Vitamin D deficiency is highly prevalent in COPD and correlates with variants in the vitamin D-binding gene

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ABSTRACT

Introduction Vitamin D deficiency has been associated with many chronic illnesses, but little is known about its relationship with chronic obstructive pulmonary disease (COPD).

Objectives Serum 25-hydroxyvitamin D (25-OHD) levels were measured in 414 (ex)-smokers older than 50 years and the link between vitamin D status and presence of COPD was assessed. The rs7041 and rs4588 variants in the vitamin D-binding gene (*GC*) were genotyped and their effects on 25-OHD levels were tested.

Results In patients with COPD, 25-OHD levels correlated significantly with forced expiratory volume in 1 s (FEV_1) ($r=0.28$, $p<0.0001$). Compared with 31% of the smokers with normal lung function, as many as 60% and 77% of patients with GOLD (Global Initiative for Obstructive Lung Disease) stage 3 and 4 exhibited deficient 25-OHD levels <20 ng/ml ($p<0.0001$). Additionally, 25-OHD levels were reduced by 25% in homozygous carriers of the rs7041 at-risk T allele ($p<0.0001$). This correlation was found to be independent of COPD severity, smoking history, age, gender, body mass index, corticosteroid intake, seasonal variation and rs4588 ($p<0.0001$). Notably, 76% and 100% of patients with GOLD stage 3 and 4 homozygous for the rs7041 T allele exhibited 25-OHD levels <20 ng/ml. Logistic regression corrected for age, gender and smoking history further revealed that homozygous carriers of the rs7041 T allele exhibited an increased risk for COPD (OR 2.11; 95% CI 1.20 to 3.71; $p=0.009$).

Conclusion Vitamin D deficiency occurs frequently in COPD and correlates with severity of COPD. The data warrant vitamin D supplementation in patients with severe COPD, especially in those carrying at-risk rs7041 variants.

INTRODUCTION

Deficiency of vitamin D is common and represents a major health problem. Early in life, vitamin D deficiency causes growth retardation and rickets, whereas in adults it is well known to accelerate osteopenia and osteoporosis. Accumulating evidence also links a low vitamin D nutritional status to highly prevalent chronic illnesses, including common cancers, autoimmune diseases, infectious and cardiovascular diseases.[1–3] Similar to other chronic diseases, we recently speculated that vitamin D deficiency might also be linked to chronic obstructive pulmonary disease (COPD),[4] which by the year 2030 is expected to rank among the top five chronic diseases in terms of global mortality and morbidity.[5]

Surprisingly, very few studies have assessed the relevance of vitamin D deficiency in COPD by measuring serum levels of 25-hydroxyvitamin D (25-OHD), which is the principal circulating vitamin D metabolite and recognised as the best short-term biomarker of total exposure to vitamin D.[6] Forli *et al* reported that in a small sample of patients with advanced COPD awaiting lung transplantation, the majority suffered from vitamin D deficiency (25-OHD <20 ng/ml).[7] Similarly, >50% of a community-dwelling COPD cohort of 250 patients exhibited insufficient 25-OHD levels.[8] Unfortunately, both studies failed to compare vitamin D status in patients with COPD with those from an age-, gender- and smoking-matched control population. Such a matched control population is, however, of crucial importance since insufficient or deficient vitamin D levels may also occur in up to 40–70% of the elderly in the USA and Europe.[9–11]

As patients with COPD mostly exhibit increased skin ageing induced by smoking and reduced sun exposure due to less outdoor activity, reductions in vitamin D serum levels are generally perceived as a consequence, rather than a cause, of COPD.[4] Intriguingly, however, epidemiological studies in healthy subjects have reported a strong relationship between 25-OHD serum levels and pulmonary function, as assessed by forced expiratory volume in 1 s (FEV_1) and forced vital capacity (FVC).[12] This suggests that vitamin D levels are not only reduced as a consequence of COPD, but might also be important for pulmonary function in health and disease.[13] Interestingly, genetic variants in the vitamin D-binding gene (*GC*) have also been linked to COPD risk.[14–17] However, these studies have remained somewhat controversial, mostly because only small COPD populations consisting of <100 patients with COPD were examined and associations were not always replicated.[14–17] Recently, the rs7041 and rs4588 variants were found to determine circulating 25-OHD levels in 741 healthy premenopausal women, with decreasing concentrations for the less frequent alleles of the rs7041 and rs4588 variants.[18] Whereas seasonal variation was the most important explanatory variable of 25-OHD concentrations in this study, rs7041 and rs4588 explained 25-OHD variation as much as vitamin D food intake, calcium intake and body mass index (BMI),[19] thus indicating that their effect was considerably large and important.

In light of the emerging role of vitamin D deficiency in chronic disease and the novel insights into the functional effect of rs7041 and rs4588, we

measured 25-OHD serum levels in an ambulatory population of 414 individuals not taking vitamin D supplements, consisting of patients with COPD and healthy control subjects of similar age, gender distribution and smoking history. We first analysed whether 25-OHD levels correlated with COPD and the severity thereof, and then performed a joint analysis of the rs7041 and rs4588 genotypes, 25-OHD serum levels and the risk of COPD.

MATERIALS AND METHODS

Patient population

All 414 individuals were prospectively recruited at the University Hospital of Leuven (Belgium). Inclusion criteria were the following: a smoking history of at least 15 pack-years, a minimal age of 50 years and no oral supplementation with vitamin D. Patients with symptomatic COPD were recruited during their routine follow-up at the outpatient clinic in stable clinical condition, which means that they were receiving stable treatment and had no exacerbation 6 weeks before inclusion in the study. Smoking controls with normal spirometry (post-bronchodilator FEV_1/FVC ratio ≥ 0.7) as well as patients with early non-diagnosed COPD (postbronchodilator FEV_1/FVC ratio <0.7) were participants in the Dutch–Belgian randomised lung cancer screening trial (NELSON).[20] In this trial, participants who were recruited via population registries were invited to participate by mail. Only NELSON individuals recruited by the University Hospital of Leuven, and therefore derived from the same geographical and ethnical region as the patients with COPD, were included in this study. Subjects with suspicion or diagnosis of asthma were excluded, as were patients with other respiratory diseases affecting pulmonary function. From all consenting patients an extensive list of demographic variables (including age, gender, BMI in kg/m^2), a complete pulmonary function assessment and questionnaires determining smoking history was collected. The study was approved by the local ethics committee (Leuven, Belgium) and submitted to www.clinicaltrials.gov.

Pulmonary function

All pulmonary function measurements were performed with standardised equipment (Whole Body Plethysmograph, Acertys, Belgium) according to American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. Spirometric values were postbronchodilator measurements, and absolute values were expressed as percentage predicted of reference values.[21] Presence of COPD was defined by a postbronchodilator FEV_1/FVC ratio <0.7 and severity of disease was staged by FEV_1 expressed as percentage predicted according to the latest GOLD (Global Initiative for Obstructive Lung Disease) classification.[22] Diffusing capacity of the lung was determined by the single breath carbon monoxide gas transfer method (DL_{CO}), corrected for alveolar ventilation (K_{CO}) and expressed as percentage predicted of reference values.[23]

Determination of 25-OHD serum levels

Serum total 25-OHD was measured in multiple batches by radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA) in all study participants, as previously described.[24] All 25-OHD samples were measured in one laboratory, which takes part in, and meets the performance targets for the vitamin D external quality assessment scheme (DEQAS).[25] Total 25-OHD measures are mean values of duplicate measures referred to appropriate positive controls. Levels are expressed in ng/ml (conversion factor 2.5 for nmol/l) and reflect the vitamin D status, as represented by the non-vertebral vitamin D2 and vertebral vitamin D3 status.

DNA analysis

Peripheral blood was sampled in K_2EDTA plastic vacutainer tubes and, after centrifugation, germline DNA was extracted from the precipitated leucocyte cell fraction according to standard procedures. Germline DNA was obtained from 404 out of 414 patients (97%). All patients were of Caucasian origin and self-reported Belgian–Flemish origin for three generations. Genotyping for the rs7041 and rs4588 single nucleotide polymorphism (SNP) genetic variants was carried out at the Vesalius Research Institute using the Sequenom iPLEX platform, as described by the manufacturer (Sequenom, San Diego, California, USA). Genotype success rates for the rs7041 and rs4588 SNPs were 99.8%. No significant deviations from Hardy–Weinberg equilibrium were observed.

Statistical analysis

All statistical analyses were performed by SAS version 9.1 (SAS Institute, Cary, North Carolina, USA). For all tests, two-sided p levels <0.05 were considered as statistically significant. Demographic, pulmonary function and smoking characteristics in patients with COPD and healthy controls were first assessed for normal distribution (Shapiro–Wilk normality test) and, depending on their distribution, compared by standardised Student t test or Pearson χ^2 test. Non-parametric data such as pack-years, years since quitting smoking and age were analysed by Wilcoxon statistics. Differences in mean 25-OHD levels between patients with COPD and controls over the different GOLD stages and between *GC* genotypes were assessed by analysis of variance (ANOVA) after correction for testing multiple subgroups by the Tukey–Kramer posthoc test. To assess determinants of serum 25-OHD in the COPD and healthy subgroups, a stepwise multivariate analysis was performed with FEV_1 and K_{CO} % predicted, age (log transformed) and BMI as continuous variables, and seasonal variation, gender, previous and current use of oral corticosteroids, current smoking status, rs7041 and rs4588 as categorical variables. Pack-years were calculated as the number of years a patient had smoked 20 cigarettes per day. Quit-years were the number of years since a patient stopped smoking. Previous use of oral corticosteroids 3 months before inclusion, as well as current use of oral corticosteroids in maintenance treatment was noted. Season of blood sampling was categorised into three 4-month periods during which the vitamin D load by sun exposure is known to range from low (December–March), to intermediate (April, May, October and November) to high (June–September). The Pearson χ^2 test (1 degree of freedom (1 df)) was also used to test for deviations from Hardy–Weinberg equilibrium. Linkage disequilibrium strength was evaluated with r and the Lewontin D' statistic between rs7041 and rs4588. To test the association between rs7041 and rs4588 allelic variants and COPD, a standard χ^2 test (1 df) was used. Logistic regression analysis with COPD as a response variable was used, while considering rs7041 as a dependent variable and correcting for age, gender, pack-years, quit-years and 25-OHD concentrations.

RESULTS

Population characteristics

In total, 414 individuals were included. All baseline characteristics of these individuals are summarised in table 1. Two hundred and sixty-two patients had COPD according to GOLD criteria; 152 individuals had a normal postbronchodilator pulmonary function and were considered as controls. Both groups exhibited a similar gender distribution and an equal percentage of current

Table 1 Baseline characteristics of the study population

	Patients with COPD (n=262)	Healthy smokers (n=152)
Demographics		
Age† (years)	66 (60–72)	61 (58–65)*
Gender (M/F)	215/49	120/32
Body mass index (kg/m^2)	25.3±4.8	26.9±4.1*
Previous steroid use, n (%)	81 (31)	0
Current steroids, n (%)	35 (13)	0
Smoking		
Current smoker, n (%)	125 (47)	68 (44)
Pack-years†	47 (33–63)	39 (30–52)*
Quit-years†	1 (0–8)	2 (0–8)
Pulmonary function tests		
FEV_1, litres	1.8±0.9	3.2±0.8*
FEV_1, % predicted	61±27	104±15*
FVC, litres	3.4±1.0	4.1±0.9*
FVC, % predicted	93±22	110±14*
DL_{CO}, % predicted	60±21	85±15*
K_{CO}, % predicted	78±23	96±16*
25-OHD (ng/ml)	19.9±8.2	24.6±8.7*
COPD		
GOLD class 1, n (%)	70 (27)	0
GOLD class 2, n (%)	87 (33)	0
GOLD class 3, n (%)	75 (29)	0
GOLD class 4, n (%)	30 (11)	0

Demographic, smoking and pulmonary function characteristics of patients with COPD and smoking controls are shown. Data are expressed as numbers (group percentages in parentheses) for categorical variables and mean values±SD for continuous variables. When variables were not normally distributed, median values (Q1–Q3 IQR in parentheses) are given instead (indicated by †). Significant p values are indicated by an asterisk (*p<0.05). COPD, chronic obstructive pulmonary disease; DL_{CO}, diffusing capacity of the lung for carbon monoxide; F, female; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; GOLD, Global Initiative for Obstructive Lung Disease; KCO, alveolar ventilation; M, male; 25-OHD, 25-hydroxyvitamin D.

smokers. However, patients with COPD were slightly older and were characterised by an increased number of pack-years (p<0.001; table 1).

25-OHD levels correlate with COPD and severity of COPD

Circulating 25-OHD serum levels were found to correlate significantly with FEV_1 in the COPD subgroup (Pearson r=0.28, p<0.0001; figure 1a). There was no correlation with FEV_1 in the subgroup of healthy smokers. Mean 25-OHD levels also differed significantly between patients with COPD and healthy smokers (19.9±8.2 ng/ml in patients with COPD vs 24.6±8.7 ng/ml in healthy smokers; p<0.0001). Although the mean 25-OHD levels in GOLD 1 (22.4 ng/ml) were not significantly different from those of the control population, average 25-OHD levels further decreased with increasing GOLD stage. Indeed, from GOLD 2 onwards, 25-OHD levels differed significantly from those of healthy smokers (20.35, 18.8 and 16.0 ng/ml for GOLD 2, 3 and 4, respectively; p<0.0001; figure 1b). Mean 25-OHD levels were almost 33% lower in patients with COPD with GOLD 4 compared with healthy smokers (figure 1b).

Stratification of all 414 participants into the three subgroups—that is, participants deficient for vitamin D (<20 ng/ml), participants with 25-OHD levels between 20 and 30 ng/ml and participants with 25-OHD levels >30 ng/ml—revealed that only 31% of the healthy smokers exhibited vitamin D deficiency, whereas 39, 47 and 60%, respectively, of the patients with GOLD stage 1, 2 and 3, and as many as 77% of GOLD stage 4 patients were deficient for vitamin D (figure 1c). In addition, only 2.0% of the healthy controls compared with 4.3, 8.1, 8.0 and 13.3%, respectively, of the patients with a GOLD stage ranging from 1 to 4 were severely vitamin D deficient (<10 ng/ml). Overall, these data clearly indicate that reduced 25-OHD levels are correlated with severity of COPD.

Variants in *GC* determine 25-OHD levels

Since two genetic variants in *GC*—that is, rs7041 and rs4588—have been associated with 25-OHD levels in premenopausal women, we genotyped all study participants for both variants and stratified for COPD. In patients with COPD, TT carriers for rs7041 and AA carriers for rs4588 were associated with significantly lower 25-OHD serum concentrations compared with homozygous carriers of the wild-type alleles (p<0.0001 and p=0.01, respectively; table 2). Intermediate reductions in 25-OHD were noted for heterozygous carriers of the rs7041 allele and the rs4588 allele, but these did not reach the level of significance. In the control population, similar trends were seen, with a significant reduction in 25-OHD levels detectable in rs7041 TT carriers when compared with wild-type GG carriers (p=0.03; table 2).

Since many environmental and disease-associated factors may determine vitamin D status in healthy smokers or patients with COPD, we used a stepwise multivariate analysis to assess whether rs7041 and rs4588 variants were independently associated with 25-OHD levels. We first assessed the effect of rs7041 and rs4588 in separate multivariate analyses. In the healthy smokers subgroup, both rs7041 and rs4588 were associated with 25-OHD when correcting for age, gender, current smoking status, BMI and seasonal variation (p=0.02 and p=0.03; data not shown). A similar analysis in the subgroup of patients with COPD revealed that rs7041 and rs4588 were also associated with 25-OHD concentrations (p=0.0001 and p=0.05; data not shown) after correction for age, gender, smoking status, BMI, seasonal variation, previous and current oral corticosteroid use, and severity of disease (both FEV_1 and K_{CO} expressed as % predicted). We then combined rs7041 and rs4588 in a single multivariate model and assessed whether their effects were independent from each other. In the COPD subgroup, rs7041 was still significantly associated with 25-OHD levels (p<0.0001; table 3), but the association between rs4588 and 25-OHD had disappeared. This is most probably due the fact that the linkage disequilibrium between rs7041 and s4588 is almost complete (D'=1.00, r^2=0.74). The rs4588 variant thus contributes the same information to the multivariate model as the rs7041 variant. The latter can therefore be regarded as the major variant predicting 25-OHD levels.

Since FEV_1 and the rs7041 variant were both independent predictors of reduced 25-OHD levels in patients with COPD, we also related 25-OHD levels to the rs7041 variant after stratification for GOLD severity. TT carriers of rs7041 with COPD were at a higher risk of being vitamin D deficient compared with GG and GT carriers (41/60 vs 94/193; p=0.008). Mean 25-OHD levels in TT carriers for rs7041 decreased significantly with increasing GOLD stage: 19.5±7.2, 17.9±7.9, 15.3±6.4 and 10.9±2.7 ng/ml for GOLD stage 1, 2, 3 and 4, respectively (p=0.03). Strikingly, in GOLD stage 3–4, as many as 73% and 100% of the homozygous carriers were found to be vitamin D deficient (data not shown).

The rs7041 variant in *GC* increases the risk for COPD

Since 25-OHD levels are reduced in patients with COPD and since the rs7041 variant determined 25-OHD levels independently of COPD severity, we assessed whether rs7041 may also constitute a genetic risk for COPD. Homozygosity for the rs7041 T allele increased the risk for COPD significantly in a χ^2 analysis (23/150 vs 60/253; p=0.04). A logistic regression analysis

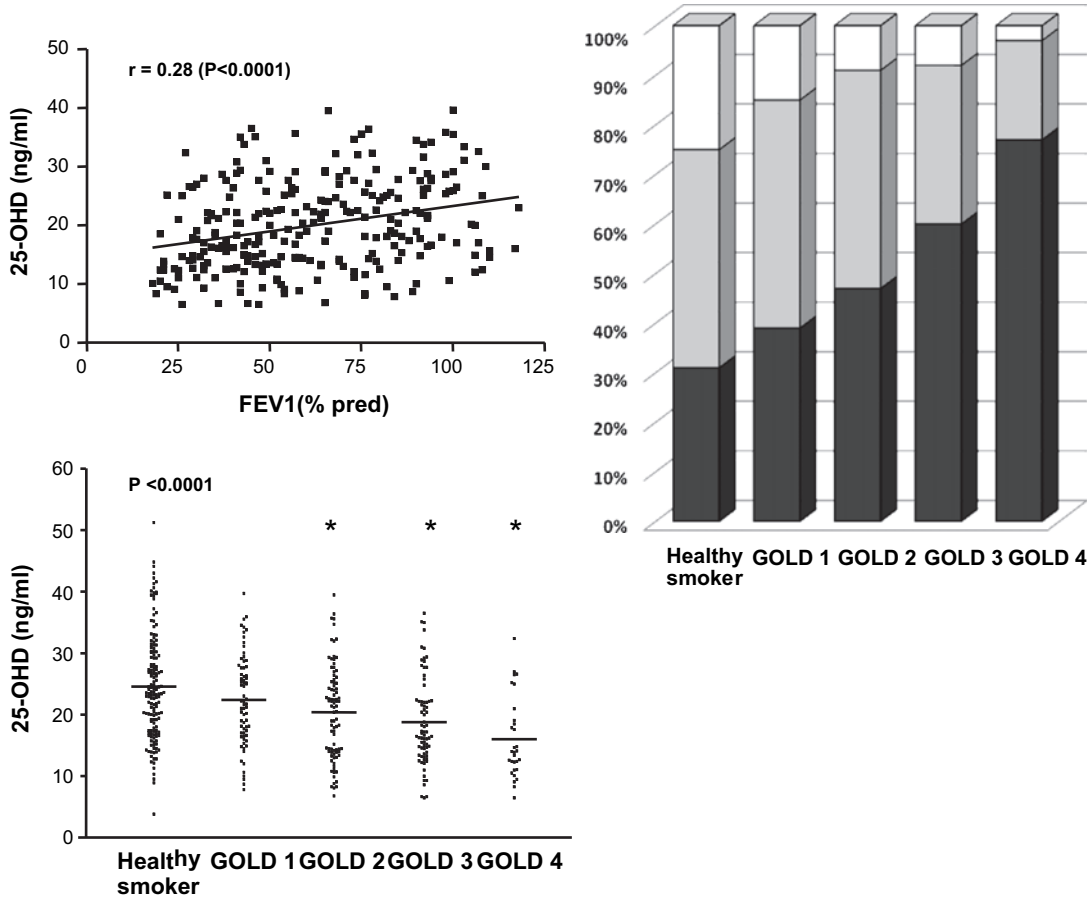

Figure 1 (a) 25-Hydroxyvitamin D (25-OHD) serum levels plotted as a function of the forced expiratory volume in 1 s (FEV_1) levels (% predicted FEV_1 was used). The Pearson coefficient of determination was calculated as indicated. (b) Plot showing 25-OHD levels according to the various GOLD (lobal Initiative for Obstructive Lung Disease) stages. An analysis of variance (ANOVA) univariate test followed by a Tukey–Kramer posthoc test for testing multiple groups was used to calculate significances. Asterisks indicate $p<0.0001$. (c) Percentage of patients having 25-OHD levels >30 ng/ml (white), serum levels between 30 and 20 ng/ml (grey), and deficient serum levels <20 ng/ml (black).

correcting for age, gender and history of smoking also revealed that homozygous carriers of the rs7041 T allele exhibited a significantly increased risk for COPD (OR 2.11; 95% CI 1.20 to 3.71; p=0.009; table 4). Heterozygous carriers of a single at-risk rs7041 T allele did not exhibit an increased risk, which is consistent with the much weaker association of heterozygous rs7041 carriers with reduced 25-OHD levels. In agreement with the observation that rs4588 was not an independent determinant of 25-OHD levels, there was also no association with the rs4588 variant and the risk for COPD (data not shown). Intriguingly, when correcting the logistic regression model for 25-OHD levels, homozygosity for the rs7041 risk variant was also no longer a significant predictor of COPD. Furthermore, logistic regression allowing for an interaction between rs7041 and pack-years, years of smoking, quit-years, BMI and seasonal variation failed to

Table 2 Univariate analysis of variance comparing mean 25-OHD serum levels according to the different genotypes of the vitamin D-binding gene (GC)

	rs Number	Genotype	Subjects, n (%)	Serum 25-OHD mean±SD	p Value
COPD	rs7041	GG	85 (34)	22.1±7.8	Reference
		GT	108 (43)	20.0±7.0	0.12
		TT	60 (23)	16.7±7.2	<0.0001
	rs4588	CC	128 (51)	21.0±7.9	Reference
		CA	109 (43)	19.2±7.0	0.15
		AA	16 (6)	15.6±7.2	0.01
Controls	rs7041	GG	54 (36)	26.3±8.7	Reference
		GT	73 (49)	24.6±9.1	0.5
		TT	23 (15)	20.9±5.0	0.03
	rs4588	CC	88 (59)	25.8±8.8	Reference
		CA	49 (33)	23.8±8.6	0.09
		AA	13 (8)	20.6±4.6	0.09

Univariate analysis of variance (ANOVA) with a Tukey–Kramer posthoc test was performed to calculate the mean 25-hydroxyvitamin D (25-OHD) serum levels (ng/ml) stratified according to rs7041 and rs4588 genotypes in patients with chronic obstructive pulmonary disease (COPD) (n=253) and controls (n=150). p Values indicate significance of the genotypes relative to wild-type genotypes.

Table 3 Stepwise multivariate analysis assessing the variance of serum 25-OHD levels in COPD

Explanatory variables	Parameter estimate (β) (95% CI)	P value
Intercept	15 (9.8 to 21)	<0.0001
FEV_1, % predicted	0.087 (0.05 to 0.12)	<0.0001
Seasonal variation	1.9 (0.79 to 3.0)	0.0009
rs7041 variant	−4.5 (−6.5 to −2.4)	<0.0001
Body mass index	−0.27 (−0.47 to −0.07)	0.009
K_{CO}, % predicted	0.043 (0.01 to 0.08)	0.04

Only variables which contributed significantly to the variance of serum 25-OHD in patients with COPD (n=252) are shown. Non-significant variables were removed from the model and are not shown. These include: current smoking, current and previous use of oral corticosteroids, age, gender and rs4588 variants.
COPD, chronic obstructive pulmonary disease; FEV_1, forced expiratory volume in 1 s; K_{CO}, alveolar ventilation; 25-OHD, 25-hydroxyvitamin D.

Table 4 Binary logistic regression analysis to assess the association between presence or absence of COPD and the rs7041 genetic variant (under a recessive model of inheritance) (n=403)

	Risk of COPD			
	Model without 25-OHD		Model with 25-OHD	
	OR (CI)	p Value	OR (CI)	p Value
25-OHD			0.94 (0.91 to 0.96)	<0.0001
Age	1.10 (1.06 to 1.13)	<0.0001	1.09 (1.06 to 1.14)	<0.0001
Pack-years	1.02 (1.01 to 1.03)	0.003	1.01 (1.00 to 1.02)	0.05
rs7041	2.11 (1.20 to 3.71)	0.009	–	–
Quit-years	–	–	–	–
Gender	–	–	–	–

A model without 25-OHD (ng/ml) as an explanatory variable is compared with a model in which 25-OHD was included. Non-significant covariates quit-years, gender and rs7041 (in the model with 25-OHD) are listed in the table but not included in the final represented model. COPD, chronic obstructive pulmonary disease; 25-OHD, 25-hydroxyvitamin D.

reveal any significant interaction (not shown). Overall, this suggests that the rs7041 variant, through its association with reduced 25-OHD levels, was correlated with the presence of COPD.

DISCUSSION

This study demonstrates that vitamin D deficiency, as assessed by 25-OHD levels in serum, is common in patients with COPD and correlates with the severity of disease as measured by FEV_1. In a large cohort, we show that patients with COPD are more likely to suffer from vitamin D deficiency than matched healthy smokers. Indeed, a non-significant trend towards reduced 25-OHD levels was already apparent in GOLD 1, and 25-OHD levels further decreased significantly from GOLD 2 onwards, resulting in vitamin D deficiency in the majority of patients with severe COPD. Comparable results were found in a population-based study—the third National Health and Nutrition Examination Survey (NHANES; cross-sectional survey on 14 091 healthy US civilians >20 years of age).[12] After adjustment for potential confounders, a strong relationship between serum levels of 25-OHD and pulmonary function, as assessed by FEV_1 and FVC, was found in this study. Although the authors did not report a significant correlation with COPD, the association between FEV_1 and 25-OHD levels tended to be slightly stronger in the smoking subgroup. Our current data therefore extend the population-based association between FEV_1 and 25-OHD levels from NHANES to a cohort of patients with COPD and, most importantly, establish that a significant subgroup of patients with COPD is deficient for vitamin D.

Although there is no consensus regarding the optimal 25-OHD serum levels, vitamin D deficiency is defined by most experts as a 25-OHD level <20 ng/ml. Levels below this threshold are experienced by the parathyroids as being insufficient.[26] Since vitamin D deficiency increases the risk for osteoporosis and osteoporotic fractures, vitamin D supplementation, at least to levels >20 ng/ml, should be strongly considered for all patients suffering from vitamin D deficiency in order to prevent osteoporosis.[27] As patients in GOLD stages 3 and 4 were vitamin D deficient in >60–77% of cases, standard vitamin D supplementation without routine measurement of 25-OHD levels might be considered appropriate in these patients. When vitamin D supplementation is also being considered for its beneficial anti-inflammatory, anti-infectious and muscle-enforcing effects,[3 28 29] one would have to speculate about the ideal target range of 25-OHD levels. Several experts have suggested that much higher levels than 20 ng/ml might be needed.[30] If this is true, the vast majority of our patients would then become candidates for intervention.

Vitamin D status measured by circulating 25-OHD reflects the dynamic equilibrium between vitamin D synthesis in the skin by sun exposure, vitamin D intake via food or dietary supplements and vitamin D degradation by catabolising enzymes. Up to 99% of 25-OHD is bound to plasma proteins, of which >90% is bound to the specific vitamin D-binding protein (DBP).[31] Patients with COPD can be considered at high risk for vitamin D deficiency because they are more prone to skin ageing (due to smoking), are involved in reduced outdoor activity, exhibit reduced food intake, and are often treated with corticosteroids, which increases vitamin D catabolism.[3] As such, vitamin D deficiency should be regarded as the consequence of COPD, and more severe types of COPD would be expected to result in more severe reductions in vitamin D. Multivariate analysis in our COPD group indeed demonstrated that 25-OHD levels are determined not only by oral substitution, seasonal variation and BMI, but also by FEV_1 and diffusion capacity, which reflect the severity and duration of the disease. However, after adjustment for FEV_1 and diffusion capacity, we found that genetic variants in *GC* still correlated significantly with 25-OHD levels. Similar associations have recently been shown in premenopausal women, in which the rs7041 and rs4588 polymorphisms were associated with reduced 25-OHD levels after correction for leisure time physical activity (as a surrogate for sun exposure), calcium/vitamin D intake and education.[18] Compared with premenopausal and postmenopausal women,[18 32] our study predominantly evaluated men of older age, whose 25-OHD levels were far more reduced, indicating that the observed association was population independent. Overall, this indicates that patients with COPD, especially those who carry two T alleles of the rs7041 variant, were at high risk for vitamin D deficiency. An important question is whether oral vitamin D supplementation will work for patients with such a genetic defect in *GC*. A recent study revealed that vitamin D supplementation in homozygous carriers of the rs4588 A allele, which are in near complete linkage with the at-risk T allele of rs7041, resulted in large increases of 25-OHD levels.[33] These data suggest that TT carriers of the rs7041 variant are indeed sensitive to vitamin D supplementation.

Epidemiological and mechanistic evidence in humans indicates that vitamin D deficiency is associated with many chronic diseases, such as cardiovascular disease, autoimmune disease, cancer and chronic infections.[1 3] Many of these diseases are also considered to be co-morbidities of COPD. Possibly, chronically reduced 25-OHD serum levels in patients with COPD may negatively affect these co-morbidities, once COPD is established, thereby promoting disease progression.[4] However, evidence also indicates that vitamin D may causally contribute to different chronic disorders.[34 35] Moreover, given the population-based association between vitamin D deficiency and incidence of upper respiratory tract infections in healthy individuals,[36] and given its association with asthma severity in childhood,[37] it is tempting also to consider vitamin D deficiency as a risk factor for COPD by dysregulation of adaptive and innate immunity. In our sample we found that the percentage of patients carrying two at-risk rs7041 alleles significantly increased from 15% in the control group to 24% in patients with COPD. The association between the risk rs7041 alleles and COPD was more pronounced after adjustment for others risk factors of COPD. When corrected for 25-OHD levels, however, it was no longer significant, suggesting that the risk effect of rs7041 was determined by reduced 25-OHD levels.

Our population is a homogeneous selected patient cohort, which limits the general applicability of the data. Genetic replication studies, involving genome-wide analyses and population-based sample cohorts, are needed to confirm the

association of rs7041 with the risk for COPD.[38–40] Furthermore, since the observed association between *GC* genetic variants and COPD does not prove causality between vitamin D deficiency and COPD, population-based prospective follow-up studies[39] and placebo-controlled intervention studies,[4] which are properly designed to do so, should be conducted. Obviously, it is important to assess this, since vitamin D supplementation could represent an attractive therapeutic option to prevent disease progression, well before the consequences of COPD and ageing further reduce 25-OHD levels.

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Competing interests None.

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