Vitamin D-binding protein gene polymorphisms and chronic obstructive pulmonary disease susceptibility: A meta-analysis

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Abstract. The vitamin D-binding protein (*VDBP*) genetic polymorphisms have been associated with chronic obstructive pulmonary disease (COPD). A number of studies have been conducted to investigate the combined effects of the *VDBP* gene (GC) rs7041 and rs4588 polymorphisms on the COPD risk. However, the results obtained are inconclusive. The present meta-analysis aimed to investigate whether GC polymorphisms may be a potential risk factor for COPD. The Web of Science, PubMed, Google Scholar, Embase, Cochrane Library, China National Knowledge Infrastructure and Wanfang Database were searched from inception until June 1, 2014. The meta-analysis was performed using the STATA 12.0 software. Twelve case-control studies, including 2,937 subjects, met the inclusion criteria. Overall, a significantly increased risk was detected in populations of GC*1F homozygotes, whereas no associations between other GC polymorphisms and COPD risk were detected. According to ethnicity, the results demonstrated that the GC*1F homozygotes may be a risk factor for COPD and the GC*2 homozygotes may be a protective factor against COPD in the Asian population. However, similar associations were not observed among the Caucasian population. In conclusion, the current meta-analysis indicates that the GC*1F homozygotes may be a risk factor for COPD and the GC*2 homozygotes may be a protective factors against COPD in the Asian population.

Introduction

Chronic obstructive pulmonary disease (COPD) is one of the leading causes of morbidity and mortality worldwide, which is characterized by irreversible or partly reversible progressive limitation of the airflow (1,2). Cigarette smoking is the major risk factor, but only 10-15% of long-term smokers develop symptomatic airflow obstruction (3,4). This indicates that the genetic factors may contribute to the individual susceptibility for the development of COPD. Numerous studies have shown that genetic factors are likely to have a role in determining the susceptibility of an individual to COPD (1,2,5-8), and genetic variants in the vitamin D-binding protein (*VDBP*) gene have also been linked to COPD risk (7,9,10).

VDBP (also known as Gc-globulin) is expressed in a number of tissues, such as liver, kidney, gonads and fat (11). Notably, VDBP is also expressed by human neutrophils (12), contributes to macrophage activation (13), augments monocyte and neutrophil chemotaxis to C5-derived peptides and acts as a scavenger protein to clear extracellular G-actin released from necrotic cells (14,15). Additionally, VDBP is the major plasma carrier protein of vitamin D, deficiency of which may also be linked to COPD (7,16,17). The human *VDBP* gene (GC) has been localized to chromosome 4q11-q13 and is highly polymorphic, with three commonly recognized variants (GC*1F, GC*1S and GC*2) caused by non-synonymous single-nucleotide polymorphisms (SNPs; rs7041 and rs4588) and >120 rarer variants (18). GC*1F, GC*1S and GC*2 are not alleles as such, but haplotypes composed of combinations of the SNPs at these loci, so an individual may be homozygous or heterozygous for each variant, depending on the two haplotypes present. The role of GC polymorphisms in COPD has been explored in a number of studies by investigating the association of GC variants and susceptibility of COPD (9,10,19-25). However, the results of these studies were inconclusive and may not be powerful enough due to the limited sample size. To evaluate the overall effect of the haplotypic association between the GC polymorphisms (rs7041 and rs4588) and COPD risk, a meta-analysis was conducted in the present study by pooling all the available data together.

Materials and methods

Identification of eligible studies. To identify all the eligible studies that investigated the haplotypic association of the GC rs7041 and rs4588 polymorphisms with COPD risk, a comprehensive electronic search of Web of Science, PubMed, Google Scholar, Embase, Cochrane Library, China

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Table I. Main characteristics of all the eligible studies.

First author	Year	Country	Ethnicity	Source of controls	No. of case/ control	Genotype frequencies of GC haplotypes (rs7041-rs4588)												(Refs.)
						Cases						Control						
						1F-1F	1F-1S	1S-1S	2-1S	2-1F	2-2	1F-1F	1F-1S	1S-1S	2-1S	2-1F	2-2	
Wood	2011	UK	Caucasian	HS	93/351	0	2	39	41	1	10	0	0	145	150	0	56	(24)
Shen	2010	China	Asian	HS	100/100	35	20	7	13	22	3	13	26	4	12	29	16	(10)
Huang	2007	China	Asian	HS	75/69	24	21	2	8	18	2	8	18	3	7	20	13	(19)
Korytina	2006	Rep of Bashk	Caucasian	HS	131/106	8	26	30	29	25	13	12	39	14	25	9	7	(21)
Korytina	2006	Russia	Caucasian	HS	166/130	14	31	42	49	20	10	14	29	25	45	12	5	(21)
Lu	2004	China	Asian	HS	69/52	23	15	5	9	16	1	6	16	3	8	14	5	(22)
Laufs	2004	Iceland	Caucasian	HS	102/183	1	11	39	35	5	11	2	24	68	67	8	14	(25)
Ito	2004	Japan	Asian	HS	103/88	33	29	3	11	25	2	15	27	5	10	30	1	(9)
Ishii	2001	Japan	Asian	HS	63/82	23	15	1	6	16	2	17	27	5	8	18	7	(20)
Schellenberg	1998	UK	Caucasian	HS	75/64	41			32		2	31			24		9	(23)
Horne	1990	USA	Caucasian	HS	104/413	6	24	40	23	3	8	5	66	141	134	25	42	(32)
Kueppers	1977	USA	Caucasian	HS	109/109	62			46		1	57			47		5	(31)

GC, vitamin D-binding protein gene; 1F, GC*1F; 1S, GC*1S; 2, GC*2; HS, healthy smokers; Bashk, Bashkortostan.

National Knowledge Infrastructure and Wanfang Database was performed until June 1, 2014. Various combinations of the following medical subject headings and key words were applied in order to include the maximum number of studies associated as possible: Vitamin D-binding protein, VDBP, GC or Gc-globulin; rs7041 or rs4588; and chronic obstructive pulmonary disease, COPD; polymorphisms, variants or haplotypes. Furthermore, the reference lists of the reviews and retrieved studies were manually screened for additional studies. There were no restrictions for language and only published studies with full-text studies were included.

Inclusion and exclusion criteria. The studies identified from the above-mentioned databases were screened by two independent investigators according to the following predesigned inclusion criteria: i) Case-control design; ii) evaluating the correlation of GC haplotypes (at position rs7041 and rs4588) with COPD risk; and iii) providing sufficient data to calculate the odds ratio (OR) and its corresponding 95% confidence interval (CI). When several studies with overlapping data were eligible, those with smaller sample size or less reliability were excluded. In addition, the studies without detailed information were excluded, following the efforts to extract data from the original study or failure of contact with the corresponding authors.

Data extraction. Data from the eligible studies were extracted by two investigators independently and in duplicate according to the predesigned data-collection form. The following information was extracted: Last name of the first author, publication year, country of origin, ethnicity, source of controls, number of the two COPD cases, control subjects and phenotypic distribution in the two groups. Different ethnicities were categorized as Asian and Caucasian. Discrepancies occurring during the process of study inclusion and data extraction were resolved by discussion with a third investigator and consensus on each item was achieved eventually.

Data analysis. The statistical analysis was conducted using STATA software (version 12.0; Stata Corporation, College Station, TX, USA). The ORs of COPD were recalculated and compared between GC*1F-1F and non GC*1F-1F, GC*1F-1S and non GC*1F-1S, GC*1S-1S and non GC*1S-1S, GC*2-1S and non GC*2-1S, GC*2-1F and non GC*2-1F, and GC*2-2 and non GC*2-2. Heterogeneity among studies was examined with the χ^2-based Q testing and I^2 statistics (26). When there was a significant heterogeneity ($P<0.10$), a random-effects model (the DerSimonian and Laird method) was selected to pool the data (27). Otherwise, a fixed-effects model (the Mantel-Haenszel method) was selected to pool the data (28). Publication bias was examined with funnel plots Begg and Egger tests (29,30). When no publication bias existed, the funnel plot was considered as symmetrical. The statistical significance of the pooled OR was assessed with the Z test and $P<0.05$ was considered to indicate a statistically significant difference. For the Egger's tests, the significance level was also set at 0.05. Subgroup analyses were also conducted to assess any moderating effects of ethnicity (Caucasian and Asian) on ORs derived from each study.

Results

Characteristics of the eligible studies. A total of 2,307 studies were obtained with the initial search of databases. Following screening, 12 case-control studies in 11 eligible studies with a total of 2,937 participants (1,190 cases and 1,747 controls) fulfilled the inclusion criteria (9,10,19-25,31,32), from which genotype data of the GC polymorphisms (rs7041 and rs4588)

Table II. Results of the meta-analysis for haplotypes (rs7041-rs4588).

	Meta-analysis		Heterogeneity		
Genetic model	Pooled OR (95% CI)	P_{OR}	I^2, %	P-value	Bias P-value
1F-1F vs. non 1F-1F	2.052 (1.227-3.431)[a]	0.006[a]	65.0	0.004	0.883
	1.115 (0.402-3.090)[b]	0.834	67.7	0.026	0.667
	2.922 (2.064-4.136)[a,c]	1.47E-09[a]	0.0	0.760	0.287
1F-1S vs. non 1F-1S	0.808 (0.651-1.003)	0.054	0.0	0.629	0.427
	0.830 (0.617-1.117)[b]	0.220	28.6	0.246	0.671
	0.784 (0.572-1.074)[c]	0.130	0.0	0.801	0.563
1S-1S vs. non 1S-1S	1.174 (0.947-1.456)	0.144	0.0	0.604	0.327
	1.223 (0.974-1.536)[b]	0.083	0.0	0.566	0.059
	0.837 (0.434-1.615)[c]	0.595	0.0	0.470	0.088
2-1S vs. non 2-1S	0.864 (0.707-1.056)	0.152	0.0	0.935	0.463
	0.835 (0.666-1.047)[b]	0.118	0.0	0.554	0.853
	0.980 (0.633-1.515)[c]	0.927	0.0	0.995	0.540
2-1F vs. non 2-1F	0.937 (0.726-1.209)	0.616	30.0	0.179	0.739
	1.340 (0.869-2.067)[b]	0.185	44.8	0.142	0.269
	0.774 (0.563-1.062)[c]	0.113	0.0	0.753	0.174
2-2 vs. non 2-2	0.610 (0.308-1.207)	0.155	71.5	0.000	0.112
	1.033 (0.505-2.129)[b]	0.929	68.1	0.004	0.443
	0.212 (0.105-0.428)[a,c]	1.51E-05[a]	3.5	0.387	0.285

[a]Bold print denotes significant results of pooled ORs. [b]Studies with Caucasian samples were included. [C]Studies with Asian samples were included. OR, odds ratio; CI, confidence interval; 1F, GC*1F; 1S, GC*1S; 2, GC*2.

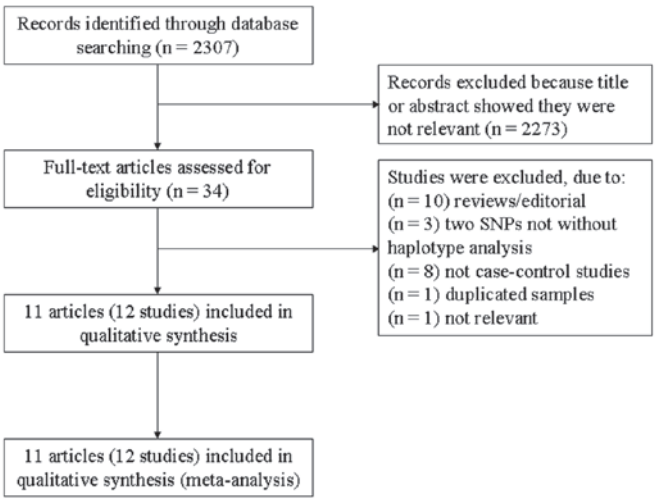

Figure 1. Flowchart showing the study selection procedure. SNPs, single-nucleotide polymorphisms.

were obtained (shown in Table I). The flowchart of reviews demonstrates the detailed process of selection (Fig. 1). As for ethnicity, seven studies investigated the Caucasian population (21,23-25,31,32) and five studies investigated the Asian population (9,10,19,20,22). The qualities of these studies were considered accessible for the meta-analysis.

Meta-analysis of haplotypes and the association with COPD. Overall, among the six haplotypes of the *VDBP* gene (GC*1F-1F, GC*1F-1S, GC*1S-1S, GC*2-1S, GC*2-1F and GC*2-2), a significantly increased risk was observed in GC*1F homozygotes (GC*1F-1F vs. non GC*1F-1F: OR=2.02, 95% CI: 1.23-3.43, P=0.006) and no association between other GC haplotypes and COPD risk were detected (Fig. 2 and Table II).

According to ethnicity, the results demonstrated a markedly increased risk in Asians with GC*1F homozygotes, but not in Caucasians (GC*1F-1F vs. non GC*1F-1F; OR, 2.92; 95% CI, 2.06-4.14; P=1.47E-09; OR, 1.12; 95% CI, 0.40-3.09; P=0.834, respectively) (Fig. 3 and Table II). In addition, GC*2 homozygotes in Asians were at lower risk of COPD (GC*2-2 vs. non GC*2-2: OR, 2.21; 95% CI, 0.11-0.43; P=1.51E-05) (Fig. 4). No significant association was observed in the other haplotypes (Table II).

Sensitivity analysis. Sensitivity analysis was also performed to explore the potential influence of each individual study on the overall results by deleting one single study each time from the pooled analysis. No substantial change was demonstrated in the overall studies, indicating that no individual study affected the pooled OR significantly (data not shown).

Heterogeneity and publication bias. Among the six haplotypes, significant heterogeneity was observed in the GC*1F homozygotes and GC*2 homozygotes with P<0.10; subsequent to stratifying for populations, no significant heterogeneity was observed in Asians, but the heterogeneity remained significant in Caucasians. For publication bias, no significant results were observed with all P>0.05 of Egger's test. Additionally, funnel plots of haplotypes did not show significant publication bias either. Results of heterogeneity and publication bias are shown in Table II.

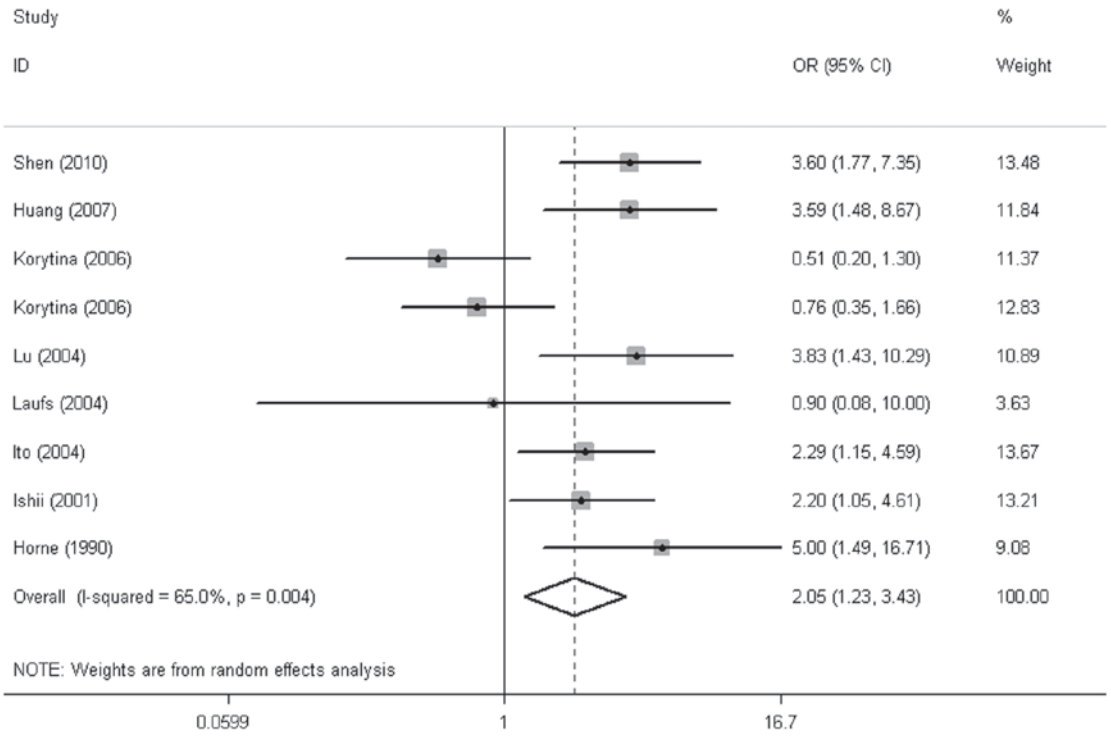

Figure 2. Forest plot for the COPD risk associated with *VDBP* gene polymorphisms in total analysis (GC*1F-1F vs. non GC*1F-1F). COPD, chronic obstructive pulmonary disease; *VDBP*, vitamin D-binding protein; OR, odds ratio; CI, confidence interval.

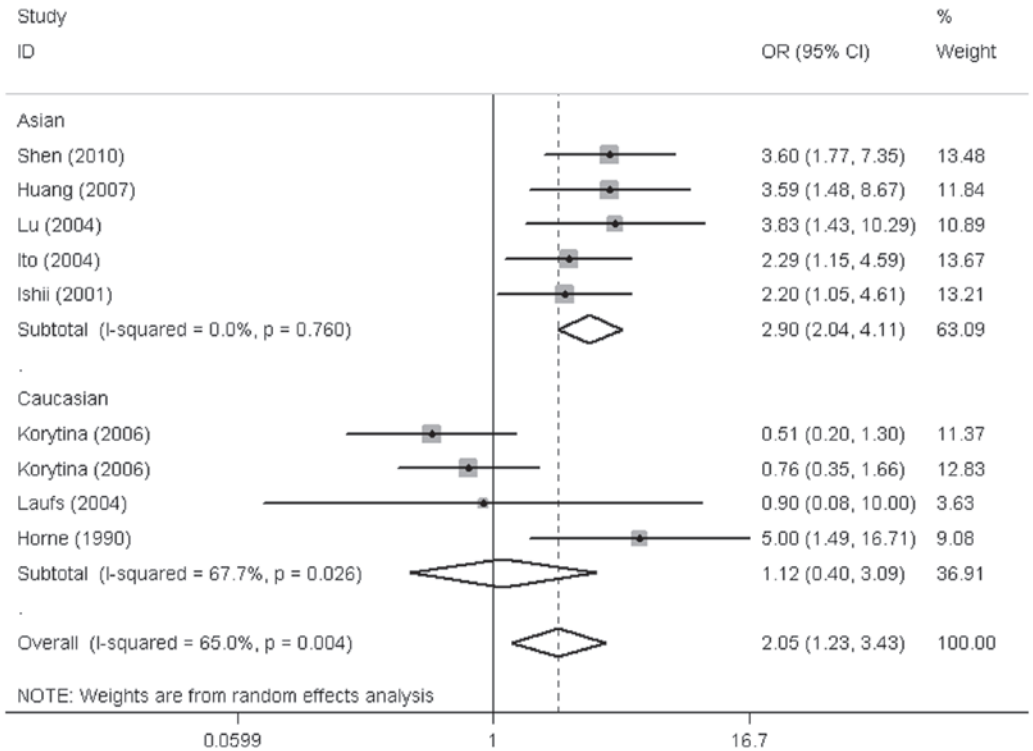

Figure 3. Forest plot for the COPD risk associated with *VDBP* gene polymorphisms stratified by ethnicity (GC*1F-1F vs. non GC*1F-1F). COPD, chronic obstructive pulmonary disease; *VDBP*, vitamin D-binding protein; OR, odds ratio; CI, confidence interval.

Discussion

In the present study, the results indicated that the GC*1F-1F homozygote carriers have an increased risk of COPD. However, following subgroup analysis by ethnicity, a significant increased risk of COPD was found in Asians, but not in Caucasians. The GC*2-2 homozygote carriers in Asians may have a decreased risk of COPD.

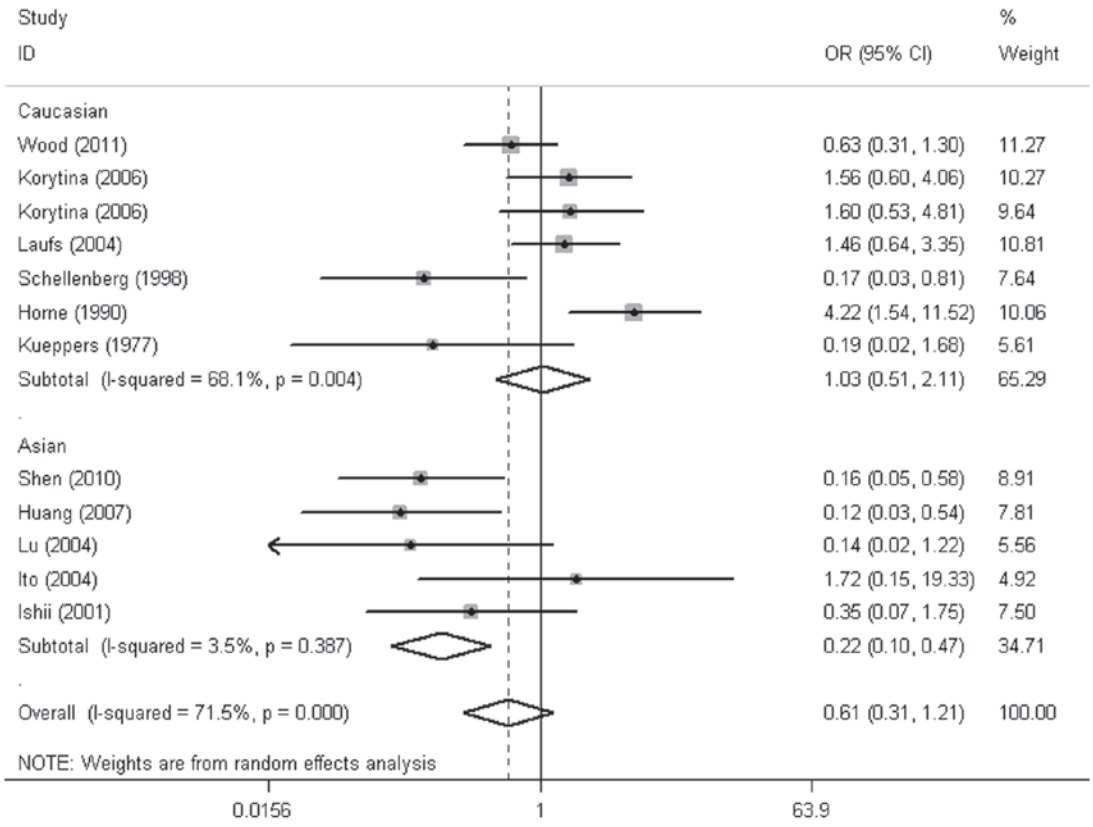

Figure 4. Forest plot for risk the COPD risk associated with *VDBP* gene polymorphisms stratified by ethnicity (GC*2-2 vs. non GC*2-2). COPD, chronic obstructive pulmonary disease; *VDBP*, vitamin D-binding protein; OR, odds ratio; CI, confidence interval.

To the best of our knowledge, this is the first meta-analysis of the *VBDP* gene polymorphsims and COPD susceptibility. In the present meta-analysis, 12 eligible studies were combined with 1,190 cases and 1,747 controls to yield summary statistics, indicating that the GC*1F-1F homozygotes of the *VDBP* gene were associated with the COPD risk among the overall population (Table II). According to ethnicity, the GC*1F homozygotes were considered as a risk factor for COPD in Asian populations, while the presence of the GC*2 homozygotes may be one of the protective factors against COPD in Asians (Table II). These findings on the correlation between GC polymorphisms and COPD were indirectly supported by the evidence that the *VDBP* gene influenced vitamin D concentrations and was associated to forced expiratory volume in one second (7,24). However, the results from the present analyses failed to reveal the significant association between the GC polymorphisms and COPD susceptibility among Caucasians, this was extremely different from the findings by Horne *et al* (32) and Schellenberg *et al* (23), but consistent with the findings by other studies (21,25,31,33). This may be due to the fact that other factors, such as the differences in age, gender, lifestyle factors, clinical characteristics or the analysis method of the *VDBP* genotype may have influenced the results. The frequency difference between Caucasians and Asians may be a cause of variable association with COPD between these two ethnic groups and studies based on larger well-designed populations are required to clarify the associations between the *VDBP* gene polymorphism and COPD susceptibility.

The heterogeneity is of note when interpreting the meta-analysis results. For the significant and stable results of the GC polymorphisms in the majority of models, there was no or low-to moderate heterogeneity (indicated by I^2-statistics) among studies (see Table II). However, relatively clear heterogeneity was observed in the models of GC*1F-1F and GC*2-2 in Caucasians, which may have been responsible for the negative results.

The current meta-analysis focused on the combined effects of the *VDBP* gene rs7041 and rs4588 polymorphisms rather than one single SNP on COPD risk (7,34), which may help to derive a precise estimation of the roles of *VDBP* SNPs in the development of COPD episodes. However, several limitations should be considered when interpreting the results. First, the limited number of COPD cases and control subjects may lead to a relatively small power. Second, the heterogeneity was not resolved following subgroup analyses in certain cases, indicating that other factors, such as the differences in age, gender, lifestyle factors or clinical characteristics, may have caused heterogeneity. Third, only published studies with sufficient data were included, and therefore, publication bias may have occurred even though results of the Begg's and Egger's tests did not detect it.

In conclusion, the present meta-analysis indicated that the GC*1F homozygotes of the *VDBP* gene may be a risk factor for COPD among Asian populations, while the presence of the GC*2 homozygotes may be one of the protective factors against COPD in Asians. Larger and well-designed studies based on different ethnic groups are required to confirm our results.

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