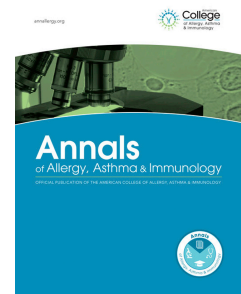


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Vitamin D Receptor Gene Polymorphism and susceptibility to Asthma: meta-analysis based on 17 case-control studies

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Title: Vitamin D Receptor Gene Polymorphism and susceptibility to Asthma: meta-analysis
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Running title: VDR gene polymorphism and Asthma risk

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VDR: vitamin D receptor

SNP: Single nucleotide polymorphisms

IL: Interleukin

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NOS: Newcastle-Ottawa Scale

RXR: Retinoid X receptor

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Background: During the last decade, several studies have evaluated the potential association between vitamin D receptor (VDR) gene polymorphism and susceptibility to asthma. In spite of valuable finding, the results are still contradictory. Therefore, a comprehensive meta-analysis not only solves discrepancies but provides a clue for future projects.

Objective: This meta-analysis was performed to identify whether VDR gene polymorphisms (FokI (rs2228570) or / and TaqI (rs731236) or / and BsmI (rs1544410) or / and ApaI (rs7975232)) play role in the risk of asthma.

Methods: The electronic search of web of science, Scopus, and PubMed databases were systematically conducted from their inception until Jun 2019, to identify all published studies. Eligibility of studies was confirmed by precise inclusion and exclusion criteria and the resultant studies were analyzed.

Results: A total of 17 studies concerning to VDR gene polymorphisms and asthma risk were included in this meta-analysis. The results of pooled analysis revealed a statistical significant association between FokI SNP [dominant model (OR= 0.78, 95% CI, 0.62-0.98, random effect model) and allelic model (OR= 0.81, 95% CI, 0.67-0.98, random effect model)] and TaqI SNP [homozygote contract model (OR= 0.70, 95% CI, 0.54 - 0.89)] with asthma risk. Moreover, subgroup analysis showed that ethnicity influences asthma risk in Asian, African and American populations. The sensitivity analyses confirmed the stability of the results.

Conclusion: This meta-analysis suggests that VDR gene polymorphism is associated with the risk of asthma.

1

2 Introduction

3 Asthma is a common clinical disease that occurs as a result of a complex interaction between
4 different environmental and genetic factors ⁽¹⁾. The main pathophysiological process involved
5 in asthma are airway inflammation, airway hyper-responsiveness, and airflow obstruction in
6 response to specific triggers ⁽²⁾. However, majority of clinical manifestations attribute to
7 airway inflammation. In this regards, the immune dysfunction especially imbalance of helper
8 T (Th)1/Th2 cytokine, like increased levels of interleukin (IL)-4, IL-5, and IL-13, may be
9 responsible for the inflammatory status in asthma ⁽³⁾. In recent years, vitamin D has emerged
10 as a new factor that contributes to the development of asthma disease. Vitamin D has potent
11 immunomodulatory properties by regulation of the adaptive and innate immune system's
12 functions that may inhibit the airway inflammation ⁽⁴⁾. Moreover, vitamin D directly
13 influences the function of T cell, by inhibiting the production of Th1 cytokines, Th17
14 cytokines, induction of Th2 cytokine (such as IL-4 and IL-13), and regulatory T (Treg) cell
15 differentiation ⁽⁵⁾. Vitamin D and its analogs bind to the vitamin D receptor (VDR) and
16 regulate the expression of multiple genes associated with inflammation and immune
17 modulation ^(6, 7). Moreover, it has been demonstrated that the biological effects of vitamin D
18 can be altered by single nucleotide polymorphisms (SNPs) of the *VDR* gene ⁽⁸⁾.

19 The human *VDR* gene is located on chromosome 12q12-14 ⁽⁹⁾. There are many validated
20 SNPs in the *VDR* gene in the dbSNP database, but only four SNPs, including *BsmI*
21 (rs1544410), *ApaI* (rs7975232), *FokI* (rs2228570), and *TaqI* (rs731236) restriction sites have
22 been extensively studied ⁽¹⁰⁾. Among them, *BsmI* and *ApaI* polymorphism located in intron 8,
23 and *TaqI* located in exon 9 of the *VDR* gene which lead to silent codon change ⁽¹¹⁾. Besides,

the *FokI* is located in the exon 2 of the *VDR* gene which leads to a protein with different size (424 amino acids)⁽¹²⁾.

Many studies investigated the associations between these polymorphisms of the *VDR* gene and asthma risk⁽¹³⁻²⁸⁾. However, the results were inconclusive. The reason for this disparity possibly due to small sample sizes, clinical heterogeneity, low statistical power, or a combination of the above factors. Therefore, the aim of the present study was to summarize the available evidence to address the precise relationship between the *VDR* gene polymorphisms and asthma risk.

Previously, three meta-analyses worked on the association of *VDR* gene SNP and asthma risk⁽²⁹⁻³¹⁾. But some limitations made it difficult to generalize their results; first, only 9 studies were included in these investigations; seconds, subgroup analysis based on ethnicity, which has a direct effect on diet, seems necessary but such analyses were not performed for two of previous meta-analysis articles. With the accumulating evidence, we performed an updated meta-analysis to evaluate the association between *VDR* gene *BsmI*, *FokI*, *ApaI*, and *TaqI* polymorphisms and asthma risk with more sample size in different ethnicities with the aim of providing a much more reliable finding on the significance of the association.

Methods

This study conducted in accordance with the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA)⁽³²⁾ statement including; literature review, study selection, inclusion and exclusion criteria, data extraction and quality assessment, and statistical analysis.

Literature review

A comprehensive systematic search process was exploited on the web of science, Scopus, and PubMed databases to retrieve all eligible publications concerning to polymorphisms of *Vitamin D Receptor* [*FokI* (rs2228570) or / and *TaqI* (rs731236) or / and *BsmI* (rs1544410) or / and *ApaI* (rs7975232)] and susceptibility to asthma from their inception until Jun 2019. The main key words and Medical Subject Headings (Mesh) were as follow: (“asthma” [Mesh] OR “asthmatic”) AND (“VDR” OR “vitamin D receptor”) AND (“single nucleotide polymorphism” OR “SNP” OR “polymorphisms” OR “mutation” OR “variation”). In spite of detailed search, manual cross-check of eligible studies and reviews was carried out to include other potential studies. In cases that one article with the same subject published in different journals only the most recent full-text article was included. Ultimately, the search strategy was restricted to the English language.

Study selection

Primary search strategy generates 289 studies that exported to Endnote X8. The title and abstract of all studies were reviewed by two investigators and irrelevant studies were excluded. Full-text verification was performed if we could not categorize studies based on title and abstract. Any disagreements during study selection were discussed and resolved by consensus.

Inclusion and exclusion criteria

Studies must meet the following criteria to be included: a) case-control studies evaluating the association between *FokI* (rs2228570) or / and *TaqI* (rs731236) or / and *BsmI* (rs1544410) or / and *ApaI* (rs7975232) and susceptibility to asthma. Basically, the patients were those who were diagnosed as having asthma and control group were healthy individuals who had no autoimmune or allergic diseases neither in themselves nor family members; b) studies

containing the frequencies of alleles and genotypes for each SNP to extract; c) studies with calculable or extractable data for odds ratio (OR) and 95% confidence intervals (CIs); d) articles published in English language. Major exclusion criteria were; a) studies on animals or cell-lines; b) case reports, duplicated, reviews, editorials, book chapter, and comments. The application of these criteria results in 17 qualified studies for the meta-analysis.

Data extraction and Quality assessment

All required information was extracted conforming to standardized extraction checklist for the following data; the first author, journal and year of publication, country of origin, ethnicity, number of subjects in the case and the control groups for every gender separately, mean or range of age, genotyping method, genotype counts for each polymorphism in the case and the control groups. The quality of each study was assessed using the Newcastle-Ottawa Scale (NOS) criteria⁽³³⁾. Studies with scores 0-3, 4-6 or 7-9 were low, moderate or high-quality, respectively.

Statistical analysis

The strength of the association between different polymorphism of *VDR* gene and asthma risk was estimated by calculating pooled ORs and their 95% CIs. For each SNP, the dominant model, recessive model, allelic model, homozygous model, and heterozygous model were examined to estimate its effect. In details: Defined model for *FokI*, *TaqI*, *BsmI*, *ApaI* are as follow: **FokI**; dominant model (ff+Ff vs FF), recessive model (ff vs Ff+FF), allelic model (f vs F), homozygote (ff vs FF), and heterozygote (Ff vs FF):**TaqI**; dominant model (tt+Tt vs TT), recessive model (tt vs Tt+TT), allelic model (t vs T), homozygote (tt vs TT), and heterozygote (Tt vs TT):**BsmI**; dominant model (bb+Bb vs BB), recessive model (bb vs Bb+BB), allelic model (b vs B), homozygote (bb vs BB), and heterozygote (Bb vs BB):**ApaI**;

dominant model (aa+Aa vs AA), recessive model (aa vs Aa+AA), allelic model (a vs A), homozygote (aa vs AA), and heterozygote (Aa vs. AA). The between-study heterogeneity was assessed with a Chi-square-based Q-test and I². A P-value of < 0.1 for the Q-test or I² > 50% indicated heterogeneity and random-effects model(REM) was used; otherwise, the fixed-effect model(FEM) was exploit⁽³⁴⁾. Potential publication bias was estimated using both Begg's test and Egger's test^(35, 36). Furthermore, the funnel plot asymmetry was assessed with the Egger's test. Practically, in condition with no evidence of publication bias, considered that studies with high precision (large study effects) will be located near the average line, and studies with low precision (small study effects) will be spread equally on both sides of the average line, any deviation from this shape can indicate publication bias. All statistical tests for this meta-analysis were performed with Stata statistical software (version 14.0; Stata Corporation, College Station, TX, USA) and SPSS (version 23.0; SPSS, Inc. Chicago, IL, USA)

Results

Study characteristics

Search and screening process based on the PRISMA statement is depicted in Figure 1⁽³²⁾. According to the combination of the keywords and MeSH terms a total of 289 studies were identified. Subsequently, exerting the inclusion and exclusion criteria resulted in the exclusion of 272 studies (90 duplicates studies, 132 and 50 studies excluded according to title abstract and full-text examination, respectively). Eventually, 17 qualified case-control studies were included in quantitative analysis. All included studies had an overall good methodological quality with NOS scores ranging from 6 to 8. The RFLP, Taq-man, and Mass spectrometry were three genotyping methods used in included studies. Table 1 and 2 summarized the characteristics and genotype frequency of the included studies.

Quantitative synthesis

FF for FokI SNP, TT for TaqI SNP, BB for BsmI SNP and AA for ApaI SNP were used as the reference category.

Meta-analysis of the association between FokI (rs2228570) polymorphism and asthma risk

Overall 13 case-control studies with 2450 cases and 2134 controls were analyzed for assessment of *FokI* gene polymorphism and asthma risk. Of 13 studies, five studies conducted in Asian countries^(14, 15, 25, 26, 37), three studies in European countries^(20, 23, 24), three studies in American countries^(13, 17, 21) and two studies in African countries^(18, 19) (Table 1). The results of pooled ORs, heterogeneity tests and publication bias tests in different analysis models are shown in Table 3. Our result found significant association between *FokI* gene polymorphism and asthma risk across dominant model (OR= 0.78, 95% CI, 0.62-0.98, REM) and allelic model (OR= 0.81, 95% CI, 0.67-0.98, REM); however, no significant association was observed in other genetic model (Figure 2). Additionally, subgroup analysis by ethnicity was performed. Overall, the presence of *FokI* SNP decrease the risk of asthma in the African population compared with Asian, American and European countries. No statistically significant association was observed between *FokI* gene polymorphism and risk of asthma in Asian, American, and European countries (Figure 3).

Meta-analysis of the association between TaqI (rs731236) polymorphism and asthma risk

There were 11 case-control studies with 2457 cases and 1926 controls concerning *TaqI* gene polymorphism and asthma risk. Five studies were conducted in Europe^(20, 22-24, 28), three studies in American population^(13, 17, 21), two studies in Asia^(15, 16), and only one study in Africa⁽¹⁹⁾. The pooled results revealed a significant association in homozygote contract

model (OR= 0.70, 95% CI, 0.54-0.89), but rejected any association under dominant model (OR= 0.96, 95% CI, 0.83-1.11, FEM), recessive model (OR=0.94, 95% CI, 0.60- 1.47, REM), allelic model (OR= 0.97, 95% CI, 0.82-1.15, REM), Tt vs. TT model (OR= 1.04, 95% CI, 0.89-1.22, FEM). Furthermore, the results of subgroup analysis in homozygote model remarkably showed that *TaqI* gene polymorphism increase the susceptibility of asthma in the Asian [tt vs. TT (OR= 2.94, 95% CI, 1.06 – 8.14)], but decrease in American population [tt vs. TT (OR= 0.64, 95% CI, 0.44-0.93)] Figure 3. In subgroup analysis, since there was only one study for the African populations, this study was excluded from the analysis.

Meta-analysis of the association between BsmI (rs1544410) polymorphism and asthma risk

To examining the association between *BsmI* gene polymorphism and asthma risk, 9 case-control studies with 1451 cases and 2008 controls subjects were included. Four studies were performed in Asia^(14, 15, 25, 27), four studies in Europe^(20, 22-24), and one study in Africa⁽¹⁹⁾. The pooled results indicated a negative, but not significant, association between *BsmI* gene polymorphism and asthma risk under all genotype models for the overall population and subgroup analysis. The results of pooled ORs, heterogeneity tests and publication bias tests in different analysis models are shown in Table 3.

Meta-analysis of the association between ApaI (rs7975232) polymorphism and asthma risk

A total of 13 case-control studies with 2130 cases and 2608 controls identified concerning to *ApaI* gene polymorphism and asthma risk. Among 13 studies, five studies were carried out in Europe^(20, 22-24, 28), four studies in Asia^(15, 16, 25, 27), three studies in America^(13, 17, 21), and only one study in the Africa⁽¹⁹⁾, which this study omitted from the subgroup analysis. There was no evidence of significant association between *ApaI* gene polymorphism and asthma risk in the pooled results and the subgroup analysis. The results of pooled ORs, heterogeneity tests and publication bias tests for different analysis models are shown in Table 3.

Evaluation of heterogeneity and publication bias

The between study heterogeneity was assessed for all four polymorphisms in all genetic models. Significant heterogeneity was observed in the overall estimation under all genetic models for *FokI*, *TaqI*, *BsmI*, and *ApaI* and subsequently random effect model was used (Table 3). Publication bias was evaluated by Funnel plot, and Begg's and Egger's tests (Table 3, Figure 5). No evidence of publication bias was observed.

Sensitivity analysis

Sensitivity analysis was conducted after sequentially removing each eligible study. This approach is enumerated as an inevitable step for analyzing multiple criteria. The significance of the pooled ORs was not affected by any single study in the dominant model for *FokI*, *TaqI*, *BsmI* and *ApaI* SNPs, indicating that our results were statistically robust.

Discussion

During the last years and based on many researches, four SNPs in the *VDR* gene, namely rs2228570 (*FokI*), rs731236 (*TaqI*), rs1544410 (*BsmI*), and rs7975232 (*ApaI*), have been suspected as contributing factors for the asthma risk. Here we performed a meta-analysis to investigate the consequence of these common four polymorphisms on asthma risk. Our findings indicated a statistically significant association of *FokI* and *TaqI* gene polymorphisms and asthma risk, while there was no evidence of the association between both *BsmI* and *ApaI* SNPs with asthma risk. In addition, subgroup analysis was stratified to assess potential ethnicity-specific effect, revealing that the presence of *FokI* SNP in African population decrease the asthma risk under dominant model (OR=0.60) and allelic model (OR=0.54);

furthermore, tt genotype of TaqI SNP was associated with an increased risk of asthma in Asian population (OR=2.94) and decreased risk in American populations (OR=0.64). This remarkable difference in outcome among ethnicities might originate from various dietary patterns, geographic discrepancies, ethnical diversity, and important influence of ethnicity on the VDR gene expression and serum level of vitamin D ⁽³⁸⁾.

Vitamin D, VDR, and the retinoid X receptor (RXR) construct a heterodimer structure in combination that bind to the vitamin D response element and, thereby, conferring a regulating loop on the level of vitamin D (19). It has been reported that *FokI* polymorphism can alter the ATG start codon in the VDR and the changes in the rs1544410 position can fundamentally modulate the expression of VDR protein (14, 37). Moreover, it has been revealed that *ApaI* and *TaqI* SNPs influence on the gene transcription as well as the stability of mRNA (39). *ApaI* and *FokI* are located in the restriction sites and are involved in the regulation on gene expression. Furthermore, *FokI* SNP was reported to be able to interfere with transcription factors involved in the regulation of immunoinflammatory genes (17, 18). Nonetheless, the combinational effect of these SNPs in a network on the modulation of VDR needs to be studied.

From genetically point of view, VDR is a pleiotropic gene and its protein product belongs to a nuclear protein family. Among four SNPs, *ApaI*, *BsmI*, and *TaqI* gene polymorphisms are silent codon alterations. Theoretically seems that VDR protein remains intact but since there is a sophisticated network between different SNPs in the gene, it is not clear if other functional SNPs linked with *ApaI*, *BsmI*, and *TaqI* polymorphisms increase or decrease the expression of VDR target gene. Additionally, proximity of *ApaI* and *TaqI* SNP to the regulatory region of 3'-untranslated region (3'-UTR) of mRNA could influence intronic regulatory element and splice sites for mRNA transcription and subsequently VDR mRNA

stability. Unlike to the three mentioned SNPs, *FokI* gene polymorphism is located on the exonic region and might affect both *VDR* protein structure and its transcriptional activity.

From the biological point of view, The *VDR* is the determinant factor of the biological activity of vitamin D. Hence, the receptor's expression and the receptor's concentration can regulate vitamin D functions. Accordingly, Triantos *et al.* showed that alteration in expression of *VDR* proteins, as a result of different SNPs, affect the pathophysiology of liver cirrhosis⁽⁴⁰⁾. Based on this findings, since *VDR* is widely expressed on various sites, especially on the epithelial layer of the human lung, and B and T lymphocytes; hence immunological disorders and airway remodeling disorders are not farfetched⁽⁴¹⁾. With respect to the *VDR* immunological function, any of these four SNPs can disturb *VDR* mRNA stability, which in turn affects the protein levels and ultimately change the balance of Th1 and Th2. Experimental data suggest that vitamin D and *VDR* reduce the production of IL-12 and interferon (IFN)- γ , which cause predominance of Th2 cytokines production, such as IL-4 and IL-13, to the benefit of allergic cytokine profile. Furthermore, Roy *et al.* demonstrated that in the allelic model of TaqI SNP, homozygous tt tends to produce Th1-type responses and disturb favoring cytokine profile for allergic disorder, while TT homozygous produce a Th-2 type response⁽⁴¹⁾.

Previous meta-analyses were reported by Tizaoui *et al.*⁽²⁹⁾ in 2014, Zhao *et al.*⁽³¹⁾ in 2016 and Han *et al.* in 2016⁽³⁰⁾, in which the number of included studies was 8, 9, and 9, respectively. Our results mostly confirm previous studies except for some results of our subgroup analysis, which due to more included studies (17 case-control studies); hence, seemingly our findings might be more convincing and stringent.

From the pathobiological point of view, Glucocorticoids (GCs) are highly effective in the treatment of asthma by their anti-inflammatory and immunosuppressive effects. This function is exerted by different mechanism, among which is the upregulation of Mitogen-activated

protein kinase phosphatase-1 (MKP-1) and IL-10 expression⁽⁴²⁾. Accordingly, some studies demonstrated that vitamin D improve GCs anti-inflammatory and immunosuppressive effects by increasing MKP-1 and IL-10 expression. Moreover, Xystrakis *et al.*⁽⁴³⁾ reported that the addition of vitamin D and Dexamethasone (DEX) to cultures of CD4⁺ Treg cells from steroid resistant asthmatics enhanced IL-10 secretion from these cells to levels comparable from cells of steroid sensitive patients treated only with DEX. Moreover, they reported that while three steroid resistant subjects were given small doses of vitamin D for seven days, all subjects showed enhanced IL-10 expression by CD4⁺ cells. In another study, Searing *et al.*⁽⁴⁴⁾ confirmed that vitamin D enhanced GC induction of MKP-1 and IL-10 in the peripheral blood mononuclear cells of asthmatic children. Together, since most of biological effects of vitamin D is exerted by VDR, and with knowledge to important functions of vitamin D in asthmatics (as supplementary or adjuvant), any change in VDR can lessen VD biological and pharmaceutical function⁽⁴⁵⁾.

The main strength of the present study is its large pooled sample size, enabling the determination of robust estimates for the association of the *VDR* gene polymorphisms with the risk of asthma. In addition, with knowledge to importance of environmental factors such as dietary regime and geographical location on risk of asthma, the subgroup analysis really seems beneficial and made the results understandable. However, some limitations of the current study are worth noting. First, some studies with little number of case and control subjects included in our analysis which they might cause potential bias. Second, our results were based on unadjusted parameters, in which some related parameters including, genotyping method, age, diet, and lifestyle factor should be considered for future meta-analysis. Third, although heterogeneity was detected but sensitivity analysis did not show any significant change in our results by omitting the studies contributing to the heterogeneity. It suggested that our results were credible and statistically robust. Forth, only four SNPs in the

270 VDR gene have been evaluated here, while there are several other functional SNPs in the
271 promoter region of the VDR gene.

272 In conclusion, the present meta-analysis is the most comprehensive meta-analysis to estimate
273 the association of VDR gene *FokI*, *TaqI*, *BsmI* and *ApaI* polymorphisms with the risk of
274 asthma. Strongly, decrease of asthma risk was found in association with *FokI* and *TaqI*
275 polymorphisms in the overall population, and in the Asians and African population subgroup.
276 In the future, large and well-designed researches, evaluation of VDR gene expression as
277 result of SNPs in asthmatic patient and outcomes on clinical and pathophysiological
278 conditions are required to decisively discuss about the association of VDR SNPs and
279 asthma.

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Table 1. Characteristics of studies included in meta-analysis of overall Asthma.

Study author	Year	Country	Ethnicity	Sex cases/controls	Total cases/controls	Age case/control (Mean)	Genotyping method	Quality score
FokI (rs2228570)								
Raby <i>et al.</i>	2004	Canada	American	M= NR F= NR	485 /505	NR	Mass spectrometry	7
Fang <i>et al.</i>	2009	China	Asian	M=42/96 F=59/110	101 /206	35.9/ 34.7	PCR-RFLP	6
Saadi <i>et al.</i>	2009	China	Asian	M=253/348 F=314/175	567/ 523	41.36/ 44.18	PCR-RFLP	8
Pillai <i>et al.</i>	2011	USA	American	M= NR F= NR	122 / 74	11.2/11.8	Taq -Man	7
Li <i>et al.</i>	2011	China	Asian	M=185/348 F=282/175	467 / 188	40.8± 14.7 / 45.1 ± 0.7	PCR-RFLP	8
Ismail <i>et al.</i>	2013	Egypt	African	M=28/18 F=23/15	51 /33	8.6± 2.7 / 7.8 ± 2.6	Taq -Man	6
Maalmi <i>et al.</i>	2013	Tunisia	African	M=96/85 F=59/67	155 /152	9.1 / 9.5	PCR-RFLP	7
Iordanidou <i>et al.</i>	2014	Greece	European	M=82/41 F=45/50	127 /91	8.4±0.2/ 9.5±0.4	Mass spectrometry	7
Einisman <i>et al.</i>	2015	Chile	American	M= NR F= NR	73 /50	9.1±3.5 / 10.3±7	PCR-RFLP	6
Batmaz <i>et al.</i>	2017	Turkey	European	M=20/13 F=10/17	30 / 30	11.74±2.4 / 11.4±2.27	PCR-RFLP	5
Despotovic <i>et al.</i>	2017	Serbia	European	M=32/42 F=47/47	79 / 89	47.48 ±15.8 / 46.81 ±16.7	PCR-RFLP	6
Zhang <i>et al.</i>	2017	China	Asian	M=86/87 F=57/56	143 / 143	7.56±2.39 / 7.28±2.54	Taq -Man	7
Bijanzadeh <i>et al.</i>	2018	Iran	Asian	M=23/18 F=27/32	50 / 50	NR	PCR-RFLP	6
TaqI (rs731236)								
Raby <i>et al.</i>	2004	Canada	American	M= NR F= NR	535 /510	NR	Mass spectrometry	7
Saadi <i>et al.</i>	2009	China	Asian	M=253/348 F=314/175	567/ 523	41.36/ 44.18	PCR-RFLP	8
Arababadi <i>et al.</i>	2010	Iran	Asian	M=31/27 F=69/ 73	100 / 100	48 ±12/ 43± 14	PCR-RFLP	6
Pillai <i>et al.</i>	2011	USA	American	M= NR F= NR	118 / 74	11.2/11.8	Taq -Man	7
Maalmi <i>et al.</i>	2013	Tunisia	African	M=96/126 F=59/99	155 / 225	9.1 / 9.5	PCR-RFLP	7
Iordanidou <i>et al.</i>	2014	Greece	European	M=82/41 F=45/50	127 /91	8.4±0.2/ 9.5±0.4	Mass spectrometry	7
Einisman <i>et al.</i>	2015	Chile	American	M= NR F= NR	72 /50	9.1±3.5 / 10.3±7	PCR-RFLP	6
Papadopoulou <i>et al.</i>	2015	Cyprus	European	M=72/270 F= 105/360	177/630	16- 18.1 / 15.9 - 18	PCR-RFLP	7
Batmaz <i>et al.</i>	2017	Turkey	European	M=20/13 F=10/17	30 / 30	11.74±2.4 / 11.4±2.27	PCR-RFLP	5
Despotovic <i>et al.</i>	2017	Serbia	European	M=32/42 F=47/47	79 / 89	47.48 ±15.8 / 46.81 ±16.7	PCR-RFLP	6
Hutchinson <i>et al.</i>	2018	Ireland	European	M=23/NR F=21/NR	44 / 57	8.7 / NR	Taq-Man	5
BsmI (rs1544410)								
Fang <i>et al.</i>	2009	China	Asian	M=42/96 F=59/110	101 /206	35.9/ 34.7	PCR-RFLP	6
Saadi <i>et al.</i>	2009	China	Asian	M=253/348 F=314/175	567/ 523	41.36/ 44.18	PCR-RFLP	8
Maalmi <i>et al.</i>	2013	Tunisia	African	M=96/126 F=59/99	155 / 225	9.1 / 9.5	PCR-RFLP	7
Iordanidou <i>et al.</i>	2014	Greece	European	M=82/41 F=45/50	127 /91	8.4±0.2/ 9.5±0.4	Mass spectrometry	7
Papadopoulou <i>et al.</i>	2015	Cyprus	European	M=77 /255 F= 102/376	179 / 631	16- 18.1 / 15.9 - 18	PCR-RFLP	7
Batmaz <i>et al.</i>	2017	Turkey	European	M=20/13 F=10/17	30 / 30	11.74±2.4 / 11.4±2.27	PCR-RFLP	5
Despotovic <i>et al.</i>	2017	Serbia	European	M=32/42 F=47/47	79 / 89	47.48 ±15.8 / 46.81 ±16.7	PCR-RFLP	6
Zhang <i>et al.</i>	2017	China	Asian	M=86/87 F=57/56	143 / 143	7.56±2.39 / 7.28±2.54	Taq -Man	7
Hou <i>et al.</i>	2018	China	Asian	M=43/37 F=27/33	70 / 70	8.84±3.2 / 8.04±3.01	PCR-RFLP	6
Apal (rs7975232)								
Raby <i>et al.</i>	2004	Canada	American	M= NR F= NR	482/490	NR	Mass spectrometry	7
Saadi <i>et al.</i>	2009	China	Asian	M=253/348 F=314/175	567/ 523	41.36/ 44.18	PCR-RFLP	6
Arababadi <i>et al.</i>	2010	Iran	Asian	M=31/27	100 / 100	48 ±12/ 43± 14	PCR-RFLP	6

Pillai <i>et al.</i>	2011	USA	American	M= NR F= NR	125 / 72	11.2/11.8	Taq -Man	7
Maalmi <i>et al.</i>	2013	Tunisia	African	M=28/18 F=23/15	155 / 225	16- 18.1 / 15.9 - 18	PCR-RFLP	7
Iordanidou <i>et al.</i>	2014	Greece	European	M=82/41 F=45/50	127 /91	8.4±0.2/ 9.5±0.4	Mass spectrometry	7
Einisman <i>et al.</i>	2015	Chile	American	M= NR F= NR	70 /50	9.1±3.5 / 10.3±7	PCR-RFLP	6
Papadopoulou <i>et al.</i>	2015	Cyprus	European	M=76/256 F=100/377	176 / 633	16- 18.1 / 15.9 - 18	PCR-RFLP	7
Batmaz <i>et al.</i>	2017	Turkey	European	M=NR F=NR	28 / 29	11.74±2.4 / 11.4±2.27	PCR-RFLP	5
Despotovic <i>et al.</i>	2017	Serbia	European	M=32/42 F=47/47	79 / 89	47.48 ±15.8 / 46.81 ±16.7	PCR-RFLP	6
Zhang <i>et al.</i>	2017	China	Asian	M=86/87 F=57/56	143 / 143	7.56±2.39 / 7.28±2.54	Taq -Man	7
Hutchinson <i>et al.</i>	2018	Ireland	European	M=23/NR F=21/NR	44 / 57	8.7 / NR	Taq-Man	5
Hou <i>et al.</i>	2018	China	Asian	M=43/37 F=27/33	70 / 70	8.84±3.2 / 8.04±3.01	PCR-RFLP	6

NR, not reported; M, male; F, female;

Table 2. Distribution of genotype and allele among asthma patients and controls.

Study author	Asthma cases					Healthy control					P-HWE	MAF
	FF	Ff	ff	F	f	FF	Ff	ff	F	f		
FokI (rs2228570)												
Raby <i>et al.</i>	175	248	62	598	372	191	245	69	627	383	0/49	0/379
Fang <i>et al.</i>	24	56	21	104	98	66	94	46	226	186	0/25	0/451
Saadi <i>et al.</i>	193	258	116	644	490	152	265	106	569	477	0/62	0/456
Pillai <i>et al.</i>	76	41	5	193	51	42	29	3	113	35	0/46	0/236
Li <i>et al.</i>	152	230	85	534	400	60	91	37	211	165	0/81	0/438
Ismail <i>et al.</i>	29	22	0	80	22	12	14	7	38	28	0/44	0/424
Maalmi <i>et al.</i>	88	56	11	232	78	70	59	23	199	105	0/08	0/345
Iordanidou <i>et al.</i>	67	54	6	188	66	38	45	8	121	61	0/29	0/335
Einisman <i>et al.</i>	11	62	0	84	62	5	45	0	55	45	<0.01	0/45
Batmaz <i>et al.</i>	19	11	0	49	11	12	12	6	36	24	0/36	0/4
Despotovic <i>et al.</i>	37	36	6	110	48	62	25	2	149	29	0/77	0/162
Zhang <i>et al.</i>	92	49	2	233	53	74	63	6	211	75	0/09	0/262
Bijanzadeh <i>et al.</i>	32	15	3	79	21	12	34	4	58	42	<0.01	0/42
Study author	Asthma cases					Healthy control					P-HWE	MAF
	TT	Tt	tt	T	t	TT	Tt	tt	T	t		
TaqI (rs731236)												
Raby <i>et al.</i>	230	237	68	697	373	181	237	92	599	421	0/34	0/412
Saadi <i>et al.</i>	510	53	4	1073	61	471	51	1	993	53	0/75	0/05
Arababadi <i>et al.</i>	6	48	46	60	140	18	35	47	71	129	0/02	0/645
Pillai <i>et al.</i>	52	55	11	159	77	40	31	3	111	37	0/31	0/25
Maalmi <i>et al.</i>	59	81	15	199	111	79	101	45	259	191	0/74	0/424
Iordanidou <i>et al.</i>	43	68	16	154	100	35	38	18	108	74	0/18	0/406
Einisman <i>et al.</i>	35	34	3	104	40	24	24	2	72	28	0/17	0/28
Papadopoulou <i>et al.</i>	69	74	34	773	487	224	325	81	212	142	0/08	0/401
Batmaz <i>et al.</i>	18	9	3	45	15	15	10	5	40	20	0/17	0/333
Despotovic <i>et al.</i>	35	34	10	104	54	32	43	14	107	71	0/94	0/398
Hutchinson <i>et al.</i>	17	21	6	55	33	34	23	0	91	210	0/05	0/201
Study author	Asthma cases					Healthy control					P-HWE	MAF
	BB	Bb	bb	B	b	BB	Bb	bb	B	b		
BsmI (rs1544410)												
Fang <i>et al.</i>	74	25	2	173	29	167	35	4	369	43	0/19	0/104

Journal Pre-proof												
Saadi <i>et al.</i>	515	49	3	1079	55	472	51	0	995	51	0/24	0/048
Maalmi <i>et al.</i>	34	72	49	140	170	26	119	80	171	279	0/06	0/62
Iordanidou <i>et al.</i>	20	67	40	107	147	19	39	33	77	105	0/24	0/576
Papadopoulou <i>et al.</i>	38	103	38	179	179	127	327	177	581	681	0/28	0/539
Batmaz <i>et al.</i>	2	12	16	16	44	5	13	12	23	37	0/64	0/616
Despotovic <i>et al.</i>	11	40	28	62	96	18	37	34	73	105	0/18	0/589
Zhang <i>et al.</i>	56	74	13	186	100	72	65	6	209	77	0/06	0/269
Hou <i>et al.</i>	66	4	0	136	4	65	5	0	135	5	0/75	0/035
Study author	Asthma cases					Healthy control					P-HWE	MAF
	AA	Aa	aa	A	a	AA	Aa	aa	A	a		
ApaI (rs7975232)												
Raby <i>et al.</i>	139	217	126	495	469	162	227	101	551	429	0/19	0/437
Saadi <i>et al.</i>	310	217	40	837	297	247	216	60	710	336	0/22	0/321
Arababadi <i>et al.</i>	7	58	35	72	128	17	56	27	90	110	0/18	0/55
Pillai et al <i>et al.</i>	55	59	11	169	81	35	33	4	103	41	0/28	0/284
Maalmi <i>et al.</i>	92	57	6	241	69	142	70	13	354	96	0/27	0/213
Iordanidou <i>et al.</i>	35	41	15	111	71	41	63	23	145	109	0/88	0/429
Einisman <i>et al.</i>	21	35	14	77	63	10	28	12	48	52	0/38	0/52
Papadopoulou <i>et al.</i>	68	91	17	227	125	232	312	89	776	490	0/32	0/387
Batmaz <i>et al.</i>	3	16	9	22	34	4	16	9	24	34	0/45	0/586
Despotovic <i>et al.</i>	22	41	16	85	73	32	43	14	107	71	0/94	0/398
Zhang <i>et al.</i>	54	75	14	183	103	8	69	66	85	201	0/06	0/702
Hutchinson <i>et al.</i>	11	23	10	45	43	5	20	32	30	84	0/47	0/893
Hou <i>et al.</i>	36	30	4	102	38	65	5	0	135	5	<0.01	0/035

Table 3. Main results of pooled ORs in meta-analysis of VDR gene polymorphisms.

Subgroup	Sample size		Test of association		Test of heterogeneity		Test of publication bias (Begg's test)		Test of publication bias (Egger's test)	
	Genetic model	Case/Control	OR	95% CI	I ² (%)	P	Z	P	T	P
FokI (rs2228570)										
Overall	Dominant model	2450 / 2134	0.78	0.62 – 0.98	56.4	≤0.001	-1.34	0.18	-1.84	0.09
	Recessive model	2450 / 2134	0.90	0.74 - 1.09	0	0.60	-0.80	0.42	-0.95	0.37
	Allelic model	2450 / 2134	0.81	0.67 – 0.98	68.4	≤0.001	-1.59	0.11	-1.67	0.12
	ff vs. FF	2450 / 2134	0.86	0.69 – 1.07	16.6	0.02	-0.45	0.65	-0.78	0.45
	Ff vs. FF	2450 / 2134	0.84	0.65 – 1.08	61	≤0.001	-0.12	0.90	-0.92	0.37
European	Dominant model	236 / 210	0.78	0.39 – 1.58	61	0.07	-0.52	0.60	-0.58	0.66
	Recessive model	236 / 210	0.88	0.30 – 2.57	60.4	0.11	1	0.31	-	-
	Allelic model	236 / 210	0.84	0.31– 2.27	87.5	≤0.001	-0.52	0.60	-0.29	0.82
	ff vs. FF	236 / 210	0.86	0.28 – 2.59	74.5	0.04	1	0.31	-	-
	Ff vs. FF	236 / 210	1.03	0.40 – 2.65	75.7	0.01	0.52	0.60	-0.13	0.91
Asian	Dominant model	1328 / 1110	0.74	0.49– 1.14	74.9	≤0.001	-0.49	0.62	-0.78	0.49
	Recessive model	1328 / 1110	0.95	0.75 – 1.19	0	0.85	-1.96	0.05	-3.01	0.05
	Allelic model	1328 / 1110	0.83	0.65 – 1.06	65.1	0.02	-0.98	0.32	-1.37	0.26
	ff vs. FF	1328 / 1110	0.88	0.67– 1.15	0	0.52	-0.98	0.32	-1.30	0.28
	Ff vs. FF	1328 / 1110	0.74	0.47 – 1.17	77	≤0.001	-0.49	0.62	-0.63	0.57
African	Dominant model	206 / 185	0.60	0.39 – 0.92	0	0.49	-	-	-	-
	Allelic model	206 / 185	0.54	0.33 – 0.87	35.4	0.21	-	-	-	-
	Ff vs. FF	206 / 185	0.73	0.46 – 1.16	0	0.81	-	-	-	-
American	Dominant model	680 / 629	1.01	0.79 – 1.28	0	0.52	-0.52	0.60	-3.89	0.16
	Recessive model	680 / 629	0.92	0.63 – 1.36	0	0.92	1	0.31	-	-
	Allelic model	680 / 629	0.98	0.83 – 1.16	0	0.78	-0.52	0.60	-3.93	0.15
	ff vs. FF	680 / 629	0.98	0.65 – 1.47	0	0.97	-1	0.31	-	-
	Ff vs. FF	680 / 629	1.02	0.79 – 1.32	0	0.48	-0.52	0.60	-3.64	0.17
TaqI (rs731236)										
Overall	Dominant model	2457 / 1926	0.96	0.83 - 1.11	46.5	0.04	1.01	0.31	2.16	0.06
	Recessive model	2457 / 1926	0.94	0.60- 1.47	63.3	≤0.001	1.52	0.12	1.55	0.16
	Allelic model	2457 / 1926	0.97	0.82 – 1.15	50.3	0.02	1.32	0.18	2.07	0.06
	tt vs. TT	2457 / 1926	0.70	0.54 – 0.89	32.2	0.15	1.16	0.24	-1.14	0.28
	Tt vs.TT	2457 / 1926	1.04	0.89 – 1.22	37	0.10	1.01	0.31	2.07	0.06
European	Dominant model	910 / 444	1.11	0.79 – 1.55	27	0.24	0	1	-0.13	0.90
	Recessive model	910 / 444	1.15	0.38 – 3.50	81.3	≤0.001	0.68	0.49	0.63	0.59
	Allelic model	910 / 444	0.99	0.73– 1.34	51.4	0.08	0	1	0.39	0.72
	tt vs. TT	910 / 444	0.70	0.47– 1.05	0	0.98	-2.04	0.04	-3.40	0.07
	Tt vs.TT	910 / 444	1.26	0.95 – 1.66	0	0.48	-0.49	0.62	-0.41	0.70
Asian	Dominant model	667 / 623	1.67	0.51 – 5.42	75.9	0.04	-	-	-	-
	Recessive model	667 / 623	1.10	0.48 – 2.50	10.1	0.29	-	-	-	-
	Allelic model	667 / 623	1.15	0.86 – 1.55	0	0.53	-	-	-	-
	tt vs. TT	667 / 623	2.94	1.06 – 8.14	90.5	≤0.001	-	-	-	-
	Tt vs.TT	667 / 623	1.14	0.77 – 1.70	82.3	0.01	-	-	-	-
American	Dominant model	725 / 634	0.95	0.59- 1.51	56.3	0.10	0.52	0.60	1.47	0.38
	Recessive model	725 / 634	0.88	0.42 – 1.86	27.2	0.23	0.52	0.60	1.36	0.40
	Allelic model	725 / 634	0.98	0.64 – 1.50	67.4	0.04	0.52	0.60	1.47	0.38
	tt vs. TT	725 / 634	0.64	0.44 – 0.93	46.6	0.15	0.52	0.60	-1.33	0.41
	Tt vs.TT	725 / 634	0.86	0.67 – 1.10	18.8	0.29	0.52	0.60	1.41	0.39
BsmI (rs1544410)										
Overall	Dominant model	1451/2008	1.13	0.91– 1.39	40.7	0.09	0	1	0.26	0.80

	Recessive model	1451/2008	0.85	0.67 – 1.08	0	0.49	7	2.25	2.50	0.05
	Allelic model	1451/2008	1.36	0.88– 2.11	88.9	≤0.001	0.63	0.53	0.92	0.38
	bb vs. BB	1451/2008	0.86	0.62 – 1.21	40.9	0.11	1.65	0.09	2.02	0.1
	Bb vs.BB	1451/2008	1.08	0.87– 1.34	39.6	0.10	0	1	0.62	0.55
European	Dominant model	415 / 841	1.36	0.94– 1.95	0	0.95	2.04	0.04	5.25	0.03
	Recessive model	415 / 841	0.80	0.59 – 1.07	0	0.50	2.04	0.04	6.73	0.02
	Allelic model	415 / 841	1.02	0.81 – 1.29	24	0.26	1.36	0.17	3.59	0.06
	bb vs. BB	415 / 841	0.93	0.62– 1.40	0	0.40	2.04	0.04	5.14	0.36
	Bb vs. BB	415 / 841	1.26	0.88 – 1.81	0	0.61	0	1	2.49	0.13
Asian	Dominant model	881 / 942	1.23	0.93 – 1.63	11.8	0.33	0	1	-0.06	0.95
	Recessive model	881 / 942	1.91	0.70 – 5.24	0	0.51	-1	0.31	-	-
	Allelic model	881 / 942	1.98	0.83 – 4.71	89.7	≤0.001	0.68	0.49	-0.3	0.79
	bb vs. BB	881 / 942	2.29	0.81– 6.46	0	0.48	-1	0.31	-	-
	Bb vs. BB	881 / 942	1.17	0.87 – 1.56	16.8	0.30	0.68	0.49	0.11	0.92
ApaI (rs7975232)										
Overall	Dominant model	2130 / 2608	0.97	0.68 – 1.38	79.2	≤0.001	0.24	0.80	0.22	0.82
	Recessive model	2130 / 2608	0.63	0.43 – 1.08	80.3	≤0.001	0.14	0.89	-1.04	0.32
	Allelic model	2130 / 2608	1.07	0.76– 1.52	91	≤0.001	0.61	0.54	0.75	0.47
	aa vs. AA	2130 / 2608	0.66	0.36 – 1.21	83.6	≤0.001	-0.27	0.78	-0.84	0.42
	Aa vs. AA	2130 / 2608	1	0.73– 1.36	70.2	≤0.001	0.49	0.62	0.40	0.69
European	Dominant model	418 / 935	0.89	0.63 – 1.27	21.7	0.27	0	1	-0.36	0.73
	Recessive model	418 / 935	0.64	0.37 – 1.10	50.1	0.09	0	1	-0.2	0.98
	Allelic model	418 / 935	1.24	0.57 – 2.71	91.2	≤0.001	0.42	0.62	0.60	0.59
	aa vs. AA	418 / 935	0.72	0.37 – 1.40	50.6	0.08	0.49	0.62	-0.23	0.83
	Aa vs. AA	418 / 935	0.96	0.72– 1.29	0	0.68	0	1	-0.22	0.83
Asian	Dominant model	880 / 836	1.17	0.25 – 5.45	93.3	≤0.001	1.36	0.17	0.39	0.73
	Recessive model	880 / 836	0.47	0.13 – 1.64	92.6	≤0.001	-0.52	0.60	-0.29	0.82
	Allelic model	880 / 836	1.06	0.42 – 2.64	95.3	≤0.001	1.36	0.17	0.50	0.66
	aa vs. AA	880 / 836	0.36	0.04 – 3.08	94.7	≤0.001	0.52	0.60	-0.20	0.87
	Aa vs. AA	880 / 836	1.24	0.34- 4.50	91	≤0.001	1.36	0.17	0.42	0.69
American	Dominant model	677 / 612	1.15	0.89 – 1.48	1.4	0.36	-1.57	0.11	-1.31	0.41
	Recessive model	677 / 612	1.31	0.98 – 1.74	0	0.55	-0.52	0.60	-0.46	0.72
	Allelic model	677 / 612	1.12	0.89 – 1.42	23.3	0.27	-1.15	0.11	-1.04	0.48
	aa vs. AA	677 / 612	1.30	0.81 – 2.08	14.3	0.31	-0.52	0.60	-0.51	0.65
	Aa vs. AA	677 / 612	1.07	0.82 – 1.39	0	0.49	-1.57	0.11	-1.18	0.44

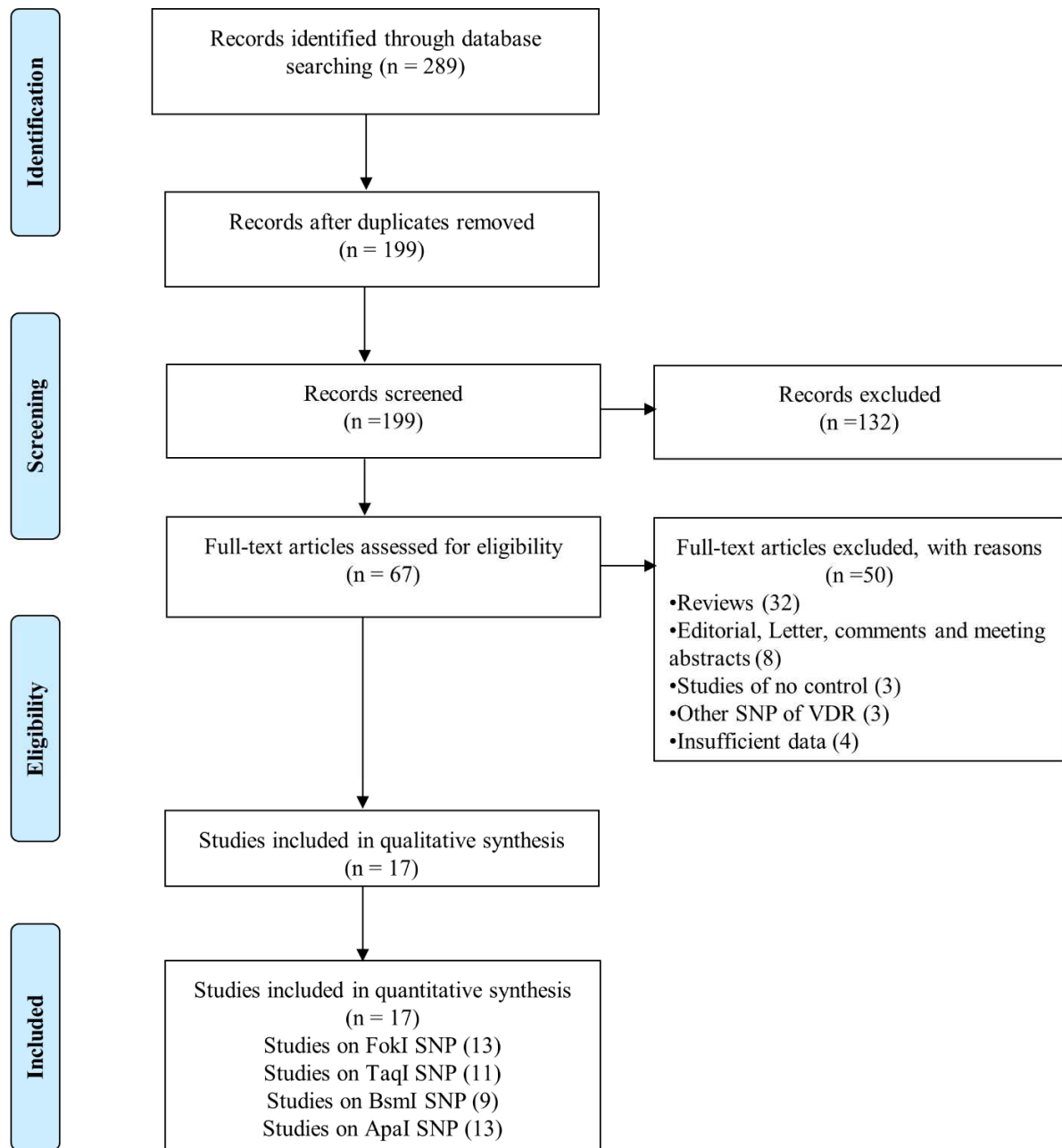
Figure 1. Flow diagram of study selection process.

Figure 2. Pooled OR and 95% CI of individual studies and pooled data for the association between *FokI* polymorphism and Asthma risk in A; Dominant Model (*FokI*) and B; Recessive Model (*FokI*).

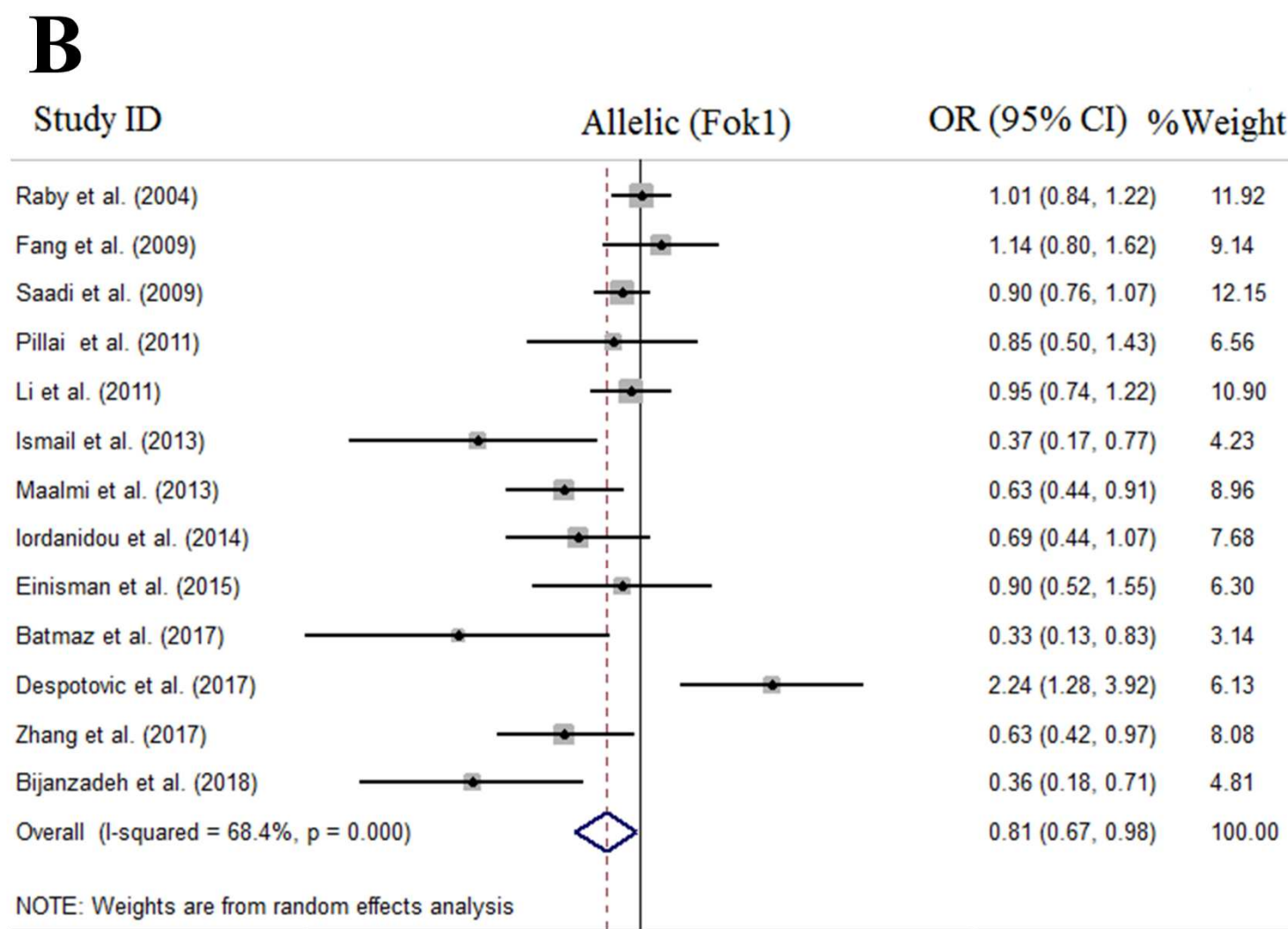
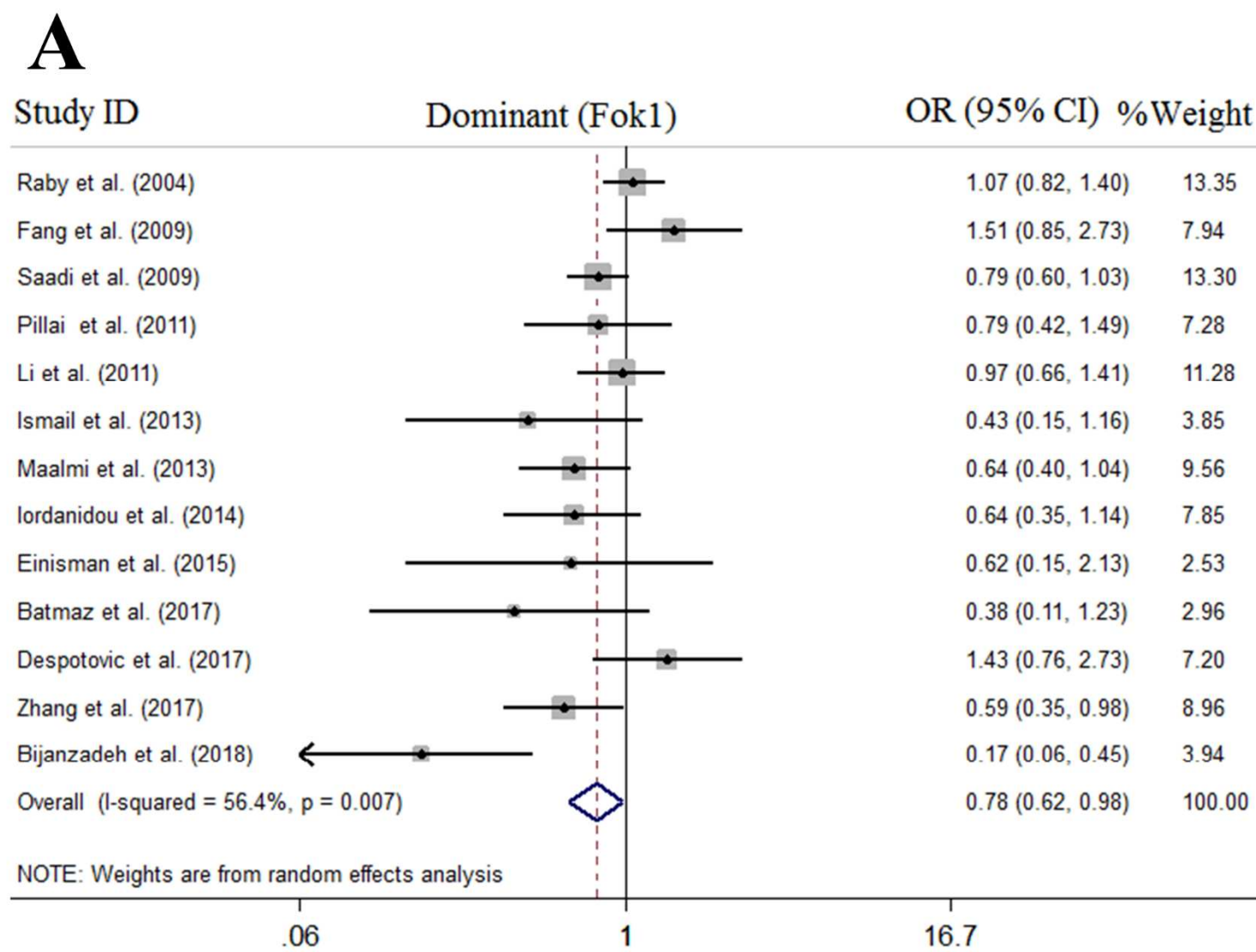
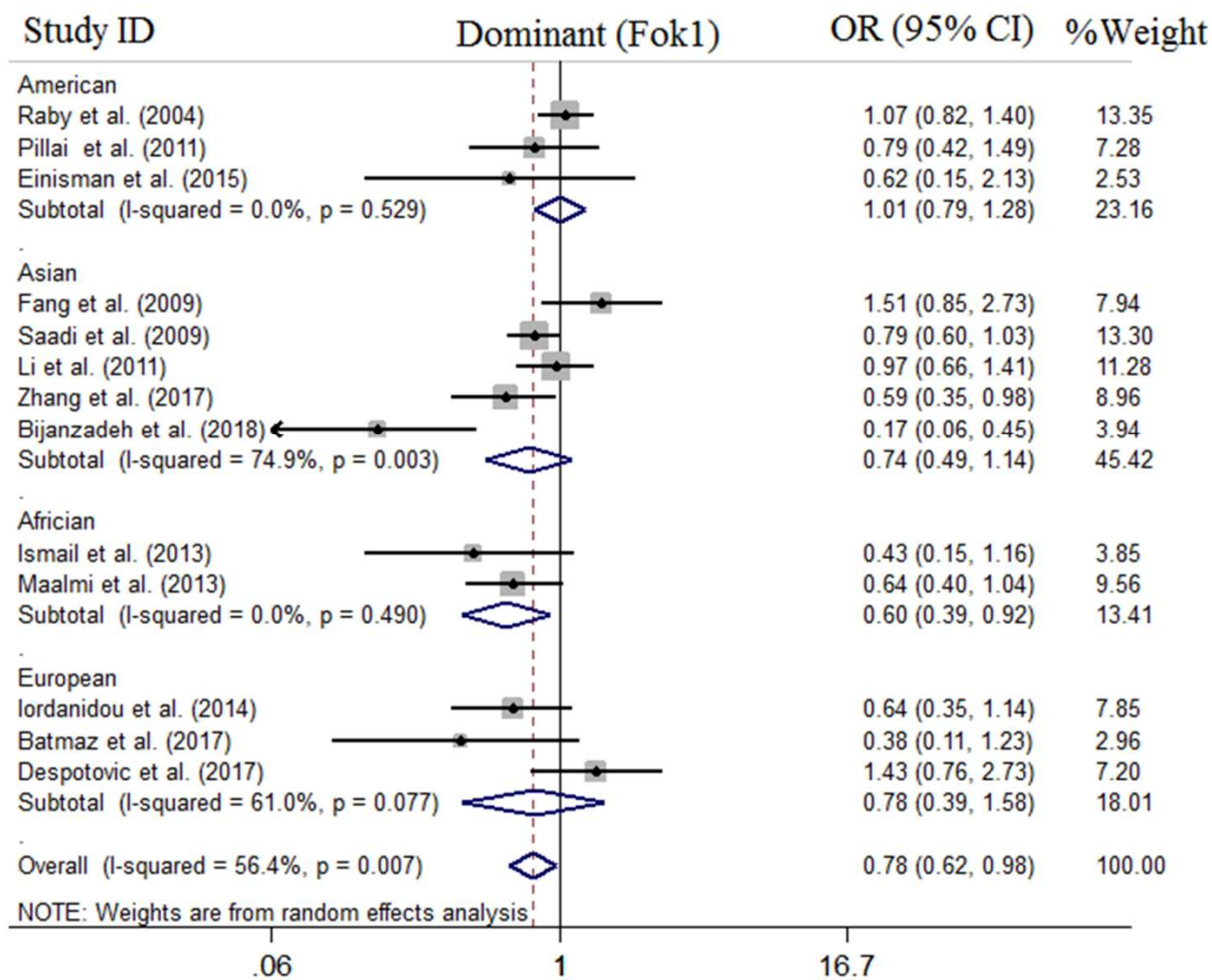


Figure 3. Pooled odds ratio (OR) and 95% confidence interval of individual studies and pooled data for the association between *FokI* polymorphism and Asthma risk in different ethnicity subgroups and overall populations for A; Dominant Model (*FokI*) and B; Allelic Model (*FokI*).

A



B

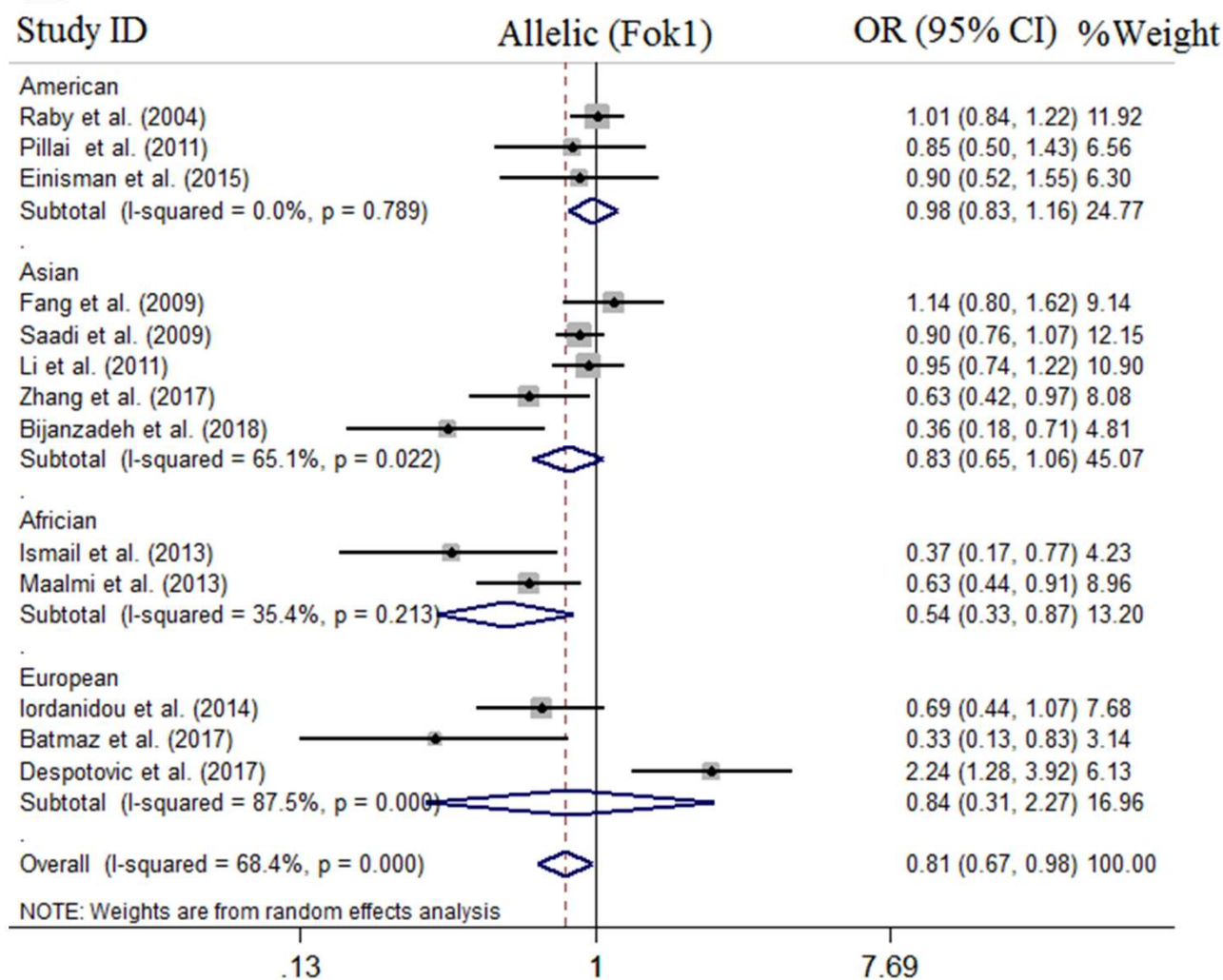


Figure 4. Pooled odds ratio (OR) and 95% confidence interval of individual studies and pooled data for the association between *TaqI* polymorphism and Asthma risk in A; overall populations and B; different ethnicity subgroups for tt vs. TT Model (*TaqI*).

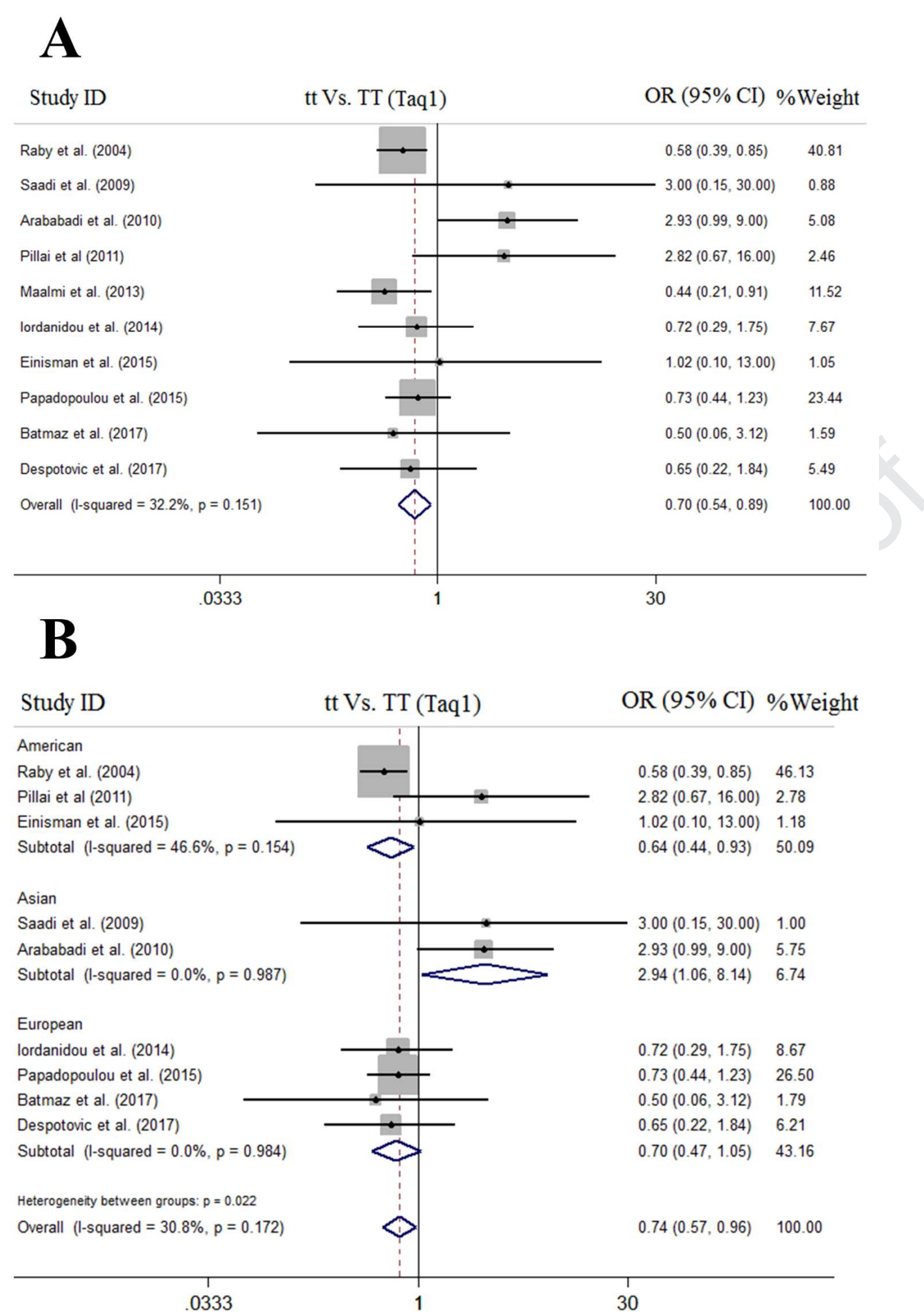
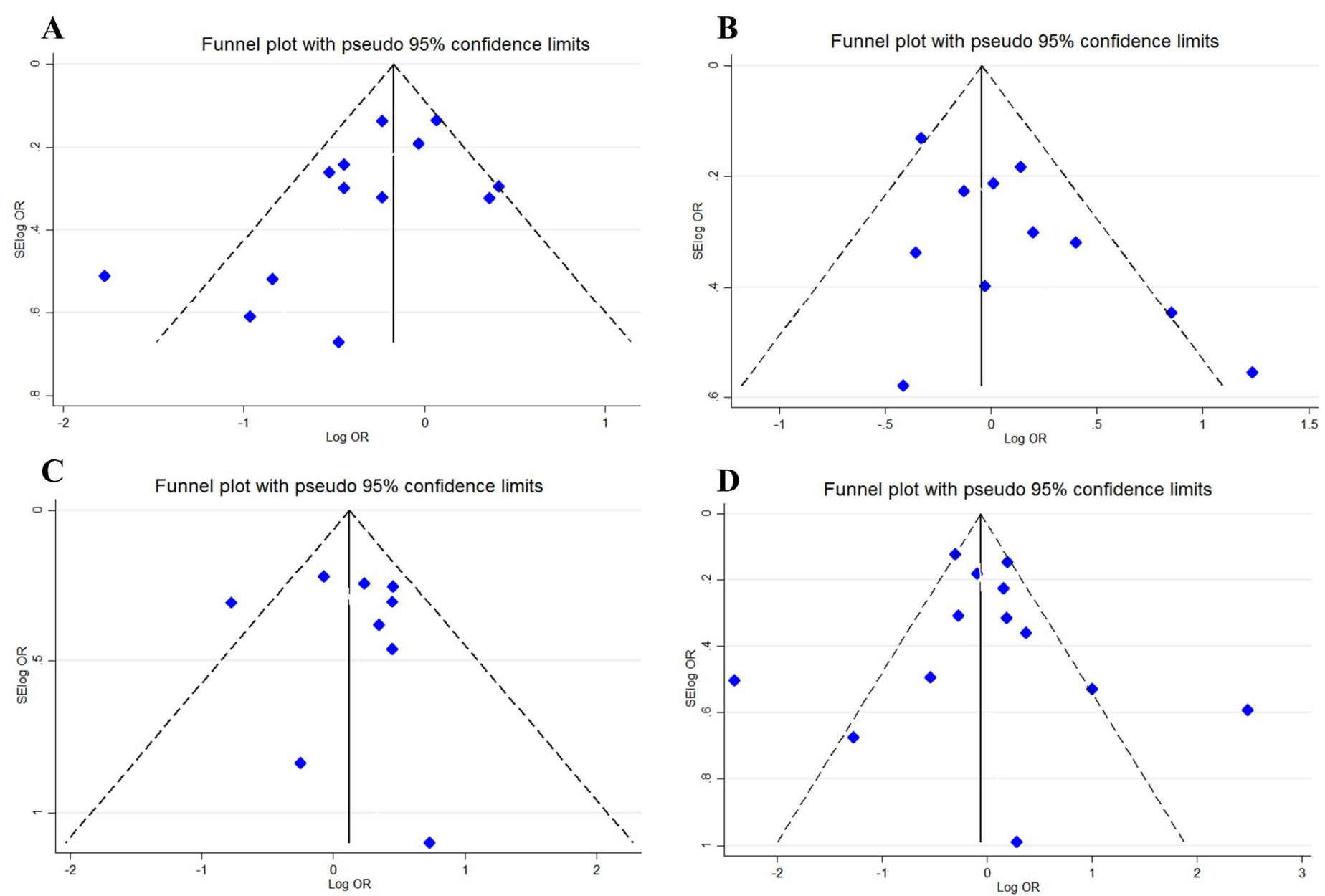


Figure 5. Begg's funnel plot for publication bias test. A; Dominant Model *FokI*, B; Dominant Model *TaqI*, C; Dominant Model *BsmI*, D; Dominant Model *ApaI*. Each point represents a separate study for the indicated association.



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