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RESEARCH

A systematic review of the relationship between the distributions of aggrecan gene VNTR polymorphism and degenerative disc disease/osteoarthritis

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Objectives

Degenerative disc disease (DDD) and osteoarthritis (OA) are relatively frequent causes of disability amongst the elderly; they constitute serious socioeconomic costs and significantly impair quality of life. Previous studies to date have found that aggrecan variable number of tandem repeats (VNTR) contributes both to DDD and OA. However, current data are not consistent across studies. The purpose of this study was to evaluate systematically the relationship between aggrecan VNTR, and DDD and/or OA.

Methods

This study used a highly sensitive search strategy to identify all published studies related to the relationship between aggrecan VNTR and both DDD and OA in multiple databases from January 1996 to December 2016. All identified studies were systematically evaluated using specific inclusion and exclusion criteria. Cochrane methodology was also applied to the results of this study.

Results

The final selection of seven studies was comprehensively evaluated and includes results for 2928 alleles. The most frequent allele among all the studies was allele 27. After comparing the distributions of each allele with others, statistically significant differences have been found in the distribution of the alleles by the two groups, with an over-representation of allele (A)21 (disease: 3.22%, control: 0.44%). Thus, carrying A21 increased the risk of DDD. Such an association was not found to be statistically significant when considering the risk of OA.

Conclusions

The findings suggest that VNTR A21 seems to be associated with higher risk to DDD, however, such an association may not be statistically significant regarding the risk of OA.

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Keywords: Degenerative disc disease, Osteoarthritis, Aggrecan variable number of tandem repeats, Meta-analysis

Article focus

- The purpose of this study was to evaluate systematically the relationship between aggrecan VNTR and the risk of DDD and OA.

Key messages

- The findings suggest that when considering the relationship between aggrecan VNTR and DDD specifically, allele 21 has a tendency to increase the risk of DDD.

Strengths and limitations

- The major strength of this systematic review is that we conducted a comprehensive search of multiple databases, selected and appraised studies by independent pairs of reviewers, and followed an *a priori* planned protocol that included several hypotheses for the role of aggrecan VNTR in DDD and/or OA.
- There are several limitations, namely the quantity of included studies was comparatively small, and thus carrying this allele

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may not have a statistically significant effect on the risk of OA.

Introduction

Degenerative disc disease (DDD) and osteoarthritis (OA) are prevalent diseases, which have staggering socioeconomic effects on today's society and place a heavy burden on global health care. Osteoarthritis is a relatively frequent musculoskeletal problem that causes stiffness and significant pain in the joints.[1] While DDD is considered an inevitable consequence of ageing, and is thought to be one of the most common causes of chronic back pain,[2] together with OA, it results in a significant impairment to quality of life. Despite numerous studies of their aetiology and pathogenesis, it is not clear why the susceptibility to DDD and OA is low in some individuals while high in others.

Intervertebral discs (IVD) and articular cartilage assist load transfer and movement in the spine and joints. In both of these chondroid tissues, an extensive matrix of collagen and aggrecan is maintained by a small population of cells, and although the chondroid tissues are essentially lacking in nerves and blood vessels, both can lead to disability and pain when influenced by degenerative changes. Matrix biology research involving both types of cartilage shows some striking parallels and considerable overlap. The traditional aetiology of DDD and OA and their links with smoking, occupation, age, and obesity, have been well described.[3] Recently, however, our understanding of genetic influences on the risk of DDD and OA indicate that genetic factors may have a significant role in the pathogenesis of DDD and OA.[4]

Recent literature suggests that the aggrecan content of the IVD and articular cartilage intimately affect their functions.[5] Aggrecan consists of globular domains G1, G2, and G3. There is a long glycosaminoglycan (GAG) attachment region between domains G2 and G3 that consists of adjacent domains of chondroitin sulfate (CS) and keratan sulfate (KS).[5] The glycosaminoglycan chain structures vary throughout life as the CS chains become shorter and KS chains become longer in the adult. This may reflect reduced oxidation of glucose to glucuronic acid, which is needed for CS synthesis, because of the avascular nature of the IVD and articular cartilage,[6] Under normal circumstances, both disc cells and articular chondrocytes maintain a dynamic equilibrium between degradation and synthesis of extracellular matrix components, containing collagen fibrils that form a network surrounding and restraining huge, hydrated aggregates of aggrecan.[7]

The aggrecan gene variable number of tandem repeats (VNTR) polymorphism in a human being has repeats of 57 nucleotides; these encode each 19-amino acid unit. The described alleles of aggrecan VNTR range from 13 to 34 repeats.[8] It has been previously suggested that there is a relationship between aggrecan gene VNTR and DDD and/or OA. Horton et al[9] identified that the allele containing 27 repeats (A27) was statistically associated with bilateral hand OA. Solovieva et al[10] also found that the A26 was statistically associated with lumbar disc degeneration. Other studies reported that A18,[11] A21,[12] and A25[13] were risk factors for lumbar disc degeneration, whereas A29 was a protective factor for DDD.[11] Considering these ambiguous results, it is still uncertain which aggrecan VNTR allele is the main risk factor for DDD and/or OA. We have therefore reviewed the literature and performed a meta-analysis to assess systematically the relationship between the aggrecan gene VNTR polymorphism and DDD and/or OA.

Materials and Methods

Search strategy. This study was completed in accord with the guidance outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement.[14] The databases used for the search were PubMed, Ovid EMBASE, Ovid MEDLINE, Cochrane Database of Systematic Reviews, Cochrane Central Register of Controlled Trials, ACP Journal Club, Scopus and Web of Science, and the Database of Abstracts of Review of Effectiveness. The search covered data from 1996 to December 2016. A combination of text words and controlled vocabulary were used. MEDLINE uses a single term, aggrecan, but EMBASE and others use the terms AGC1 or ACAN, but include more specific terms for aggrecan. To be as inclusive as possible, the search also included text words: agcan, cspgcp, and cspg1 (Aggrecan core protein). The same approach was used for osteoarthritis: osteoarthritis is used by MEDLINE, however, EMBASE and others use arthritis with more specific terms including cartilage or chondrocyte. There are similar differences for chondrolysis, remodelling of the subchondral bone *versus* degeneration of the cartilage. Additionally, the same approach was also used for degenerative disc disease: intervertebral disc is used by MEDLINE, however, EMBASE and others use intervertebral disc, with more specific terms including nucleus pulposus, endplate or annulus fibrosus. It is a similar story for intervertebral disc disease, degeneration, spinal stenosis, and displacement *versus* herniation. Text words were also used to be inclusive. The results were downloaded into EndNote (EndNote X7, Bld 7072; Thomson ResearchSoft, Stamford, Connecticut), and duplicates removed. The reference lists of all identified studies without language restrictions were reviewed for further identification of potentially relevant articles.

Selection criteria. We systematically identified published articles regarding the relationship between the aggrecan gene VNTR polymorphism and DDD and/or OA according to the following inclusion criteria: Assessing the relationship between aggrecan VNTR polymorphism and

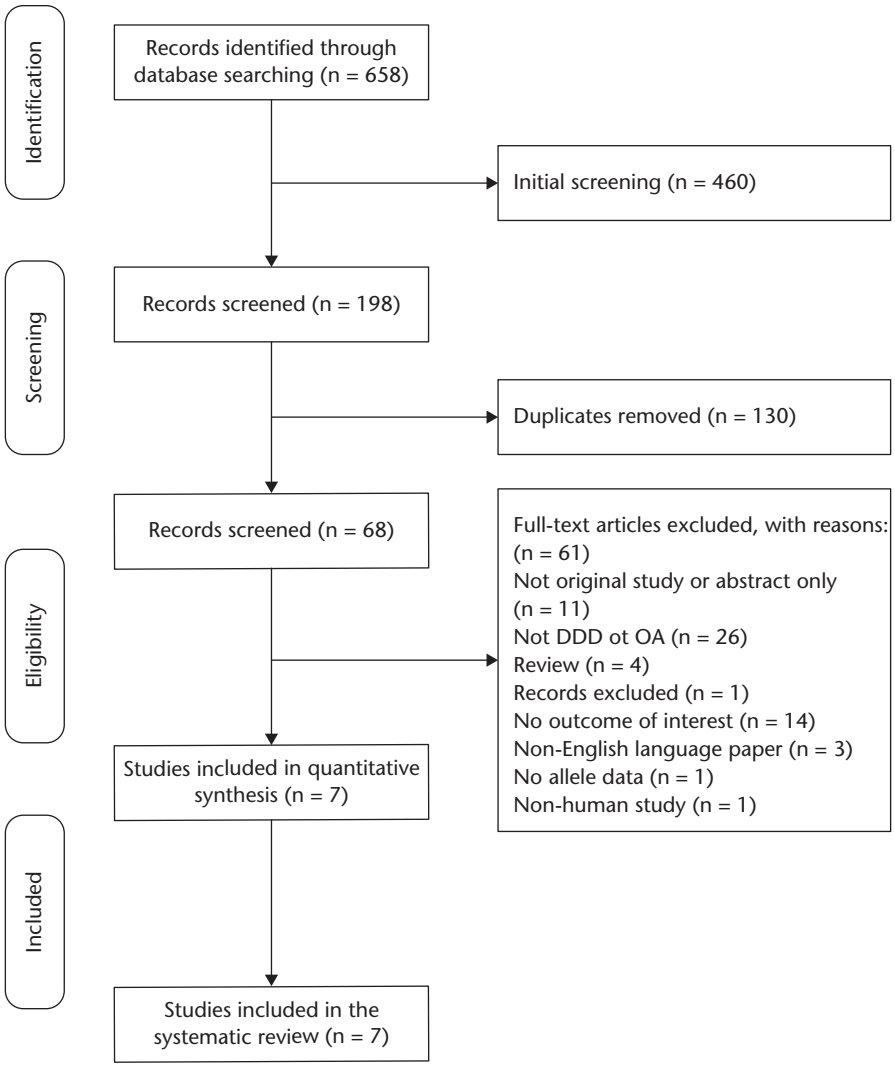

Fig. 1

Flow chart showing the the selection of studies for meta-analysis. DDD, degenerative disc disease; OA, osteoarthritis.

DDD and/or OA; cohort design (case-controlled or cross-sectional); full articles only (accordingly, animal studies, case reports, abstracts, conference presentations, reviews, expert opinions, and editorials were excluded); and articles must contain information on allele frequency of the aggrecan VNTR polymorphism or sufficient data for computation of OR (odds ratio) with the corresponding 95% confidence interval (CI). When the relevant information was not available, we contacted the authors to request it.

Data collection. The two investigators (LC and GT) independently extracted data from the text, figures and tables of the included studies using a standardized datasheet. They then selected the eligible cohorts according to the inclusion and exclusion criteria. Disagreements would be dealt with by discussion with the two investigators and, if necessary, by further discussion with another independent co-author. The categories of the extracted data were as follows: author's name; publication year; participant characteristics (country, source of control subjects, age and ethnicity of the investigated population) and number of participants; study characteristics; numbers of allele frequency in cases and controls; and OR and 95% CI of the comparisons.

Statistical analysis. For each study, we compared every single allele of aggrecan VNTR with other alleles to find the risk allele for DDD and/or OA. This study statistically pooled the data of included studies in order to discover the distribution of aggrecan VNTR. The calculated results were expressed in terms of OR and 95% CI for dichotomous outcomes. Two reviewers checked the collected data, entered them into the computer, and then analyzed the data using Review Manager (RevMan, Version 5.3. Copenhagen, Denmark). The Laird Q test was performed for heterogeneity and the I^2 statistic was also calculated for each analysis.[15] If a study had a p value $\leqslant 0.05$ and $I^2 \geqslant 50\%$, indicating obvious heterogeneity between studies, the random effects model was performed to evaluate

Table I. Characteristics of included studies when examining the relationship between aggrecan variable number of tandem repeats and degenerative disc disease (DDD) and/or osteoarthritis (OA)

Author	Cong[11]	Eser[25]	Horton (hand)[9]	Horton (knee)[9]	Kawaguchi[12]	Kim[13]	Solovieva[10]	Kämäräinen[26]
Year	2010	2010	1998	1998	1999	2011	2007	2006
Country	China	Turkey	USA	USA	Japan	South Korea	Finland	Finland
Setting	Hospital case-control	Hospital case-control	Hospital case-control	Hospital case-control	Hospital case-control	Hospital case-control	Hospital case-control	Hospital case-control
Gender	Men	Men	Men	Men	Women	Both	Men	Women
Age	14 to 49	20 to 30	72.0 (SD 7.1)	72.0 (SD 7.1)	20 to 29	13 to 73	41 to 46	45 to 63
Disease group	LDH (n = 70)	DDD (n = 150)	Hand OA (43)	Knee OA (n = 28)	DDD (n = 32)	IDD (n = 43)	IDD (n = 116)	Hand OA (n = 281)
Control group	Trauma patient (n = 14); Healthy (n = 113)	None DDD (n = 150)	None hand OA (n = 50)	None knee OA (n = 65)	Normal (n = 32)	Normal (n = 12)	None IDD (n = 16)	Normal (n = 249)
Risk allele	A21 and A25	A13 to A27	A27	None	A18 and A21	A21	A26	A27
Protect allele	A29	None	None	None	None	None	None	None
Size of participants	197	300	93	93	64	55	132	530

LDH, lumbar disc herniation; IDD, intervertebral disc degeneration.

the pooled OR.[16,17] Otherwise, the fixed effects model was used.[18] We also performed a sensitivity analysis by omitting each study in turn to assess the stability of the results. A p-value $\leqslant 0.05$ was considered statistically significant. All p-values presented are two-tailed. All authors had access to all of the data. We then used the rating system (with levels of evidence 1 through 5) of the Cochrane Back Review Group to assess the level of evidence.[19,20]

Results

Description of studies. We identified 658 eligible studies by filtering through the inclusion and exclusion criteria (Fig. 1). After initial screening, 460 studies were removed that examined genes, but did not focus on aggrecan. Upon further evaluation of titles and abstracts, 130 additional studies were omitted (Fig. 1). Of the remaining 68 candidate studies, we excluded a further 61 because assessment of the full-text versions revealed that these studies were either reviews,[5,21-23] or lacked the required allele data[24] (Fig. 1). The final selection of seven studies was comprehensively evaluated and encompassed results for 2928 alleles, with one set of hand OA and knee OA[9] discussed in a single multidisciplinary cohort (Tables I and II).[10-13,25,26]

Table I summarizes the characteristics of the seven included cohorts. Five cohorts were performed on the association between aggrecan VNTR and DDD,[10-13,25] and the other two were performed on the association between aggrecan VNTR and OA.[9,26] All seven cohorts were case-control studies which used standard polymerase chain reaction (PCR) genotyping methods, and investigated male and/or female populations. The distribution of aggrecan gene VNTR polymorphism allele frequencies in disease and controls in each of the identified cohorts is summarized in Table II.

Meta-analysis results. The described aggrecan VNTR alleles range from 13 to 36 repeats.[9-13,25,26] The most frequent allele in all of the studies was A27. After comparing the distributions of each allele with others, there was a statistically significant difference in the distribution of the alleles between the two groups, with an over-representation of A21 (disease group: 3.22%, control group: 0.44%). Thus, carrying A21 increased the risk of DDD (OR 5.43; 95% CI 2.10 to 13.58; p = 0.0004; I^2 = 43%). No OA-related studies identified A21. The result is similar to that of previous studies.[11-13] Additionally, no significant difference was found in the frequency of A18, A19, A22, A23, A24, A25, A26, A27, A28, A29, A32 or A33 for either DDD or OA. Figure 2 shows the results of the meta-analysis.

Sensitivity analyses. We performed sensitivity analyses and found that there was no single study which influenced the pooled ORs' quantitative polymorphism. Pooled ORs were steadily significant in the overall population by omitting any one study under each comparison of allele, which indicates robustness of this meta-analysis.

Publication bias. The number of included studies was less than ten, therefore, we did not assess publication bias. We cannot identify unpublished research with negative results. Publication bias may exist, which could lead to an overestimation of the effectiveness of aggrecan VNTR.

Discussion

Intervertebral discs (IVD) and articular cartilage assist load transfer and movement in the spine and joints. Aggrecan is the major proteoglycan of IVDs and articular cartilage, and it is present in very high concentrations in the form of aggregates which create osmotic swelling pressure gradients that draw water into the tissue. Recent literature indicates that the aggrecan content of the IVD and articular cartilage intimately affect their functions. The loss of aggrecan has a major effect on both DDD and OA.[27,28] It has been previously suggested that there is a relationship between aggrecan gene VNTR and loss of aggrecan. Thus, there could be a common genetic predisposition to both DDD and OA with certain aggrecan

Table II. Distribution of the aggrecan VNTR alleles among all included studies

	Cong[11]		Eser[25]		Horton (hand)[9]		Horton (knee)[9]		Kawaguchi[12]		Kim[13]		Solovieva[10]			Kämäräinen[26]	
Observed allele repeat, n	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**	**Observed allele repeat, n**	**Frequency of the disease group, n**	**Frequency of the control group, n**
13	0	0	4	0	0	0	0	0	0	0	0	0	0	0	**13**	0	0
18	2	0	0	0	0	0	0	0	1	0	0	0	0	0			
19	0	1	2	0	0	1	0	1	0	0	0	0	0	0			
20	1	4	0	0	0	0	0	0	0	0	0	0	0	0			
21	8	1	13	2	0	0	0	0	3	0	5	0	2	1			
22	4	10	26	24	1	3	1	3	1	3	4	0	2	0			
23	8	14	0	0	0	0	0	0	0	0	2	1	0	0			
24	14	31	0	0	0	0	0	0	0	0	4	1	1	0			
25	33	31	28	29	3	3	1	5	6	2	7	1	42	10			
26	19	36	43	69	14	17	12	19	20	15	21	5	81	7	**18 to 26**	110	138
27	34	57	90	74	45	35	25	55	25	35	30	14	74	13	**27**	150	259
28	14	30	63	68	21	38	16	43	6	6	11	2	26	1			
29	3	20	30	33	2	2	1	3	2	3	0	0	3	0			
30	0	9	0	0	0	0	0	0	0	0	0	0	0	0			
31	0	4	0	0	0	0	0	0	0	0	0	0	0	0			
32	0	3	1	0	0	0	0	0	0	0	0	0	1	0			
33	0	3	0	1	0	1	0	1	0	0	1	0	0	0			
34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	**28 to 34**	188	215
36	0	0	0	0	0	0	0	0	0	0	1	0	0	0	**36**	0	0
Total	140	254	300	300	86	100	56	130	64	64	86	24	232	32	**Total**	448	612

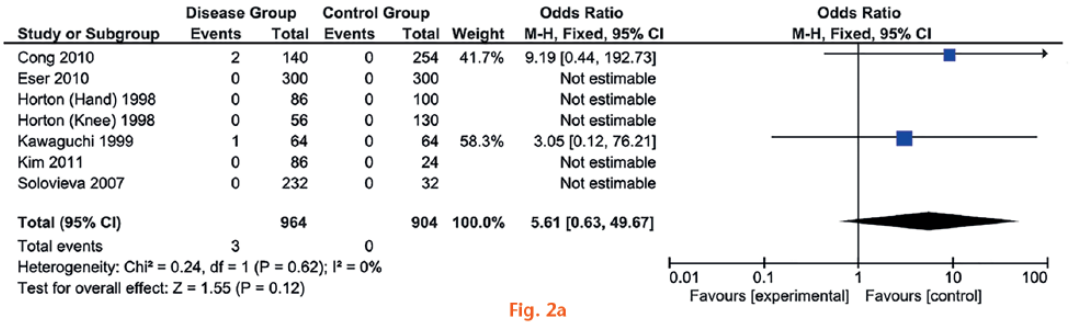

Fig. 2a

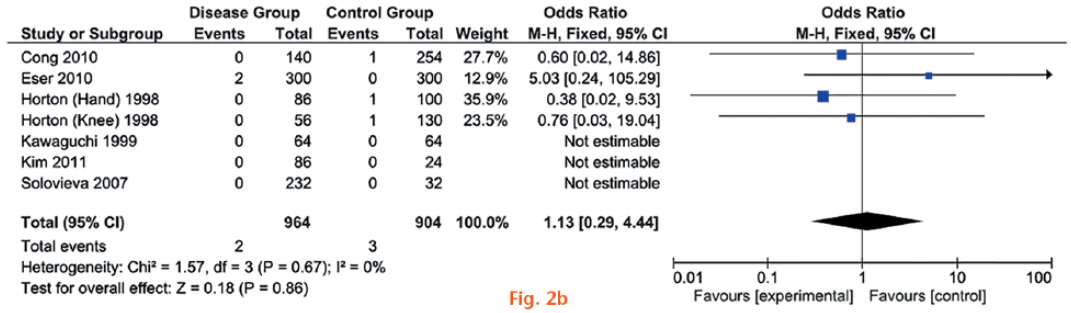

Fig. 2b

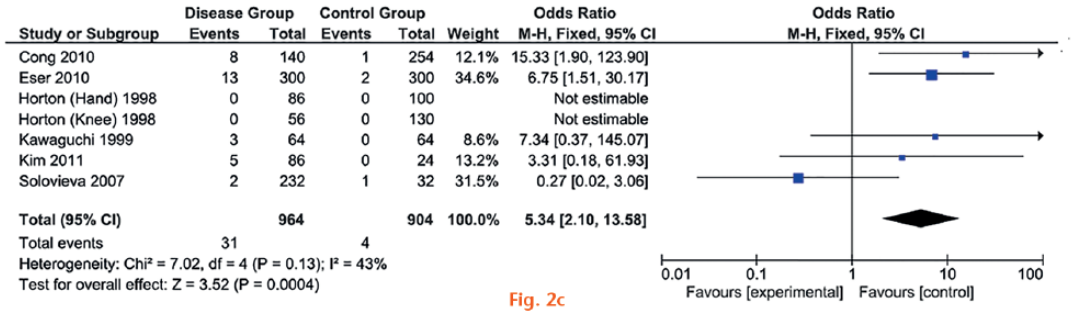

Fig. 2c

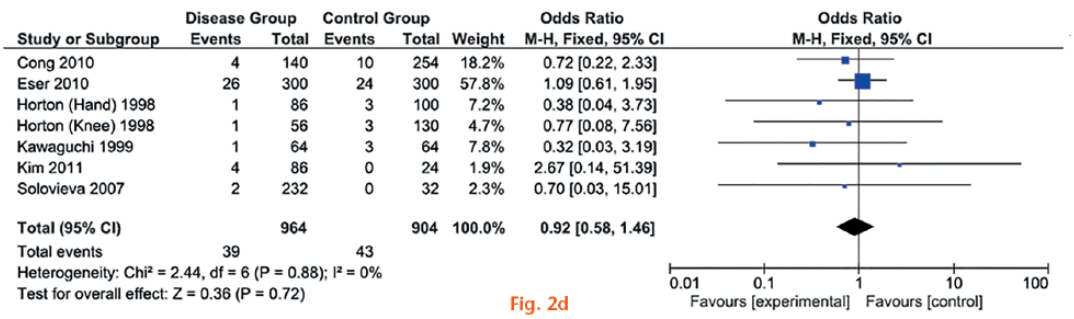

Fig. 2d

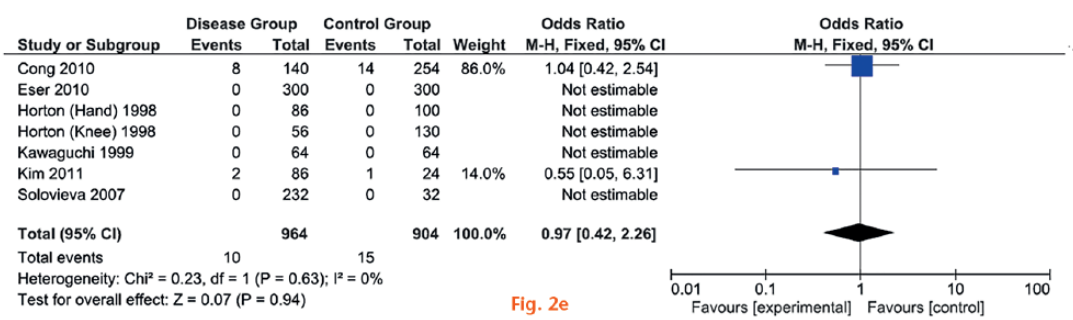

Fig. 2e

(continued)

Study or Subgroup	Disease Group Events	Total	Control Group Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cong 2010	14	140	31	254	89.4%	0.80 [0.41, 1.56]
Eser 2010	0	300	0	300		Not estimable
Horton (Hand) 1998	0	86	0	100		Not estimable
Horton (Knee) 1998	0	56	0	130		Not estimable
Kawaguchi 1999	0	64	0	64		Not estimable
Kim 2011	4	86	1	24	6.7%	1.12 [0.12, 10.53]
Solovieva 2007	1	232	0	32	3.9%	0.42 [0.02, 10.56]
Total (95% CI)		**964**		**904**	**100.0%**	**0.81 [0.43, 1.51]**
Total events	19		32			

Heterogeneity: Chi² = 0.24, df = 2 (P = 0.89); I² = 0%
Test for overall effect: Z = 0.67 (P = 0.50)

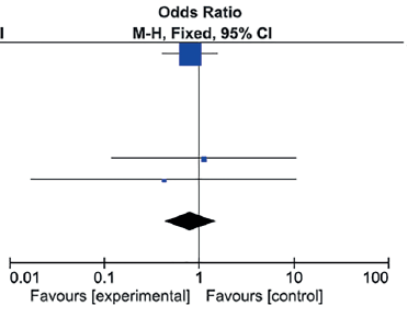

Fig. 2f

Study or Subgroup	Disease Group Events	Total	Control Group Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Cong 2010	33	140	31	254	25.5%	2.22 [1.29, 3.81]
Eser 2010	28	300	29	300	25.4%	0.96 [0.56, 1.66]
Horton (Hand) 1998	3	86	3	100	9.0%	1.17 [0.23, 5.95]
Horton (Knee) 1998	1	56	5	130	5.7%	0.45 [0.05, 3.98]
Kawaguchi 1999	6	64	2	64	8.9%	3.21 [0.62, 16.53]
Kim 2011	7	86	1	24	5.8%	2.04 [0.24, 17.43]
Solovieva 2007	42	232	10	32	19.7%	0.49 [0.21, 1.10]
Total (95% CI)		**964**		**904**	**100.0%**	**1.18 [0.67, 2.09]**
Total events	120		81			

Heterogeneity: Tau² = 0.26; Chi² = 12.61, df = 6 (P = 0.05); I² = 52%
Test for overall effect: Z = 0.57 (P = 0.57)

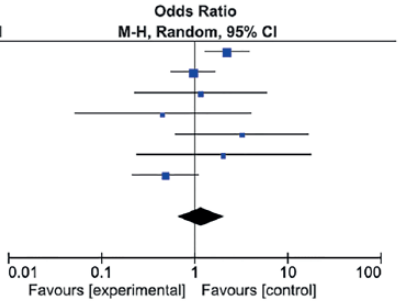

Fig. 2g

Study or Subgroup	Disease Group Events	Total	Control Group Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cong 2010	19	140	36	254	17.3%	0.95 [0.52, 1.73]
Eser 2010	43	300	69	300	46.3%	0.56 [0.37, 0.85]
Horton (Hand) 1998	14	86	17	100	10.3%	0.95 [0.44, 2.06]
Horton (Knee) 1998	12	56	19	130	7.0%	1.59 [0.71, 3.56]
Kawaguchi 1999	20	64	15	64	8.1%	1.48 [0.68, 3.25]
Kim 2011	21	86	5	24	4.6%	1.23 [0.41, 3.69]
Solovieva 2007	81	232	7	32	6.3%	1.92 [0.79, 4.62]
Total (95% CI)		**964**		**904**	**100.0%**	**0.93 [0.73, 1.20]**
Total events	210		168			

Heterogeneity: Chi² = 11.53, df = 6 (P = 0.07); I² = 48%
Test for overall effect: Z = 0.56 (P = 0.58)

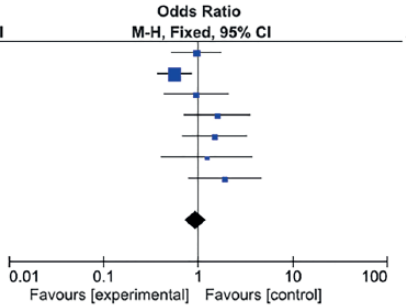

Fig. 2h

Study or Subgroup	Disease Group Events	Total	Control Group Events	Total	Weight	Odds Ratio M-H, Random, 95% CI
Cong 2010	34	140	57	254	14.0%	1.11 [0.68, 1.80]
Eser 2010	90	300	74	300	16.1%	1.31 [0.91, 1.88]
Horton (Hand) 1998	45	86	35	100	12.2%	2.04 [1.13, 3.68]
Horton (Knee) 1998	25	56	55	130	11.6%	1.10 [0.58, 2.07]
Kamainen 2006	150	448	259	612	17.9%	0.69 [0.53, 0.88]
Kawaguchi 1999	25	64	35	64	10.5%	0.53 [0.26, 1.07]
Kim 2011	30	86	14	24	7.8%	0.38 [0.15, 0.96]
Solovieva 2007	74	232	13	32	9.8%	0.68 [0.32, 1.46]
Total (95% CI)		**1412**		**1516**	**100.0%**	**0.91 [0.66, 1.27]**
Total events	473		542			

Heterogeneity: Tau² = 0.14; Chi² = 22.98, df = 7 (P = 0.002); I² = 70%
Test for overall effect: Z = 0.53 (P = 0.59)

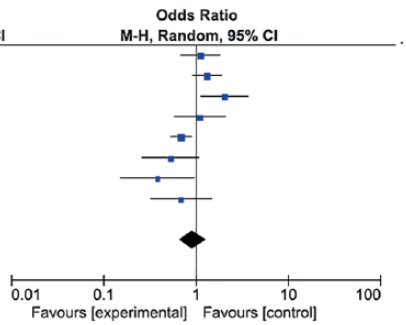

Fig. 2i

Study or Subgroup	Disease Group Events	Total	Control Group Events	Total	Weight	Odds Ratio M-H, Fixed, 95% CI
Cong 2010	14	140	30	254	15.0%	0.83 [0.42, 1.62]
Eser 2010	63	300	68	300	42.1%	0.91 [0.62, 1.34]
Horton (Hand) 1998	21	86	38	100	20.8%	0.53 [0.28, 1.00]
Horton (Knee) 1998	16	56	43	130	14.5%	0.81 [0.41, 1.61]
Kawaguchi 1999	6	64	6	64	4.3%	1.00 [0.30, 3.28]
Kim 2011	11	86	2	24	2.1%	1.61 [0.33, 7.83]
Solovieva 2007	26	232	1	32	1.2%	3.91 [0.51, 29.87]
Total (95% CI)		**964**		**904**	**100.0%**	**0.86 [0.66, 1.11]**
Total events	157		188			

Heterogeneity: Chi² = 5.18, df = 6 (P = 0.52); I² = 0%
Test for overall effect: Z = 1.18 (P = 0.24)

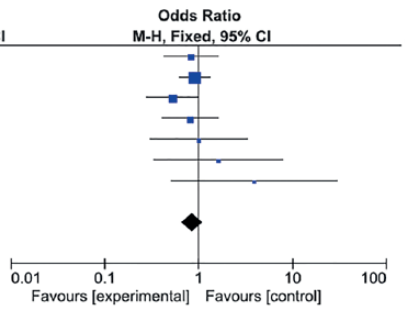

Fig. 2j

(continued)

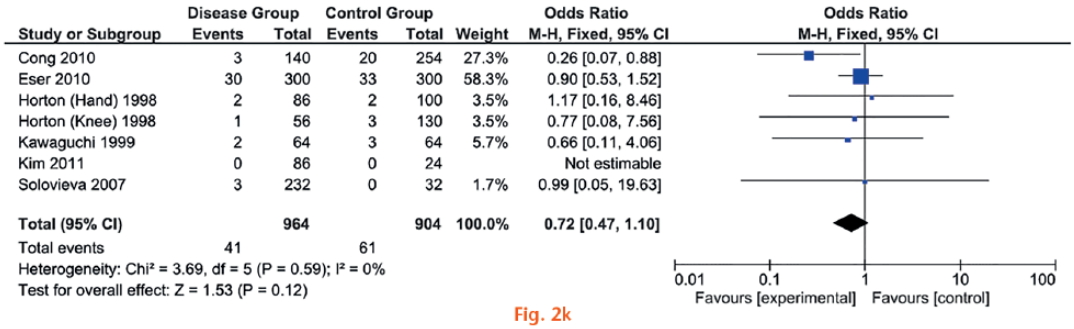

Fig. 2k

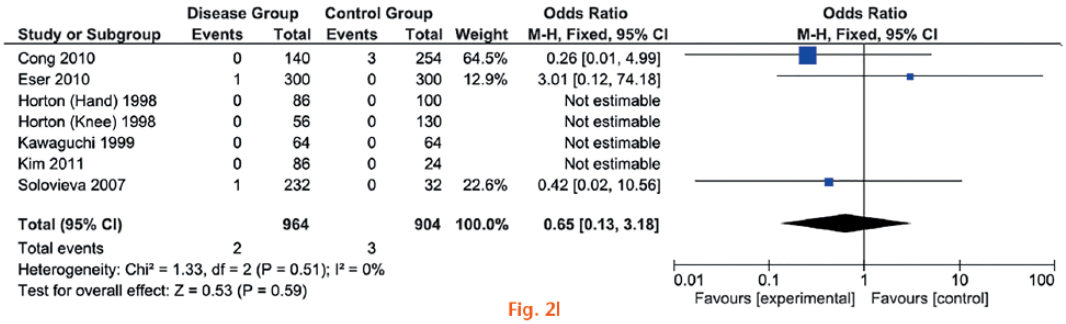

Fig. 2l

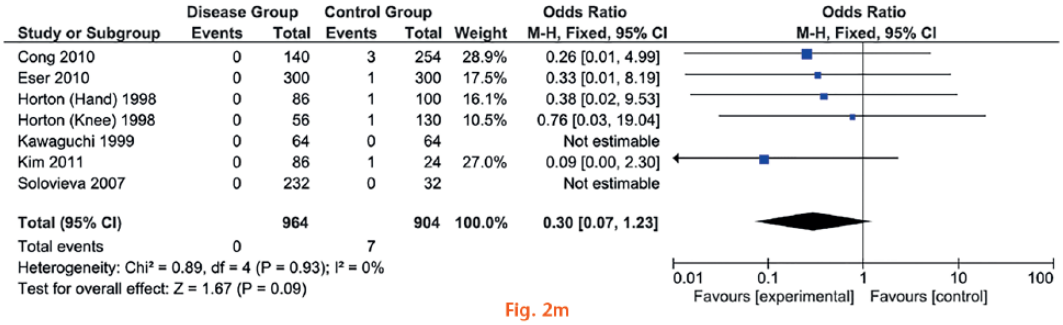

Fig. 2m

Forest plots of aggrecan variable number of tandem repeats (VNTR) associated with degenerative disc disease and/or osteoarthritis in overall populations: a) A18, b) A19, c) A21, d) A22), e) A23, f) A24, g) A25, h) A26), i) A27, j) A28, k) A29, l) A32, and m) A33. The squares and horizontal lines correspond to the study-specific odds ratio (OR) and 95% confidence interval (CI). The area of the squares reflects the study-specific weight (inverse of the variance). Diamonds represent the pooled OR and 95% CI. M-H, Mantel–Haenszel method.

gene VNTR, which is why we have considered them both in this meta-analysis. The human aggrecan gene VNTR is unique among the species evaluated to date as human genes possess VNTR polymorphism on exon 12, which encodes the CS1 domain.[29] Both DDD and OA are multi-stage processes, in which several environmental or genetic factors dominate each stage, and may be affected by the interaction of environmental and genetic events. Therefore, there may be a relationship between gene polymorphisms and intermediate phenotypes rather than the end stage of this process.[10,11]

This study has systematically assessed the relationship between the aggrecan gene VNTR polymorphism and DDD and or/OA. Xu et al[23] previously reviewed the association between aggrecan gene/vitamin D receptor gene polymorphisms and intervertebral disc degeneration in 2012, and subsequently Gu et al[22] reviewed aggrecan VNTR and LDD in 2013. However, both studies confused the number of participants and alleles. Thus, the results of their research are unconvincing.

In this meta-analysis, seven studies looking at the distribution of aggrecan VNTR have been identified, which assessed a total of 2928 alleles on OA and/or DDD. After pooling the data from these seven studies, we found that A21 (containing 21 repeats) was over-represented and increased the risk of DDD, which is similar to the findings of previous research.[11-13] The results suggest that human beings carrying the shorter aggrecan VNTR alleles would possess a lower number of CS chains or the special G3 domain of the aggrecan molecular structure, and this will lead to impaired function of aggrecan. The aggrecan protein core is adjusted with GAG chains, including domains of CS and KS. The polyelectrolyte nature of these GAG chains maintain the high osmotic pressure of aggrecan. Thus, it is suggested that the shorter GAG chains or the special G3 domain of aggrecan will lead to less water

holding capacity of the IVD, which could result in a lower ability to withstand compression and an increased susceptibility to DDD. However, the other crucial consideration is whether the existence of the special G3 domain and one allele with a short-encoding domain of CS1 would be enough to have a detrimental effect on intervertebral disc and articular cartilage function. So far, there is no clear mechanism as to how a difference in a single repeat, or the length of the repeated sequence, can affect the overall length of the aggrecan core protein.

Previous studies found that alleles 18, 25, 26 and 27 were statistically associated with either DDD or OA.[9-13] However, qualitative analysis reveals that no significant difference was found in the frequency of alleles 18, 19, and 22 to 33. The difference in the distribution of alleles between populations is one possible explanation for such inconsistent findings. Humans carrying the extreme form of the gene were not observed in the current sample, probably because of the low frequency of the alleles (A13 to A20 and A30 to A36) in all of the studies.[30] Although there does appear to be an influence of aggrecan VNTR on DDD and/or OA, it is still unclear whether this is because of the contributions of other associated genes, or is due to a specific gene effect of a comparatively large magnitude.

Aggrecan VNTR screening, that is coordinated and sustained at a national level, will be of benefit to precision medicine and increase the chances of discovering early DDD and OA in a patient, thus improving the chance of possible preventive therapies. Early diagnosis, or even primary prevention of these conditions would be more valuable than complicated treatments, both financially, and for patient quality of life. High-level screening also provides doctors with the tools to comprehend fully the complicated mechanisms underlying a patient's health, DDD or OA disease, or situation, and to anticipate better which treatments would be most useful.

This study systematically reviewed the relationship between aggrecan gene VNTR and DDD and/or OA, and could be applied to patients at risk. The patients could be advised on how their lifestyle might affect potential development of DDD and/or OA. Moreover, patients could be provided with further clinical direction on whether they will benefit from surgical treatments. Screening will also give DDD and OA patients the opportunity to evaluate the prognosis of the clinical treatments.[8,31]

Meta-analysis is a statistical method which uses pooled data from multiple surveys and research on the same problem in order to reach a more scientific and impartial conclusion. Ideally, a meta-analysis will include only studies that have a similar validated design, and contain large populations.[32] The major strength of this systematic review is that we conducted a comprehensive search of multiple databases, selected and appraised studies by independent pairs of reviewers, and followed an *a priori* planned protocol that included several hypotheses for the role of aggrecan VNTR in DDD and/or OA. When drawing conclusions regarding the role of aggrecan VNTR in DDD/OA, we should consider several limitations of this meta-analysis. First, the number of included studies was comparatively small; only seven studies were evaluated. This limited our ability to perform the asymmetry test with the Stata software and produce a funnel plot to assess potential publication bias visually. Thus, we cannot identify unpublished research with negative results. The potential publication bias could lead to an overestimation of the association between aggrecan VNTR and DDD and/or OA. Second, the included studies had a small population size; indeed, three of the seven studies included in the meta-analysis have less than 100 participants. Due to these limitations, we should accept the combined results of this study with caution. Furthermore, the conclusion of this meta-analysis needs to be validated in a larger population sample under different settings. In addition, a better understanding of the association between aggrecan VNTR and DDD and/or OA will, it is hoped, accelerate biomedical discoveries and improve clinical care based on new knowledge of gene-related disease mechanisms.

In conclusion, this study demonstrates that the most frequent allele in all of the studies was A27. Comprehensive analysis of all seven selected studies revealed a relationship between aggrecan VNTR and DDD, which identifies that A21 may have an association with DDD. However, such an association may not be statistically significant for OA.

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Author Contributions

- L. Cong: Analysis and interpretation of the data, Drafting of the article.
- G. Tu: Conception and design of study.
- D. Liang: Conception and design, Collection and assembly of data.

Conflicts of Interest Statement

- None declared

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