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Vitamin D receptor gene *Fok*I, *Taq*I, *Bsm*I, and *Apa*I polymorphisms and susceptibility to pulmonary tuberculosis: a meta-analysis

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ABSTRACT. The aim of this study was to determine whether vitamin D receptor (*VDR*) genetic polymorphisms are associated with the susceptibility to pulmonary tuberculosis (PTB). MEDLINE and Embase databases and manual literature searches were used. A meta-analysis was conducted on the associations between the *VDR Fok*I, *Taq*I, *Bsm*I, and *Apa*I polymorphisms and PTB susceptibility. A total of 16 studies comprising 3231 patients and 3670 controls met the study inclusion criteria, consisting of 14 studies on the *VDR Fok*I polymorphism, 13 on the *VDR Taq*I polymorphism, 8 on the *VDR Bsm*I polymorphism, and 5 on the *VDR Apa*I polymorphism. Meta-analysis of the *VDR Fok*I polymorphism showed no association between PTB and the f allele of the *VDR Fok*I polymorphism (long variant) in all subjects (OR = 1.070, 95%CI = 0.979-1.169, P = 0.134). In contrast, after stratification by ethnicity, meta-analysis indicated that the *VDR Fok*I F allele (short variant) was associated with PTB risk in an East Asian population (OR = 1.507, 95%CI = 1.192-1.906, P = 0.001). Meta-analysis revealed no association between PTB susceptibility and the *VDR Taq*I t allele in all study subjects (OR = 0.986, 95%CI = 0.839-1.159, P = 0.866)

or in individual ethnic populations. Furthermore, a risk of PTB was not associated with the *Bsm*I and *Apa*I polymorphisms. This meta-analysis suggested that the VDR *Fok*I polymorphism is associated with a susceptibility to PTB in East Asians.

Key words: Vitamin D receptor; Meta-analysis; Polymorphism; Pulmonary tuberculosis

INTRODUCTION

Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, affects several organs, but most frequently attacks the lungs. Pulmonary tuberculosis (PTB) caused by *M. tuberculosis* is a common infection worldwide; it is a granulomatous disease of the lungs causing high mortality and morbidity, particularly in developing countries (Lawn and Zumla, 2011). Susceptibility to PTB is influenced by host genetic background (Bellamy, 2003), and approximately 10% of patients who are infected with *M. tuberculosis* are known to progress to clinical disease (Comstock, 1982).

Although vitamin D is primarily involved in the maintenance of bone mineral homeostasis, it is also involved in interleukin (IL)-2 inhibition, antibody production, and lymphocyte proliferation (Maruotti and Cantatore, 2010). It has been reported that 1,25-dihydroxy vitamin D_3 ($1,25(OH)_2 D_3$) inhibits interferon secretion and negatively regulates IL-12 production by down-regulating nuclear factor-kappa B (Boonstra et al., 2001). Vitamin D plays a role in human innate immunity to infectious agents including *M. tuberculosis* (Haussler et al., 1998). The activity of vitamin D is dependent on the vitamin D receptor (VDR), a member of the nuclear hormone receptor superfamily. Thus, *VDR* is one of the most frequently studied genes with respect to PTB susceptibility. *VDR* is located on chromosome 12q13.11 (Miyamoto et al., 1997), and three polymorphisms, *Bsm*I (rs1544410) and *Apa*I (rs7975232), both in intron 8, and *Taq*I (rs731236) in exon 9, have been identified at the 3'-end of the gene, and have been shown to be in strong linkage disequilibrium (LD) (Morrison et al., 1992). Another polymorphism, *Fok*I (rs2228570), is located at the *VDR* start codon (Miyamoto et al., 1997). Although the functional significance of these four polymorphisms remains unknown, it is believed that they might be in LD with one or more functional polymorphisms elsewhere in *VDR*, contributing to the observed association between these polymorphisms and PTB susceptibility.

Multiple studies have examined the potential contribution made by *VDR* polymorphisms to PTB susceptibility, but the findings of these studies have been contradictory (Bellamy et al., 1999; Wilkinson et al., 2000; Delgado et al., 2002; Bornman et al., 2004; Liu et al., 2004; Roth et al., 2004; Chen et al., 2006; Babb et al., 2007; Olesen et al., 2007; Søborg et al., 2007; Banoei et al., 2010; Ates et al., 2011; Singh et al., 2011; Joshi et al., 2014). Individual studies based on small sample sizes have insufficient statistical power to detect positive associations and are incapable of demonstrating the absence of an association. Furthermore, the low statistical power of individual studies might explain the contradictory published results. A meta-analysis, on the other hand, can integrate previous research results and can increase statistical power and resolution by pooling the results of independent analyses (Merza et al., 2009; Selvaraj et al., 2009; Lee et al., 2007, 2010, 2012). In the present study, we explored whether the *VDR Fok*I, *Taq*I, *Bsm*I, and *Apa*I polymorphisms are associated with the susceptibility to PTB, using a meta-analysis approach.

MATERIAL AND METHODS

Identification of eligible studies and data extraction

A literature search using the MEDLINE and Embase citation databases was used to identify available articles in which *VDR* polymorphisms were analyzed in patients with PTB (until May 2014). Combinations of key words, such as "vitamin D receptor", "VDR", "polymorphism", and "pulmonary tuberculosis" were entered as medical subject headings (MeSH) or text words. References in the identified studies were also investigated to identify additional studies not indexed by MEDLINE and Embase. No language or country restrictions were applied. Studies were included if: 1) they involved case-control studies; 2) they contained original data (independent of other studies); and 3) they provided enough data to allow calculation of an OR. Studies were excluded if: 1) they contained data overlapping with that of other studies; 2) the number of genotypes or alleles could not be ascertained; or 3) family members were also included in the study, because such analyses are based on linkage considerations.

Following the specified selection criteria, data were extracted from the original studies by two independent reviewers. Any discrepancies between reviewer observations were resolved by consulting a third reviewer. The information extracted from each study included the author, year of publication, ethnicity of the study population, demographics, and number of patients and controls for each of the *Fok*I, *Taq*I, *Bsm*I, and *Apa*I genotypes. Allele frequencies were calculated from the corresponding genotype distributions.

Evaluation of statistical associations

We performed meta-analyses using: 1) allelic contrast, 2) homozygote contrast, 3) recessive models, and 4) dominant models. Point estimates of risk, ORs, and 95%CIs were determined for each study. Furthermore, within- and between-study variations or heterogeneities were assessed using Cochran's Q-statistic. This heterogeneity test assesses the null hypothesis that all studies under evaluation have the same effect. The effect of heterogeneity was quantified using I^2, which ranges between 0 and 100%, and which represents the proportion of between-study variability that can be attributed to heterogeneity rather than to chance (Higgins and Thompson, 2002). I^2 values of 25, 50, and 75% were nominally assigned as low, moderate, and high estimates, respectively. The fixed-effect model assumed that the effect of genetic factors on PTB susceptibility across all studies investigated was similar, and that the variations observed among studies were caused by chance alone (Egger et al., 1997b). The random-effect model assumed that different studies were substantially diverse and assessed both within-study sampling error and between-study variance (DerSimonian and Laird, 1986). When study groups are homogeneous, the use of fixed- or random-effect models generates similar results, and when this is not the case, the random-effect model usually provides wider CIs than does the fixed-effect model. The random-effect model is therefore used when there is significant between-study heterogeneity (DerSimonian and Laird, 1986). Statistical analyses were conducted using a comprehensive meta-analysis computer program (Biosta, Englewood, NJ, USA).

Evaluation of publication bias

While funnel plots are often used to detect publication bias, funnel plotting requires

a range of studies with varying sizes, and involves subjective judgments. Accordingly, we evaluated publication bias by using the Egger linear regression test (Egger et al., 1997a), which measures funnel plot asymmetry using a natural logarithm scale of ORs.

RESULTS

Studies included in the meta-analysis

Eighty-two studies were identified after electronic and manual literature searches. Of these, 27 were selected for a full-text review based on title and abstract details. Eleven studies were excluded because they included no extractable genotype data, were reviews, or included studies of tuberculosis (TB) other than PTB, or contained duplicate data.

Thus, a total of 16 relevant studies were considered in this meta-analysis (Bellamy et al., 1999; Wilkinson et al., 2000; Delgado et al., 2002; Bornman et al., 2004; Liu et al., 2004; Roth et al., 2004; Chen et al., 2006; Babb et al., 2007; Olesen et al., 2007; Søborg et al., 2007; Merza et al., 2009; Selvaraj et al., 2009; Banoei et al., 2010; Ates et al., 2011; Singh et al., 2011; Joshi et al., 2014), comprising 3231 patients and 3670 controls in total (Table 1 and Figure 1). These 16 studies encompassed 5 studies from Africa, 4 from South Asia, 3 from the Middle East, 2 from East Asia, 1 from Southeast Asia, and 1 from South America. Thus, an ethnicity-specific meta-analysis was conducted for the African, South Asian, Middle Eastern, and East Asian populations. Fourteen studies examined the *VDR Fok*I polymorphism, 13 the *VDR Taq*I polymorphism, 8 the *VDR Bsm*I polymorphism, and 5 the *VDR Apa*I polymorphism. Selected characteristics of the relationships found between the *VDR* polymorphisms and PTB are summarized in Table 1.

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

Reference	Country	Ethnicity	Numbers		Polymorphism(s)	Association findings
			Patients	Controls		
Joshi et al. (2014)	India	South Asian	110	115	*Fok*I, *Bsm*I	*Fok*I (NS); *Bsm*I (P = 0.002)
Singh et al. (2011)	India	South Asian	101	225	*Fok*I, *Taq*I, *Bsm*I	NS
Ates et al. (2011)	Turkey	Middle Eastern	128	80	*Fok*I, *Taq*I, *Bsm*I	*Fok*I, *Taq*I (NS); *Bsm*I (P = 0.0006)
Banoei et al. (2010)	Iran	Middle Eastern	60	62	*Fok*I, *Taq*I, *Bsm*I	*Fok*I, *Taq*I (P < 0.0001); *Bsm*I (P < 0.0001)
Merza et al. (2009)	Iran	Middle Eastern	117	60	*Fok*I, *Bsm*I	*Fok*I (NS); *Bsm*I (P = 0.001)
Selvaraj et al. (2009)	India	South Asian	65	60	*Fok*I, *Taq*I, *Bsm*I, *Apa*I	NS
Olesen et al. (2007)	Gambia	African	321	347	*Fok*I, *Taq*I, *Bsm*I, *Apa*I	*Fok*I, *Taq*I, *Bsm*I (NS); *Apa*I (P = 0.028)
Babb et al. (2007)	South Africa	African	249	352	*Fok*I, *Taq*I, *Apa*I	NS
Chen et al. (2006)	China	East Asian	140	139	*Fok*I	*Fok*I (P = 0.021)
Søborg et al. (2007)	Tanzania	African	443	426	*Fok*I, *Taq*I, *Apa*I	NS
Roth et al. (2004)	Peru	South American	103	206	*Fok*I, *Taq*I	NS
Liu et al. (2004)	China	East Asian	120	240	*Fok*I, *Taq*I	*Fok*I (P = 0.002); *Taq*I (NS)
Bornman et al. (2004)	South Africa	African	417	722	*Fok*I, *Taq*I, *Bsm*I, *Apa*I	NS
Delgado et al. (2002)	Cambodia	Southeast Asian	358	106	*Taq*I	NS
Wilkinson et al. (2000)	India	South Asian	91	116	*Fok*I, *Taq*I	NS
Bellamy et al. (1999)	Gambia	African	408	414	*Taq*I	*Taq*I (P = 0.01)

NS = not significant.

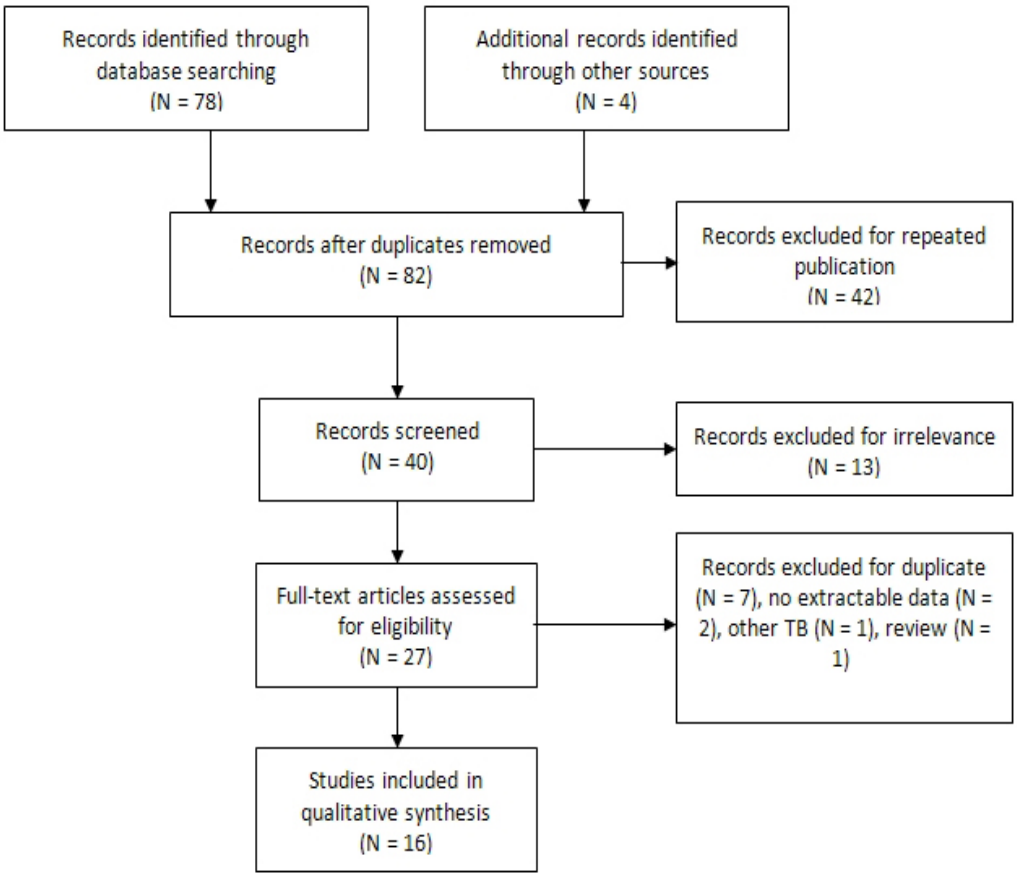

Figure 1. Study flow chart. TB, tuberculosis.

Meta-analysis of the association between the *VDR Fok*I polymorphism and PTB susceptibility

The results of meta-analyses of the association between the *VDR Fok*I polymorphism and PTB are summarized in Table 2. Meta-analysis of the *VDR Fok*I polymorphism showed no association between PTB and the f allele (long variant) when all subjects were considered (OR = 1.070, 95%CI = 0.979-1.169, P = 0.134; Table 2 and Figure 2). After stratification by ethnicity, meta-analysis indicated that the f allele was associated with PTB risk in East Asians (OR = 1.507, 95%CI = 1.192-1.906, P = 0.001; Table 2 and Figure 2). Furthermore, meta-analysis using either the dominant model or homozygote contrast showed the same pattern of results as with the *VDR Fok*I f allele (Table 2). In addition, with meta-analysis, an association was found between the *VDR Fok*I ff+fF genotype and PTB risk in the overall cohort and in East Asian populations (OR = 1.232, 95%CI = 1.004-1.512, P = 0.046; OR = 2.104, 95%CI = 1.333-3.323, P = 0.001, respectively; Table 2).

Table 2. Meta-analysis of the association between the *VDR Fok*I polymorphism and pulmonary tuberculosis .

Polymorphism	Population	No. of studies	Subject No.		Test of association			Test of heterogeneity		
			Patients	Controls	OR	95%CI	P value	Model	P value	I^2
*Fok*I	Overall	14	2452	3128	1.070	0.979-1.169	0.134	F	0.119	32.0
f *vs* F allele	Africa	4	1420	1830	0.989	0.878-1.115	0.862	F	0.708	0
	South Asia	4	367	516	1.102	0.777-1.563	0.586	R	0.061	59.3
	Middle East	3	305	202	1.011	0.763-1.341	0.938	F	0.813	0
	East Asia	2	260	379	1.507	1.192-1.906	0.001	F	0.969	0
ff+fF *vs* FF	Overall	14	2452	3128	1.038	0.928-1.162	0.511	F	0.317	12.4
(Dominant)	Africa	4	1420	1830	0.996	0.862-1.150	0.953	F	0.600	0
	South Asia	4	367	516	1.057	0.699-1.599	0.791	R	0.086	54.5
	Middle East	3	305	202	0.956	0.666-1.372	0.807	F	0.935	0
	East Asia	2	260	379	1.517	1.078-2.134	0.017	F	0.490	0
ff *vs* fF+FF	Overall	14	2452	3128	1.232	1.004-1.512	0.046	F	0.661	7.2
(Recessive)	Africa	4	1420	1830	0.946	0.685-1.306	0.737	F	0.954	0
	South Asia	4	367	516	1.284	0.736-2.241	0.378	F	0.221	31.9
	Middle East	3	305	202	1.167	0.575-2.370	0.668	F	0.368	0
	East Asia	2	260	379	2.104	1.333-3.323	0.001	F	0.301	6.68
ff *vs* FF	Overall	14	2452	3128	1.224	0.974-1.537	0.083	F	0.106	33.5
	Africa	4	1420	1830	0.950	0.685-1.318	0.761	F	0.943	0
	South Asia	4	367	516	1.288	0.726-2.284	0.387	F	0.118	48.9
	Middle East	3	305	202	1.108	0.529-2.321	0.786	F	0.430	0
	East Asia	2	260	379	2.608	1.558-4.364	$2.6x10^{-5}$	F	0.602	0

OR = odds ratio; CI = confidence interval; F = fixed-effect model; R = random-effect model; *Fok*I f, F alleles = long, short variants, respectively.

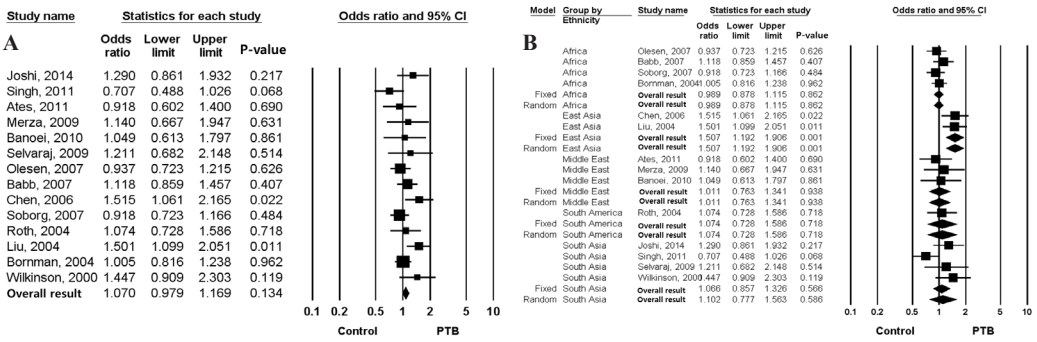

Figure 2. Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for allelic associations between the *VDR Fok*I polymorphism and pulmonary tuberculosis (PTB) in the overall cohort (**A**) and in each ethnic group (**B**). The size of the boxes means the weight assigned to each study.

Meta-analysis of the association between the *VDR Taq*I polymorphism and PTB susceptibility

Meta-analysis showed no association between PTB susceptibility and the *VDR Taq*I t allele in the study subjects as a whole (OR = 0.986, 95%CI = 0.839-1.159, P = 0.866; Table 3 and Figure 3). Furthermore, ethnicity-specific meta-analysis indicated that the *VDR* t allele was not associated with PTB risk in any individual ethnic population analyzed (Table 3). Analyses using the dominant model and homozygote contrast showed the same pattern as for the t allele (Table 3). However, meta-analysis revealed an association between the tt genotype of the *VDR Taq*I polymorphism and PTB susceptibility in South Asians (OR = 0.596, 95%CI

= 0.357-0.994, P = 0.048; Table 3). On the other hand, when a study in which Hardy-Weinberg equilibrium (HWE) deviation was present was excluded, there was no longer any association detected between the tt genotype and PTB in South Asians (OR = 0.560, 95%CI = 0.278-1.129, P = 0.105).

Table 3. Meta-analysis of the association between the *VDR Taq*I polymorphism and pulmonary tuberculosis.

Polymorphism	Population	No. of studies	Subject No.		Test of association			Test of heterogeneity		
			Patients	Controls	OR	95%CI	P value	Model	P value	I^2
*Taq*I t *vs* T allele	Overall	13	2781	3260	0.986	0.839-1.159	0.866	R	0.001	65.1
	Africa	5	1758	2170	0.944	0.855-1.043	0.260	F	0.224	29.5
	South Asia	3	257	401	0.872	0.683-1.115	0.275	F	0.816	0
	Middle East	2	188	142	1.864	0.463-7.502	0.381	R	0.000	94.1
tt+tT *vs* TT (Dominant)	Overall	13	2781	3260	0.998	0.837-1.190	0.980	R	0.045	51.7
	Africa	5	1758	2170	0.956	0.841-1.085	0.484	F	0.794	0
	South Asia	3	257	401	0.979	0.708-1.354	0.899	F	0.533	0
	Middle East	2	188	142	2.595	0.354-19.03	0.348	R	0.000	92.8
tt *vs* tT+TT (Recessive)	Overall	13	2781	3260	0.084	0.640-1.223	0.458	R	0.014	52.4
	Africa	5	1758	2170	0.845	0.565-1.266	0.415	R	0.022	64.8
	South Asia	3	257	401	0.596	0.357-0.994	0.048	F	0.969	0
	Middle East	2	188	142	1.961	0.297-12.93	0.484	R	0.005	87.0
tt *vs* TT	Overall	13	2781	3260	0.951	0.643-1.407	0.802	R	0.001	64.1
	Africa	5	1758	2170	0.837	0.556-1.260	0.394	R	0.027	63.5
	South Asia	3	257	401	0.644	0.377-1.100	0.107	F	0.902	0
	Middle East	2	188	142	3.385	0.179-64.07	0.416	R	0.000	93.0

OR = odds ratio; CI = confidence interval; F = fixed-effect model; R = random-effect model.

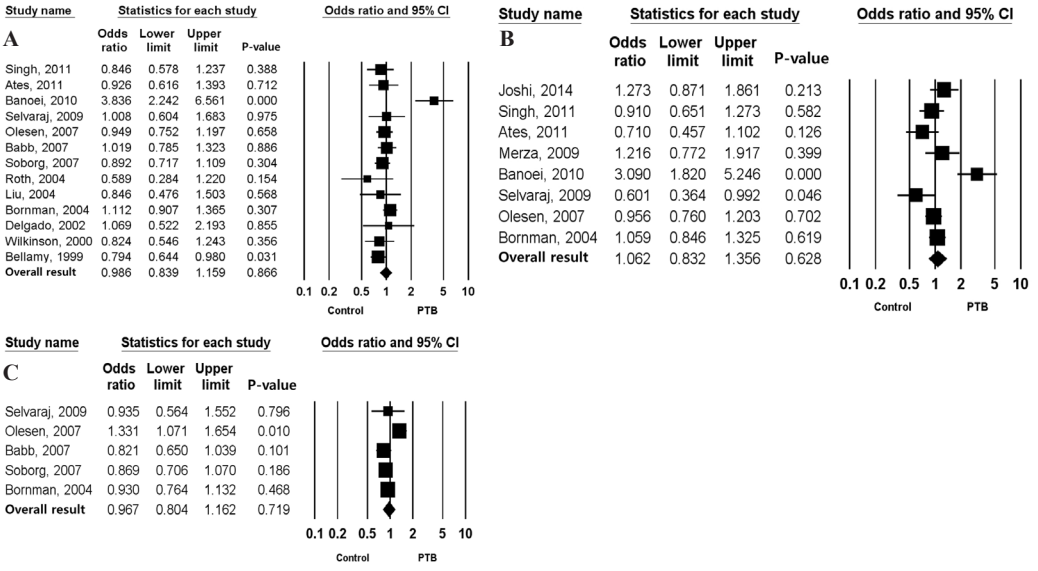

Figure 3. Odds ratios (ORs) and 95% confidence intervals (CIs) of individual studies and pooled data for the allelic associations between the *VDR Taq*I (**A**), *Bsm*I (**B**), and *Apa*I (**C**) polymorphisms and pulmonary tuberculosis (PTB) in all subjects. The size of the boxes means the weight assigned to each study.

Meta-analysis of the association between the *VDR Bsm*I and *Apa*I polymorphisms and PTB susceptibility

Meta-analysis revealed no association between PTB and the *Bsm*I b allele in the study subjects as a whole (OR = 1.062, 95%CI = 0.832-1.356, P = 0.628; Table 4 and Figure 3). Likewise, stratification by ethnicity indicated no association between the *VDR Bsm*I b allele and PTB in any individual ethnic group (Table 4). Analyses using recessive and dominant models and homozygote contrast failed to reveal any association between the *Bsm*I polymorphism and PTB (Table 4). Meta-analysis also indicated no association between PTB and the *Apa*I a allele in either the overall study population or in the African population (OR = 0.967, 95%CI = 0.804-1.162, P = 0.719 and OR = 0.970, 95%CI = 0.789-1.193, P = 0.774, respectively; Table 4). The recessive and dominant models and homozygote contrast analyses also failed to reveal any association between the *Apa*I polymorphism and PTB (Table 4).

Table 4. Meta-analysis of the association between the *VDR Bsm*I and *Apa*I polymorphisms and pulmonary tuberculosis.

Polymorphism	Population	No. of studies	Subject No.		Test of association			Test of heterogeneity		
			Patients	Controls	OR	95%CI	P value	Model	P value	I^2
*Bsm*I b *vs* B allele	Overall	8	1244	1558	1.062	0.832-1.356	0.628	R	0.000	73.5
	Africa	2	663	976	1.007	0.858-1.183	0.930	F	0.534	0
	South Asia	3	276	400	0.911	0.621-1.338	0.636	R	0.062	63.9
	Middle East	3	305	182	1.371	0.610-3.084	0.445	R	0.000	88.6
bb+bB *vs* BB	Overall	8	1244	1558	1.166	0.730-1.864	0.520	R	0.000	79.0
(Dominant)	Africa	2	663	976	0.977	0.747-1.278	0.865	F	0.747	0
	South Asia	3	276	400	0.926	0.423-2.026	0.847	R	0.006	80.7
	Middle East	3	305	182	0.749	0.369-8.294	0.482	R	0.000	88.2
bb *vs* bB+BB	Overall	8	1244	1558	0.990	0.830-1.200	0.982	R	0.047	50.7
(Recessive)	Africa	2	663	976	1.035	0.810-1.312	0.778	F	0.594	0
	South Asia	3	276	400	0.828	0.555-1.236	0.357	F	0.427	0
	Middle East	3	305	182	0.416	0.494-4.056	0.517	R	0.004	82.0
bb *vs* BB	Overall	8	1244	1558	1.143	0.672-1.951	0.624	R	0.001	72.6
	Africa	2	663	976	0.982	0.670-1.439	0.927	F	0.644	0
	South Asia	3	276	400	0.824	0.524-1.295	0.402	F	0.278	21.9
	Middle East	3	305	182	2.135	0.326-13.97	0.429	R	0.000	88.3
*Apa*I a *vs* A allele	Overall	5	1411	1809	0.967	0.804-1.162	0.719	R	0.023	64.6
	Africa	4	1346	1749	0.970	0.789-1.193	0.774	R	0.010	73.4
aa+aA *vs* AA	Overall	5	1411	1809	0.985	0.764-1.269	0.904	R	0.030	62.6
(Dominant)	Africa	4	1346	1749	0.985	0.740-1.311	0.916	R	0.014	71.9
aa *vs* aA+AA	Overall	5	1411	1809	0.909	0.674-1.226	0.530	R	0.095	49.3
(Recessive)	Africa	4	1346	1749	0.914	0.650-1.285	0.605	R	0.050	61.5
aa *vs* AA	Overall	5	1411	1809	0.905	0.596-1.376	0.642	R	0.013	68.3
	Africa	4	1346	1749	0.912	0.566-1.470	0.706	R	0.006	76.1

OR = odds ratio; CI = confidence interval; F = fixed-effect model; R = random-effect model.

Heterogeneity, subgroup analysis, and publication bias

Some heterogeneity was observed in the meta-analysis of the *VDR Taq*I, *Bsm*I, and *Apa*I polymorphisms, but between-study heterogeneity was not found during the meta-analysis of the *VDR Fok*I polymorphisms (Tables 3 and 4). Deviation from HWE among controls implies potential bias during control selection or might be due to genotyping errors; however, excluding studies deviating from HWE did not affect our *VDR* polymorphism association results (Ates et al., 2011; Singh et al., 2011; Joshi et al., 2014), except for those related to the

*Taq*I polymorphism in South Asians. It was difficult to correlate publication bias with the funnel plot, which is usually used to detect publication bias, as the number of studies included in the analysis was relatively small. However, the Egger regression test showed no evidence of publication bias (Egger regression test P values >0.1; Figure 4).

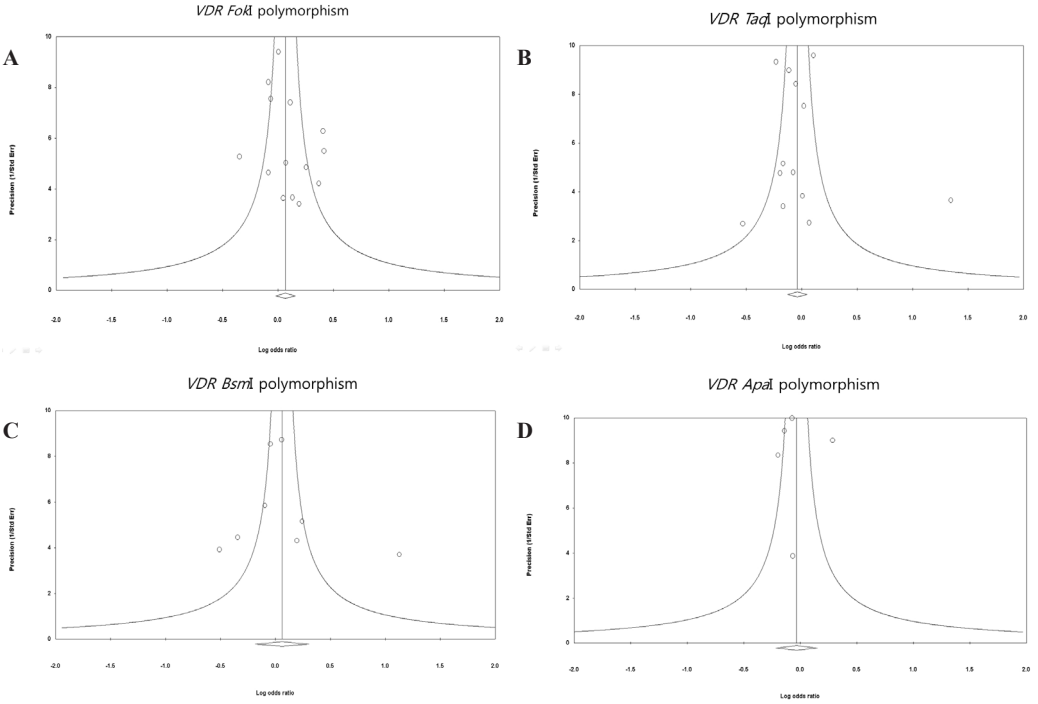

Figure 4. Funnel plot of studies of the associations between the *VDR Fok*I (**A**), *Taq*I (**B**), *Bsm*I (**C**) and *Apa*I (**D**) polymorphisms and pulmonary tuberculosis in all subjects (Egger's regression P values = 0.3225, 0.581, 0.645, and 0.944, respectively).

DISCUSSION

The host immune response against *M. tuberculosis* is mediated by cellular immunity (Bellamy, 2003). The compound $1,25(OH)_2$ D_3 restricts the growth of *M. tuberculosis* in monocytes by inducing the expression of the antimicrobial peptide cathelicidin (Denis, 1991). Vitamin D regulates the differentiation and growth of various immune cells as an immunomodulatory hormone (Baeke et al., 2010). Low serum vitamin D levels have been associated with active TB and with an increased risk of active TB (Nnoaham and Clarke, 2008). Vitamin D acts via its receptor, encoded by *VDR*, a candidate gene for immune pathways; VDR is activated to recognize and kill bacteria present inside macrophage cells. *VDR* polymorphisms might therefore result in an altered immune response.

In this meta-analysis, we combined data from published studies to evaluate the genetic associations between the most commonly studied polymorphisms of *VDR*, namely, the *VDR Fok*I, *Taq*I, *Bsm*I, and *Apa*I polymorphisms, and PTB susceptibility. Our meta-analysis of the *VDR Taq*I, *Bsm*I, and *Apa*I polymorphisms showed no association with PTB risk. In contrast,

meta-analysis of the *Fok*I polymorphism revealed a significant association between PTB risk and the f allele in the East Asian population (OR = 1.507, 95%CI = 1.192-1.906, P = 0.001). In addition, meta-analysis using both recessive and dominant models, and homozygote contrast, found an association of this allele with PTB susceptibility in the East Asian population. Our meta-analysis suggested that the *VDR Fok*I f allele might be a risk factor for PTB infection in East Asians.

Our findings are consistent with a previous functional analysis of the *VDR Fok*I polymorphism. The *Fok*I polymorphism is located in an alternative *VDR* start site, resulting in the synthesis of a protein of an alternate length (Arai et al., 1997). The long variant (f allele) is transcriptionally less active and is associated with a lower transactivation of *VDR* than is the short variant (F allele). The variant protein might have a decreased capacity to enhance phagocytosis via the activation of macrophages. Thus, the *VDR Fok*I f allele may increase the risk of PTB infection.

The functional effects of the *VDR Taq*I, *Bsm*I, and *Apa*I polymorphisms, which are all located near the 3'-untranslated region, are not clear. However, some studies have suggested that these polymorphisms might alter polyadenylation of the *VDR* mRNA transcript, and thus affect mRNA stability (Uitterlinden et al., 2004). The *VDR Taq*I, *Bsm*I, and *Apa*I polymorphisms are located in regions with strong LD. Our study did not identify any association between PTB and the *Bsm*I, *Apa*I, and *Taq*I polymorphisms. The reason for this lack of association might be explained using further haplotype studies.

Our results should be interpreted with caution, as only two studies were included in the East Asian group. It is very important to substantially increase this number so that subgroup analysis for various ethnic populations can be performed. This information will increase the depth of meta-analysis and add new information to the existing field.

It should also be explained why the *VDR Fok*I polymorphism was found to be associated with PTB risk only in the East Asian population. First, genetic heterogeneity for PTB might exist in different populations. In fact, genetic association studies on PTB have demonstrated genetic heterogeneity in different ethnic groups. Second, clinical heterogeneities and differences between patient populations might be responsible. Third, discrepancies might be caused by different LD patterns; for example, these polymorphisms might be in LD with a nearby causal variant in one ethnic group, but not in another. Fourth, a lack of association might be due to the small number of studies included, low statistical power, or type II errors (false-negative results).

The present study had some limitations. First, patient heterogeneity and confounding factors might have distorted the analysis. Second, haplotype analysis might have provided more information and would have been more powerful than single polymorphism analysis. Furthermore, LD was found between the *Taq*I, *Bsm*I, and *Apa*I polymorphisms (Morrison et al., 1992); however, no meta-analysis of haplotypes was possible due to inadequate haplotype data. Third, the *VDR* polymorphisms might be associated with clinical features of PTB, but the limited amount of available data did not allow us to investigate this association.

In conclusion, this meta-analysis demonstrated that the *VDR Fok*I polymorphism is associated with susceptibility to PTB in an East Asian population, suggesting that this polymorphism might play an important role in risk of PTB. However, no similar association was found between the *VDR Taq*I, *Bsm*I, and *Apa*I polymorphisms and PTB susceptibility. Further large-scale studies in populations of different ethnicities are necessary to explore the roles of *VDR* polymorphisms in the susceptibility to PTB.

Conflicts of interest

The authors declare no conflict of interest.

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