caucasian	102	44.0±2.0	30	44.0±2.0	MRI+CT	PCR-SSCP
Cong et al.	2010	China	Asian	140	32.5 (14-41)	254	38 (20-49)	MRI+CT	PCR-RFLP
Eser et al.	2010	Turkey	Caucasian	300	20-30	300	20-30	MRI+CT	PCR
Mashayekhi et al.	2010	Iran	Caucasian	71	36.0±3.2	108	NA	MRI+CT	Illumina
Eser et al.	2011	Turkey	Caucasian	100	22.3 (20-30)	100	22.3 (20-30)	MRI+CT	PCR-RFLP
Kim et al.	2011	Korea	Asian	86	39.2 (13-73)	24	39.2 (13-73)	MRI+CT	PCR
Cong et al.	2014	China	Asian	61	34.3±5.9	198	37.3±5.9	MRI+CT	PCR-RFLP
Present study	2015	China	Asian	98	33.5±5.6	94	33.2±4.9	MRI+CT	PCR-RFLP

NA, Not available; PCR-RFLP, Polymerase chain reaction restriction fragment-length polymorphism; PCR-SSCP, Polymerase chain reaction-single strand conformation polymorphism; SD, Standard deviation.

Table 4. Allele frequencies for the aggrecan VNTR Polymorphism of included studies

Author	Year	Group	Sample size	Allele distribution		
				Shorter alleles	Normal alleles	Longer alleles
Kawaguchi et al.	1999	Case	64	11	45	8
		Control	64	5	50	9
Solovieva et al.	2007	Case	102	22	62	18
		Control	30	11	18	1
Cong et al.	2010	Case	140	70	53	17
		Control	254	92	93	69
Eser et al.	2010	Case	300	73	133	94
		Control	300	55	143	102
Mashayekhi et al.	2010	Case	71	33	27	11
		Control	108	20	65	23
Eser et al.	2011	Case	100	27	34	39
		Control	100	19	37	44
Kim et al.	2011	Case	86	22	51	13
		Control	24	3	19	2
Cong et al.	2014	Case	61	28	12	21
		Control	198	57	60	81
Present study	2015	Case	98	50	37	11
		Control	94	33	35	26

eligibility criteria (**Figure 1**). All of the included studies investigated the relation between aggrecan VNTR polymorphism and the risk of IDD. We, therefore, performed meta-analysis by combination the previous studies and our present case-control study. Ultimately, 9 studies investigated the relationship between aggrecan VNTR polymorphism and IDD risk with a total of 1022 IDD cases and 1172 healthy controls. Characteristics of the studies that were included in the meta-analysis are presented in **Table 3**. For the aggrecan polymorphism, 4 and 5 studies were on Caucasians and Asians, respectively. All the included studies used blood samples for DNA extraction. Magnetic resonance images (MRI) was used for the detection of IDD in almost all the studies. No studies included provided the genotypes of each subjects, thus we only compared the difference of allele distribution. Allele frequencies for the aggrecan VNTR polymorphism were present in **Table 4**.

Quantitative data synthesis

First, we compared the allele frequency difference in IDD patients and controls. A significant relationship was observed between the shorter and longer alleles and IDD susceptibility in all subjects (shorter: OR = 1.45, 95% CI = 1.19-1.77; longer: OR = 0.79, 95% CI = 0.65-0.97). The common alleles had no effect on increasing the risk of IDD (OR = 0.89, 95% CI = 0.76-1.05). After stratification by ethnicity, subgroup analysis indicated that the shorter alleles was significantly increased in IDD patients in Asians (OR = 1.50, 95% CI = 1.17-1.93). Longer alleles was significantly decreased in IDD patients in Asians (OR = 0.62, 95% CI = 0.45-0.85). No statistical significance was found in Asians between the common and longer alleles distribution and risk of IDD (OR = 0.91, 95% CI = 0.72-1.15). The distribution of the 3 allele groups showed no significant association between aggrecan VNTR polymorphism and IDD in Cau-

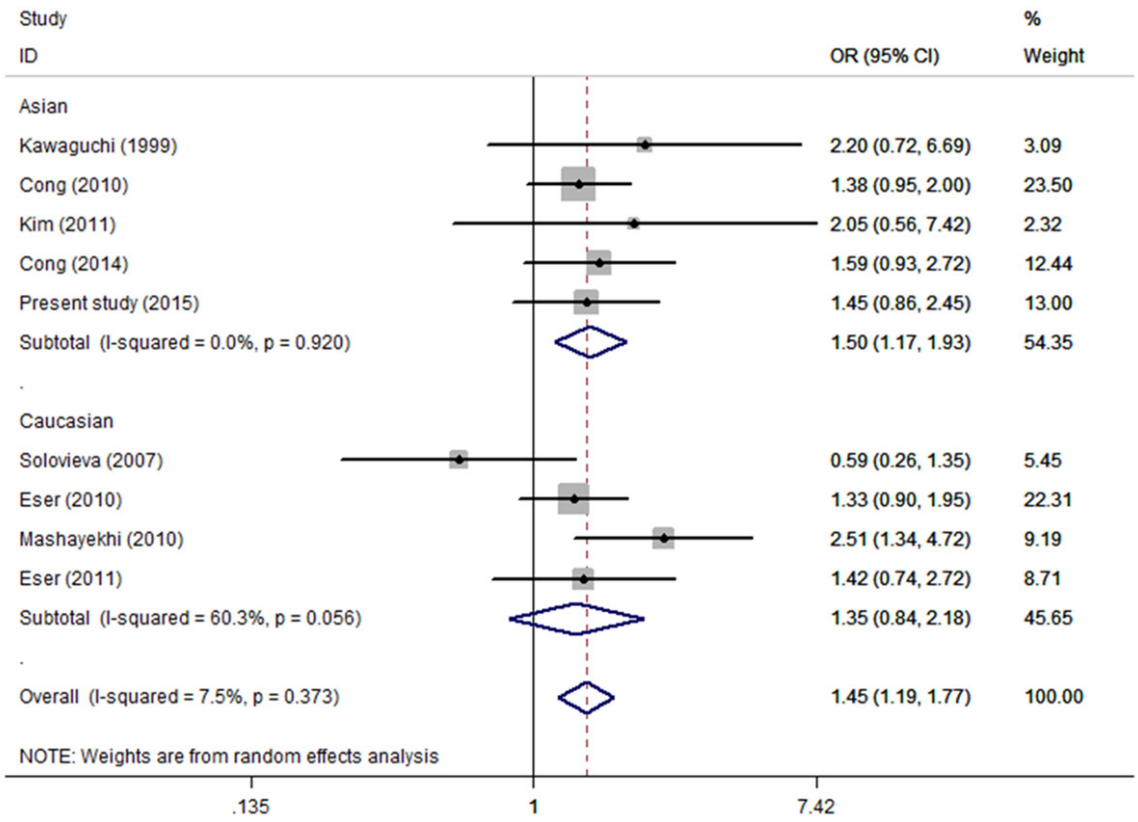

Figure 2. Forest plot of IDD risk associated with the aggrecan VNTR polymorphism (shorter alleles versus common and longer alleles).

casians (shorter: OR = 1.35, 95% CI = 0.84-2.18; common: OR = 0.88, 95% CI = 0.71-1.09; longer: OR = 0.94, 95% CI = 0.73-1.21; **Figures 2-4**).

Sensitivity analyses

Sensitivity analyses indicated that one study conducted by Mashayekhi *et al.* was the main origin of heterogeneity for the aggrecan VNTR polymorphism in the overall population. No single study influence the pooled results qualitatively, as showed by sensitivity analyses. Pooled ORs were consistent in Asians or Caucasians respectively by omitting one study at a time under allele comparison, suggesting robustness of the present results.

Publication bias

The publication bias test was performed for overall populations in shorter allele group. No significant publication bias was shown for overall populations by the Begg rank correlation method (P = 0.35) (**Figure 5**) and the Egger weighted regression method (P = 0.70) (**Figure 6**).

Discussion

IDD was traditionally regarded as a result of mechanical overloading and senescence. However, recent studies have demonstrated that genetic factors may play an important role in developing IDD [24]. Although several gene polymorphisms have been investigated widely in the risk of IDD, aggrecan VNTR polymorphism is a recently studied polymorphism. Several case-control studies have evaluated the role of aggrecan VNTR polymorphism in the development of IDD among various populations, the results still remain controversial. The controversial results regarding the effects of aggrecan VNTR polymorphism on IDD risk may be attributed to the differences in race, the age, the weight, and the occupation of the subjects. Moreover, the smaller sample size of individual studies may lower the statistical power.

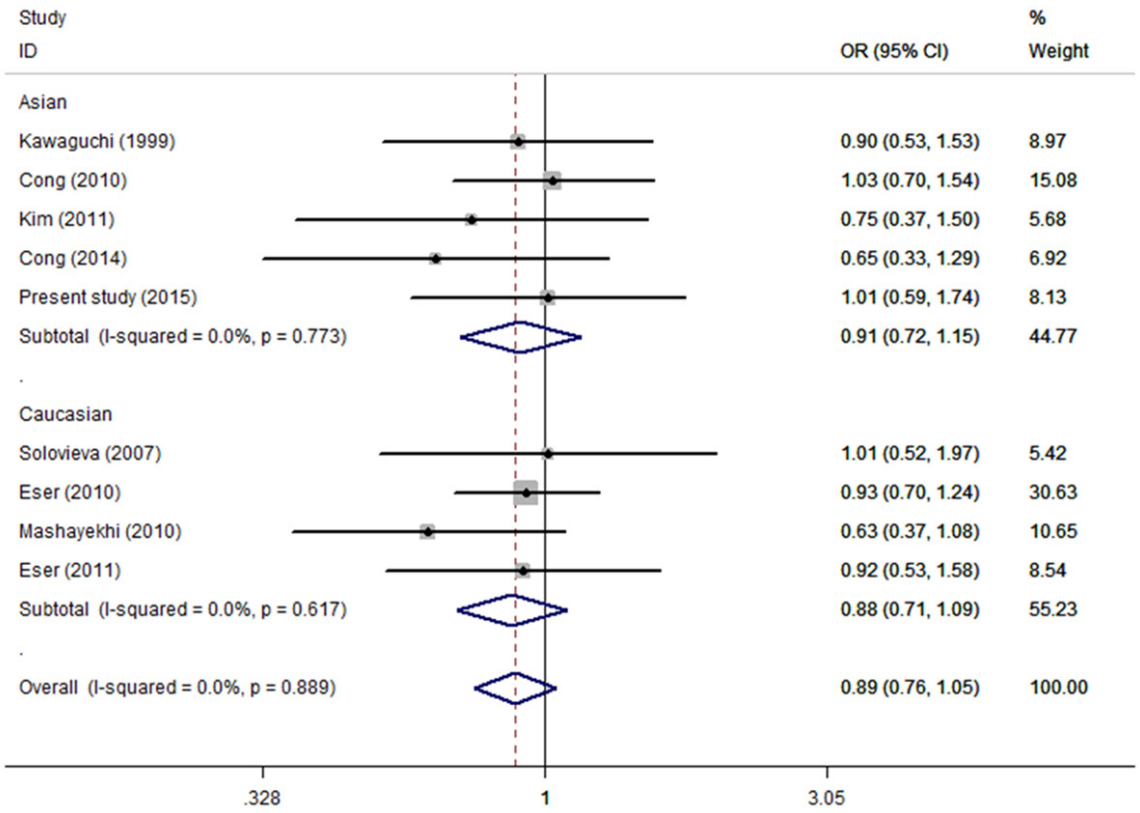

Figure 3. Forest plot of IDD risk associated with the aggrecan VNTR polymorphism (common alleles versus shorter and longer alleles).

Therefore, a meta-analysis should be an appropriate method to obtain a more definitive conclusion based on the current evidence.

GWAS is a powerful method for the detecting genetic contributions to polygenic diseases, and has been increasingly used to study genetic predisposition in IDD. However, this method may produce spurious association [27, 28]. Therefore, replications of the associations in different ethnic groups are important to confirm the results of GWAS [29]. In the present study, we identified that aggrecan VNTR polymorphism was associated with risk of IDD in case-control study. The present case-control study provided powerful evidence to support that aggrecan VNTR polymorphism may account for disease pre-disposition of IDD. Our study confirmed the earlier finding of positive association in Asian population [30]. Moreover, the results of meta-analysis implicated that aggrecan VNTR polymorphism was associated with increased IDD susceptibility in the Asian population but not in Caucasians. The present case-control study provides a more comprehensive summary of the currently available evidence on the association between the aggrecan VNTR polymorphism and the risk of IDD.

IDD is a multistage process, in which several genetic or environmental events underlie each stage, and may result from the interplay of genetic and environmental factors. There might be an association between gene polymorphisms and intermediate phenotypes rather than end stage of this process. However, we did not evaluate the association between the following factors like stage and severity of IDD, symptomatology and radiological finding of IDD and genetic predisposition, due to the limited information and sample size. In the further study, several studies can be conducted based on the present limitations.

Association studies are a useful tool to identify genetic factors conferring susceptibility to diseases [31]. However, the original association studies are not powerful enough to detect the

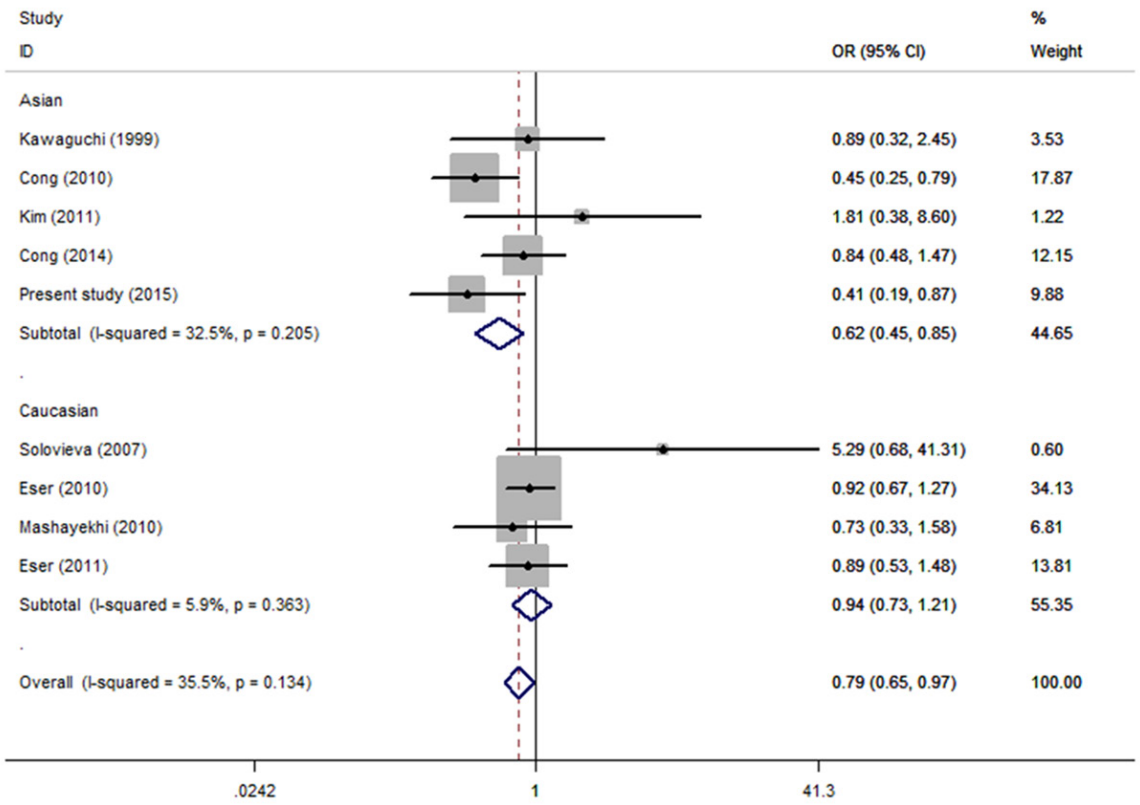

Figure 4. Forest plot of IDD risk associated with the aggrecan VNTR polymorphism (longer alleles versus common and shorter alleles).

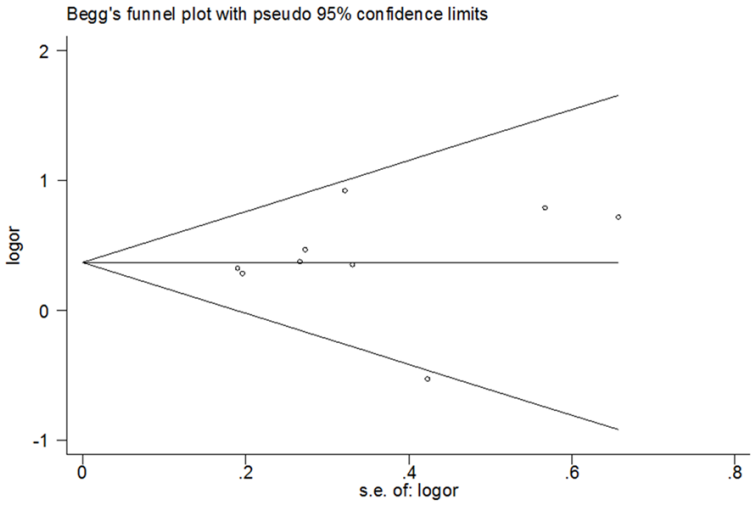

Figure 5. Begg funnel plot for publication bias of aggrecan VNTR polymorphism and risk of IDD.

genetic effects underlying the genetic susceptibility to develop diseases. This disadvantage has resulted in the reporting of inconsistent findings in the literature. The reasons for inconsistent results include the following items: limited sample size, poorly designed studies, false-positive studies, and different ethnicities. A meta-analysis method may permit the estimation of population-wide effects and the identification of sources of variability of genetic risk factors [32]. Therefore, we combined the genetic data from the included studies and present case-control study to evaluate the association of aggrecan VNTR polymorphism and IDD with the help of a meta-analysis approach. Although the meta-analysis could not increase the quality of the present case-control study, it can strength the current results based on the current evidence. The above mentioned is the merit of the meta-analysis method. Although 9 comparisons were in-

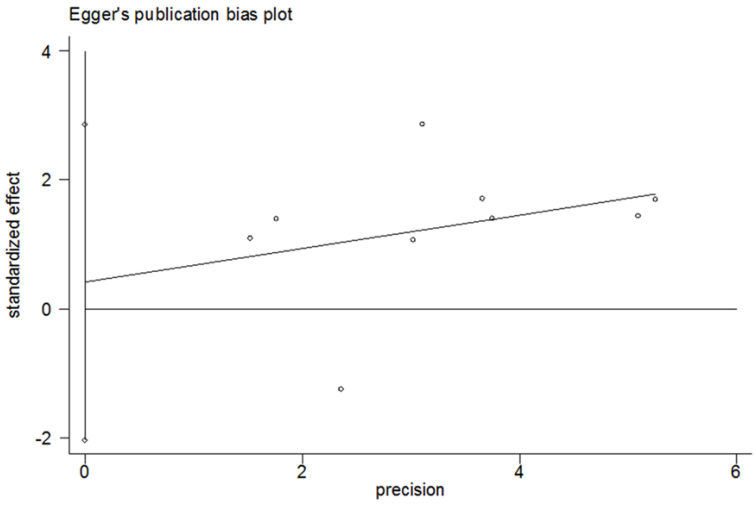

Figure 6. Egger test plot for publication bias of aggrecan VNTR polymorphism and risk of IDD.

cluded in the present meta-analysis, the study design of the original studies would be a limiting factor that exerts positive bias on the results. Genetic heterogeneity in the form of differences in aggrecan VNTR polymorphism and the presence of admixture within the study populations could also be a source of heterogeneity influencing the conclusion of the present meta-analysis. Another potential explanation for the differences in allele distribution is genotyping error, caused by different genotyping methods. To a certain degree, this could contribute to the variation in the allele distribution that we observed across the studies. Despite stratification by ethnicity, heterogeneity could not be absolutely omitted.

In the present study, stratification analyses were conducted to evaluate heterogeneity between studies. In the stratified analysis by ethnicity, a significant association was found in Asians for the polymorphism under shorter and longer allele groups. However, no significant result was detected among Caucasians. The following items may account for such difference. (1) Different populations usually have different linkage disequilibrium patterns. The aggrecan VNTR polymorphism may be in close linkage with different nearby causal variants in different populations. (2) The distribution of the allele varies extensively between different races. Further studies are still required to validate ethnic differences in the effect of this polymorphism on IDD. (3) Other clinical heterogeneity such as age, body mass index, years from onset, and disease severity may also explain the difference.

Although stratification analyses were conducted, clinical heterogeneity cannot be resolved completely. Some degree of clinical heterogeneity was induced by the different genotyping method, severity of IDD, medical co-morbidities, nutritional status of patients and diagnostic criteria for IDD. Heterogeneity may also have been caused by different study design. Because of limited information got from original studies, heterogeneity cannot be completely resolved. Accordingly, although the results of the meta-analysis should be considered appropriate, methodological quality defects and clinical heterogeneity should be considered when interpreting the findings.

The limitations of this meta-analysis mainly include the following items: (1) The efficacy of the statistics may be further improved by including more studies in the future. The statistical power may be lower after subgroup analysis. Further studies are still required in the future. (2) In the current meta-analysis, we only included published English-language association studies of aggrecan VNTR polymorphism and IDD. Thus, language bias may be an issue in the present study because it is exclusively based on English-language reports. In addition, we may have missed some grey studies, including negative results or unpublished studies. Our reasons for the reluctance to include grey literature included the absence of peer-review of such unpublished literature. Meta-analysis of unpublished data from interested sources is a controversial issue. (3) Although a meta-analysis can extract several similar studies to increase the statistical power, heterogeneity among studies can introduce some bias. Stratification by ethnicity may help to improve homogeneity among studies, but it may also influence statistical power. (4) Further individual studies are needed to investigate the association between aggrecan VNTR polymorphism

and severity, symptomatology and radiology of IDD.

Conclusion

This study suggested that aggrecan VNTR polymorphism was associated with the risk of IDD in Chinese population. The further meta-analysis provides additional evidence supporting the above result that the shorter allele of the VNTR polymorphism may increase IDD risk in Asians. Due to the limited data currently available for Caucasians and Asians, further studies with large sample sizes are required.

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Disclosure of conflict of interest

None.

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