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# Associations between vitamin D receptor polymorphisms and susceptibility to Behcet's disease: A meta-analysis

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**ABSTRACT**

**Background**: The vitamin D receptor (VDR) gene polymorphisms have been reported to be related to the development of Behcet's disease (BD). However, the results have been inconsistent among diverse populations. Therefore, this comprehensive meta-analysis has been designed to assess a more accurate association between VDR polymorphisms and BD susceptibility.

**Methods**: An electronic literature search was conducted to identify eligible studies. Pooled odds ratios (OR) with corresponding 95% confidence interval (CI) were calculated in different genetic models to assess this association.

**Results**: A total of six separate comparisons comprised of 468 cases and 516 controls were included in the meta-analysis model. The meta-result demonstrated that A allele of ApaI (A vs. a: 1.54 95% CI = 1.04–2.26, $P = 0.029$), and F allele of FokI (F vs. f: OR = 0.58, 95% CI = 0.45–0.76, $P = 0.007$) polymorphisms were associated with the risk of BD in total and African populations, respectively. This significant association was also found in recessive and homozygotes models. Subgroup analysis indicated that FokI variant among Africans and ApaI variant among Caucasian were significantly associated with the risk of BD. No relationship was found between BsmI and TaqI polymorphisms and BD risk.

**Conclusion**: This meta-analysis demonstrated the association between FokI and ApaI polymorphisms in VDR gene with the risk of BD, providing insights into the potential role of vitamin D receptor in the pathogenesis of BD.

**KEYWORDS**
Behcet's disease; meta-analysis; polymorphism; vitamin D receptor

## Introduction

Behcet's disease (BD) is a systemic inflammatory disorder characterized by recurrent episodes of oral aphthae, uveitis, skin lesions, and genital ulceration (Sakane et al., 1999). It is also proposed that BD is associated with a wide range of manifestations, including gastrointestinal ulceration, arthritis, thrombophlebitis, and central nervous system involvement (Kokturk, 2012). Although the exact cause and pathogenesis of BD remains poorly understood, a combination of genetic, environmental, and immunological factors may contribute to the development of the disease (Takeuchi et al., 2015). Genome-

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wide association studies (GWAS) and candidate gene studies have reported several genetic variants associated with susceptibility to BD (Takeuchi et al., 2015).

Beside the genetic and environmental factors, vitamin D with its immunomodulatory effect has newly been proposed as an important factor in the pathogenesis of BD. Existing data, demonstrated a decreased level of vitamin D in serum of patients with active BD (Hamzaoui et al., 2010; Karatay et al., 2011; Khabbazi et al., 2014). The immunomodulatory function of vitamin D is mediated through binding to vitamin D receptor (VDR) located on various type of immune cells, mainly T lymphocytes and antigen presenting cells (Morgan et al., 2000; Veldman et al., 2000). This binding leads to an inhibition in T cell activation, B cell immunoglobulin production and secretion of cytokines like IL-1, 2, 6, 12, TNF, and IFN-γ (Bhalla et al., 1986; D'Ambrosio et al., 1998; Rigby et al., 1987; Tsoukas et al., 1989).

While a growing body of evidence supports the important role of vitamin D in the development of BD, some studies have reported no correlation between serum levels of vitamin D and BD activity (Do et al., 2008; Hamzaoui et al., 2010; Karatay et al., 2011). This conflict may be related to the functional differences in immunomodulatory action of vitamin D, due to genetic variations in both conserved DNA binding or ligand binding domains of VDR or/and downstream genes, which lead to an altered capacity for signal transduction (Zhang et al., 2011).

The VDR is encoded by the VDR gene located on chromosome 12q12-q22 region which contains over 63 single nucleotide polymorphisms (SNPs), some of which leading to a decrease in VDR binding capacity to vitamin D (Uitterlinden et al., 2004; Zmuda et al., 2000). Among them, the four most common SNPs, including FokI (rs10735810), BsmI (rs1544410), TaqI (rs731236), and ApaI (rs7975232), have extensively been studied in various autoimmune disorders (Hitchon et al., 2012; Mao and Huang, 2014; Mostowska et al., 2013; Stefanic et al., 2005). Recently, more attention has been paid toward the possible role of these polymorphisms in the development of BD, but the results are still contradictory (Al-Nahas et al., 2017; Erten et al., 2016; Kamal et al., 2016; Karray et al., 2012; Khabbazi et al., 2014; Kolahi et al., 2015; Tizaoui et al., 2014b). Therefore, we performed a meta-analysis to explore whether the four common polymorphisms of VDR gene confer susceptibility to BD.

## Methods

### *Literature search and data extraction*

A literature search of electronic databases including, PubMed, EMBASE, Web of Science and Scopus was conducted to identify eligible studies, in English, examining the association of VDR gene polymorphisms with BD, using multiple search strategies up to December 2017. Search terms such as "Behcet disease" or "BD", "Behcet syndrome", "vitamin D", "VDR", "genetic" and "polymorphism" were used individually or/and in various combinations. The reference lists of all identified eligible articles were also searched manually to find additional relevant publications not found in the database search. Studies which met the following criteria were included in this meta-analysis; (1) case–control design studies published as original study; (2) those investigated and reported the association of VDR four common polymorphisms including BsmI, TaqI,

FokI, and ApaI with BD; (3) studies provided enough data to calculate odds ratios (ORs); and (4) the genotype distribution in controls being consistence with the Hardy–Weinberg equilibrium (HWE). Studies that failed to meet the inclusion criteria and those with overlapping data were excluded. Two investigators (HMM and ST) extracted and reviewed the essential information of each eligible study independently and any discordance was resolved through discussion and consensus in collaboration with a third reviewer (ZMF). Briefly, the following information was extracted from each study: first author's surname, year of publication, ethnicity, numbers of cases and controls, genotyping technique and the genotype and allele frequencies of each VDR gene polymorphism. All analyses were based on previously published studies, thus no ethical approval or patient consent was required. Quality assessment for each study was done independently by two authors using the Newcastle-Ottawa scale (NOS) which uses a star rating system (a score of 0–9) to evaluate the quality of each study (Wells et al., 2013). Studies with six or more stars were considered good-quality.

### *Data analysis*

The pooled odds ratios (ORs) and 95%CIs were used to measure the strength of the association between VDR polymorphisms (FokI, BsmI, ApaI, and TaqI) and the risk of BD in four genetic models. The statistical significance of the pooled OR was determined by the Z-test and considered significant for $P < 0.05$. Statistical heterogeneity among different studies was measured using the $\chi^2$-based Q and $I^2$ statistic which ranges from 0 to 100%, and represents the proportion of inter-study variability that is attributable to heterogeneity rather than to chance alone. If the value of $I^2$ was less than 50% and heterogeneity was not statistically significant, the fixed effects model was used to estimate the pooled OR; otherwise, the random effect model was applied. Sensitivity analyses were performed to assess the stability of the results, by which each study was omitted in turn and meta-analysis was repeated with the rest. Publication bias was also checked by visually inspection of Begg's funnel plots symmetry and Egger's regression test. Significant publication bias existed if $P < 0.05$. The HWE for control subjects was tested by Pearson's $\chi 2$ test. A p value $> 0.05$ indicated no deviation from HWE. Statistical analysis was carried out using the Comprehensive Meta-Analysis (CMA) computer program (Biostat, Englewood, NJ, USA).

## Results

### *Literature search analysis*

A summary of the study identification and selection process is provided in Figure 1. A total of seven relevant studies (four African and three Caucasian population studies) on VDR polymorphisms in BD met the study inclusion criteria (Al-Nahas et al., 2017; Erten et al., 2016; Kamal et al., 2016; Karray et al., 2012; Khodadadi et al., 2013; Kolahi et al., 2015; Tizaoui et al., 2014b) (Table 1). One of the eligible studies was excluded because of overlapping population with another paper written by the same author (Khodadadi et al., 2013). Thus, a total of six separate comparisons comprised of 468 cases and 516 controls were considered for meta-analysis. Departure of HWE was observed in the cases and

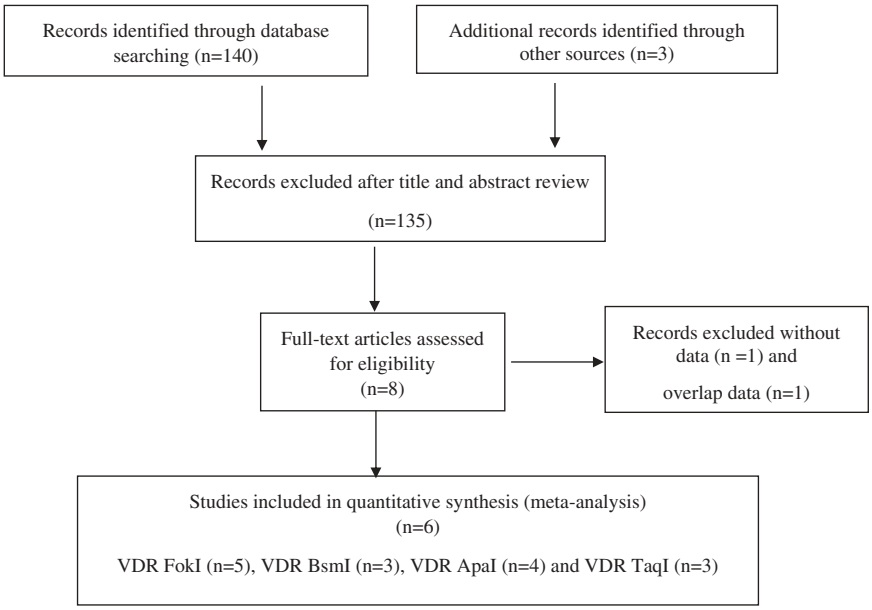


**Figure 1.** Process flowchart of study selection.

**Table 1.** Characteristics of the individual studies included in the meta-analysis.

| Study | Country | Population | Numbers | | VDR SNPs | Technique | Findings for association | NOS assessment Score |
|---|---|---|---|---|---|---|---|---|
| | | | Case | Control | | | | |
| Al-Nahas et al., 2017 | Egypt | African | 45 | 45 | BsmI, FokI | RFLP | BsmI ($P$ = 0.001) and FokI ($P$ = 0.128) | 6 |
| Erten et al., 2016 | Turkey | Caucasian | 37 | 30 | FokI, TaqI, ApaI | RT-PCR | FokI ($P$ = 0.794), TaqI ($P$ = 0.128)and ApaI ($P$ = 0.0942) | 6 |
| Kamal et al., 2016 | Egypt | African | 54 | 60 | ApaI, TaqI | RFLP | ApaI ($P$ = 0.019) and TaqI ($P$ = 0.059) | 6 |
| Karray et al., 2012 | Tunisian | African | 131 | 152 | FokI, BsmI | RFLP | FokI ($P$ = 0.002)and BsmI ($P$ = 0.78) | 7 |
| Kolahi et al., 2015 | Iran | Caucasian | 50 | 50 | FokI, BsmI, TaqI, ApaI | RFLP | FokI ($P$ = 0.04), BsmI ($P$ = 0.97),TaqI ($P$ = 0.68) and ApaI ($P$ = 0.16) | 7 |
| Tizaoui et al., 2014b | Tunisian | African | 151 | 179 | TaqI, ApaI | RFLP | TaqI ($P$ = 0.165)and ApaI ($P$ = 0.934) | 7 |
| Khodadadi et al., 2013 | Iran | Caucasian | 50 | 50 | FokI, BsmI | RFLP | FokI ($P$ = 0.04)and BsmI ($P$ = 0.97) | 7 |

controls of only one study for BsmI polymorphism (Kolahi et al., 2015). We also performed ethnicity-specific meta-analysis in African and Caucasian populations.

Of all included publications, three studies examined the VDR BsmI polymorphism (Al-Nahas et al., 2017; Karray et al., 2012; Kolahi et al., 2015), three the VDR TaqI polymorphism (Erten et al., 2016; Kolahi et al., 2015; Tizaoui et al., 2014b), five the VDR FokI polymorphism (Al-Nahas et al., 2017; Erten et al., 2016; Kamal et al., 2016; Karray et al., 2012; Kolahi et al., 2015), and four the ApaI polymorphism (Erten et al., 2016; Kamal et al., 2016; Kolahi et al., 2015; Tizaoui et al., 2014b). The main characteristics of these

studies related to the association between VDR polymorphisms and BD are summarized in Tables 1 and 2. We performed meta-analysis on the association between polymorphisms and the disease when there were at least two relevant studies.

## Meta-analysis of VDR FoKI, BsmI, ApaI and TaqI polymorphisms and BD susceptibility

Meta-analyses findings regarding the associations of VDR FokI, BsmI, ApaI and TaqI polymorphisms with BD are summarized in Table 3.

### FokI polymorphism

In terms of FokI polymorphism, a total of five eligible studies comprised of 317 cases and 337 controls were included in the meta-analysis. Random effect models were used in the two genetic models (allele contrast and Dominant model), as heterogeneity across studies was statistically significant; otherwise, fixed effect models were applied in homozygote contrast and recessive model (Table 3). Moreover, both recessive (OR = 0.37, 95% CI = 0.22–0.61, $P < 0.001$) and homozygotes genetic models (OR = 0.34, 95% CI = 0.20–0.60, $P < 0.001$) were found to be associated with the risk of BD. However, no significant consequence was observed for other genotype comparisons: F vs. f: OR with a 95% CI 0.80 (0.50–1.28), $P = 0.35$; Ff + FF vs. ff: 1.57 (0.73–3.41), $P = 0.25$ (Table 3, Figure 2a). To trace the possible source of heterogeneity, we performed sub-group analysis based on ethnicity in which, the obtained results revealed no statistically significant heterogeneity and a significant association between FokI alleles with the risk of BD in African population (OR = 0.58, 95% CI = 0.45–0.76, $P = 0.007$). The same pattern was observed for African group in other genotype comparison (Table 3).

### BsmI polymorphism

For the BsmI variant, three studies with 226 cases and 247 controls were included. There was significant between-study heterogeneity in the meta-analysis of BsmI polymorphism in all genetic models; therefore, random models were used during data synthesis. This polymorphism was not associated with BD in four genetic models; B vs. b: 0.77 (0.39–1.52), $P = 0.45$; BB vs. Bb + bb: 0.60 (0.20–1.85), $P = 0.37$; BB + Bb vs. bb: 0.85 (0.45–1.61), $P = 0.61$; BB vs. bb: 0.56 (0.15–2.12), $P = 0.40$, neither in the overall population, nor when stratified by ethnicity in all genetic models (Table 3). Since a deviation from HWE was present for genotype distribution among controls in one study of BsmI polymorphism (Kolahi et al., 2015), meta-analysis was performed by excluding the Kolahi et al study, to gain insight on the reliability of the results, in which no alteration was found in the results (Table 3).

### ApaI polymorphism

Four studies with 292 cases and 319 controls were included for the ApaI polymorphism. Some heterogeneity was found in the meta-analyses of ApaI polymorphism in allele contrast and recessive model. So, random models were used during data synthesis for these two models, otherwise fixed models were applied. Meta-analysis of the A allele, and

**Table 2.** Allele and genotypic frequencies of VDR polymorphisms in cases and control.

| | | | BD patients | | | | | Controls | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number | Genotype | | | Allele | | Genotype | | | Allele | | |
| Study | Country | Case/Control | I/I | I/II | II/II | I | II | I/I | I/II | II/II | I | II | HWE |
| **FokI (rs10735810), f = I & F = II** | | | | | | | | | | | | | |
| Al-Nahas et al., 2017 | Egypt | 45/45 | 21 (46.7) | 22 (48.9) | 2 (4.4) | 64 (71.1) | 26 (28.9) | 17 (37.8) | 20 (44.4) | 8 (17.8) | 54 (60) | 36 (40) | 0.40 |
| Erten et al., 2016 | Turkey | 37/30 | 20 (45.1) | 15 (40.5) | 2(5.4) | 55 (74.3) | 19 (25.7) | 15 (50) | 12 (40) | 3 (10) | 42 (70) | 18 (30) | 0.09 |
| Kamal et al., 2016 | Egypt | 54/60 | 14 (25.9) | 33 (61.1) | 7 (13) | 61 (56) | 47 (44) | 16 (26.7) | 26 (43.3) | 18 (30) | 58 (48) | 62 (52) | 0.31 |
| Karray et al., 2012 | Tunisian | 131/152 | 61 (46.9) | 57 (43.8) | 12 (9.3) | 179 (68.8) | 81 (31.9) | 46 (30.2) | 72 (47.3) | 34 (22.3) | 164 (53.9) | 140 (46) | 0.56 |
| Kolahi et al., 2015 | Iran | 50/50 | 26 (52) | 22 (44) | 2 (4) | 74 (74) | 26 (26) | 37(74) | 13 (26) | 0 | 87 (87) | 13 (13) | 0.29 |
| ***BsmI (rs1544410), b = I & B = II*** | | | | | | | | | | | | | |
| Al-Nahas et al., 2017 | Egypt | 45/45 | 15 (33.3) | 27 (60) | 3 (6.7) | 57 (63.3) | 33 (36.7) | 7 (15.6) | 20 (44.4) | 18 (40) | 34 (37.2) | 56 (62.2) | 0.51 |
| Karray et al., 2012 | Tunisian | 131/152 | 26 (19.8) | 55 (42.0) | 50 (38.8) | 107 (40.8) | 155 (59.2) | 35 (23) | 64 (42.1) | 53 (34.8) | 134 (44) | 170 (55.9) | 0.07 |
| Kolahi et al., 2015 | Iran | 50/50 | 17 (34) | 14 (28) | 19 (38) | 48 (48) | 52 (52) | 17 (34) | 15 (30) | 18 (36) | 49 (49) | 51 (51) | 0.005 |
| ***ApaI (rs7975232), a = I & A = II*** | | | | | | | | | | | | | |
| Erten et al., 2016 | Turkey | 37/30 | 6 (17.1) | 15(42.9) | 14 (40) | 27 (38.5) | 43(61.5) | 13 (43.3) | 10 (33.3) | 7 (23.4) | 36 (60) | 24 (40) | 0.09 |
| Kamal et al., 2016 | Egypt | 54/60 | 17 (31.5) | 32 (59.3) | 5 (9.3) | 66 (61.0) | 42 (39.0) | 34 (56.7) | 24 (40.0) | 2 (3.3) | 92 (77.0) | 28 (23.0) | 0.36 |
| Kolahi et al.,2015 | Iran | 50/50 | 17 (34) | 24 (48) | 9 (18) | 58 (58) | 42 (42) | 22 (44) | 25 (50) | 3 (6) | 69 (69) | 31 (31) | 0.23 |
| Tizaoui et al., 2014b | Tunisian | 151/179 | 47 (31.1) | 79 (52.3) | 25 (16.6) | 137 (57.2) | 129 (42.7) | 57 (31.8 | 95 (53.1) | 27 (15.1) | 209 (58.37) | 149 (41.62) | 0.22 |
| ***TaqI (rs731236), t = I & T = II*** | | | | | | | | | | | | | |
| Erten et al., 2016 | Turkey | 37/30 | 19(51.4) | 14(37.8) | 4(10.8) | 52 (70.2) | 22(29.8) | 13 (43.3) | 11 (36.7) | 6 (20) | 37 (61.7) | 23 (38.3) | 0.22 |
| Kolahi et al., 2015 | Iran | 50/50 | 21 (42) | 21 (42) | 8 (16) | 58 (58) | 42 (42) | 25 (50) | 17 (34) | 8 (16) | 67 (67) | 33 (33) | 0.10 |
| Tizaoui et al., 2014b | Tunisian | 151/179 | 73 (48.3) | 64 (42.4) | 14 (9.3) | 210(69.5) | 92 (30.4) | 68 (38) | 90 (50.3) | 21 (11.7) | 226 (63.1) | 132 (36.8) | 0.28 |

NS: not significant, HWE: Hardy-Weinberg equilibrium of genotypes of controls, I & II indicated the wild and risk allele respectively.

**Table 3.** Meta-analysis of associations between the VDR FokI, BsmI, ApaI and TaqI polymorphisms and BD.

| | | | Test of association | | Test of heterogeneity | | |
|---|---|---|---|---|---|---|---|
| VDR polymorphism | Population | No. of studies | OR & 95% CI | *P*-value | Model | $I^2$ | *P*-value |
| **FokI (rs10735810)** | | | | | | | |
| F versus f (allele comparison) | Overall | 5 | 0.80 (0.50–1.28) | 0.353 | R | 70.31 | 0.009 |
| | Caucasian | 2 | 1.39 (0.76–2.57) | 0.286 | R | 74.65 | 0.047 |
| | African | 3 | **0.58 (0.45–0.76)** | **0.007** | F | 0.00 | 0.60 |
| | Without Kolahi study | 4 | **0.60 (0.47–0.77)** | **0.001** | F | 0.00 | 0.65 |
| FF versus ff+Ff (recessive model) | Overall | 5 | **0.37 (0.22–0.61)** | **0.000** | F | 0.00 | 0.484 |
| | Caucasian | 2 | 0.96 (0.196–4.70) | 0.959 | F | 37.80 | 0.21 |
| | African | 3 | **0.33 (0.19–0.57)** | **0.000** | F | 0.000 | 0.86 |
| Ff+FF versus ff (Dominant model) | Overall | 5 | 0.90 (0.49–1.63) | 0.718 | R | 66.78 | 0.017 |
| | Caucasian | 2 | 1.57 (0.73–3.41) | 0.25 | R | 66.53 | 0.084 |
| | African | 3 | **0.60 (0.41–0.87)** | **0.007** | F | 2.00 | 0.36 |
| | Without Kolahi study | 4 | **0.63 (0.44–0.89)** | **0.008** | F | 0.00 | 0.477 |
| FF versus ff (homozygote model) | Overall | 5 | **0.343 (0.20–0.60)** | **0.000** | F | 17.96 | 0.300 |
| | Caucasian | 2 | 1.41 (0.11–17.84) | 0.789 | R | 51.371 | 0.152 |
| | African | 3 | **0.30 (0.16–0.53)** | **0.000** | F | 0.000 | 0.681 |
| Ff versus ff (heterozygote model) | Overall | 5 | 1.06 (0.63–1.80) | 0.82 | R | 53.56 | 0.07 |
| | Caucasian | 2 | 1.63 (0.85–3.13) | 0.14 | F | 48.93 | 0.16 |
| | African | 3 | 0.78 (0.52–1.15) | 0.21 | F | 33.622 | 0.22 |
| ***BsmI (rs1544410)*** | | | | | | | |
| B versus b (allele comparison) | Overall | 3 | 0.77 (0.39–1.52) | 0.453 | R | 82.61 | 0.003 |
| | Overall in HWE | 2 | 0.65 (0.21–2.06) | 0.47 | R | 91.05 | 0.001 |
| | Caucasian | 1 | 1.04 (0.60–1.81) | 0.89 | - | - | - |
| | African | 2 | 0.65 (0.21–2.06) | 0.47 | R | 91.05 | 0.001 |
| | Without Al-Nahas | 2 | 1.11 (0.84–1.48) | 0.46 | F | 0 | 0.78 |
| bb versus BB+Bb (recessive model) | Overall | 3 | 0.60 (0.20–1.85) | 0.373 | R | 82.27 | 0.004 |
| | Caucasian | 1 | 1.09 (0.48–2.46) | 0.84 | - | - | - |
| | African | 2 | 0.38 (0.037–3.89) | 0.42 | R | 90.95 | 0.001 |
| | Without Al-Nahas | 2 | 1.14 (0.75–1.722) | 0.55 | F | 0 | 0.91 |
| Bb+BB versus bb (Dominant model) | Overall | 3 | 0.85 (0.45–1.61) | 0.61 | R | 50.24 | 0.13 |
| | Caucasian | 1 | 1.00 (0.44–2.29) | 1.000 | - | - | - |
| | African | 2 | 0.72 (0.23–1.29) | 0.58 | R | 74.88 | 0.46 |
| BB versus bb (homozygote model) | Overall | 3 | 0.56 (0.15–2.12) | 0.40 | R | 82.22 | 0.004 |
| | Caucasian | 1 | 1.06 (0.42–2.68) | 0.91 | - | - | - |
| | African | 2 | 0.34 (0.02–5.27) | 0.44 | R | 90.97 | 0.001 |
| | Without al-nahas | 2 | 1.19 (0.71–2.026) | 0.50 | F | 0 | 0.75 |
| Bb versus bb (heterozygote model) | Overall | 3 | 0.98 (0.61–1.57) | 0.93 | F | 0 | 0.62 |
| | Caucasian | 1 | 0.93 (0.35–2.51) | 0.89 | - | - | - |
| | African | 2 | 0.99 (0.58–1.70) | 0.98 | F | 0 | NS |
| ***ApaI (rs7975232)*** | | | | | | | |
| A **versus** a (allele comparison) | Overall | 4 | 1.54 (1.04–2.26) | 0.029 | R | 55.17 | 0.082 |
| | Caucasian | 2 | **1.79 (1.15–2.80)** | **0.01** | F | 0 | 0.58 |
| | African | 2 | 1.38 (0.81–2.34) | 0.23 | R | 76.96 | 0.037 |
| AA versus aa+Aa (recessive model) | Overall | 4 | 1.53 (0.96–2.44) | 0.07 | F | 7.63 | 0.36 |
| | Caucasian | 2 | **2.46 (1.05–5.73)** | **0.04** | F | 0 | 0.54 |
| | African | 2 | 1.24 (0.71–2.18) | 0.45 | F | 12.64 | 0.29 |
| | Without Tizaoui study | 3 | 2.55 (1.20–5.44) | 0.015 | F | 0 | 0.82 |
| Aa+AA versus aa (Dominant model) | Overall | 4 | 1.71 (0.996–2.94) | 0.052 | R | 53.10 | 0.094 |
| | Caucasian | 2 | 1.89 (0.0.99–3.61) | 0.051 | F | 0 | 0.38 |
| | African | 2 | 1.61 (0.70–3.70) | 0.30 | R | 79.50 | 0.03 |
| | Without Tizaoui study | 3 | **2.24 (1.37–3.68)** | **0.001** | F | 0 | 0.50 |

(*Continued*)

**Table 3.** (Continued).

| VDR polymorphism | Population | No. of studies | Test of association | | Test of heterogeneity | | |
|---|---|---|---|---|---|---|---|
| | | | OR & 95% CI | *P*-value | Model | $I^2$ | *P*-value |
| AA versus aa (homozygote model) | Overall | 4 | **1.84 (1.09–3.10)** | **0.023** | F | 45.70 | 0.14 |
| | Caucasian | 2 | **3.79 (1.44–9.94)** | **0.007** | F | 0 | 0.96 |
| | African | 2 | 1.52 (0.66–3.49) | 0.33 | R | 59.47 | 0.12 |
| | Without Tizaoui study | 3 | **4.043 (1.74–9.40)** | **0.001** | F | 0 | 0.96 |
| Aa versus aa (heterozygote model) | Overall | 4 | 1.61 (0.91–2.82) | 0.09 | R | 50.95 | 0.11 |
| | Caucasian | 2 | 1.68 (0.83–3.38) | 0.15 | F | 35.53 | 0.21 |
| | African | 2 | 1.55 (0.64–3.96) | 0.34 | R | 76.40 | 0.04 |
| | Without Tizaoui study | 3 | **2.07 (1.17–3.65)** | **0.012** | F | 12.87 | 0.32 |
| ***TaqI (rs731236)5*** | | | | | | | |
| T versus t (allele comparison) | Overall | 3 | 0.89 (0.57–1.41) | 0.64 | R | 54.66 | 0.11 |
| | Caucasian | 2 | 1.03 (0.49–2.19) | 0.93 | R | 62.67 | 0.102 |
| | Overal without kolahi | 2 | **0.74 (0.55–0.99)** | **0.045** | F | 0 | 0.81 |
| | African | 1 | 0.75 (0.54–1.04) | 0.08 | - | - | - |
| TT versus tt+Tt (recessive model) | Overall | 3 | 0.77 (0.44–1.32) | 0.34 | F | 0 | 0.71 |
| | Caucasian | 2 | 0.76 (0.33–1.77) | 0.49 | F | 0 | 0.41 |
| | African | 1 | 0.77 (0.38–1.57) | 0.47 | - | - | - |
| Tt+TT versus tt (Dominant model) | Overall | 3 | 0.77 (0.54–1.01) | 0.16 | F | 24.38 | 0.27 |
| | Caucasian | 2 | 1.07 (0.58–1.97) | 0.83 | F | 2.43 | 0.31 |
| | Overall without Kolahi | 2 | 0.66 (0.45–0.99) | 0.046 | F | 0 | 0.85 |
| | African | 1 | 0.66 (0.42–1.02) | 0.059 | - | - | - |
| TT versus tt (homozygote model) | Overall | 3 | 0.70 (0.39–1.24) | 0.22 | F | 0 | 0.53 |
| | Caucasian | 2 | 0.83 (0.34–2.02) | 0.67 | F | 3.96 | 0.31 |
| | African | 1 | 0.62 (0.29–1.32) | 0.22 | - | - | - |
| Tt versus tt (heterozygote model) | Overall | 3 | 0.80 (0.55–1.17) | 0.25 | F | 22.50 | 0.28 |
| | Caucasian | 2 | 1.19 (0.61–2.33) | 0.61 | F | 0 | 0.45 |
| | African | 1 | 0.66 (0.42–1.05) | 0.079 | - | - | - |

BD: Behcet disease, HWE Hardy-Weinberg equilibrium, OR: Odds ratio, CI: confidence interval, R: random effects model, F: fixed effects model.

AA versus aa genotype of the ApaI polymorphism showed significant association with BD (Table 3). The overall risk of developing BD conferred by the ApaI A allele was 1.54 (95% CI = 1.04–2.26, $P = 0.029$), and the pooled OR of the AA vs. aa genotype also showed the same trend (1.84, 95% CI = 1.09–3.10, $P = 0.023$). In addition sub-group analysis based on ethnicity, indicated a significant association of ApaI allele and genotypes distribution in different genetic models with BD (Table 3).

## *TaqI polymorphism*

For the TaqI polymorphism, three relevant studies with 238 cases and 259 controls were included. Except for allele contrast, no significant heterogeneity was observed for meta-analysis of TaqI polymorphism. This polymorphism was not associated with BD risk in all genetic models in overall, or in sub-group populations (Table 3).

## *Sensitivity analysis*

To assess the influence of each study on the pooled OR, sensitivity analysis was performed by omitting each study, one at a time. In term of FokI polymorphism, following the exclusion of

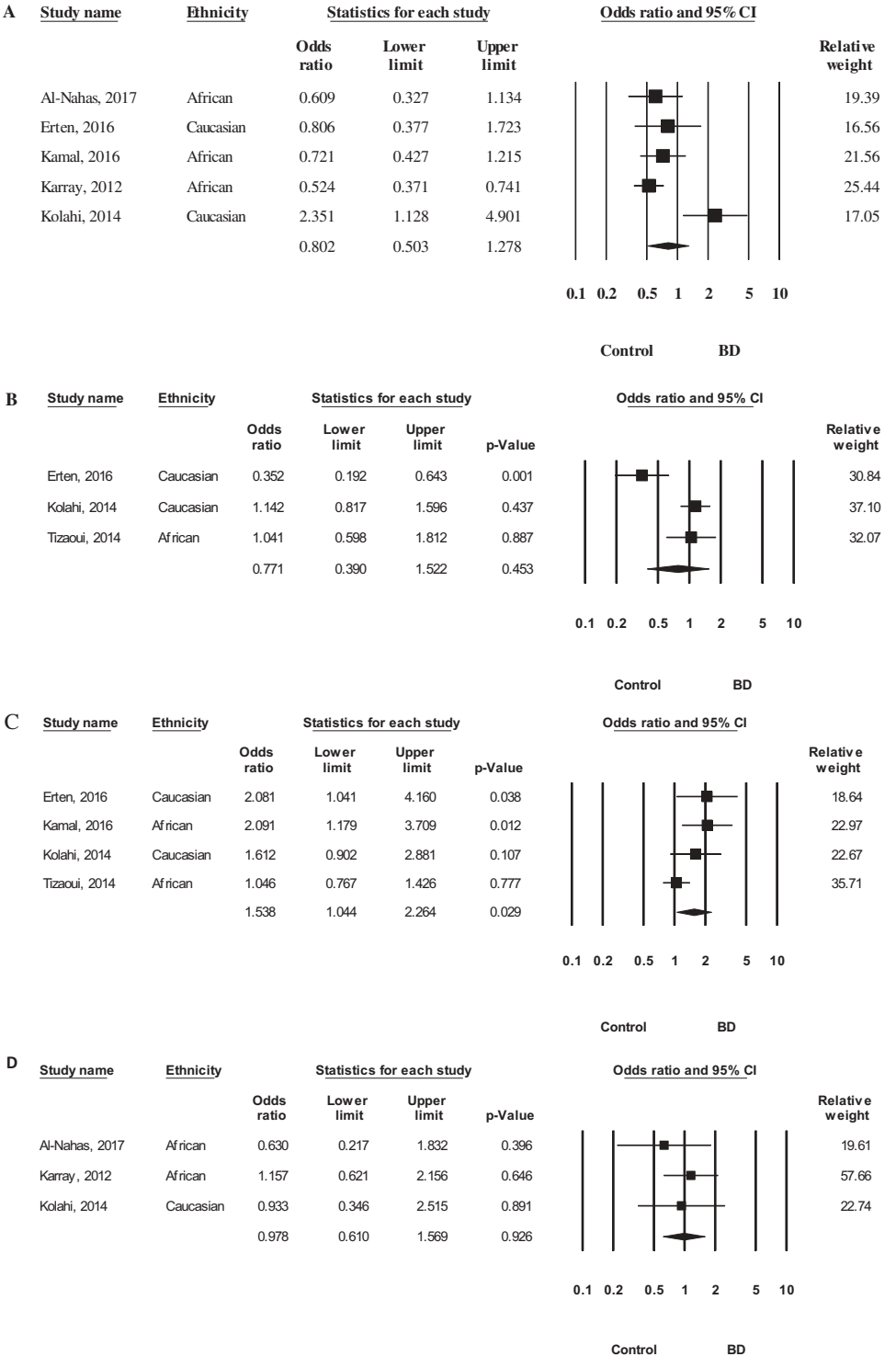


**Figure 2.** ORs and 95% CIs of individual studies and pooled data for allelic associations of the VDR FokI (A), BsmI (B), ApaI (C), and TaqI (D) polymorphisms and Behcet's disease.

Kolahi et al study, heterogeneity was no longer statistically significant and pooled OR in both allele contrast and dominant model were changed toward the significant values (Kolahi et al., 2015). For BsmI polymorphism, the exclusion of Al-Nahas et al (Al-Nahas et al., 2017) removed the heterogeneity, and pooled OR was also materially altered in all genetic models. In the case of ApaI, when Tizaoui et al study (Tizaoui et al., 2014b) was excluded, heterogeneity was materially altered, and pooled ORs with 95% CI were changed to a significant value in homozygote contrast, recessive and dominant models (Table 3).

## Publication bias

Begg's funnel plot and Egger's test are often used to detect publication bias of the selected articles. However due to limitation of the number and sample size of studies included in our analysis, publication bias was evaluated using only Egger's linear regression test, except in FokI polymorphism. As shown in Figure 3A, visual inspection of Begg's funnel

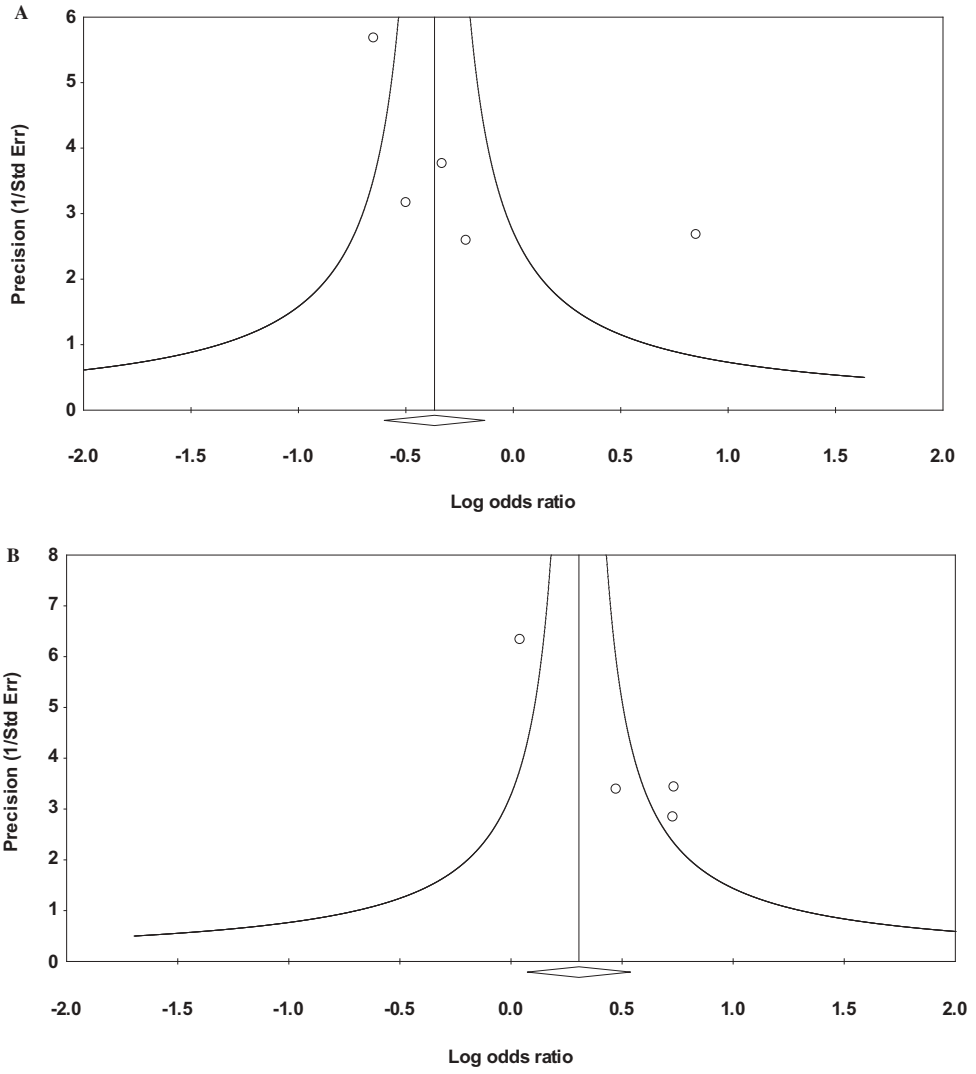


**Figure 3.** Begg's funnel plots of the alleles of FokI (A), and ApaI (B) and Behcet's disease comparison.

plot with regard to the FokI polymorphism reveals no evidence of an obvious asymmetry, neither in allele comparison models nor in other genetic models (data not shown). Moreover, the results of Egger's test also indicated no publication bias for FokI, BsmI and TaqI polymorphisms (corresponding to the $P$ -values > 0.05). However, publication bias was observed in the ApaI polymorphism in the allele contrast (A vs. a: $P = 0.038$, Figure 3B), homozygote contrast (AA vs. aa, $P = 0.014$) and recessive genetic models (AA vs. Aa+aa, $P = 0.03$).

## Discussion

Like other autoimmune disorders, BD appears to be caused by complex interactions between endogenous and exogenous factors such as certain genetic background and several environmental factors (Marson et al., 2015). Among them, vitamin D, with its specific nuclear receptor and potent immunomodulatory function has been recognized as both an exogenous and endogenous player (Arnson et al., 2007). An increasing number of epidemiological, genetic, and basic studies have provided insight into the potential role of vitamin D in the pathogenesis of certain autoimmune diseases. In this case, the presence of VDR polymorphisms, which can modify the immunomodulatory action of vitamin D, may further support such a plausible pathogenic link. Being highly polymorphic, many SNPs have been reported for VDR gene (Uitterlinden et al., 2004; Zmuda et al., 2000). Findings of several studies considering the role of VDR polymorphisms in the development of BD have led to various conclusions. These discrepancies may reflect the limited number of cases in some studies, difference in study design or the analysis of different ethnic groups.

The present meta-analysis addresses the association of four common polymorphisms of VDR gene with BD susceptibility. Available data from six eligible published articles comprised of 468 cases and 516 unrelated healthy controls were combined to evaluate genetic contribution of the most studied polymorphisms, including FokI, BsmI, ApaI, and TaqI in the development of BD. Although the meta-analysis of the VDR BsmI and TaqI polymorphisms revealed no association with BD in all subjects or in ethnicity-based sub-groups, a strong relationship was found between ApaI and FokI polymorphisms with the risk of BD in various genetic comparison models. The results suggested that the TaqI-t allele may be a risk factor of BD, with a pooled OR of 1.54 (95% CI = 1.04–2.26, $P = 0.029$), while the FokI-f allele carriage may be protective for BD with an OR of 0.60 (95% CI = 0.47–0.77, $P = 0.001$). Importantly, the FokI-f allele was associated with BD in a protective manner in African sub-group and in overall subject when the Kolahi et al. study was excluded (Kolahi et al., 2015). This association between VDR polymorphisms and the risk of BD, observed in this meta-analysis, indicates that vitamin D deficiency may play a role in susceptibility to disease.

VDR is an intracellular receptor protein, expressed by human immune cells including macrophages, dendritic cells, and T and B lymphocytes. After ligation with vitamin D, the vitamin D/VDR complex is translocated into the nucleus, and forms a heterodimer with the retinoid X receptor (RXR), which finally regulates transcriptionally vitamin D response genes (Pike et al., 2012). Most of the genetic abnormalities result in a VDR with decreased capacity in binding to RXR, contributing to immunity-related diseases (Gallone et al., 2017). VDR gene polymorphisms, due to any alteration in gene expression

or function, results in an abnormal function of VDR which may affect immune cells interaction with vitamin D, influence regulation of immune cells proliferation/differentiation and lead to an uncontrolled increment in immune response responsible for T-cell mediated autoimmune diseases (Uitterlinden et al., 2004; Whitfield et al., 2001). Several studies have shown the contribution of VDR polymorphisms in development of autoimmune disease due to modification of the immunomodulatory action of vitamin D (Bizzaro et al., 2017; Song et al., 2016; Tizaoui et al., 2014a, 2015).

The exact mechanism by which VDR polymorphisms are contributed to the pathogenesis of autoimmune disorders may be attributed to their biological effect (Uitterlinden et al., 2004). It has been suggested that ApaI, BsmI and TaqI polymorphisms located in the region of intron 8/exon 9 of the VDR gene do not affect the VDR protein structure, and may not have any functional effect (Tizaoui et al., 2015). However, at the molecular level, they may influence gene expression through the regulation of mRNA stability and/or translation efficiency. One potential exception is the FokI polymorphism located in the coding area (exon 2) of the VDR gene, which has been most analyzed because of its functional significance (Uitterlinden et al., 2004). The FokI polymorphism leads to a VDR protein lacking three amino acids, which results in an increased VDR transcriptional activity (Van Etten et al., 2007). Our results are consistent with these functional properties of these polymorphisms, as the short isoform of FokI was associated with a decreased risk of BD, while the ApaI polymorphism was implicated in BD risk.

To the best of our knowledge, no prior meta-analysis had been conducted to access the association of VDR gene polymorphisms with BD. This is the first study, to better understand the potential relationship of VDR polymorphisms with the risk of BD and to make a relatively comprehensive conclusion. However, due to the limited number of studies included and small number of cases in each study the results of this meta-analysis should be interpreted with caution. Furthermore, our results indicated that based on ethnic groups, VDR polymorphisms may have a different relative importance during the development of BD. However, this study could not conduct any ethnic specific meta-analysis in Asian and European BD patients due to the lack of relevant studies.

This study has some limitations that need to be considered. First, there was a significant inter-study heterogeneity that might have distorted the results, which is a common problem in meta-analysis for genetic associations (Munafo and Flint, 2004). However, following the sensitivity analysis or subgroups analysis based on ethnicity, heterogeneity was disappeared in most of the subgroups, suggesting the region (ethnicity) as a main source of heterogeneity. Second, any potential confounding factors and gene–environment interaction were not considered due to the lack of sufficient data. Third, as only studies published in English were included, publication bias may also have affected the analysis. Although Egger's regression test was performed, the possibility of bias was not eliminated. Finally, the small sample size of the included studies was another important limitation which could affect the power of detection a causal variant in genetic association studies. Also, the lack of European and Asian studies prevented us to have a more comprehensive analysis for assessing these polymorphisms association; thus, our results are restricted to specific ethnic groups. The major strength of this study is conducting a comprehensive assessment along with extensive sensitivity analyses, excluding studies that did not meet specific criteria. Taken together, the interpretation of the results should be done cautiously considering its limitations.

In conclusion, despite the undeniable limitations, this meta-analysis demonstrated that alleles of ApaI confer a strong susceptibility for developing BD especially in Caucasian

population, and can be treated as a risk factor for BD. However, FokI polymorphism can be regarded as a protective factor, especially in the African sub-group. Further epidemiologic studies with larger sample-size in populations with different ethnicities are required to investigate more accurately the role of these polymorphisms in the development of BD.

## Declaration of interest

The authors report no conflict of interests in this work.

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