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Original Article

Association of Vitamin D receptor gene TaqI polymorphisms with tuberculosis susceptibility: a meta-analysis

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Abstract: Vitamin D receptor (VDR) is a receptor of vitamin D3, which plays a pivotal role in regulating cell proliferation and differentiation, lymphocyte activation and cytokine production, and is associated with TB susceptibility. Growing studies explored the association of TaqI polymorphism of VDR with tuberculosis (TB) susceptibility. However, the results were inconsistent and conflicting. To assess the relationship between the VDR TaqI gene polymorphism and the risk of TB, a meta-analysis was performed. Databases including PubMed and EMbase were systematically searched for genetic association studies of TaqI polymorphism of VDR and tuberculosis until February 15, 2015. Data were extracted by two independent authors and pooled odds ratio (OR) with 95% confidence interval (CI) was calculated to assess the strength of the association between VDR TaqI gene polymorphism and TB risk, meta-regression and subgroup analyses were performed to identify the source of heterogeneity. Thirty-eight studies with a total of 6881 cases and 7511 controls were reviewed in the present meta-analysis. A statistically significant correlations were observed between VDR TaqI gene polymorphism and TB risk in South and West Asians (t vs. T: OR=1.27, 95% CI=1.07-1.51, P=0.007; tt vs. TT: OR=1.59, 95% CI=1.11-2.26, P=0.011; tt vs. Tt + TT: OR=1.43, 95% CI=1.17-1.73, P=0.000; tt + Tt vs. TT: OR=1.32, 95% CI=1.05-1.67, P=0.019). Heterogeneity between studies was not pronounced, and meta-regression found no source contributed to heterogeneity. However, after stratified analysis with respect to genotyping methods and sample size, significant association was found in "small" studies (<500 participants) and studies with "PCR-RFLP" methods. Synthesis of the available studies suggests that t allele of the VDR TaqI polymorphism is significantly associated with an increased TB risk in South and West Asians.

Keywords: Tuberculosis, Vitamin D receptor, TaqI polymorphisms

Introduction

Tuberculosis (TB) is one of the most common infectious diseases and the leading cause of mortality worldwide, with an estimated 9 million new cases and 1.5 million deaths occurred in 2013. According to World Health Organization (WHO) report, approximately 56% of new cases occurred in the South-East Asia and Western Pacific Regions [1]. It is well known that tuberculosis susceptibility may be influenced by multiple genetic, socio-economic and environmental factors [2, 3], which contains single nucleotide polymorphisms (SNP) as a major factor.

Vitamin D (VitD) is well known to play a critical role in modulating monocyte and macrophage activity and influencing human innate immunity to certain infectious agents including *M. tuberculosis* [4]. Vitamin D receptor (VDR) is a nuclear hormone receptor, which upon binding to vitamin D3, interacts with Vitamin D response elements and signals other target genes. It is highly expressed on dendritic cells, activated T lymphocytes and macrophages. After binding with Vitamin D, VDR could modulate cytokine responses by T cells [5, 6], and thus represents antibacterial responses in innate immunity [7]. Many recent studies have demonstrated the critical role of VDR in the inflammatory-related immune response to active TB disease [8-10]. Human VDR gene is located on chromosome 12q13.11 and contains 14 exons [11]. The VDR polymorphisms located in coding region and 3' untranslated region are FokI, TaqI, ApaI and BsmI. Accumulated evidence has suggested

that polymorphisms in the VDR gene may influence the expression and function of VDR and subsequent downstream vitamin D-mediated effect [12]. To date, several meta-analyses focused on the association of VDR TaqI polymorphisms with tuberculosis risk across different ethnicities [13-15], however, due to the limitations of sample size and broadly statistical analysis, the results were varied and inconsistent among different employed genetic models. In addition, the previous studies did not cover all eligible publications related to tuberculosis and thus resulted in biased effect sizes. To ascertain the authentic effect of VDR TaqI polymorphisms on susceptibility to tuberculosis, we conducted a meta-analysis including all eligible case-control studies focused on the relationship between the VDR TaqI polymorphism and tuberculosis risk.

Materials and methods

Literature Search

A systematic search was conducted using the databases of the US National Institutes of Health (PubMed), Web of Science and Embase databases (last search was updated on February 15 2015), with the combination of terms like: 'VDR'OR 'Vitamin D receptor' OR 'FokI' OR 'rs10735810' AND 'polymorphism' OR 'mutation' OR 'SNP' OR 'Single Nucleotide Polymorphism' AND 'tuberculosis'. To identify the extra eligible studies, the relevant published studies and review articles were manually examined. The search in these databases was limited to articles relating to humans. No language restrictions were applied.

The identified studies in our meta-analysis met all of the following criteria: (1) studies had to assess the association between Vitamin D receptor TaqI polymorphisms and tuberculosis risk; (2) case-control studies or cohort design, and studies included available genotype frequencies to calculate odds ratio (OR) and 95% confidence interval (CI); (3) independent studies using original data. Studies were excluded for the following criteria: (1) the studies not providing genotype distribution or allele frequency data; (2) reviews or case reports, case studies without control subjects; (3) duplicated previous publications. At last, 38 case-control published studies from PubMed, Web of Science and Embase were available. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) checklist was available as supplementary material, as displayed in Checklist S1.

Data extraction

Two investigators (CY and WXJ) independently performed the required data extraction, and then conducted group discussion to resolve the disagreements. The following data were extracted from each study: publication year, the name of first author, country, ethnicity, study design, genotyping method, diagnosis method of cases, the tuberculosis type, number of cases and controls, genotype and allele frequencies for cases and controls, HIV status of cases and controls, and source of genotyping. According to the source of controls, all eligible studies were defined as hospital-based (HB) and population-based (PB). If data concerning the genotype distributions were not displayed regarding the included studies, the primary author was contacted via electronic mail to obtain the missing data.

Statistical analysis

Minor allele frequency was calculated manually based on genotypic distribution among cases and controls. We assessed Hardy-Weinberg Equilibrium (HWE) among control population for each study using Hardy-Weinberg Equilibrium Online Calculator (http://www.changbioscience.com/genetics/hardy.html). And a *P* value of >0.05 was considered to meet HWE.

All statistical analyses in this meta-analysis were carried out using the software Stata 12.0 (Stata Corporation, College Station, TX, USA), with two-sided *p* values. The extracted data from all publications were tested using five genetic models i.e. allele model (t vs. T), homozygote model (tt vs. TT), heterozygote model (Tt vs. TT), dominant model (tt + Tt vs. TT) and recessive model (tt vs. Tt + TT). Odds ratios (ORs) with a corresponding 95% confidence interval (CI) were calculated (for all five genetic models) to assess the strength of association between VDR TaqI polymorphism and the TB risk. The significance of pooled OR was measured by the Z-test ($P<0.05$ was considered statistically significant). Heterogeneity assumption was assessed by the x^2-based Q-statistics and Higgins I^2 test. Those resulting with $I^2>50\%$

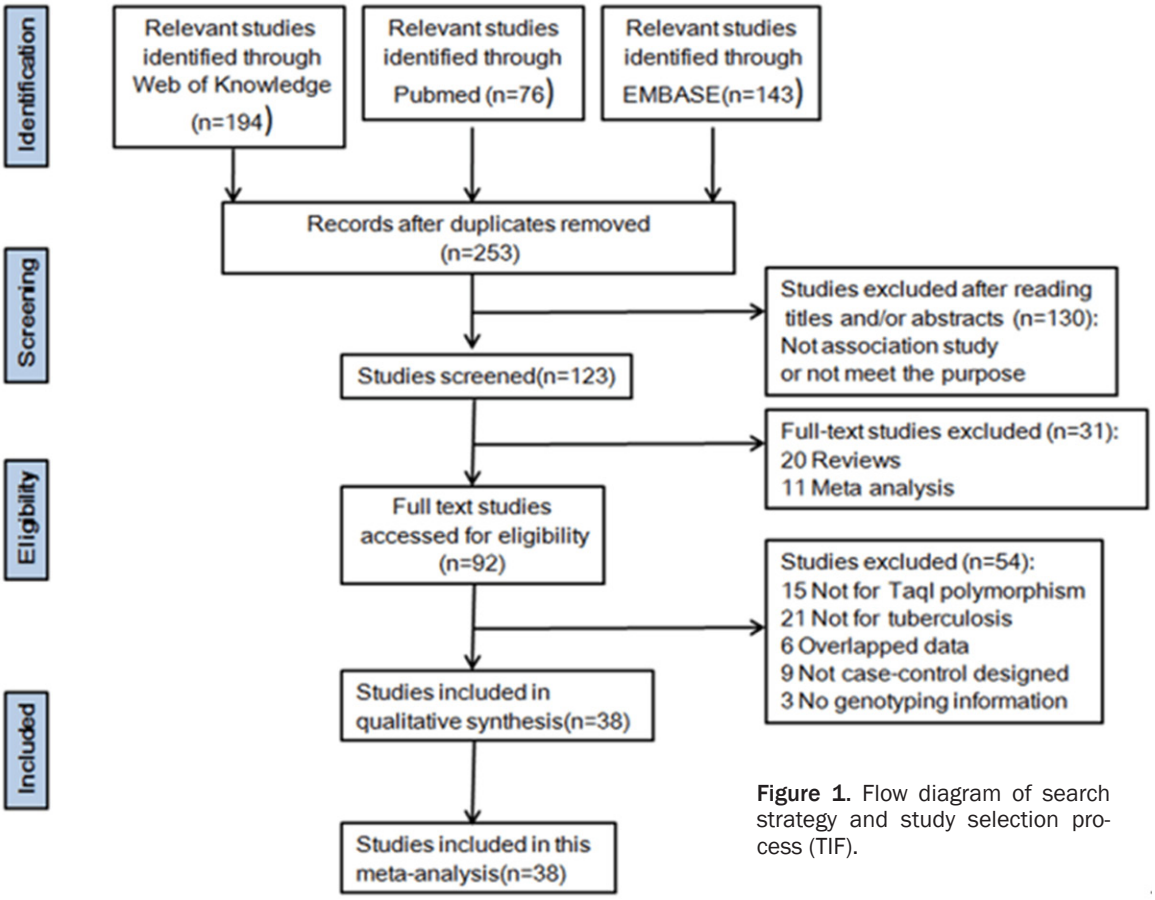

Figure 1. Flow diagram of search strategy and study selection process (TIF).

were identified as a heterogeneous group, then ORs were pooled according to random effect model (Mantel-Haenszel method) [16]. Otherwise the fixed effect model was adopted (DerSimonian-Laird method) [17].

Subgroup analyses were conducted based on these genetic models to define the sources of heterogeneity, according to ethnicities, sample size, tuberculosis type, HWE, the source of controls as well as for the genotyping methods. A meta-regression was used to illustrate the potential reasons for heterogeneity between the studies. We classified the studies that were conducted in Asia into two groups: East and Southeast Asia (China, South Korean, South Sumatera, Indonesia and Cambodia) and South and West Asia (India and Iran). As a result, the enrolled studies were classified into five subgroups based on ethnicity: East and Southeast Asians, South and West Asians, Africans, Europeans and Americans. Studies with more than 500 participants were defined as "large", and studies with less 500 participants were defined as "small".

Sensitivity analysis was conducted to evaluate stability of the results by deleting of a single study at a time, the pooled ORs were recalculated to determine whether individual study could influence the overall results. Furthermore, publication bias was identified by examining the Begg's funnel plots [18] and Egger's regression test [19].

Results

Characteristics of eligible studies

A total of 413 potential studies were identified by preliminary searching PubMed, Embase and Web of Science, among which 38 case-control studies were selected according to the inclusion and exclusion criteria, involving a total of 6881 tuberculosis patients and 7511 control subjects in this meta-analysis [20-57]. The detailed literature search strategy and included or excluded studies were explained in **Figure 1**. The baseline characteristics, such as author name, publication year, region, ethnicity, design, genotyping method, numbers about

Table 1. Main characteristics of included studies summarized for the meta-analysis

Year	First Author	Country	Ethnicity	Study design	Tuberculosis				controls		HIV status	Source of genotyping
					Part of the body	Sample size	Diagnosis method	Genotyping method		Sample size		
2014	Arji N	Morocco	African	PB	Pulmonary tuberculosis	274	AFB smear and culture	PCR-RFLP	Healthy persons	203	Negative	blood
2013	Wu	China	ES Asian	PB	Pulmonary tuberculosis	213	Clinical symptoms bacteriology X-ray	PCR-RFLP	Healthy persons	211	Negative	blood
2013	Alexandra	Romania	European	PB	Pulmonary tuberculosis	68	Not available	ARMS-PCR	Healthy persons	110	Negative	blood
2012	Rathored	India	SW Asian	PB	MDR tuberculosis and drug sensitive pulmonary tuberculosis	692	AFB smear and culture	PCR-RFLP	Healthy persons	205	Negative	blood
2011	J Kim	South Korean	ES Asian	PB	Pulmonary (98) and extra pulmonary tuberculosis (62)	160	*AFB smear and culture*	Pyro sequencing	Healthy persons	156	Not available	blood
2011	T Kang	South Korean	ES Asian	PB	Pulmonary tuberculosis	103	AFB smear and culture	PCR-RFLP	Healthy persons	105	Not available	blood
2011	Sudarto	South Sumatera, Indonesia	ES Asian	PB	Pulmonary tuberculosis	40	positive acid-fast bacilli sputum examination	PCR-RFLP	Healthy persons	40	Not available	blood
2011	A Singh	India	SW Asian	PB	Pulmonary tuberculosis	101	AFB smear or culture	PCR-RFLP	Healthy persons	225	Negative	blood
2011	Sharma	India	SW Asian	PB	Pulmonary tuberculosis	474	AFB smear or culture	PCR-RFLP	Healthy persons	607	Not available	blood
2011	Wang X	China	ES Asian	PB	Pulmonary tuberculosis	213	AFB smear or culture	PCR-RFLP	Healthy persons	211	Not available	blood
2011	Ates	Turkey	European	PB	Pulmonary (98) and extra pulmonary tuberculosis (30)	128	AFB smear or culture	PCR-RFLP	Healthy persons	80	Not available	blood
2010	Marashian	Iran	SW Asian	PB	Pulmonary tuberculosis	164	AFB smear and X-ray	PCR-RFLP	contacts	50	Not available	blood
2009	Banoei	Iran	SW Asian	PB	Pulmonary tuberculosis	60	Confirmed in Massih Danes hvari	PCR-RFLP	Healthy subjects	62	Negative	blood
2009	Vidyarani	India	SW Asian	PB	Pulmonary tuberculosis	40	AFB smear and culture	PCR-RFLP	Healthy subject	49	Not available	blood
2009	Selvaraj	India	SW Asian	PB	Pulmonary tuberculosis	65	Clinical symptom, AFB smear and culture	PCR-RFLP	Healthy subjects	60	Negative	blood
2009	Alagarasu	India	SW Asian	PB	Pulmonary (187) and extra pulmonary tuberculosis (30)	217	AFB smear, clinical criteria and X-ray	PCR-RFLP	Healthy controls	144	Cases (51%), Controls (0)	blood
2009	Meng XJ	China	ES Asian	PB	Pulmonary (185 and extra pulmonary tuberculosis (39)	224	AFB smear or culture	PCR-RFLP	Healthy controls	225	Negative	blood
2009	Jiao WW	China	ES Asian	HB	Pulmonary tuberculosis	125	Clinical symptom, AFB smear and X-ray	PCR-RFLP	Healthy controls	446	Negative	blood

2008	Selvaraj	India	SW Asian	PB	Pulmonary tuberculosis	51	AFB smear and culture	PCR-RFLP	Normal health subjects	60	Negative	blood
2008	Liu Y.-D	China	ES Asian	PB	Pulmonary tuberculosis	60	AFB smear and culture	SNaPshot	Normal health subjects	30	Negative	blood
2007	Wilbur	Paraguay	American	PB	Pulmonary tuberculosis	54	Clinical symptoms, PPD test	PCR-RFLP	No symptoms	124	Not available	blood
2007	Olesen	Guinea-Bissau	African	PB	Pulmonary tuberculosis	320	AFB smear and clinical criteria	TaqMan	Healthy controls	344	HIV positive in 33% case sand negative in controls	blood
2007	Babb	South Africa	African	PB	Pulmonary tuberculosis	249	AFB smear and XRay	PCR-RFLP	No clinical history or symptoms of TB	352	Negative	blood
2007	Soborg	Tanzanian	African	PB	Pulmonary tuberculosis	435	Culture	PCR-SSP	Culture negative	416	HIV positive in 44% cases and 18% controls	blood
2006	Chen XR	China	ES Asian	PB	Pulmonary tuberculosis	140	Clinical symptoms, AFB smear and XRay	PCR-RFLP	household contacts	139	Negative	blood
2006	Lombard	Venda	African	HB	Pulmonary and meningeal tuberculosis	66	AFB smear	ARMS-PCR	Healthy controls with no history of TB	86	Negative	blood
2004	Bornman	Gambia, Guinea-Bissau, Guinea	African	HB	Pulmonary tuberculosis	416	AFB or culture	PCR-RFLP	Healthy community control subjects	718	Cases (12.5%), controls (6.8%)	blood
2004	Fitness	Malawi	African	PB	Pulmonary tuberculosis	386	AFB smear, culture and histology	PCR-RFLP	Healthy controls	624	Cases (67.6%), Controls (13.1%)	blood
2004	Selvaraj[a]	India	SW Asian	PB	Spinal tuberculosis patients	64	X-ray and Clinical criteria	PCR-RFLP	77 were contacts and 26 were normal healthy subjects.	103	Not available	blood
2004	Selvaraj[b]	India	SW Asian	PB	Pulmonary tuberculosis	46	AFB smear, culture and and radiographic abnormalities	PCR-RFLP	clinically normal	64	Negative	blood
2004	Roth	Peru	American	PB	Pulmonary tuberculosis	100	AFB smear	PCR-RFLP	Two healthy controls, 1PPD+ and 1PPD-	201	Negative	blood
2004	Liu	China	ES Asian	PB	Pulmonary tuberculosis	120	AFB smear, culture and X-ray	PCR-RFLP	normal controls	240	Negative	blood
2002	Delgado	Cambodia	ES Asian	PB	Pulmonary tuberculosis	358	AFB smear	PCR-RFLP	contacts with no TB	106	Negative	Blood
2000	Selvaraj[a]	India	SW Asian	PB	Spinal tuberculosis	66	Culture and X-ray	PCR-RFLP	contacts with no TB	80	Not available	Blood
2000	Selvaraj[b]	India	SW Asian	PB	Pulmonary tuberculosis	44	AFB smear and culture	PCR-SSOP	contacts with no TB	66	Not available	Blood
2000	Selvaraj[c]	India	SW Asian	PB	Pulmonary tuberculosis	200	X-ray and Clinical criteria	PCR-SSOP	patient contacts	108	Not available	Blood
2000	Wilkinson	India	SW Asian	PB	Pulmonary tuberculosis (27) and military tuberculosis (64)	91	Biopsy or culture Tuberculosis	PCR-RFLP	contacts with no TB	116	Negative	Blood
1999	Bellamy	Gambia	African	PB	Pulmonary tuberculosis	408	AFB smear	PCR-SSCP	Male donors	414	Negative	Blood

[a,b,c]: To differentiate the different articles by the same author (Selvaraj) in the same year (2004, 2000). PB: population-based; HB: hospital-based; AFB, Acid-fast bacilli; HIV, human immunodeficiency virus; MDR, multi-drug resistance for isoniazide and rifampicin; PPD, purified protein derivative; SNPs, single nucleotide polymorphism; TB, tuberculosis; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism.

Table 2. Distribution of gene polymorphism of studies included in the meta-analysis

Year	First Author	Case				Control				HWE
		genotype			Minor allele	genotype			Minor allele	
		TT	Tt	tt	MAF	TT	Tt	tt	MAF	*p*-value
2014	Arji N	137	79	58	0.36	109	48	46	0.34	0.9185
2013	Fang Wu	191	19	3	0.06	183	23	5	0.08	0.0004
2013	Alexandra	16	52	0	0.38	43	48	19	0.39	0.3802
2012	J. Rathored	290	285	117	0.38	97	79	29	0.33	0.0002
2011	J Kim	143	16	1	0.06	137	18	1	0.06	0.6319
2011	T Kang	134	14	1	0.05	148	85	8	0.05	0.0022
2011	Sudarto	14	12	14	0.50	17	9	14	0.46	0.0120
2011	A. Singh	61	30	10	0.25	132	60	33	0.28	0.0563
2011	Sharma	138	95	42	0.33	258	275	47	0.32	0.0002
2011	Wang X	191	19	3	0.06	183	23	5	0.08	0.0004
2011	Ates	49	65	14	0.36	30	39	11	0.38	0.7659
2010	Marashian	63	93	8	0.33	26	24	0	0.24	0.0256
2009	Banoei	8	33	19	0.59	33	24	5	0.27	0.8288
2009	Vidyarani	15	18	7	0.40	27	18	4	0.27	0.6863
2009	Selvaraj	24	33	8	0.38	27	21	12	0.38	0.0497
2009	Alagarasu	82	95	38	0.40	70	62	14	0.31	0.9597
2009	Meng XJ	154	66	4	0.17	170	50	5	0.13	0.5640
2009	Jiao WW	113	12	0	0.05	387	58	1	0.07	0.4423
2008	Selvaraj	18	23	10	0.42	34	22	4	0.25	0.8633
2008	Liu Y.-D	54	5	1	0.06	24	6	0	0.10	0.5428
2007	Wilbur	22	28	4	0.33	59	58	5	0.28	0.0438
2007	Olesen	150	145	25	0.30	161	150	34	0.32	0.9132
2007	Babb	136	94	19	0.27	190	140	22	0.26	0.5723
2007	Soborg	247	172	19	0.24	233	162	30	0.26	0.7997
2006	Chen XR	137	3	0	0.01	134	5	0	0.02	0.8290
2006	Lombard	51	30	5	0.23	47	34	1	0.22	0.0571
2004	Bornman	174	132	37	0.30	331	253	50	0.28	0.8644
2004	Fitness	261	154	22	0.23	384	241	47	0.25	0.2791
2004	Selvaraj[a]	27	28	9	0.36	40	48	14	0.37	0.9470
2004	Selvaraj[b]	13	23	10	0.47	27	27	10	0.37	0.8388
2004	Roth	90	10	0	0.05	169	31	1	0.08	0.9928
2004	Liu	105	12	3	222/18	203	32	5	438/42	0.4821
2002	Delgado	325	30	3	680/36	96	10	0	202/10	0.6103
2000	Selvaraj[a]	27	30	9	84/48	32	38	10	102/58	0.8042
2000	Selvaraj[b]	15	21	8	51/37	22	37	7	81/51	0.1386
2000	Selvaraj[c]	79	90	31	248/152	51	42	15	144/72	0.1939
2000	Wilkinson	39	46	6	124/58	45	58	13	148/84	0.3750
1999	Bellamy	204	177	27	585/231	188	177	49	553/275	0.4603

[a,b,c]: To differentiate the different articles by the same author (Selvaraj) in the same year (2004, 2000). HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

cases and controls were depicted in **Table 1**. The publication year of eligible studies ranged from 1999 to 2014. The study participants were broadly classified according to the predominant ancestry, including East and Southeast Asians, South and West Asians, Africans, Europeans and Americans. Three studies adopted hospital-based control [37, 45, 46], while the other thirty-five studies adopted population-based control. Thirty were pulmonary

Table 3. Meta-analysis results

		t vs. T		tt vs. TT		tt vs. Tt + TT		Tt vs. TT		tt + Tt vs. TT	
	N	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2,P_Q)
Total	38	1.03 (0.97, 1.09)[F]	47.5%; 0.001	1.09 (0.96, 1.25)[F]	46.9%, 0.001	1.05 (0.92, 1.19)[F]	42.2%, 0.004	1.03 (0.96, 1.12)[F]	37%, 0.013	1.04 (0.96, 1.12)[F]	40.3%, 0.006
Ethnicities											
ES Asians	11	0.93 (0.77, 1.11)[F]	0%; 0.707	0.93 (0.55, 1.57)[F]	0%, 0.996	0.89 (0.53, 1.48)[F]	0%, 0.998	0.94 (0.76, 1.16)[F]	0%, 0.494	0.93 (0.76, 1.14)[F]	0%, 0.587
SW Asians	15	1.27 (1.07, 1.51)[*]	63.0%; 0.001	1.59 (1.11, 2.26)[*]	56.5%, 0.004	1.43 (1.17, 1.73)[*,F]	46%, 0.026	1.25 (0.99, 1.59)	57.8%, 0.003	1.32 (1.05, 1.67)[*]	61.6%, 0.001
Africans	8	0.95 (0.87, 1.03)[F]	0%; 0.432	0.86 (0.64, 1.16)	51.2%, 0.045	0.86 (0.64, 1.15)	51.9%, 0.042	0.98 (0.88, 1.10)[F]	0%, 0.927	0.96 (0.86, 1.07)[F]	0%, 0.884
Americans	2	0.92 (0.43, 1.97)	67.9%; 0.078	1.69 (0.48, 5.96)[F]	0%, 0.488	1.56 (0.46, 5.33)[F]	0%, 0.559	0.91 (0.43, 1.91)	54.2%, 0.14	0.92 (0.40, 2.09)	63.7%, 0.097
Europeans	2	0.94 (0.70, 1.27)[F]	0%; 0.894	0.32(0.03,3.85)	66.2%, 0.086	0.20 (0.01, 6.66)	82.5%, 0.017	1.70 (0.61, 4.74)	79.9%, 0.026	1.39 (0.66, 2.95)	65.1%, 0.091
Sample size											
Large[a]	9	0.97 (0.89, 1.04)[F]	29.0%; 0.187	0.96 (0.70, 1.31)	61.7%, 0.008	0.98 (0.70, 1.37)	68.7%, 0.001	0.94 (0.85, 1.04)[F]	12.1%, 0.334	0.94 (0.85, 1.04)[F]	0%, 0.530
Small[b]	29	1.12 (1.03, 1.23)[*,F]	47.2%; 0.003	1.24 (1.02, 1.52)[*,F]	39.1%, 0.019	1.09 (0.91, 1.31)[F]	25.6%, 0.109	1.18 (1.05, 1.33)[*,F]	33.4%, 0.043	1.17 (1.05, 1.31)[*,F]	41%, 0.012
Genotyping method											
PCR-RFLP	29	1.10 (0.98, 1.23)	51.8%; 0.001	1.26 (1.08, 1.46)[*,F]	40.6%, 0.015	1.20 (1.04, 1.39)[*,F]	29.5%, 0.073	1.04 0.94, 1.14)[F]	37.3%, 0.024	1.06 (0.97, 1.16)[F]	45.1%, 0.005
Other methods	9	0.92 (0.83, 1.03)[F]	0%; 0.594	0.73 (0.56, 0.95)[*,F]	19.4%, 0.27	0.69 (0.54, 0.90)[*,F]	45.6%, 0.065	1.03 (0.89, 1.19)[F]	42.8%, 0.082	0.97 (0.85, 1.12)[F]	19.4%, 0.270
Source of controls											
Contacts[c]	11	1.06 (0.93, 1.21)[F]	0.0%; 0.526	1.15 (0.83, 1.60)[F]	0%, 0.857	1.13 (0.82, 1.55)[F]	0%, 0.875	1.05 (0.88, 1.25)[F]	0%, 0.510	1.06 (0.90, 1.25)[F]	0%, 0.478
Healthy[d]	27	1.06 (0.95, 1.18)	57.5%; 0.000	1.16 (0.89, 1.53)	58.8%, 0.000	1.07 (0.84, 1.38)	55%, 0.000	1.03 (0.94, 1.12)[F]	47.4%, 0.004	1.08 (0.94, 1.23)	50.2%, 0.002
HWE											
P_{HWE}>0.05	29	1.05 (0.94, 1.17)	54.9%; 0.000	1.14 (0.87, 1.48)	53.9%, 0.000	0.97 (0.84, 1.12)[F]	43.3%, 0.008	1.05 (0.96, 1.15)[F]	34.9%, 0.034	1.04 (0.95, 1.13)[F]	45.1%, 0.005
P_{HWE}<0.05	9	1.09 (0.96, 1.23)[F]	0%; 0.476	1.35 (1.02, 1.78)[*,F]	0%, 0.569	1.33 (1.02, 1.73)*[,F]	27.3%, 0.201	0.97 (0.82, 1.16)[F]	47.2%, 0.056	1.03 (0.87, 1.21)[F]	27.5%, 0.200
Tuberculosis type											
pulmonary	29	1.05 (0.95, 1.17)	53.2%; 0.000	1.15 (0.90, 1.48)	52.6%, 0.001	1.03 (0.90, 1.18)[F]	48.5%, 0.002	1.03 (0.94, 1.12)[F]	46%, 0.003	1.02 (0.94, 1.11)[F]	47.9%, 0.002
Extra and pulmonary	7	1.13 (0.95, 1.33)[F]	30%; 0.21	1.29 (0.84, 1.96)[F]	39.6%, 0.141	1.22 (0.81, 1.82)[F]	35.7%, 0.169	1.12 (0.90, 1.39)[F]	0%, 0.545	1.14 (0.93, 1.41)[F]	0.9%, 0.410
extra	2	0.97 (0.70, 1.36)[F]	0%; 0.855	1.00 (0.49, 2.04)[F]	0%, 0.876	1.06 (0.55, 2.06)[F]	0%, 0.915	0.90 (0.55, 1.46)[F]	0%, 0.873	0.92 (0.58, 1.46)[F]	0%, 0.855

Abbreviations: N: number of studies included; OR: odds ratio; Ph: *p* value for heterogeneity; P_Q: Cochran's Q statistics; I^2: Higgin's I^2 statistics. F: Results derived using Fixed effects for analysis. Random effects were used for all other calculations.
*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 person.

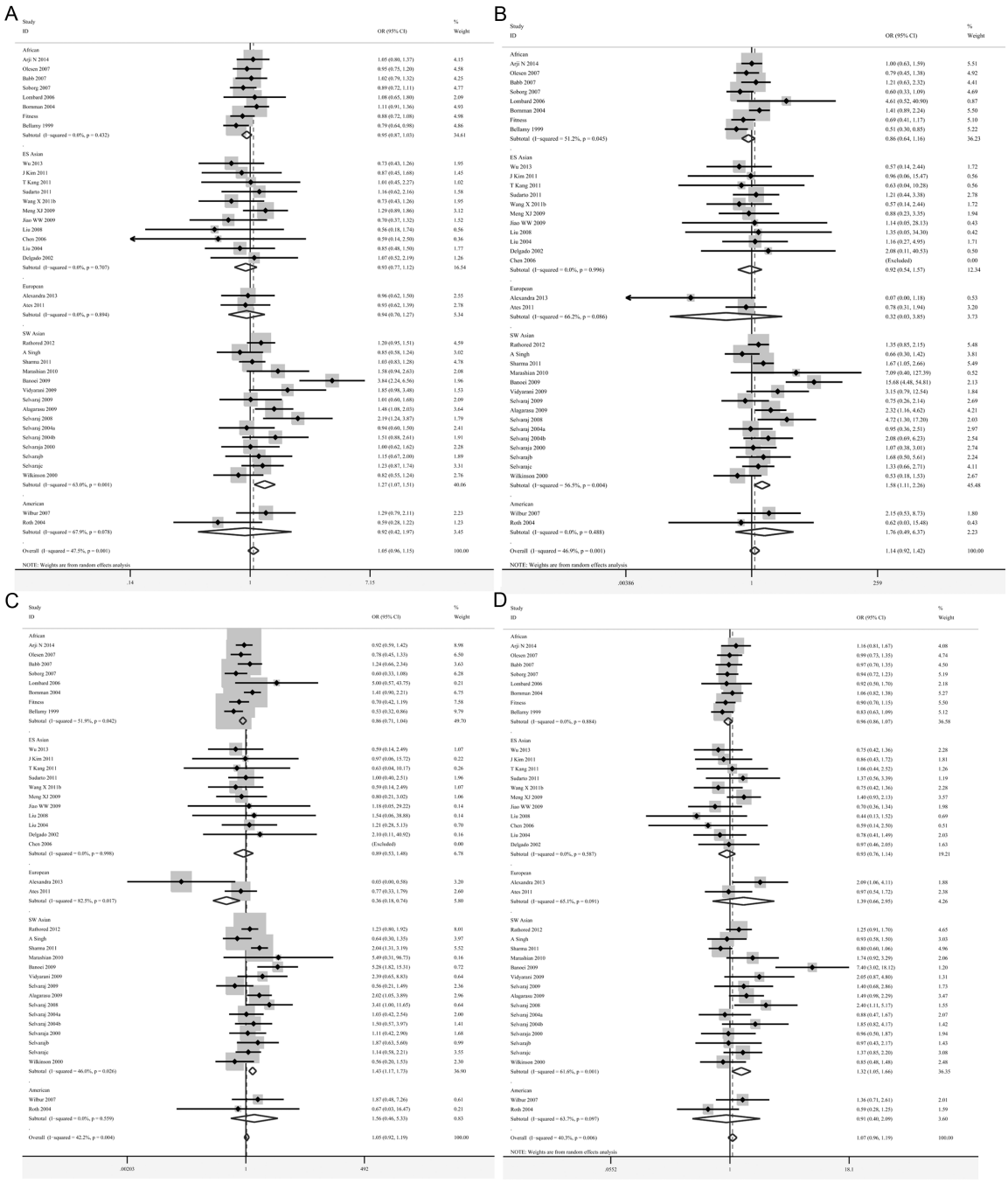

Figure 2. Forest plots showing the association of the TaqI polymorphisms with risk of tuberculosis for five ancestral subgroups. A. t vs. T; B. tt vs. TT; C. tt vs. Tt + TT; D. tt + Tt vs. TT (TIF).

TB studies, two were extra-pulmonary TB studies [48, 53] and the other six studies were pulmonary and extra-pulmonary merged studies [23, 30, 35, 36, 45, 56]. HIV status of the studied population was considered in twenty-six studies. All of them adopted blood samples for genotyping. Genotyping for Vitamin D receptor TaqI polymorphism across all studies, twenty-nine were conducted using PCR-RFLP assay [20, 21, 23, 25-38, 40, 42, 44, 46-53, 56], and the other nine studies were merged into the "other methods" group. The results of HWE test in the control population and genotype frequencies of TaqI polymorphisms were recalculated and extracted from all eligible publications, and were shown in **Table 2**. Twenty-nine of the eligible studies met the HWE (P>0.05), except for nine study [21, 23, 25, 26, 28, 29, 31, 34, 40].

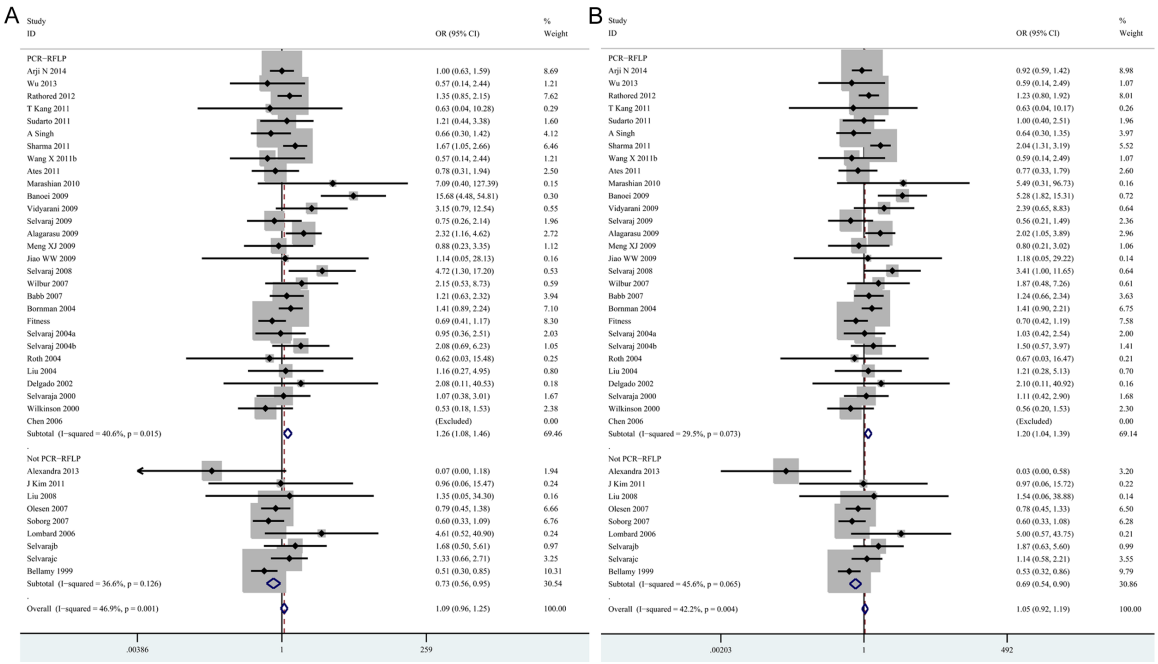

Figure 3. Forest plots showing the association of the TaqI polymorphisms with risk of tuberculosis for genotyping methods. A. tt vs. TT; B. tt vs. Tt + TT (TIF).

Quantitative data synthesis

Pooled analysis. **Table 2** shows the genotype distribution and allele frequencies in the original 38 studies. The overall frequency of t allele in TaqI polymorphism was 25.3% in cases and 23.9% in controls. This analysis, using five different genetic models showed low heterogeneity (I^2 range =37-47.5% for all comparisons). The significant association has not been detected in all the five genetic models between TaqI polymorphism and risk of tuberculosis (**Table 3**).

Subgroup analysis. Subgroup meta-analyses according to different races (Africans, East and Southeast Asians, South and West Asians, Americans and Europeans) have been conducted based on the five genetic models. 15 studies belonged to the South and West Asians [23, 27, 28, 31-35, 38, 48, 49, 53-56], 11 to the East and Southeast Asians [21, 24-26, 36, 37, 39, 44, 51, 52], 8 to the Africans [20, 41-43, 45-47, 57], 2 to the Americans [40, 50] and 2 to the Europeans [22, 30] group. Appropriate effects were used for further analysis according to the heterogeneity. We found significant positive correlations between the t allele polymorphisms and increased risks of tuberculosis in South and West Asians (t vs. T: OR=1.27, 95% CI=1.07-1.51, P=0.007; tt vs. TT: OR=1.59, 95% CI=1.11-2.26, P=0.011; tt vs. Tt + TT: OR=1.43, 95% CI=1.17-1.73, P=0.000; tt + Tt vs. TT: OR=1.32, 95% CI=1.05-1.67, P=0.019) (**Table 3**; **Figure 2A-D**). However, the Africans, East and Southeast Asians, Americans and Europeans groups showed no significant difference in all five genetic models (**Table 3**).

In a further stratified analysis by genotyping methods, significant associations were observed in the studies using PCR-RLFP for homozygote model (tt vs. TT: OR=1.26, 95% CI=1.08-1.46, P=0.004) and recessive model (tt vs. Tt + TT: OR=1.20, 95% CI=1.04-1.39, P=0.014). In contrast, decreased risks of tuberculosis was found in studies using other methods for homozygote model (tt vs. TT: OR=0.73, 95% CI=0.56-0.95, P=0.019) and recessive model (tt vs. Tt + TT: OR=0.69, 95% CI=0.54-0.90, P=0.005) (**Table 3**; **Figure 3A**, **3B**). In the stratified analysis by sample size, statistically significant associations were found in the "small" studies for allele model (t vs. T: OR=1.12, 95% CI=1.03-1.23, P= 0.010), homozygote model (tt vs. TT: OR=1.24, 95% CI=1.02-1.52, P=0.030), heterozygote model (Tt vs. TT: OR=1.18, 95% CI=1.05-1.33, P=0.007) and dominant model (tt + Tt vs. TT: OR=1.17, 95% CI=1.05-1.31, P=0.006), respectively (**Table 3**; **Figure 4A-D**). However, there was no significant

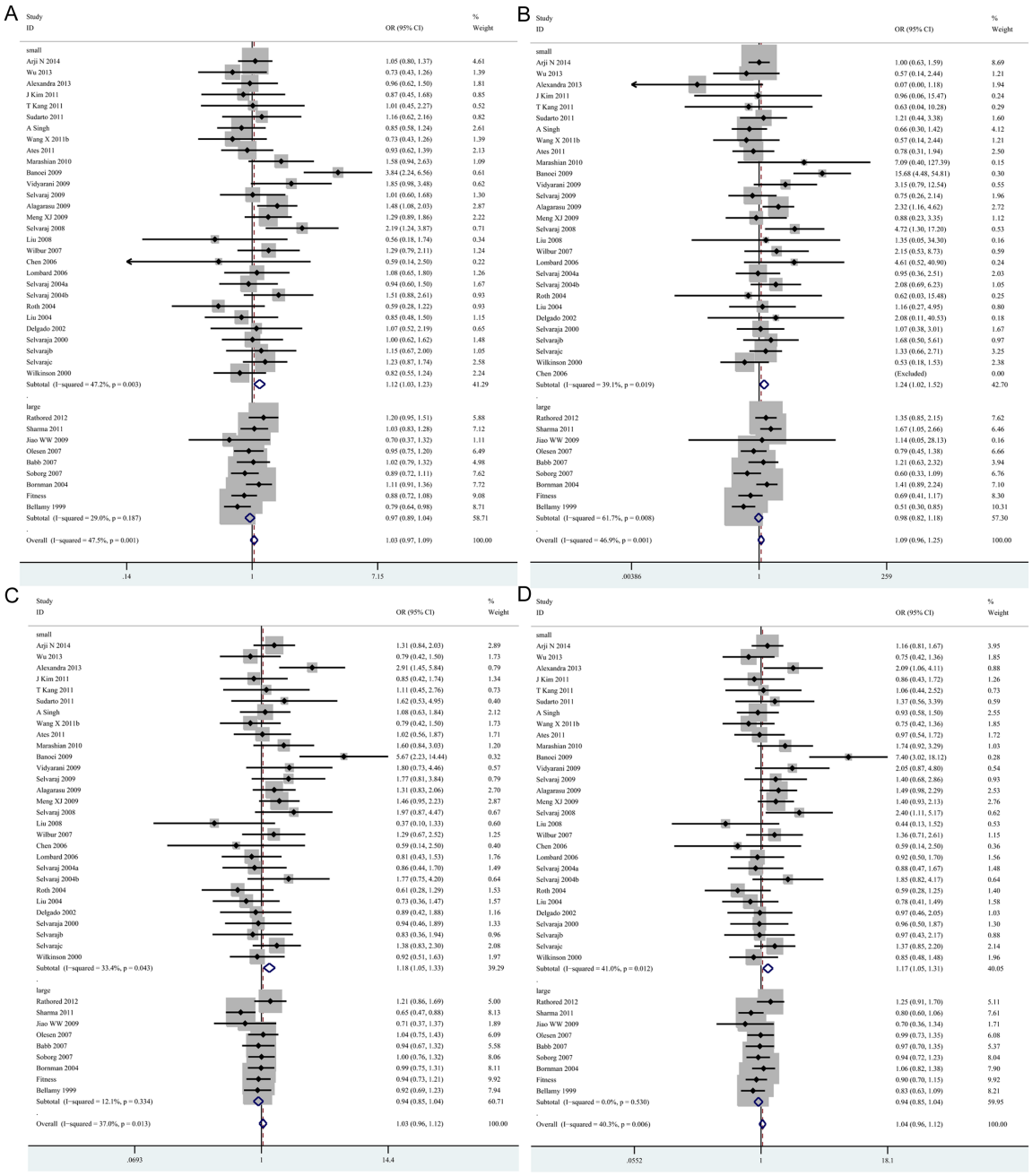

Figure 4. Forest plots showing the association of the TaqI polymorphisms with risk of tuberculosis for sample sizes. A. t vs. T; B. tt vs. TT; C. Tt vs. TT; D. tt + Tt vs. TT (TIF).

difference in "big" studies [23, 28, 37, 41-43, 46, 47, 57] for all five models. In subgroup analyses according to HWE in controls, significant associations were observed in the studies not in HWE for homozygote model (tt vs. TT: OR=1.35, 95% CI=1.02-1.78, P=0.034) and recessive model (tt vs. Tt + TT: OR=1.33, 95% CI=1.02-1.73, P=0.035) (**Table 3**; **Figure 5A**, **5B**). However, when stratified by source of control and tuberculosis type, statistical significant association was not detected in all subgroups.

Sensitivity analysis

Sensitivity analysis was conducted to evaluate the root of heterogeneity in every genetic model. The pooled OR in none of the studied genetic models affected by excluding studies

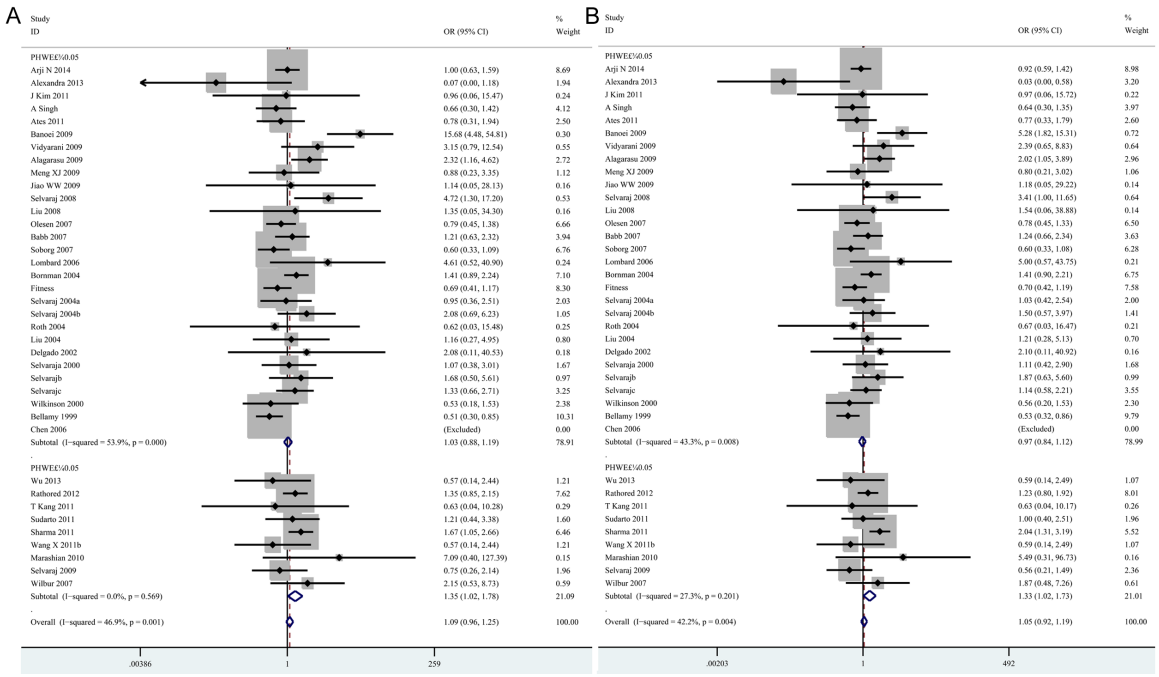

Figure 5. Forest plots showing the association of the TaqI polymorphisms with risk of tuberculosis for HWE. A. tt vs. TT; B. tt vs. Tt + TT (TIF).

one after another (**Figure 6**). This indicates that this meta-analysis is reliable in nature.

Publication bias

The Begg's funnel plot and the Egger's linear regression test were performed to evaluate the publication bias of all included studies. The funnel plots seemed symmetrical under all the five genetic models (**Figure 7A**. t vs. T: z=0.16, P=0.870; **Figure 7B**. tt vs. TT: z=0.55, P=0.583; **Figure 7C**. Tt vs. TT: z=0.49, P=0.624; **Figure 7D**. tt + Tt vs. TT: z=1.29, P=0.195; **Figure 7E**. tt vs. Tt + TT: z=0.58, P=0.565). Egger's test also suggested that there was no significant publication bias under all the genetic models (**Figure 8A**. t vs. T: t=0.85, P=0.402; **Figure 8B**. tt vs. TT: t=0.83, P=0.413; **Figure 8C**. Tt vs. TT: t=1.41, P=0.168; **Figure 8D**. tt + Tt vs. TT: t=1.58, P=0.124; **Figure 8E**. tt vs. Tt + TT: t=0.89, P=0.377)

Discussion

Extensive evidence regarding the potential association between VDR TaqI polymorphism and TB risk, however, the results from published studies remain controversial. The discrepancies may be partly attributed to differing genetic backgrounds and environment among various populations. Up till now, there were three meta-analyses investigating the correlation between VDR TaqI polymorphism and TB risk. Nevertheless, due to the limitations of relatively smaller sample size, it failed detect any genetic associations either in the overall analysis or in subgroup analyses stratified by ethnicity [13-15]. To adjust for potential confounding factors, we examined the data in this meta-analysis, adding more recently published studies, by regional stratification, sample size and genotyping methods, to evaluate the association between VDR TaqI polymorphisms and TB risk more comprehensively and rigorously. Our pooled meta-analysis found there to be no significant evidence on the association between the VDR TaqI polymorphism and human tuberculosis risk, but subgroup results suggested an ethnic-specific increased risk in genotypes carrying the minor (t) allele in the SW Asian population, whereas the tuberculosis risk was positively related to the "small" sample size and negatively related to the "others" genotyping methods.

TB is a serious public health problem in worldwide which prevention and control dependent on many factors such as early diagnosis, drug resistance, vaccine and HIV co-infection. In the

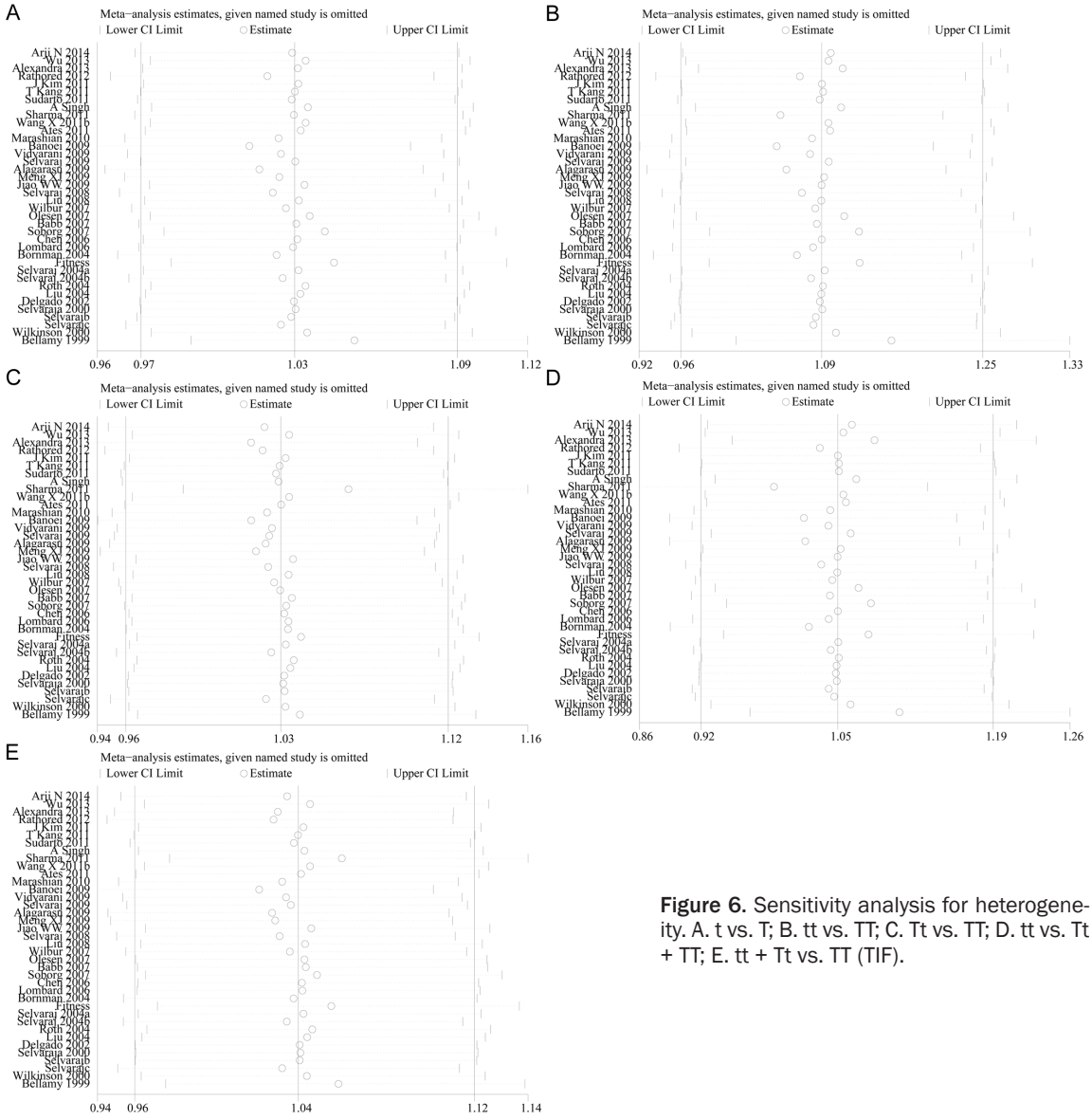

Figure 6. Sensitivity analysis for heterogeneity. A. t vs. T; B. tt vs. TT; C. Tt vs. TT; D. tt vs. Tt + TT; E. tt + Tt vs. TT (TIF).

past decades, the association between genetic factors and host susceptibility to TB has been widely studied. VDR is known as an intracellular hormone receptor. This receptor exerts immune modulatory effects in regulating cell proliferation and differentiation, lymphocyte activation and cytokine production, and is associated with TB susceptibility [4]. Underlying mechanisms have been proposed based on its function as vitamin D receptor. Vitamin D is an immunomodulator hormone that enhances macrophage phagocytosis of live *M. tuberculosis* and induces the expression of antimicrobial peptide cathelicidin which restricts the growth of *M. tuberculosis* in monocytes [7]. Vitamin D exerts its actions through vitamin D receptor (VDR), which variants may influence VDR activity and subsequent downstream vitamin D-mediated effect [12]. One of the most widely studied polymorphism in human VDR gene is TaqI, which is located in exon 9. This gene polymorphism is located within the 3' untranslated region which is known to be involved in regulation of gene expression, especially through regulation of VDR mRNA stability, thus affects the circulating 25-hydroxyvitamin D levels. Positive association between VDR TaqI polymorphisms and the tuberculosis infections has provided strong evidence for this hypothesis. However, this polymorphism might have diverse

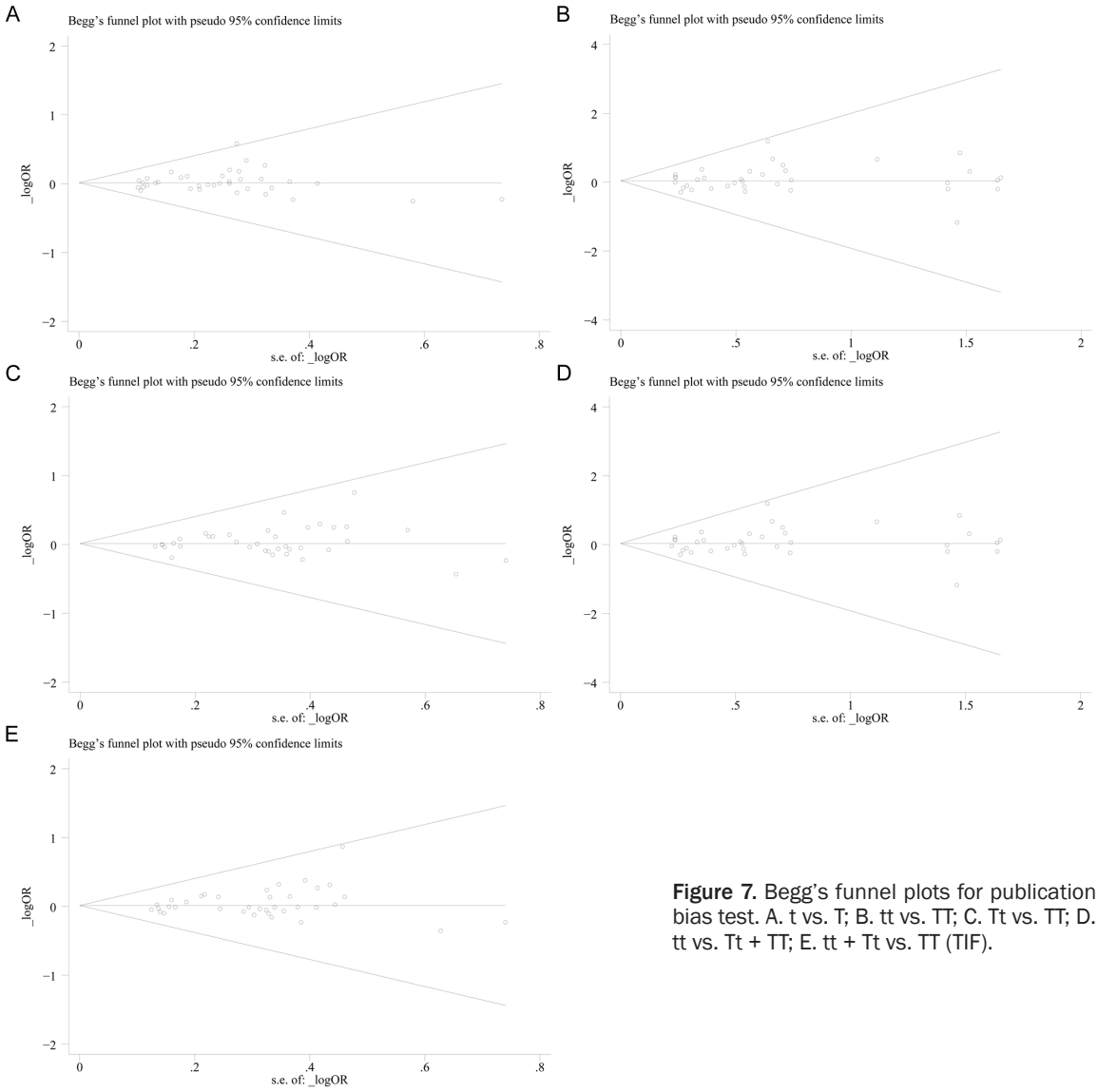

Figure 7. Begg's funnel plots for publication bias test. A. t vs. T; B. tt vs. TT; C. Tt vs. TT; D. tt vs. Tt + TT; E. tt + Tt vs. TT (TIF).

roles in different ethnic populations: significantly associations with TB were observed in SW Asian population, but not among ES Asians, Africans, Europeans and Americans. It might be owing to pertinent environmental factors which were able to influence serum vitamin D concentrations on different populations, including dietary factors, intensity and hours of sunlight. Additionally, the different genotype frequencies of VDR TaqI polymorphisms between populations may contribute to inconsistent associations with tuberculosis risk. The other stratified analyses suggested sample size might partly affect the association between VDR TaqI gene polymorphism and TB risk. There was a significantly increased TB risk of four genetic models of VDR TaqI gene polymorphism in "small" studies, but insignificant association was found in "large" studies. It was worth noting that seven out of nine of the "large" studies were based on Africans in which a trend of reduced TB risk was observed in t allele genotype. Additionally, small sample size studies tend to overestimate the influence of genetic factors [58]. In this meta-analysis, we also found an increased TB risk of two genetic models in studies with P_{HWE}<0.05. It is probable that studies without HWE in controls hint a non-random inclusion or genotyping error, which may led to misleading results. Interestingly, we observed that significant association was reversed in "other methods" studies relative to in "PCR-RFLP" studies. It may be due to high detection rate of PCR-RFLP methods. Thus, more "large" studies in

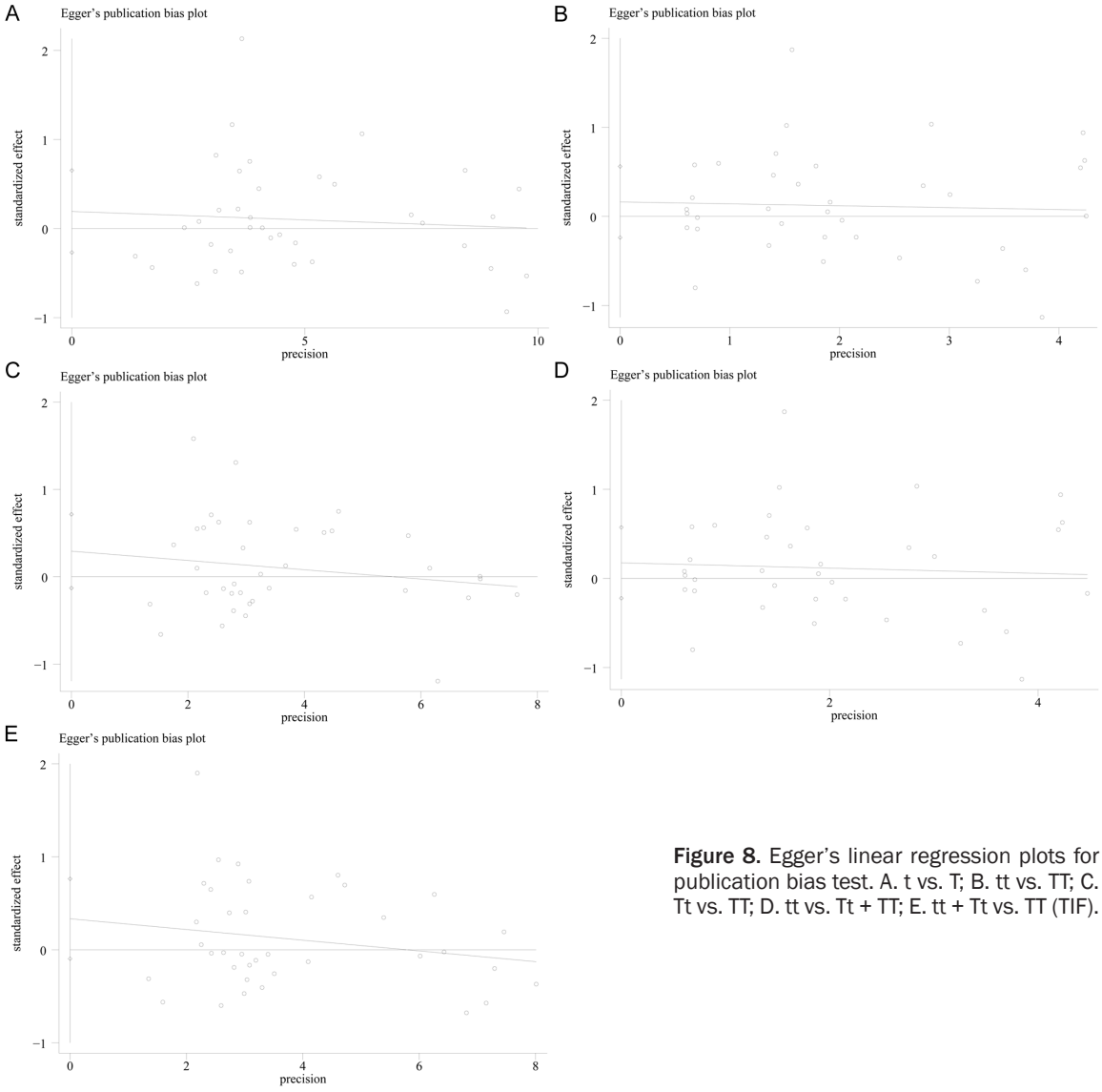

Figure 8. Egger's linear regression plots for publication bias test. A. t vs. T; B. tt vs. TT; C. Tt vs. TT; D. tt vs. Tt + TT; E. tt + Tt vs. TT (TIF).

agreement with HWE based on SW Asians which used PCR-RFLP methods are required to quantify this effect size reliably.

In the present study, the subgroup analyses suggested ethnicities, source of controls and tuberculosis type might partly explain the moderate heterogeneity between studies observed under some genetic models. Moreover, the heterogeneity was not remarkably decreased upon exclusion of the studies that deviated from the HWE (Table S2). Further meta-regression analyses were performed as well to identify potential sources of the heterogeneity (Table S1). However, we could not find the source among publication years, ethnicity, sample sizes, HWE, genotype methods and source of controls. We could not further explore the source of heterogeneity because not all necessary information could be obtained from all the studies included. However, eligible studies were conducted in 19 countries in this meta-analysis, thus the cause of heterogeneity may partly due to the environmental factors quite different in these countries which may influence VDR gene expression and modulate genotype-related risk, gene-environment interaction. Additionally, the different experimental designs, diagnosis standards, ages of participants and HIV status also may contribute to the heterogeneity.

Current systematic review has several limitations that require careful consideration. First, the interactions of environmental risk factors,

other co-variables and host cells might elucidate the mechanism by which TaqI polymorphism increase TB risk. More original data need to be obtained to interpret the gene environment interactions. Second, only articles in English or Chinese were included, which may impeded the completeness of evidence and deviate the results. Third, relevant stratifications could not be made for many studies due to incomplete information (e.g., by diagnosis standards, ages of participants or HIV status). In addition, in the subgroup analysis according to regional geography, only 2 studies concerning the relationship between the TaqI polymorphism and the Americans and Europeans were included; such a small sample size makes the analyses be prone to bias. Thus, further studies on the association of the TaqI polymorphism with TB risk are warranted to verify current findings. Fourth, some of the included studies did not mention whether their study populations were in HWE. Based on the data supplied by the articles and own calculations, significant deviations from HWE ($P<0.05$) in controls were observed for nine studies on TaqI polymorphisms. Their results should be interpreted with greater caution. We therefore repeated the meta-analyses after exclusion of these studies. However, this exclusion did not materially affect the results (Table S2).

In conclusion, results from this meta-analysis demonstrate that VDR TaqI polymorphism is associated with increased TB risk in SW Asians, while the relationship between tuberculosis risk and Americans and Europeans need to be proved in future large scale studies. 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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Disclosure of conflict of interest

None.

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Table S1. Meta-regression analysis results

		t vs. T		tt vs. TT		tt vs. Tt + TT		Tt vs. TT		tt + Tt vs. TT	
	N	95% CI	P	95% CI	P	95% CI	P	95% CI	P	OR	P
Publication years	38	-50, 82, 23.22	0.45	-87.42, 38.08	0.43	-83.56, 40.63	0.49	-52.32, 27.24	0.53	-50.82, 23.22	0.45
Ethnicities	38	-0.33, 0.58	0.58	-1.11, 0.70	0.65	-1.11, 0.70	0.65	-0.27, 0.68	0.39	-0.33, 0.58	0.58
Sample size	38	-0.05, 0.19	0.27	-0.12, 0.31	0.38	-0.13, 0.30	0.43	-0.06, 0.20	0.27	-0.05, 0.19	0.27
Genotyping method	38	-0.07, 0.12	0.60	-0.07, 0.26	0.26	-0.08, 0.25	0.29	-0.08, 0.11	0.77	-0.07, 0.12	0.60
Source of controls	38	-0.15, 0.20	0.75	-0.30, 0.40	0.77	-0.30, 0.40	0.77	-0.16, 0.20	0.81	-0.15, 0.20	0.75
HWE	38	-0.07, 0.10	0.75	-0.15, 0.17	0.89	-0.16, 0.16	0.94	-0.07, 0.11	0.67	-0.07, 0.10	0.75
The type of tuberculosis	38	-0.16, 0.28	0.59	-0.36, 0.56	0.67	-0.36, 0.56	0.67	-0.18, 0.28	0.67	-0.16, 0.28	0.59

Table S2. Sensitivity analyses of study with controls not in HWE excluded

		t vs. T		tt vs. TT		tt vs. Tt + TT		Tt vs. TT		tt + Tt vs. TT	
	N	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)	OR (95% CI)	Heterogeneity (I^2, P_Q)
Total	38	1.05 (0.94, 1.17)	54.9%; 0.000	1.14 (0.87, 1.48)	53.9%, 0.000	0.97 (0.84, 1.12)^F	43.3%, 0.008	1.05 (0.96, 1.15)^F	34.9%, 0.034	1.04 (0.95, 1.13)^F	45.1%, 0.005
Ethnicities											
ES Asians	7	0.97 (0.77, 1.22)^F	0%; 0.541	1.10 (0.49, 2.48)^F	0%, 0.997	1.09 (0.48, 2.44)^F	0%, 0.993	0.94 (0.73, 1.22)^F	23.2%, 0.252	0.96 (0.75, 1.23)^F	8.9%, 0.361
SW Asians	11	1.33 (1.04, 1.72)*	70.4%; 0.000	1.72 (1.03, 2.86)*	65.4%, 0.001	1.40 (1.08, 1.82)^{*,F}	46.2%, 0.046	1.29 (1.06, 1.56)^{*,F}	40.3%, 0.080	1.41 (1.03, 1.91)*	61.6%, 0.004
Africans	8	0.95 (0.87, 1.03)^F	0%; 0.432	0.86 (0.64, 1.16)	51.2%, 0.045	0.86 (0.64, 1.15)	51.9%, 0.042	0.98 (0.88, 1.10)^F	0%, 0.927	0.96 (0.86, 1.07)^F	0%, 0.884
Americans	1	0.59 (0.28, 1.22)	-	0.62 (0.03, 15.48)^F	-	0.67 (0.03, 16.47)^F	-	0.91 (0.43, 1.91)^F	-	0.59 (0.28, 1.25)^F	-
Europeans	2	0.94 (0.70, 1.27)^F	0%; 0.894	0.32 (0.03, 3.85)	66.2%, 0.086	0.20 (0.01, 6.66)	82.5%, 0.017	1.70 (0.61, 4.74)	79.9%, 0.026	1.39 (0.66, 2.95)	65.1%, 0.091
Sample size											
Large[a]	7	0.93 (0.85, 1.01)^F	11.8%; 0.339	0.81 (0.65, 1.01)^F	47.9%, 0.074	0.82 (0.67, 1.02)^F	49.6%, 0.064	0.96 (0.85, 1.08)^F	0%, 0.971	0.93 (0.84, 1.04)^F	0%, 0.849
Small[b]	22	1.14 (0.97, 1.33)	54.3%; 0.001	1.28 (1.04, 1.59)^{*,F}	48.7%, 0.007	1.13 (0.92, 1.38))^F	34.9%, 0.059	1.18 (1.03, 1.36)^{*,F}	42.9%, 0.018	1.19 (1.05, 1.35)^{*,F}	49.0%, 0.005
Genotyping method											
PCR-RFLP	20	1.11 (0.96, 1.29)	62.4%; 0.000	1.30 (0.95, 1.79)	53.1%, 0.003	1.15 (0.96, 1.37)^F	31.7%, 0.092	1.06 (0.95, 1.19)^F	34.3%, 0.067	1.11 (0.94, 1.32)	52.3%, 0.003
Other methods	9	0.92 (0.83, 1.03)^F	0%; 0.594	0.73 (0.56, 0.95)^{*,F}	36.6%, 0.126	0.69 (0.54, 0.90)^{*,F}	45.6%, 0.065	1.03 (0.89, 1.19)^F	42.8%, 0.082	0.97 (0.85, 1.12)^F	19.4%, 0.270
Source of controls											
Contacts[c]	10	1.03 (0.90, 1.18)^F	0.0%; 0.674	1.15 (0.83, 1.60)^F	0%, 0.927	1.09 (0.79, 1.49)^F	0%, 0.915	1.01 (0.85, 1.21)^F	0%, 0.591	1.03 (0.86, 1.22)^F	0%, 0.620
Healthy[d]	19	1.08 (0.93, 1.25)	67.5%; 0.000	1.20 (0.84, 1.73)	67.4%, 0.000	1.07 (0.79, 1.46)	59%, 0.001	1.06 (0.96, 1.17)^F	49.2%, 0.008	1.11 (0.94, 1.32)	58.9%, 0.001
Tuberculosis type											
pulmonary	21	1.00 (0.93, 1.07)	62.4%; 0.000	1.16 (0.83, 1.60)	61.5%, 0.000	1.05 (0.79, 1.38)	51.4%, 0.004	1.04 (0.94, 1.15)^F	47.7%, 0.008	1.08 (0.92, 1.27)	55.2%, 0.001
Extra and pulmonary	6	1.13 (0.95, 1.33)^F	30%; 0.21	1.29 (0.84, 1.96)^F	39.6%, 0.141	1.22 (0.81, 1.82)^F	35.7%, 0.169	1.12 (0.90, 1.39)^F	0%, 0.545	1.14 (0.93, 1.41)^F	0.9%, 0.410
extra	2	0.97 (0.70, 1.36)^F	0%; 0.855	1.00 (0.49, 2.04)^F	0%, 0.876	1.06 (0.55, 2.06)^F	0%, 0.915	0.90 (0.55, 1.46)^F	0%, 0.873	0.92 (0.58, 1.46)^F	0%, 0.855

Abbreviations: N: number of studies included; OR: odds ratio; Ph: *p* value for heterogeneity; P_Q: Cochran's Q statistics; I^2: Higgin's I^2 statistics. F: Results derived using Fixed effects for analysis. Random effects were used for all other calculations. *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 person.