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REVIEW

Vitamin D binding protein genotype variants and risk of chronic obstructive pulmonary disease: A meta-analysis

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ABSTRACT

Background and objective: **Genetic susceptibility for development of chronic obstructive pulmonary disease (COPD) is under intensive investigation. Among the three alleles of vitamin D binding protein, or group-specific (GC) components, some have suggested that having GC-1F and GC-2 alleles was associated with a risk of COPD. Although previous studies have shown considerable variance, no meta-analysis has been conducted.**

Methods: **Through four databases, two independent investigators searched for case-control studies providing sufficient data to calculate odds ratios by the vitamin D binding protein allele variant and genotype variant for a case of COPD. Studies whose control did not satisfy the Hardy–Weinberg equilibrium (Chi-square $P \geq 0.05$) were excluded. We used a fixed-model to estimate the pooled odds ratio at both allele and genotype level.**

Results: **Of 141 candidate studies, six were included. We analysed 1712 subjects, consisting of 466 Asians, 1246 Caucasians, 531 COPD cases and 1181 non-COPD controls. The prevalence of each allele among the 1181 controls was as follows: GC-1F 14.0%, GC-1S 53.8% and GC-2 31.9%. When compared to GC-1S, the GC-1F allele and GC-2 allele were associated with COPD risk with pooled odds ratios of 1.44 (95% CI 1.14–1.83, $P = 0.002$) and 0.83 (95% CI 0.69–0.996, $P = 0.045$), respectively. When compared to the 1S-1S genotype, the 1F-1F genotype was a risk factor of COPD with pooled odds ratio of 2.64 (95% CI 1.29–5.39, $P = 0.008$).**

Conclusion: **The GC-1F allele of the vitaminD binding protein was a risk for COPD in recessive mode.**

Key words: allele, group-specific component, inheritance, Mendelian model, single nucleotide polymorphism.

Abbreviations: COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; GC, group-specific component; GWAS, genome-wide association studies); HWE, Hardy–Weinberg equilibrium; OR, odds ratio; SNP, single nucleotide polymorphism; VDBP, vitamin D binding protein.

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INTRODUCTION

Chronic obstructive pulmonary disease (COPD), a complex systemic inflammatory disease characterized by chronic airflow limitation, is expected to become the third leading cause of death worldwide by 2020.[1] Although by far the strongest risk factor is smoking, genetic susceptibility for COPD is believed to play a considerable role in the development of the disease. This hypothesis is supported by the fact that only 10% or 15% of persons with a substantial smoking history eventually develop COPD.[2] Alpha-1 antitrypsin protein deficiency is an established genetic disease resulting in COPD; however, it can explain only a small number of COPD cases. Up to now, more than 100 candidate genes have been identified as having an association with a risk for COPD development.[3]

Vitamin D binding protein (VDBP), which is often called, vitamin D receptor, group-specific component (GC) protein, or GC-globulin, is coded in chromosome 4q. VDBP has three major allele-level polymorphisms, that is, GC-1F, GC-1S and GC-2, according to the combination of two single nucleotide polymorphisms (SNP), rs7041 (p.Glu416Asp) and rs4588 (p.Thr420Lys). These SNP correspond to allele-level polymorphisms as follows: rs7041 (p.416 = Asp) + rs4588 (p.420 = Thr) to GC-1F, s7041 (p.416 = Asp) + rs4588 (p.420 = Lys) to GC-2 and rs7041 (p.416 = Glu) + rs4588 (p.420 = Thr) to GC-1S. The combination of rs7041 (p.416 = Glu) + rs4588 (p.420 = Lys) does not correspond to any of the three major alleles.[4,5] There are six genotypes that are combinations of the three alleles, 1F-1F, 1F-1S, 1F-2, 1S-1S, 1S-2 and 2-2. VDBP has important immunemodulatory functions in addition to vitamin D transport. Recent studies have suggested that VDBP plays a role in respiratory diseases such as asthma, COPD, tuberculosis and lung cancer through neutrophil chemotaxis and macrophage activation.[6]

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The association between VDBP variants and risk of COPD has been evaluated.[7–18] Some of these studies have suggested that the GC-2 allele reduces the risk of COPD and that the GC-1F allele increases the risk. However, previous studies have shown considerable variance. This may be partly due to sample size, race difference or insufficient evaluation of the Hardy–Weinberg equilibrium (HWE). There have been meta-analyses concerning many other genes to estimate the precise risk of developing COPD. However, the VDBP variant has not yet been subject to meta-analysis. Therefore, we designed this meta-analysis study to obtain more precise evidence for the association of the VDBP variant and the risk for COPD.

METHODS

Study search

Two investigators independently searched for eligible articles using the MEDLINE, EMBASE, Web of Science and Cochrane Databases as of February 2014. The following search formula was used for MEDLINE: ('COPD' OR 'chronic obstructive airway disease' OR 'emphysema' OR 'chronic bronchitis' OR 'chronic airflow obstruction') AND ('vitamin D' OR 'group specific component' OR 'GC-globulin' OR 'rs7041' OR 'rs4588').

Articles in authors' reference files were also regarded as candidates.

The eligibility criteria for the current meta-analysis were case-control studies providing sufficient data to calculate odds ratio (OR) by the VDBP allele and genotype for a case of COPD. Any definition for COPD was generally accepted after consideration by the authors. One study published in 1990 that evaluated cases of chronic obstructive airway disease (chronic bronchitis and emphysema) was included,[7] because this disease category matched the COPD definition according to the American Thoracic Society statement published in 1995.[19] A language restriction was not set. Duplicate use of the same data was carefully evaluated. Studies whose control did not satisfy HWE (chi-square $P \geq 0.05$) were excluded.[20]

Statistical analysis

HWE was evaluated by chi-square test with three degrees of freedom for studies with three types of allele.[20] If at least one cell in the 2 by 2 contingency was null, 0.5 was added to all cells to estimate OR.[21] For the allele-level analysis, GC-1S was used as the reference, because the GC-1S allele was the most prevalent among the three types of alleles. Similarly, the 1S-1S genotype was used as the reference to conduct genotype-level analysis. The fixed model with confidence interval method was used for meta-analysis to estimate pooled OR,[22] after we confirmed that the heterogeneity was not substantial ($I^2 < 50\%$). The heterogeneity among the original studies was evaluated with (i) the chi-square distribution test with a rejection region of $P = 0.1$, and (ii) the I^2 statistics test whereby $I^2 = 0\%$ indicates no heterogeneity, $I^2 = 25\%$ indicates mild heterogeneity, $I^2 = 50\%$ indicates moderate heterogeneity and $I^2 = 75\%$ indicates strong heterogeneity.[23] A funnel plot and Begg's test with the Kendall's rank correlation test with a rejection region of $P = 0.1$ were used to evaluate the publication bias.[24] A test for interaction was performed to compare pooled OR by Asians and Caucasians.[25] All analyses were performed using Excel Toukei version 2012 (SSRI, Tokyo, Japan) and GraphPad PRISM 5.0 (GraphPad Software Inc., CA, USA).

RESULTS

Study search

We screened 134 potentially eligible articles from database searches and seven potentially eligible articles from authors' reference lists. Of the 141 articles, 114 were excluded based on title and abstract, 21 were excluded after considering the full text. Six articles were not used for qualitative synthesis, even though they evaluated the associations between VDBP genotype and COPD risks.[13–18] This was because HWE in the controls was not confirmed. A total of six articles were finally included in our analysis (Fig. 1).[7–12] Each of the six articles provided detailed method for genotyping. However, the smoking history of controls was not sufficiently described in four studies.

The eligible six articles were published between 1990 and 2011. Of the six articles, three were on Asians, three on Caucasians, five were written in English, and one in Chinese. The smoking history of the controls was not sufficiently described in some studies (Table 1). We analysed 1712 subjects, consisting of 466 Asians, 1246 Caucasians, 531 COPD subjects and 1181 non-COPD controls (Table 1). The prevalence of each

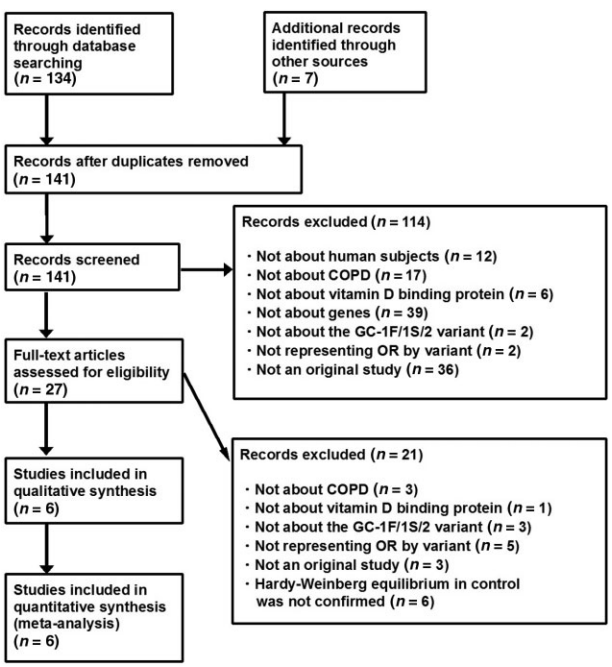

Figure 1 Flow chart for study search (Preferred Reporting Items for Systematic Reviews and Meta-Analyses diagram).

Table 1 Summary of included studies

Author, year, reference	Language	Race		Smoking history of control	Recruitment criteria	GC allele (n) *N*				GC genotype *n*							HWE in control χ2 (P)
						1F	1S	2	Sum	1F-1F	1F-1S	1F-2	1S-1S	1S-2	2-2	Sum	
<<Asian>>																	
Ishii '01[8]	English	Asian (Japanese)	subject	Not specified	ATS	77	23	26	126	23	15	16	1	6	2	63	3.6 (0.313)
			control		Healthy volunteer	79	45	40	164	17	27	18	5	8	7	82	
Lu '04[10]	Chinese	Asian (Chinese)	subject	Smoker only	CARD	77	34	27	138	23	15	16	5	9	1	69	2.6 (0.452)
			control		Without COPD	42	30	32	104	6	16	14	3	8	5	52	
Shen '10[11]	English	Asian (Chinese)	subject	Not specified	ATS	112	47	41	200	35	20	22	7	13	3	100	5.9 (0.119)
			control		Healthy volunteer	81	46	73	200	13	26	29	4	12	16	100	
<<Caucasian> >																	
Horne '90[7]	English	Caucasian	subject	Not specified	COAD	39	127	42	208	6	24	3	40	23	8	104	3.3 (0.342)
			control		Town resident	101	482	243	826	5	66	25	141	134	42	413	
Laufs '04[9]	English	Caucasian (Icelandic)	subject	Mostly not	ATS	18	124	62	204	1	11	5	39	35	11	102	0.85 (0.837)
			control		Healthy volunteer	36	227	103	366	2	24	8	68	67	14	183	
Wood '11[12]	English	Caucasian	subject	Not specified	Spirometry	3	121	62	186	0	2	1	39	41	10	93	2.6 (0.105)
			control		Geographically matched	0	440	262	702	0	0	0	145	150	56	351	

Spirometry: FEV1 < 80% predicted and FEV1/FVC < 70%.

ATS, American Thoracic Society; CARD, Chinese Association of Respiratory Disease; COAD, chronic obstructive airway disease; COPD, chronic obstructive pulmonary disease; GC, group-specific components; FEV1/FVC, forced expiratory volume in 1 s/forced vital capacity; HWE, Hardy–Weinberg Equilibrium.

allele was as follows: GC-1F 43.0%, GC-1S 25.9% and GC-2 31.0% in Asian; GC-1F 7.2%, GC-1S 60.7% and GC-2 32.1% in Caucasians. In short, the 1F allele was much more prevalent in Asians. This difference of prevalence was compatible with a previous report.[5]

Allele-level meta-analyses

A fixed-model meta-analyses for the GC-1F allele involving both Asians and Caucasians suggested that the GC-1F allele was a risk factor of COPD with a pooled OR of 1.44 (95% CI 1.14–1.83, $P = 0.002$) compared to the GC-1S allele (Fig. 2). There was a weak heterogeneity with I^2 of 26% ($P = 0.236$), no race interaction ($z = 0.77$, $P = 0.444$) (Fig. 2) and no evidence of publication bias with τ of 0.33 ($P = 0.348$) (Fig. 3).

A fixed-model meta-analyses for the GC-2 allele involving both Asians and Caucasians suggested that the GC-2 allele was a preventive factor of COPD with a pooled OR of 0.83 (95% CI 0.69–0.996, $P = 0.045$) compared to the GC-1S allele (Fig. 2). There was a weak heterogeneity with I^2 of 31% ($P = 0.207$), no race interaction ($z = 0.56$, $P = 0.577$) and no publication bias with τ of 0.07 ($P = 0.851$) (Fig. 3).

Genotype-level meta-analyses

Genotype-level fixed-model meta-analyses involving both Asians and Caucasians, using the 1S-1S genotype as reference, yielded pooled OR as follows: 1F-1F genotype, OR of 2.64 (95% CI 1.29–5.39, $P = 0.008$); 1F-1S genotype, OR of 1.04 (95% CI 0.68–1.59, $P = 0.847$); 1F-2 genotype, OR of 0.80 (95% CI 0.43–1.49, $P = 0.486$); 1S-2 genotype, OR of 0.84 (95% CI 0.63–1.14, $P = 0.261$); and 2-2 genotype, OR of 0.69 (95% CI 0.44–1.08, $P = 0.101$) (Fig. 2, Supplementary Fig. S1). There was no or weak heterogeneity ($I^2 < 50\%$) among the studies throughout the five analyses (Fig. 2, Supplementary Fig. S2). Interaction between Asians and Caucasians was observed only for 2-2 genotype analysis ($z = 2.06$, $P = 0.040$), and Asian subgroup analysis suggested that the 2-2 genotype worked as a preventive factor with OR of 0.20 (95% CI 0.06–0.71, $P = 0.012$) (Fig. S1). Publication bias for 1F-2 genotype analysis with a marginal significance with τ of 0.60 ($P = 0.091$) was indicated (Fig. S2).

Model of inheritance

Figure 4 suggested that only the 1F-1F homo-genotype was a significant risk factor and the 1F hetero-genotype (1F-1S, 1F-2) were not.

Therefore, the GC-1F allele contributed as a risk only when homogeneous, which suggested that the GC-1F allele was a risk factor in a recessive model.

DISCUSSION

We performed meta-analyses to evaluate the association between the VDBP variant and the risk of COPD. The GC-1F allele is a risk factor of COPD with pooled OR of 1.44 (95% CI 1.14–1.83, $P = 0.002$) compared to the GC-1S allele (Fig. 2). The 1F-1F homo-genotype was a risk for COPD with OR of 2.64 (95% CI 1.29–5.39,

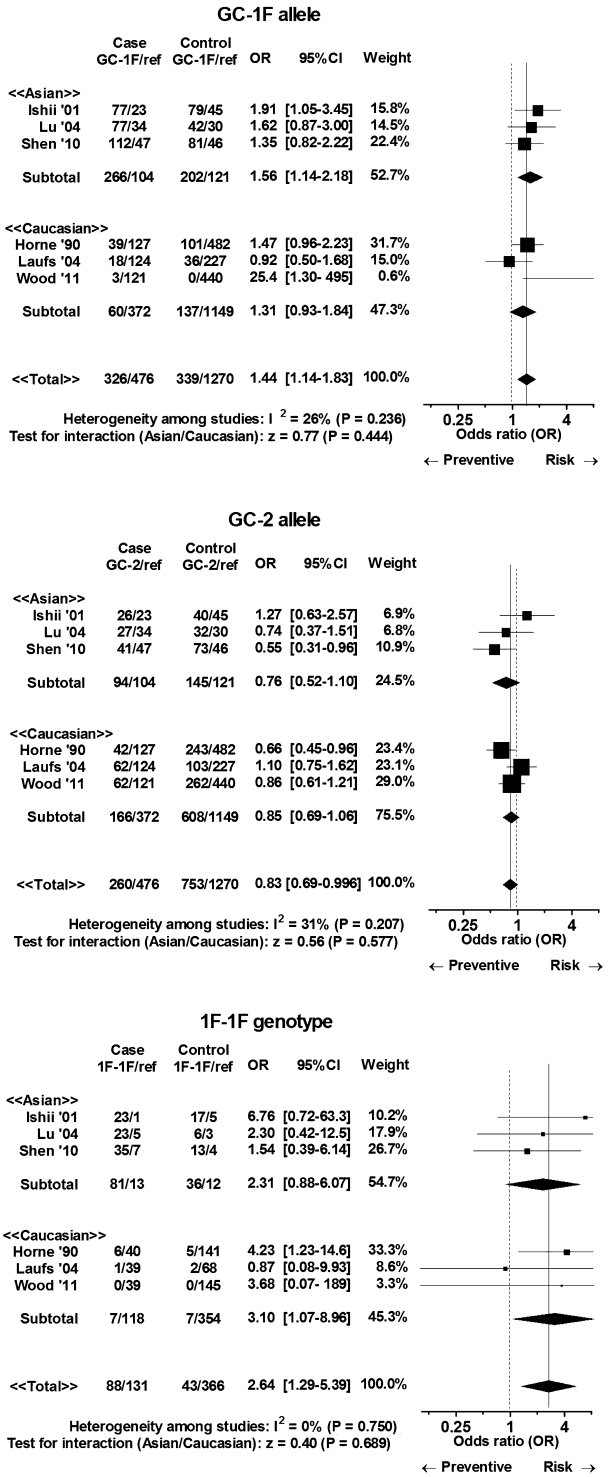

Figure 2 Risk of chronic obstructive pulmonary disease (COPD) by vitamin D binding protein variant group-specific (GC)-1F, GC-2 and 1F-1F. Meta-analysis was conducted with a fixed model. ref: reference. GC-1S allele and 1S-1S genotype were used as references for allele-level, and genotype-level analysis, respectively.

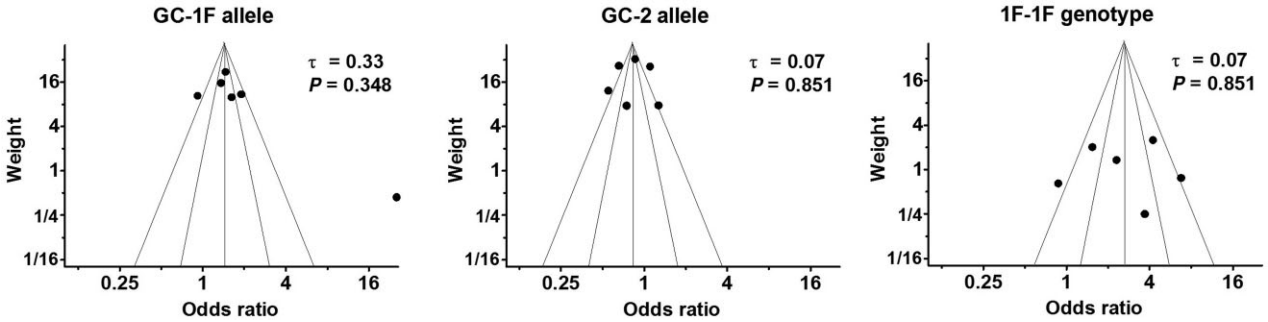

Figure 3 Funnel plots for vitamin D binding protein variant group-specific (GC)-1F, GC-2 and 1F-1F. τ: correlation coefficient for Begg's test.

	GC-1F	GC-1S	GC-2
GC-1F	1F-1F OR 2.64 (95% CI 1.29–5.39) P = 0.003	1F-1S OR 1.04 (95% CI 0.68–1.59) P = 0.847	1F-2 OR 0.80 (95% CI 0.43–1.49) P = 0.486
GC-1S	1F-1S	1S-1S OR 1 (reference)	1S-2 OR 0.84 (95%CI 0.63–1.14) P = 0.261
GC-2	1F-2	1S-2	2-2 OR 0.69 (95% CI 0.44–1.08) P = 0.101

Figure 4 Group-specific (GC) component protein genotype and risk of chronic obstructive pulmonary disease (COPD). OR: odds ratio. 95% CI: 95% confidence interval. Left lower cells are left blank, as right upper cells indicate same genotypes. ■ Risk factor for COPD; ■ Not related to COPD risk.

$P = 0.008$), but the 1F hetero-genotypes (1F-1S, 1F-2) were not (Fig. 4). This suggested a recessive model. Given the fact there was only weak heterogeneity, no publication bias and no race interaction, we believe that the results for the GC-1F allele and the 1F-1F genotype were solid. The GC-2 allele was a weak preventive factor with marginal significance with a pooled OR of 0.83 (95% CI 0.69–0.996, $P = 0.045$) compared to the GC-1S allele. The impact of GC-2 may be different for Asians and Caucasians (Fig. 2). Observed evidence of possible publication bias regarding the 1F-2 genotype might limit the conclusion about the 1F-2 genotype.

Here, we discuss some studies that were not included in the analysis, though they reported on the association between the VDBP variant and COPD risk. Schellenberg's case-control study, which included 75 COPD subjects and 64 non-COPD controls, revealed that the 2-2 genotype was a strong preventive factor for COPD with OR of 0.17 (95% CI 0.03–0.83). However, this study observed peripheral lung carcinoma patients who underwent lobar or lung resection surgery. This particular kind of patient background may affect the observed link between the 2-2 genotype and COPD.[13] Kasuga's report in 2003 concluded that the VDBP genotype did not contribute to reduced lung function in a cohort of smokers. In the study, the authors first recruited smokers with spirometric signs of early COPD, and then compared smokers with high forced expiratory volume in 1 s (FEV_1) and smokers with low FEV_1.[14] Sandford's study compared smokers with per cent predicted FEV_1 decline ≥ 3.0%/year and those with per cent predicted FEV_1 increase ≥ 0.4%/year and concluded that the VDBP genotype was not associated with rate of FEV_1 decline among smokers.[15] Kasuga's study and Sandford's study may have relevant suggestions for the issue addressed in the current meta-analysis, but these reports apparently did not compare COPD subjects and non-COPD controls. Furthermore, controls did not satisfy HWE (chi-square 34.6, $P < 0.001$ for Kasuga's study. Chi-square 10.6, $P = 0.014$ for Sandford's study).[14,15] Korytina's article published in the Russian language concluded that the distributions of alleles and genotypes of the GC gene were similar in COPD patients and healthy individuals, among the sufficiently large number of subjects, 594 subjects and 472 controls. Unfortunately, the controls did not satisfy the HWE (chi-square 14.8, $P = 0.002$); thus, we excluded the study from the analysis.[16] Ito reported, in 2004, that there was an increased proportion of 1F-1F homozygotes in the COPD subjects (32%) compared with healthy smokers (17%) with OR of 2.3 (95% CI 1.2–4.6, $P = 0.01$). This observation supports the results of the current meta-analysis but was not included because the controls did not satisfy the HWE (chi-square 10.3,

$P = 0.016$).[17] Ohkura's report in 2006 was similarly compatible with the current study but was not included for the same reason.[18]

Genome-wide association studies (GWAS) are often used to identify SNP that are associated with the aetiology of a specific disease. Some SNP have been found to be genetic risk factors for COPD. Although GWAS is a powerful tool to identify the SNP that may cause a disease, the results from GWAS are not always consistent. Wilk conducted a GWAS to evaluate 70 798 autosomal SNP using a large-scale Framingham Heart Study cohort, and suggested that SNP nearby VDBP have an association with COPD,[26] while some other GWAS could not replicate the impact of SNP at VDBP for COPD.[27,28] GWAS were useful for finding out candidate SNP that may cause COPD but did not provide information on the term of OR. Thus, we have not included results from GWAS in our meta-analysis.

The current meta-analysis, together with previous case-control studies, showed the association between the VDBP variant and COPD risk; however, the mechanism has not yet been completely investigated. Despite the facts that GC genotypes are associated with serum vitamin D level[29] and that vitamin D deficiency is highly prevalent in COPD,[30] the genetic association of VDBP with COPD may be mediated by different mechanisms.[12] VDBP has two distinct functions related to inflammation.[6,13,14,17] First, VDBP enhances the chemotactic activity of C5 for neutrophils, but there are no differences in potency to induce neutrophil chemotaxis among GC-1F, GC-1S and GC-2. Second, VDBP is known to undergo conversion to a potent macrophage activating factor by the removal of specific glycosylated moieties. The GC-2 protein cannot be converted to macrophage activating factor due to the lack of glycosylated Lys. This second immune-mediating function may explain the COPD preventive effect by GC-2, but the mechanism of association between GC-1F polymorphism and COPD has not yet been clarified.[6]

A limitation of the current research is that the meta-analysis included a limited number of studies. After eliminating some studies that did not satisfy HWE, only six remained. However, we believe that including only studies with HWE is essential to precisely evaluate OR, because deviation from HWE in random samples may suggest that the control does not represent the general population or that the assays are problematic.[31] The impact of the 2-2 genotype among Asians needs to be further clarified. In addition, the limited number of studies might diminish the power to detect publication bias. Another limitation is that most of the included original studies did not provide data in sufficient depth about smoking history or the process for selecting control subjects.

In conclusion, we performed a meta-analysis to evaluate the association between the VDBP variant and the risk of COPD including studies whose control satisfied HWE. The GC-1F allele and the 1F-1F homogenotype were risk factors of COPD with pooled OR of 1.44 (95% CI 1.14–1.83, $P = 0.002$) and 2.64 (95% CI 1.29–5.39, $P = 0.008$), respectively, but the 1F heterogenotype was not, which suggested a recessive model. The GC-2 allele was a weak preventive factor of COPD. The GC-2 allele may be a stronger preventive factor for Asians compared to Caucasian.

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Supplementary Information

Additional Supplementary Information can be accessed via the *html* version of this article at the publisher's web-site:

Supplementary Figure S1 Risk of COPD by vitamin D binding protein variant 1F-1S, 1F-2, 1S-2 and 2-2. Meta-analysis was conducted with a fixed model. GC-1S allele and 1S-1S genotype were used as references for allele-level, and genotype-level analysis, respectively.

Supplementary Figure S2 Funnel plots for vitamin D binding protein variant 1F-1S, 1F-2, 1S-2 and 2-2. τ: correlation coefficient for Begg's test.

