Vitamin D receptor gene FokI polymorphisms and tuberculosis susceptibility: a meta-analysis

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Abstract

Introduction: The association between FokI polymorphism of vitamin D receptor (VDR) and tuberculosis (TB) susceptibility has been investigated previously; however, the results were inconsistent and conflicting. In the present study, a meta-analysis was performed to assess the relationship between VDR FokI gene polymorphism and the risk of TB.

Material and methods: Databases including PubMed and Embase were searched for genetic association studies of FokI polymorphism of vitamin D receptor (VDR) and TB. Data were extracted by two independent authors and the pooled odds ratio (OR) with 95% confidence interval (CI) was calculated to assess the strength of the association between VDR FokI gene polymorphism and TB risk. Meta-regression and subgroup analyses were performed to identify the source of heterogeneity.

Results: Thirty-four studies with a total of 5669 cases and 6525 controls were reviewed in the present meta-analysis. A statistically significant correlation was found between VDR FokI gene polymorphism and increased TB risk in two comparison models: the homozygote model (ff vs. FF: OR = 1.37, 95% CI: 1.17–1.60; $P_{\text{heterogeneity}} = 0.001$) and the recessive model (ff vs. Ff + FF: OR = 1.32, 95% CI: 1.14–1.52; $P_{\text{heterogeneity}} = 0.006$). Meta-regression found no source contributing to heterogeneity. However, sub-group analyses revealed that there was a statistically increased TB risk in the East and Southeast Asian population.

Conclusions: Synthesis of the available studies suggests that homozygosity for the FokI polymorphism of the VDR gene might be associated with an increased TB risk, especially in the East and Southeast Asian population. Additional well-designed, larger-scale epidemiological studies among different ethnicities are needed.

Key words: vitamin D receptor, FokI polymorphisms, tuberculosis susceptibility.

Introduction

Tuberculosis (TB) is one of the most important infectious diseases, with an estimated 9.0 million new cases and 1.5 million deaths worldwide in 2013, and more than half were in the South-East Asia and Western Pacific Regions [1]. It is suggested that the susceptibility to disease after infection with *Mycobacterium tuberculosis* is influenced by many risk factors, such as malnutrition, HIV infection, and environmental and host genetic factors [2–5]. Host genetic factors implicated in human susceptibility

to TB include NRAMP, HLA-DQB1, interleukin (IL) genes and the vitamin D receptor (VDR) [6–8].

Vitamin D deficiency seems to be involved in susceptibility to TB and severity of the disease [9], and 1,25-dihydroxyvitamin D_3, the activated form of vitamin D, is a potent immune modulator. Expression and nuclear activation of the VDR are essential for these activities of vitamin D. The VDR gene is located on the long arm of chromosome 12, and several polymorphisms occur in the 5′ regulatory region, coding region and 3′ untranslated region (UTR) [10]. Among all the gene loci, the one most studied recently is FokI [11–15], which can regulate the transcriptional activity of the gene [16]. FokI polymorphism in combination with low serum vitamin D_3 may attenuate VDR functions, which in turn is strongly associated with TB [11]. Several studies have tried to investigate the role of FokI gene polymorphism on susceptibility to TB, but they have not reached a consensus. To date, two analyses on the FokI polymorphism and TB risk across different ethnicities have been reported [17, 18], but they failed to identify a significant association of FokI polymorphism in overall populations. In addition, more recent studies concerning the association between the polymorphism and TB risk in different populations have not included the two analyses [8, 12–15, 19–24]. Furthermore, several important factors which may bias the results were not clearly addressed, such as Hardy-Weinberg equilibrium (HWE). Thus, it is necessary to evaluate the true association of the VDR FokI gene polymorphism and the risk of TB. In the present study, we performed an updated meta-analysis to address these discrepancies and to explore the risk factors associated with TB.

Material and methods

Literature search strategy

We performed a literature search of the PubMed, Web of Science and Embase web databases with a combination of the key words ''VDR'' or "Vitamin D receptor"; "FokI", "rs10735810"; "polymorphism" AND "Tuberculosis'' up to January 2015. Furthermore, we evaluated potentially relevant genetic association studies by manual searching of references of relative articles and reviews. Search results were limited to human populations. No language restrictions were applied.

Inclusion and exclusion criteria

Published articles included in the current meta-analysis were selected according to the following criteria: (1) appraisal of the association between VDR FokI gene polymorphism and TB risk, (2) case-control study design, (3) with clearly described and confirmed TB patients and TB-free controls, (4) containing available genotype frequency in cases and controls. The major reasons for study exclusion were data overlapping, case-only studies, reviews, repeated literature, and without genotype frequencies.

Data extraction and quality assessment

Data of each retrieved publication were independently abstracted in duplicate by two independent investigators with a standard procedure. Data extracted from the retrieved publications included the name of the first author, publication year, the country of origin, ethnicity, source of controls, number of cases and controls, study type, diagnosis method of cases, the selection of controls and genotype frequencies. The Hardy-Weinberg equilibrium (HWE) was examined by χ^2 test ($p < 0.05$ was considered as significant disequilibrium) based on FokI genotyping distribution in controls.

Statistical analysis

Data from the meta-analysis were analyzed using STATA software (Version 12.1; Stata Corp, College Station, Texas, USA). The significance of the association for five comparison models – allele model (f vs. F), homozygote model (ff vs. FF), heterozygote model (Ff vs. FF), dominant model (ff + Ff vs. FF) and recessive model (ff vs. Ff + FF) – was evaluated for 34 studies separately. All associations were evaluated by calculating odds ratios (ORs) with the 95% confidence interval (CI). The statistical heterogeneity between studies was checked using the χ^2-based Q test and considered significant at $p < 0.05$. When there was no significant heterogeneity, the fixed effects model (Mantel-Haenszel method) was used; otherwise, the random-effects model (the DerSimonian and Laird method) was used. Sensitivity analyses were performed to identify an individual study's effect on pooled results and test the reliability of results. Meta-regression analysis was performed to explore the source of potential heterogeneity. Stratification analyses were performed to further identify the possible source of heterogeneity among variables, such as ethnicity and sample size (studies with more than 500 participants were defined as ''large'', and studies with less than 500 participants were defined as ''small''). Publication bias was assessed with both Egger's test and Begg's funnel plot, and the statistical significance was defined as $p < 0.05$. All p values were two-sided.

Results

Characteristics of enrolled studies

A flow chart of the study selection process is shown in Figure 1. According to the inclusion crite-

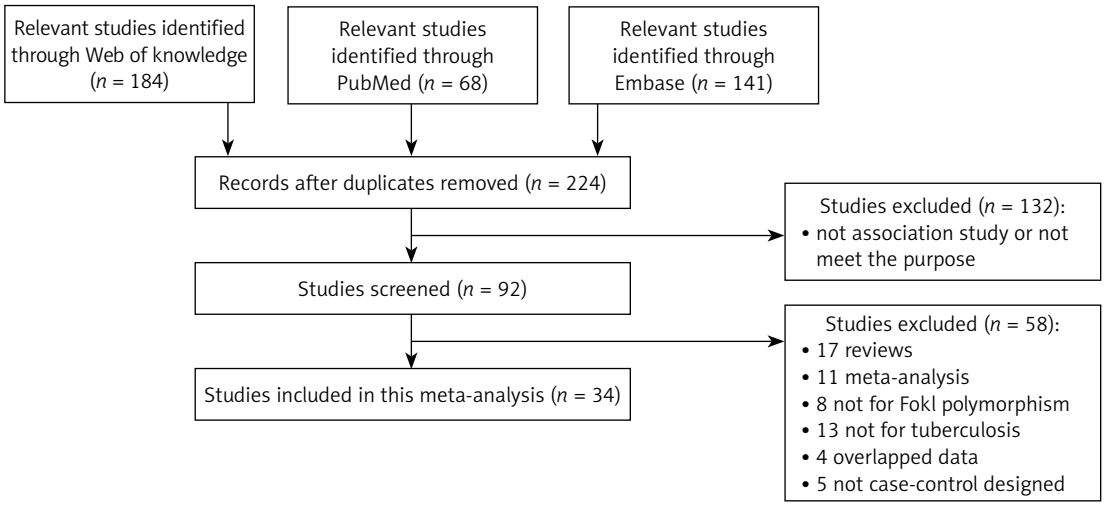

Figure 1. Flow diagram of search strategy and study selection process (TIF)

ria, 34 qualified case-control studies were selected in the final analysis after the literature search from the PubMed (Medline), Web of Science and Embase web databases [8, 10–15, 19–45]. Twenty-four studies were based on Asian populations [8, 10–13, 19–37], seven were based on African populations [14, 15, 38–42] and the remaining three were conducted in Europe and America [43–45]. The eligible studies contained 4 "large" studies [19, 29, 40, 42] and 30 "small" studies [8, 10–15, 21–28, 30–39]. Thirty studies were genotyped by restriction fragment length polymorphism (RFLP) analysis and five were conducted by other methods [19, 23, 38, 40, 41]. The detailed characteristics of the enrolled studies are listed in Table I. A total of 5669 TB cases were obtained in the 34 studies, including 5126 (92.3%) with pulmonary TB and 426 (7.7%) with extra-pulmonary TB. The corresponding controls for the TB cases numbered 6525. Distribution of genotypes and HWE *p*-values in the controls are shown in Table II. Among the controls, the genotype distribution for 31 studies of the assessed polymorphisms was in HWE, except for 3 studies from India and Iran [31, 32, 35].

Sensitivity analyses and publication bias

In the sensitivity analysis, the influence of each individual data set on the pooled OR was assessed by deleting one single study each time. The results showed that the corresponding pooled ORs were not materially varied, suggesting stability of this meta-analysis (data not shown). Begg's funnel plot and Egger's test were used to evaluate the publication bias of the selected studies for the meta-analysis (Figure 2). Begg's funnel plot seemed symmetrical in all genetic models. Furthermore, the statistical results from Egger's test supported the result of Begg's funnel plot indicating that there was no publication bias among all genetic models ($p > 0.05$) (Table III).

Meta-analysis results

We pooled all 34 studies together for the assessment of the relationship between the VDR FokI polymorphism and the risk of TB. The pooled ORs from overall studies indicated a significantly increased risk of TB in the homozygote model (ff vs. FF: OR = 1.37, 95% CI: 1.17–1.60; $P_{\text{heterogeneity}}$ = 0.001, Figure 3) and recessive model (ff vs. Ff + FF: OR = 1.32, 95% CI: 1.14–1.52; $P_{\text{heterogeneity}}$ = 0.006, Figure 4). However, no significant association was found in the allele model (f vs. F: OR = 1.09, 95% CI: 0.97–1.21; $P_{\text{heterogeneity}}$ = 0.000, Figure 5) and in the dominant model (ff + Ff vs. FF: OR = 1.08, 95% CI: 0.99–1.17; $P_{\text{heterogeneity}}$ = 0.000, Figure 6). The heterozygote model (Ff vs. FF: OR = 1.03, 95% CI: 0.95–1.13; $P_{\text{heterogeneity}}$ = 0.001, Figure 7) failed to show any association with the risk of TB. The strength of the association between VDR FokI gene polymorphism and TB risk is shown in Table IV.

To account for the sources of heterogeneity, we performed meta-regression by publication years, ethnicity, sample size, genotyping methods, as well as source of controls and type of TB. However, no significant source was found to substantially contribute to heterogeneity (Table V).

To further investigate the heterogeneity, we performed subgroup analyses (Table IV). To evaluate the possible effect of the geographical differences on the variability of overall estimates, we classified the studies conducted in Asia into two groups: East and Southeast Asia (China, Indonesian and South Korean) and South and West Asia (India and Iran). As a result, the enrolled studies were divided into five subgroups including Africans, East and Southeast Asians, South and West

Table I. Main characteristics of included studies summarized for the meta-analysis

Year	First author	Country	Ethnicity	Study design	Tuberculosis Part of the body	Sample size Cases/ controls	Diagnosis method	Geno-typing method	Controls source	HIV status	Age, gender	Diabetes status
2014	Arji	Morocco	Arab or Berber	PB	Pulmonary tuberculosis	274/203	AFB smear and culture	PCR-RFLP	Healthy persons	Negative	Matched	Negative
2014	Mahmoud	Egypt	Egyptian	PB	Pulmonary tuberculosis	40/25	AFB smear and culture	PCR-RFLP	Healthy persons	Not available	Matched	Negative
2014	Sinaga	Indonesian	Indonesian Batak	PB	Pulmonary tuberculosis	76/76	Clinical evaluation, AFB smear and chest radiography	PCR-RFLP	Healthy health workers, tuberculin skin test positivity (61.7%)	Negative	Matched	Negative
2013	Wu	China	Chinese Kazakh	PB	Pulmonary tuberculosis	213/211	Clinical symptoms bacteriology X-ray	PCR-RFLP	Healthy persons	Negative	Matched	Negative
2013	Joshi	India	Indian	PB	Pulmonary tuberculosis	110/225	AFB smear	PCR-RFLP	Household contacts (110) and healthy persons (115)	Negative	Matched	Negative
2012	Rathored	India	Indian	PB	MDR tuberculosis and drug-sensitive pulmonary tuberculosis	692/205	AFB smear and culture	PCR-RFLP	Healthy persons	Negative	Matched	Negative
2011	Kim	South Korean	Korean	PB	Pulmonary (98) and extra-pulmonary tuberculosis (62)	160/156	AFB smear and culture	Pyro sequencing	Healthy persons	Not available	Matched	Not available
2011	Kang	South Korean	Korean	PB	Pulmonary tuberculosis	103/105	AFB smear and culture	PCR-RFLP	Healthy persons	Not available	Matched	Not available
2011	Singh	India	Indo-Caucasian Brahmin caste	HB, PB	Pulmonary tuberculosis	101/225	AFB smear or culture	PCR-RFLP	Healthy persons	Negative	Not matched	Not available
2011	Sharma	India	Indian	PB	Pulmonary tuberculosis	474/607	AFB smear or culture	PCR-RFLP	Healthy persons	Not available	Matched	Not available

Table I. Cont.

Year	First author	Country	Ethnicity	Study design	Tuberculosis Part of the body	Sample size Cases/ controls	Diagnosis method	Geno-typing method	Controls source	HIV status	Age, gender	Diabetes status
2011	Ates	Turkey	Anatolian	PB	Pulmonary (98) and extra-pulmonary tuberculosis (30)	128/80	AFB smear or culture	PCR-RFLP	Healthy persons	Not available	Matched	Not available
2010	Marashian	Iran	Iranian	HB	Pulmonary tuberculosis	164/50	AFB smear and X-ray	PCR-RFLP	Contacts	Not available	Matched	Not available
2010	Zhang	China	Chinese Han	PB	Spinal tuberculosis	110/102	Postoperative pathology	PCR-RFLP	Unrelated contacts	Negative	Matched	Negative
2009	Banoei	Iran	Iranian	PB	Pulmonary tuberculosis	60/62	Confirmed in Massih Daneshvari	PCR-RFLP	Healthy subjects	Negative	Matched	Negative
2009	Merza	Iran	Iranian	HB	Pulmonary tuberculosis	117/60	AFB smear and X-ray	PCR-RFLP	Contacts	Not available	Matched	Not available
2009	Vidyarani	India	Dravidian	PB	Pulmonary tuberculosis	40/49	AFB smear and culture	PCR-RFLP	Normal healthy subjects	Not available	Matched	Not available
2009	Selvaraj	India	Indian	HB	Pulmonary tuberculosis	65/60	Clinical symptom, AFB smear and culture	PCR-RFLP	Healthy subjects	Negative	Matched	Not available
2009	Alagarasu	India	Dravidian	HB	Pulmonary (187) and extra-pulmonary tuberculosis (30)	217/144	AFB smear, clinical criteria and X-ray	PCR-RFLP	Healthy controls	Cases (51%), controls (0)	Matched	Not available
2008	Selvaraj	India	Dravidian	HB	Pulmonary tuberculosis	51/60	AFB smear and culture	PCR-RFLP	Normal healthy subjects	Negative	Matched	Not available
2008	Liu	China	Chinese Han	PB	Pulmonary tuberculosis	60/30	AFB smear and culture	SNaPshot	Normal healthy subjects	Negative	Matched	Negative
2007	Wilbur	Paraguay	Ache, Chiripa, Guaraní	PB	Pulmonary tuberculosis	54/124	Clinical symptoms, PPD test	PCR-RFLP	No symptoms	Not available	Not available	Not available

Table I. Cont.

Year	First author	Country	Ethnicity	Study design	Tuberculosis Part of the body	Sample size Cases/ controls	Diagnosis method	Geno-typing method	Controls source	HIV status	Age, gender	Diabetes status
2007	Olesen	Guinea-Bissau	Papel, Manjaco, Mancanha, Balanta, Fulani, Mandinka and others	PB	Pulmonary tuberculosis	320/344	AFB smear and clinical criteria	TaqMan	Healthy controls	HIV positive in 33% of cases and negative in controls	Gender not matched	Not available
2007	Babb	South Africa	South African	HB	Pulmonary tuberculosis	249/352	AFB smear and X-ray	PCR-RFLP	No clinical history or symptoms of TB	Negative	Not available	Not available
2007	Soborg	Tanzania	Tanzanian	HB	Pulmonary tuberculosis	435/416	Culture	PCR-SSP	Culture negative	HIV positive in 44% of cases and 18% of controls	Gender not matched	Not available
2006	Chen XR	China	Chinese Tibetans	PB	Pulmonary tuberculosis	140/139	Clinical symptoms, AFB smear and X-ray	PCR-RFLP	Household contacts	Negative	Matched	Negative
2006	Lombard	Venda	Venda	HB	Pulmonary and meningeal tuberculosis	66/86	AFB smear	ARMS-PCR	Healthy controls with no history of TB	Negative	Not available	Not available
2004	Bornman	Gambia, Guinea-Bissau, Guinea	Gambia, Guinea-Bissau, Guinea	HB	Pulmonary tuberculosis	416/718	AFB or culture	PCR-RFLP	Healthy community control subjects	Cases (12.5%), controls (6.8%)	Matched	Not available

Table I. Cont.

Year	First author	Country	Ethnicity	Study design	Tuberculosis Part of the body	Sample size Cases/ controls	Diagnosis method	Geno-typing method	Controls source	HIV status	Age, gender	Diabetes status
2004	Selvaraj[a]	India	Indian	HB	Spinal tuberculosis patients	64/103	X-ray and clinical criteria	PCR-RFLP	77 were contacts and 26 were normal healthy subjects	Not available	Matched	Not available
2004	Selvaraj[b]	India	Indian	HB	Pulmonary tuberculosis	46/64	AFB smear, culture and radiographic abnormalities	PCR-RFLP	Clinically normal	Negative	Matched	Not available
2004	Roth	Peru	Amerindian	PB	Pulmonary tuberculosis	100/201	AFB smear	PCR-RFLP	Two healthy controls, 1 PPD + and 1 PPD–	Negative	Matched	Not available
2004	Liu	China	Chinese Han	PB	Pulmonary tuberculosis	120/240	AFB smear, culture and X-ray	PCR-RFLP	Normal controls	Negative	Not available	Negative
2004	Liu	China	Chinese Han	PB	Pulmonary tuberculosis	76/171	Culture and X-ray	PCR-RFLP	Normal controls	Not available	Matched	Negative
2003	Selvaraj	India	Indian	HB	Pulmonary tuberculosis	120/80	Culture	PCR-RFLP	Patient contacts	Not available	Matched	Not available
2000	Wilkinson	India	Gujarati	HB	Pulmonary tuberculosis (27) and military tuberculosis (64)	91/116	Biopsy or culture Tuberculosis	PCR-RFLP	Contacts with no TB	Negative	Gender not matched	Not available

PB – population-based, HB – hospital-based, AFB – acid-fast bacilli, HIV – human immunodeficiency virus, MDR – multi-drug resistance for isoniazid and rifampicin, PPD – purified protein derivative, SNPs – single nucleotide polymorphism, TB – tuberculosis, PCR-RFLP – polymerase chain reaction–restriction fragment length polymorphism.

Table II. Distribution of gene polymorphism of studies included in the meta-analysis

Year	First author	Case				Control				
		Genotype			Minor allele	Genotype			Minor allele	HWE
		FF	Ff	ff	MAF	FF	Ff	ff	MAF	*P*-value
2014	Arji	151	103	20	0.26	109	82	12	0.26	0.5038
2014	Mahmoud	12	20	8	0.45	10	10	5	0.4	0.404
2014	Sinaga	27	42	7	0.37	30	34	12	0.38	0.6497
2013	Fang	72	96	45	0.44	101	88	22	0.31	0.6642
2013	Joshi	51	46	13	0.33	118	85	22	0.29	0.252
2012	Rathored	319	298	75	0.32	118	80	7	0.23	0.1356
2011	Kim	47	75	38	0.47	46	73	37	0.47	0.4463
2011	Kang	30	58	15	0.43	41	43	21	0.40	0.1240
2011	Singh	55	40	6	0.26	96	110	19	0.33	0.1069
2011	Sharma	77	67	10	0.28	395	197	36	0.21	0.0880
2011	Ates	58	60	10	0.31	35	37	8	0.33	0.6945
2010	Marashian	97	57	10	0.23	15	30	5	0.40	0.0771
2010	Zhang	16	43	51	0.66	26	47	29	0.51	0.4330
2009	Banoei	30	21	9	0.33	29	27	6	0.31	0.9375
2009	Merza	67	46	4	0.23	35	25	0	0.21	0.0415
2009	Vidyarani	23	14	3	0.25	20	29	0	0.30	0.0033
2009	Selvaraj	33	29	3	0.27	33	26	1	0.23	0.1019
2009	Alagarasu	138	66	13	0.21	81	59	4	0.23	0.0766
2008	Selvaraj	31	16	4	0.24	27	33	0	0.28	0.0033
2008	Liu	16	25	19	0.53	11	17	2	0.35	0.1789
2007	Wilbur	35	19	0	0.18	81	42	1	0.18	0.0740
2007	Olesen	198	106	16	0.22	207	118	19	0.23	0.6862
2007	Babb	132	104	13	0.26	203	129	20	0.24	0.9337
2007	Soborg	288	128	19	0.19	267	128	21	0.20	0.2734
2006	Chen	60	56	24	0.37	70	60	9	0.28	0.4144
2006	Lombard	43	21	2	0.19	64	18	2	0.13	0.5917
2004	Bornman	258	138	20	0.21	444	242	32	0.21	0.8932
2004	Selvaraj[a]	47	15	2	0.15	55	39	9	0.28	0.5834
2004	Selvaraj[b]	28	15	3	0.23	38	23	3	0.23	0.8388
2004	Roth	9	32	59	0.75	14	78	109	0.74	0.9928
2004	Liu	29	63	28	0.50	85	120	35	0.40	0.4821
2004	Liu W	29	34	13	0.39	90	70	11	0.27	0.5930
2003	Selvaraj	78	36	6	0.20	43	29	8	0.28	0.3551
2000	Wilkinson	52	31	8	0.26	74	39	3	0.19	0.4178

HWE – Hardy-Weinberg equilibrium, MAF – minor allele frequency, [a,b]the different articles by the same author in the same year.

Asians, Americans and Europeans. As for ethnicities, an increased TB risk was found in the East and Southeast Asia population in five comparison models: allele model (f vs. F: OR = 1.42, 95% CI: 1.20–1.69; $P_{heterogeneity}$ = 0.055), homozygote model (ff vs. FF: OR = 1.98, 95% CI :1.53–2.56; $P_{heterogeneity}$ = 0.012), recessive model (ff vs. Ff + FF: OR = 1.64, 95% CI: 1.31–2.06; $P_{heterogeneity}$ = 0.003), heterozygote model (Ff vs. FF: OR = 1.37, 95% CI: 1.13–1.65; $P_{heterogeneity}$ = 0.853) and dominant model (ff + Ff vs. FF: OR = 1.52, 95% CI: 1.27–1.82; $P_{heterogeneity}$ = 0.695). In South and West Asians, however, no significant association was found in the heterozygote model (ff vs. Ff + FF: OR = 1.33, 95% CI: 1.00–1.78; $P_{heterogeneity}$ = 0.045).

Further subgroup analyses were stratified by the source of the controls. Studies were divided into healthy persons-based and patient

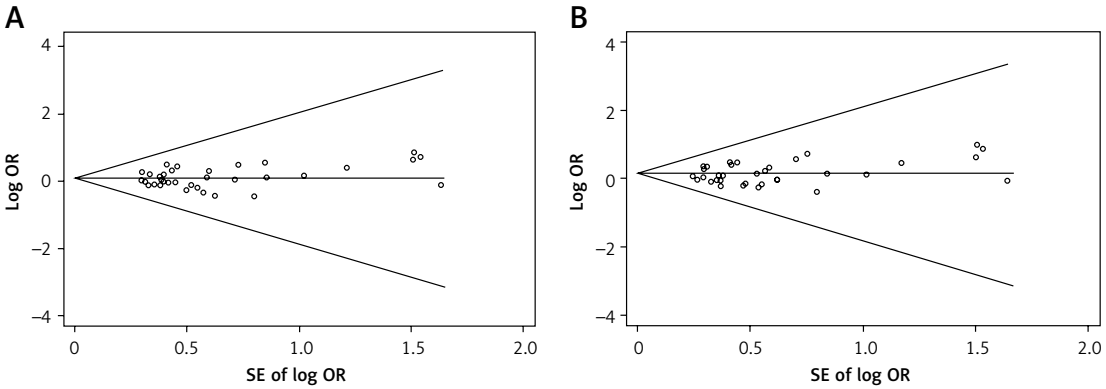

Figure 2. Funnel plot analysis to detect publication bias in 34 eligible studies. **A** – Funnel plot analysis of homozygote model (ff vs. FF). Egger's test $p = 0.567$, Begg's test $p = 0.423$; **B** – Funnel plot analysis of recessive model (ff vs. Ff + FF). Egger's test $p = 0.419$, Begg's test $p = 0.343$; the circles represent the weight of individual study. log – logarithm, SE – standard error (TIF)

Table III. Statistics to test the publication bias and heterogeneity in the meta-analysis

Comparisons	Begg's regression analysis	Egger's regression analysis			Heterogeneity analysis		Model used for the meta-analysis
	P-value	95% confidence interval	*P*-value	Q-value	$P_{heterogeneity}$	I^2 (%)	
f vs. F	0.614	(–1.133)–0.404	0.341	88.47	0.000	62.7	Random
ff vs. FF	0.441	(–0.327)–0.574	0.580	65.90	0.001	49.9	Random
Ff vs. FF	0.313	(–0.949)–0.241	0.234	66.35	0.001	50.3	Random
ff + Ff vs. FF	0.459	(–0.918)–0.409	0.440	79.44	0.000	58.5	Random
ff vs. Ff + FF	0.495	(–0.327)–0.640	0.514	57.01	0.006	42.1	Random

contacts-based studies, and importantly the association in healthy persons-based studies was reinforced in the allele model (f vs. F: OR = 1.13, 95% CI: 1.01–1.27; $P_{heterogeneity} = 0.001$), the homozygote model (ff vs. FF: OR = 1.42, 95% CI: 1.18–1.70; $P_{heterogeneity} = 0.019$) and the recessive model (ff vs. Ff + FF: OR = 1.31, 95% CI: 1.10–1.56; $P_{heterogeneity} = 0.028$), which conferred a significantly increased risk of TB, whereas this risk was reversed in patient contacts-based studies with no significance in each model (Table IV).

In addition, when categorized by the sample size with a cutoff of 500 individuals, 30 out of 34 studies had sample sizes less than 500 and conferred an increased risk of TB for two comparison models: the homozygote model (ff vs. FF: OR = 1.38, 95% CI: 1.15–1.64; $P_{heterogeneity} = 0.002$) and the recessive model (ff vs. Ff + FF: OR = 1.33, 95% CI: 1.14–1.56; $P_{heterogeneity} = 0.012$). For the subgroup analysis by the genotyping methods, the homozygote model (ff vs. FF: OR = 1.47, 95% CI: 1.23–1.75; $P_{heterogeneity} = 0.001$), recessive genetic model (ff vs. Ff + FF: OR = 1.39, 95% CI: 1.19–1.63; $P_{heterogeneity} = 0.010$) and dominant model (ff + Ff vs. FF: OR = 1.10, 95% CI: 1.00–1.20; $P_{heterogeneity} = 0.000$) remained statistically significant in PCR-RFLP studies (Table IV).

Discussion

Tuberculosis is one of the leading causes of morbidity and mortality, and the VDR gene might be important in modulating host susceptibility to TB because of the potential roles of VDR in the immune response to TB. However, many studies generated conflicting association data concerning the association between VDR FokI gene polymorphism and the risk of TB.

Our present meta-analysis, based on 34 eligible studies until January 2015, provides evidence to propose a consistent effect of VDR FokI polymorphism. We found that the f allele was associated with a significantly increased risk of TB in the homozygote model (ff vs. FF) and the recessive model (ff vs. Ff + FF), especially in the East and Southeast Asian population. However, an insignificant association was found in South and West Asians, Africans, Americans and Europeans for all comparison models. To a certain extent, this finding could reflect the existence of racial differences, suggesting that this polymorphism might have a multifunctional role in the pathogenesis of TB or interact with other genetic and environmental factors. Previous studies including the WHO TB report suggested that the yellow race was more susceptible to TB than the black and white race [1].

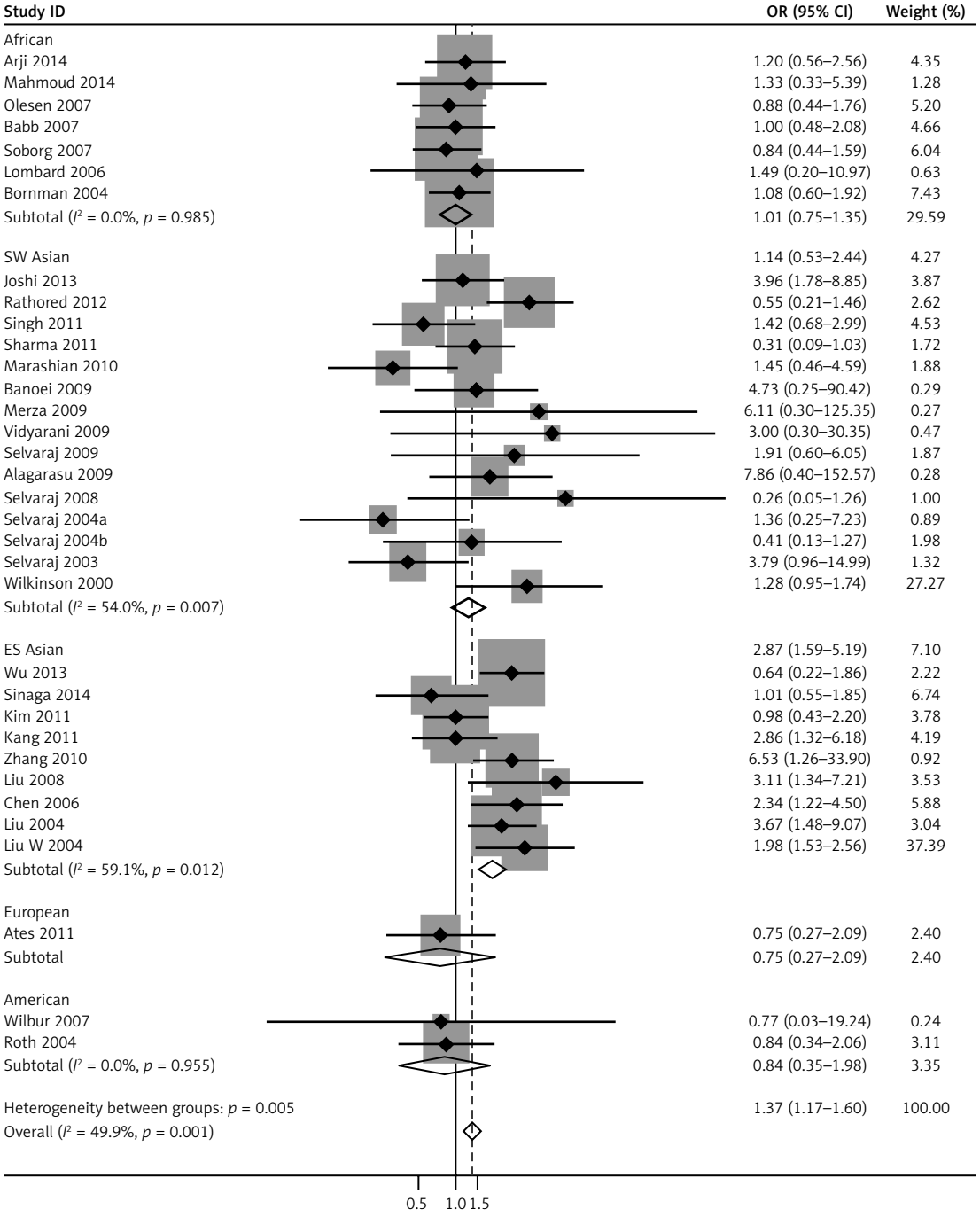

Figure 3. Forest plot of homozygote model for overall comparison (ff vs. FF) (TIF)

Additionally, it was reported that the f allele frequency was higher in Asians than Africans [17]. Thus, the finding of this meta-analysis might be attributed to the racial differences.

There are some limitations to this systematic review. First, some individual information such as age, sex, HIV status and environmental factors could not be obtained, which makes the detailed sub-grouping analyses and interpretation of the results difficult. Second, considering that diabetes, hypertension and any other medical problem may affect vitamin D level, the confounding effect should be taken into account. VDR FokI polymorphisms have been suggested to be related to diabetes in Asians [46]. Diabetes status in the study population may therefore influence the association observed for VDR polymorphisms and TB incidence. Therefore, the stratification of diabetes status would further reveal the relationship between VDR gene SNPs and TB. However, diabetes status was not reported in two-thirds of the enrolled studies. Therefore, it was not possible to

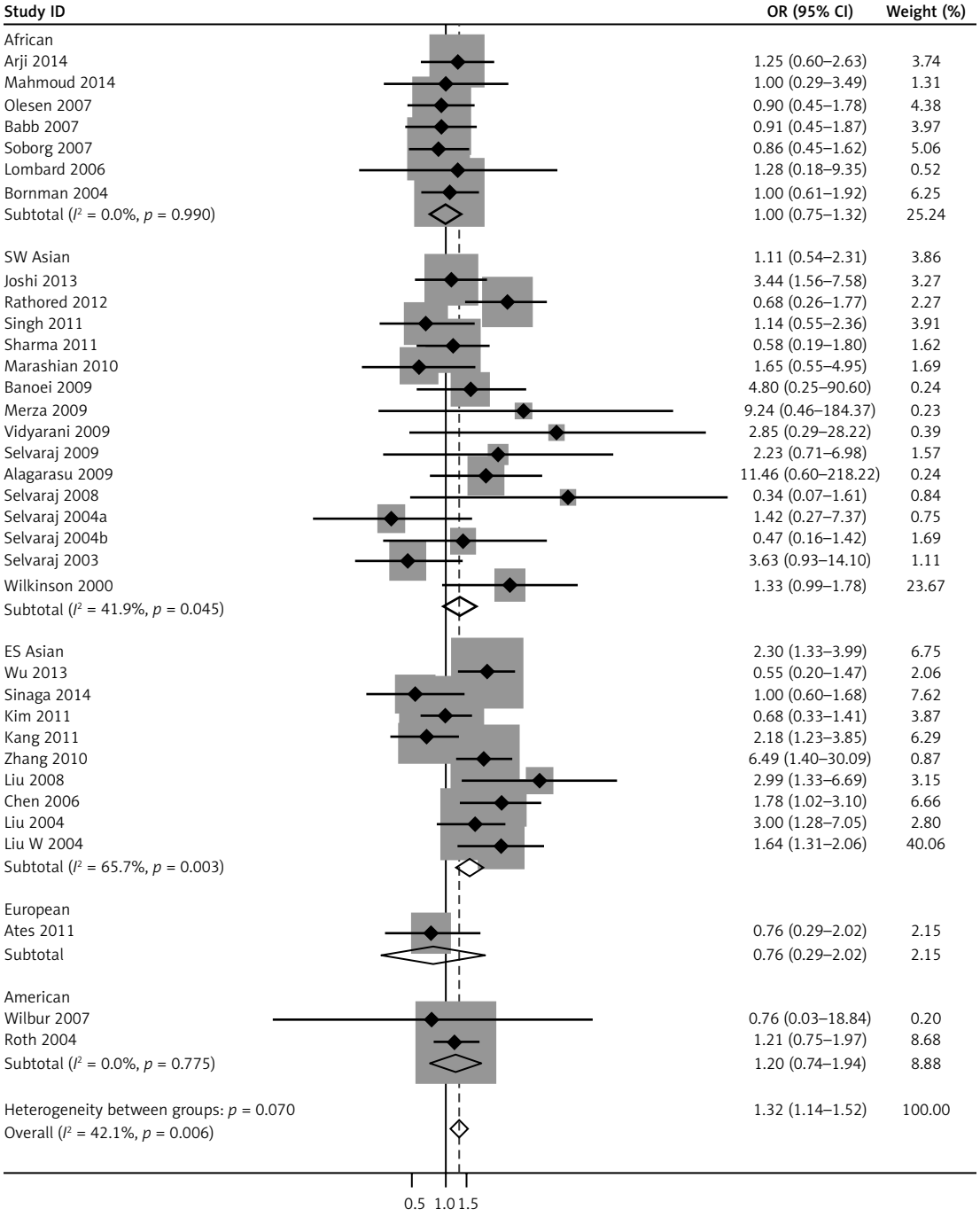

Figure 4. Forest plot of recessive model for overall comparison (ff vs. Ff + FF) (TIF)

apply stratification according to diabetes status. Third, the small sample sizes in some subgroup analyses may not comprehensively represent the population. More studies are needed to confirm the association of FokI polymorphisms and TB risk, especially in different ethnic populations. Fourth, the different experimental designs and diagnostic standards make the analyses prone to bias. Fifth, included studies were restricted to those published in English or Chinese in our study, which might introduce potential bias into data analysis as well. Sixth, based on the data provided by the articles and our own calculations, significant deviations from HWE ($p < 0.05$) in controls were observed for three studies based on Asians [31, 32, 35]. Thus, their results should be interpreted with more caution. We therefore repeated the meta-analyses after exclusion of these studies. However, this exclusion did not materially affect the results (Table VI). Although genome-wide association studies (GWAS) are important for the discovery of genetic variations, we did not identify

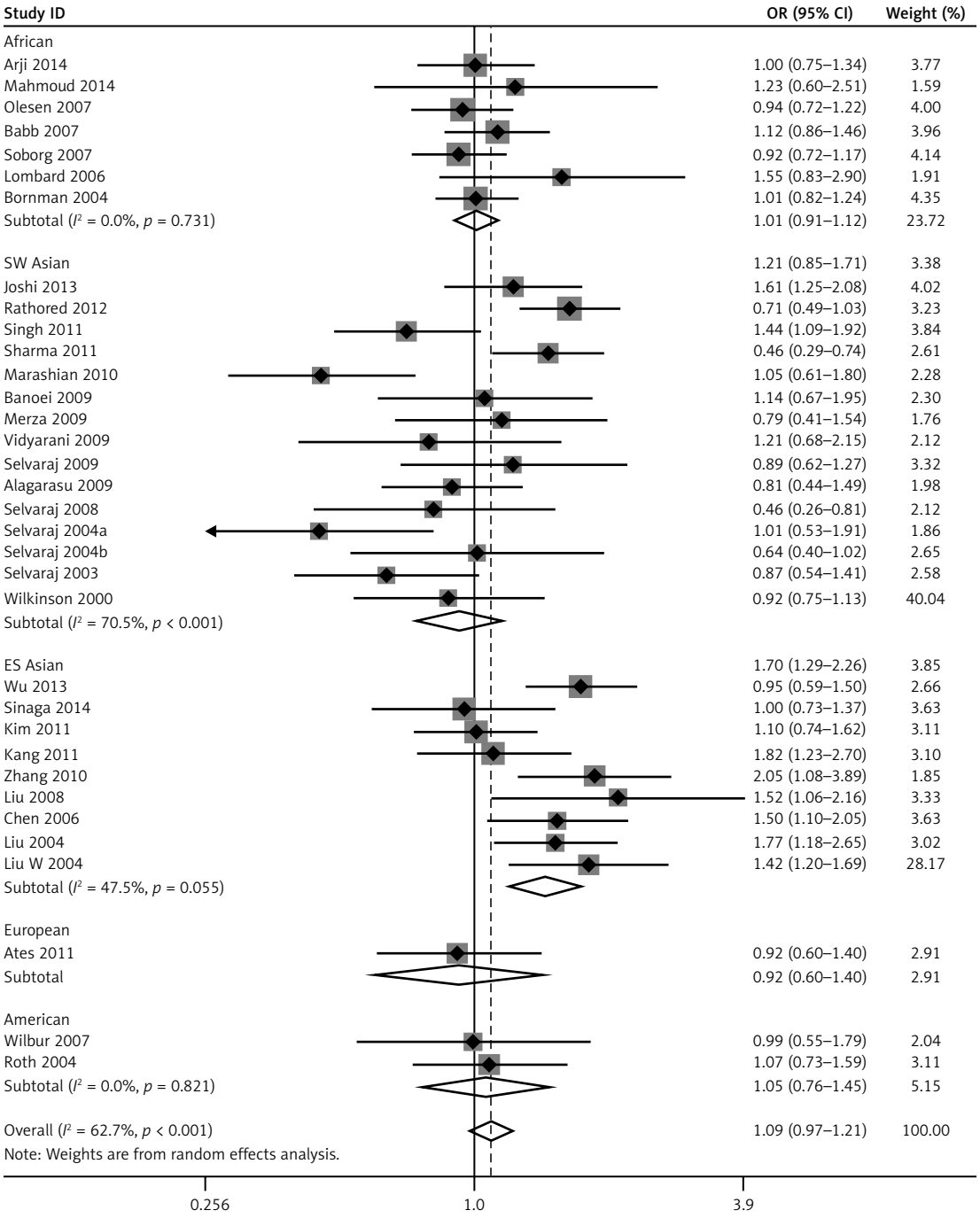

Figure 5. Forest plot of allele model for overall comparison (f vs. F) (TIF)

any published GWAS on this subject. In conclusion, the results from this meta-analysis demonstrate that VDR FokI polymorphism is associated with increased TB risk, especially in East and Southeast Asians, which supports the hypothesis that VDR might play an important role in the host defense against TB. However, due to the moderate strength of the associations, their values to be used for risk prediction should be considered cautiously, and future large scale case-control studies are required to validate these findings.

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Conflict of interest

The authors declare no conflict of interest.

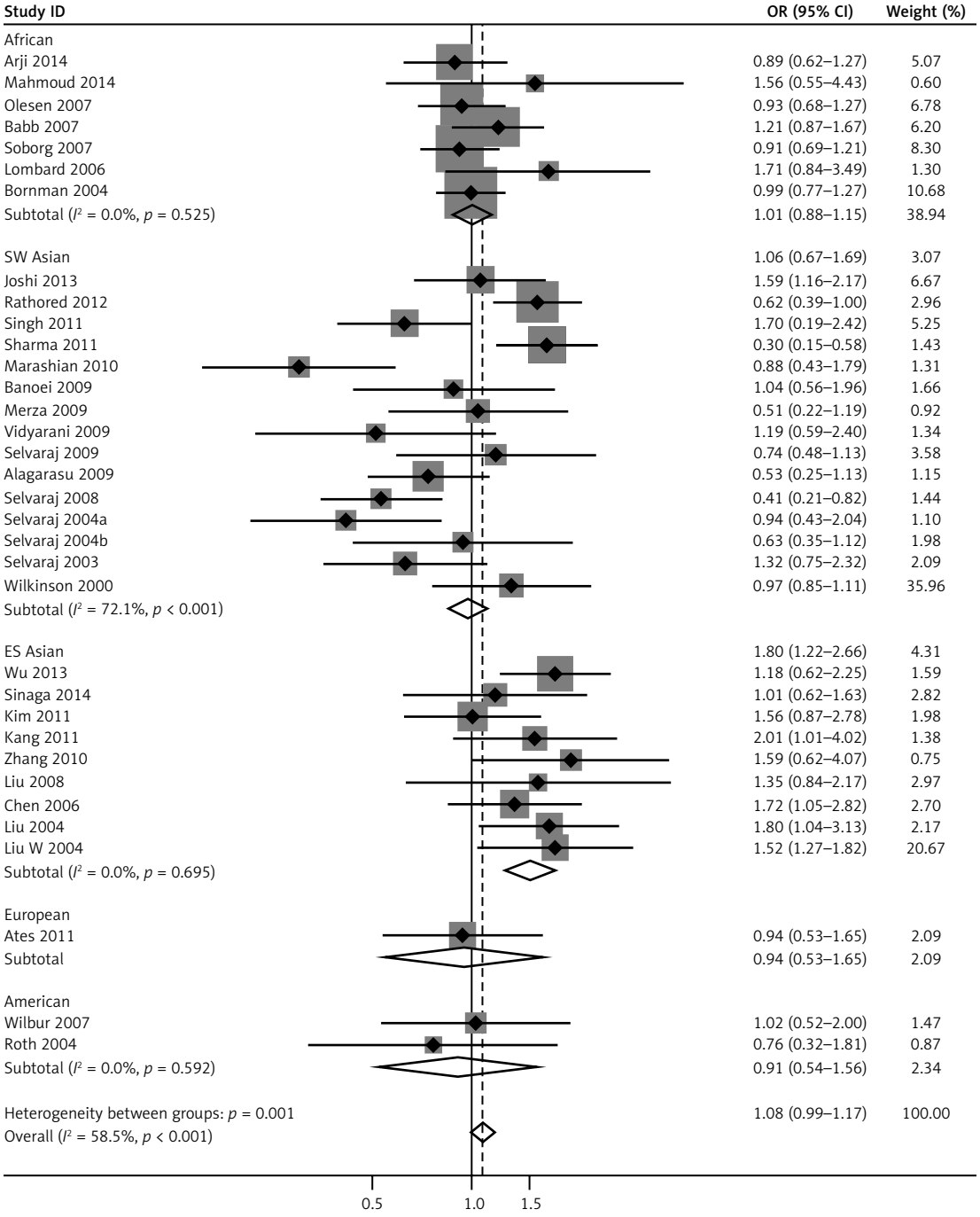

Figure 6. Forest plot of dominant model for overall comparison (ff + Ff vs. FF) (TIF)

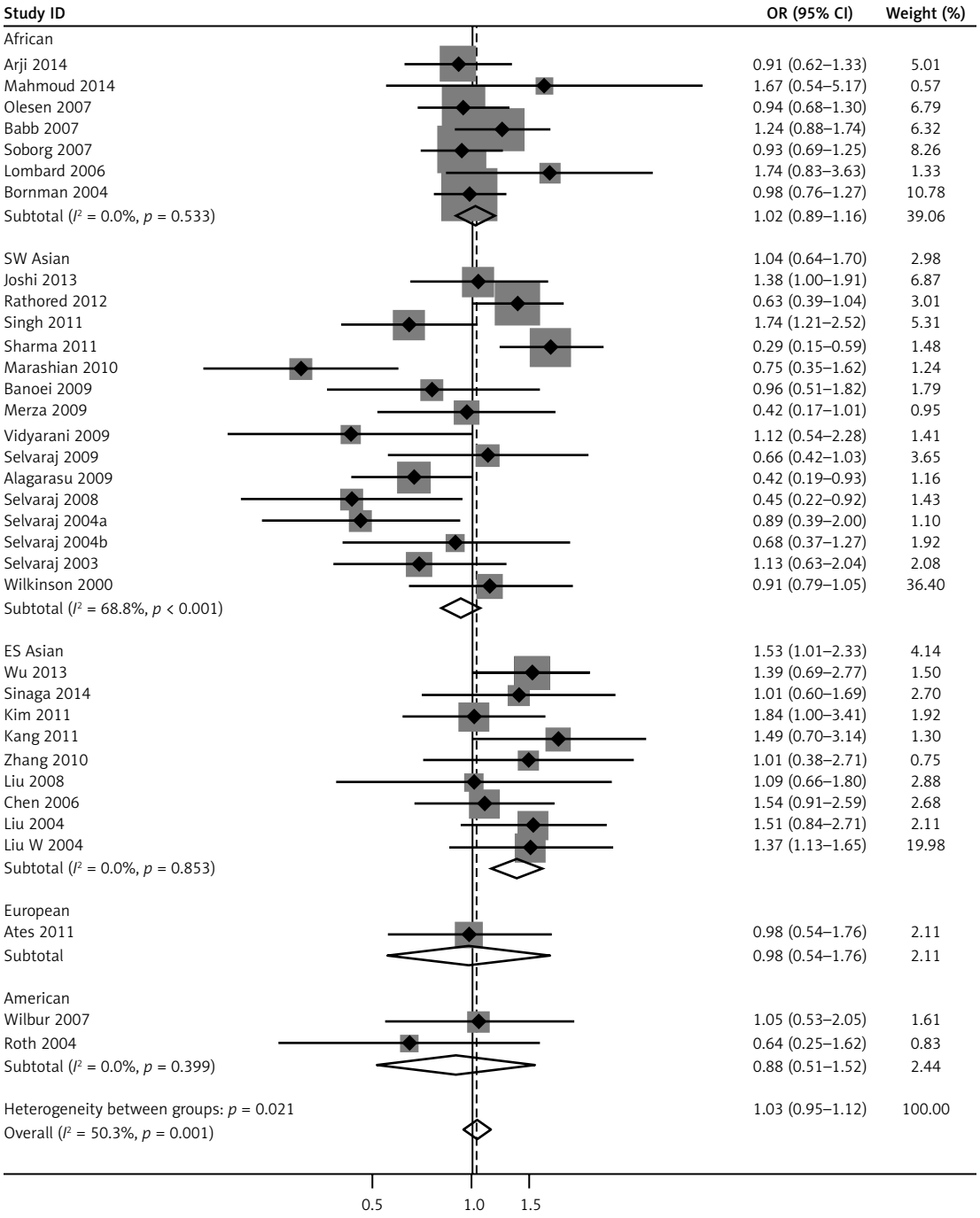

Figure 7. Forest plot of heterozygote model for overall comparison (Ff vs. FF) (TIF)

Table IV. Meta-analysis results

Variable	f vs. F		ff vs. FF		ff vs. Ff + FF		ff vs. FF		ff + Ff vs. FF	
	N	OR	P_h	OR	P_h	OR	P_h	OR	P_h	OR	P_h
Total	34	1.09 (0.97–1.21)	0.000	1.37 (1.17–1.60)*	0.001	1.32 (1.14–1.52)*	0.006	1.03 (0.95–1.13)	0.001	1.08 (0.99–1.17)	0.000
Ethnicities:											
ES Asians	9	1.42 (1.20–1.69)*	0.055	1.98 (1.53–2.56)*	0.012	1.64 (1.31–2.06)*	0.003	1.37 (1.13–1.65)	0.853	1.52 (1.27–1.82)*	0.695
SW Asians	15	0.92 (0.75–1.13)	0.000	1.28 (0.95–1.74)	0.007	1.33 (1.00–1.78)*	0.045	0.91 (0.79–1.05)	0.000	0.97 (0.85–1.11)	0.000
Africans	7	1.01 (0.91–1.12)	0.731	1.01 (0.75–1.35)	0.985	0.10 (0.75–1.32)	0.990	1.02 (0.89–1.17)	0.533	1.01 (0.88–1.15)	0.525
Americans	2	1.05 (0.76–1.45)	0.821	0.84 (0.35–1.98)	0.955	1.20 (0.74–1.94)	0.775	0.88 (0.51–1.53)	0.399	0.92 (0.54–1.56)	0.592
Europeans	1	0.92 (0.60–1.40)	–	0.75 (0.27–2.09)	–	0.76 (0.29–2.02)	–	0.98 (0.54–1.76)	–	0.94 (0.54–1.65)	–
Sample size:											
Large[a]	4	1.09 (0.97–1.21)	0.003	1.34 (0.96–1.88)	0.022	1.26 (0.90–1.76)	0.048	1.15 (0.99–1.34)	0.023	1.18 (1.02–1.36)*	0.000
Small[b]	30	1.06 (0.94–1.20)	0.000	1.38 (1.15–1.64)*	0.002	1.33 (1.14–1.56)*	0.012	0.98 (0.89–1.09)	0.003	1.04 (0.94–1.14)	0.006
Genotyping method:											
PCR-RFLP	29	1.08 (0.95–1.22)	0.000	1.47 (1.23–1.75)*	0.001	1.39 (1.19–1.63)*	0.010	1.05 (0.95–1.15)	0.000	1.10 (1.00–1.20)*	0.000
Other methods	5	1.07 (0.86–1.33)	0.114	1.02 (0.71–1.45)	0.235	1.03 (0.74–1.44)	0.196	0.99 (0.81–1.19)	0.642	0.99 (0.83–1.19)	0.447
Source of controls:											
Contacts[c]	10	0.97 (0.75–1.26)	0.000	1.24 (0.91–1.68)	0.002	1.33 (1.03–1.71)*	0.022	0.93 (0.78–1.11)	0.012	0.99 (0.83–1.17)	0.001
Healthy[d]	24	1.13 (1.01–1.27)*	0.001	1.42 (1.18–1.70)*	0.019	1.31 (1.10–1.56)*	0.028	1.07 (0.97–1.17)	0.006	1.11 (1.01–1.21)*	0.001

*N – number of studies included, OR – odds ratio, P_h – p-value for heterogeneity, *OR with statistical significance, [a]studies with more than 500 participants, [b]studies with less than 5000 participants, [c]studies with controls from patient contacts, [d]studies with controls from healthy persons.*

Table V. Meta-regression analysis results

Variable	f vs. F			ff vs. FF		ff vs. Ff + FF		Ff vs. FF		ff + Ff vs. FF	
	N	95% CI	*P*-value	95% CI	*P*-value	95% CI	*P*-value	95% CI	*P*-value	OR	*P*-value
Publication years	34	(–52.14)–23.05	0.44	(–92.13)–90.56	0.99	(–76.13)–88.01	0.88	(–65.78)–36.31	0.56	(–61.78)–35.21	0.58
Ethnicities	34	(–0.48)–0.40	0.86	(–1.17)–0.96	0.85	(–1.13)–0.90	0.82	(–0.62)–0.60	0.98	(–0.614)–0.56	0.92
Sample size	34	(–0.06)–0.19	1.08	(–0.24)–0.46	0.52	(–0.25)–0.45	0.56	(–0.10)–0.22	0.44	(–0.08)–0.220	0.35
Genotyping method	34	(–0.15)–0.15	0.96	(–0.38)–0.37	0.98	(–0.33)–0.36	0.94	(–0.21)–0.19	0.95	(–0.19)–0.19	0.97
Source of controls	34	(–0.11)–0.15	0.76	(–0.28)–0.37	0.78	(–0.14)–0.39	0.36	(–0.22)–0.15	0.74	(–0.18)–0.17	0.95
Type of tuberculosis	34	(–0.21)–0.99	0.97	(–0.45)–0.51	0.90	(–0.40)–0.46	0.89	(–0.32)–0.25	0.79	(–0.29)–0.25	0.87

Table VI. Sensitivity analyses of study with controls not in HWE excluded

Study with controls not in HWE excluded	Summarized odds ratio (95% CI)	No. of included studies	I^2 (%)	*P*-value
f vs. F	1.097 (0.978–1.229)	31	65.3	0.113
ff vs. FF	1.323 (1.037–1.689)	31	52.3	0.025
ff vs. Ff + FF	1.320 (1.083–1.608)	31	39.7	0.006
Ff vs. FF	1.042 (0.917–1.185)	31	47.4	0.526
ff + Ff vs. FF	1.085 (0.945–1.246)	31	58.8	0.246

CI – confidence interval.

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