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Association of Vitamin D Receptor genepolymorphism with the risk of Type 2 diabetes mellitus: an update meta-analysis

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Abstract:

Backgroud and aims: Two well-known polymorphisms (*Bsm*I and *Fok*I) in Vitamin D receptor (*VDR*) have been implicated in susceptibility of type 2 diabetes mellitus (T2DM), but the results to date have been inconclusive. The aim of this study was to quantitatively summarize the association between polymorphisms of *Bsm*I and *Fok*I in *VDR* gene and T2DM risk by an update meta-analysis.

Methods: Statistical analysis was performed using the software STATA 12. 0. A total of 30 case-control studies in 23 published articles were included.

Results: Overall, marginal significant associations between *Bsm*I polymorphism and T2DM risk were found for Bb *vs.* bb (*OR*=1.36, 95%*CI*: 1.02-1.83, *P*=0.038) and BB+Bb *vs.* bb (*OR*=1.36, 95%*CI*: 1.00-1.84, *P*=0.049). And significant increasedassociations of *Fok*I polymorphism with T2DM were detected for three genetic models (ff *vs.* FF: *OR*=1.57, 95%*CI*: 1.28-1.93, *P*<0.0001; Ff *vs.* FF: *OR*=1.54, 95%*CI*: 1.31-1.81, *P*<0.0001; ff+Ff *vs.* FF: *OR*=1.57, 95%*CI*: 1.35-1.83, *P*<0.0001, respectively). Subgroup analysis shown that significantly increased susceptibility of T2DM were only found for *VDRB*smI polymorphism among the studies with small sample size (<200) in 2 genetic models (Bb *vs.* bb: *OR*=2.12, 95%*CI*: 1.24-3.62, *P*=0.006; BB+ Bb *vs.* bb: *OR*=2.38, 95%*CI*: 1.33-4.25, *P*=0.003, respectively). Interestingly, a significantly increased susceptibility was only found in T2DM patients among Chinese for all genetic models (ff *vs.* FF: *OR*=1.78, 95%*CI*: 1.40-2.27, *P*<0.0001; Ff *vs.* FF: *OR*=1.66, 95%*CI*: 1.38-1.99, *P*<0.0001; ff+Ff *vs.* FF: *OR*=1.70, 95%*CI*: 1.43-2.02, *P*<0.0001; ff *vs.* FF+Ff: *OR*=1.27, 95%*CI*: 1.03-1.57, *P*<0.0001, respectively). In contrast, no significant association was observed among Caucasians in three studies (*P*>0.05).

Conclusions: Ethnicity and sample size might be the possible factors of the heterogeneity. This meta-analysis suggests that the *Fok*I polymorphism of the *VDR* gene could bea risk factor for T2DM, especially in Chinese population. Further studies are needed to confirm our results.

Keywords: Vitamin D receptor; type 2 Diabetes mellitus; Genetic polymorphism; Association; Meta analysis.

Introduction

Diabetes mellitus (DM) is a metabolic disorder of multiple etiologies caused by the defects in insulin secretion and insulin action, characterized by ahigh sugar (glucose) level in blood and urine. Diabetes mellitus, especially type 2 diabetes mellitus (T2DM) is becoming one of the most prevalent endocrine diseases. It is reported that more than 300 million cases worldwide suffered from type 2 diabetes mellitus[1]. Over time, T2DM can induce many chronic complications such as retinopathy, renal failure, diabetes foot, and nerve damage[1,2]. Furthermore, T2DM has a major effect on accelerating cardiovascular disease[3] and eventually lead to death.

Recently, Many investigations indicate the genetic predispositions also play crucial roles in the development mechanisms of T2DM[2]. Many potential genes have been find to be associated with T2DM, including TCF7L2[4], CD36[5], WFS1, CDKN2A/B, KCNJ11, CDC123/CAMK1D, JAZF1, SLC30A8, FTO, CDKAL1, HHEX/IDE[6]. Vitamin D is famous for its effect on maintaining healthy bones and regulating serum calcium/phosphate homeostasis. Epidemiological studies have showed that vitamin D deficiency is widespread in the diabetes patients[7], those taking vitamin D supplementation in early childhood will have a lower risk of type 2 diabetes in adulthood[8]. The activated form of vitamin D, 1, 25-$(OH)_2D_3$, can enhances the b-cell function, protects the b-cell from detrimental immune attacks, improves insulin sensitivity and amends insulin resistance. Exerts these functions, 1, 25-$(OH)_2D_3$ must be banded with the intracellular vitamin D receptor (*VDR*), which belongs to the superfamily of steroid/thyroid hormone receptor. Therefore, the *VDR* gene is involved in the pathogenesis and progression of T2DM and may be a good candidate gene for the T2MD.

The *VDR* gene is located on human chromosome 12q12-q14, whose frequent polymorphisms have been reported to be associated with a variety of physiological and pathological phenotypes in many populations. At present, two common single nucleotide polymorphisms (SNPs) have been most often investigated may

be associated with T2DM in different ethnic populations, which are *Fok*I (rs10735810, in exon 2) and *Bsm*I (rs1544410, in intron 8), respectively. But the results of these studies have not been consistent with each other. Hence, considering an isolated study may not have enough statistical power to ascertain the association between *VDR* polymorphisms and T2DM, two meta-analyses have been performed by WangQ.[9] and Li L.[10] to detect the association between allelic variants of *VDR* polymorphisms and T2DM. However, the terminology used in different papers describing the genotype of *VDR* is confusing because of the different SNP analysis methods. Some utilized the bases directly to describe the different allele, such as G or C is for the presence of *Bsm*I restriction enzyme sites, while T or A is for the absence of *Bsm*I restriction enzyme sites; T or A is for the presence of FokI restriction enzyme site, while G or C is for the absence of *Fok*I restriction enzyme site. The most researcherswere used to applying the initial letter of the restriction enzyme to designate the different alleles; someone defined the presence of the restriction enzyme site by a lowercase letter and the acapital letter for its absence[11], while others are quite opposite[12]. Misnomers will lead to misunderstanding and erroneous interpretation of data. Therefore, it needs urgently a unified expression of the allelic gene before a meta-analysis. In this paper, we selected the two most controversial *VDR* gene loci, namely *Bsm*I and *Fok*I sites, using the initial letter of the restriction enzyme to name the different alleles, the capital letter for absence of the restriction enzyme site, whereas a lower-case letter indicates its presence. Then, the data were gathered correctly from the different studies, and an updated, strict and more comprehensive meta-analysis was carried out to evaluate the association between type 2 diabetes susceptibility and the two above-mentioned polymorphisms in *VDR*.

Materials and methods

Literature and search strategy

All the originalliteraturesfrom 1999 to July 2013 on the association of *VDR* and T2DM were identified through computer-based searches from the followingdatabases, Pubmed, ISI Web of Science, CNKI (China NationalKnowledge Infrastructure), Chinese Wanfang data, and Chinese Biomedical Literature Database. The searching keywords were as follows: vitamin D receptor, *VDR*, *Fok*I, *Bsm*I, type 2 diabetes mellitus, T2DM, NIDDM, polymorphism, genotype, as well as theircombinations. Besides, the references of the original literatures and the related articles were also searched for potential complements.

Inclusion criteria and exclusion criterion

To make the accuracy of the analysis results, the studies involved in this meta-analysis should meet the following criteria: (1) original article about the association of *VDR* polymorphisms (*Fok*I and *Bsm*I) with T2DM risk, (2) case-control study, (3) performed in a human population, (4) sufficient data for estimating an odds ratio (*OR*) with 95% confidence interval (*CI*). Accordingly, the following exclusion criteria were also used: (1) no healthy population as control subjects, (2) abstracts, reviews, and repeated publications, (3) no exact genotype frequency, (4) apart from T2DM, the cases alsosuffered from other diseases (such as cardiovasculardiseases, osteoporosis, psoriasis and so on.).

Data extraction

In meta-analysis, the date extraction of genotype distribution in cases and controls was

very crucial, which is the bases of the statistical analysis. In our study, each study was checked and assessed by two independent authors (Fei Yu and Lingling Cui) usingthe method reported by Xu et al[13], and reached conformity on all items through consultation. According to the recommendations of the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines[13] and other relevant meta-analytic papers, the following information was extracted from the selected literatures: year of publication, name of first author, region/country where the study was performed, ethnicity and region of population, sex ratios, mean age or range of subjects, as well as genotype distribution, source of controls; genotype distribution in cases and controls; genotyping methods and diagnostic criteria. If the same study data was used by more than one publication, the data were only collected from a largest sample size or a more authoritative scientific magazine.

Statistical analysis

The combined odds ratios (*ORs*) together with their corresponding 95% confidence intervals (95% *CIs*) were used to calculate and assess the strength of association between the polymorphism of *VDR* gene and T2DM risk for two polymorphism. For the polymorphism *Fok*I, the codominant model (ff *vs*. FF, Ff *vs*. FF), the dominant model (ff+Ff *vs*. FF) and the recessive model (ff *vs*. FF+Ff) were estimated, respectively. Similarly, for the polymorphism BsmI, the codominant model (BB *vs*. bb, Bb *vs*. bb), the dominant model (BB+Bb *vs*. bb) and the recessive model (BB *vs*. Bb+bb) were estimated, respectively. In searching for possible factors that might have impacted the results, we performed further analysis by meta-regression and subgroup analysis by ethnicity, sample size (the sum of the case and control), match (by age, gender, region and ethnicity) and HWE.

Heterogeneity assumption was examined by the chi-square based on *Q*-test. The pooled *OR* estimation of each study was calculated with a random-effect model using the DerSimonian and Laird method when $P<0.10$, otherwise with a fixed-effect model using the Mantel-Haenszel method[14]. Publication bias was evaluated through the Begg's test, the Egger's Asymmetry test, and visual inspection of funnel plots, in which the standard error was plotted against the Log (*OR*) to form a simple scatterplot. The distribution of genotypes in controls of each individual population was tested for a departure from Hardy Weinberg equilibrium (HWE) by using online software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The sensitive analysis was performedby omitting one study at a time to assess the stability of the meta-analysis results. If the pooled *OR* was not changed meant the result would be stability.

The statistical analyses were performed using STATA version 12.0 (Stata Corporation, College Station, TX). All the P values were for a two-sided test and $P<0.05$ was considered as statistically significant.

Results

Characteristics of the eligible studies

Apart from the completely irrelevant, a total of 292 publications were chosen from the five electronic databases. Then, 234 reports were denied for not the association of *VDR* polymorphisms and type 2 diabetes mellitus by screening the titles and reading the abstracts. Examined the full-text of the left 52 potential articles, 3 duplicated results[15-17], 10 reviews or comments and 6 articles studied on *Apa*I and *Taq*I were excluded. In the left 33 articles, 3 articles were excluded for non-healthy control

group[18-20], and another 7 articles were excluded because of the cases complicatedby other diseases. Therefore, only 23 eligible articles (8 written in English and 15 written in Chinese) involving 30 independent case-control studies qualified for this meta-analysis on the association between *VDR* polymorphism (*Bsm*I and *Fok*I) and T2DM risk. The detailed steps of our literature search are shown in Figure1.

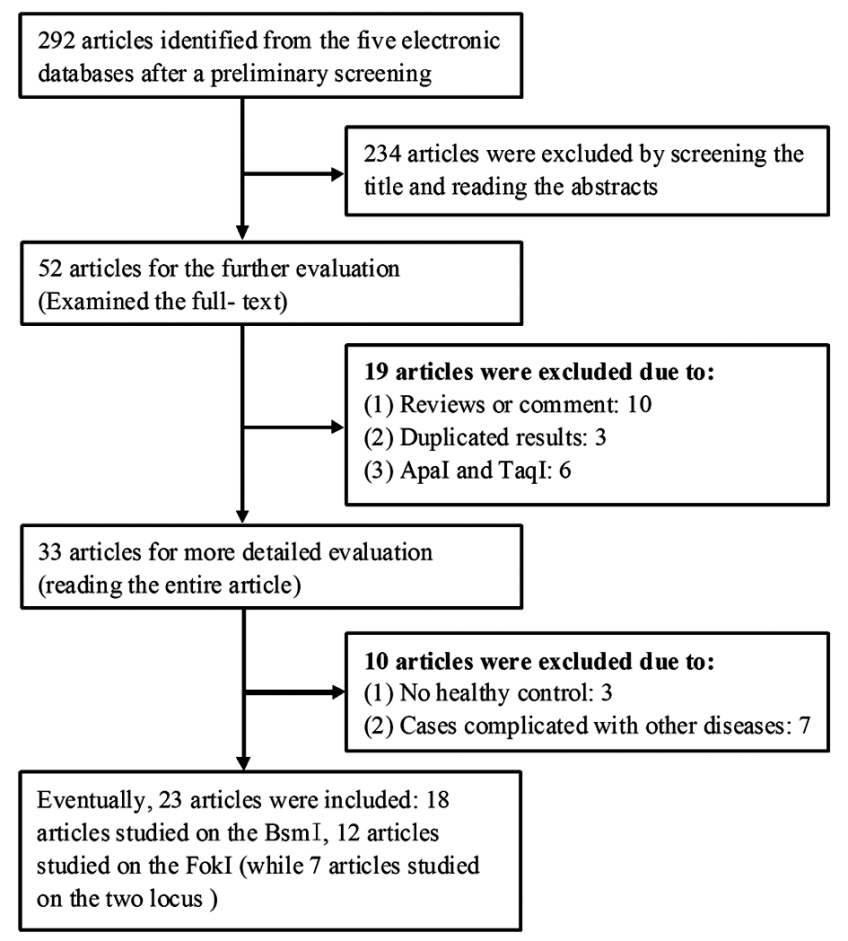

Fig. 1 A flow diagram for selection of studies and specific reasons for exclusion in this meta-analysis.

There are 18 articles studied on the BsmI polymorphisms including 2757 cases and 3517 controls, and 12 articles studied on the FokI polymorphisms including 2218 cases and 1859 controls. The detailed characteristic and genotype allele distributions for each case-control study is listed in Table 1-2, including first author, published year, reference, original country, ethnicity, gender, age, genotype distribution and HWE test of controls.

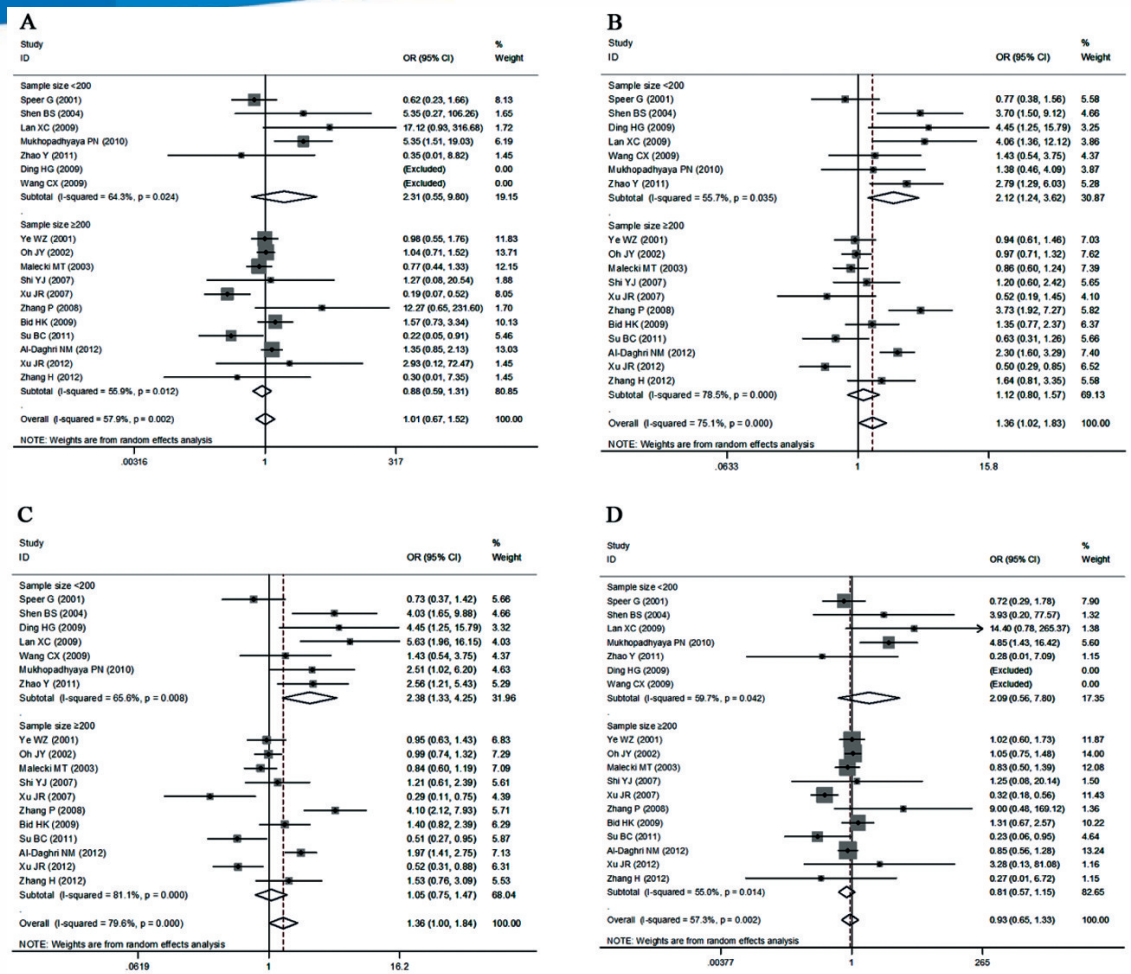

Fig. 2 Forest plots for the overall association between *VDR Bsm*I polymorphism and T2DM risk.
A. BB *vs.* bb; B. Bb *vs.* bb; C. BB *vs.* Bb+bb. D. BB+Bb *vs.* bb

Table 1 Main characteristic of included studies about *Bsm*I genotype polymorphism in the meta-analysis

First author/Year(Refference)	Country	Ethnicity	Gender(Female/male)		Age		Genotype distribution(case/control)			HWE(*P*)*
			Case	Control	Case	Control	bb	Bb	BB	
Speer G,2001[21]	Hungary	Caucasian	27/22	66/72			20/46	22/66	7/26	0.7874
Ye WZ,2001[22]	France	Caucasian	130/179	72/71	62±12	61±16	119/54	135/65	52/24	0.5574
Oh JY,2002[23_ENREF_30]	USA	Caucasian	NR	NR	71.7±8.6	68.8±9.26	86/460	107/590	49/253	0.0104
Malecki MT,2003[24_ENREF_31]	Poland	Caucasian	165/143	142/98	59.8±9.2	54.0±15.1	131/92	142/116	35/32	0.6298
Shen BS,2004[25]	China	Chinese	40/56	21/31	65.6±10.5	36.0±4.9	59/45	34/7	3/0	0.6028
Shi YJ,2007[26]	China	Chinese	65/92	67/129	58±11	NR	139/177	17/18	1/1	0.4699
Xu JR,2007[27]	China	Chinese	61/45	60/42	62±11	58±10	19/6	46/28	41/68	0.1917
Zhang P,2008[28_ENREF_35]	China	Chinese	50/66	51/61	55.6±10.5	56.0±8.9	71/97	41/15	4/0	0.4475
Bid HK,2009[19]	India	Caucasian	NR	NR	49.32±10.97	NR	30/60	52/77	18/23	0.8309
Ding HG,2009[29]	China	Chinese	15/17	14/16	42±8	40±6	19/26	13/4	0/0	0.6956
Lan XC,2009[30]	China	Chinese	34/32	41/39	53.0±14.1	51.3±13.3	48/75	13/5	5/0	0.7729
Wang CX,2009[31]	China	Caucasian	40/24	83/38	48.69±8.54	48.02±8.56	56/110	8/11	0/0	0.6004
Mukhopadhyaya PN,2010[32]	India	Caucasian	21/19	21/19	47.25±12.24	42.45±12.14	17/26	9/10	14/4	0.0733
Su BC,2011[33]	China	Chinese	129/159	63/76	53.75±11.94	54.02±11.62	264/118	21/15	3/6	<0.0001
Zhao Y,2011[34_ENREF_41]	China	Chinese	45/51	40/43	55.7±11.4	55.7±11.7	67/71	29/11	0/1	0.4553
Al-Daghri NM,2012[35_ENREF_42]	Saudi Arabia	Caucasian	NR	NR	51.5±8.6	44.1±9.9	105/114	201/95	62/50	0.0004
Xu JR,2012[36]	China	Chinese	77/124	131/88	NR	NR	176/172	24/47	1/0	0.0753
Zhang H,2012[18]	China	Chinese	54/68	47/53	57.0±10.8	55.3±8.8	96/85	26/14	0/1	0.6247

注：* *P* value for HardyeWeinberg equilibrium in control group; NR: not reported

Table 1 Main characteristic of included studies about *Bsm*I genotype polymorphism in the meta-analysis

First author/Year(Refference)	Country	Ethnicity	Gender(Female/male)		Age		Genotype distribution(case/control)			HWE(*P*)*
			Case	Control	Case	Control	bb	Bb	BB	
Speer G,2001[21]	Hungary	Caucasian	27/22	66/72			20/46	22/66	7/26	0.7874
Ye WZ,2001[22]	France	Caucasian	130/179	72/71	62±12	61±16	119/54	135/65	52/24	0.5574
Oh JY,2002[23_ENREF_30]	USA	Caucasian	NR	NR	71.7±8.6	68.8±9.26	86/460	107/590	49/253	0.0104
Malecki MT,2003[24_ENREF_31]	Poland	Caucasian	165/143	142/98	59.8±9.2	54.0±15.1	131/92	142/116	35/32	0.6298
Shen BS,2004[25]	China	Chinese	40/56	21/31	65.6±10.5	36.0±4.9	59/45	34/7	3/0	0.6028
Shi YJ,2007[26]	China	Chinese	65/92	67/129	58±11	NR	139/177	17/18	1/1	0.4699
Xu JR,2007[27]	China	Chinese	61/45	60/42	62±11	58±10	19/6	46/28	41/68	0.1917
Zhang P,2008[28_ENREF_35]	China	Chinese	50/66	51/61	55.6±10.5	56.0±8.9	71/97	41/15	4/0	0.4475
Bid HK,2009[19]	India	Caucasian	NR	NR	49.32±10.97	NR	30/60	52/77	18/23	0.8309
Ding HG,2009[29]	China	Chinese	15/17	14/16	42±8	40±6	19/26	13/4	0/0	0.6956
Lan XC,2009[30]	China	Chinese	34/32	41/39	53.0±14.1	51.3±13.3	48/75	13/5	5/0	0.7729
Wang CX,2009[31]	China	Caucasian	40/24	83/38	48.69±8.54	48.02±8.56	56/110	8/11	0/0	0.6004
Mukhopadhyaya PN,2010[32]	India	Caucasian	21/19	21/19	47.25±12.24	42.45±12.14	17/26	9/10	14/4	0.0733
Su BC,2011[33]	China	Chinese	129/159	63/76	53.75±11.94	54.02±11.62	264/118	21/15	3/6	<0.0001
Zhao Y,2011[34_ENREF_41]	China	Chinese	45/51	40/43	55.7±11.4	55.7±11.7	67/71	29/11	0/1	0.4553
Al-Daghri NM,2012[35_ENREF_42]	Saudi Arabia	Caucasian	NR	NR	51.5±8.6	44.1±9.9	105/114	201/95	62/50	0.0004
Xu JR,2012[36]	China	Chinese	77/124	131/88	NR	NR	176/172	24/47	1/0	0.0753
Zhang H,2012[18]	China	Chinese	54/68	47/53	57.0±10.8	55.3±8.8	96/85	26/14	0/1	0.6247

注：* *P* value for HardyeWeinberg equilibrium in control group；NR：not reported

Overall and subgroup meta-analysis results

*Bsm*I

Overall, marginal significant associations with T2DM risk were found for Bb *vs*. bb (*OR*=1.36, 95%*CI*: 1.02-1.83, *P*=0.038) and BB+Bb *vs*. bb (*OR*=1.36, 95%*CI*: 1.00-1.84, *P*=0.049). Whereas no significant associations were observed for BB *vs*. bb (*OR*=1.01, 95%*CI*: 0.67-1.52, *P*=0.956) and BB *vs*. Bb+bb (*OR*=0.93, 95%*CI*: 0.65-1.33, *P*=0.692) (Table 3). With significant heterogeneity between studies for all contrast model (*P*<0.05), a random-effect model was used (Supplementary). In searching for the source of heterogeneity, meta-regression was performed using the possible factors, such as ethnicity, sample size, match and HWE of the included studies. The results of meta-regression revealed that ethnicity and sample size might be the possible factors of the heterogeneity (*P*<0.05, Table 3). Thus, a further analysis was performed on data stratified by ethnicity and sample size, in which Indian was classified as Caucasians. Significantly increased susceptibility of T2DM wereonly found for *VDR Bsm*I polymorphism among the studies with small sample size (<200) in 2 genetic models (Bb *vs*. bb: *OR*=2.12, 95%*CI*: 1.24-3.62, *P*=0.006; BB+ Bb *vs*. bb: *OR*=2.38, 95%*CI*: 1.33-4.25, *P*=0.003, respectively). However, we failed to detect any association in the subgroup analysis by ethnicity in all genetic models (Figure 2, Table 4).

Table 3 Results of Meta-regression (*P* value)

Locus	Models	Ethnicity	Sample size	Match	HWE
*Bsm*I	BB *vs* bb	0.036	0.511	0.993	0.897
	Bb *vs* bb	0.229	0.055	0.387	0.454
	BB+Bb *vs* bb	0.285	0.031	0.371	0.258
	BB *vs* Bb+bb	0.013	0.306	0.850	0.689
*Fok*I	ff *vs*. FF	0.003	0.071	0.547	0.304
	Ff *vs* FF	0.003	0.412	0.703	0.241
	ff+Ff *vs* FF	0.003	0.232	0.575	0.273
	ff *vs*. FF +Ff	0.169	0.066	0.746	0.033

Table 4 Results of meta-analysis for *VDR BsmI* and *FokI* polymorphism and T2DM

Locus	Groups	Case/control	Codominant model				Dominant model		Recessive model	
			BB *vs.* bb		Bb *vs.* bb		BB+Bb *vs.* bb		BB *vs.* Bb+bb	
			P	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)
BsmI	Total	2757/3517	0.956	1.01(0.67,1.52)	0.038	1.36(1.02,1.83)	0.049	1.36(1.00,1.84)	0.692	0.93(0.65,1.33)
	Ethnicity									
	Chinese	1413/2283	0.940	1.05(0.30,3.65)	0.068	1.62(0.97,2.72)	0.130	1.57(0.88,2.80)	0.881	0.92(0.32,2.66)
	Caucasians	1344/1234	0.447	1.13(0.83,1.54)	0.414	1.15(0.83,1.59)	0.309	1.17(0.86,1.89)	0.874	1.02(0.79,1.32)
	Sample size									
	<200	443/544	0.256	2.31(0.55,9.80)	0.006	2.12(1.24,3.62)	0.003	2.38(1.33,4.25)	0.272	2.09(0.56,7.80)
	≥200	2314/2973	0.527	0.88(0.59,1.31)	0.498	1.12(0.80,1.57)	0.786	1.05(0.75,1.47)	0.234	0.81(0.57,1.15)
			ff *vs.* FF		Ff *vs.* FF		ff+Ff *vs.* FF		ff *vs.* FF+Ff	
			P	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)	*P*	*OR*(95%*CI*)
FokI	Total	2218/1859	<0.0001	1.57(1.28,1.93)	<0.0001	1.54(1.31,1.81)	<0.0001	1.57(1.35,1.83)	0.055	1.16(1.00,1.36)
	Ethnicity									
	Chinese	1446/1201	<0.0001	1.78(1.40,2.27)	<0.0001	1.66(1.38,1.99)	<0.0001	1.70(1.43,2.02)	0.025	1.27(1.03,1.57)
	Caucasians	772/658	0.440	1.16(0.79,1.70)	0.281	1.20(0.86,1.68)	0.229	1.21(0.89,1.66)	0.682	1.05(0.84,1.31)
	Sample size									
	<200	425/404	0.001	2.16(1.40,3.33)	<0.0001	1.53(1.31,1.81)	<0.0001	1.68(1.25,2.24)	0.007	1.74(1.16,2.60)
	≥200	1793/1455	0.002	1.44(1.14,1.81)	<0.0001	1.55(1.28,1.86)	<0.0001	1.53(1.28,1.83)	0.354	1.08(0.92,1.28)

FokI

Overall, significant increased associations with T2DM were detected for three genetic models (ff *vs.* FF: *OR* = 1.57, 95% *CI*: 1.28 - 1.93, *P* < 0.0001; Ff *vs.* FF: *OR* = 1.54, 95%*CI*: 1.31 - 1.81, *P* < 0.0001; ff + Ff *vs.* FF: *OR* = 1.57, 95% *CI*: 1.35 - 1.83, *P* < 0.0001, respectively), andmarginal significant association were found for ff *vs.* FF+Ff (*OR* = 1.16, 95%*CI*: 1.00-1.36, *P* = 0.055) (Table 4). Since there was no indication of heterogeneity among the included studies for all contrast model (*P* > 0.05), a fixed-effect model was used (Supplementary). Further analysis by meta-regression revealed that ethnicity and sample size might affect the overall results (Table 3). Interestingly, a significantly increased susceptibility was only found in T2DM patients among Chinese for all genetic models (ff *vs.* FF: *OR* = 1.78, 95% *CI*: 1.40 - 2.27, *P* < 0.0001; Ff *vs.* FF: *OR* = 1.66, 95% *CI*: 1.38-1.99, *P*<0.0001; ff+Ff *vs.* FF: *OR* = 1.70, 95% *CI*: 1.43 - 2.02, *P* < 0.0001; ff *vs.* FF+Ff: *OR* = 1.27, 95%*CI*: 1.03-1.57, *P* < 0.0001, respectively). In contrast, no significant association was observed among Caucasians in three studies (*P*>0.05). Subgroup analysis by sample size found significantly increased susceptibility of T2DM for ff *vs.* FF, Ff *vs.* FF and ff+Ff *vs.* FF (*P*<0.01) both in the studies with small and large sample size. However, significantly increased risk was only observed in the studies with small sample size for ff *vs.* FF+Ff (*OR* = 1.74, 95%*CI*: 1.16-2.60, *P* = 0.007), and no significant association was detected in the studies with large sample size for ff *vs.* FF+Ff (*OR* = 1.08, 95%*CI*: 0.92-1.28, *P* = 0.354) (Figure 3, Table 4).

Sensitivity analyses and publication bias

Sensitivity analyses were conducted to assess whether each individual study affected the final results. The sensitivity analyses did not detect any individual study affected the results in all subjects using the exclusion method step by step (data not shown). Neither the Begg's test nor Egger's test provided any obvious evidence of publication bias (Table 5, *P* ≥ 0.05). The shapes of the funnel plots appeared to be symmetrical in all genetic models (Figure. 4, 5).

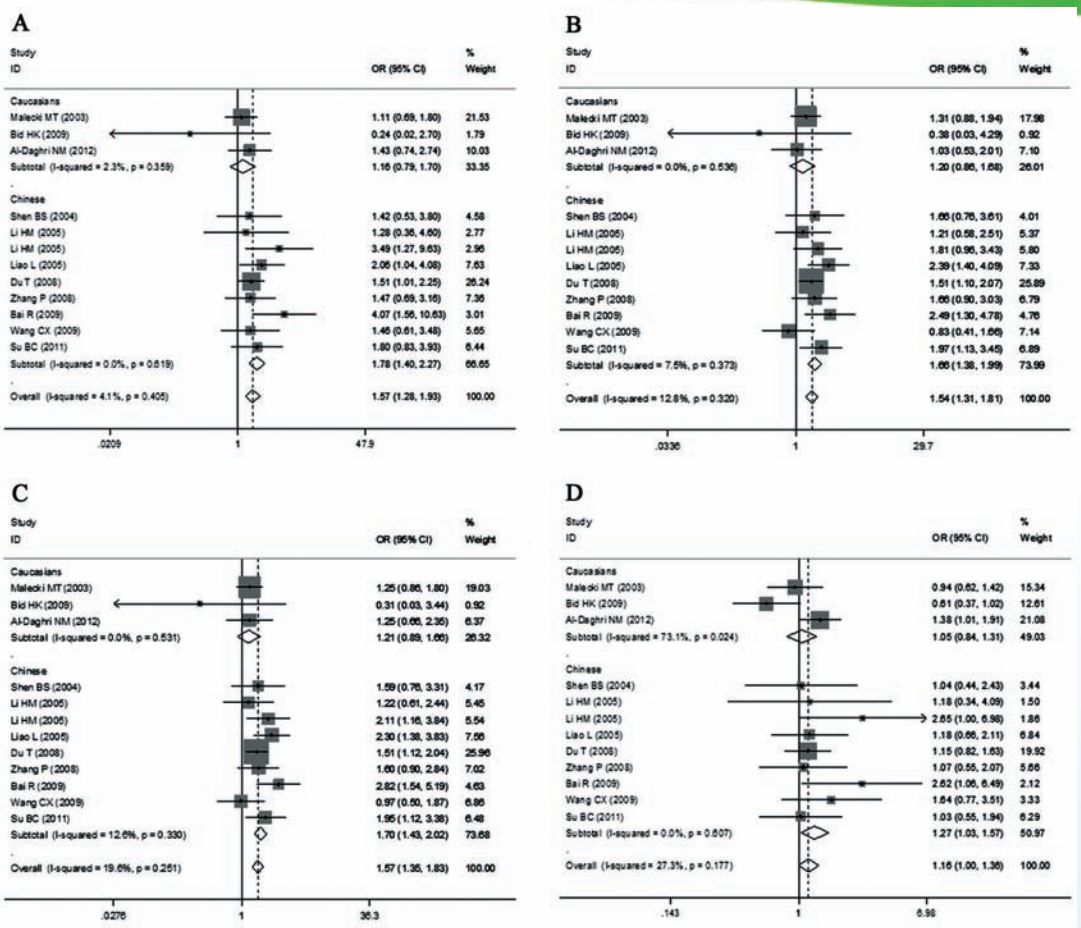

Fig. 3 Forest plots for the overall association between *VDR Fok*I polymorphism and T2DM risk. A. ff *vs.* FF; B. Ff *vs.* FF; C. ff *vs.* Ff+FF. D. ff+Ff *vs.* FF

Discussion

Vitamin D is essential for the function of the endocrine pancreas, and the *VDR* gene may be involved in the pathogenesis and progression of T2DM. In recent years, there have been many articles studying on the links between vitamin D status and T2DM in different ethnicities and regions. When we went through these papers, we found it was very confusing in describing the genotype of *VDR*. For example, Al-Daghri NM [35] and Dilmec F [42] used the bases to describe the different alleles, while Bid HK[12] and Malecki MT[24], Zhang H[11] andNosratabadi R[16] applied the initial letter of the restriction enzyme to designate the different alleles; Nosratabadi R and Bid HKemployed a capital letter for the presence of the restriction enzyme site, while Malecki MT and Zhang H employed a lowercase letter for its presence. If the readers read these papers with no enough care, the data were too apt to be extracted incorrectly. Also, several studies focused on the population of Indian were categorized as Caucasians, which was often classified as Asians in previous studies. So, in this present work, we unified the definition of the alleles and clarified the definitions of the alleles in different papers to make sure that the data of genotype distribution were extracted exactly for the meta-analysis.

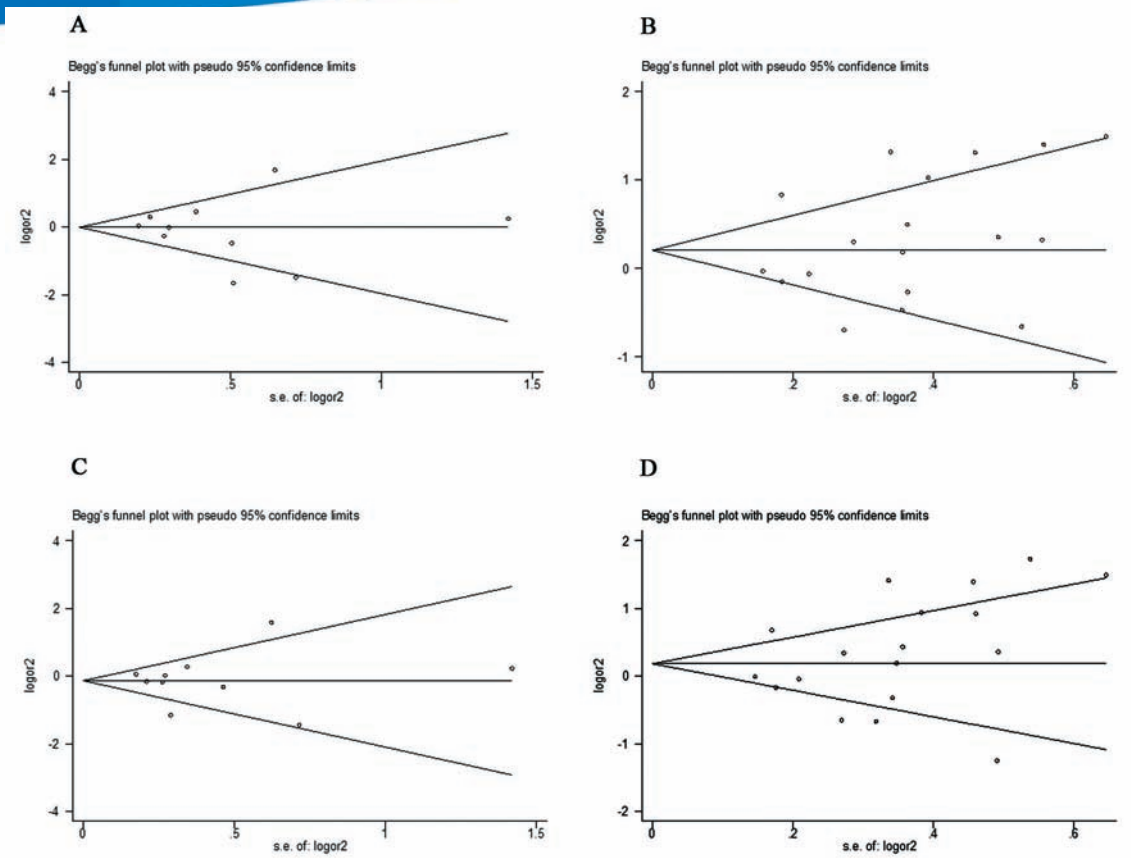

Fig. 4 Funnel plots for *Bsm*I polymorphism of *VDR* in T2DM patients.
A. BB *vs.* bb; B. Bb *vs.* bb; C. BB *vs.* Bb+bb. D. BB+Bb *vs.* bb

In our meta-analysis, marginal significant associations between *Bsm*I polymorphism and T2DM risk were found for Bb *vs.* bb and BB+ Bb *vs.* bb, which is similar with the results of another meta-analysis by Wang Q.[9]. It seems that the allele B and the variant homozygote BB of *Bsm*I weretherisk factorsfor T2DM patients. But the heterogeneity was significant between studies for all contrast models. A meta-regression was performed to searchfor the source of heterogeneity and the results revealed that ethnicity and sample size, especially the later one might be the possible factors of the heterogeneity. The further subgroup analysis revealedsignificant associationsbetween *Bsm*I polymorphism and T2DM among the studies with small sample size (<200) in 2 genetic models (Bb *vs.* bb; BB+ Bb *vs.* bb respectively), and no association between them among large sample size studies. Therefore, the marginal significant associations between *Bsm*I polymorphism and T2DM risk might be induced by small sample size populations, which counld not reflect the genuine-association between *Bsm*I polymorphism and T2DM risk. Thesubgroup analysis did not detect any significant associationsbetween *Bsm*I polymorphism and T2DM in different ethnicities in all genetic models, which indicated geneticbackground was not an important effectfactor for *Bsm*I polymorphism and T2DM risk. Therefore, furtherstudies includinglarger sample sizes are necessary in different ethnicity to confirm the relationship of *Bsm*I polymorphism in the *VDR* gene and T2DM.

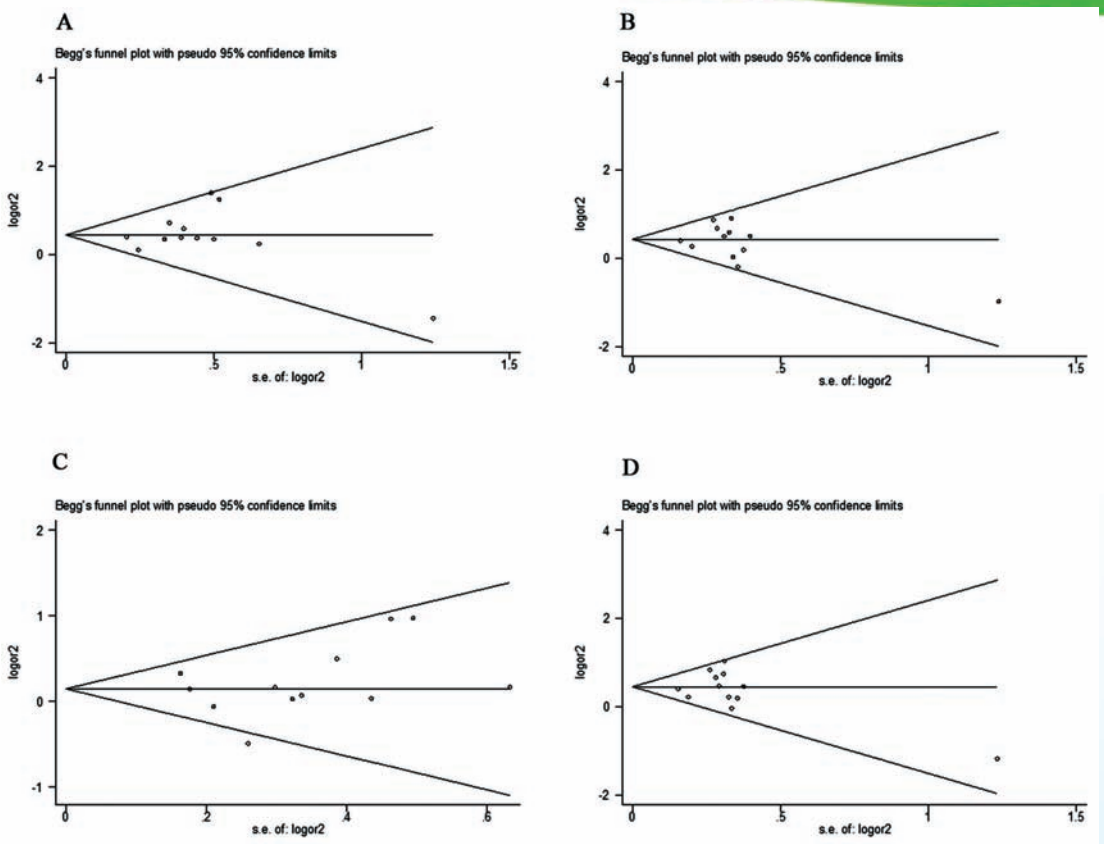

Fig. 5 Funnel plots for *Fok*I polymorphism of *VDR* in T2DM patients.
A. ff *vs.* FF; B. Ff *vs.* FF; C. ff *vs.* Ff+FF. D. ff+Ff *vs.* FF

In the meta-analysis of WangQ. and Li L., the results indicated that *Fok*I polymorphism in the *VDR* gene was significantly associatedwith T2DM risk, and the allele f and variant homozygoteff of *Fok*I may be the risk factors for T2DM. We also found significant associations with T2DM for three genetic models (ff *vs.* FF, Ff *vs.* FF, ff+Ff *vs.* FF, respectively) and marginal significant association were found for ff *vs.* FF +Ff in the overall analysis. However, it is interesting that this significantly increased susceptibility was only found in T2DM patients among Chinese for all genetic models. In contrast, no significant association was observed among Caucasians in three studies. So, we infer that the significant association obtained from overall analysis might be arisen from the Chinese population. In addition, subgroup analysis by sample size showed that the marginal significant association disappeared for ff *vs.* FF+Ffin the studies with large sample size.

In conclusion, the correct date extractionwas very crucial for meta-analysis, which is the base of the statistical analysis. Therefore, the authors should be carefully when they reviewed associationstudies, and make clear the meaning of the symbol of gene type. The results of our meta-analysis indicate that the evidence of significantassociation between the *Bsm*I polymorphism and T2DM was weak and the sample size is the main source of the heterogeneity. The *Fok*I polymorphism in the *VDR* gene is significantly associatedwith T2DM risk only in Chinese people and no significant association was ob-

served among Caucasians. Meanwhile, the sample size may be an important factor that influences the result of case-control studies. Future larger sample size studies are needed to investigate theassociations between *VDR* polymorphism and T2DM. Besides, the gene-environmentinteractions and the molecular evidence of *VDR* polymorphism with T2DM should be studied.

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