Meta-Analysis of Observational Studies in Epidemiology

Medicine

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Gene locus polymorphisms and expression levels of interleukin-1 in lumbar disc disease

A MOOSE-compliant meta-analysis and immunohistochemical study

Kunxue Yang, MD[a], Qianyi Xiao, MS[a], Ruijun Zhang, MS[a], Dihua Meng, MD[a], Jiaqi Wang, MD[a], Qingjun Wei, MD[b], Hua Jiang, MD, PhD[a]

Abstract

Objective: To investigate the association between interleukin (IL)-1α (rs1800587), IL-1β (rs1143634) and IL-1 receptor antagonist (RN) variable number tandem repeat 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. 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, variable number tandem repeat in interleukin-1 receptor antagonist) and LDD susceptibility. Statistical analysis was conducted by Review Manager (Revman) 5.31 software (Nordic Cochrane Centre, Cochrane Collaboration, Copenhagen, Denmark). Furthermore, qRT-PCR and immunohistochemistry were performed to evaluate IL-1α, IL-1β and interleukin-1 receptor antagonist 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* = .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* = .03). Results of qRT-PCR and immunohistochemistry demonstrated that expressions of IL-1α and IL-1β were significantly increased in the degenerated disc. (all *P* < .05)

Conclusion: IL-1α rs1800587 and IL-1β rs1143634 polymorphisms were significantly associated with LDD in overall population and in Asian population, respectively. The increased expression levels of IL-1α and IL-1β may be the important risk factors for LDD.

Abbreviations: CI = confidence intervals, HWE = Hardy-Weinberg Equilibrium, IHC = immunohistochemistry, IL-1 = interleukin-1, IL-1RN = interleukin-1 receptor antagonist, LBP = low back pain, LDD = lumbar disc degeneration, ORs = odds ratios, RoB = risk of bias, SNP = single nucleotide polymorphism, VNTR = variable number tandem repeat polymorphism.

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

1. Introduction

Low back pain (LBP) is a most common musculoskeletal disorder. The annual prevalence of LBP ranges from 15% to 45%, and 70% to 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

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The authors have no conflicts of interest to disclose.

The datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

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 involved in qRT-PCR and immunohistochemical studies.

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[a] *Department of Spine Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, China,* [b] *Department of Orthopedics Surgery, The First Affiliated Hospital of Guangxi Medical University, Nanning, China.*

** Correspondence: Hua Jiang, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning 530021, China (e-mail: jianghua@gxmu.edu.cn, drjianghua@163.com).*

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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 interleukin-1 (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 tandem repeat polymorphism (VNTR) of the interleukin-1 receptor antagonist (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, 2 studies showed a positive association,[11,18] whereas 7 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.[14,17] 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 of case-control studies to provide the comprehensive data on the association between IL-1 gene locus polymorphisms and LDD risk in Asian and Caucasian populations. 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.

2. Methods

The meta-analysis conformed to the Meta-Analysis of Observational Studies in Epidemiology 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 the participants involved in qRT-PCR and immunohistochemical studies. All procedures were performed in accordance with the guidelines the institutional research committees and with the Declaration of Helsinki.

2.1. Search strategy

Four electronic databases, including PubMed, Embase, Web of Science and China National Knowledge Infrastructure, were searched by 2 reviewers (KY 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 LBP). Additional studies were identified through a hand search of references listed in the reports and reviews. If necessary, we may contact the corresponding authors to request detail information or unpublished data. The articles published in non-English languages should be translated to English. The final literature search was conducted on December 18, 2021.

2.2. Inclusion and exclusion criteria

Eligible studies were included in meta-analysis according to the following inclusion criteria: case-control design; LDD diagnosed on the basis of clinical or/and radiologic examinations; the study evaluated the association between IL-1α rs1800587 or IL-1β rs1143634 or IL-1RN (VNTR) polymorphisms and LDD risk; genotype of control group conformed to the Hardy-Weinberg balance; and sufficient data were provided to calculate the odds ratios (ORs) and 95% confidence intervals (CI). The exclusion criteria were as follows: study without available or sufficient genotype data; studies whose allele and genotype frequencies of control group deviated from Hardy-Weinberg Equilibrium (HWE) ($P < .05$); comments, reviews, case reports, and letter to editor. For repeated publications, only the most complete or recent 1 was included. Based on the inclusion and exclusion criteria, eligible studies were identified independently by 2 reviewers (KY and QX). The disagreements were resolved by a third reviewer (HJ).

2.3. Data extraction

According to a standardized form, 2 investigators (KY and QX) independently extracted data on outcomes for each study. The following information were collected from the included studies: first author; publication year; country of enrollment; ethnicity; study design; numbers of cases and controls; characteristics of participants (gender and age); diagnostic criteria; source of control group; allele or/and genotype frequencies; *P* value for HWE of control.

2.4. Methodological quality assessment

Methodological quality of studies was assessed using Critical Appraisal Skills Programme (CASP).[26] For each question, there were 3 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 to 20 represented a high-quality study.

2.5. Study population

Based on our previous study,[27–29] we collected degenerative disc tissues (n = 34) and normal disc tissues (n = 21) from the LDD patients and the control subjects, respectively (Table S1, Supplemental Digital Content, http://links.lww.com/MD/H614). 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 LBP. According to Schneiderman's classification,[30] 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).

2.6. 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 ($\Delta\Delta$Ct) method ($2^{-\Delta\Delta Ct}$ with logarithm transformation). For profiling gene expressions, qRT-PCR was performed, using the primer pairs for IL-1α (5'-CCTCACCTTCCAGGAGAATGTG- 3'

and 5'-GCATCGCCCAGATTTTGTAG TG-3'), IL-1β (5'-CTGTCCTGCGTGTT GAAAGAT-3' and 5'-TTCTGCTTGAGAG GTGCTGAT-3'), IL-1RN (5'-TTGTCCT GCTTTCTGTTCTCG-3' and 5'-CTGTCC TGTGTCAAGTCTGG-3'), and GAPDH (5'-GACATGCCGCCTGGAGAAAC-3' and 5'-AGCCCAGGATGCCCTTTAGT-3').

2.7. 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.[27–29] 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.

2.8. 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 5 different genetic models. The heterogeneity of statistics was calculated by chi-square-based Q statistics and I^2 statistics. Heterogeneity was considered to be effective, when $P < .10$ and I^2 was greater than 50%. If there was significant heterogeneity ($I^2 > 50\%$), 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 1 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 2 groups was evaluated using unpaired Student t test. $P < .05$ 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).

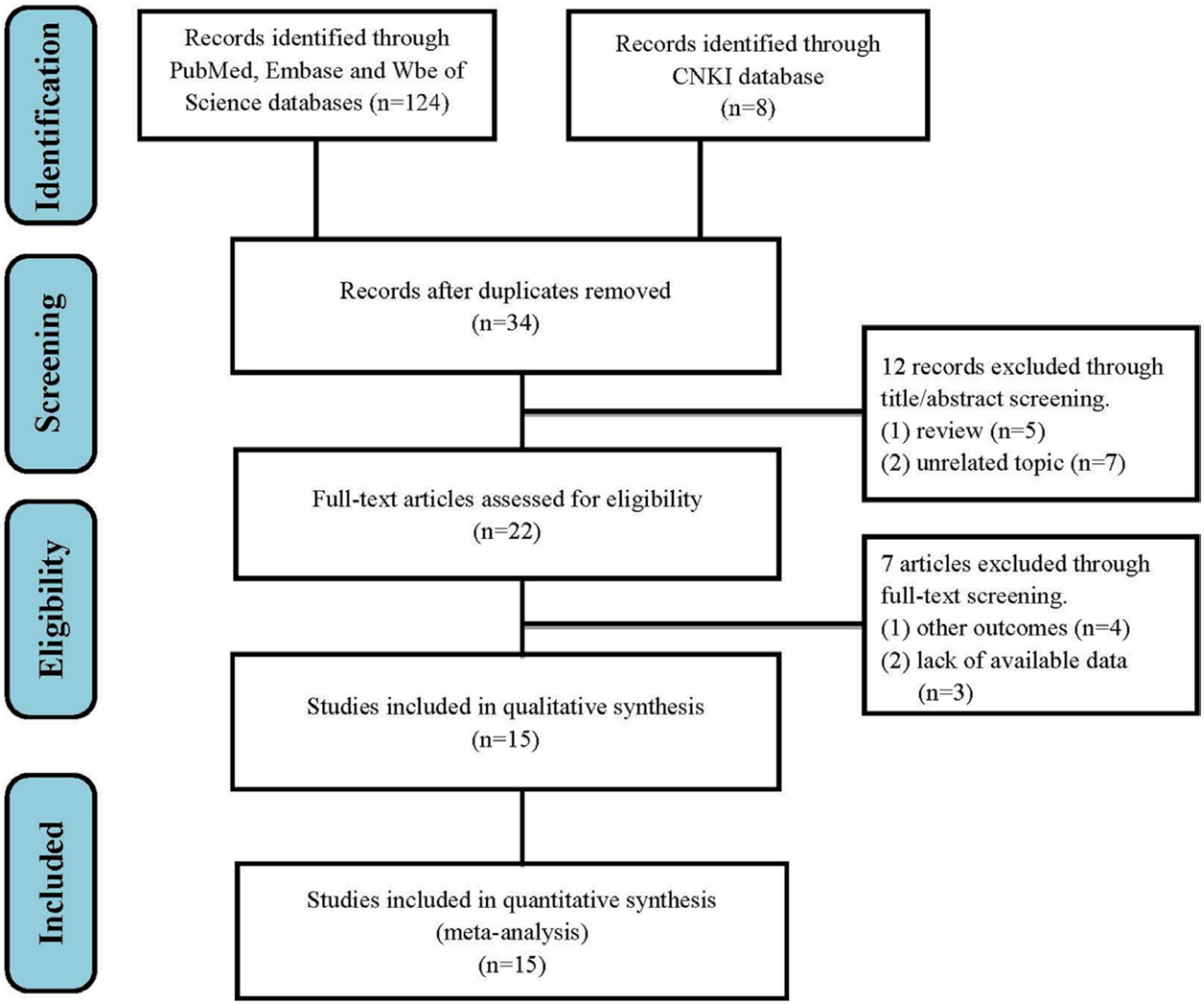

Figure 1. Literature search strategy and selection of articles. A total of 113 articles were selected for the meta-analysis by searching PubMed, Embase, Web of Science and CNKI, of which 79 articles were excluded after reviewing the title and abstract, 19 articles were excluded after reviewing the full publications. Finally, a total of 15 studies were included in the meta-analysis. CNKI = China National Knowledge Infrastructure.

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)	CASP
				Cases	Controls					
Solovieva[9]	2004	Finland	Caucasian	41–45	41–45	38/95	MRI	volunteers without LDD	rs1800587, rs1143634	17
Noponen-Hietala[10]	2005	Finland	Caucasian	44 ± 13	39 ± 13	155/179	sciatica, MRI	NM	rs1800587, rs1143634,IL-1RN (VNTR)	18
Ye[11,12]	2007	China	Asian	42.7	38.4	81/101	sciatica, MRI	volunteers without sciatica	rs1143634,IL-1RN (VNTR)	18
Karppinen[13]	2009	Finland	Caucasian	44	44	45/63	MRI	volunteers without modic changes	rs1800587, rs1143634	16
Eskola[14]	2010	Denmark	Caucasian	13.1 ± 0.4	13.1 ± 0.4	66/154	MRI	volunteers without LDD	rs1800587, rs1143634	16
Kim[15]	2010	Korea	Asian	49.7 ± 15.4	51.7 ± 9.9	54/227	symptoms, MRI	volunteers without LDD	IL-1RN (VNTR)	16
Aparicio[16]	2011	Spain	Caucasian	43.9 ± 11.9	68.7 ± 9.2	50/129	symptoms, MRI	patients without LDD	rs1800587, rs1143634	17
Kelempisioti[17]	2011	Finland	Caucasian	19	19	150/246	MRI	volunteers without LDD	rs1800587, rs1143634	18
Duan[18]	2013	China	Asian	46.5 ± 7.3	48.3 ± 7.7	42/85	symptoms, MRI	patients without LDD	rs1800587	15
Loncar[19]	2013	China	Asian	48.35 ± 5.14	41.44 ± 5.20	93/96	symptoms, MRI	veterans without LBP	rs1143634	17
Mu[20]	2013	Croatia	Caucasian	21.94 ± 1.60	22.09 ± 1.68	305/587	symptoms, MRI	soldiers without LBP	rs1143634	18
Serrano[21]	2014	Mexico	Caucasian	39.22 ± 6.88	39.13 ± 6.80	100/100	MRI	volunteers without LDD	rs1800587	17
Abdollahzade[22]	2018	Iran	Asian	39.1 ± 10.6	NM	76/100	symptoms, MRI	volunteers without LDD	rs1800587, rs1143634	17
Chen[23]	2018	China	Asian	42.51 ± 4.42	41.93 ± 4.03	200/200	MRI, pathological analyses	volunteers without LDD	rs1800587	16

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										*P*-value for HWE of control
				Case					Control					
				11	12	22	1	2	11	12	22	1	2	
IL-1α (rs1800587)														
Solovieva[9]	2004	Finland	Caucasian	11	21	6	43	33	34	51	8	119	67	.07
Noponen-Hietala[10]	2005	Finland	Caucasian	62	72	21	196	114	85	77	17	247	111	.94
Karppinen[13]	2009	Finland	Caucasian	12	26	7	50	40	30	28	5	88	38	.66
Eskola[14]	2010	Denmark	Caucasian	23	35	8	81	51	72	65	17	209	99	.69
Aparicio[16]	2011	Spain	Caucasian	22	25	3	69	31	63	61	5	187	71	.04
Kelempisioti[17]	2011	Finland	Caucasian	64	67	19	195	105	106	114	26	326	166	.57
Duan[18]	2013	China	Asian	22	17	3	61	23	47	33	5	127	43	.80
Serrano[21]	2014	Mexico	Caucasian	51	45	4	147	53	55	35	10	145	55	.22
Abdollahzade[22]	2018	Iran	Asian	33	33	10	99	53	62	62	12	185	86	.53
Chen[23]	2018	China	Asian	87	78	28	252	134	102	81	14	285	109	.70
IL-1β (rs1143634)														
Solovieva[9]	2004	Finland	Caucasian	19	12	4	50	20	42	42	9	126	60	.75
Noponen-Hietala[10]	2005	Finland	Caucasian	81	62	12	224	86	101	67	11	269	89	.98
Ye[11]	2007	China	Asian	77	4	0	158	4	89	12	0	190	12	.53
Karppinen[13]	2009	Finland	Caucasian	20	20	5	60	30	32	24	6	88	36	.63
Eskola[14]	2010	Denmark	Caucasian	31	29	6	91	41	82	53	19	217	91	.03
Aparicio[16]	2011	Spain	Caucasian	4	16	30	24	76	3	50	76	56	202	.11
Kelempisioti[17]	2011	Finland	Caucasian	82	53	15	217	83	140	91	15	371	121	.97
Loncar[19]	2013	China	Asian	283	22	0	145	41	525	61	1	147	45	.57
Mu[20]	2013	Croatia	Caucasian	55	35	3	588	22	55	37	4	1111	63	.47
Solovieva[9]	2018	Iran	Asian	39	33	3	111	39	70	58	12	192	82	.99
IL-1RN (VNTR)														
Noponen-Hietala[10]	2005	Finland	Caucasian	77	65	13	219	91	86	76	17	248	110	.97
Ye[12]	2007	China	Asian	55	25	1	135	27	83	17	1	183	19	.90
Kim[15]	2010	Korea	Asian	38	4	0	80	4	193	24	1	410	26	.78

11, 12 and 22 respectively represent CC, CT and TT for rs1800587, rs1143634; A_1A_1, A_1A_2 and A_2A_2 for IL-1RN VNTR.
HWE = Hardy-Weinberg's equilibrium.

3. Results

3.1. 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 Tables 1 and 2. All eligible studies were categorized as high quality, with scores > 15.

3.2. 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 = .01) (Fig. 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 > .05). After

Table 3

Association test and heterogeneity test of IL-1 gene locus polymorphisms.

			Test of association				Test of heterogeneity	
SNPs	Allele contrast	N	OR	95% CI	*P*-value	Statistical Model	*P*-value	I^2 (%)
IL-1α (rs1800587)								
	Overall	10						
	T vs C		1.23	[1.08, 1.40]	.001	FEM	.79	0
	CT vs CC		1.22	[1.02, 1.46]	.03	FEM	.82	0
	TT vs CC		1.60	[1.19, 2.14]	.002	FEM	.51	0
	CT/TT vs CC		1.28	[1.08, 1.52]	.004	FEM	.82	0
	TT vs CC/CT		1.42	[1.08, 1.88]	.01	FEM	.55	0
	Caucasian	6						
	T vs C		1.26	[1.06, 1.48]	.007	FEM	.65	0
	CT vs CC		1.29	[1.03, 1.60]	.02	FEM	.64	0
	TT vs CC		1.46	[1.02, 2.08]	.04	FEM	.33	13
	CT/TT vs CC		1.30	[1.06, 1.61]	.01	FEM	.59	0
	TT vs CC/CT		1.25	[0.89, 1.76]	.19	FEM	.45	0
	Asian	3						
	T vs C		1.28	[1.02, 1.60]	.04	FEM	.70	0
	CT vs CC		1.09	[0.79, 1.49]	.60	FEM	.95	0
	TT vs CC		1.93	[1.15, 3.26]	.01	FEM	.68	0
	CT/TT vs CC		1.24	[0.92, 1.67]	.15	FEM	.79	0
	TT vs CC/CT		1.86	[1.13, 3.08]	.01	FEM	.70	0
IL-1β (rs1143634)								
	Overall	9						
	T vs C		0.99	[0.85, 1.16]	.94	FEM	.44	0
	CT vs CC		0.93	[0.76, 1.14]	.48	FEM	.20	28
	TT vs CC		1.10	[0.74, 1.64]	.63	FEM	.66	0
	CT/TT vs CC		0.95	[0.78, 1.16]	.63	FEM	.21	26
	TT vs CC/CT		1.13	[0.80, 1.60]	.48	FEM	.92	0
	Caucasian	7						
	T vs C		1.07	[0.91, 1.26]	.43	FEM	.90	0
	CT vs CC		1.04	[0.83, 1.30]	.75	FEM	.38	6
	TT vs CC		1.11	[0.74, 1.67]	.60	FEM	.56	4
	CT/TT vs CC		1.09	[0.88, 1.36]	.42	FEM	.81	0
	TT vs CC/CT		1.14	[0.81, 1.61]	.45	FEM	.87	0
	Asian	2						
	T vs C		0.61	[0.39, 0.96]	.03	FEM	.44	0
	CT vs CC		0.61	[0.38, 0.97]	.04	FEM	.40	0
	TT vs CC		0.62	[0.03, 15.22]	.77	FEM	NA	NA
	CT/TT vs CC		0.60	[0.38, 0.95]	.03	FEM	0.39	0
	TT vs CC/CT		0.64	[0.03, 15.76]	.78	FEM	NA	NA
IL-1RN (VNTR)								
	Overall	3						
	2 vs 1		1.15	[0.68, 1.94]	.59	REM	.12	53
	12 vs 11		1.24	[0.68, 2.26]	.48	REM	.12	53
	22 vs 11		0.92	[0.44, 1.91]	.82	FEM	.87	0
	12/22 vs 11		1.21	[0.66, 2.22]	.53	REM	.10	56
	22 vs 11/12		0.92	[0.45, 1.88]	.82	FEM	.90	0
	Asian	2						
	2 vs 1		1.51	[0.90, 2.25]	.12	FEM	.16	49
	12 vs 11		1.52	[0.60, 3.82]	.38	REM	.15	51
	22 vs 11		1.57	[0.19, 13.10]	.68	FEM	.96	0
	12/22 vs 11		1.47	[0.57, 3.79]	.42	REM	.14	54
	22 vs 11/12		1.41	[0.17, 11.88]	.75	FEM	.89	0

95% CI = 95% confidence interval, FEM= fixed effect model, OR = odds ratio, N = number of studies included, NA = not applicable, REM = random effect model, SNPs = single nucleotide polymorphisms.

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 = .03) (Fig. 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

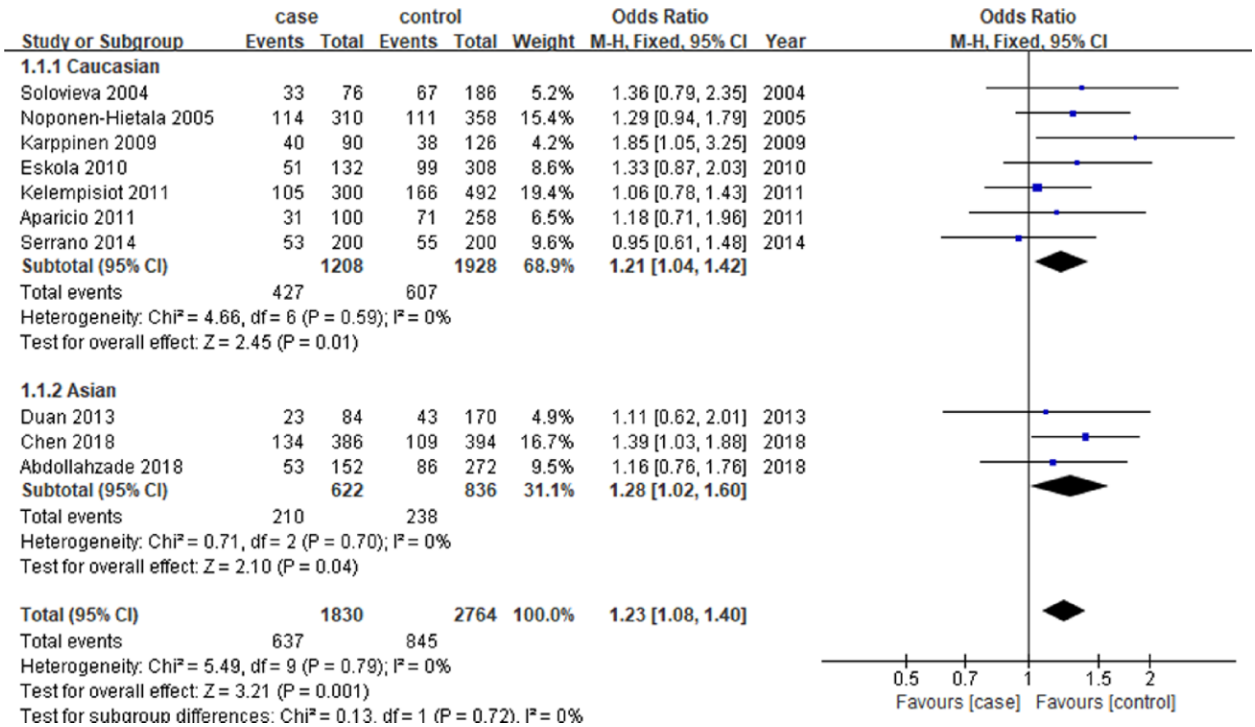

Figure 2. Forest plot of association between IL-1α rs1800587 polymorphism and LDD risk under allelic contract model (T vs. C). There was a significant association between rs1800795 and LDD risk in overall population (T vs. C, OR: 1.23, 95% CI: 1.08–1.40, P = .001). CI = confidence interval, LDD = lumbar disc disease, OR = odds ratio.

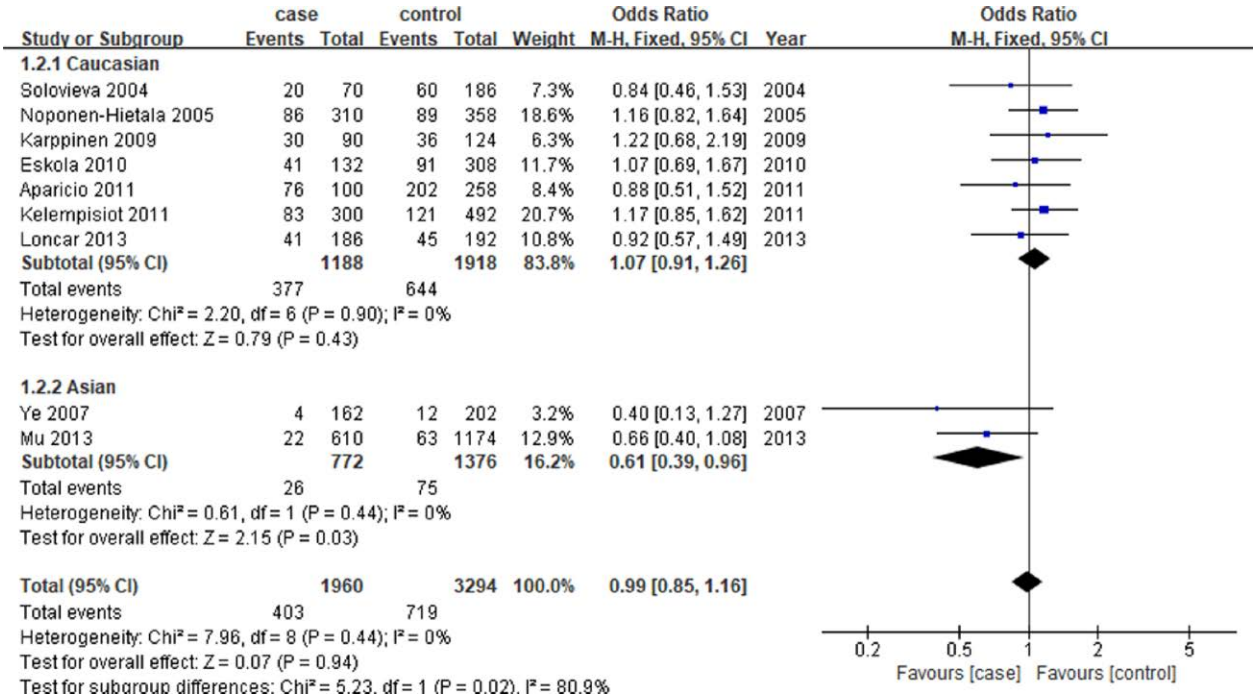

Figure 3. Forest plot of association between IL-1β rs1143634 polymorphism and LDD risk under allelic contract model (T vs. C). There was a significant association between rs1800797 and LDD risk in Asian population (T vs. C, OR: 0.61, 95% CI: 0.39–0.96, P = .03), but not in Caucasian population (T vs. C, OR: 1.07, 95% CI: 0.91–1.26, P = .43). CI = confidence interval, LDD = lumbar disc disease, OR = odds ratio.

Table 4

Sensitivity analysis with each study eliminated for IL-1α (rs1800587), IL-1β (rs1143634) and IL-1RN (VNTR) polymorphisms.

		Test of heterogeneity	
Study	**OR (95% CI)**	***P*-value**	***I*²(%)**
IL-1α (rs1800587) T/C			
Solovieva[9]	1.23 [1.07, 1.40]	.72	0
Noponen-Hietala[10]	1.22 [1.06, 1.40]	.72	0
Karppinen[13]	1.21 [1.06, 1.37]	.91	0
Eskola[14]	1.22 [1.07, 1.40]	.72	0
Aparicio[16]	1.24 [1.08, 1.41]	.71	0
Kelempisioti[17]	1.27 [1.11, 1.47]	.83	0
Duan[18]	1.24 [1.09, 1.41]	.72	0
Serrano[21]	1.26 [1.10, 1.44]	.85	0
Abdollahzade[22]	1.24 [1.08, 1.42]	.71	0
Chen[23]	1.20 [1.04, 1.38]	.78	0
IL-1β (rs1143634) T/C			
Solovieva[9]	1.01 [0.86, 1.18]	.37	8
Noponen-Hietala[10]	0.96 [0.80, 1.14]	.42	1
Ye[11]	1.01 [0.87, 1.19]	.60	0
Karppinen[13]	0.98 [0.83, 1.15]	.38	6
Eskola[14]	0.98 [0.83, 1.16]	.35	11
Aparicio[16]	1.01 [0.86, 1.18]	.36	9
Kelempisioti[17]	0.95 [0.79, 1.13]	.45	0
Loncar[19]	1.00 [0.85, 1.18]	.35	11
Mu[20]	1.04 [0.89, 1.23]	.67	0
IL1-RN (VNTR) A2/A1			
Noponen-Hietala[10]	1.38 [0.59, 3.22]	.16	49
Ye[12]	0.92 [0.67, 1.27]	.76	0
Kim[15]	1.28 [0.63, 2.57]	.05	75

95% CI = confidence interval, OR = odds ratio.

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 (Fig. 4A and B) (all $P > .05$)

3.3. IL-1α, IL-1β and IL-1rn expressions and LDD

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 (Fig. 5D and H). IL-1RN mRNA levels were not significant difference between the 2 groups (Fig. 5L) (all $P > .05$). IHC analysis showed that significantly higher IL-1α and IL-1β expression levels in the LDD group than those in the control group (Fig. 5A–C and E–G) (IL-1α immunopositive cells: 43.8 ± 4.9% *vs.* 22.5 ± 2.8%, $P < .001$; IL-1β immunopositive cells: 36.4 ± 3.7% *vs.* 21.6 ± 2.3%, $P < .001$). However, there were no significant differences in IL-1RN expression between the 2 groups (Fig. 5I–K) (IL-1RN immunopositive cells: 21.4 ± 3.3% *vs.* 24.8 ± 2.3%, $P = .09$).

4. 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.[31] 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, 3 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,[32] 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. Furthermore, the Risk Of Bias In Non-randomized Studies-of Interventions (ROBINS-I)[33] was used to identify the risk of bias (RoB) in the included studies. 12 studies were rated as moderate RoB, and 3 studies were rated as high RoB. Selection bias and information bias may be the main sources of the high RoB in these studies. In the present study, only English-language articles were included which may lead to publication bias. Thus Begg's funnel plots were used to evaluate the potential publication bias. The results indicated that no obvious asymmetry in Begg's funnel plot (Fig. 4). We also evaluated the methodological quality of studies via CASP. There was high quality in all the included studies. The overall quality of evidence is high and imparts confidence in the contribution of the IL-1 gene locus polymorphisms to risk of LDD.

IL-1 has extensively been studied among proinflammatory mediators, and is believed to play a critical role in the etiology of LDD.[8,34] 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.[35,36] 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

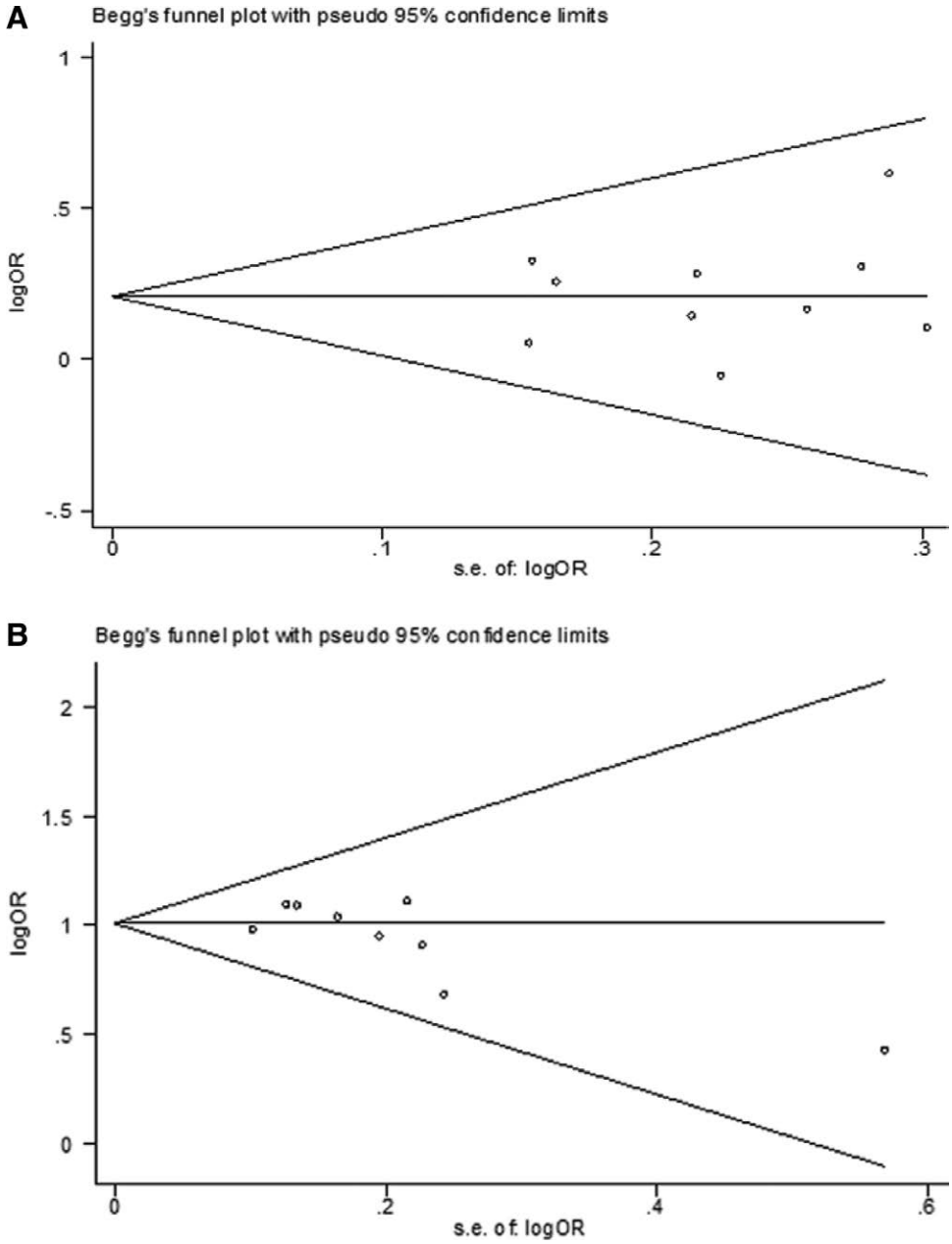

Figure 4. Funnel plot analysis for publication bias in selection of studies on IL-1α rs1800587 (a) and IL-1β rs1143634 (b) polymorphisms under allelic contract model. There was no significant publication bias ($P > .05$).

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.[37] 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.[38,39] 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.[40–42] 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

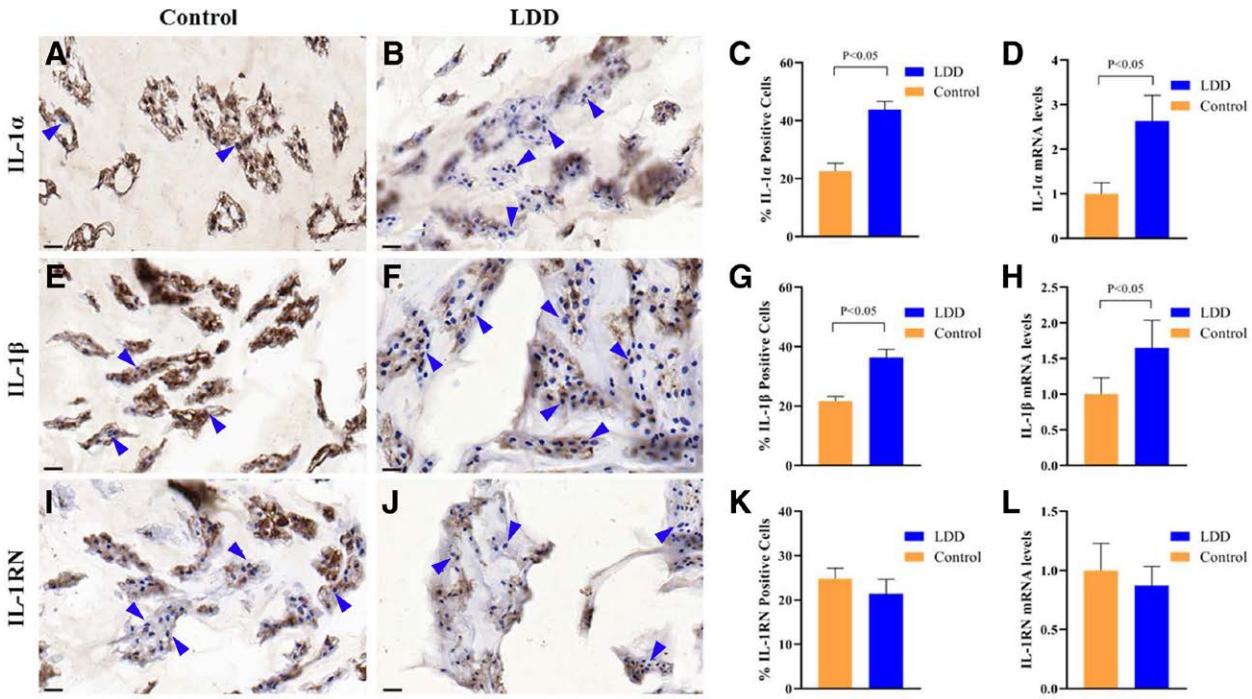

Figure 5. The expression levels of IL-1α, IL-1β and IL-1RN in the LDD group and the control group. The positive signals were identified in the cytoplasm of nucleus pulpous cells in the LDD group (B, F, J) and the control group (A, E, I). (Scale bar = 20 μm) Bar charts (C, G, K) show the expression levels of IL-1α, IL-1β and IL-1RN in the 2 groups. Bar charts (E, H, L) show the mRNA levels of IL-1α, IL-1β and IL-1RN in the 2 groups. Results are mean ± SD. LDD = lumbar disc disease.

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.

5. 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. Elevated IL-1α and IL-1β expression levels may be the important risk factors for LDD.

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Author contributions

Conceptualization: Hua Jiang, Qingjun Wei.
Data curation: Kunxue Yang.
Formal analysis: Qianyi Xiao, Ruijun Zhang.
Funding acquisition: Hua Jiang.
Investigation: Hua Jiang, Kunxue Yang.
Methodology: Dihua Meng.
Resources: Jiaqi Wang.
Software: Kunxue Yang, Qianyi Xiao, Ruijun Zhang.
Supervision: Hua Jiang, Qingjun Wei.
Validation: Hua Jiang.
Visualization: Kunxue Yang.
Writing – original draft: Hua Jiang.
Writing – review & editing: Hua Jiang.

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