Acta Orthopaedica et Traumatologica Turcica 53 (2019) 497–501

Contents lists available at ScienceDirect

Acta Orthopaedica et Traumatologica Turcica

journal homepage: https://www.elsevier.com/locate/aott

Meta-analysis of the association of IL1-RN variable number of tandem repeats polymorphism with osteoarthritis risk

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ARTICLE INFO

Article history:
Received 3 January 2019
Received in revised form
11 April 2019
Accepted 19 July 2019
Available online 21 August 2019

Keywords:
IL1-RN
VNTR
Polymorphism
Osteoarthritis
Meta-analysis

ABSTRACT

Objective: The aim of this meta-analysis was to clarify the role of Interleukin-1 receptor antagonist gene (IL1-RN) Variable Number of Tandem Repeats (VNTR) polymorphism on the risk of OA by means of meta-analysis.

Methods: Eligible articles were retrieved from PubMed, Web of science and Google scholar with a total of 1187 OA cases and 2659 controls. The strength of the association between the IL1-RN VNTR polymorphism and the risk of OA was assessed by odds ratios (ORs) with the corresponding 95% confidence interval (CI) for each study.

Results: The meta-analysis of seven published studies retrieved from the literature search showed a significantly increased OA risk in the recessive model analysis (22 vs 2L + LL: $P^b = 0.18$, $I^2 = 32.8$, OR(95% CI) = 1.50(1.12, 2.02), P = 0.007), the additive model analysis (22 vs LL: $P^b = 0.08$, $I^2 = 46.8$, OR(95% CI) = 1.56(1.15, 2.12), P = 0.004) and in the allele contrast model (2 vs L: $P^b = 0.02$, $I^2 = 58.8$, OR(95% CI) = 1.20(1.05, 1.36), P = 0.007). By subgroup analysis, the IL1-RN VNTR polymorphism was found to be significantly associated with OA susceptibility in Caucasian and Hospital based case-control study (HCC) groups.

Conclusion: This meta-analysis showed that IL1-RN VNTR polymorphism may increase the susceptibility to OA. More studies with detailed information are needed to validate our conclusion.

Level of evidence: Level III, diagnostic study.

Introduction

Osteoarthritis (OA) is a multifactorial disease and often occurs among middle-aged and elderly people.[1] OA is characterized by chronic grinding loss of articular cartilage, which causes many symptoms, such as pain, stiffness and loss of joint function.[2] A large body of evidence have shown that OA may be a chronic inflammatory disease, and the levels of Interleukin 1 (IL1), Interleukin 6 (IL6), Tumor necrosis factor alpha (TNF-α), and other acute phase proteins are higher in OA patients than controls.[3,4]

IL1 family plays an important role in the destruction of articular cartilage, by decreasing synthesis of matrix components and increasing synthesis of matrix metalloproteinases. There are 11 members of the IL-1 family of cytokines and 10 members of the IL-1 family of receptors.[5,6] IL1 receptor antagonist (IL1-RA) is an important anti-inflammatory molecule, which can bind to IL1 receptors in competition with IL1α and IL1β, thus inhibit their activities and modulate a variety of IL1-related immune and inflammatory activities.[7] The IL1-RA gene (IL1-RN) has a variable number of tandem repeats (VNTR) polymorphism of 86 base pairs (bp) in intron 2. There are five alleles, corresponding to allele 1 (four repeats), allele 2 (two repeats), allele 3 (five repeats), allele 4 (three repeats) and allele 5 (six repeats), which can be further summed up as a short allele (2:2 repeats) and a long allele (S: 3–6repeats). Thus, the genotypes are divided into LL, 2L and 22.[8] Some studies have investigated the association between the

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Peer review under responsibility of Turkish Association of Orthopaedics and Traumatology.

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https://doi.org/10.1016/j.aott.2019.07.004

VNTR polymorphism and the susceptibility to large-joint OA, but the results are conflicting.

Therefore, we performed a meta-analysis, comprising a total of 1187 OA cases and 2659 controls, to clarify the association between the IL-1RN VNTR polymorphism and OA risk.

Methods

Search strategy

Qualified articles were achieved by retrieving the Web of science, Embase, Google scholar and Pubmed (up to August 16, 2017) using a combination of the following keywords: interleukin-1 receptor antagonist or IL1-RN; osteoarthritis or OA; variants or variant or polymorphisms or polymorphism. Moreover, the references in the articles of inclusion and reviews were checked to avoid missing any relevant studies. To minimize bias and human error, Xu and Shi completed the article selecting independently.

Inclusion and exclusion criteria

The inclusion criteria were: (1) case-control design; (2) studies investigating the relationship between the IL1-RN VNTR polymorphisms and OA susceptibility; (3) the number of cases and controls were provided; (4) genotype frequency and(or) allele frequency of the cases and controls were provided; (5) the research sample was independent of other research report; (6) other important information for the analysis was provided, such as ethnicity, design type, phenotype. The exclusion criteria were: (1) studies with overlapping populations; and (2) studies with insufficient data.

Data extraction

Two independent researchers collected the following information from all eligible articles: (1) the first author; (2) journal name; (3)publication year; (4) population information; (5) sample size; (6) phenotype information; (7) the number of genotypes in cases and controls; (8) and the results of the study.

Statistical analysis

Samples collected from those studies were subdivided into Asian and Caucasian, HCC and PCC (population based case-control study) and different phenotypes. The data of genotype and allele distributions were summarized by data table. The Hardy-Weinberg equilibrium (HWE) were used to assess the distribution of the genotypes in the control populations. $P < 0.05$ was considered that the genotype distribution of this sample deviated from HWE. Meta-analysis was used to analyze the general data. First of all, heterogeneity test were conducted by a chi-squared($\chi 2$) test. If $P < 0.05$, the random effect model was used. If $P > 0.05$, the fixed effect model was used. Meta-regression analysis was used to look for sources of any heterogeneity. Funnel plots were used to assess the publication bias, and the Begg's and Egger's tests could help get a clear conclusion. After determining the effect model, the ORs and CI were used to aseess the strength of the association between the IL1-RN VNTR polymorphism and risk of OA. Two authors independently completed the analysis and obtained the same results. The above statistical analysis used

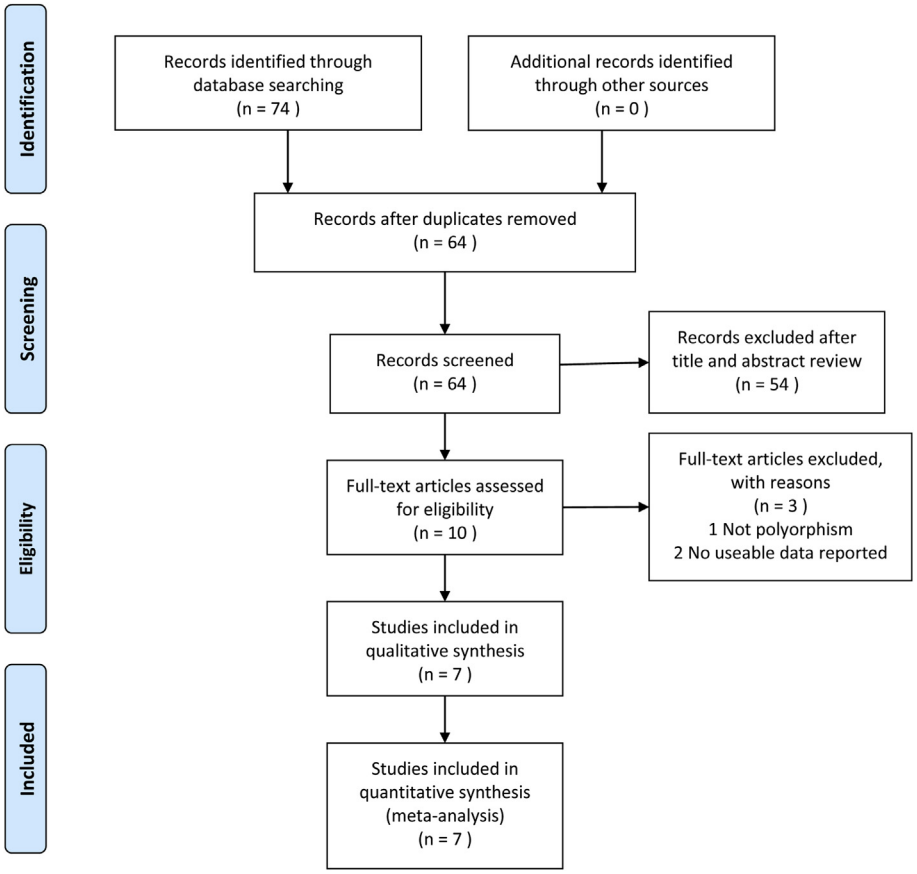

Fig. 1. Flowchart of the study selection.

Table 1
Characteristics of studies included in IL1-RN VNTR polymorphism and OA.

Study	Ethnicity	Design	Phenotype	Quality score	Patients					Controls					P_{HWE}
					22	2L	LL	2	L	22	2L	LL	2	L	
Sezgin 2007[9]	Caucasian	PCC	Knee	5	4	20	83	28	186	3	13	51	19	115	0.09
Jotanovic 2012[10]	Caucasian	HCC	Knee	5	23	86	108	132	302	45	184	266	265	709	0.11
Jotanovic 2011[11]	Caucasian	PCC	Hip	4	25	81	126	131	333	44	177	266	265	709	0.06
Ni 2009[12]	Asian	HCC	Knee	3	4	56	393	64	842	2	65	420	69	905	0.75
Meulenbelt 2004[13]	Caucasian	PCC	Hip	5	8	32	25	48	82	50	311	475	411	1261	0.92
Stern 2003[14]	Caucasian	HCC	Hand	6	10	22	36	42	94	4	23	23	31	69	0.59
Moos 2000[15]	Caucasian	HCC	Mixed	4	9	17	19	35	55	12	80	144	104	368	0.83

Quality score was determined by using the Newcastle–Ottawa quality assessment scale.
HWE: Hardy-Weinberg equilibrium.
HCC: Hospital-based case-control study; PCC: Population-based case-control study.

STATA software (version14; Stata Corporation, College Station, TX, USA). When $P < 0.05$, there was a significant difference.

Results

Characteristics of the studies

As shown in Fig. 1, according to the search terms, a total of 64 studies were included. Among these, 54 studies were excluded after title and abstract review and 3 studies were excluded according to the inclusion and exclusion criteria. The 7 eligible articles included 1187 OA cases and 2659 controls. The first author's surname, publication year, ethnicity, quality score, sex distribution, frequencies of various genotypes in OA cases and controls and HWE in controls for each study were listed in Table 1. The average score of articles quality evaluation was 4.57. The genotype distributions of the control groups were all consistent with the HWE.

Quantitative synthesis

The pooled analysis based on all included studies showed significant associations in the recessive model analysis (22 vs 2L + LL: $P^b = 0.18$, $I^2 = 32.8$, OR(95% CI) = 1.50(1.12, 2.02), $P^d = 0.007$), the additive model analysis (22 vs LL: $P^b = 0.08$, $I^2 = 46.8$, OR(95% CI) = 1.56(1.15, 2.12), $P^d = 0.004$) and in the allele contrast model (2 vs L: $P^b = 0.02$, $I^2 = 58.8$, OR(95% CI) = 1.20(1.05, 1.36), $P^d = 0.007$) (Fig. 2). When grouped by OA types, there was no significant association in all the models of all subgroups. When grouped by ethnicity, significant associations were found in Caucasian group (2 vs L: $P^b = 0.02$, $I^2 = 62.3$, OR(95% CI) = 1.23(1.07, 1.42), $P^d = 0.004$).In the genetic model analysis, significant associations were found in Caucasian group (22 vs 2L + LL: $P^b = 0.12$, $I^2 = 43.1$, OR(95% CI) = 1.48(1.10, 2.00), $P^d = 0.01$; 22 vs LL: $P^b = 0.05$, $I^2 = 55.3$, OR(95% CI) = 1.54(1.13, 2.11), $P^d = 0.006$; 22+2L vs LL: $P^b = 0.06$, $I^2 = 53.6$, OR(95% CI) = 0.83(0.69, 0.99), $P^d = 0.04$). When grouped by study design, significant associations were found in HCC group (2 vs L: $P^b = 0.04$, $I^2 = 63.6$, OR(95% CI) = 1.20(1.01, 1.43), $P^d = 0.04$). In the genetic model analysis, significant associations were found in HCC group (22 vs 2L + LL: $P^b = 0.09$, $I^2 = 53.4$, OR(95% CI) = 1.66(1.10, 2.50), $P^d = 0.02$; 22 vs LL: $P^b = 0.07$, $I^2 = 56.8$, OR(95% CI) = 1.72(1.12, 2.64), $P^d = 0.01$). However, the IL1-RN VNTR polymorphism showed no significant association with OA susceptibility in the Asian and PCC group. The detailed results are shown in Table 2.

Tests of heterogeneity

There was heterogeneity in the whole allele contrast model analysis ($P = 0.02$). Therefore, a random effects model should be adopted. Meanwhile, a meta-regression analysis was conducted to

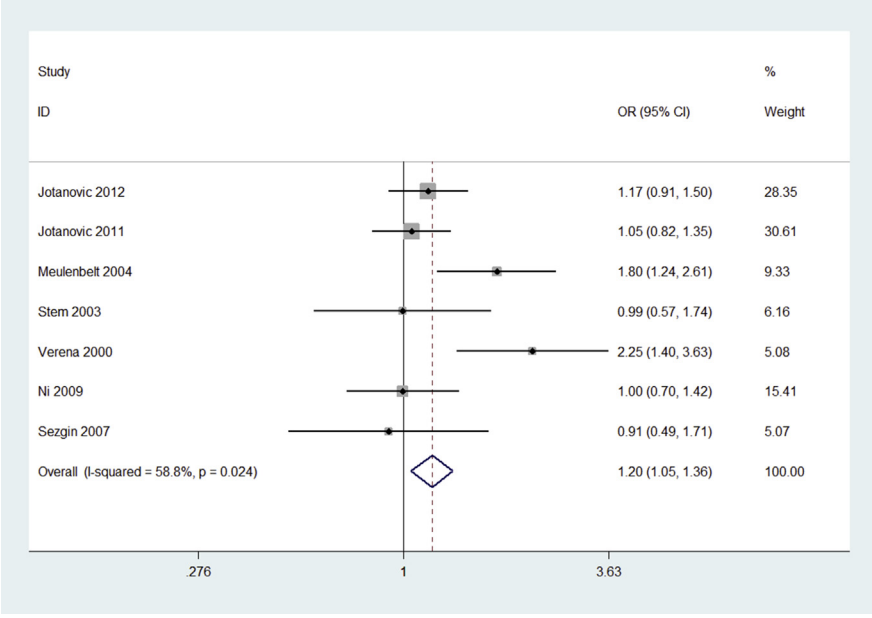

Fig. 2. Forest plot of the association between IL1-RN VNTR polymorphism and OA (2 vs L).

Table 2
Stratification analyses of genetic susceptibility of IL1-RN VNTR polymorphism to OA.

Category	n[a]	Additive (22 vs LL)				Dominant (22+2L vs LL)				Recessive (22 vs 2L + LL)				Allelic contrast (2 vs L)			
		I^2(%)	P[b,c]	OR (95%CI)	P[d]	I^2(%)	P[b,c]	OR (95%CI)	P[d]	I^2(%)	P[b,c]	OR (95%CI)	P[d]	I^2(%)	P[b,c]	OR (95%CI)	P[d]
Total	7	46.8	0.08	1.56 (1.15, 2.12)	0.004	49.9	0.06	0.87 (0.73,1.02)	0.08	32.8	0.18	1.50 (1.12,2.02)	0.007	58.8	0.02	1.20 (1.05,1.36)	0.007
Phenotype																	
Knee	3	0.0	0.72	1.26 (0.77, 2.07)	0.35	0.0	0.67	0.94 (0.75,1.19)	0.62	0.0	0.71	1.22 (0.76, 1.97)	0.44	0.0	0.65	1.09 (0.90, 1.32)	0.39
Hip	2	70.2	0.07	1.49 (0.94, 2.35)	0.09	82.2	0.02	0.81 (0.62,1.05)	0.12	34.8	0.22	1.41 (0.91,2.19)	0.12	81.8	0.02	1.23 (1.00, 1.51)	0.05
Ethnicity																	
Asian	1	/	/	2.14 (0.39, 11.73)	0.38	/	/	1.04 (0.72,1.52)	0.82	/	/	2.16 (0.39,11.85)	0.38	/	/	1.00 (0.70, 1.42)	0.99
Caucasian	6	55.3	0.05	1.54 (1.13, 2.11)	0.006	53.6	0.06	0.83 (0.69,0.99)	0.04	43.1	0.12	1.48 (1.10,2.00)	0.01	62.3	0.02	1.23 (1.07, 1.42)	0.004
Design																	
HCC	4	56.8	0.07	1.72 (1.12,2.64)	0.01	47.5	0.13	0.89 (0.71,1.10)	0.27	53.4	0.09	1.66 (1.10,2.50)	0.02	63.6	0.04	1.20 (1.01, 1.43)	0.04
PCC	3	49.7	0.14	1.41 (0.91,2.20)	0.12	67.5	0.05	0.84 (0.65,1.07)	0.16	0.0	0.36	1.35 (0.89,2.07)	0.16	68.0	0.04	1.19 (0.98, 1.45)	0.08

I^2 0–25, no heterogeneity; 25–50, modest heterogeneity; 50 high heterogeneity.
[a] Number of studies.
[b] P value for heterogeneity test.
[c] Random effect model was used when a P value < 0.05 for heterogeneity test; otherwise, fixed effect model was used.
[d] P value for each test.

find potential sources of heterogeneity. However, no source of heterogeneity was found. Based on the types of OA, ethnicity and study type, we still carry out subgroup analyses, because this made sense.

Sensitivity analysis

Sensitivity analysis was used to evaluate the sensitivity of each study on the pooled ORs, by successive culling of each individual study. The results showed that heterogeneity was reduced after some studies were eliminated: Ingrid et al. 2004 (Pb = 0.09, I^2 = 48.0%); Verena et al. 2000 (Pb = 0.19, I^2 = 33.0%).

Publication bias

The potential publication bias was assessed qualitatively by funnel plots. Taking the allele contrast model (2 vs L) as an example, the results of the funnel plots did not show any apparent asymmetry (Fig. 3). Moreover, the potential publication bias was also tested by the Begg's and Egger's tests. The p values were all greater than 0.05 (Egger's: P = 0.63; Begg's: P = 0.54), which represented no publication bias.

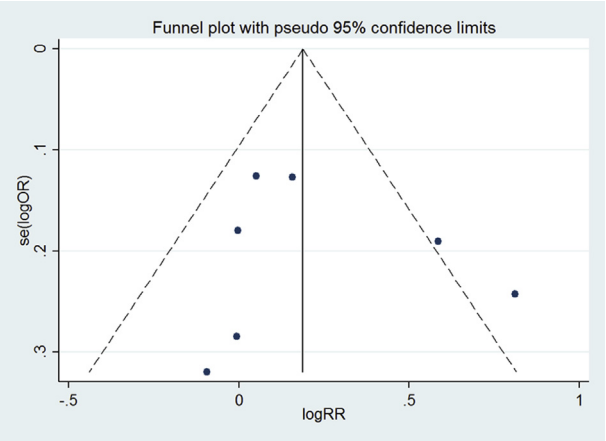

Fig. 3. Funnel plot for publication bias test (2 vs L).

Discussion

More and more attention has been paid to the studies of the relationship between genetic polymorphisms and OA. As far as we know, this is the first meta-analysis to evaluate the relationship between the IL1-RN VNTR polymorphism and OA risk. In this meta-analysis, we discovered an increased OA risk in the additive, recessive genetic model analysis and in the 2 allele vs L allele analysis. Furthermore, by subgroup analysis, we found that the relationship of IL-1-RN VNTR polymorphism and OA risk only exist among Caucasian and HCC groups, instead of Asian and PCC groups.

There are two main types of OA: (1) primary, the etiology is still uncertain, (2) secondary, which has the characteristics of early onset and clear etiology, such as the developmental abnormalities, trauma and so on.[16] Although many factors are believed to be related to OA, the pathogenesis of osteoarthritis is not fully understood.[17,18] Recent studies have shown that inflammatory processes play a very important role in the pathogenesis of OA. Pro-inflammatory cytokines are very important mediators in OA, and the major cytokines include TNF-α, IL1 and IL6.[19] IL1 is considered to be one of the strongest pro-inflammatory cytokines. When tissue is inflamed, IL1 seems to play an significant role in cell signal transmission.[20] IL1-RA is a natural antagonist of IL1, and its anti-inflammatory function is mediated by several different pathways.[21–23] The IL1-RN VNTR 2 allele is associated with an increase in production of IL1β in vitro. Meanwhile, the concentration of IL1RA was proved to be bound up with IL1β.[24] The IL1-RN VNTR 2 allele had a high circulating IL1-RA level and an even more elevated IL1β level. Binding of IL1-RA to the IL1 receptor inhibits IL1 mediated signaling, resulting in a strengthened and prolonged inflammatory response.[25] Meulenbelt et al. found that a predisposing effect for hip OA was associated with allele 2 of the IL1-RN VNTR polymorphisms.[13] Moos et al. investigated the distribution of polymorphic alleles of four different genes and found that the quantity of homozygous for allele 2 in OA cases was more than controls (9). Moreover, this meta-analysis identified carriage of the 2 allele as a risk factor for OA susceptibility (2 vs L: Pb = 0.02, I^2 = 58.8, OR(95% CI) = 1.20(1.05, 1.36), P = 0.007). In support of this, the IL1-RN VNTR contains three potential protein-binding sites: an acute phase response element, an α-interferon silencer A and a β-interferon silencer B. The 2 allele of IL1-RN VNTR only has 2

repeats. This may affect the length of mRNA and the subsequent protein synthesis and processing, which is likely to affect the production of IL1-RA.[26]

Considering the diversity of OA etiology, its pathogenesis is likely to be affected by factors such as age, gender, ethnicity, environmental factors, and other variables. Thus, a subgroup analysis based on ethnicity was carried out. Associations between OA susceptibility and IL1-RN VNTR were only found in individuals of Caucasian group, but not in Asian group, which was in accordance with studies of Sezgin et al[9] and Ni et al.[12] This may be due to genetic heterogeneity among different populations. Differences in life style and environmental factors in different populations may also interact with genetic variants, which can affect the pathogenesis of OA.[7] Meanwhile, another potential reason is that the number and sample size of the studies for Asian group were small. When grouped by study design, significant associations were found in HCC group. We found that the OA severity in HCC patients was more significant than PCC patients, which may partly provide the explanation for the above result.

There were some unavoidable limitations in this meta-analysis. First of all, heterogeneity still existed between studies of the IL-RN VNTR polymorphism, although the potential sources of heterogeneity failed to be found by meta-regression analysis, which may cause a misunderstanding to this meta analysis. Secondly, the total sample size of the eligible studies is not large enough, and it is not enough to summarize a convincing conclusion. Especially when stratified analysis of OA type, ethnicity or study design are conducted, this problem will be more obvious. Next, the conclusion of the meta-analysis was based on unadjusted estimates. In recent years, a number of risk factors come to light, such as advance age, obesity, previous damage, smoking habit and using of hormone. If these data can be obtained, then a more accurate analysis can be made. In the end, the quality scores of certain studies were not high, which can increase the risk of misinterpretation to the meta-analysis.

Conclusions

Taken together, this meta-analysis shown that the IL1-RN VNTR 2 allele and the 22 genotype may increase the susceptibility to OA, especially in individuals of Caucasian and HCC populations. However, due to considerable heterogeneity, this conclusion should be interpreted with caution. Moreover, more relevant studies are needed to explore the functions of these alleles. We need more well-designed studies among different races, which can provide more detailed information, such as age, sex and age of onset, to validate our findings.

Conflicts of interest

All authors declare that they have no competing interests.

Funding

This work was supported by the National Natural Science Foundation of China (No.81573993, No.81774334). Jiangsu Provincial Bureau of traditional Chinese Medicine program (No. YB2017023, No. 2015NL-068-02), Jiangsu provincial health and Family Planning Commission program (No. BJ15019), Jiangsu Key R & D project (No. BE2017774), Postgraduate Research and Practice Innovation Program of Jiangsu Province (No. KYCX19-1193).

Acknowledgements

Not applicable.

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