Gene locus polymorphisms and expression levels of interleukin-1 in lumbar disc disease: a meta-analysis and immunohistochemical study

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Research article

Keywords: gene expression, interleukin-1, lumbar disc disease, meta-analysis, polymorphism

Posted Date: October 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-18060/v3

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Abstract

Background: To investigate the association between interleukin (IL)-1α (rs1800587), IL-1β (rs1143634) and IL-1 receptor antagonist (RN) variable number tandem repeat (VNTR) polymorphisms, expression levels and lumbar disc disease (LDD).

Methods: All relevant articles were searched from 4 databases including PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI). Odds ratios (OR) with 95% confidence intervals (CI) were calculated to evaluate the association between IL-1 gene locus polymorphisms (rs1800587 in IL-1α, rs1143634 in IL-1β, VNTR in IL-1RN) and LDD susceptibility. Statistical analysis was conducted by Review Manager (Revman) 5.3.1 software. Furthermore, qRT-PCR and immunohistochemistry (IHC) were performed to evaluate IL-1α, IL-1β and IL-1RN expressions in the normal and degenerated disc.

Results: A total of 15 case-control studies (1455 cases and 2362 controls) were included in our meta-analysis. The pooled results suggested that IL-1α rs1800587 polymorphism was associated with an increased risk of LDD in overall population (T vs. C, OR=1.21, 95%CI=1.04-1.40, P=0.01). The subgroup analysis found a significant association between IL-1β rs1143634 polymorphism and LDD in Asian population (T vs. C, OR=0.61, 95%CI=0.39-0.96, P=0.03). Results of qRT-PCR and IHC demonstrated that expressions of IL-1α and IL-1-β were significantly increased in the degenerated disc. (all P<0.05)

Conclusion: IL-1α rs1800587 and IL-1β rs1143634 polymorphisms were significantly associated with LDD in overall population and in Asian population, respectively.

Introduction

Low back pain (LBP) is a most common musculoskeletal disorder. The annual prevalence of LBP ranges from 15% to 45%, and 70-85% of all people have LBP at some time in life[1]. The consequences of LBP and related disability are substantial, affecting individuals, families, and health-care systems[2]. It is accepted that lumbar disc degeneration (LDD) is a main risk factor for LBP[3]. Although the pathogenesis of LDD is not fully understood, immune system has been proved to play an important role in development of LDD[4-6]. Interleukin (IL)-1 cytokines are key mediators of immune responses and apoptosis of intervertebral disc cells[7,8]. Recently, increasing evidence showed that IL-1 gene cluster, including IL-1, IL-1β and IL-1 receptor antagonist (RN), could be responsible for the appearance or the severity of LDD[9,10].

To date, several single nucleotide polymorphisms (SNPs) have been identified in the IL-1 gene cluster. The most widely studied of these are IL-1α rs1800587, IL-1β rs1143634 polymorphisms and variable number repeat polymorphism (VNTR) of the IL-1RN gene.[11-25] In 2004, Solovieva et al. first reported that IL-1α rs1800587 and IL-1β rs1143634 polymorphisms were associated with LDD risk in a Caucasian population[11]. The association of the IL-1α rs1800587 polymorphism to LDD was subsequently confirmed in Finnish and Danish population studies[15,16]. However, Spanish, Chinese and Mexican cohort studies were unable to replicate this initial finding[12,18-20,23]. For IL-1β rs1143634 polymorphism, two studies showed a positive association[11,18], whereas seven demonstrated the null association[12,13,15,16,19,21,22]. A significant association between IL-1RN (VNTR) polymorphism and LDD risk was supported by Ye’s and Kim’s studies[12,16]. However, the studies reported by Noponen-Hietala et al.[12] showed no such association. As the conclusions of the available studies were not consistent, we conducted a meta-analysis to provide the comprehensive data on the association between IL-1 gene locus polymorphisms and LDD risk. Furthermore, we used quantitative reverse-transcription PCR (qRT-PCR) and immunohistochemistry (IHC) to evaluate IL-1α, IL-1β and IL-1RN expression levels in intervertebral disc between the LDD patients and the control subjects.

Methods

The meta-analysis conformed to the Preferred Reporting Items for Systematic Review and Meta-Analysis Protocols (PRISMA-P) guidelines, and registered in PROSPERO International Prospective Register of Systematic Reviews (CRD42019124118). This study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (2018-KY-NSFC-025). Written informed consent was obtained from all the participants involved in this study. All procedures were performed in accordance with the guidelines the institutional research committees and with the Declaration of Helsinki.

Search strategy

Four electronic databases, including PubMed, Embase, Web of Science and China National Knowledge Infrastructure (CNKI), were searched by two reviewers (HJ and QX). The following keyword search string was used to identify studies: (IL-1 OR IL 1 OR interleukin-1 OR interleukin 1 OR IL-1RN) AND (polymorphism OR variant OR mutation OR SNPs) AND (disc degeneration OR disc disease OR low back pain). Additional studies were identified through a hand search of references listed in the reports and reviews. No language or publication date restrictions were used. The final literature search was conducted on February 5, 2020.

Inclusion and exclusion criteria

Eligible studies were included in meta-analysis according to the following inclusion criteria: (1) case-control design; (2) LDD diagnosed on the basis of clinical or/radiologic examinations; (3) the study evaluated the association between IL-1α rs1800587 or IL-1β rs1143634 or IL-1RN (VNTR) polymorphisms and LDD risk; (4) genotype of control group conformed to the Hardy-Weinberg balance; and (5) sufficient data were provided to calculate the odds ratios (ORs) and 95% confidence intervals (CI). The exclusion criteria were as follows: (1) comments, reviews, or meta-analysis; (2) repeated publications; (3) study without...
available data. Based on the inclusion and exclusion criteria, eligible studies were identified independently by two reviewers (HJ and QX). The disagreements were resolved by a third reviewer (QW).

Data extraction

According to a standardized form, two investigators (HJ and QX) independently extracted data on outcomes for each study. The following information were collected from the included studies: (1) first author; (2) publication year; (3) country of enrollment; (4) ethnicity; (5) study design; (6) numbers of cases and controls; (7) characteristics of participants (gender and age); (8) diagnostic criteria; (9) source of control group; (10) allele or genotype frequencies; (11) P value for Hardy-Weinberg equilibrium (HWE) of control.

Methodological quality assessment

Methodological quality of studies was assessed using Critical Appraisal Skills Programme (CASP) [26]. For each question, there were three answers including “no”, “can’t tell” and “yes”, which respectively indicate scored 0, scored 1 and scored 2. The quality score ranged from 0 to 20, and a study with scored 15-20 represented a high-quality study.

Study population

Based on our previous study [27], we collected degenerative disc tissues (n=34) and normal disc tissues (n=21) from the LDD patients and the control subjects, respectively (Table S1). These disc samples were used to evaluate gene expressions via qRT-PCR and IHC. LDD patients were diagnosed as lumbar disc herniation by physical examination and MRI scan. The final diagnosis was verified by histopathology. The control subjects were the patients with traumatic lumbar vertebral fracture, who had no history of low back pain. According to Schneiderman's classification [28], MRI evaluation of the control subjects showed no significant disc damage and degeneration before surgery (Schneiderman's classification, Grade 1: 19 cases; Grade 2: 2 cases).

RNA extraction and qRT-PCR analysis

Nucleus pulposus tissues harvested from 55 subjects (34 cases and 21 controls) were lysed in TRIzol® (Invitrogen Inc, Carlsbad, CA, USA) and total RNA was extracted using an RNeasy® Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. The reverse transcriptions (RT) were performed using PrimeScript RT Master Mix kit (Takara, Japan), with 1 μg total RNA used for the synthesis of the complementary DNA (cDNA) via using iScripts cDNA Synthesis kit (Quanta Biosciences, MD, USA). SYBR Green real-time PCR kit (Quanta Biosciences, MD, USA) was used to measure the relative mRNA levels, and samples normalized for GAPDH expression. All reactions were run on a real-time PCR system (Applied Biosystems) and analyzed using the comparative Ct (ΔΔCt) method (2^{-ΔΔCt} with logarithm transformation). For profiling gene expressions, qRT-PCR was performed, using the primer pairs for IL-1α (5'-CCTCACCTTCCAGGAGAATGTG-3' and 5'-GCATCGCCCAGATTGTGATAAGAT-3'), IL-1β (5'-CTGTCCTGCGTGTTGAAAGAT-3' and 5'-TTCTGCTTGAGAGGTATG-3'), IL-1RN (5'-TTGTCCTGCTTTCTGTTCTG-3' and 5'-CTGTCCGTGTCAAGTCTGG-3'), and GAPDH (5'-GACATGCCGCGAGAAGAC-3' and 5'-AGCCCAGATGCCCTTGTAG-3').

Immunohistochemical assay

Nucleus pulposus tissues were obtained from case and control groups (34 cases and 21 controls). Immunohistochemical assay was performed using a standard protocol as previously reported[27]. The sections were treated with 1/200 IL-1α antibody (ab7632, Cambridge, MA, USA), 1/200 IL-1β antibody (#2022, Cell Signaling, Danvers, MA, USA), 1/400 IL-1RN antibody (ab123235, Abcam, Cambridge, MA, USA) overnight at 4 °C and incubated with 1/400 secondary biotinylated goat anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA) for 30 min, followed by treatment with VECTASTAIN Elite ABC Kit (Vector Laboratories, Burlingame, CA, USA). Based on stained slides, the number of positive cells was manually counted using Olympus BX43 upright microscope.

Statistical analysis

The association between IL-1α rs1800587, IL-1β rs1143634 and IL-1RN (VNTR) gene polymorphisms and LDD were estimated by odds ratio (OR) and 95% confidence intervals (CI). We evaluated the pooled ORs in five different genetic models. The heterogeneity of statistics was calculated by chi-square-based Q statistics and I² statistics. Heterogeneity was considered to be effective, when P was less than 0.10 and I² was greater than 50%. If there was significant heterogeneity (I²>50%), the random effect model was used. Otherwise, the fixed effect model was applied. Subgroup analysis of ethnicity was conducted to identify the source of heterogeneity. HWE was detected in control groups using the chi-square test. We performed the sensitivity test to assess the possible influence of one study on the pooled OR. In sensitivity test, studies were removed, in turn, from the overall analysis. Publication bias was tested by Begg’s funnel plot and Egger's test. The qRT-PCR and IHC assay were calculated by the mean±standard error. Statistical difference between two groups was
evaluated using unpaired Student t test. P<0.05 was considered to be statistically significant. Statistical analysis was performed by STATA 12.0 software (Stata, College Station, TX) and Revman 5.31 software (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark).

**Results**

**Characteristics of included studies**

A flow chart of article selection process is described in Figure 1. A total of 15 studies that met the inclusion criteria were identified in our meta-analysis, including 1455 cases and 2362 controls (IL-1α rs1800587: 10 studies, 922 cases and 1351 controls), (IL-1β rs1143634: 10 studies, 1056 cases and 1747 controls), and (IL-1RN VNTR: 3 studies, 290 cases and 507 controls). LDD was diagnosed by MRI scan in all the studies. The characteristics of included studies were shown in Table 1 and 2. All eligible studies were categorized as high quality, with scores >15.

**IL-1α rs1800587, IL-1β rs1143634 and IL-1RN (VNTR) polymorphisms and LDD susceptibility**

The meta-analysis of IL-1α rs1800587, IL-1β rs1143634 and IL-1RN (VNTR) polymorphisms are presented in Table 3. The pooled result showed rs1800587 was significantly associated with LDD risk in overall population (T vs. C, OR=1.21, 95%CI=1.04-1.40, P=0.01) (Figure 2). This significance was across the ethnicity. It was proposed that IL-1α rs1800587 T allele increased the susceptibility to LDD, and in contrast, IL-1α rs1800587 C allele was protective. However, IL-1β rs1143634, IL-1RN (VNTR) polymorphisms were not associated with LDD susceptibility in overall population (all P>0.05). After stratification by ethnicity, the result showed C allele of IL-1β rs1143634 may be a risk allele for LDD in Asian population (T vs. C, OR=0.61, 95%CI=0.39-0.96, P=0.03) (Figure 3).

Sensitivity analysis was performed to examine the impact of each study on the pooled ORs by removing each study in turn (Table 4). For rs1143634, when the study reported by Mu et al. was omitted in turn, the heterogeneity was obviously reduced under allele contrast genetic models [13, 19, 22]. For IL-1RN (VNTR), when we omitted the study reported by Ye et al. [14], the heterogeneity was significantly reduced under allele contrast genetic models. When the studies deviated from HWE were excluded, our results were robust and consistent. Funnel plots showed no significant evidence of publication bias (Figure 4a and 4b) (all P>0.05)

**IL-1α, IL-1β and IL-1RN expressions and LDD**

Hematoxylin & eosin stained sections were shown in Figure 5. In contrast to control group, IL-1α and IL-1β mRNA levels were increased 2.6 fold and 1.7 fold in LDD group, respectively (Figure 6d and 6h). IL-1RN mRNA levels were not significant difference between the two groups (Figure 6l) (all P>0.05). IHC analysis showed that significantly higher IL-1α and IL-1β expression levels in the LDD group than those in the control group (Figure 6a-c and Figure 6e-g) (IL-1α immunopositive cells: 43.8±4.9% vs. 22.5±2.8%, P<0.001; IL-1β immunopositive cells: 36.4±3.7% vs. 21.6±2.3%, P<0.001). However, there were no significant differences in IL-1RN expression between the two groups (Figure 6i-k) (IL-1RN immunopositive cells: 21.4±3.3% vs. 24.8±2.3%, P=0.09).

**Discussion**

Our meta-analysis 15 studies, involving 1455 cases and 2362 controls, found the statistically significant associations between IL-1α rs1800587, IL-1β rs1143634 polymorphisms and LDD. The pooled analysis indicated that T allele of rs1800587 was significant associated with increased risk of LDD in overall population. This significance was across the ethnicity. Subgroup analysis revealed that IL-1β rs1143634 polymorphism was associated with LDD in Asian population but not in Caucasian population. The C allele of rs1143634 was identified as a risk allele in the patients with LDD. Furthermore, the results of qRT-PCR and IHC analysis demonstrated that increased IL-1α and IL-1β expression levels were found in the degenerated disc. Wang et al. initially reported a meta-analysis on IL-1α rs1800587 and IL-1β rs1143634 polymorphisms and LDD, which suggested that IL-1α rs1800587 polymorphism was significantly associated with the risk of disc degeneration [29]. There were several weaknesses in the previous meta-analysis. First, some data extraction errors were found in meta-analysis, such as the allele and genotype frequencies from Solovieva's and Aparicio's studies [11, 18]. Second, the result of pooled analysis was limited by the marginal p values. This may lead to inflate the chance of a false positive association. More importantly, three genetic studies focusing on this topic were published in recent years [23-25], which were not included in Wang's study. Based on the Cochrane guidelines [30], an overlapping meta-analysis is necessary to be updated in time with latest studies. To the best of our knowledge, the current study is the largest sample size of meta-analysis to investigate the association between IL-1 gene locus polymorphisms and LDD.

IL-1 has extensively been studied among proinflammatory mediators, and is believed to play a critical role in the etiology of LDD [8, 31]. There are 3 major members in the IL-1 gene family: IL-1α, IL-1β, and IL-1RN. IL-1α and IL-1β have a strong influence on apoptosis of intervertebral disc cells, whereas IL-1RN suppresses the effect of IL-1 by competitively inhibiting the binding of IL-1 to the IL-1 receptor [32, 33]. The genetic control of the cytokine function may have an impact on the occurrence and the severity of LDD [8]. We performed an in silico analysis for evaluating the possible functional implication of IL-1α rs1800587, IL-1β rs1143634 and IL-1RN (VNTR) polymorphisms by using rSNPase (http://rsnp3.psych.ac.cn/). The results indicated that rs1800587 and rs1143634 were located within the promoter and exon 5 of IL-1 gene, which may affect the normal production, secretion or function of IL-1. The C to T polymorphism at position-889 of IL-1α gene (rs1800587) could increase gene expression at mRNA and at protein levels by enhanced promoter activity [34].
Regarding the IL-1β rs1143634 (T/C) polymorphism, earlier data suggested that a shift from T to C lead to a disruption of the TATA box in exon 5 sequences. The C allele conferred higher expression of the IL1β gene compared to the T allele [35, 36]. However, the relationship between IL-1α, IL-1β polymorphisms and IL-1α, IL-1β expressions in LDD patients has not been reported. The results of qRT-PCR and IHC analysis demonstrated that elevated IL-1α and IL-1β expression levels were found in the degenerated disc. Compared with other genotypes, TT genotype of rs1800587 and CC genotype of rs1143634 were associated with higher expression levels of IL-1α and IL-1β respectively. Thus we postulated that rs1800587 TT and rs1143634 CC genotypes were the genetic risk factors for the progression of LDD, probably by increasing the expression of IL-1α and IL-1β [37-39]. Mutations in the introns may affect gene expression and function via effects on mRNA splicing or RNA stability. IL-1RN (VNTR) gene polymorphism, located within a non-regulatory region (intron 2), could not be part of RNA-binding protein site. Thus the functional influence of IL-1RN (VNTR) polymorphism remains unclear. The molecular mechanisms of IL-1 gene locus polymorphisms and expressions in LDD are likely to be more complicated, which need be investigated in the future.

Some limitations in our study should be taken into consideration. First, the sample size is relatively small, which may exert an impact on the statistical power. There is much to be done to ensure the accuracy of this result. Second, we only acquired suitable studies published in English or Chinese. The potential publication bias could not be eliminated as the exclusion of unpublished articles, or articles published in another language. Third, the function information provided by qRT-PCR and IHC is limit to assess the exact mechanisms of disc degeneration. It is necessary to accumulate further evidence to clarify the impact of IL-1 gene locus polymorphisms on LDD.

**Conclusion**

IL-1α rs1800587 polymorphism was significantly associated with LDD in overall population, while IL-1β rs1143634 polymorphism was significantly associated with LDD in Asian population but not in Caucasian population. IL-1α rs1800587 T allele and IL-1β rs1143634 C allele could increase the susceptibility to LDD.

**Abbreviations**

IL-1: interleukin-1; IL-1RN: interleukin-1 receptor antagonist; IHC: immunohistochemistry; LBP: low back pain; LDD: lumbar disc degeneration; SNP: single nucleotide polymorphism; VNTR: variable number tandem repeat polymorphism.

**Declarations**

**Ethical Approval and Consent to participate**

The histology study was approved by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. All the experimental protocol and the methods were carried out in accordance with the relevant guidelines and regulations, and complied with the principles of the Declaration of Helsinki. Written informed consent was achieved from each participant.

**Consent for publication**

Not applicable.

**Availability of supporting data**

Please contact authors for data requests.

**Competing interests**

The authors declare that they have no competing interests.

**Funding**

This work was supported by the Natural Science Foundation of China (81860406/ 81460353/81560371), Guangxi Natural Science Foundation (2018GXNSFAA281127/ 2015GXNSFBA139167), and Medical Excellence Award Funded by the Creative Research Development Grant from the First Affiliated Hospital of Guangxi Medical University.

**Authors' contributions**
HJ and QJW conceived and designed the study. HJ and QX were involved in the data search and selection of data, analyzed the data and wrote the manuscript. RZ was involved in the data search and selection of data and analyzed the data. JW and DM were involved in the data search and analyzed the data. QX and RZ were involved in the data search and selection of data. XZ analyzed the data and contributed analysis tools. HJ was involved in IHC study. All authors read and approved the final manuscript.

Acknowledgements

The authors thank all the participants in this study.

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**Tables**
Table 1. Characteristics of the case-control studies included in meta-analysis

| First author      | Year | Country   | Ethnicity | Age (year, mean±sd) | Number cases/controls | Diagnostic criteria | Control group                  | Outcome measure (SNP genotyping) |
|-------------------|------|-----------|-----------|---------------------|-----------------------|---------------------|--------------------------------|----------------------------------|
| Solovieva [9]     | 2004 | Finland   | Caucasian | 41–45               | 38/95                 | MRI                 | volunteers without LDD          | rs1800587, rs114                  |
| Noponen-Hietala [10] | 2005 | Finland   | Caucasian | 44±13               | 155/179               | sciatica, MRI       | NM                             | rs1800587, rs114 IL-1RN (VNTR)   |
| Ye [11,12]        | 2007 | China     | Asian     | 42.7                | 81/101                | sciatica, MRI       | volunteers without sciatica     | rs1143634, IL-1R (VNTR)          |
| Karppinen [13]    | 2009 | Finland   | Caucasian | 44                  | 45/63                 | MRI                 | volunteers without modic changes| rs1800587, rs114                  |
| Eskola [14]       | 2010 | Denmark   | Caucasian | 13.1±0.4            | 66/154                | MRI                 | volunteers without LDD          | rs1800587, rs114                  |
| Kim [15]          | 2010 | Korea     | Asian     | 49.7±15.4           | 54/227                | symptoms, MRI       | volunteers without LDD          | IL-1RN (VNTR)                     |
| Aparicio [16]     | 2011 | Spain     | Caucasian | 43.9±11.9           | 50/129                | symptoms, MRI       | patients without LDD            | rs1800587, rs114                  |
| Kelempisioti [17] | 2011 | Finland   | Caucasian | 19                  | 150/246               | MRI                 | volunteers without LDD          | rs1800587, rs114                  |
| Duan [18]         | 2013 | China     | Asian     | 46.5±7.3            | 42/85                 | symptoms, MRI       | patients without LDD            | rs1800587                         |
| Loncar [19]       | 2013 | China     | Asian     | 48.35±5.14          | 93/96                 | symptoms, MRI       | veterans without LBP            | rs1143634                         |
| Mu [20]           | 2013 | Croatia   | Caucasian | 21.94±1.60          | 305/587               | symptoms, MRI       | soldiers without LBP            | rs1143634                         |
| Serrano [21]      | 2014 | Mexico    | Caucasian | 39.22±6.88          | 100/100               | MRI                 | volunteers without LDD          | rs1800587                         |
| Abdollahzade [22] | 2018 | Iran      | Asian     | 39.1 ±10.6          | 76/100                | symptoms, MRI       | volunteers without LDD          | rs1800587, rs114                  |
| Chen [23]         | 2018 | China     | Asian     | 42.51±4.42          | 200/200               | MRI, pathological analyses | volunteers without LDD          | rs1800587                         |

CASP: Critical Appraisal Skills Programme; CT: computerized tomography; LBP: low back pain; LDD: lumbar disc degeneration; NM: not mentioned; MRI, magnetic resonance imaging
Table 2. Genotype frequency of IL-1 gene locus polymorphisms in case-control studies

| Authors            | Year | Country | Ethnicity | Genotype | Case | Control | P-value for HWE of control |
|--------------------|------|---------|-----------|----------|------|---------|---------------------------|
| IL-1α (rs1800587) |      |         |           |          |      |         |                           |
| Solovieva [9]      | 2004 | Finland | Caucasian | 11 12 22 | 1 2  | 11 12 22 | 1 2 | 0.07                     |
| Noponen-Hietala [10]| 2005 | Finland | Caucasian | 62 72 21 | 196 114 85 | 77 17 247 | 111 | 0.94                     |
| Karppinen [13]     | 2009 | Finland | Caucasian | 12 26 7 50 | 40 30 28 5 88 | 38 | 0.66                     |
| Eskola [14]        | 2010 | Denmark | Caucasian | 23 35 8 81 | 51 72 65 17 209 | 99 | 0.69                     |
| Aparicio [16]      | 2011 | Spain   | Caucasian | 22 25 3 69 | 31 63 61 5 187 | 71 | 0.04                     |
| Kelempisioti [17]  | 2011 | Finland | Caucasian | 64 67 19 | 195 105 106 114 26 326 | 166 | 0.57                     |
| Duan [18]          | 2013 | China   | Asian     | 22 17 3 61 | 23 47 33 5 127 | 43 | 0.80                     |
| Serrano [21]       | 2014 | Mexico  | Caucasian | 51 45 4 147 | 53 55 35 10 145 | 55 | 0.22                     |
| Abdollahzade [22]  | 2018 | Iran    | Asian     | 33 33 10 99 | 53 62 62 12 185 | 86 | 0.53                     |
| Chen [23]          | 2018 | China   | Asian     | 87 78 28 252 | 134 102 81 14 285 | 109 | 0.70                     |
| IL-1β (rs1143634)  |      |         |           |          |      |         |                           |
| Solovieva [9]      | 2004 | Finland | Caucasian | 19 12 4 50 | 20 42 42 9 126 | 60 | 0.75                     |
| Noponen-Hietala [10]| 2005 | Finland | Caucasian | 81 62 12 | 224 86 101 67 11 269 | 89 | 0.98                     |
| Ye [11]            | 2007 | China   | Asian     | 77 4 0 158 | 4 89 12 0 190 | 12 | 0.53                     |
| Karppinen [13]     | 2009 | Finland | Caucasian | 20 20 5 60 | 30 32 24 6 88 | 36 | 0.63                     |
| Eskola [14]        | 2010 | Denmark | Caucasian | 31 29 6 91 | 41 82 53 19 217 | 91 | 0.03                     |
| Aparicio [16]      | 2011 | Spain   | Caucasian | 4 16 30 24 | 76 3 50 76 56 202 | 0.11 |
| Kelempisioti [17]  | 2011 | Finland | Caucasian | 82 53 15 | 217 83 140 91 15 371 | 121 | 0.97                     |
| Loncar [19]        | 2013 | China   | Asian     | 283 22 0 145 | 41 525 61 1 147 | 45 | 0.57                     |
| Mu [20]            | 2013 | Croatia | Caucasian | 55 35 3 588 | 22 55 37 4 1111 | 63 | 0.47                     |
| Solovieva [9]      | 2018 | Iran    | Asian     | 39 33 3 111 | 39 70 58 12 192 | 82 | 0.99                     |
| IL-1RN VNTR        |      |         |           |          |      |         |                           |
| Noponen-Hietala [10]| 2005 | Finland | Caucasian | 77 65 13 | 219 91 86 76 17 248 | 110 | 0.97                     |
| Ye [12]            | 2007 | China   | Asian     | 55 25 1 135 | 27 83 17 1 183 | 19 | 0.90                     |
| Kim [15]           | 2010 | Korea   | Asian     | 38 4 0 80 | 4 193 24 1 410 | 26 | 0.78                     |

11, 12 and 22 respectively represent CC, CT and TT for rs1800587, rs1143634; A1A1, A1A2 and A2A2 for IL-1RN VNTR; HWE: Hardy-Weinberg's equilibrium
### Table 3. Association test and heterogeneity test of IL-1 gene locus polymorphisms

| SNPs       | Allele contrast | N  | Test of association | Test of heterogeneity |
|------------|-----------------|----|---------------------|-----------------------|
|            |                 |    | OR                  | 95%CI                 | P-value   | Statistical Model | P-value | I² (%) |
| IL-1α (rs1800587) |                 |    |                     |                       |           |                   |         |
| Overall    |                 | 10 |                     |                       |           |                   |         |
| T vs C     | 1.23            | 1.08,1.40 | 0.001 | FEM | 0.79 | 0 |
| CT vs CC   | 1.22            | 1.02,1.46 | 0.03  | FEM | 0.82 | 0 |
| TT vs CC   | 1.60            | 1.19,2.14 | 0.002 | FEM | 0.51 | 0 |
| CT/TT vs CC| 1.28            | 1.08,1.52 | 0.004 | FEM | 0.82 | 0 |
| TT vs CC/CT| 1.42            | 1.08,1.88 | 0.01  | FEM | 0.55 | 0 |
| Caucasian  |                 | 6  |                     |                       |           |                   |         |
| T vs C     | 1.26            | 1.06,1.48 | 0.007 | FEM | 0.64 | 0 |
| CT vs CC   | 1.29            | 1.03,1.60 | 0.02  | FEM | 0.64 | 0 |
| TT vs CC   | 1.46            | 1.02,2.08 | 0.04  | FEM | 0.33 | 13 |
| CT/TT vs CC| 1.30            | 1.06,1.61 | 0.01  | FEM | 0.59 | 0 |
| TT vs CC/CT| 1.25            | 0.89,1.76 | 0.19  | FEM | 0.45 | 0 |
| Asian      |                 | 3  |                     |                       |           |                   |         |
| T vs C     | 1.28            | 1.02,1.60 | 0.04  | FEM | 0.70 | 0 |
| CT vs CC   | 1.09            | 0.79,1.49 | 0.60  | FEM | 0.95 | 0 |
| TT vs CC   | 1.93            | 1.15,3.26 | 0.01  | FEM | 0.68 | 0 |
| CT/TT vs CC| 1.24            | 0.92,1.67 | 0.15  | FEM | 0.79 | 0 |
| TT vs CC/CT| 1.86            | 1.13,3.08 | 0.01  | FEM | 0.70 | 0 |
| IL-1β (rs1143634) |             |    |                     |                       |           |                   |         |
| Overall    |                 | 9  |                     |                       |           |                   |         |
| T vs C     | 0.99            | 0.85,1.16 | 0.94  | FEM | 0.44 | 0 |
| CT vs CC   | 0.93            | 0.76,1.14 | 0.48  | FEM | 0.20 | 28 |
| TT vs CC   | 1.10            | 0.74,1.64 | 0.63  | FEM | 0.66 | 0 |
| CT/TT vs CC| 0.95            | 0.78,1.16 | 0.63  | FEM | 0.21 | 26 |
| TT vs CC/CT| 1.13            | 0.80,1.60 | 0.48  | FEM | 0.92 | 0 |
| Caucasian  |                 | 7  |                     |                       |           |                   |         |
| T vs C     | 1.07            | 0.91,1.26 | 0.43  | FEM | 0.90 | 0 |
| CT vs CC   | 1.04            | 0.83,1.30 | 0.75  | FEM | 0.38 | 6 |
| TT vs CC   | 1.11            | 0.74,1.67 | 0.60  | FEM | 0.56 | 4 |
| CT/TT vs CC| 1.09            | 0.88,1.36 | 0.42  | FEM | 0.81 | 0 |
| TT vs CC/CT| 1.14            | 0.81,1.61 | 0.45  | FEM | 0.87 | 0 |
| Asian      |                 | 2  |                     |                       |           |                   |         |
| T vs C     | 0.61            | 0.39,0.96 | 0.03  | FEM | 0.44 | 0 |
| CT vs CC   | 0.61            | 0.38,0.97 | 0.04  | FEM | 0.40 | 0 |
| TT vs CC   | 0.62            | 0.03,1.52 | 0.77  | FEM | NA   | NA |
| CT/TT vs CC| 0.60            | 0.38,0.95 | 0.03  | FEM | 0.39 | 0 |
| TT vs CC/CT| 0.64            | 0.03,1.57 | 0.78  | FEM | NA   | NA |
| IL-1RN (VNTR) |                |    |                     |                       |           |                   |         |
| Overall    |                 | 3  |                     |                       |           |                   |         |
| 2 vs 1     | 1.15            | 0.68,1.94 | 0.59  | REM | 0.12 | 53 |
| Study                | Test of heterogeneity | $\text{OR (95\%CI)}$ | $P$-value | $I^2$(%) |
|----------------------|-----------------------|------------------------|-----------|----------|
| **IL-1$\alpha$ (rs1800587) T/C** |                       |                        |           |          |
| Solovieva [9]        |                       | 1.23[1.07,1.40]        | 0.72      | 0        |
| Noponen-Hietala [10] |                       | 1.22[1.06,1.40]        | 0.72      | 0        |
| Karppinen [13]       |                       | 1.21[1.06,1.37]        | 0.91      | 0        |
| Eskola [14]          |                       | 1.22[1.07,1.40]        | 0.72      | 0        |
| Aparicio [16]        |                       | 1.24[1.08,1.41]        | 0.71      | 0        |
| Kelempisioti [17]    |                       | 1.27[1.11,1.47]        | 0.83      | 0        |
| Duan [18]            |                       | 1.24[1.09,1.41]        | 0.72      | 0        |
| Serrano [21]         |                       | 1.26[1.10,1.44]        | 0.85      | 0        |
| Abdollahzade [22]    |                       | 1.24[1.08,1.42]        | 0.71      | 0        |
| Chen [23]            |                       | 1.20[1.04,1.38]        | 0.78      | 0        |
| **IL-1$\beta$ (rs1143634) T/C** |                       |                        |           |          |
| Solovieva [9]        |                       | 1.01[0.86,1.18]        | 0.37      | 8        |
| Noponen-Hietala [10] |                       | 0.96[0.80,1.14]        | 0.42      | 1        |
| Ye [11]              |                       | 1.01[0.87,1.19]        | 0.60      | 0        |
| Karppinen [13]       |                       | 0.98[0.83,1.15]        | 0.38      | 6        |
| Eskola [14]          |                       | 0.98[0.83,1.16]        | 0.35      | 11       |
| Aparicio [16]        |                       | 1.01[0.86,1.18]        | 0.36      | 9        |
| Kelempisioti [17]    |                       | 0.95[0.79,1.13]        | 0.45      | 0        |
| Loncar [19]          |                       | 1.00[0.85,1.18]        | 0.35      | 11       |
| Mu [20]              |                       | 1.04[0.89,1.23]        | 0.67      | 0        |
| **IL1-RN (VNTR) A_2/A_1** |                       |                        |           |          |
| Noponen-Hietala [10] |                       | 1.38[0.59,3.22]        | 0.16      | 49       |
| Ye [12]              |                       | 0.92[0.67,1.27]        | 0.76      | 0        |
| Kim [15]             |                       | 1.28[0.63,2.57]        | 0.05      | 75       |

OR: odds ratio; 95%CI: confidence interval

Table 4. Sensitivity analysis with each study eliminated for IL-1$\alpha$ (rs1800587), IL-1$\beta$ (rs1143634) and IL-1RN (VNTR) polymorphisms

SNPs: single nucleotide polymorphisms; N: number of studies included; FEM: fixed effect model; REM: random effect model; OR: odds ratio; 95%CI: 95% confidence interval; NA: not applicable
Figure 1

Literature search strategy and selection of articles. A total of 113 articles were selected for the meta-analysis by searching PubMed, Embase, Web of Science and CNKI, of which 79 articles were excluded after reviewing the title and abstract, 19 articles were excluded after reviewing the full publications. Finally, a total of 15 studies were included in the meta-analysis.
Figure 2

Forest plot of association between IL-1α rs1800587 polymorphism and LDD risk under allelic contrast model (T vs. C). There was a significant association between rs1800795 and LDD risk in overall population (T vs. C, OR: 1.23, 95% CI: 1.08-1.40, P=0.001). OR, odds ratio; CI=confidence interval.
Figure 3

Forest plot of association between IL-1β rs1143634 polymorphism and LDD risk under allelic contract model (T vs. C). There was a significant association between rs1800797 and LDD risk in Asian population (T vs. C, OR: 0.61, 95%CI: 0.39-0.96, P=0.03), but not in Caucasian population (T vs. C, OR: 1.07, 95%CI: 0.91-1.26, P=0.43). OR, odds ratio; CI=confidence interval
Figure 4
Funnel plot analysis for publication bias in selection of studies on IL-1α rs1800587 (a) and IL-1β rs1143634 (b) polymorphisms under allelic contract model. There was no significant publication bias (P>0.05).

Figure 5
Hematoxylin & eosin stained sections from the control group (a) and the LDD group (b). (Scale bar=20 μm)
Figure 6

The expression levels of IL-1α, IL-1β and IL-1RN in the LDD group and the control group. The positive signals were identified in the cytoplasm of nucleus pulpos cells in the LDD group (b, f, j) and the control group (a, e, i). (Scale bar=20 μm) Bar charts (c, g, k) show the expression levels of IL-1α, IL-1β and IL-1RN in the two groups. Bar charts (e, h, l) show the mRNA levels of IL-1α, IL-1β and IL-1RN in the two groups. Results are mean ± SD.

Supplementary Files

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- TableS1.pdf