



## Vitamin D receptor polymorphisms may contribute to asthma risk

Ji-Chang Han MM, Jin Du MM, Ya-Jun Zhang MB, Guan-Bin Qi MM, Hong-Bing Li MB, Yi Jie Zhang MB & Xiao-ling Yu MB

To cite this article: Ji-Chang Han MM, Jin Du MM, Ya-Jun Zhang MB, Guan-Bin Qi MM, Hong-Bing Li MB, Yi Jie Zhang MB & Xiao-ling Yu MB (2016): Vitamin D receptor polymorphisms may contribute to asthma risk, Journal of Asthma, DOI: [10.3109/02770903.2016.1158267](https://doi.org/10.3109/02770903.2016.1158267)

To link to this article: <http://dx.doi.org/10.3109/02770903.2016.1158267>



Accepted author version posted online: 18 Apr 2016.  
Published online: 18 Apr 2016.



Submit your article to this journal [↗](#)



Article views: 35



View related articles [↗](#)



View Crossmark data [↗](#)

## Vitamin D receptor polymorphisms may contribute to asthma risk

Ji-Chang Han, MM, Jin Du, MM, Ya-Jun Zhang, MB, Guan-Bin Qi, MM, Hong-Bing Li, MB, Yi Jie Zhang, MB, and Xiao-ling Yu, MB

Department of Respiration, Huaihe Hospital of Henan University, Kaifeng, China

### Abstract

**Background:** Asthma is a common chronic airway disorder associated with significant morbidity and mortality. **Objective:** Current study aims at investigating the correlation between four vitamin D receptor (VDR) gene polymorphisms and asthma susceptibility by conducting a meta-analysis. **Methods:** PubMed, EBSCO, Ovid, Wiley, Web of Science, Wanfang, CNKI and VIP databases were searched using combinations of keywords relating to VDR and asthma. The published studies were filtered using our stringent inclusion and exclusion criteria, and the resultant high-quality data from final selected studies were analyzed using Stata 12.0 software. **Results:** A total of 77 studies were initially retrieved, and after further selection, 9 studies were eligible in current analysis. The selected studies contained 2,116 patients with asthma and 1,884 healthy controls. Our results demonstrated that rs2228570, rs7975232 and rs731236 in both allele models and dominant models, and rs3782905 in allele model in the VDR gene were linked with a high risk of asthma. No significant association between VDR gene rs3782905 in dominant model and risk of asthma was detected. **Conclusions:** This meta-analysis provides convincing evidence that rs2228570, rs7975232, rs731236 and rs3782905 gene polymorphisms in VDR are associated with increased susceptibility to asthma, indicating VDR polymorphisms could be developed as biomarkers for asthma susceptibility.

### Keywords

Asthma, Vitamin D receptor, polymorphism, allele model, dominant model, susceptibility, meta-analysis, case and control study

### History

Received 15 April 2016  
Revised 22 January 2016  
Accepted 21 February 2016

### Introduction

Asthma is a universal chronic airway disorder associated with significant morbidity and mortality affecting more than 300 million people worldwide, and the numbers are expected to increase substantially in the next few decades [1,2]. It is believed that 5%~10% of populations in developed countries are affected by asthma, which causes a large socio-economic burden [3]. Despite inhaled glucocorticoid treatment, many patients still need more intensive therapy [4]. It was believed that asthma is the result of interactions between multiple genetic and environmental factors [5,6]. Recently, associations between asthma and gene polymorphisms have become an attractive way to explain the heterogeneity observed in asthma and several studies have adopted this approach to understand asthma-related biological responses [7,8]. A recent study reported that genetic alterations of the vitamin D receptor (VDR) and vitamin D deficiency could initiate several diseases, including asthma [9].

VDR gene was cloned in 1988 and maps to chromosome 12q13.11 consisting of 9 exons, with at least 6 isoforms of exon 1, and encoding a 427-amino acid protein [10,11]. As an intracellular hormone receptor, VDR can mediate the biological

responses to the 1,25(OH)<sub>2</sub>D<sub>3</sub> hormone and activate vitamin D metabolite [12,13]. Four single nucleotide polymorphisms (SNPs) in VDR gene have been known through genetic associated studies, namely rs7975232 (ApaI), rs731236 (TaqI), rs2228570 (FokI) and rs3782905 [14,15]. The ApaI and TaqI polymorphisms are located near the 3' end of the VDR gene, and FokI is present in exon 2 and rs3782905 in intron 2 [16,17]. It was believed that low vitamin D status strongly correlates with poor pulmonary function and increased incidence of respiratory infections, asthma, and chronic obstructive pulmonary disease [18].

Furthermore, an epidemiological study confirmed a positive link between vitamin D deficiency and clinical features found in asthma [19]. Nevertheless, published studies on the associations between VDR polymorphisms and asthma are contradictory with each other [20]. On one hand, studies demonstrated that VDR polymorphisms may be implicated in asthma susceptibility [15, 17,19], while on the other hand, a study concluded that one of the VDR gene polymorphisms, FokI, does not significantly contribute to the development of asthma [21]. To solve the conflicting results, the four SNPs (ApaI, TaqI, FokI and rs3782905) were selected for our current study from a SNP database (dbSNP; <http://www.ncbi.nlm.nih.gov/SNP>) for analysis because those four SNPs are known to have a specified role for asthma and/or other pulmonary diseases described in the earlier researches among different populations [22–27]. Therefore, to clarify the associations between VDR gene polymorphisms and asthma susceptibility completely, we analyzed

Ji-Chang Han and Jin Du are co-first authors.

Correspondence: Ji-Chang Han, Department of Respiration, Huaihe Hospital of Henan University, Ximen Street No. 115, Kaifeng 475000, China. E-mail: [hanjichang1231@126.com](mailto:hanjichang1231@126.com)

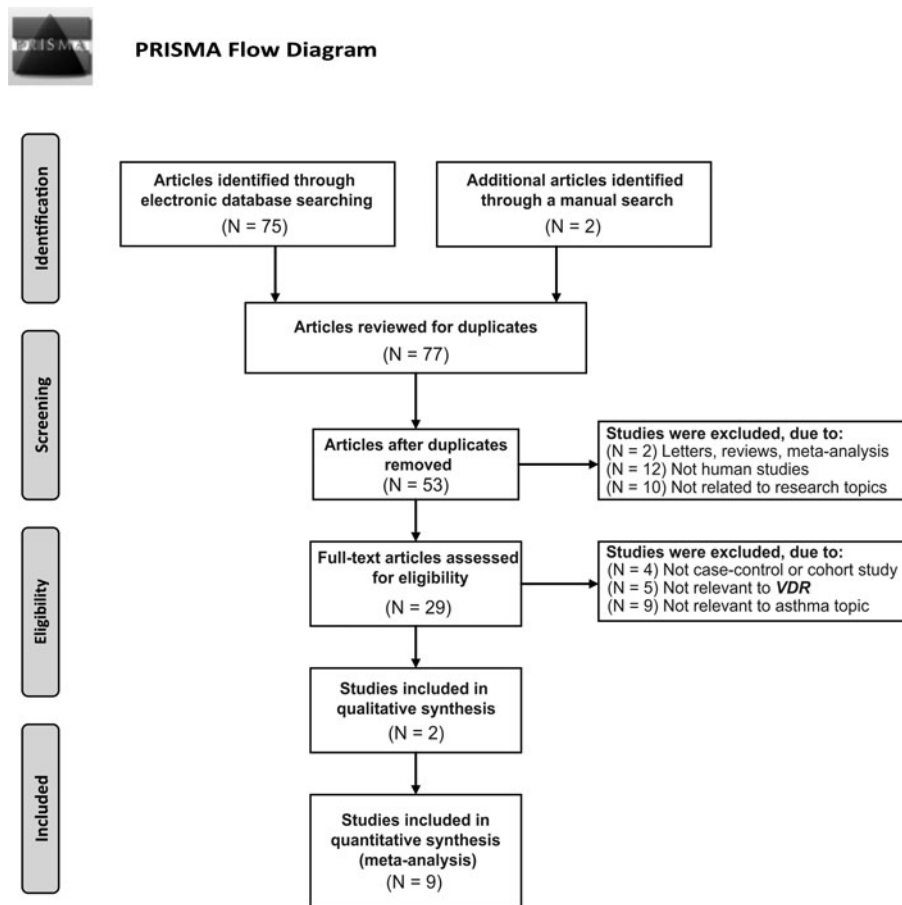


Figure 1. Flow chart of literature search and study selection. Nine studies were included in this meta-analysis.

Fok I, Apa I, Taq I, rs3782905 polymorphisms in *VDR* gene by meta-analysis.

## Materials and methods

### Literature search

Computerized bibliographic databases: PubMed, EBSCO, Ovid, Wiley, Web of Science, Wanfang, China national knowledge infrastructure (CNKI), VIP databases updated to September 2014, were searched to identify relevant studies

with the combinations of (“receptors, calcitriol” or “calcitriol receptors” or “*VDR*” or “vitamin D receptor” or “1,25-Dihydroxycholecalciferol receptors”) and (“asthma” or “bronchial asthma”). Although there were no restrictions for language, we only obtained studies with English and Chinese for the convenience of data collection. Manual research was also applied for potential relevant literature.

### Study selection

Studies that met the following criteria were enrolled in present meta-analysis: (1) case-controls design; (2) studies investigated the association of *VDR* gene polymorphism and asthma; (3) asthma patients and healthy controls that do not have allergic response to asthma allergen were respectively arranged as the subjects in case and control group; (4) literatures should provide sufficient information, such as the number in cases and controls, gender, age and gene loci (Fok I, Apa I, Taq I, rs3782905); (5) individuals in the control group were selected from the healthy crowd underwent the normal physical examination or patients from surgical department or neurology department. Included individuals in control group were excluded from asthma or any respiratory diseases, typical allergic diseases or rheumatic immune diseases, such as allergic rhinitis, conjunctivitis and rheumatoid. According to the included studies, serum IgE antibody level from each individual in control group was detected to determine allergic response using ELISA and radioimmunosorbent assays. The studies were excluded if they were (1) duplicate publication; (2) provide incomplete data; (3) provide ambiguous diagnostic criteria.

|                 | CASP01 | CASP02 | CASP03 | CASP04 | CASP05 | CASP06 | CASP07 | CASP08 | CASP09 | CASP10 | CASP11 |
|-----------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| Maalmi H(2013)  | +      | +      | +      | +      | +      | ?      | +      | +      | +      | +      | ?      |
| Ismail MF(2013) | +      | +      | +      | +      | ?      | +      | +      | +      | +      | +      | ?      |
| Pillai DK(2011) | +      | +      | +      | +      | +      | ?      | +      | +      | +      | +      | +      |
| Li F(2011)      | +      | +      | +      | +      | +      | ?      | +      | +      | +      | +      | +      |
| Zhen YF(2010)   | +      | +      | +      | +      | +      | +      | +      | +      | +      | ?      | ?      |
| Zhao M(2010)    | +      | +      | +      | +      | +      | ?      | +      | +      | +      | ?      | +      |
| Saadi A(2009)   | +      | +      | +      | +      | ?      | ?      | +      | +      | +      | +      | +      |
| Fang WL(2009)   | +      | +      | +      | +      | ?      | +      | +      | +      | +      | ?      | +      |
| Raby BA(2007)   | +      | +      | +      | +      | +      | ?      | +      | +      | +      | +      | ?      |

Figure 2. Critical Appraisal Skill Program scores for 9 included studies investigate the association of Vitamin D Receptor polymorphisms and susceptibility to asthma.

Table 1. Baseline characteristics for included studies in present meta-analysis investigating the association between Vitamin D receptor polymorphisms and increase risk for asthma susceptibility.

| First author | Year | Country | Ethnicity | Source            | SNP           |
|--------------|------|---------|-----------|-------------------|---------------|
| Maalmi H-a   | 2013 | Tunisia | Africa    | PCR-RFLP          | rs2228570 C>T |
| Maalmi H-b   | 2013 | Tunisia | Africa    | PCR-RFLP          | rs1544410 A>G |
| Maalmi H-c   | 2013 | Tunisia | Africa    | PCR-RFLP          | rs731236 A>G  |
| Maalmi H-d   | 2013 | Tunisia | Africa    | PCR-RFLP          | rs7975232 A>C |
| Ismail MF    | 2013 | Egypt   | Asian     | TaqMan array      | rs2228570 C>T |
| Pillai DK-a  | 2011 | USA     | Caucasian | TaqMan array      | rs731236 A>G  |
| Pillai DK-b  | 2011 | USA     | Caucasian | TaqMan array      | rs7975232 A>C |
| Pillai DK-c  | 2011 | USA     | Caucasian | TaqMan array      | rs2239185 A>G |
| Pillai DK-d  | 2011 | USA     | Caucasian | TaqMan array      | rs2107301 A>G |
| Pillai DK-e  | 2011 | USA     | Caucasian | TaqMan array      | rs1540339 C>T |
| Pillai DK-f  | 2011 | USA     | Caucasian | TaqMan array      | rs3782905 G>C |
| Pillai DK-g  | 2011 | USA     | Caucasian | TaqMan array      | rs2228570 C>T |
| Li F         | 2011 | China   | Asian     | PCR-RFLP          | rs2228570 C>T |
| Zhen YF      | 2010 | China   | Asian     | PCR-RFLP          | rs7975232 A>C |
| Zhao M-a     | 2010 | China   | Asian     | Direct sequencing | rs7975232 A>C |
| Zhao M-b     | 2010 | China   | Asian     | Direct sequencing | rs1544410 A>G |
| Saadi A-a    | 2009 | China   | Asian     | PCR-RFLP          | rs2228570 C>T |
| Saadi A-b    | 2009 | China   | Asian     | PCR-RFLP          | rs3782905 G>C |
| Saadi A-c    | 2009 | China   | Asian     | PCR-RFLP          | rs1544410 A>G |
| Saadi A-d    | 2009 | China   | Asian     | PCR-RFLP          | rs7975232 A>C |
| Saadi A-e    | 2009 | China   | Asian     | PCR-RFLP          | rs731236 A>G  |
| Fang WL-a    | 2009 | China   | Asian     | PCR-RFLP          | rs2228570 C>T |
| Fang WL-b    | 2009 | China   | Asian     | PCR-RFLP          | rs1544410 A>G |
| Raby BA-a    | 2007 | Germany | Caucasian | TaqMan array      | rs2228570 C>T |
| Raby BA-b    | 2007 | Germany | Caucasian | TaqMan array      | rs3782905 G>C |
| Raby BA-c    | 2007 | Germany | Caucasian | TaqMan array      | rs2239185 A>G |
| Raby BA-d    | 2007 | Germany | Caucasian | TaqMan array      | rs7975232 A>C |
| Raby BA-e    | 2007 | Germany | Caucasian | TaqMan array      | rs731236 A>G  |

Note: PB: population-based; PCR-RFLP: polymerase chain reaction–restriction fragment length polymorphism; VDR: vitamin D receptor.

## Data extraction and quality assessment

After all enrolled articles were assessed by two investigators according to the inclusion criteria, the following information were collected from each: first author's name, publication year, country, total numbers of cases and controls, ethnicity, age, detection method, gene loci (Fok I, Apa I, Taq I, rs3782905) and genotype frequencies. Discrepancies between the two investigators were settled by discussion with a third investigator. The quality assessment was conducted using Critical Appraisal Skill Program (CASP) (<http://www.casp-uk.net/#!/casp-tools-checklists/c18f8>).

## Statistical analysis

Data analysis was conducted using STATA software (Version 12.0, Stata Corp., College Station, TX, USA) for present meta-analysis. The correlations between VDR gene polymorphisms and asthma were estimated by risk ratio (RR) with 95% confidence interval (95% CI). Z test was conducted to evaluate the overall effect size RRs. Both Cochran's Q-statistic and  $I^2$  tests were applied to assess the heterogeneity [28]. The selection between fixed effects model and random effects model was based on the absence and presence of heterogeneity. Fixed effects model was selected if there was no heterogeneity, while random effects model was used for obvious heterogeneity between studies ( $P < 0.05$  or  $I^2$  test exhibited  $> 50\%$ ) [29,30]. The potential sources of heterogeneity were assessed by meta-regression analysis and Monte Carlo simulation [31]. Sensitivity analysis was conducted to determine whether the removal of one single study could influence the overall outcomes. Funnel

plots and Egger's test were used for determine the publication bias [32,33]. A bilateral test was conducted with  $P$  value of less than 0.05 considering being significant.

## Results

### Study characteristics

The procedures of study selection are shown in Figure 1. Utilizing our predefined search strategy, we identified a total of 77 potential publications from our initial search. After removing 2 duplicated studies, 24 studies were excluded either because they were letters, reviewer studies, meta-analysis, irrelevant, or not about human studies, 51 studies were remained for further evaluations, resulting in exclusion of an additional 40 studies. Eleven relevant publications were selected for inclusion in our analysis. After further examination of the data from these 11 studies, 2 more studies were excluded because of insufficient data. Ultimately 9 published studies (between 2007 and 2013) met the eligibility criteria, and contained 2,116 asthma patients and 1,884 healthy controls [14,15, 17,18, 21,34–37]. Detection methods included in those eligible studies were direct sequencing, polymerase chain reaction with the restriction fragment length polymorphism (PCR-RFLP) and TaqMan assay. CASP scores and baseline characteristics for eligible studies are presented in Figure 2 and Table 1, respectively.

### FokI polymorphism

A total of 7 studies measured the correlation between FokI polymorphism in VDR gene and asthma. No significant

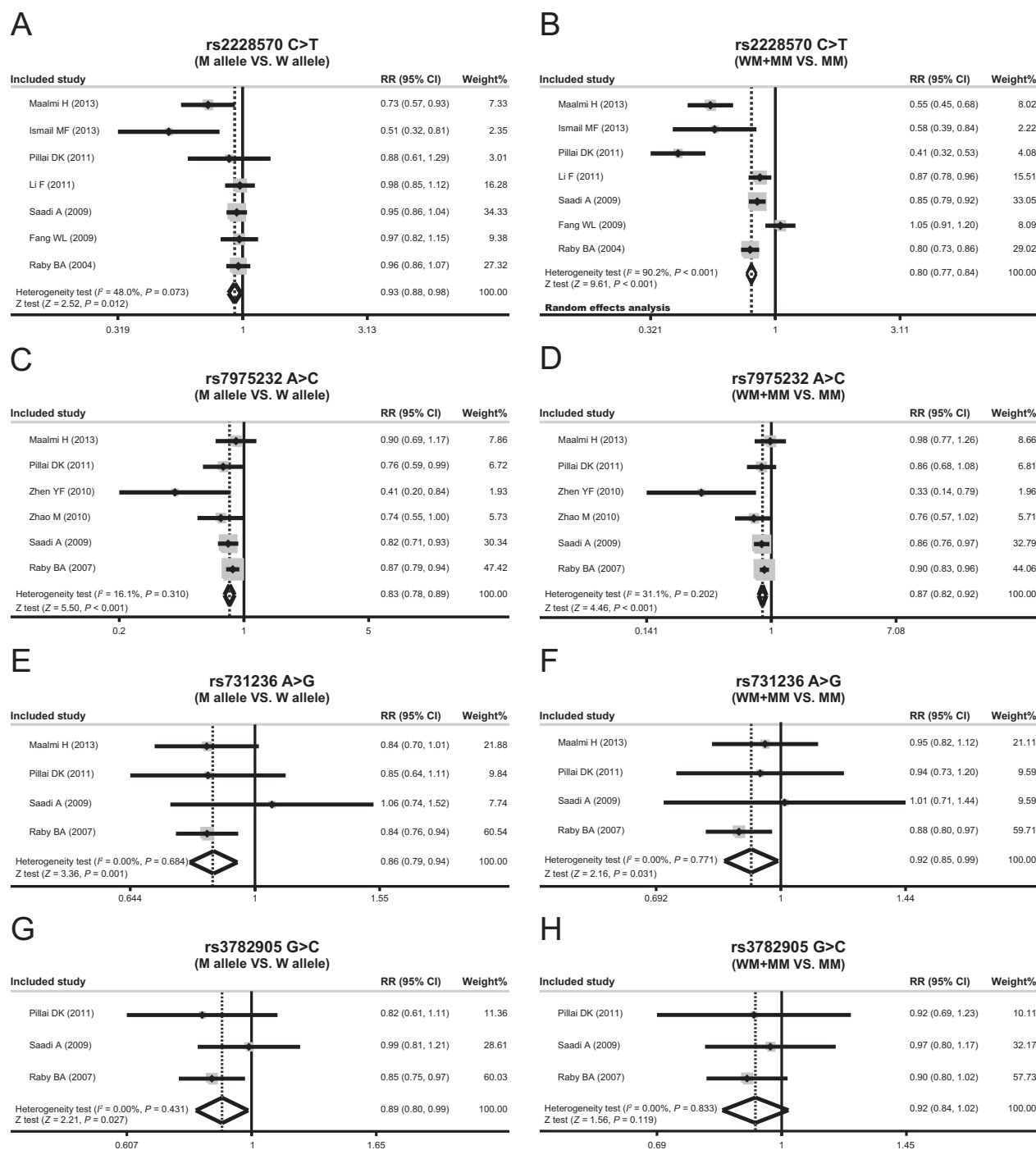


Figure 3. Forest plot in present meta-analysis investigates the single nucleotide polymorphisms of Vitamin D Receptor contribute to an increase risk for asthma susceptibility in fixed effect model (A, rs2228570 C>T; B, rs2228570 C>T; C, rs7975232 A>C; D, rs7975232 A>C; E, rs731236 A>G; F, rs731236 A>G; G, rs3782905 G>C; H, rs3782905 G>C).

heterogeneity was found on allele model ( $I^2 = 48\%$ ,  $P = 0.073$ ), thus fixed effects model was applied, whereas random effects model was applied in dominant model for presence of significant heterogeneity ( $I^2 = 90.2\%$ ,  $P < 0.001$ ). The result in our meta-analysis demonstrated that FokI polymorphism in *VDR* gene may contribute to asthma susceptibility (allele model:  $RR = 0.93$ , 95%  $CI = 0.88-0.98$ ,  $P = 0.012$ ; dominant model:  $RR = 0.81$ , 95%  $CI = 0.77-0.84$ ,  $P < 0.001$ ) (Figures 3A, 3B and Table 2).

### Apal polymorphism

Among the nine included literature, 6 studies explored the association between Apal polymorphism in *VDR* gene and asthma. Fixed effects model was utilized on both allele model and dominant model with no significant heterogeneity being discovered (allele model:  $I^2 = 16.1\%$ ,  $P = 0.310$ ; dominant model:  $I^2 = 31.1\%$ ,  $P = 0.202$ ). A significant association was found between Apal polymorphism and asthma, which implying that Apal polymorphism in *VDR* gene may be associated with asthma susceptibility (allele model:  $RR = 0.83$ , 95%

Table 2. Comparisons of genotype and allele frequencies between the case and the control groups investigating the association between Vitamin D Receptor polymorphisms and increase risk for asthma susceptibility.

|                                      | rs2228570 |           |        | rs7975232 |           |        | rs731236 |           |       | rs3782905 |           |       |
|--------------------------------------|-----------|-----------|--------|-----------|-----------|--------|----------|-----------|-------|-----------|-----------|-------|
|                                      | RR        | 95%CI     | P      | RR        | 95%CI     | P      | RR       | 95%CI     | P     | RR        | 95%CI     | P     |
| M allele vs. W allele (Allele model) | 0.93      | 0.88–0.98 | 0.012  | 0.83      | 0.78–0.89 | <0.001 | 0.86     | 0.79–0.94 | 0.001 | 0.89      | 0.80–0.99 | 0.027 |
| WM + MM vs. WW (Dominant model)      | 0.81      | 0.77–0.84 | <0.001 | 0.87      | 0.82–0.93 | <0.001 | 0.92     | 0.85–0.99 | 0.031 | 0.92      | 0.84–1.02 | 0.119 |
| MM vs. WW (Homozygous model)         | 0.85      | 0.77–0.84 | 0.014  | 0.68      | 0.59–0.78 | <0.001 | 0.66     | 0.53–0.82 | 0.001 | 0.67      | 0.49–0.92 | 0.014 |
| MM vs. WM (Heterozygous model)       | 1.04      | 0.99–1.09 | 0.101  | 1.09      | 1.02–1.15 | 0.006  | 1.1      | 1.03–1.17 | 0.003 | 1.05      | 1.00–1.11 | 0.058 |
| MM vs. WW + WM (Recessive model)     | 0.85      | 0.73–0.99 | 0.033  | 0.7       | 1.02–1.15 | <0.001 | 0.65     | 0.51–0.83 | 0.001 | 0.68      | 0.49–0.94 | 0.019 |

Note: RR: relative risk; 95%CI: 95% confidential intervals.

CI = 0.78–0.89,  $P < 0.001$ ; dominant model: RR = 0.87, 95% CI = 0.82–0.92,  $P < 0.001$ ) (Figures 3C, 3D and Table 2).

TaqI polymorphism

A total of 4 studies measured the association between TaqI polymorphism in *VDR* gene and asthma in this meta-analysis. There is no significant heterogeneity on both allele model and dominant model (allele model:  $I^2 < 50\%$ ,  $P = 0.684$ ; dominant model:  $I^2 < 50\%$ ,  $P = 0.771$ ). Therefore, the fixed effects model was utilized on both allele model and dominant model. The result in present meta-analysis showed that TaqI polymorphism in *VDR* gene may increase the susceptibility of asthma (allele model: RR = 0.86, 95% CI = 0.79–0.94,  $P = 0.001$ ; dominant model: RR = 0.92, 95% CI = 0.85–0.99,  $P = 0.031$ ) (Figures 3E, 3F and Table 2).

rs3782905 polymorphism

Altogether 3 studies measured the correlation between *VDR* gene rs2228570 polymorphism and asthma. No significant heterogeneity was found on both allele model and dominant model, thus fixed effects model was utilized (allele model:  $I^2 < 50\%$ ,  $P = 0.431$ ; dominant model:  $I^2 < 50\%$ ,  $P = 0.833$ ). The result in our meta-analysis implied that rs3782905 polymorphism in *VDR* gene may contribute to asthma susceptibility (allele model: RR = 0.89, 95% CI = 0.80–0.99,  $P = 0.027$ ), however, a similar correlation was not found in dominant model: RR = 0.92, 95% CI = 0.84–1.02,  $P = 0.119$ ) (Figures 3G, 3H and Table 2).

Regression analyses, sensitivity analyses and publication bias

According to the meta-regression analysis, years of publication, sample size, ethnicity, country, SNP and detection method were not the key resources for heterogeneity (all  $P > 0.05$ ) (Figure 4 and Table 3). Sensitivity analysis was performed, and the results in our study demonstrated that no single study had the weight to impact on the association between polymorphism

in *VDR* gene and an increased risk of asthma (Figure 5). The graphical funnel plots of case and control group for *VDR* gene FokI, ApaI and TaqI in both the allele and dominant models, as well as rs3782905 in dominant models presented asymmetric and Egger’s test suggested for significant heterogeneity (all  $P < 0.05$ ) (Figure 6). The funnel plots for *VDR* gene rs3782905 in allele model were symmetrically distributed and the Egger’s test indicated no publication bias existed ( $P = 0.144$ ).

Discussion

Previous studies involving genetic associations between *VDR* polymorphisms and asthma are conflicting, and the role of *VDR* polymorphisms remained unclear, likely due to limited sample size, lifestyle differences and geographic variations [20,38]. To overcome the limitations of individual studies, we performed meta-analysis by combining data from published studies to estimate the associations of Fok I, Apa I, Taq I, rs3782905 polymorphisms in *VDR* gene with asthma susceptibility. Moreover, the clear association between *VDR* polymorphisms and asthma risk contributes to the increased understanding of genetic and asthma pathogenesis and sheds a little light for further studies. Our analyses suggested that Fok I, Apa I, Taq I, rs3782905 polymorphisms in *VDR* gene may contribute to asthma susceptibility, which implied that *VDR* polymorphisms might be, directly or indirectly, involved in mechanisms related to asthma.

*VDR* gene is considered as a pleiotropic gene, associated with multiple autoimmune, inflammatory, and allergy diseases [39]. Our conclusions are supported by previous studies which identified the linkage between *VDR* polymorphisms and asthma and suggested that *VDR* plays a role in the development of asthma [18]. FokI, ApaI, TaqI and rs3782905 have previously been associated with atopic diseases, such as allergic asthma [40,41]. Several articles regarding the *VDR* SNPs with lung function showed that FokI polymorphism in *VDR* may contribute to increased risk of tuberculosis and TaqI, BsmI and ApaI gene polymorphism are associated with the risk of lung cancer [42,43]. As the common polymorphic sites in *VDR*,

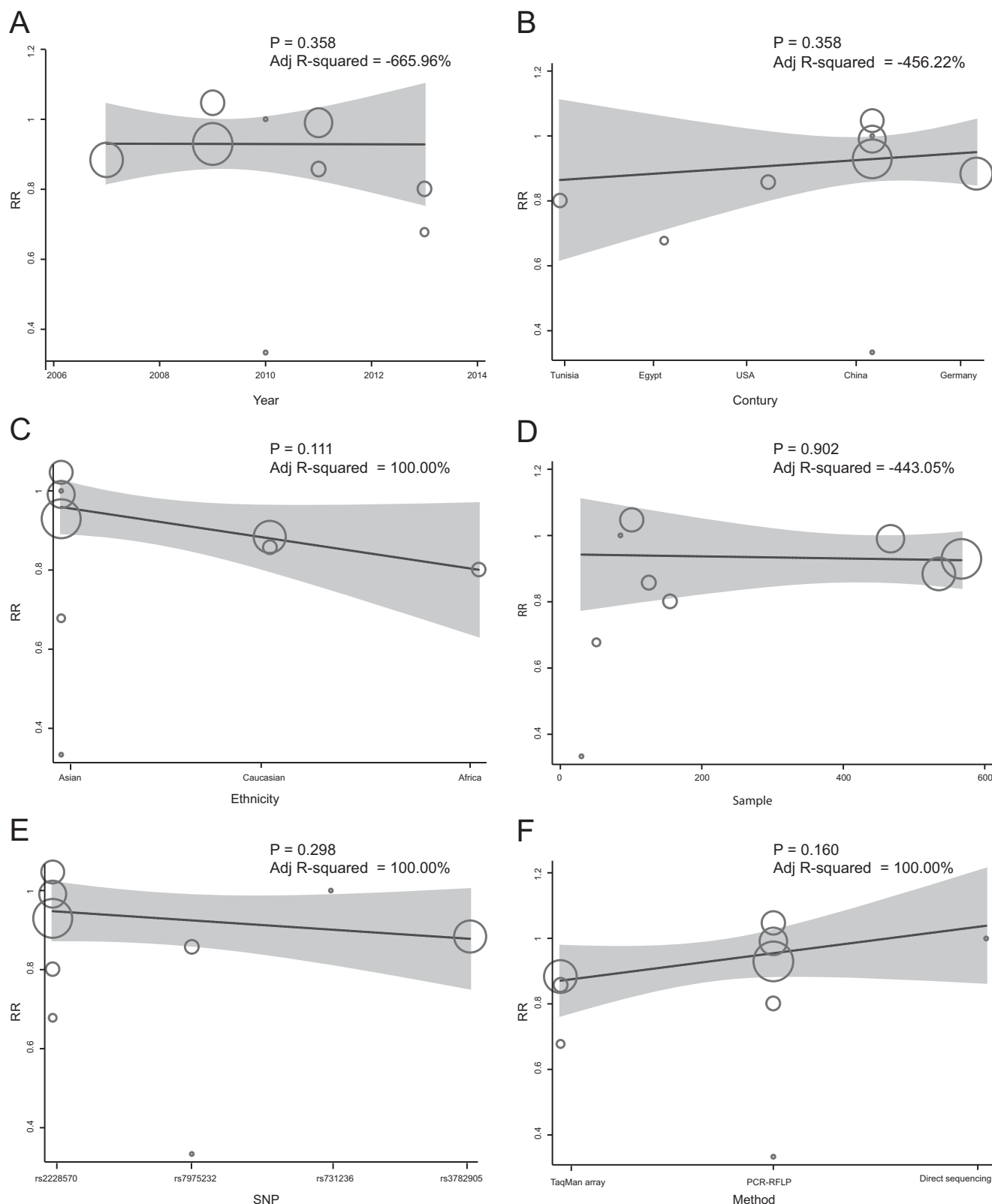


Figure 4. Meta-regression analysis on potential sources of heterogeneity in present meta-analysis investigates Vitamin D Receptor polymorphisms contribute to an increase risk for asthma susceptibility (A, Year; B, Country; C, Ethnicity; D, Sample; E, SNP; F, Method).

ApaI and TaqI sites have been shown to influence gene transcription and mRNA stability [44]. For the TaqI polymorphism, our findings are also in concordance with the results of Maalmi H, which reported association between this polymorphism and asthma in African [19].

A previous study identified that the *VDR* Taq1 'T' allele was related with obesity, as well as elevated triglyceride levels and HDL levels, both of those two increased levels suggest an exacerbation of lipid accumulation and development of atherosclerosis [45]. The polymorphisms of ApaI and

Table 3. Meta-regression analysis of potential sources of heterogeneity in present meta-analysis investigating the association between Vitamin D Receptor polymorphisms and increase risk for asthma susceptibility.

| Heterogeneity factors | Coefficient | SE    | <i>t</i> | P (Adjusted) | 95% CI  |         |
|-----------------------|-------------|-------|----------|--------------|---------|---------|
|                       |             |       |          |              | LL      | UL      |
| Year                  | 0.015       | 0.034 | 0.45     | 0.961        | − 0.093 | 0.124   |
| Country               | 0.226       | 0.122 | 1.85     | 0.248        | − 0.162 | 0.614   |
| Ethnicity             | 0.194       | 0.16  | 1.21     | 0.626        | − 0.316 | 0.704   |
| Method                | 0.033       | 0.15  | 0.22     | 0.999        | − 0.611 | − 0.679 |
| SNP                   | − 0.149     | 0.085 | − 1.73   | 0.311        | − 0.423 | 0.124   |
| Sample                | − 0.001     | 0.001 | − 1.3    | 0.563        | − 0.001 | 0.001   |

Notes: SE: Standard Error; LL: Lower Limit; UL: Upper Limit.

FokI are known restriction sites, suggesting they exert their effect through regulation on gene expression [17]. Studies demonstrated that FokI polymorphisms could interfere with transcription factors in immune-mediated diseases and were found to be significantly associated with decreased pulmonary functions, increased asthma severity, and uncontrolled disease status in addition to elevated IgE levels and hypovitaminosis D, suggesting that FokI mutation conveys an immunoregulatory effect through alternation in vitamin D metabolism [14,17].

VDR and its ligand 1.25 (OH)2D and the retinoid X receptor (RXR) generate an heterodimer which bind to the vitamin D response element play a regulating loop on vitamin D metabolites levels [19]. The change of a single Fok I restriction site can result in the alteration of ATG start cordon in the *VDR* and the alternations happened in the rs1544410 site can lead to significant influence on protein expression [15,21]. The potential mechanism for the association between polymorphisms in *VDR* and asthma risk may be explained by the fact that both vitamin D and *VDR* have significant anti-inflammatory and anti-infectious functions [46,47]. Specifically, genes encoding pro-inflammatory and anti-inflammatory cytokines involved in the development of airway inflammation are the targets of vitamin D and reduced activity of *VDR*-dependent signaling pathways caused by *VDR* polymorphisms would ultimately influence the rate of transcription of these genes and then affect asthma [15,48].

The TaqI and ApaI SNPs were considered to be associated with other functional polymorphisms, thus involving in a complicated gene network, which may enhance or inhibit the expression of *VDR* target genes. Moreover, *VDR* polymorphisms may influence the concentration of Vitamin D metabolites, which may contribute to the regulation of vitamin D pathway [49]. Vitamin D insufficiency is correlated with lower pulmonary function, including forced expiratory volume in 1 second and forced vital capacity [50]. Furthermore, Vitamin D deficiency is considered as a risk factor for asthma and genetic factors may also contribute to vitamin D deficiency status by feedback loop regulation [51]. The vitamin D exerts its protective effect in asthma could by its immunomodulatory properties, which was proved to have effects on epithelial cell, T-cell, B-cell, and dendritic cell functions, which are important for innate and adaptive immunity [52].

In contrast to our results, a study by Kalthoum Tizaoui demonstrated that no significant association was found between ApaI polymorphism and asthma risk [20]. The ApaI

and TaqI were found had no relation with asthma, which was disagreement with our results [53]. The expressions of gene depend on not only the variations of genetics, but also on ethnicity, environment factors and their complicated interactions [54]. Despite environmental, cultural differences, the racial morbidity and mortality disparities that exist in asthma suggest a genetic influence [17]. The interactions between different genes and/or environmental factors play a role in the action of *VDR* [55]. Therefore, a possible explanation for these discrepancies could be that ethnic characteristics, geographical differences and living habits might be the important reasons for these inconsistent results.

Although genome-wide association studies found limited information on the association between the four SNPs and pathogenesis of asthma [46,48], our study found that significant role of *VDR* SNP in pathogenesis of asthma. There are several limitations that should be noted while interpreting the results. First, only four polymorphisms in the *VDR* gene have been studied, although there are several other functional *VDR* polymorphisms in the complex promoter region of the *VDR* gene. Second, asthma is a disease in which both genetic and environmental factors play important roles, and we could not account for the environmental risk factors in this study. Third, the sample sizes in case group was slightly larger than that in the control group, which may have minimum influence on our overall results. Moreover, although we investigated the four SNPs of *VDR* in asthma, no cumulative effect of the combined SNPs in asthma was investigated, which can be considered as a topic for future studies. In addition, although IgE data were obtained from each individual, we double-checked the included studies and failed to find any information regarding the atopy stratification on patients, which might confound SNP analysis and limit the distinction of multiple phenotypes of asthma. Finally, selection bias may have been introduced because the majority of included studies were in Asian population.

In summary, we have identified that multiple *VDR* polymorphisms may contribute or combine together to increase the susceptibility to asthma. We propose that *VDR* polymorphisms may be considered as biomarkers for asthma susceptibility. Further studies on the interactions between *VDR* polymorphisms and related risk factors or the cumulative effect of combined SNPs with asthma, involving a large sample size and comprehensive knowledge of genetic and environmental factors, may strengthen our conclusions and elucidate new approaches for asthma prevention and treatment.

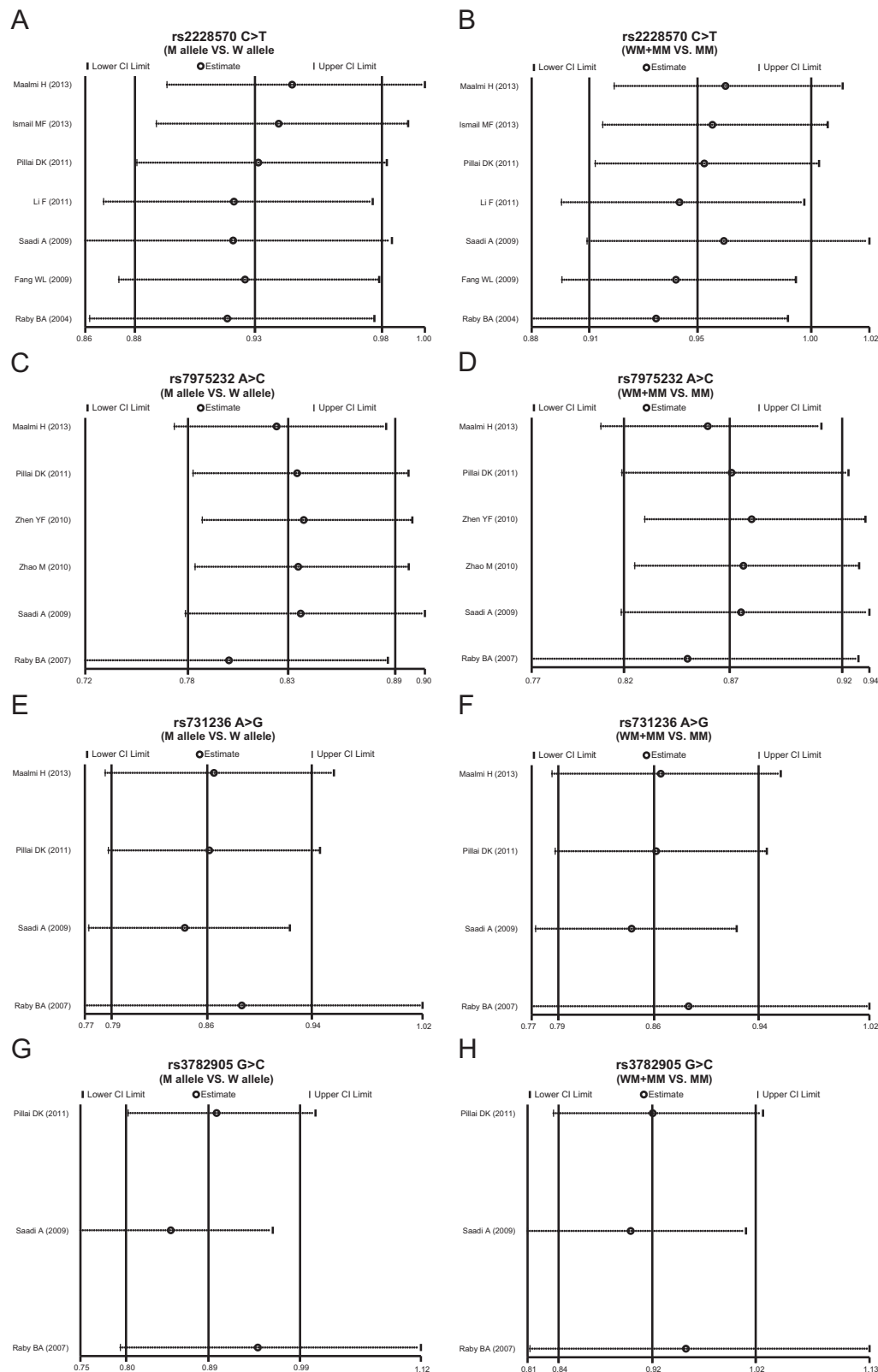


Figure 5. Sensitivity analysis in present meta-analysis investigates the single nucleotide polymorphisms of Vitamin D Receptor contribute to an increase risk for asthma susceptibility (A, rs2228570 C>T; B, rs2228570 C>T; C, rs7975232 A>C; D, rs7975232 A>C; E, rs731236 A>G; F, rs731236 A>G; G, rs3782905 G>C; H, rs3782905 G>C).

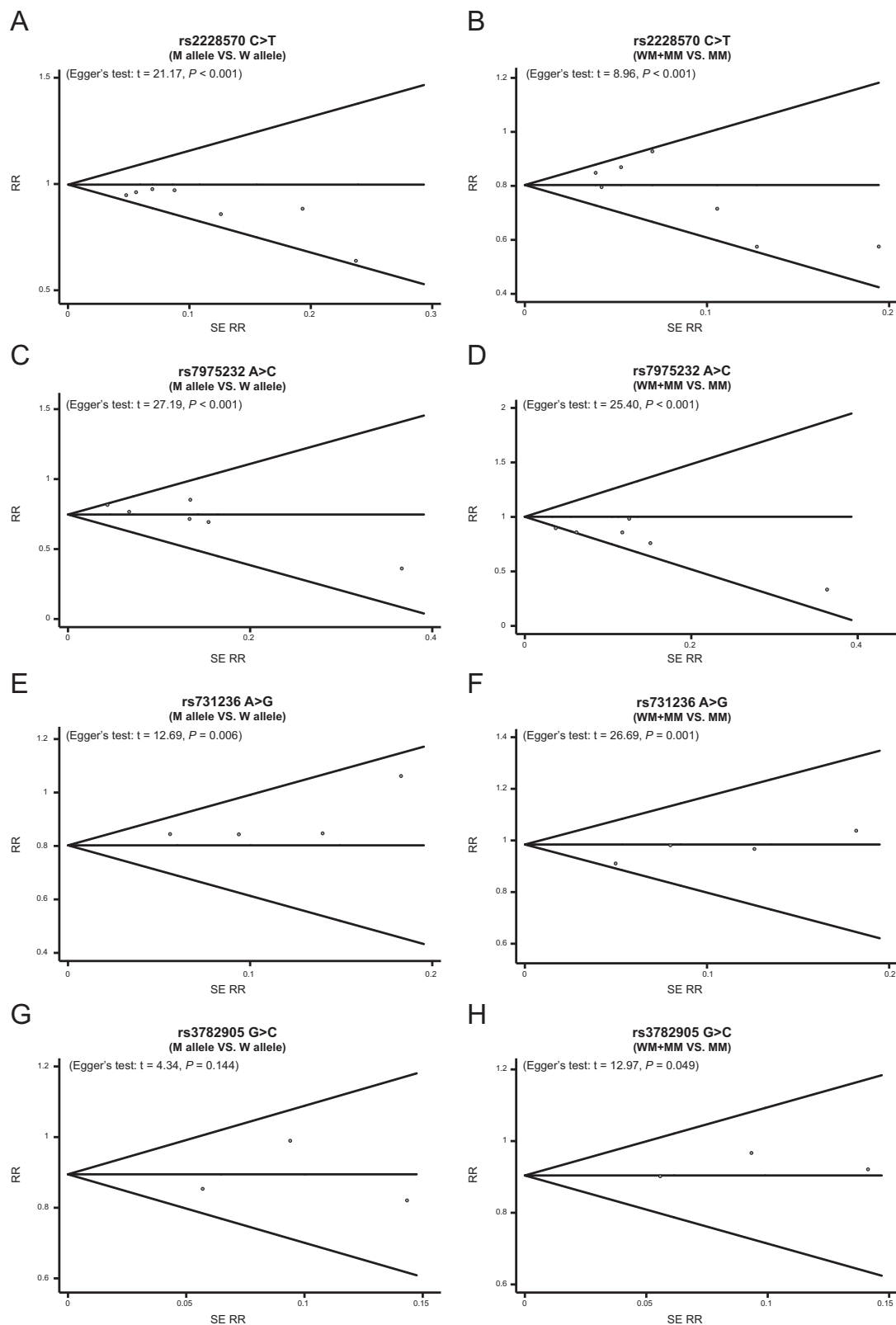


Figure 6. Publication biases in present meta-analysis investigate the single nucleotide polymorphisms of Vitamin D Receptor contribute to an increase risk for asthma susceptibility (A, rs2228570 C>T; B, rs2228570 C>T; C, rs7975232 A>C; D, rs7975232 A>C; E, rs731236 A>G; F, rs731236 A>G; G, rs3782905 G>C; H, rs3782905 G>C).

## Acknowledgements

We would like to acknowledge the reviewers for their helpful comments on this manuscript.

## Funding

This study was supported by a grant from Henan Provincial Health Department of Science and Technology Innovation in Young Talent Project.

## References

- Wang C, Rose-Zerilli MJ, Koppelman GH, Sandling JK, Holloway JW, Postma DS. Evidence of association between interferon regulatory factor 5 gene polymorphisms and asthma. *Gene* 2012;504:220–225.
- Chung LP, Waterer G, Thompson PJ. Pharmacogenetics of beta2 adrenergic receptor gene polymorphisms, long-acting beta-agonists and asthma. *Clin Exp Allergy* 2011;41:312–326.
- Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716–725.
- Song CH, Lee JK. Lebrikizumab treatment in adults with asthma. *N Engl J Med* 2011;365:2433; author reply 2433–2434.
- Mukherjee AB, Zhang Z. Allergic asthma: influence of genetic and environmental factors. *J Biol Chem* 2011;286:32883–32889.
- Bijanzadeh M, Mahesh PA, Ramachandra NB. An understanding of the genetic basis of asthma. *Indian J Med Res* 2011;134:149–161.
- Binia A, Van Stiphout N, Liang L, Michel S, Bhavsar PK, Fan Chung K. A polymorphism affecting MYB binding within the promoter of the PDCD4 gene is associated with severe asthma in children. *Hum Mutat* 2013;34:1131–1139.
- Harada M, Hirota T, Jodo AI, Hitomi Y, Sakashita M, Tsunoda T. Thymic stromal lymphopoietin gene promoter polymorphisms are associated with susceptibility to bronchial asthma. *Am J Respir Cell Mol Biol* 2011;44:787–793.
- Ben-Zvi I, Aranow C, Mackay M, Stanevsky A, Kamen DL, Marinescu LM. The impact of vitamin D on dendritic cell function in patients with systemic lupus erythematosus. *PLoS One* 2010;5:e9193.
- Baig S, Moatter T. Vitamin D is not just about healthy bones anymore. *J Coll Phys Surg Pak* 2010;20:355–356.
- Gezen-Ak D, Dursun E, Bilgic B, Hanagasi H, Ertan T, Gurvit H. Vitamin D receptor gene haplotype is associated with late-onset Alzheimer's disease. *Tohoku J Exp Med* 2012;228:189–196.
- Touvier M, Chan DS, Lau R, Aune D, Vieira R, Greenwood DC. Meta-analyses of vitamin D intake, 25-hydroxyvitamin D status, vitamin D receptor polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2011;20:1003–1016.
- Haussler MR, Jurutka PW, Mizwicki M, Norman AW. Vitamin D receptor (VDR)-mediated actions of 1 $\alpha$ ,25(OH) $_2$ vitamin D(3): genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab* 2011;25:543–559.
- Ismail MF, Elnady HG, Fouda EM. Genetic variants in vitamin D pathway in Egyptian asthmatic children: a pilot study. *Hum Immunol* 2013;74:1659–1664.
- Li F, Jiang L, Willis-Owen SA, Zhang Y, Gao J. Vitamin D binding protein variants associate with asthma susceptibility in the Chinese Han population. *BMC Med Genet* 2011;12:103.
- Wang S, Wang X, Wu J, Lin Y, Chen H, Zheng X. Association of vitamin D receptor gene polymorphism and calcium urolithiasis in the Chinese Han population. *Urol Res* 2012;40:277–284.
- Pillai DK, Iqbal SF, Benton AS, Lerner J, Wiles A, Foerster M. Associations between genetic variants in vitamin D metabolism and asthma characteristics in young African Americans: a pilot study. *J Invest Med* 2011;59, 938–946.
- Berraies A, Hamzaoui K, Hamzaoui A. Link between vitamin D and airway remodeling. *J Asthma Allergy* 2014;7:23–30.
- Maalmi H, Sassi FH, Berraies A, Ammar J, Hamzaoui K, Hamzaoui A. Association of vitamin D receptor gene polymorphisms with susceptibility to asthma in Tunisian children: A case control study. *Hum Immunol* 2013;74:234–240.
- Tizaoui K, Berraies A, Hamdi B, Kaabachi W, Hamzaoui K, Hamzaoui A. Association of Vitamin D receptor gene polymorphisms with asthma risk: systematic review and updated meta-analysis of case-control studies. *Lung* 2014;192:955–965.
- Fang WL, Gao LB, Liang WB, Xue H, Bai P, Lv ML. Association analysis of vitamin D receptor gene polymorphisms in chinese population with asthma. *Iran J Allergy Asthma Immunol* 2009;8:141–147.
- Poon AH, Laprise C, Lemire M, Montpetit A, Sinnett D, Schurr E, Hudson TJ. Association of vitamin d receptor genetic variants with susceptibility to asthma and atopy. *Am J Respir Crit Care Med* 2004;170:967–973.
- Raby BA, Lazarus R, Silverman EK, Lake S, Lange C, Wjst M, Weiss ST. Association of vitamin d receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med* 2004;170:1057–1065.
- Wjst M. Variants in the vitamin D receptor gene and asthma. *BMC Genet* 2005;6:2.
- Liu W, Cao WC, Zhang CY, Tian L, Wu XM, Habbema JD, Zhao QM, et al. Vdr and nramp1 gene polymorphisms in susceptibility to pulmonary tuberculosis among the chinese han population: A case-control study. *Int J Tuberc Lung Dis* 2004;8:428–434.
- Bornman L, Campbell SJ, Fielding K, Bah B, Sillah J, Gustafson P, Manneh K, et al. Vitamin D receptor polymorphisms and susceptibility to tuberculosis in west africa: A case-control and family study. *J Infect Dis* 2004;190:1631–1641.
- Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, Wright D, et al. Influence of vitamin D deficiency and vitamin d receptor polymorphisms on tuberculosis among gujarati asians in west london: A case-control study. *Lancet* 2000;355:618–621.
- Zintzaras E, Ioannidis JP. HEGESMA: genome search meta-analysis and heterogeneity testing. *Bioinformatics* 2005;21:3672–3673.
- Zintzaras E, Ioannidis JP. Heterogeneity testing in meta-analysis of genome searches. *Genet Epidemiol* 2005;28:123–137.
- Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002;21:1539–1558.
- Ferrenberg AM, Swendsen RH. New Monte Carlo technique for studying phase transitions. *Phys Rev Lett* 1988;61:2635–2638.
- Peters JL, Sutton AJ, Jones DR, Abrams KR, Rushton L. Contour-enhanced meta-analysis funnel plots help distinguish publication bias from other causes of asymmetry. *J Clin Epidemiol* 2008;61:991–996.
- Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997;315:629–634.
- Raby BA, Lazarus R, Silverman EK, Lake S, Lange C, Wjst M. Association of vitamin D receptor gene polymorphisms with childhood and adult asthma. *Am J Respir Crit Care Med* 2004;170:1057–1065.
- Saadi A, Gao G, Li H, Wei C, Gong Y, Liu Q. Association study between vitamin D receptor gene polymorphisms and asthma in the Chinese Han population: a case-control study. *BMC Med Genet* 2009;10:71.
- Zhao M, Zhao J, Dai Y. [Effects of VDR gene on the association between blood lead level and childhood asthma]. 2010;04:267–271.
- Zhen Y, Wang L. Association of vitamin D receptor gene polymorphisms with children asthma and wheezy bronchitis. *China Prevent Med* 2010;11:1055–1058.
- Cantorna MT, Zhao J, Yang L. Vitamin D, invariant natural killer T-cells and experimental autoimmune disease. *Proc Nutr Soc* 2012;71:62–66.
- Tuller T, Atar S, Ruppel E, Gurevich M, Achiron A. Common and specific signatures of gene expression and protein-protein interactions in autoimmune diseases. *Genes Immun* 2013;14:67–82.
- Heine G, Hoefer N, Franke A, Nothling U, Schumann RR, Hamann L. Association of vitamin D receptor gene polymorphisms with severe atopic dermatitis in adults. *Br J Dermatol* 2013;168:855–858.
- Grant DJ, Hoyo C, Akushevich L, Iversen ES, Whitaker R, Marks J. Vitamin D receptor (VDR) polymorphisms and risk of ovarian cancer in Caucasian and African American women. *Gynecol Oncol* 2013;129:173–178.
- Huang L, Liu C, Liao G, Yang X, Tang X, Chen J. Vitamin D receptor gene foki polymorphism contributes to increasing the risk of tuberculosis: An update meta-analysis. *Medicine (Baltimore)* 2015;94:e2256.

43. Zhong H, Zhou R, Feng Y, Zheng GX, Liang Y, Zhang JY, Qin XQ, et al. Association of vitamin d receptor gene polymorphism with the risk of lung cancer: A meta-analysis. *J Recept Signal Transduct Res* 2014;34:500–505.
44. Zhou TB, Jiang ZP, Huang MF, Su N. Association of vitamin D receptor FokI (rs2228570), TaqI (rs731236) and ApaI (rs7975232) gene polymorphism with the risk of chronic kidney disease. *J Recept Signal Transduct Res* 2014;35:1–5.
45. Vasilopoulos Y, Sarafidou T, Kotsa K, Papadimitriou M, Goutzelas Y, Stamatis C. VDR TaqI is associated with obesity in the Greek population. *Gene* 2013;512:237–239.
46. Sun J. Vitamin D and mucosal immune function. *Curr Opin Gastroenterol* 2010;26:591–595.
47. Van Belle TL, Gysemans C, Mathieu C. Vitamin D in autoimmune, infectious and allergic diseases: a vital player?. *Best Pract Res Clin Endocrinol Metab* 2011;25:617–632.
48. Khoo AL, Joosten I, Michels M, Woestenenk R, Preijers F, He XH. 1,25-Dihydroxyvitamin D3 inhibits proliferation but not the suppressive function of regulatory T cells in the absence of antigen-presenting cells. *Immunology* 2011;134:459–468.
49. Papadopoulou A, Kouis P, Middleton N, Kolokotroni O, Karpathios T, Nicolaidou P, Yiallourou PK. Association of vitamin D receptor gene polymorphisms and vitamin d levels with asthma and atopy in cypriot adolescents: A case-control study. *Multidiscip Respir Med* 2015;10:26.
50. Ginde AA, Mansbach JM, Camargo CA, Jr. Vitamin D, respiratory infections, and asthma. *Curr Allergy Asthma Rep* 2009;9:81–87.
51. Maalmi H, Sassi FH, Berraies A, Ammar J, Hamzaoui K, Hamzaoui A. Association of vitamin D receptor gene polymorphisms with susceptibility to asthma in tunisian children: A case control study. *Hum Immunol* 2013;74:234–240.
52. Man L, Zhang Z, Zhang M, Zhang Y, Li J, Zheng N, Cao Y, et al. Association between vitamin d deficiency and insufficiency and the risk of childhood asthma: Evidence from a meta-analysis. *Int J Clin Exp Med* 2015;8:5699–5706.
53. Einisman H, Reyes ML, Angulo J, Cerda J, Lopez-Lastra M, Castro-Rodriguez JA. Vitamin D levels and vitamin D receptor gene polymorphisms in asthmatic children: a case-control study. *Pediatr Allergy Immunol*, vol., pp., 2015;26:545–550.
54. van Etten E, Verlinden L, Giulietti A, Ramos-Lopez E, Branisteanu DD, Ferreira GB. The vitamin D receptor gene FokI polymorphism: functional impact on the immune system. *Eur J Immunol* 2007;37:395–405.
55. Bossé Y, Lemire M, Poon AH, Daley D, He JQ, Sandford A. Asthma and genes encoding components of the vitamin D pathway. *Respir Res* 2009;10:98.
56. Moffatt MF, Gut IG, Demenais F, Strachan DP, Bouzigon E, Heath S. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010;363:1211–1221.
57. Fang WL, Gao LB, Liang WB, Xue H, Bai P, Lv ML, Wang YY, et al. Association analysis of vitamin d receptor gene polymorphisms in chinese population with asthma. *Iran J Allergy Asthma Immunol* 2009;8:141–147.
58. Pillai DK, Iqbal SF, Benton AS, Lerner J, Wiles A, Foerster M, Ozedime T, et al. Associations between genetic variants in vitamin D metabolism and asthma characteristics in young african americans: A pilot study. *J Investig Med* 2011;59:938–946.