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ORIGINAL RESEARCH

Association of Single Nucleotide Polymorphism rs2228570 with Lumbar Disc Degeneration: A Case–Control Study and Meta-Analysis

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Objective: To examine the association between single nucleotide polymorphisms (SNPs) rs2228570, rs731236, rs7975232, and rs1544410 and lumbar disc degeneration (LDD) predisposition.

Methods: A search strategy was carried out, and the data were extracted after being chosen by the inclusion and exclusion criteria. Pooled odds ratios and 95% confidence intervals were calculated to assess the association between the aforementioned SNPs and LDD under allelic, dominant, recessive, heterozygous, and homozygous genetic models. In addition, a case–control study involving 46 LDD cases and 45 controls was also performed in the analysis to verify the result.

Results: A total of 17 studies were included in this meta-analysis. The pooled results did not show any association between vitamin D receptor (VDR) gene polymorphisms and LDD. But, interestingly, in subgroup analysis, the rs2228570 polymorphism was associated with LDD under the allelic (OR = 0.70, 95% CI = 0.56–0.87, p = 0.002), recessive (OR = 0.60, 95% CI = 0.43–0.84, p = 0.003), and homozygous (OR = 0.47, 95% CI= 0.28–0.79, p = 0.004) genetic models in the Asian population. SNPs rs731236 and rs7975232 still did not show any obvious association. We obtained a similar result from the case–control study: rs2228570 had an obvious relationship with LDD under allelic and homozygous genetic models. At the same time, we found that rs2228570 was also associated with the degree of low back pain (visual analogue scale, VAS score) in LDD population.

Conclusion: SNP rs2228570 was significantly associated with LDD predisposition and the degree of low back pain in the Asian population.

Keywords: vitamin D receptor, single nucleotide polymorphism, lumbar disc degeneration, visual analogue scale, meta-analysis, case-control study

Introduction

Low back pain (LBP) is the third most common disorder in neurology, following peripheral neuropathy and migraine, and thus affects a large population. The pain might arise from nerves, muscles,[1] or bones in the back, and various etiological factors may contribute to the pain. Among these factors, lumbar disc degeneration (LDD) is considered a significant cause.[2]

In the pathogenesis of LDD, both environmental and genetic factors play important roles. Environmental factors include physical factors or psychosocial factors,[3] and in terms of genetic factors, single nucleotide polymorphisms (SNPs) have been recognized to be significant recently. It seems that SNPs in the genes of disc proteins,

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proteoglycans, cytokines, enzymes, and vitamin D receptor (*VDR*) would contribute considerably to an LDD attack.[4] Among them, *VDR* gene polymorphisms have been widely explored to assess the association with LDD.

VDR is a nucleoprotein, and it is crucial for maintaining the body's calcium and phosphorus metabolism and regulating cell proliferation and differentiation. As a candidate gene for LDD, *VDR* gene, which is located on human chromosome 12 with a length of 100 kb, has been widely studied by many scientific research groups. Up till now, the association of several *VDR* gene polymorphisms, including rs2228570, rs731236, rs7975232, and rs1544410, with LDD predisposition has been reported.[5] However, the results are contradictory. Several studies have suggested that there are obvious associations between *VDR* gene polymorphisms and the risk of LDD,[6–8] whereas some other studies have reported contrary conclusions.[9–11] With inconsistent results from these studies based on relatively small sample sizes, we cannot draw a valid conclusion. Although several systematic reviews and meta-analysis have summarized, integrated, and analyzed previous studies, considering that there are several new studies and some deviations in collection of data from previous studies, it is necessary to update the meta-analysis and obtain a more reliable result with a more accurate and comprehensive analysis. In this study, we conducted a systematic review and meta-analysis of *VDR* gene polymorphisms and risk of LDD. We used normal people's and patients' genetic information to verify the meta-analysis result.

Materials and Methods

Study Subjects

From March 2019 to May 2019, 46 patients with confirmed LDD and 45 normal controls were recruited from the Qilu Hospital of Shandong University. A total of 2 mL peripheral venous blood from the selected population was collected and used to isolate and extract genome-wide DNA from individuals for further detection. Basic information like age, gender, height, weight and special information in case group like VAS score and imaging data were also collected. This study was approved by the Ethics Committee of Qilu Hospital of Shandong University. Inclusion criteria were as follows: (1) The age of the participants should be greater than 18 years. (2) The patients in the case group were definitely diagnosed with lumbar disc degeneration, with single central disc herniation and required surgical treatment. (3) The selected candidate could undergo surgery under general anesthesia. (4) Candidates should have normal mental consciousness and be able to cooperate with relevant research. (5) Candidates should be willing to participate in the study and sign the informed consent. Exclusion criteria were as follows: (1) Pregnant and lactating patients, infants, and adolescents; (2) Cannot cooperate with researchers; (3) A history of spinal trauma; (4) Patients with scoliosis, spinal deformity, metabolic diseases, and malignant tumors; (5) Have undergone surgery in the same period.

Sample Size Calculation

$$n = \frac{K[\pi 1(1-\pi 1)+\pi 2(1-\pi 2)]}{\delta},$$

where[12]

n = the sample size in one group and K = a function of the chosen significance level and chosen power.

The power of the study will be 80% and the two-sided significance level will be 0.05. The K value is for the power and the significance level is 7.8.

The characteristic of interest—polymorphism of the *VDR* gene.

$\pi 1$ = Proportion of subjects with the disease who are having the characteristic of interest.

$\pi 2$ = Proportion of normal subjects who are having the characteristic of interest.

$\delta = (\pi 1 - \pi 2)$.

n= 45 (each in test and control).

DNA Extraction and Genotyping

Blood samples were digested by SDS/proteinase K (QIAGEN, Duesseldorf, Germany), the activity of the proteinase K solution is 600 mAU/mL. The DNA of samples was extracted by phenol/chloroform, and then precipitated with ethanol. Finally, the DNA was dissolved in a Reduced EDTA TE Buffer at a concentration of no less than 50 ng/μL and a total of no less than 1 μg. Agarose gel electrophoresis was performed on the sample DNA, and the result showed clear electrophoresis bands with a length more than 10 kb and there was no significant DNA degradation. OD 260/280 of DNA sample was between 1.7 and 2.1. In this study, genotyping was performed by using Capital Biotechnology Precision Medicine Research Array Kit (Thermo Fisher Scientific, Waltham, MA, USA), which is a customized chip based on Axiom 2.0 platform. This microarray contains over 787,400 SNPs, which includes 50,000 novel markers covering East and South Asian populations based on the human genome version 19 (GRCH 37).

Low Back Pain VAS Score Assessment

Low back pain degree of 46 patients with confirmed LDD was assessed before blood collection by using the Visual Analogue Scale (VAS): 0 points, no pain; 1–3 points, slight pain, tolerable; 4–6 points, moderate pain, interfere with sleep, tolerable; 7–10 points, severe pain, interfere with sleep and appetite, unbearable.

Statistical Analysis

For the case–control study, the χ^2 test was performed to assess the Hardy–Weinberg equilibrium (HWE) in control cases. For each analysis, the results were considered to be statistically significant when two-sided P was <0.05.

Meta-Analysis

Search Strategy

PubMed, EMBASE, COCHRANE, and three Chinese databases—CNKI, Wanfang, and VIP—were searched up to May 31, 2019. The reference lists of some related articles were screened, as well as those of conference articles and clinical trials. The detailed search strategy is shown in the supplement.

Study Selection and Data Extraction

This work was independently completed by three authors (Lu Chen, Ziyu Wang and Fuan Wang). Any disagreement was settled by consultation. Data obtained included the first author, year of publication, country where the study was conducted, number of participants in the case and control groups, number of male and female participants, age and BMI of the participants, and the method for diagnosis and genotyping. The criteria for inclusion were as follows: (1) Case–control study or cohort study, (2) Patients selected on the basis of specific diagnostic criteria, (3) Complete data. The criteria for exclusion were as follows: (1) Duplication, (2) Defect of the study design, (3) No specific diagnostic criteria, (4) Errors in the statistical method or no supplement for the data that was or could be converted to OR and 95% CI.

Statistical Analysis

Meta-analysis was performed by Review Manager 5.3 (the Cochrane Collaboration, UK) and STATA 12.0 (StataCorp LLC, US). The HWE test was performed in the control group of each included study, and p value >0.05 was regarded as fitting the genetic equilibrium. Summary Odds Ratios (ORs) with 95% confidence intervals (CIs), which were calculated using a fixed-effect model or a random-effect model according to the heterogeneity, were estimated for the association between the risk of LDD and SNP. Heterogeneity was reflected in I^2 statistics, which is on the basis of Q test and has higher sensitivity. If $I^2 > 50\%$, the meta-analysis has obvious heterogeneity, whereas $I^2 < 50\%$ means lower heterogeneity that can be accepted. Sensitivity analysis was performed using STATA 12.0, and the results showed a change in summary ORs after removing each study. Finally, the funnel plots, Begg's test, and Egger's test were used to measure the publication bias. The funnel plots were recommended to be performed only if more than 10 studies were included. For the case-control study, the Chi-square test was used to assess the relationship between two groups.

Results

Search Results and Characteristics of Included Studies

The literature search yielded 149 records (Figure 1), and 17 records met the inclusion criteria. Among these included studies, sample size ranged from 60 to 538 (age range, 18 to 86 years). All the studies were case–control studies and aimed at LDD. Patients were diagnosed by computed tomography (CT), magnetic resonance imaging (MRI) or both, and polymerase chain reaction (PCR) or PCR-restriction fragment length polymorphism (PCR-RFLP) was a common method for genotyping. In terms of ethnicity, eight studies were based on the Asian population, and the other studies were based on Caucasian population. Ten studies of rs2228570, nine studies of rs731236, and six studies of rs7975232 were included in the meta-analysis, and the characteristics of each study are listed in Table 1. All the records had good quality after assessment by the Newcastle−Ottawa Scale (Table S1 in the Supplement). The number of participants in the case and control groups and the types of polymorphisms in each study are listed in Table 2.

Quantitative Synthesis and Sensitivity Analysis

As shown in Table 3, we performed the data synthesis of the above three polymorphisms according to the following five genetic models: allelic model, dominant model, recessive model, heterozygous model, and homozygous model. All the pooled results suggested that the three polymorphisms rs2228570, rs731236, and rs7975232

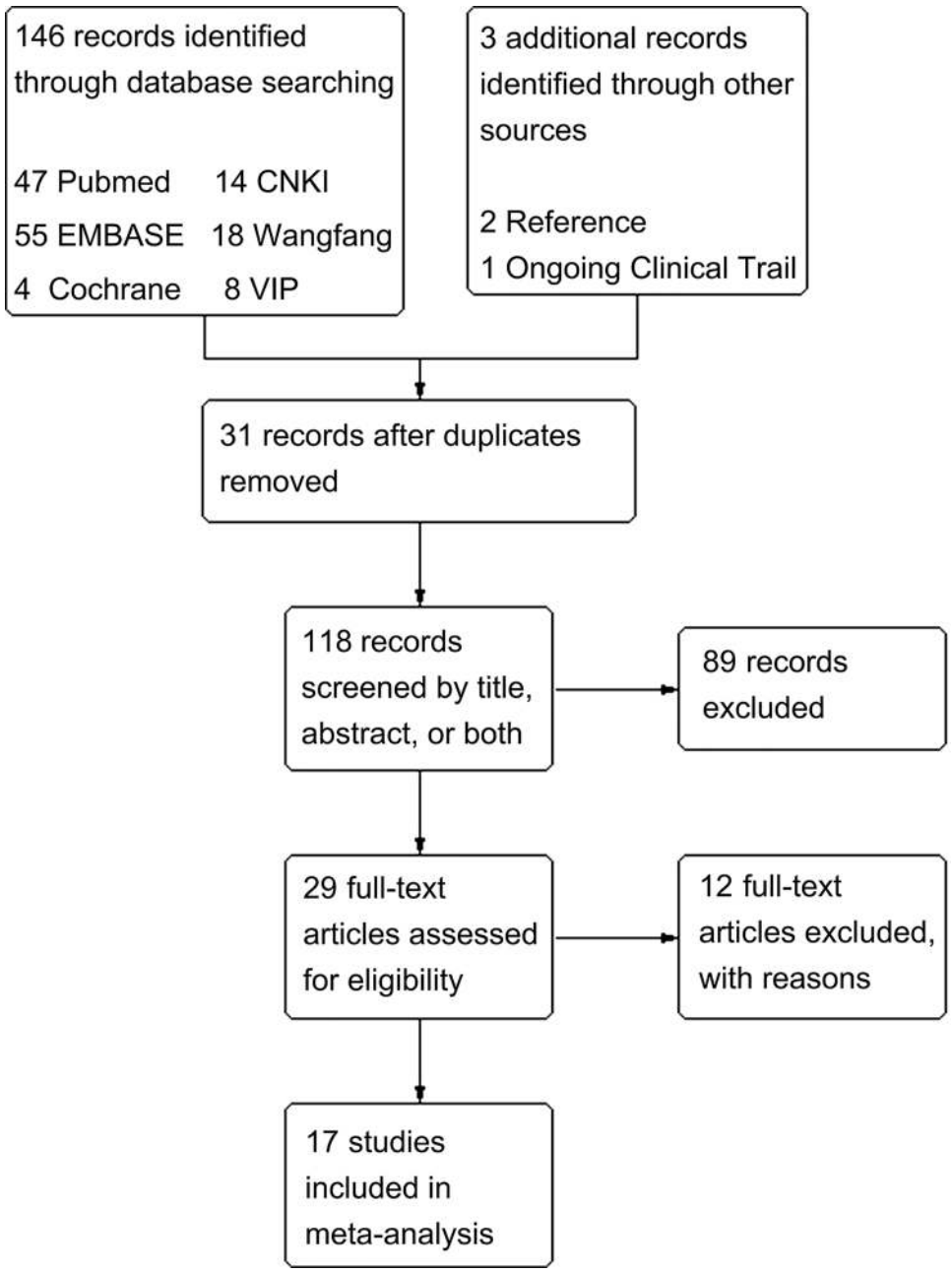

Figure 1 Flow diagram of studies selection.

had no association with LDD, but they had obvious heterogeneity except the rs2228570 heterozygous model (I^2 = 34%) and the rs731236 homozygous model (I^2= 0%). It is worth mentioning that there were four studies related to rs731236 that had two groups with no person under dominant, heterozygous, and homozygous genetic models; therefore, they could not be included in the pooled OR calculation. Next, we performed sensitivity analysis (Figure 2), and the results showed that the removal of either one could affect the pooled ORs significantly, which proved the high stability of the meta-analysis. That was the reason why we included the studies that did not fit the HWE.

Subgroup Analysis

To further explore the cause of heterogeneity, subgroup analysis according to the ethnicity was performed after sensitivity analysis. The data were stratified into two ethnic groups: Asian and Caucasian. For rs2228570, the heterogeneity in the Asian population decreased significantly

Table 1 Characteristics of Studies Included in Meta-Analysis

First Author	Year	Country	Gender	Age (Years)	BMI	Sample Size (Case/Control)	Study Design	Diagnostic Criteria	Genotyping Method
Cauci[19]	2017	Italy	Male	19–62	25.9 ± 3.8	53/79	Case-control	MRI	PCR
Cervin Serrano[20]	2014	Mexico	Both	Case:39.22±6.8 Control:39.13±6.8	Case:25.99±4.33 Control:26.23 ±3.72	100/100	Case-control	MRI	PCR-RFLP
Colombini[21]	2015	Italy	Both	NA	NA	267/254	Case-control	MRI	PCR-RFLP
Kawaguchi[10]	2002	Japan	Both	20–29	≤20	87/118	Case-control	MRI	PCR-RFLP
Kelempisioti[22]	2011	Finland	Both	Mean=19	Case: mean=22.6 Control: mean=23.4	150/246	Case-control	MRI	PCR
Kitis[23]	2018	Turkey	Both	Case:43.9±10.2 Control:42.6±11.8	Case:26.9±4.3 Control:26.1±4.4	199/197	Case-control	MRI	PCR
Mashayekhi[24]	2018	Iran	Both	Case:37.32±8.81 Control:35.9 ±7.63	Case:25.66±2.98 Control:25.93 ±2.31	180/230	Case-control	MRI	PCR-RFLP
Oishi[25]	2003	Japan	Female	61–86	16.9–39.1	39/21	Case-control	MRI	PCR
Ozdogan[26]	2019	Turkey	Both	Case:37.33±8.26 Control:34.81±4.53	NA	45/49	Case-control	MRI	PCR
Vieira[7]	2018	Brazil	Both	Case:38.0–42.0 Control:31.0–35.0	Case:25.2–27.0 Control:24.5–26.6	119/112	Case-control	MRI	PCR-RFLP
Yuan[27]	2010	China	Both	Case:48.5±13.1 Control:40.6±15.8	Case:22.8±2.8 Control:22.5±3.3	178/284	Case-control	CT	PCR-RFLP
Vieira[28]	2014	Brazil	Both	Case:Male:46.0±5.4; Female:45.2±5.9 Control:Male:33.8±8.2 Female:33.9±8.1	NA	121/131	Case-control	MRI	PCR-RFLP
Xu[29]	2014	China	Both	30–71	≤27.5	78/79	Case-control	MRI/CT	PCR-RFLP

(*Continued*)

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Table 1 (Continued).

First Author	Year	Country	Gender	Age (Years)	BMI	Sample Size (Case/Control)	Study Design	Diagnostic Criteria	Genotyping Method
Zawilla[8]	2014	Egypt	Both	Case:44.2±11.28 Control:43.3±10.58	Case:43.3±10.6 Control:23.5±3.6	84/60	Case-control	MRI	PCR-RFLP
Chen[30]	2007	China	Both	Case:42.7±13.7 Control:38. 4 ±12.6	Case:22.5±2.9 Control:22.5±2.5	81/101	Case-control	MRI	PCR-RFLP
Cheung[5]	2006	China	Both	18–55	NA	167/190	Case-control	MRI	PCR
Withanage[12]	2018	Sri Lanka	Both	Case:41.3±14.6 Control:43.3±15.4	Case:28.2±5.7 Control:23.5±3.4	51/68	Case-control	MRI	PCR-RFLP

Abbreviations: BMI, body mass index; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction polymerase chain reaction followed by restriction fragment length polymorphism; MRI, magnetic resonance imaging; CT, computed tomography; NA, not available.

while that in the Caucasian population showed minimal change, as well as in the rs7975232 subgroup analysis. Regarding rs731236, a contrary result was obtained; the heterogeneity in the Caucasian population decreased drastically, but it did not show any obvious association with LDD in either subgroup. It should be noted that subgroup analysis showed that rs2228570 polymorphism was associated with LDD under the allelic (Figure 2A), recessive (Figure 2B), and homozygous (Figure 2C) genetic models in the Asian population.

Publication Bias

With respect to rs2228570, 10 studies were included in this meta-analysis; therefore, funnel plots, Begg's test, and Egger's test were used to assess the publication bias. With respect to rs731236 and rs7975232, the number of studies was less than 10; therefore, the Begg's test and Egger's test were only used. All the results showed that there was no obvious publication bias in the meta-analysis of three *VDR* gene polymorphisms under every genetic model.

Basic Information of Research Subjects in Case–Control Study

A total of 46 patients were included in the case group, and 45 patients were included in the control group. There were no significant differences in gender, age, height, weight, and BMI between the two groups (Table S2 in the Supplement). According to the results of HWB test, P values of SNPs rs2228570, rs731236, rs7975232, and rs1544410 of the *VDR* gene were greater than 0.05, suggesting that their distribution in the study population met the Hardy −Weinberg balance (Table S3 in the Supplement), and the samples were representative of the population.

Distribution of Single Nucleotide Polymorphisms of *VDR*s and Their Relationship with Degeneration of the Lumbar Intervertebral Disc

Single Nucleotide Polymorphism rs2228570

The genotype frequency in the control group was (1 for the main allele and 2 for the suballele) 11:13.3%, 12:48.9%, and 22:37.8%. The genotype frequency in the case group was 11:28.3%, 12:52.2%, and 22:19.6%. The results showed that in the allele model (OR = 0.51, P= 0.02497) and the homozygous model (OR = 0.63, P = 0.02504), SNP rs2228570 was significantly associated with the risk of LDD (Table 4).

Table 2 Distribution of Genotype and Allele of Different VDR Gene Polymorphism

Author	Year	Ethnicity	Cases (n) [b]			Controls (n) [a]			HWE (p)
			11	12	22	11	12	22	
rs2228570									
Cauci	2017	Caucasian	27	22	4	32	34	13	0.44
Cervin Serrano	2014	Caucasian	15	65	20	17	51	32	0.66
Colombini	2015	Caucasian	117	120	30	101	117	36	0.42
Kelempisioti	2011	Caucasian	81	57	12	111	119	16	0.03
Mashayekhi	2018	Asian	30	86	64	20	104	106	0.44
Ozdogan	2019	Caucasian	3	11	31	6	22	21	0.95
Vieira	2018	Caucasian	53	49	17	61	41	10	0.42
Vieira	2014	Caucasian	54	50	17	75	46	10	0.43
Chen	2007	Asian	12	51	18	17	48	36	0.88
Withanage	2018	Asian	34	16	1	38	26	4	0.87
rs731236									
Cauci	2017	Caucasian	9	22	22	13	40	26	0.72
Cervin Serrano	2014	Caucasian	69	27	4	62	35	3	0.46
Kawaguchi	2002	Asian	0	34	53	0	20	98	0.31
Oishi	2003	Asian	0	8	31	0	5	16	0.54
Vieira	2018	Caucasian	27	42	50	14	46	52	0.45
Yuan	2010	Asian	0	22	156	0	28	256	0.38
Xu	2014	Asian	0	3	75	0	15	64	0.35
Cheung	2006	Asian	150	16	1	182	8	0	0.77
Withanage	2018	Asian	31	16	4	25	39	4	0.03
rs7975232									
Cauci	2017	Caucasian	19	30	4	32	34	13	0.44
Kawaguchi	2002	Asian	13	35	39	13	52	53	0.96
Kitis	2018	Caucasian	22	67	110	38	81	78	0.05
Vieira	2018	Caucasian	37	64	18	39	59	14	0.25
Yuan	2010	Asian	20	100	58	27	129	128	0.50
Zawilla	2014	Caucasian	17	48	19	34	22	4	0.86
rs1544410									
Cauci	2017	Caucasian	12	25	16	14	39	26	0.92
Vieira	2018	Caucasian	52	67	0	51	61	0	<0.0001

Notes: [a]11 presents common homozygote, 12 presents heterozygote, 22 presents rare homozygote, 1 presents common allele, 2 presents rare allele.
Abbreviations: VDR, vitamin D receptor; HWE, Hardy–Weinberg Equilibrium.

Single Nucleotide Polymorphism rs731236

The genotype frequency in the control group was 11:86.7%, 12:13.3%, and 22:0%. The genotype frequency in the case group was 11:9 5.7%, 12:4.3%, and 22:0%. Under the five gene models, the distribution of rs731236 and the χ^2 test value of LDD were greater than 0.05, indicating that there was no significant correlation between rs731236 and LDD (Table 4).

Single Nucleotide Polymorphism rs7975232

The genotype frequency in the control group was 11:68.9%, 12:31.1%, and 22:0%. The genotype frequency in the case group was 11:69.6%, 12:28.3%, and 22:2.2%. Under the five gene models, the distribution of rs7975232 and the χ^2 test value of LDD were greater than 0.05, indicating that there was no significant correlation between rs7975232 and LDD (Table 4).

Table 3 Meta-Analysis of VDR Gene Polymorphisms Under Different Genetic Model

Genetic Model [a]	Analysis Model	Overall Effect		Test for Heterogeneity		Test for Publication Bias	
		OR(95% CI)	p	I^2	p	Begg's (p)	Egger's (p)
rs2228570							
Allelic model							
1 vs 2	REM	0.94(0.75, 1.17)	0.58	68	0.0008	0.85	0.41
Dominant model							
12+22 vs 11	REM	0.93(0.71, 1.22)	0.60	52	0.03	0.72	0.68
Recessive model							
11+12 vs 22	REM	0.91(0.62, 1.34)	0.63	63	0.004	0.21	0.70
Codominant model							
12 vs 22	FEM	0.93(0.77, 1.12)	0.43	34	0.13	0.72	0.55
11 vs 22	REM	0.88(0.57, 1.36)	0.57	56	0.02	0.99	0.41
rs731236							
Allelic model							
1 vs 2	REM	0.94(0.64, 1.37)	0.74	70	0.0009	0.34	0.11
Dominant model							
12+22 vs 11	REM	0.77(0.42, 1.42)	0.41	69	0.01	0.80	0.46
Recessive model							
11+12 vs 22	REM	1.06(0.63, 1.77)	0.33	63	0.006	0.17	0.13
Codominant model							
12 vs 22	REM	0.71(0.38, 1.33)	0.29	67	0.02	0.80	0.64
11 vs 22	FEM	0.78(0.47, 1.30)	0.34	0	0.51	0.46	0.12
rs7975232							
Allelic model							
1 vs 2	REM	1.23(0.82, 1.84)	0.31	85	<0.00001	0.45	0.49
Dominant model							
12+22 vs 11	REM	1.43(0.84, 2.41)	0.19	74	0.002	0.99	0.91
Recessive model							
11+12 vs 22	REM	1.13(0.64, 1.99)	0.67	80	0.0002	0.99	0.80
Codominant model							
12 vs 22	REM	1.40(0.90, 2.18)	0.14	59	0.03	0.99	0.79
11 vs 22	REM	1.35(0.64, 2.85)	0.43	78	0.0003	0.99	0.86

Notes: [a]11 presents common homozygote, 22 presents rare homozygote, 12 presents heterozygote, 1 presents common allele, 2 presents rare allele.
Abbreviations: VDR, vitamin D receptor; OR, odds ratio; CI, confidence interval; I^2, inconsistency index; FEM, fixed-effect model; REM, random-effect model.

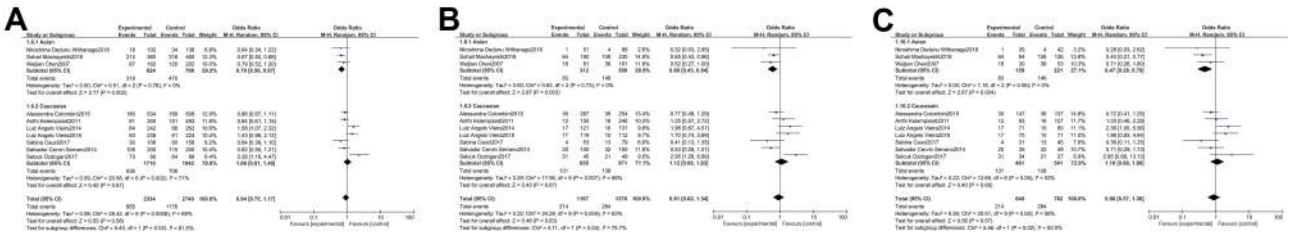

Figure 2 Subgroup analysis based on ethnicity of rs2228570 and LDD under allelic (**A**), recessive (**B**) and homozygous (**C**) model. Box sizes are proportional to study weight (box center positioned at point estimate of effect). Horizontal lines indicate 95% CIs. The I^2 value indicates the percentage of variability across the pooled estimates attributable to heterogeneity beyond chance. The *P* value is for a test of heterogeneity across all studies, P <0.05 indicates likely variation across pooled estimates beyond chance.

Single Nucleotide Polymorphism rs1544410

The genotype frequency in the control group was 11:91.1%, 12:8.9%, and 22:0%. The genotype frequency in the case group was 11:9 7.8%, 12:2.2%, and 22:0%. Under the five gene models, the distribution of rs1544410 and the χ^2 test value of LDD were greater than 0.05, indicating that there was no significant correlation between rs1544410 and LDD (Table 4).

Relationship Between Single Nucleotide Polymorphisms of *VDR*s and Low Back Pain

According to the VAS score, 46 patients were divided into two groups (VAS < 4 and VAS ≥ 4). There were no significant differences in gender, age, height, weight, BMI, spinal canal occupation ratio (SCOR) and decrease in spinal canal ratio (DSCR) between the two groups

Table 4 The Relationship Between VDR Gene Polymorphisms and Intervertebral Disc Degeneration

Model Type [a]	Control Group(n)		Case Group(n)		P value
rs2228570					
Allelic model (1 vs 2)	34	56	50	42	0.02
Dominant model (11 vs.12+22)	6	39	9	37	0.42
Recessive model (11+12 vs 22)	28	17	37	9	0.05
Heterozygous model (12 vs 11)	22	6	24	13	0.23
Homozygous model (22 vs 11)	17	6	9	13	0.03
rs731236					
Allelic model (1 vs 2)	84	6	90	2	0.13
Dominant model (11 vs.12+22)	39	6	44	2	0.13
Recessive model (11+12 vs 22)	45	0	46	0	1
Heterozygous model (12 vs 11)	6	39	2	44	0.13
Homozygous model (22 vs 11)	0	39	0	44	0.93
rs7975232					
Allelic model (1 vs 2)	76	14	77	15	0.89
Dominant model (11 vs.12+22)	31	14	32	14	0.94
Recessive model (11+12 vs 22)	45	0	45	1	0.32
Heterozygous model (12 vs 11)	14	31	13	32	0.82
Homozygous model (22 vs 11)	0	31	1	32	0.33
rs1544410					
Allelic model (1 vs 2)	86	4	91	1	0.17
Dominant model (11 vs.12+22)	41	4	45	1	0.16
Recessive model (11+12 vs 22)	45	0	46	0	1
Heterozygous model (12 vs 11)	4	41	1	45	0.16
Homozygous model (22 vs 11)	0	41	0	45	0.95

Notes: [a]11 presents common homozygote, 22 presents rare homozygote, 12 presents heterozygote, 1 presents common allele, 2 presents rare allele.
Abbreviation: VDR, vitamin D receptor.

(Table S4 in the Supplement). Low back pain VAS scores are shown in Table 5. SNPs rs2228570 (P=0.033) and rs7975232 (P=0.046) of the *VDR* gene had an obvious relationship with low back pain VAS score. However, SNPs rs731236 (P=0.23) and rs1544410 (P=0.40) did not show any significant association.

Discussion

Over the past few years, many researchers have paid attention to the association between SNPs and diseases. In terms of the association between SNP rs2228570, rs731236, rs7975232, and rs1544410 and LDD, some studies have shown that there is no association between them, whereas other studies have reported contradictory conclusions. Therefore, we performed this meta-analysis.

From the result of this meta-analysis, it can be seen that rs2228570 was likely associated with LDD in the Asian population, and rs731236 had no association with LDD. However, we did not obtain any specific conclusion about rs7975232 from the result, because the results under different genetic models were contradictory to each other even though the heterogeneity was decreased after subgroup analysis. The small sample size might be the main cause. Studies about rs1544410 polymorphism were also collected, but there were only two studies, and one of them did not fit the HWE ($p<0.0001$). Therefore, the meta-analysis about rs1544410 was abandoned.

In this work, no publication bias was found and the results were very stable after sensitivity analysis. The main problem was heterogeneity. With respect to rs2228570 and rs7975232, there was no heterogeneity in the Asian population, while little change in the heterogeneity was found in the Caucasian population after subgroup meta-analysis. Besides, subgroup analysis based on other aspects, such as continents or the number of total participants, was also performed, but the heterogeneity of the Caucasian population did not decrease either, and meta-analysis based on the ethnicity seemed to be the best method. We guess that the main reason may be that some of the characteristics of Caucasian population are affected by other races due to its high mobility. After subgroup analysis, the result in the Asian population is available as a suggestion. With respect to some previous meta-analysis on this subject, the process to explore and explain the heterogeneity cannot be found.

For determination of the genetic model, we tried to use the method introduced by Thakkinstian et al.[13] But the standard is very rigorous, and the realistic model may not fit very well; therefore, we have summarized and explained all the results under the five genetic models above.

In this meta-analysis, the number of included studies was the highest and the source of heterogeneity was also explored further. The whole process was integrated, clear, and logical. It is more important that we obtained a new conclusion, the rs2228570 polymorphism was associated with LDD in the Asian population, after sorting out the included studies and checking the methods of genotype specification.

Next, we conducted a case–control study on the correlation between single nucleotide polymorphism of *VDR*s (rs2228570, rs731236, rs7975232, and rs1544410) and the risk of LDD in the population. After calculating the sample size, we recruited 46 patients with LDD and 45 normal control volunteers, collected their basic information and blood samples, and assessed 46 patients' low back pain VAS scores. We determined the genotype of each participant at the four polymorphic sites described above by using a single nucleotide polymorphic chip. Under the five genetic models of the allele model, dominant model, recessive model, heterozygous model, and homozygous model, we used the χ^2 test to analyze the association between the genotype of polymorphism and the risk of LDD. The results showed that under the allelic model and the dominant model, the single nucleotide polymorphism rs2228570 was significantly associated with the risk of LDD, while the other three models showed no significant association with LDD. The participants included in the case–control study were all Asians; therefore, the conclusion of the case–control study was consistent with our meta-analysis: in the Asian population, rs2228570 SNP of *VDR* was significantly associated with the risk of LDD. In addition, after analyzing the VAS scores of patients, we found that SNP rs2228570 was also significantly correlated with the degree of low back pain.

Table 5 The Relationship Between VDR Gene Polymorphisms and Back Pain VAS Score

Polymorphism	VAS<4(n) [a]			VAS≥4(n) [a]			P value
	11	12	22	11	12	22	
Rs2228570	4	14	1	9	10	8	0.033
Rs731236	19	0	0	25	2	0	0.23
Rs7975232	17	2	0	15	11	1	0.046
Rs1544410	19	0	0	26	1	0	0.40

Notes: [a]11 presents common homozygote, 12 presents heterozygote, 22 presents rare homozygote, 1 presents common allele, 2 presents rare allele.
Abbreviations: VDR, vitamin D receptor; VAS, Visual Analogue Scale.

It is worth mentioning that intervertebral disc degeneration is not an isolated process,[14–17] it's always associated with Modic change in end plate, which is also related with *VDR* polymorphisms,[18] and fatty infiltration in paraspinal muscles. Finally, all of this may contribute to lower back pain. This is why some SNPs of *VDR* are associated with intervertebral disc degeneration, like rs2228570, but some do not.

Based on the result of this meta-analysis and case–control study, we think that future clinical studies need to be designed to collect more basic information and the data of clinical indicators. It will be better to explore the correlation between different SNPs and LDD or its relevant clinical indicator in different subgroups, such as age, gender, pain scores, and segment of LDD.

Conclusions

In summary, we confirmed that SNP rs2228570 was significantly associated with the risk of LDD in the Asian population, while rs731236, rs7975232 and rs1544410 have no significant correlation with LDD. Meanwhile, SNP rs2228570 was also significantly associated with the degree of low back pain in patients with LDD. Further studies will help to improve the value of the results of this study.

Abbreviations

SNPs, single nucleotide polymorphisms; LDD, lumbar disc degeneration; VDR, vitamin D receptor; VAS, visual analogue scale; LBP, low back pain; HWE, Hardy–Weinberg equilibrium; ORs, odds ratios; CIs, confidence intervals; CT, computed tomography; MRI, magnetic resonance imaging; PCR, polymerase chain reaction; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SCOR, spinal canal occupation ratio; DSCR, decrease in spinal canal ratio; FEM, fixed-effect model; REM, random-effect model; BMI, body mass index.

Ethics Statement and Data Availability

This study was conducted in accordance with the Declaration of Helsinki. A written informed consent was obtained from all participants, and this study was approved by the ethics committee of Qilu Hospital and is numbered KYLL-2018 (KS)-135. All data generated or analyzed during this study are available via contacting the corresponding author.

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Disclosure

The authors declare that there is no conflict of interest regarding the publication of this paper.

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