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Prevalence, determinants and clinical correlates of vitamin D deficiency in patients with Chronic Obstructive Pulmonary Disease in London, UK

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ABSTRACT

Vitamin D deficiency is common in patients with chronic obstructive pulmonary disease (COPD), yet a comprehensive analysis of environmental and genetic determinants of serum 25-hydroxyvitamin D (25[OH]D) concentration in patients with this condition is lacking. We conducted a multi-centre cross-sectional study in 278 COPD patients aged 41–92 years in London, UK. Details of potential environmental determinants of vitamin D status and COPD symptom control and severity were collected by questionnaire, and blood samples were taken for analysis of serum 25(OH)D concentration and DNA extraction. All participants performed spirometry and underwent measurement of weight and height. Quadriceps muscle strength (QS) was measured in 134 participants, and sputum induction with enumeration of lower airway eosinophil and neutrophil counts was performed for 44 participants. Thirty-seven single nucleotide polymorphisms (SNP) in 11 genes in the vitamin D pathway (*DBP, DHCR7, CYP2R1, CYP27B1, CYP24A1, CYP27A1, CYP3A4, LRP2, CUBN, RXRA, and VDR*) were typed using Taqman allelic discrimination assays. Linear regression was used to identify environmental and genetic factors independently associated with serum 25(OH)D concentration and to determine whether vitamin D status or genetic factors independently associated with % predicted forced expiratory volume in one second (FEV_1), % predicted forced vital capacity (FVC), the ratio of FEV_1 to FVC (FEV_1:FVC), daily inhaled corticosteroid (ICS) dose, respiratory quality of life (QoL), QS, and the percentage of eosinophils and neutrophils in induced sputum. Mean serum 25(OH)D concentration was 45.4 nmol/L (SD 25.3); 171/278 (61.5%) participants were vitamin D deficient (serum 25[OH]D concentration <50 nmol/L). Lower vitamin D status was independently associated with higher body mass index ($P = 0.001$), lower socio-economic position ($P = 0.037$), lack of vitamin D supplement consumption ($P < 0.001$), sampling in Winter or Spring (P for trend = 0.006) and lack of a recent sunny holiday ($P = 0.002$). Vitamin D deficiency associated with reduced % predicted FEV_1 (P for trend = 0.060) and % predicted FVC (P for trend = 0.003), but it did not associate with FEV_1:FVC, ICS dose, QoL, QS, or the percentage of eosinophils or neutrophils in induced sputum. After correction for multiple comparisons testing, genetic variation in the vitamin D pathway was not found to associate with serum 25(OH)D concentration or clinical correlates of COPD severity.

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Vitamin D deficiency was common in this group of COPD patients in the UK, and it associated independently with reduced % predicted FEV1 and FVC. However, genetic variation in the vitamin D pathway was not associated with vitamin D status or severity of COPD.

1. Introduction

Vitamin D deficiency is consistently reported to be more common in patients with chronic obstructive pulmonary disease (COPD) than in healthy controls [1–3], and is associated with reduced forced expiratory volume in one second (FEV_1) [3–8], forced vital capacity (FVC), peak expiratory flow (PEF), respiratory quality of life (QoL) [4], increased daily inhaled corticosteroid (ICS) dose and hypoxaemia [8]. Findings from studies which have investigated the impact of vitamin D status on exercise capacity or skeletal muscle strength are conflicting, with some studies reporting inverse associations [6,9] and others studies reporting null effects [7,10].

In a recent clinical trial we found vitamin D supplementation to significantly reduce risk of COPD exacerbation in those who were deficient at baseline (<50 nmol/L) [11]. However the determinants of vitamin D deficiency in COPD patients, and the clinical correlates of disease severity are not well defined: raised neutrophil and eosinophil counts and increased concentrations of inflammatory mediators secreted by these cells have been identified in the lower airways during COPD exacerbation [12–14], but to our knowledge, there are no previous cross-sectional investigations of serum 25 (OH)D concentration and differential white cell counts in induced sputum in patients with COPD. Furthermore, environmental and genetic factors which have been shown to influence vitamin D status in healthy populations [15,16] have not yet been well characterised in COPD patients, nor have genetic factors which may associate with clinical markers of COPD severity.

We therefore conducted a cross-sectional analysis to determine the prevalence of vitamin D deficiency in COPD patients living in London, UK, and to identify genetic and environmental determinants of vitamin D status in this group. We also conducted analyses to explore whether vitamin D status or genetic variation in the vitamin D pathway associated with any clinical features of COPD, either independently or in interaction with each other.

2. Methods

2.1. Participants

Adult patients with a medical record diagnosis of COPD, emphysema or chronic bronchitis were identified by database searches at 60 general practices and COPD clinics in 4 Acute National Health Service Trusts in London, UK, and invited for screening as previously described [11]. The study was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/76) and written informed consent was obtained from all participants before enrolment.

2.2. Procedures

Respondents were asked to complete a lifestyle questionnaire detailing age, sex, ethnicity, self-reported Fitzpatrick skin-type [17], self-classified socio-economic position (SEP) using the National Statistics – Socio-Economic Classification (NS-SEC) method [18], number of hours spent outdoors each day, history of recent sunny holidays abroad (defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, up to 2 months prior to blood draw, for a duration of ≥1 week), smoking behaviour and consumption of alcohol and supplemental vitamin D. Respondents also completed the St. George's Respiratory Questionnaire (SGRQ) [19] and underwent a baseline clinical assessment including the following: spirometry before and after inhalation of 400 μg salbutamol via a spacer device, performed using a MicroLab ML3500 desktop spirometer (CareFusion GmbH, Hoechberg, Germany) with calculation of% predicted FVC and FEV_1 values according to American Thoracic Society (ATS)/European Respiratory Society (ERS) recommendations [20,21]; height measurement (using a Seca 220 Telescopic Measuring Rod, Seca, Hamburg, Germany); and weight measurement (using Marsden MMPS-250 column scales, Marsden, Rotherham, UK). A blood sample was collected for subsequent DNA extraction and determination of serum concentration of total 25(OH)D and parathyroid hormone (PTH). One sub-set of 134 participants underwent measurement of leg quadriceps strength using a Nicholas manual muscle tester (Lafayette Instruments, US). Another sub-set of 44 participants underwent sputum induction with hypertonic saline, and their samples were processed to make cytospin slides according to methods described by Pizzichini et al. [22]. Participants' COPD severity was categorised according to Global Initiative on Obstructive Lung Disease (GOLD) stages: mild/GOLD stage 1 (FEV_1 ≥80%), moderate/GOLD stage 2 (FEV_1 50–79%), severe/GOLD stage 3 (FEV_1 30–49%), and very severe/GOLD stage 4 (FEV_1 <30%).

2.3. Single nucleotide polymorphism panel selection

A literature search of the PubMed database was performed to identify SNP previously shown to associate with serum 25(OH)D concentration and/or non-skeletal disease, described here [23]. We identified 55 such SNP in 11 genes in the vitamin D pathway. Based on linkage disequilibrium (LD) relationships between these SNP, we selected 6 tagging SNP (tSNP) [24], using a r^2 threshold of 0.8, which reduced the number of genotyped SNP to 37.

2.4. Laboratory analyses

Serum concentrations of $25(OH)D_2$ and $25(OH)D_3$ were determined by isotope-dilution liquid chromatography–tandem mass spectrometry [14] in the Department of Clinical Biochemistry at Homerton Hospital, and summed to give total serum 25(OH)D concentration. This laboratory participates in the international vitamin D external quality assurance scheme (www.deqas.org/). PTH concentrations were determined using an Architect ci8200 analyser (Abbott Diagnostics, Chicago, IL, USA). DNA was extracted from whole blood using a salting-out method [15] on the Biomek FX robot (Beckman Coulter), quantified using the Nanodrop spectrophotometer and normalised to 5 ng/μl. 10 ng DNA were used as template for 2 μl TaqMan assays (Applied Biosystems, Foster City, CA, USA) performed on the ABI 7900HT platform in 384-well format and analysed with Autocaller software as previously described [25]. Typing for two SNP failed (rs6127118, CYP24A1 and rs11574010, VDR).

2.5. Statistical analyses

Using STATA 12 we performed unpaired Student's *T*-tests or one-way ANOVA tests on normally distributed dependent

variables (serum 25[OH]D concentration, % predicted FEV_1, % predicted FVC, QS, SGRQ score, and % neutrophils in induced sputum) and Mann-Whitney or Kruskal-Wallis tests on non-normally distributed dependent variables (FEV_1:FVC, ICS dose, and % eosinophils in induced sputum) to identify correlates of serum 25 (OH)D concentration, vitamin D pathway SNP, and clinical features

Table 1
Participant Characteristics.

		N = 278
Sex, n (%)	Female	109 (39.2)
	Male	169 (60.8)
Mean Age, yrs (range)		66.4 (40.8–91.9)
Mean BMI, kg/m^2 (s.d.)		27.7 (6.8)
Ethnicity, n (%)[a]	White	262 (94.2)
	Asian/Asian British	5 (1.8)
	Black/Black British	2 (0.7)
	Mixed	5 (1.8)
Fitzpatrick Skin-type, n (%)[b]	1	35 (12.8)
	2	56 (20.5)
	3	137 (50.2)
	4	29 (10.6)
	5	15 (5.5)
	6	1 (0.4)
Socio-economic Position, n (%)[c]	1	81 (29.7)
	2	24 (8.8)
	3	42 (15.4)
	4	62 (23.7)
	5	64 (23.4)
Time outdoors, hours/day (%)[d]	>2 h	130 (47.6)
	≤2 h	143 (52.4)
Daily vitamin D supplements, n (%)[e]	Any	52 (19.3)
	None	217 (80.7)
Quarter of sampling, n (%)	Q1 (Jan–Mar)	82 (29.5)
	Q2 (Apr–Jun)	55 (19.8)
	Q3 (Jul–Sep)	69 (24.8)
	Q4 (Oct–Dec)	72 (25.9)
Smoking status, n (%)	Non-current	169 (60.8)
	Current	109 (39.2)
Mean alcohol, units/week (s.d.)[f]		10.9 (18.3)
Managed exclusively in primary care, n (%)		202 (72.7)
Medication use	Short-acting bronchodilator	203 (73.0)
	Long-acting β agonist	30 (10.8)
	Inhaled corticosteroid and long-acting β agonist combination[j]	140 (50.4)
	Inhaled corticosteroid only	41 (14.7)
	Tiotropium	98 (35.3)
	Mean daily inhaled corticosteroid dose at entry in betamethasone equivalents, μg (s.d.)[k]	698.9 (723.7)
Mean serum corrected calcium (s.d.)[g]		2.25 (0.09)
Recent sunny holiday, n (%)[h]	Yes	16 (5.9)
	No	257 (94.1)
Tanning bed use in previous yr, n (%)[i]	Yes	4
	No	269
GOLD Stage	I	69 (24.8)
	II	131 (47.1)
	III	62 (22.3)
	IV	16 (5.8)
Mean serum PTH (s.d.)		5.9 (3.0)
Serum PTH >6.8 pmol/L, n (%)	Yes	68 (24.5)
	No	210 (75.5)
Serum 25(OH)D, nmol/L (%)	≥75	33 (11.9)
	50–74.9	74 (26.6)
	25–49.9	110 (39.6)
	<25	61 (21.9)
Mean serum 25(OH)D (s.d.)		45.3 (25.4)

[a] Ethnicity not reported in n = 4. Mixed ethnicity participants: n = 3 'White and Black Caribbean'; n = 1 'White and Black African'; n = 1 'White and Asian'.
[b] Fitzpatrick skin-type score not reported in n = 5. Categories defined as: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.
[c] SEP not reported in n = 5. Classes defined as: 1 = managerial, administrative and professional occupations, 2 = intermediate occupations, 3 = small employers and own account workers, 4 = lower supervisory and technical occupations, 5 = semi-routine and routine occupations, 6 = student, 7 = Never/Long-term (>5 yrs) unemployed.
[d] Time outdoors not reported in n = 5.
[e] Vitamin D supplementation consumption not reported in n = 9.
[f] Alcohol consumption not reported in n = 13.
[g] Corrected calcium not measured in n = 5.
[h] Recent sunny holiday not reported in n = 5. Defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.
[i] Tanning bed use not reported in n = 5.
[j] Includes combinations of ICS/LABA and LABA.
[k] 1 μg betamethasone assumed equivalent to 1 μg budesonide, 0.5 μg fluticasone dipropionate and 0.75 μg ciclesonide.

of COPD severity on univariate analysis. All dependent variables were continuous. Non-normally distributed dependent variables had positively skewed distributions, and were transformed to their natural logarithms for multivariate analyses. All factors with a minimum of 5 participants at each level were then fitted in a multivariate model to give adjusted regression coefficients, along with a 95% confidence interval and a P value for pairwise association in variables with 2 categories, or a P value for trend for variables with ≥3 categories. For the analysis of association between vitamin D pathway SNP and clinical correlates of COPD severity, a P value for the interaction between SNP genotype and baseline vitamin D status was also calculated. In the case of log-transformed dependent variables, the anti-logs of adjusted regression coefficients are presented. Genetic analyses were adjusted for all putative environmental determinants of serum 25(OH)D concentration investigated (for 15 SNP previously found to associate with vitamin D status), or clinical correlates of COPD severity (for 35 SNP previously found to associate with vitamin D status and/or non-skeletal disease outcomes). Multiple comparison testing was then applied using the Benjamini & Hochberg method with a false discovery rate (FDR) of 5% [16]. All environmental and genetic independent variables were classified as categorical variables. All SNP were analysed under an additive model.

3. Results

3.1. Study population

A total of 278 adult COPD patients were enrolled in the study between 11th September 2009 and 12th April 2012. All consented to undergo clinical measurements and to donate blood samples for quantification of serum 25(OH)D and PTH concentration; 277/278 consented to donate a blood sample for DNA storage and genotyping. Participant characteristics are presented in Table 1. Age range was 40.8–91.9 years, with a mean of 66.4 years (SD 9.5). Most participants (60.8%) were male. The majority of participants (94.2%) classified their ethnic origin as being White; 1.8% classified

Table 2
Environmental determinants of serum 25-hydroxyvitamin D concentration in COPD patients.

		N	Serum 25(OH)D, nmol/L		Univariate P value[a]	Multivariable model – Beta Coefficient (95% CI)	P value[b]
			Mean (s.d.)	Mean difference			
Sex	Female	109	49.1 (29.2)	referent	0.049	referent	0.52
	Male	169	42.9 (22.3)	−6.2		−2.1 (−8.6 to 4.4)	
Age quartiles	1 (40.8–60.3 yrs)	69	45.3 (27.3)	referent	0.68	referent	0.84[†]
	2 (60.5–65.3 yrs)	70	47.1 (23.8)	+1.8		+1.5 (−6.9 to 9.9)	
	3 (65.3–72.3 yrs)	69	46.6 (28.3)	+1.3		0.0 (−8.5 to 8.5)	
	4 (72.4–91.9 yrs)	70	42.3 (21.9)	−3.0		−0.4 (−9.3 to 8.4)	
BMI, kg/m^2	<25	109	48.9 (29.4)	referent	0.058	referent	0.001
	≥25	169	43.0 (22.1)	−5.9		−10.1 (−16.2 to −4.0)	
Ethnicity[c]	White	262	45.9 (25.7)	referent	0.29	referent	0.29
	Other	12	37.9 (17.2)	−8.0		−16.4 (−46.7 to 13.9)	
SEP[d]	Higher	105	50.0 (28.0)	referent	0.029	referent	0.037
	Lower	168	43.1 (23.2)	−6.9		−6.6 (−12.8 to −0.4)	
Time outdoors, h/day[e]	>2	130	50.5 (27.7)	referent	0.003	referent	0.069
	≤2	143	41.5 (22.2)	−9.0		−5.5 (−11.5 to 0.4)	
Vitamin D supplements[f]	Any	52	57.8 (27.8)	referent	<0.001	referent	<0.001
	None	217	42.8 (24.1)	−15.0		−12.4 (−19.6 to −5.2)	
Quarter of sampling	Q1 (Jan–Mar)	82	37.2 (23.4)	−15.2	0.001	−14.0 (−22.3 to −5.7)	0.006[†]
	Q2 (Apr–Jun)	55	43.7 (20.0)	−8.7		−8.0 (−16.9 to 0.8)	
	Q3 (Jul–Sep)	69	52.4 (24.5)	referent		referent	
	Q4 (Oct–Dec)	72	49.0 (29.5)	−3.4		−3.0 (−11.2 to 5.2)	
Fitzpatrick skin-type[g]	1,2	91	44.8 (24.7)	−1.9	0.73	−4.0 (−10.4 to 2.4)	0.42[†]
	3,4	166	46.7 (26.0)	referent		referent	
	5,6	16	42.3 (22.6)	−4.4		+7.2 (−19.7 to 34.0)	
Smoking status	Non-current	169	45.4 (22.2)	referent	0.97	referent	0.15
	Current	109	45.3 (29.8)	−0.1		−4.7 (−11.1 to 1.7)	
Alcohol consumption, units/wk[h]	0	121	47.4 (26.4)	referent	0.42	referent	0.49[†]
	1–20	98	44.1 (22.6)	−3.3		−5.3 (−12.0 to 1.4)	
	>20	46	42.4 (26.4)	−5.0		−2.9 (−11.2 to 5.5)	
Recent sunny holiday[i]	Yes	16	67.4 (36.0)	referent	<0.001	referent	0.002
	No	257	44.4 (24.0)	−23.0		−20.2 (−32.7 to −7.6)	
Tanning bed use, previous yr	Yes	4	90.8 (34.6)	referent	<0.001	[*]	
	No	269	45.1 (24.7)	−45.7			

[a] Univariate method: Student's *T*-test/One-way ANOVA.
[b] Adjusted for all potential determinants of 25(OH)D concentration included in univariate analysis.
[c] Ethnicity not reported in n = 4.'Other' ethnicity defined as: n = 5 Asian/Asian British, n = 2 Black/Black British, n = 5 mixed ethnicity.
[d] SEP not reported in n = 5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).
[e] Time outdoors not reported in n = 5.
[f] Vitamin D supplementation consumption not reported in n = 9.
[g] Fitzpatrick skin-type score not reported in n = 5. Categories defined as: 1 = extremely fair skin; always burn, never tan, 2 = fair skin; always burn, sometimes tan, 3 = pale skin; sometimes burn, always tan, 4 = olive skin; rarely burn, always tan, 5 = moderately pigmented brown skin; never burn, always tan, 6 = markedly pigmented black skin; never burn, always tan.
[h] Alcohol consumption not reported in n = 13.
[i] Recent sunny holiday not reported in n = 5. Defined as a trip to any location within a latitude 51° North/South of the equator, during the local sunny season, 2 months prior to blood draw, for a duration of ≥1 week.
[†] P value for trend.
[*] Tanning bed use omitted from multivariable analysis due to <5 participants in 'yes' category.

their ethnic origin as Asian/Asian British; 0.7% as Black/Black British; 1.8% as being of mixed ethnicity; and 1.4% preferred not to report their ethnicity. Participants' COPD was managed exclusively in primary care in 202/278 (72.7%). Mean serum 25(OH)D concentration for all participants was 45.3 nmol/L (SD 25.4). Sixty-one participants (21.9%) had serum 25(OH)D concentration <25 nmol/L; 110 (39.6%) had serum 25(OH)D concentration in the range 25–49.9 nmol/L; 74 (26.6%) had serum 25(OH)D concentration in the range 50–74.9 nmol/L; and 33 (11.9%) had serum 25(OH)D concentration ≥75 nmol/L.

3.2. Environmental determinants of serum 25(OH)D concentration

Environmental determinants of vitamin D status are presented in Table 2. Multiple linear regression analysis showed the following factors to independently associate with lower serum 25(OH)D concentration: higher BMI (adjusted mean difference of −10.1 nmol/L for <25 kg/m^2 vs. ≥25 kg/m^2; 95% CI −16.2 to −4.0 nmol/L; P = 0.001); lower SEP (adjusted mean difference of −6.6 nmol/L for lower vs. higher classes; 95% CI −12.8 to −0.4 nmol/L; P = 0.037); lack of vitamin D supplement consumption (adjusted mean difference of −12.4 nmol/L for those taking no supplement vs. those taking any supplement; 95% CI −19.6 to −5.2 nmol/L; P < 0.001); sampling in January–March vs. July–September (adjusted mean difference −14.0 nmol/L; 95% CI −22.3 to −5.7 nmol/L; P for trend = 0.006); and lack of a recent sunny holiday abroad (adjusted mean difference of −20.2 nmol/L for those without vs. with such a holiday; 95% CI −32.7 to −7.6 nmol/L; P = 0.002).

3.3. Genetic determinants of serum 25(OH)D concentration

Genetic determinants of vitamin D status are presented in Table 3. After adjusting for sex, age, ethnicity, BMI, SEP, number of hours spent outdoors, quarter of sampling, vitamin D supplement

Table 3
Genetic determinants of serum 25-hydroxyvitamin D concentration in COPD patients.

Gene	SNP	Genotype	N[a]	Serum 25(OH)D, nmol/L		Multivariable model – Beta Coefficient (95% CI)	P Value for trend[b]
				Mean (s.d.)	Mean difference		
CYP24A1	rs6013897	TT	180	44.3 (25.5)	referent	referent	0.54
		AT	78	47.3 (25.2)	+3.0	+3.3 (−3.1 to 9.8)	
		AA	13	43.5 (21.1)	−0.8	+4.5 (−9.8 to 18.7)	
	rs2248137	CC	96	46.4 (29.4)	referent	referent	0.49
		CG	144	44.0 (23.1)	−2.4	+2.5 (−4.2 to 9.1)	
		GG	33	45.0 (21.7)	−1.6	+3.4 (−6.2 to 13.0)	
DBP	rs16846876	AA	124	47.2 (24.3)	referent	referent	0.41
		AT	126	43.3 (26.8)	−3.9	−2.6 (−8.9 to 3.6)	
		TT	23	42.2 (22.3)	−5.0	−2.2 (−13.2 to 8.8)	
	rs7041	GG	65	50.3 (28.8)	referent	referent	0.010
		TG	147	44.0 (25.5)	−6.3	−6.3 (−13.4 to 0.9)	
		TT	57	40.9 (19.7)	−9.4	−11.6 (−20.4 to −2.9)	
	rs12512631	TT	113	44.2 (23.4)	referent	referent	0.12
		CT	130	45.3 (26.6)	+1.1	+0.2 (−6.2 to 6.6)	
		CC	28	48.7 (26.0)	+4.5	+8.2 (−2.2 to 18.5)	
	rs4588	CC	130	48.5 (26.1)	referent	referent	0.099
		CA	123	42.9 (25.4)	−5.6	−5.1 (−11.2 to 1.1)	
		AA	23	39.9 (20.3)	−8.6	−9.2 (−20.1 to 1.7)	
	rs2070741	TT	237	44.7 (25.5)	referent	referent	0.84
		TG	31	49.8 (24.1)	+5.1	+0.4 (−8.8 to 9.6)	
		GG	3	53.3 (19.1)	+8.6	−2.9 (−30.9 to 25.1)	
	rs2298849	AA	181	44.2 (24.5)	referent	referent	0.38
		AG	87	47.1 (27.5)	+2.9	+2.5 (−3.8 to 8.9)	
		GG	7	41.7 (18.1)	−2.5	−8.0 (−25.8 to 9.8)	
CYP27B1	rs4646536	AA	111	44.0 (27.2)	referent	referent	0.65
		AG	128	45.7 (25.2)	+1.7	+0.3 (−6.2 to 6.7)	
		GG	29	47.6 (22.8)	+3.6	+2.4 (−8.0 to 12.7)	
CYP2R1	rs10500804	TT	95	46.6 (28.6)	referent	referent	0.49
		GT	132	45.9 (24.7)	−0.7	−2.6 (−9.2 to 4.1)	
		GG	47	40.7 (21.1)	−5.9	−3.0 (−11.6 to 5.5)	
	rs2060793	GG	95	42.9 (23.6)	referent	referent	0.99
		AG	129	48.1 (48.1)	+5.2	+6.1 (−0.5 to 12.7)	
		AA	49	42.6 (25.2)	−0.3	0.0 (−8.6 to 8.6)	
	rs10766197	GG	79	45.8 (27.8)	referent	referent	0.53
		AG	132	46.0 (24.1)	+0.2	−2.5 (−9.4 to 4.4)	
		AA	57	41.9 (27.8)	−3.9	−2.6 (−10.8 to 5.6)	
DHCR7	rs12785878	TT	171	45.5 (25.7)	referent	referent	0.33
		GT	87	45.4 (25.8)	−0.1	−1.6 (−7.9 to 4.7)	
		GG	15	39.3 (17.9)	−6.2	−7.5 (−22.4 to 7.5)	
	rs3829251	GG	210	45.8 (25.6)	referent	referent	0.22
		AG	54	45.1 (25.1)	−0.7	−1.7 (−8.8 to 5.4)	
		AA	7	31.9 (10.1)	−13.9	−12.0 (−31.5 to 7.4)	
VDR	rs10783219	AA	106	44.6 (24.0)	referent	referent	0.026
		AT	126	46.6 (25.5)	+2.0	−3.4 (−9.8 to 3.1)	
		TT	33	37.8 (23.5)	−6.8	−10.9 (−20.4 to −1.3)	

Abbreviations: DBP: vitamin D binding protein, CYP2R1: cytochrome P450-2R1, CYP24A1: cytochrome P450-24A1, CYP27B1: cytochrome P450-27B1, DHCR7: 7-dehydrocholesterol reductase, VDR: vitamin D receptor, SNP: single nucleotide polymorphism, s.d.: standard deviation, CI: confidence interval.

[a] Genotyping not conducted in n = 1.

[b] Adjusted for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, season of blood draw, Fitzpatrick skin-type, smoking status, alcohol consumption, recent sunny holiday, tanning bed use, and GOLD stage, and corrected for multiple comparisons testing, using the Benjamini & Hochberg method with a 5% false discovery rate.

Table 4
Determinants of% predicted Forced Expiratory Volume in 1 s (FEV_1).

		N	Mean% predicted FEV_1 (SD)	Univariate P value[a]	Multivariable model – beta coefficient (95% CI)	P value[b]
Sex	Female	109	68.6 (20.2)	0.001	referent	0.029
	Male	169	60.4 (20.2)		−6.1 (−11.6 to −0.6)	
Age quartiles	1 (40.8–60.3 yrs)	69	66.8 (19.8)	0.24	referent	0.085[†]
	2 (60.5–65.3 yrs)	70	65.3 (19.2)		−3.0 (−10.3 to 4.2)	
	3 (65.3–72.3 yrs)	69	61.6 (22.5)		−7.2 (−14.6 to 0.2)	
	4 (72.4–91.9 yrs)	70	60.7 (20.3)		−5.5 (−13.0 to 1.9)	
BMI, kg/m^2	<25	109	59.3 (20.8)	0.005	referent	0.004
	≥25	169	66.4 (19.9)		+7.6 (2.4 to 12.8)	
Ethnicity[c]	White	262	63.7 (20.7)	0.52	referent	0.57
	Other	12	59.8 (13.4)		−3.6 (−16.0 to 8.9)	
SEP[d]	Higher	105	68.0 (19.7)	0.007	referent	0.089
	Lower	168	61.1 (20.6)		−4.6 (−10.0 to 0.7)	
Smoking status	Non-current	169	63.3 (21.4)	0.75	referent	0.89
	Current	109	64.1 (19.1)		+0.4 (−5.1 to 5.8)	
Alcohol consumption, units/wk[e]	0	121	63.2 (21.0)	0.76	referent	0.26[†]
	1–20	98	65.2 (18.9)		+3.2 (−2.4 to 8.8)	
	>20	46	64.8 (22.3)		+4.1 (−3.1 to 11.3)	
Influenza vaccination	Yes	255	63.7 (20.6)	0.91	referent	0.71
	No	23	63.1 (19.9)		−1.9 (−11.7 to 7.9)	
Pneumonia vaccination	Yes	179	62.9 (20.1)	0.45	referent	0.35
	No	99	64.9 (21.4)		+2.6 (−2.9 to 8.2)	
Serum 25(OH)D, nmol/L	≥75	33	69.3 (19.4)	0.16	referent	0.060[†]
	74.9–50	74	63.6 (20.0)		−5.8 (−14.4 to 2.8)	
	49.9–25	110	64.3 (20.2)		−4.0 (−12.2 to 4.3)	
	<25	61	59.4 (21.9)		−9.6 (−18.8 to −0.4)	

Definitions: 25(OH)D: 25-hydroxyvitamin D, BMI: Body mass index, SEP: Socio-economic position, CI: Confidence interval, SD: Standard deviation, nmol/L: Nanomoles per litre, SEP: Socio-economic position.
[a] Univariate analysis method:Students *T*-test/One-way ANOVA test.
[b] Adjusted for all potential determinants of FEV_1investigated in univariate analysis.
[c] Ethnicity not reported in n = 4.'Other' ethnicity defined as: n = 5 Asian/Asian British, n = 2 Black/Black British, n = 5 mixed ethnicity.
[d] SEP not reported in n = 5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).
[e] Alcohol consumption not reported in n = 13.
[†] P value for trend.

consumption, recent sunny holiday and GOLD stage, and correcting for multiple comparison testing (Benjamini & Hochberg, FDR = 0.05) none of the SNP investigated was found to associate with serum 25(OH)D concentration.

3.4. Association between vitamin D status and clinical correlates of COPD severity

After adjustment for potential environmental confounders, profound vitamin D deficiency was found to associate independently with reduced% predicted FEV_1 (adjusted mean difference of −9.6% for those with a serum 25(OH)D concentration of <25 nmol/L vs. those with serum 25(OH)D concentration ≥75 nmol/L; 95% CI −18.8 to −0.4%, Table 4), though the P value for trend across all 4 subgroups of vitamin D status was of borderline significance (P = 0.060). Vitamin D status also inversely associated with% predicted FVC (adjusted mean difference of −12.5% for those with serum 25(OH)D concentration of <25 nmol/L vs. those with serum 25(OH)D concentration ≥75 nmol/L; 95% CI −20.7 to −4.2%; P value for trend across all 4 categories of vitamin D status = 0.003, Table 5). We found no statistically significant association between vitamin D status and the other markers of COPD severity investigated, namely: FEV_1:FVC (Table S1), quadriceps strength (Table S2), St. George's respiratory questionnaire score (Table S3), daily inhaled corticosteroid dose (Table S4), percentage of eosinophils in sputum (Table S5), or percentage of neutrophils in sputum (Table S6).

Multiple linear regression analysis revealed several other factors to independently associate with various clinical features of COPD. Higher% predicted FEV_1 associated with higher BMI (Table 4, adjusted mean difference of 7.6% for those ≥25 kg/m^2 vs. <25 kg/m^2; 95% CI 2.4–12.8%; P = 0.004), and with female sex (Table S4, adjusted mean difference of −6.1% lower for males; 95% CI −11.6 to −0.6; P = 0.03). Higher FEV_1:FVC associated with higher BMI (Table S2, adjusted mean difference of 17.0% for those ≥25 kg/m^2 vs. <25 kg/m^2; 95% CI 9.0–26.0%; P < 0.001), and with decreasing age (Table S1, adjusted mean difference of 10.0% lower in the 4th vs. 1st age quartile; 95% CI 0.81–1.00; P value for trend = 0.02). Lower % predicted FVC associated with male sex (Table 5, adjusted mean difference of 11.1% lower for males; 95% CI −16.07 to −6.21; P < 0.001). Diminished quadriceps strength associated with older age (Table S2, adjusted mean difference of 9.3 kg less for 4th vs. 1st age quartile; 95% CI −14.7 to −3.8; P value for trend <0.001) and non-smoking status (Table S2, adjusted mean difference of 4.7 kg higher for smokers vs. non-smokers; 95% CI 0.1–8.5 kg; P = 0.02). Poorer respiratory quality of life, as indicated by lower St. George's Respiratory Questionnaire scores, associated with older age (Table S3, adjusted mean difference of −8.7 points for 4th vs. 1st age quartile; 95% CI −15.6 to −1.8; P value for trend = 0.013). Finally, % eosinophils in sputum positively associated with % eosinophils in peripheral blood (Table S5, adjusted mean difference of 2.7% higher in those with blood eosinophils ≥3% vs. <3%; 95% CI 1.4–5.0; P = 0.003).

3.5. Association between genetic factors and clinical correlates of COPD severity

Genetic determinants of clinical correlates of COPD severity are presented in Table S7 (FEV_1), Table S8 (FVC), Table S9 (FEV_1:FVC), and Table S10 (QS). After correcting for multiple comparisons testing none of the genetic factors which independently associated with markers of COPD severity as main effects, or by interaction with baseline vitamin D status, remained significant.

Table 5
Determinants of% predicted Forced Vital Capacity (FVC).

		N	Mean% predicted FVC (SD)	Univariate P value[a]	Multivariable model – beta coefficient (95% CI)	P value[b]
Sex	Female	109	104.0 (19.0)	<0.001	referent	<0.001
	Male	169	92.2 (17.8)		−11.14 (−16.07 to −6.21)	
Age quartiles	1 (40.8–60.3 yrs)	69	98.6 (19.3)	0.69	referent	0.52[†]
	2 (60.5–65.3 yrs)	70	97.8 (18.6)		−0.77 (−7.29 to 5.75)	
	3 (65.3–72.3 yrs)	69	95.1 (18.7)		−3.57 (−10.21 to 3.08)	
	4 (72.4–91.9 yrs)	70	95.9 (20.1)		−1.36 (−8.05 to 5.32)	
BMI, kg/m^2	<25	109	98.3 (19.7)	0.29	referent	0.40
	≥25	169	95.8 (18.8)		−2.00 (−6.64 to 2.64)	
Ethnicity[c]	White	262	97.5 (19.3)	0.009	referent	0.045
	Other	12	82.7 (9.8)		−11.41 (−22.58 to −0.24)	
SEP[d]	Higher	105	101.5 (19.3)	0.002	referent	0.12
	Lower	168	94.3 (18.6)		−3.80 (−8.59 to 0.98)	
Smoking status	Non-current	169	96.6 (19.4)	0.78	referent	0.60
	Current	109	97.2 (18.8)		−1.29 (−6.19 to 3.61)	
Alcohol consumption, units/wk[e]	0	121	97.2 (19.8)	0.89	referent	0.31[†]
	1–20	98	98.1 (17.5)		+3.45 (−1.56 to 8.46)	
	>20	46	96.7 (20.0)		+3.30 (−3.14 to 9.73)	
Influenza vaccination	Yes	255	96.8 (18.8)	0.94	referent	0.97
	No	23	96.5 (23.5)		−0.19 (−8.97 to 8.59)	
Pneumonia vaccination	Yes	179	97.1 (17.0)	0.75	referent	>0.99
	No	99	96.3 (22.7)		+0.02 (−4.95 to 4.98)	
Serum 25(OH)D, nmol/L	≥75	33	107.4 (18.4)	0.002	referent	0.003[†]
	74.9–50	74	98.2 (18.0)		−7.07 (−14.80 to 0.66)	
	49.9–25	110	95.4 (18.9)		−8.00 (−15.39 to −0.59)	
	<25	61	92.0 (19.5)		−12.46 (−20.70 to −4.21)	

Definitions: 25(OH)D: 25-hydroxyvitamin D, BMI: Body mass index, SEP: Socio-economic position, CI: Confidence interval, SD: Standard deviation, nmol/L: Nanomoles per litre, SEP: Socio-economic position.

[a] Univariate analysis method: Students *T*-test/One-way ANOVA test.

[b] Adjusted for all potential determinants of FVC investigated in univariate analysis.

[c] Ethnicity not reported in n = 4, 'Other' ethnicity defined as: n = 5 Asian/Asian British, n = 2 Black/Black British, n = 5 mixed ethnicity.

[d] SEP not reported in n = 5. 'Higher' defined as classes: 1 (managerial, administrative and professional occupations) and 2 (intermediate occupations), 'Lower' defined as classes: 3 (small employers and own account workers), 4 (lower supervisory and technical occupations), and 5 (semi-routine and routine occupations).

[e] Alcohol consumption not reported in n = 13.

[†] P value for trend.

4. Discussion

To our knowledge this study represents the most comprehensive cross-sectional analysis of environmental and genetic determinants of serum 25(OH)D concentration and clinical correlates of disease severity in patients with COPD. It is also the first to explore the influence of vitamin D deficiency on induced sputum biomarkers of disease severity in this patient group.

We found that vitamin D deficiency was present in the majority of participants, and that lower serum 25(OH)D concentrations were independently associated with increased BMI, lower SEP, lack of vitamin D supplement consumption, sampling in Winter or Spring and lack of recent sunny holidays abroad. Serum 25(OH)D concentration was positively correlated with % predicted FEV_1 (9.6% difference in % predicted FEV_1 corresponded with a difference in mean raw FEV_1 value of 0.21 l; 95% CI −0.49–0.08; P = 0.15, for <25 nmol/L vs. >75 nmol/L group) and % predicted FVC (12.5% difference in % predicted FVC corresponded with a difference in mean raw FVC value of: 0.28 l; 95% CI −0.64–0.08; P = 0.12, for <25 nmol/L vs. >75 nmol/L group), but not with any other measure of disease severity investigated. Genetic variation in the vitamin D pathway was not found to associate with vitamin D status or disease severity.

The findings we present on environmental determinants of vitamin D status are in agreement with previous findings in the healthy UK population [26]. The associations we observed between vitamin D status and indices of spirometric lung function, namely % predicted FEV_1 and % predicted FVC, are also consistent with previous reports [3–7]. Prospective studies with careful measurement of potential confounders are required to establish whether vitamin D deficiency is a cause or effect of reduced lung volumes, or whether this association is due to residual confounding. Our findings of no association between vitamin D status and FEV_1:FVC, or quadriceps strength are in agreement with the majority of previous observational findings [4,6,9,10], whilst the lack of association between vitamin D status and ICS dose we report is in contrast to a case-control study conducted in Norway which found a 5 nmol/L lower mean serum 25(OH)D concentration in COPD cases using ICS vs. those who were not (P = 0.05) [8]. However, the analysis conducted in this study was univariate, so factors which may have confounded the relationship between vitamin D status and ICS dose, such as sex, age, BMI and SEP, were not controlled for.

4.1. Study strengths

Our study has several strengths. We characterised our patients in a considerable level of detail which allowed us to investigate a wide range of potential genetic and environmental determinants of vitamin D status, and to control for a wide range of potential factors which may confound the relationship between vitamin D status and markers of COPD severity. Spirometry was performed using international guidelines and serum 25(OH)D concentrations were measured with the gold standard assay (LC–MS/MS) in a laboratory that participated in the international vitamin D external quality assurance scheme (www.deqas.org/). Our study population included COPD patients with a range of disease severity, who were recruited in both community and hospital settings and across all four seasons – all of which are factors that enhance the generalisibility of our results. Finally, to our knowledge, this was the first study of COPD patients to investigate a cross-sectional relationship between vitamin D status and sputum biomarkers.

4.2. Study limitations

Our study also has some limitations. Due to the high prevalence of vitamin D deficiency in our cohort only a little over 10% of participants had a serum 25(OH)D concentration >75 nmol/L: we may therefore have been underpowered to detect associations between the highest serum concentrations of 25(OH)D and clinical correlates of COPD severity. Type 2 error as a result of limited power may also have been an issue in our analysis of quadriceps strength, which was conducted in a subset of 134 patients, and sputum markers of COPD severity, which was conducted in a subset of 44 patients, and it may have prevented us from detecting modest effects of genetic variation on vitamin D status and disease severity.

In conclusion, our findings highlight a high prevalence of vitamin D deficiency in a UK population of COPD patients, which was independently associated with reduced % predicted FEV1 and % predicted FVC. Vitamin D deficiency was associated with classical environmental determinants as reported in healthy populations, but not with genetic variation in the vitamin D pathway. Our findings will help clinicians to identify COPD patients who are at particular risk of vitamin D deficiency, and allow correction of deficiency where appropriate – an intervention that has now been shown to reduce risk of COPD exacerbations in two clinical trials [11,27].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jsbmb.2017.01.019.

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