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Original Article

Association of rs731236 polymorphism in the vitamin D receptor gene with degenerative disc disease: evidence from a meta-analysis

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Abstract: The purpose of this study was to investigate the association between the rs731236 polymorphism in the vitamin D receptor gene and degenerative disc disease, especially in Chinese. We elaborately searched the relevant studies through China National Knowledge Infrastructure (CNKI), PubMed and EMBASE databases. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to evaluate the strength of the association. A total of 10 studies involving 1,220 cases and 1,225 controls were included in the present study. Overall, no evidence of significant risk between rs731236 polymorphism and degenerative disc disease was found in any genetic models. In addition, stratified analyses by ethnicity revealed similar results. However, stratified analyses by sample size in Chinese population show that sample size may be the primary source of heterogeneity. This meta-analysis suggested that the rs731236 polymorphism may not be associated with degenerative disc disease. However, for Asians, there existed some diversities, especially in Chinese population. Therefore, a large number of well-designed studies are still required to assess this polymorphism and degenerative disc disease.

Keywords: Degenerative disc disease, rs731236, meta-analysis, polymorphism, vitamin D receptor, gene

Introduction

Intervertebral disc degeneration (IDD) is a well-known complex musculoskeletal disorder. In recent years, low-back disorders mainly caused by lumbar disc disease have been the most common problem in the industrialized countries. Some studies show that 20% of patients with LDD require operative treatment due to persistent or aggravated leg pain [10, 24]. Also, it is playing an important contributor to absence of working and impacting on the economy worldwide [1, 9].

Many researchers have been trying to elucidate its pathogenic mechanism. To date, however, its exact etiology is still unclear. It is generally believed that age and environmental factors such as sporting activities, occupation, injury, and smoking contribute to its development [8, 23, 28]. However, over the past few decades, numerous genes have been reported to be associated with degenerative disc disease, such as Collagen I [27], Collagen IX [22], Aggrecan [15], COL9A2 [33] and Interleukins [5]. One of the susceptible genes which has been intensely investigated is vitamin D receptor gene.

The vitamin D receptor (VDR) is an endocrine member which belongs to the nuclear receptor superfamily for steroid hormones and acts as a ligand-activated transcription factor [4]. VDR is famous for playing a critical role in normal bone mineralization and remodeling. Its gene polymorphisms are thought to contribute to disorders such as osteoarthritis, osteoporosis and degenerative disc disease [7] . Until now, several locations have been studied to assess the association of VDR and degenerative disc disease in different populations. Among these polymorphisms, rs731236 polymorphism was one of the most widely studied. However, the results were often controversial and ambiguous. The inconsistency of these studies may be explained by differences in the source of

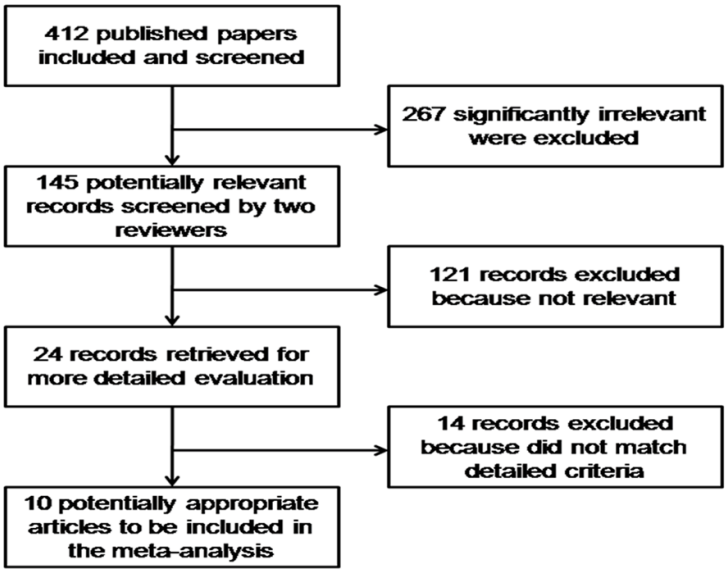

Figure 1. Study flow diagram of search strategy.

patients, sample size, population background and also by chance [16]. To our knowledge, there was only one meta-analysis once pay attention to this location three years ago [29]. But it had some weaknesses: First, perhaps because of the limitation of sample size especially from Chinese, they did not find the source of the heterogeneity; second, it only focused on the total population without performing subgroup analysis in Asian and Chinese population; Third, some studies without sufficient data were included.

Therefore, we include more relevant studies, put strict limits on inclusion and exclusion criteria so that we can provide most considerable evidence for association of the rs731236 polymorphism and degenerative disc disease.

Materials and methods

Search strategies

We elaborately searched the relevant studies that examined the association of rs731236 polymorphism and degenerative disc disease. Two authors independently retrieved China National Knowledge Infrastructure (CNKI), PubMed and EMBASE databases without restriction for language to identify available articles published up to March 2015. The following publication search strategy was performed by consecutively entering the combined free words "disc", "lumbar", "degeneration", "vitamin D receptor gene", "polymorphism", "rs731236", including all alternative locations and combinations of the terms. We also screened the reference lists of all retrieved articles and relevant reviews to confirm other undetected potentially eligible studies.

Inclusion and exclusion criteria

For the meta-analysis, studies were included if they met the following criteria: (1) case-control or cohort studies; (2) the articles had original data to assess quantitatively the relationship of rs731236 polymorphism and degenerative disc disease; (3) cases and controls were eligible regardless of country, ethnicity and age; (4) providing sufficient data for calculation of odds ratio (OR) and 95% confidence interval (CI).

While for the exclusion criteria, we provided as follows: (1) not for rs731236 polymorphism research; (2) studies containing overlapping data; (3) case-only studies, family-based studies, case reports, editorials, and review articles (including meta-analyse); (4) studies that investigated rs731236 variants as makers for response to therapy; (5) studies in which the number of genotypes or alleles were not offered.

Data extraction

Two authors carefully extracted valuable information from all eligible publications according to the inclusion criteria. Discrepancy was settled by discussion between the two authors or a third author. The following data were collected from each study: first author's surname, year of publication, original country, ethnicity, the number of cases and controls and genotype frequency information. If more than one study includes the same population, only the most complete study was included in this meta-analysis. Then we verified accuracy of information by comparing collection forms from each investigator.

Statistical analysis

As for studies investigating the association between rs731236 polymorphism and degen-

Table 1. The characteristics of the selected studies

Study type (Case/Control)				Sample Size (Case/Control)	Genotype Distribution (Case/Control)			Quality Score	P for HWE
First author	Year	Country	Ethnicity		T/T	T/t	t/t		
Kawaguchi	2002	Japan	Asian	116/89	79/72	37/17	0/0	8	0.319
Noponen-Hietala	2003	Filand	Caucasian	24/56	12/26	11/19	0/11	6	0.044
Oishi	2003	Japan	Asian	39/21	31/16	8/5	0/0	6	0.536
Cheung	2006	China	Asian	388/191	354/183	33/8	1/0	8	0.768
Eskola	2010	Danmark	Caucasian	66/154	29/57	28/74	9/23	7	0.898
Yuan	2010	China	Asian	178/284	156/256	22/28	0/0	7	0.382
Eser	2010	Turkey	Caucasian	150/150	65/67	67/67	18/16	8	0.902
Chen	2012	China	Asian	81/101	79/86	2/14	0/1	7	0.617
Xu	2014	China	Asian	78/79	75/15	3/15	0/0	8	0.351
Serrano	2014	Mexico	Caucasian	100/100	69/62	27/35	4/3	7	0.461

HWE, Hardy-Weinberg equilibrium.

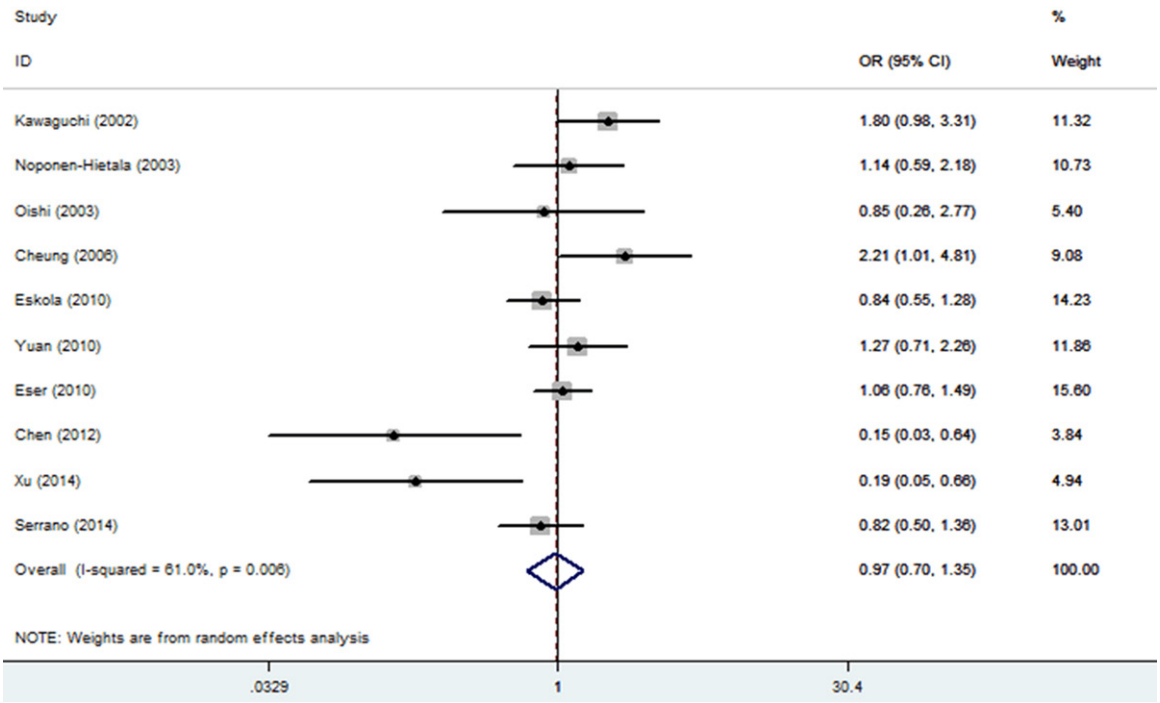

Figure 2. Forest plot for the meta analysis of the association between rs731236 polymorphism and degenerative disc disease (under allele comparison model).

erative disc disease, odds ratios (ORs) and 95% confidence intervals (CIs) were used to assess the strength of the association. The statistical significance of the pooled OR was evaluated by the Z test. Hardy-Weinberg equilibrium (HWE) in the control group for each study was determined by Chi square test; $P < 0.05$ was considered significant. We calculated the pooled ORs for allele comparison model (T vs. t), homozygote model (TT vs. tt), heterozygote model (Tt vs. tt), dominant model [(Tt+TT) vs. tt] and recessive model [TT vs. (tt+Tt)], respectively. Heterogeneity among the studies was evaluated with the chi-square-based Q test and I2 index ($P < 0.10$ was considered significant). I2 values of 25, 50, and 75 were normally reckoned low, moderate, and high heterogeneity [17]. When the heterogeneity was present, the

Table 2. Overall and subgroup meta-analysis of the association between rs731236 polymorphism and degenerative disc disease under genetic models

Groups	N	Allelic		Homozygous		Heterozygous		Dominant		Recessive	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Total	10	0.97 (0.70, 1.35)	0.006	1.01 (0.63, 1.63)	0.985	1.11 (0.69, 1.81)	0.990	1.14 (0.72, 1.79)	0.753	0.93 (0.63, 1.38)	0.003
Asian	6	0.8 (0.40, 1.75)	0.001	0.7 (0.09, 5.83)	0.530	1.16 (0.10, 14.01)	0.702	0.77 (0.10, 6.21)	0.580	0.83 (0.38, 1.81)	0.001
Caucasian	4	0.9 (0.77, 1.19)	0.716	1.03 (0.63, 1.68)	0.898	1.11 (0.68, 1.82)	0.941	1.16 (0.73, 1.85)	0.518	0.90 (0.67, 1.20)	0.622
Asian											
Chinese	4	0.61 (0.19, 1.94)	0.000	0.74 (0.09, 5.38)	0.530	1.16 (0.10, 14.01)	0.702	0.77 (0.10, 6.21)	0.580	0.60 (0.18- 1.95)	0.000
Japanese	2	1.47 (0.77, 2.82)	0.268	NA	NA	NA	NA	NA	NA	1.53 (0.69, 3.35)	0.230
Chinese											
> 200	2	1.57 (0.92, 2.67)	0.260	1.55 (0.06, 38.31)	NA	0.76 (0.03, 20.39)	NA	1.48 (0.06, 36.56)	NA	1.58 (0.95, 2.62)	0.288
< 200	2	0.17 (0.06, 0.44)	0.799	0.36 (0.01, 9.03)	NA	1.93 (0.06, 62.17)	NA	0.41 (0.02, 10.23)	NA	0.16 (0.18, 1.95)	0.872

N: total number of studies involved in the analysis; NA: the data were not available.

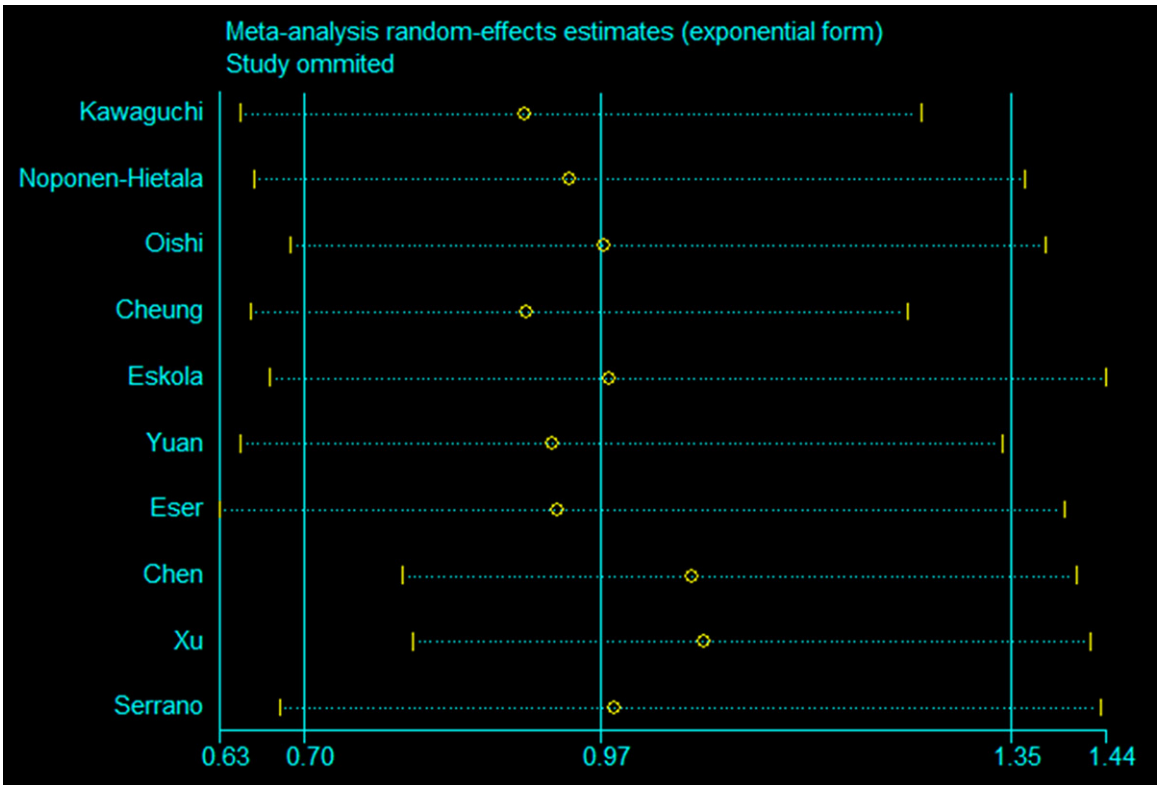

Figure 3. One-way sensitivity analysis of the pooled ORs and 95% CI for the overall analysis, omitting each dataset in the meta- analysis.

random-effect model was used to calculate the pooled OR [11], otherwise the fix-effect model was used [19]. To explore the source of the heterogeneity, we also carried out the stratified analysis by ethnicity in all population, original country in Asia and sample size in Chinese. In order to assess the stability of the results, we performed sensitivity analysis to evaluate the influence of the individual data on the overall effect by omitting each study sequentially. We utilized funnel plots to estimate the potential publication bias, in which the standard error of log (OR) of each study was plotted against its log (OR). Begg's [3] and Egger's [12] tests were also performed to assess the publication bias ($P < 0.05$ indicates a significant publication bias). All statistical tests for this meta-analysis were performed with STATA version 12.0 (Stata Corporation, College Station, TX).

Results

Characteristics of included studies

Eventually, a total of 10 eligible studies met the inclusion criteria [5-7, 13, 14, 18, 20, 21, 30, 31]. **Figure 1** shows the flow diagram of the selection process of this literature review. All the included studies were original studies. There were 4 studies performed in Caucasians, 6 studies in Asians (4 are from Chinese population). Totally, 1,220 cases and 1,225 controls were included in the final pooled analyses. The characteristics of the selected studies are summarized in **Table 1**.

Quantitative synthesis

We conducted this meta-analysis on the association between rs731236 polymorphism with degenerative disc disease. Overall, no evidence of significant risk between rs731236 polymorphism and degenerative disc disease was found in any genetic models (allelic model: OR=0.97, 95% CI=0.70-1.35, P=0.006 (**Figure 2**); homozygote model: OR=1.01, 95% CI=0.63-1.63, P=0.958; heterozygote model: OR=1.11, 95%=0.69-1.81, P=0.990; dominant model: OR=1.14, 95%=0.72-1.79, P=0.753; recessive model: OR=0.93, 95%=0.63-1.38, P=0.003). In addition, stratified analyses were shown in **Table 2**.

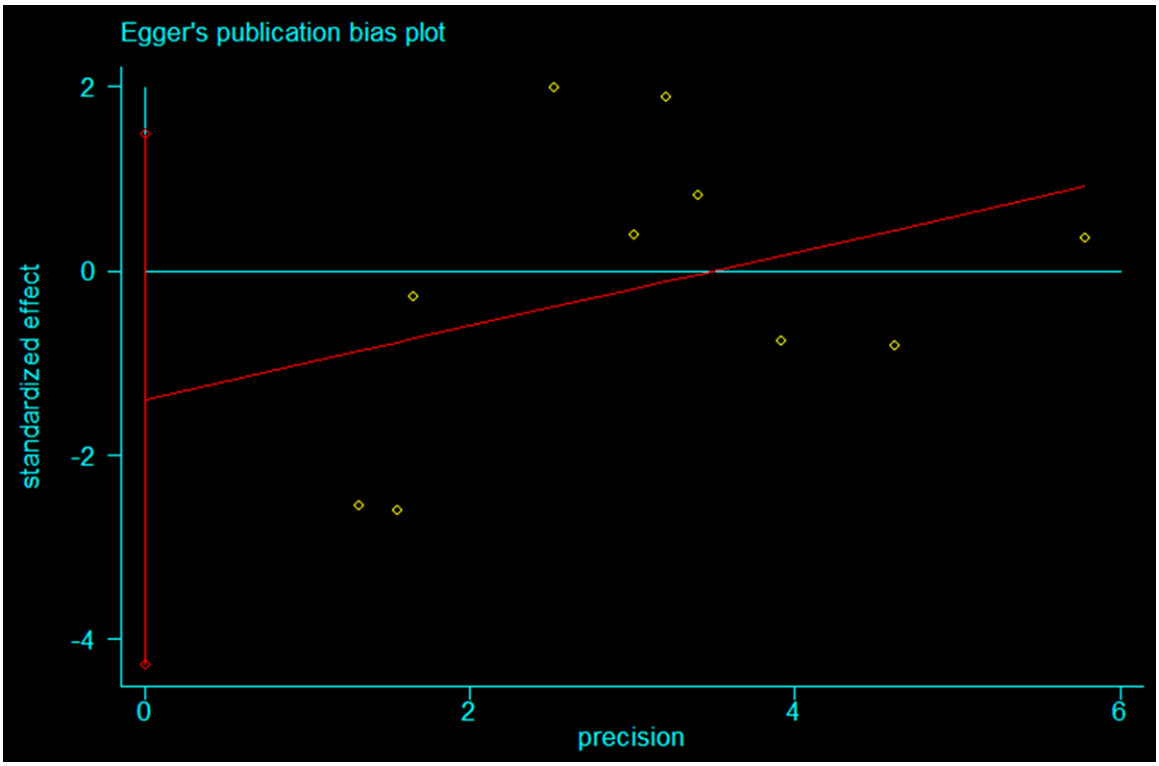

Figure 4. Egger's test was held to detect potential publication bias.

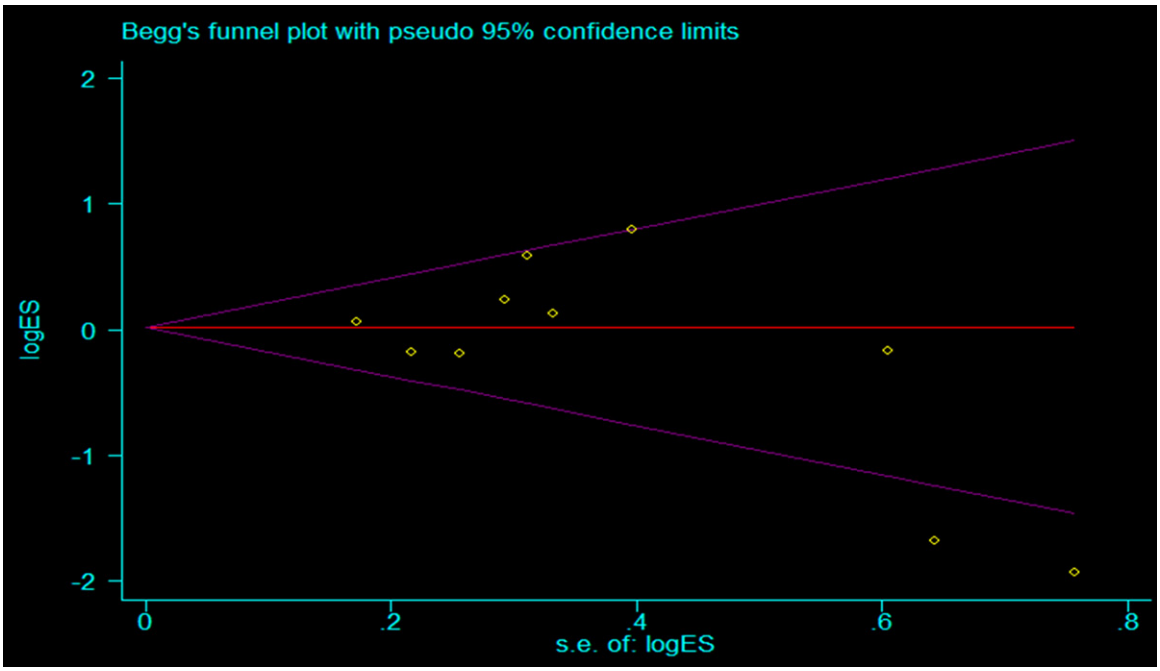

Figure 5. Begg's test was held to detect potential publication bias.

Test of heterogeneity

There was significant heterogeneity for allelic and recessive genetic model in our meta-analysis. To avoid the influence of heterogeneity among the included studies, subgroup analyses were distinctively carried out according to ethnicity, original country in Asia, sample size in

Chinese. After evaluating the source of heterogeneity, it shows that the Chinese population is a significant source of heterogeneity, especially sample size is less than 200 (**Table 2**). Other variables do not affect it.

Sensitivity analysis and publication bias

In order to evaluate the stability of the pooled results, we further conducted sensitivity analysis by sequential omission of individual studies. When omitting each study in the present meta-analysis, the pooled ORs were not materially altered , indicating that our results were statistically consistent and credible (**Figure 3**).

Both Begg's and Egger's test were performed to assess the publication bias of the literatures for the association between rs731236 polymorphism and degenerative disc disease. The shape of funnel plots did not showed obvious asymmetry. Our statistical data also did not show an evidence of publication bias (Egger's test P = 0.296; Begg's test P = 0.721) (**Figures 4**, **5**).

Discussion

Now, a variety of genetic and proteomics tools are beginning to help us to understand the molecular basis of disease. Intervertebral disc degeneration is no exception. Information gained from studies suggests that genetic factors are playing crucial roles in the onset and progression of intervertebral disc degeneration (IDD) [2, 32]. There are a lot of genes that have been associated with IDD. Vitamin D receptor gene is the first gene reported potentially related to intervertebral disc degeneration risks [26]. VDR gene is located on chromosome 12 (12q12-q14) with a length of 100 kb, and has more than 100 site polymorphisms [25].

As an important location of the VDR gene, only one meta-analysis focused on rs731236 mutation in IDD 3 years ago. It had analyzed the influence of age and ethnicity (only divided into Asian and Caucasian) on this mutation. But, perhaps because of the limitation of sample size especially from Chinese, they did not find the source of the heterogeneity. In addition, it did not perform subgroup analysis in Asian and Chinese population.

This present meta-analysis, including 1,220 patients and 1,225 controls, explored the association between rs731236 polymorphism and degenerative disc disease further. In test of heterogeneity, subgroup analyses were performed according to ethnicity, original country in Asia, sample size in Chinese. After evaluating the source of heterogeneity, we found that the Chinese population is a significant source of heterogeneity, especially sample size is less than 200. The reason for this phenomenon may be caused by the limited sample size or absence of tt genotype in South Chinese population [6, 7, 29, 31].

Although meta-analyses have been made to resolve the matter, the result should not be extrapolated blindly. We must admit that some limitations still exist in the current research. Firstly, the sample size of the included studies was not enough resulting in inadequate large-scale research on the relationship between rs731236 polymorphism and degenerative disc disease. Secondly, our result was based on unadjusted estimates, while a more precise analysis should be conducted adjusted by other factors like age, BMI, height, weight and so on. Thirdly, in the stratified analysis by ethnicity, all of them were European and Asian population. This may increase the risk of false-negative findings in all population levels. For example, original descent studies of Africans were absent in our study. Therefore, studies from other continents should be performed in the future. In addition, papers included in our articles only were written in English and Chinese, and therefore some qualified studies written in other languages were not included. Therefore, we are not sure whether there is a significant association between rs731236 polymorphism and degenerative disc disease in the whole population.

In conclusion, the results of our meta-analysis indicate that the rs731236 polymorphism may not be associated with degenerative disc disease. However, for Asians, there existed some diversities, especially in Chinese population. Hence, we cannot predict the risk of them just by the research of only one single gene but to synthesise all kinds of factors including environment, ethnicity, region and different genes. A large number of well-designed studies should be conducted to re-evaluate the relationship.

Disclosure of conflict of interest

None.

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