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## Prevalence, determinants and clinical correlates of vitamin D deficiency in adults with inhaled corticosteroid-treated asthma in London, UK.

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### Highlights

- Vitamin D deficiency is common among UK adults with ICS-treated asthma
- Classical environmental determinants of vitamin D status operate in this population
- Vitamin D status did not associate with markers of asthma severity or control
- Genetic variation in the vitamin D pathway did not influence vitamin D status or measures of asthma severity or control

### Abstract

Vitamin D deficiency is common in children with asthma, and it associates with poor asthma control, reduced forced expiratory volume in one second (FEV<sub>1</sub>) and increased requirement for inhaled corticosteroids (ICS). Cross-sectional studies investigating the prevalence, determinants and clinical correlates of vitamin D deficiency in adults with asthma are lacking. We conducted a multi-centre cross-sectional study in 297 adults with a medical record diagnosis of ICS-treated asthma living in London, UK. Details of potential environmental determinants of vitamin D status, asthma control and medication use were collected by questionnaire; blood samples were taken for analysis of serum 25(OH)D concentration and DNA extraction, and participants underwent measurement of weight, height and fractional exhaled nitric oxide concentration (FeNO), spirometry and sputum induction for determination of lower airway eosinophil counts (n=35 sub-group). Thirty-five single nucleotide polymorphisms (SNP) in 11 vitamin D pathway genes (*DBP*, *DHCR7*, *RXRA*, *CYP2R1*, *CYP27B1*, *CYP24A1*, *CYP3A4*, *CYP27A1*, *LRP2*, *CUBN*, *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 was independently associated with Asthma Control Test (ACT) score, ICS dose, FeNO,

forced vital capacity (FVC), FEV<sub>1</sub> or lower airway eosinophilia. Mean serum 25(OH)D concentration was 50.6 nmol/L (SD 24.9); 162/297 (54.5%) participants were vitamin D deficient (serum 25(OH)D concentration <50 nmol/L). Lower vitamin D status was associated with higher body mass index (P=0.014), non-White ethnicity (P=0.036), unemployment (P for trend =0.012), lack of vitamin D supplement use (P<0.001), sampling in Winter or Spring (P for trend <0.001) and lack of a recent sunny holiday abroad (P=0.030), but not with potential genetic determinants. Vitamin D status was not found to associate with any marker of asthma control investigated. Vitamin D deficiency is common among UK adults with ICS-treated asthma, and classical environmental determinants of serum 25(OH)D operate in this population. However, in contrast to studies conducted in children, we found no association between vitamin D status and markers of asthma severity or control.

### **Keywords**

Vitamin D, asthma, phenotype, environmental, cross-sectional, genetics.

### **1. Introduction**

Vitamin D deficiency has been reported to be common among children with asthma in diverse settings, and to associate with reduced forced expiratory volume in one second (FEV<sub>1</sub>), poor asthma control and increased requirement for inhaled corticosteroids (ICS) (1-5). Despite the high prevalence of both asthma and vitamin D deficiency among adults in the industrialised world, cross-sectional studies assessing the prevalence, determinants and clinical correlates of vitamin D deficiency in adults with asthma have not previously been performed in such settings to our knowledge. Moreover, despite evidence suggesting that genetic variation can influence vitamin D status in the general population (6), studies to quantify the relationship between single nucleotide polymorphisms (SNP) in the vitamin D pathway and serum 25-hydroxyvitamin D (25[OH]D) concentrations (the accepted biomarker of vitamin D status), or clinical correlates of asthma phenotype, have not previously been performed in patients with asthma.

We therefore conducted a cross-sectional study to assess the prevalence of vitamin D deficiency in a group of adults with ICS-treated asthma in London, UK, and to explore environmental and genetic determinants of vitamin D status in this group. We also conducted analyses to determine whether serum 25(OH)D

concentration or genetic variation in the vitamin D pathway associated with markers of asthma control in this population, including symptom control, FEV<sub>1</sub>, forced vital capacity (FVC), ICS requirement and fractional exhaled nitric oxide concentration (FeNO). Additional analyses were performed to determine whether serum 25(OH)D concentration and genetic variants in the vitamin D pathway interacted to influence asthma control.

## **2. Methods**

### *2.1. Participants*

Adult patients with a medical record diagnosis of asthma treated with ICS were identified by searching databases at 60 general practices and at asthma clinics in 2 Acute National Health Service Trusts in London, UK, and invited for screening as previously described (7). The study was approved by East London and The City Research Ethics Committee 1 (ref 09/H0703/67) 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 (8), self-classified socio-economic position (SEP) using the National Statistics – Socio-Economic Classification (NS-SEC) method (9), daily hours spent outdoors, 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, 2 months prior to blood draw, for a duration of  $\geq 1$  week), smoking behaviour and consumption of alcohol and supplemental vitamin D. Respondents also completed the asthma control test (ACT) questionnaire (10), and underwent a baseline clinical assessment including the following: spirometry before and after inhalation of 400  $\mu$ g salbutamol via a spacer device, performed using a MicroLab ML3500

desktop spirometer (CareFusion GmbH, Hoechberg, Germany) according to American Thoracic Society (ATS) / European Respiratory Society (ERS) recommendations (11); FeNO measurement, performed using a NIOX MINO 09-1100 (Aerocrine, Solna, Sweden) according to ATS / ERS recommendations (12); 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 DNA extraction and determination of serum concentration of total 25(OH)D and parathyroid hormone (PTH). A sub-set of 35 participants underwent sputum induction with hypertonic saline, and their samples were processed to make cytospin slides according to methods described by Pizzichini *et al* (13). Differential cell counts were performed by one operator for all specimens throughout the study; a second operator repeated cell counts on a randomly selected sub-set of 20 slides: differential cell counts were highly correlated between operators (for eosinophil count, Pearson's  $r = 0.95$ , 95% CI 0.87 to 0.98,  $P < 0.001$ ).

### 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 or non-skeletal disease outcomes, which is described elsewhere (14). 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) (15), using a  $r^2$  threshold of 0.8, which reduced the number of SNP genotyped to 37.

### 2.4. Laboratory analyses

Serum concentrations of 25(OH)D<sub>2</sub> and 25(OH)D<sub>3</sub> were determined by isotope-dilution liquid chromatography–tandem mass spectrometry (16) 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/](http://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 (17) on the Biomek FX robot (Beckman Coulter), quantified using the Nanodrop spectrophotometer and normalised to 5ng/μl. 10ng 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 (7). 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<sub>1</sub>, and % predicted FVC) and Mann-Whitney or Kruskal-Wallis tests on non-normally distributed dependent variables (ACT score, FeNO readings, sputum eosinophilia levels, ICS dose) to identify independent correlates of serum 25(OH)D concentration, vitamin D pathway SNP and measures of asthma control on univariate analysis. All dependent variables were continuous. Non-normally distributed dependent variables were transformed to their natural logarithms. All factors with a minimum of 5 participants per outcome were fitted in multiple linear regression models to give adjusted 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 where in variables with  $\geq 3$  categories. For the analyses exploring associations between vitamin D pathway SNP and clinical correlates of asthma phenotype, a P value for the interaction between SNP genotype and baseline vitamin D status was also generated. In the case of log-transformed dependent variables, the anti-log of the adjusted regression coefficient is presented. Genetic analyses were adjusted for all putative environmental determinants of serum 25(OH)D concentration (for n=15 SNP previously found to associate with vitamin D status), or clinical correlates of asthma phenotype (for n=35 SNP previously found to associate with vitamin D status and/or non-skeletal disease outcomes) that were investigated. Multiple comparison testing was then applied using the Benjamini & Hochberg method with a false discovery rate (FDR) of 5% (18). 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 297 adults with a medical record diagnosis of ICS-treated asthma were enrolled in the study between 27th August 2009 and 25th June 2012. All consented to undergo clinical measurements and to donate blood samples for quantification of serum 25(OH)D and PTH concentration; all but one agreed to



donate a blood sample for DNA storage and genotyping. Participant characteristics are presented in Table 1. Age range was 16-78 years, with a mean of 48.7 years (SD 14.4). Most participants (57.2%) were female. The majority of participants (82.5%) classified their ethnic origin as White; 8.5% classified their ethnic origin as Black/Black British, 5.7% as Asian/Asian British, and 3.0% as being of mixed ethnicity. 261/297 (87.9%) participants' asthma was managed exclusively in primary care. Mean serum 25(OH)D concentration for all participants was 50.6 nmol/L (SD 24.9). Forty participants (13.5%) had serum 25(OH)D concentration <25 nmol/L; 122 (41.1%) had serum 25(OH)D concentration 25 - 49.9 nmol/L; 80 (26.9%) had serum 25(OH)D concentration 50 – 74.9 nmol/L; and only 55 (18.5%) had serum 25(OH)D concentration  $\geq$  75 nmol/L.

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

Environmental determinants of vitamin D status are presented in Table 2 and Figure 1. Multiple linear regression analysis showed the following factors to independently associate with lower serum 25(OH)D concentration: higher BMI (adjusted mean difference of 7.2 nmol/L for BMI of  $\geq$ 25 kg/m<sup>2</sup> vs. <25 kg/m<sup>2</sup>; 95% CI -13.0 to -1.5 nmol/L; P=0.014); non-White Ethnicity (adjusted mean difference 13.3 nmol/L for White vs. non-White; 95% CI -25.8 to -0.9 nmol/L; P=0.036); lower SEP (greatest adjusted mean difference 19.6 nmol/L for SEP groups 1/2 vs. Unemployed; 95% CI -36.8 to -2.5 nmol/L; P for trend = 0.012); lack of vitamin D supplement consumption (adjusted mean difference 21.5 nmol/L for those taking a dose of any size vs. those taking no vitamin D supplement; 95% CI -28.7 to -14.2 nmol/L; P<0.001); sampling in Winter or Spring (greatest adjusted mean difference 18.5 nmol/L for those sampled in Quarter 3 vs. Quarter 1; 95% CI -26.2 to -10.8 nmol/L, P for trend <0.001); lack of a recent sunny holiday (adjusted mean difference 7.7 nmol/L for those who took one in the previous 2 months vs. those who did not; 95% CI -14.7 to -0.8 nmol/L; P=0.030); and BTS treatment step (adjusted mean difference 8.1 nmol/L for groups 2/3 vs. 4/5; 95% CI -16.0 to -0.3 nmol/L; P=0.043).

### 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, BMI, ethnicity, SEP, number of hours spent outdoors, vitamin D supplement consumption, season of blood draw, Fitzpatrick skin-type, smoking status, alcohol consumption, tanning bed use, recent sunny holiday, and BTS

treatment step, and correcting for multiple comparison testing, none of the 15 SNP investigated were found to associate independently with serum 25(OH)D concentration.

### 3.4. Association between vitamin D status and asthma phenotype

Clinical determinants of vitamin D status are presented in Table 4. After adjustment for potential confounders, we found no relationship between vitamin D status and asthma phenotype. Specifically, we found no statistically significant independent association between serum 25(OH)D concentration and Asthma Control Test score (Table S1), % predicted FEV<sub>1</sub> (Table S2), % predicted FVC (Table S3), inhaled corticosteroid dose (Table S4), FeNO (Table S5) or induced sputum eosinophilia (Table S6; n=35 sub-set of participants). However, multiple linear regression analysis did show other factors to associate with these various aspects of asthma phenotype. Poor asthma control, as indicated by lower ACT scores, was independently associated with lower alcohol consumption (P for trend = 0.003), BMI  $\geq 25\text{kg/m}^2$  (P=0.026), non-White ethnicity (P<0.001) and lower SEP (P for trend = 0.018; Table S2). Older age and non-White ethnicity associated with decreased % predicted FEV<sub>1</sub> (P<0.001 for both factors, Table S3). Alcohol consumption and previous pneumococcal vaccination associated independently with increased ICS dose (P=0.03 for both factors, Table S4). Decreased FeNO levels were associated with female sex (P<0.001) and history of current smoking (P=0.040, Table S5), while sputum eosinophilia was positively correlated with peripheral blood eosinophilia (P=0.013, Table S6).

### 3.5. Association between genetic factors and asthma phenotype

Genetic determinants of clinical correlates of asthma phenotype are presented in Table S7 (% predicted FEV<sub>1</sub>), Table S8 (% predicted FVC), Table S9 (FeNO), Table 10 (ACT), and Table S11 (ICS). After correcting for multiple comparison testing none of the genetic factors which independently associated with markers of asthma phenotype as main effects, or by interaction with baseline vitamin D status, remained significant.

## 4. Discussion

To our knowledge, this study represents the largest cross-sectional investigation of vitamin D status in adults with asthma conducted to date, and the first such study to explore the influence of both genetic and

environmental determinants of vitamin D status in this patient group. Vitamin D deficiency, defined using the 50 nmol/L 25(OH)D threshold, was present in the majority of participants, and it associated with classical environmental determinants of vitamin D status, but not with any potential genetic determinant investigated. No association was found between vitamin D status or vitamin D pathway SNP and a broad range of measures of asthma control including symptom score, % predicted FEV<sub>1</sub>, % predicted FVC, ICS requirement and FeNO concentration. The lack of association between vitamin D genotype and the outcome measures we investigated may reflect a small impact from genetic variation in this particular population.

Our findings with respect to prevalence of environmental determinants of vitamin D status are in keeping with those previously reported for other UK populations without asthma (19). By contrast, our finding of a lack of association between serum 25(OH)D concentrations and asthma phenotype - supported by the lack of association between vitamin D pathway SNP and asthma phenotype we observed - conflicts with the other studies in the literature, which have variously reported associations between lower vitamin D status and worse asthma control, more severe disease, lower % predicted FEV<sub>1</sub> and increased requirement for inhaled corticosteroids (1, 2, 4, 5, 20-22).

Why might our findings differ? First, the majority of studies in the literature have investigated children with more severe asthma (1-5, 23, 24) while our study is in adults with generally better symptom control. It may be that asthma phenotype is more readily modified by vitamin D in paediatric populations, and/or in those with more severe asthma: in keeping with the former hypothesis, randomised controlled trials of vitamin D supplementation to improve asthma control have tended to show protective effects in children (25, 26), but not in adults (7, 27). A second potential explanation is that residual confounding may have contributed to the finding of positive associations in other studies: we collected detailed information on potential confounders of the relationship between vitamin D status and asthma control and adjusted for them in multivariable analyses. A third possibility is that publication bias may have contributed to the dearth of null studies in the published literature.

Our analysis of other determinants of asthma phenotype identified a number of expected predictors, but also revealed some new information. The association between increased alcohol consumption and

improved asthma control is intriguing: a negative association between maternal alcohol consumption and risk of asthma in offspring has been previously reported (28). More research is needed to understand the relationship between alcohol intake and asthma control, and to identify potential mechanisms of protection if applicable. Our finding of an independent association between poorer asthma control and non-White ethnicity chimes with other reports (29, 30); the fact that serum 25(OH)D did not associate with ACT score in this analysis suggests that lower vitamin D status does not account for such ethnic differences in asthma control.

#### *4.1. Study strengths*

Our study has several strengths. We investigated a wide range of potential environmental and genetic determinants of vitamin D status and asthma control, recorded detailed information on potential confounders of the relationship between 25(OH)D and asthma control, and phenotyped patients in considerable detail: spirometry and measurement of FeNO were 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/](http://www.deqas.org/)). The study population included patients with mild, moderate and severe disease from both community and hospital settings, studied across all seasons: these features enhance generalisability of our results.

#### *4.2. Study limitations*

Our study also has some limitations. A minority of participants had serum 25(OH)D concentrations >75 nmol/L, so we may have been underpowered to detect effects of the highest 25(OH)D concentrations on asthma phenotype: our results do not therefore definitively preclude beneficial effects of elevating serum 25(OH)D to >75 nmol/L. However, our findings are in keeping with clinical trials of vitamin D supplementation in adults, which have been null to date with respect to asthma control indices (7, 27). Lack of effects for vitamin D on asthma control outcomes in these trials may reflect a lack of power, low prevalence of profound vitamin D deficiency at baseline, and / or sub-optimal dosing regimens; they do not exclude protective effects of vitamin D on risk of asthma exacerbation, as discordance between physiological measures of asthma control and exacerbation risk are well recognised (31); indeed, a recent

meta-analysis of randomised controlled trials of vitamin D supplementation revealed a protective effect of vitamin D against severe asthma exacerbation, but no effect on day-to-day symptom control (32)

In conclusion, we report that vitamin D deficiency is common in a UK adult population with ICS-treated asthma, and that this is influenced by the same classical environmental determinants of vitamin D status that operate in the general population. However, in this population genetic factors did not have a strong influence on vitamin D status, or on measures of asthma control.

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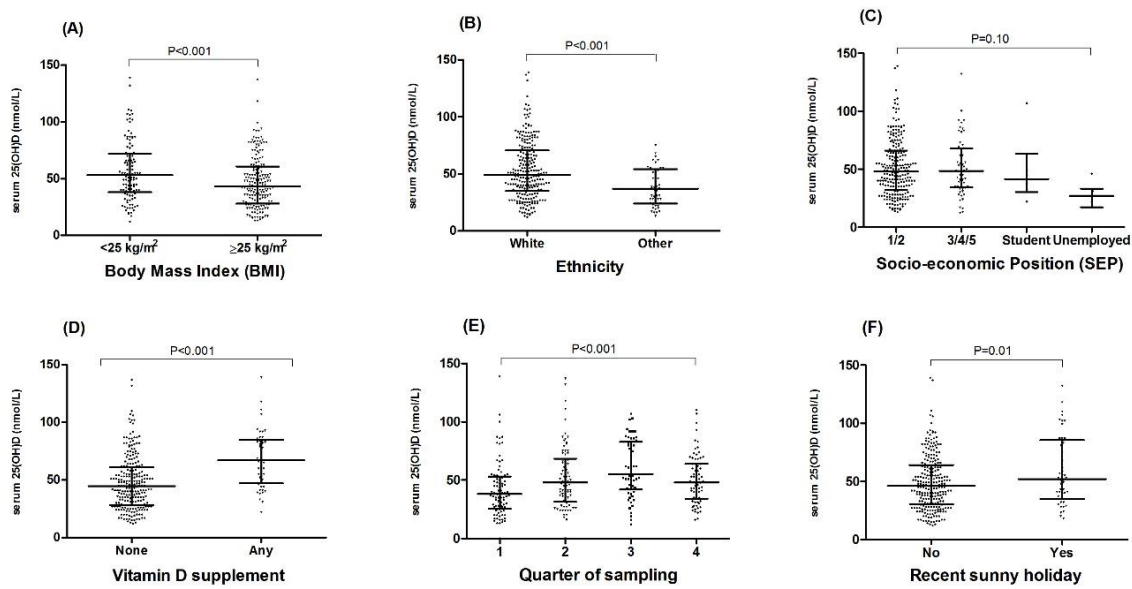
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**Figure 1: Environmental determinants of vitamin D status in adults with ICS-treated asthma.** Lower serum 25-hydroxyvitamin D (25[OH]D) concentrations were associated with higher body mass index (A), non-white ethnicity (B), lower socio-economic position (C), lack of vitamin D supplement use (D), sampling in Winter or Spring (E) and lack of a recent sunny holiday (F). Error bars represent median and interquartile ranges. P values are from linear regression adjusting for all potential environmental determinants of vitamin D status.



**Table1: Participant Characteristics (N=297)**

<b>Sex, n (%)</b>	Female	170 (57.2)
	Male	127 (42.8)
<b>Mean Age, yrs (SD)</b>		48.7 (14.4)
<b>Mean BMI, kg/m<sup>2</sup> (SD)</b>		27.6 (5.9)
<b>Ethnicity, n (%)<sup>1</sup></b>	White	245 (82.5)
	Asian / Asian British	17 (5.7)
	Black / Black British	25 (8.5)
	Mixed	9 (3.0)
<b>Fitzpatrick skin type, n (%)<sup>2</sup></b>	1	19 (6.4)
	2	57 (19.2)
	3	127 (42.8)
	4	57 (19.2)
	5	25 (8.4)
	6	12 (4.0)
<b>Socio-economic position, n (%)<sup>3</sup></b>	1	199 (67.0)
	2	29 (9.8)
	3	29 (9.8)
	4	12 (4.0)
	5	15 (5.0)
<b>Time outdoors per day, n (%)</b>	Student	6 (2.0)
	Unemployed	7 (2.4)
<b>Time outdoors per day, n (%)</b>	>2hrs	103 (34.7)
	≤2hrs	194 (65.3)
<b>Vitamin D supplement, IU/day, n (%)<sup>4</sup></b>	Any	48 (16.2)
	None	242 (81.5)
<b>Quarter of blood draw, n (%)</b>	Q1 (January – March)	88 (29.6)
	Q2 (April – June)	78 (26.3)
	Q3 (July – September)	60 (20.2)
	Q4 (October – December)	71 (23.9)
<b>Smoking status, n (%)</b>	Non-current	279 (93.9)
	Current	18 (6.1)
<b>Mean alcohol intake, units/week (SD)<sup>5</sup></b>		10.2 (11.7)
<b>Tanning bed use in previous year, n (%)</b>	Yes	19 (6.4)
	No	278 (93.6)
<b>Recent sunny holiday, n (%)<sup>6</sup></b>	Yes	52 (17.8)
	No	240 (82.2)
<b>BTS step of treatment, n (%)</b>	2: Regular preventer therapy	134 (45.1)
	3: Initial add-on therapy	125 (42.1)
	4: Persistent poor control	35 (11.8)
	5: Continuous / frequent use of OCS	3 (1.0)
<b>Managed exclusively in primary care, n (%)</b>		261 (87.9)
<b>Medication use</b>	Mean ICS dose at entry in beclometasone equivalents, µg (SD) <sup>7</sup>	725.3 (631.2)
	Inhaled LABA use, n (%) <sup>8</sup>	155 (52.2)
	Leukotriene antagonist use, n (%)	33 (11.1)
<b>Mean % predicted FEV<sub>1</sub> (SD)</b>		82.4 (20.3)
<b>Mean FeNO, ppb (SD)</b>		36.2 (26.4)
<b>Mean serum corrected calcium (SD)</b>		2.23 (0.08)
<b>Mean serum PTH (SD)</b>		5.7 (3.9)
<b>Serum PTH &gt;6.8 pmol/L, n (%)</b>	Yes	71 (23.9)
	No	226 (76.1)
<b>Serum 25(OH)D, nmol/L (%)</b>	<25	40 (13.5)
	25 – 49.9	122 (41.1)
	50 – 74.9	80 (26.9)
	≥ 75	55 (18.5)
<b>Mean serum 25(OH)D, nmol/L (SD)</b>		50.6 (24.9)

<sup>[1]</sup>Ethnicity not reported in n=1. Mixed ethnicity: n=6 White and Black Caribbean, n=1 British Mauritian, n=1 Asian Caribbean, n=1 Irish Sri Lankan. <sup>[2]</sup>Fitzpatrick skin-type scores: 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.<sup>[3]</sup> SEP classes: 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 (>5yrs) unemployed.<sup>[4]</sup> Vitamin D supplement consumption not reported in n=7.<sup>[5]</sup> One alcohol unit = 8g pure alcohol.<sup>[6]</sup> Recent sunny holiday not reported in n=5.<sup>[7]</sup> 1µg beclometasone assumed equivalent to 1µg budesonide, 0.5 mcg fluticasone dipropionate and 0.75 mcg ciclesonide.<sup>[8]</sup> Includes combinations of ICS/LABA and LABA

Table 2: Environmental determinants of vitamin D status

		N	Serum 25(OH)D, nmol/L		Univariate P value <sup>7</sup>	Multivariable model - Beta Coefficient (95% CI)	P value <sup>8</sup>
			Mean (SD)	Mean difference			
Sex	Female	170	50.7 (26.1)	referent	0.96	referent	0.13
	Male	127	50.5 (23.2)	-0.2			
Age quartiles	1 (16.0 – 37.7 yrs)	74	52.5 (25.4)	referent	0.26	referent	0.54 <sup>†</sup>
	2 (37.8 – 49.6 yrs)	74	48.4 (25.0)	-4.1			
	3 (49.7 – 60.5 yrs)	74	47.2 (22.2)	-5.3			
	4 (60.6 – 79.0 yrs)	75	54.3 (26.4)	+1.8			
BMI, kg/m <sup>2</sup>	<25	113	56.8 (26.2)	referent	<b>&lt;0.001</b>	referent	<b>0.014</b>
	≥25	184	46.8 (23.2)	-10.0			
Ethnicity <sup>1</sup>	White	245	53.3 (25.5)	referent	<b>&lt;0.001</b>	referent	<b>0.036</b>
	Non-white	51	37.7 (16.8)	-15.6			
SEP <sup>2</sup>	1,2	228	51.0 (24.9)	referent	0.099	referent	<b>0.012<sup>†</sup></b>
	3,4,5	56	51.8 (24.6)	+0.8			
	Student	6	49.0 (30.0)	-2.0			
	Unemployed	7	27.6 (10.3)	-23.4			
Hours spent outdoors/day	>2	103	56.7 (26.9)	referent	<b>0.002</b>	referent	0.17
	≤2	194	47.4 (23.2)	-9.3			
Vitamin D supplement, IU/day <sup>3</sup>	Any	48	68.5 (24.8)	referent	<b>&lt;0.001</b>	referent	<b>&lt;0.001</b>
	None	242	47.4 (23.5)	-21.1			
Quarter of blood draw	Q1 (January – March)	88	42.2 (23.6)	-17.7	<b>&lt;0.001</b>	referent	<b>&lt;0.001<sup>†</sup></b>
	Q2 (April – June)	78	52.5 (27.1)	-7.4			
	Q3 (July – September)	60	59.9 (24.2)	referent			
	Q4 (October – December)	71	51.0 (21.2)	-8.9			
Fitzpatrick skin-type <sup>4</sup>	1,2	76	48.7 (24.4)	-5.3	<b>&lt;0.001</b>	referent	0.81 <sup>†</sup>
	3,4	184	54.0 (25.4)	referent			
	5,6	37	37.6 (17.9)	-16.4			
Smoking status	Non-current	279	50.1 (24.6)	referent	0.22	referent	0.92
	Current	18	57.5 (28.8)	+7.4			
Alcohol consumption, units/wk	0	51	45.0 (23.4)	referent	0.21	referent	0.25 <sup>†</sup>
	1-20	198	51.7 (24.8)	+6.7			
	>20	48	52.0 (26.2)	+7.0			
Tanning bed use, previous year	Yes	19	62.3 (17.9)	referent	<b>0.033</b>	referent	0.19
	No	278	49.8 (25.1)	-12.5			
Recent sunny holiday <sup>5</sup>	Yes	52	58.6 (30.0)	referent	<b>0.012</b>	referent	<b>0.030</b>
	No	240	49.0 (23.5)	-9.6			
BTS treatment step <sup>6</sup>	2/3	259	50.2 (24.0)	-3.1	0.47	referent	<b>0.043</b>
	4/5	38	53.3 (30.4)	referent			

<sup>[1]</sup>Ethnicity not reported in n=1. Other ethnicities: n=17 Asian, n=25 Black, n=9 Mixed ethnicity. One participant declined to report ethnicity.

<sup>[2]</sup> Socio-economic Position (SEP) classes: 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 (>5yrs) unemployed.

<sup>[3]</sup>Vitamin D supplement consumption not reported in n=7.

<sup>[4]</sup>Fitzpatrick skin-type scores: 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.

<sup>[5]</sup>Recent sunny holiday not reported in n=5.

<sup>[6]</sup> BTS treatment step definitions: 2. Regular preventer therapy, 3. Initial add-on therapy, 4. Persistent poor control, 5. Continuous / frequent use of OCS

<sup>[7]</sup>Univariate method: Student's T-test/One-way ANOVA

<sup>[8]</sup>Adjusted for all investigated potential determinants of 25(OH)D concentration : Sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplementation, quarter of blood draw, skin-type, smoking status, alcohol consumption, tanning bed use, recent sunny holiday, British Thoracic Society treatment step.

†P-value for trend

Table 3: Genetic determinants of vitamin D status

Gene	SNP	Genotype	N	Serum 25(OH)D, nmol/L		Multivariable model - Beta Coefficient (95% CI)	P value for trend <sup>1</sup>	
				Mean (SD)	Mean difference			
<b>CYP24A1</b>	rs6013897	TT	174	53.5 (26.6)	referent	referent	0.13	
		AT	96	47.0 (22.5)	-6.5	-3.6 (-9.2 to 2.0)		
		AA	14	41.9 (19.3)	-11.6	-9.4 (-21.4 to 2.6)		
	rs2248137	CC	97	53.4 (25.7)	referent	referent	0.24	
		CG	129	51.2 (26.4)	-2.2	+1.1 (-4.9 to 7.1)		
		GG	60	45.4 (19.6)	-8.0	-4.4 (-11.9 to 3.0)		
<b>DBP</b>	rs16846876	AA	138	54.9 (26.6)	referent	referent	<b>0.015</b>	
		AT	127	47.7 (23.1)	-7.2	-7.8 (-13.2 to -2.4)		
		TT	15	47.5 (19.3)	-7.4	-8.2 (-20.0 to 3.6)		
	rs12512631	TT	113	49.2 (24.9)	referent	referent	0.087	
		CT	137	50.3 (24.2)	+1.1	+2.6 (-3.2 to 8.4)		
		CC	41	56.6 (27.4)	+7.4	+7.2 (-1.0 to 15.4)		
	rs2070741	TT	240	50.2 (24.3)	referent	referent	<b>0.038</b>	
		TG	46	53.7 (25.3)	+3.5	+6.7 (-0.4 to 13.8)		
		GG	1	118.0 (0)	+67.8	+45.7 (2.6 to 88.8)		
	rs2298849	AA	175	50.1 (25.1)	referent	referent	0.47	
		AG	101	51.8 (24.9)	+1.7	+2.9 (-2.8 to 8.5)		
		GG	12	52.2 (24.8)	+2.1	+4.9 (-8.3 to 18.1)		
	rs4588	CC	160	56.0 (25.8)	referent	referent	<b>0.005</b>	
		CA	113	45.0 (23.0)	-11.0	-10.5 (-15.9 to -5.2)		
		AA	16	36.6 (16.6)	-22.4	-16.8 (-28.4 to -5.2)		
rs7041		GG	91	58.8 (26.7)	referent	referent		0.053
		TG	132	47.9 (23.1)	-10.9	-8.4 (-14.4 to -2.4)		
TT	TT	63	47.1 (24.1)	-11.7	-7.6 (-15.2 to 0.1)			
	<b>CYP27B1</b>	rs4646536	AA	132	50.9 (25.9)	referent	referent	0.34
		AG	118	51.8 (25.2)	+0.9	+0.1 (-5.6 to 5.7)		
GG		30	47.7 (21.4)	-3.2	-4.4 (-13.3 to 4.6)			
<b>CYP2R1</b>	rs10500804	TT	104	54.4 (24.9)	referent	Referent	0.77	
		GT	143	46.6 (24.3)	-7.8	-8.1 (-13.8 to -2.4)		
		GG	44	56.5 (25.3)	+2.1	-1.2 (-9.1 to 6.7)		
	rs2060793	GG	107	50.3 (23.9)	referent	referent	0.84	
		AG	129	52.5 (26.3)	+2.2	+2.5 (-3.4 to 8.3)		
		AA	41	45.7 (23.5)	-4.6	-0.8 (-9.0 to 7.4)		
	rs10766197	GG	86	55.0 (24.9)	referent	referent	0.51	
		AG	143	47.4 (24.6)	-7.6	-9.4 (-15.5 to -3.4)		
		AA	43	54.5 (25.4)	-0.5	-2.8 (-11.0 to 5.5)		
<b>DHCR7</b>	rs12785878	TT	154	52.7 (25.3)	referent	referent	<b>0.011</b>	
		GT	98	52.2 (25.1)	-0.5	+1.4 (-4.5 to 7.3)		
		GG	38	38.9 (21.3)	-13.8	-12.2 (-21.5 to -2.8)		
	rs3829251	GG	220	50.1 (23.9)	referent	referent	0.96	
		AG	69	52.6 (27.4)	+2.5	+0.5 (-5.8 to 6.7)		
		AA	2	42.0 (31.1)	-8.1	+0.7 (-30.0 to 31.4)		
<b>VDR</b>	rs10783219	AA	136	50.8 (24.6)	referent	referent	0.28	
		AT	116	50.4 (24.9)	-0.4	-1.8 (-7.6 to 3.9)		
		TT	34	49.3 (28.3)	-1.5	-4.6 (-13.0 to 3.8)		

<sup>[1]</sup> Adjusted for sex, age, BMI, ethnicity, SEP, hours outdoors, vitamin D supplement consumption, quarter of sampling, Fitzpatrick skin-type, smoking status, alcohol consumption, tanning bed use, recent sunny holiday, and BTS treatment step. After correction for multiple comparisons testing using the Benjamini & Hochberg method with a 5% false discovery rate, none of the above P values remained significant.

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, SD: standard deviation, CI: confidence interval.

Table 4. Clinical correlates of vitamin D status

	Serum 25-hydroxyvitamin D, nmol/L	N	Mean (SD) / Median (IQR) <sup>1</sup>	Univariate P value <sup>2</sup>	Multivariable model			
					Beta coefficient, (95% CI) <sup>3</sup>	P value <sup>4</sup>	Antilog of beta coefficient, (95% CI) <sup>3</sup>	P value <sup>4</sup>
<b>Asthma Control Test (ACT) score</b>	<25	40	19.5 (16.5 to 22)	0.87	-	-	1.05 (0.96 to 1.16)	0.25
	25 – 49.9	122	20 (17 to 22)	-	-	-	1.02 (0.94 to 1.09)	-
	50 – 74.9	80	20 (17 to 22)	-	-	-	0.99 (0.92 to 1.07)	-
	≥ 75	55	20 (17 to 22)	-	-	-	referent	-
<b>% Predicted Forced Expiratory Volume in 1 second (FEV<sub>1</sub>)</b>	<25	40	85.07 (17.60)	0.48	-1.11 (-8.67 to 6.45)	0.75	-	-
	25 – 49.9	122	89.55 (21.90)	-	+1.30 (-4.44 to 7.03)	-	-	-
	50 – 74.9	80	90.33 (15.99)	-	+1.74 (-4.48 to 7.96)	-	-	-
	≥ 75	55	90.59 (17.02)	-	referent	-	-	-
<b>% Predicted Forced Vital Capacity (FVC)</b>	<25	40	0.98 (0.13)	0.079	-0.01 (-0.07 to 0.05)	0.81	-	-
	25 – 49.9	122	1.04 (0.18)	-	+0.01 (-0.04 to 0.06)	-	-	-
	50 – 74.9	80	1.04 (0.15)	-	+0.00 (-0.05 to 0.05)	-	-	-
	≥ 75	55	1.07 (0.13)	-	referent	-	-	-
<b>Inhaled corticosteroid (ICS) dose in beclometasone equivalents, µg<sup>5</sup></b>	<25	40	400 (400 to 500)	0.87	-	-	0.98 (0.72 to 1.34)	0.93
	25 – 49.9	120	400 (200 to 800)	-	-	-	1.00 (0.79 to 1.27)	-
	50 – 74.9	80	400 (200 to 700)	-	-	-	1.00 (0.77 to 1.29)	-
	≥ 75	54	400 (200 to 800)	-	-	-	referent	-
<b>Fractional exhaled nitric oxide (FeNO), ppb</b>	<25	40	30 (20.5 to 42)	0.55	-	-	1.04 (0.80 to 1.35)	0.84
	25 – 49.9	120	29 (20 to 44.5)	-	-	-	1.10 (0.90 to 1.34)	-
	50 – 74.9	80	31 (19 to 45)	-	-	-	1.13 (0.91 to 1.40)	-
	≥ 75	54	24.5 (18 to 37)	-	-	-	referent	-
<b>% Eosinophils in induced sputum</b>	<50	19	1.50 (0.63 to 4.42)	0.97	-	-	1.03 (0.58 to 1.84)	0.91
	≥ 50	16	1.50 (0.63 to 3.25)	-	-	-	referent	-

<sup>[1]</sup> Mean values and standard deviations reported for normally distributed outcome data; median values and inter-quartile ranges reported for non-normally distributed outcome data

<sup>[2]</sup> Univariate analysis method: One-way ANOVA test for normally distributed outcome data; Kruskal-Wallis test for non-normally distributed outcome data.

<sup>[3]</sup> Beta coefficients and 95% confidence intervals reported for normally distributed outcome data; antilog of beta coefficients reported for log transformed outcome data

<sup>[4]</sup> ACT, % predicted FEV<sub>1</sub>, % predicted FVC, ICS, and FeNO adjusted for sex, age, BMI, ethnicity, SEP, smoking status, alcohol consumption, influenza vaccination, and pneumonia vaccination; % eosinophils in induced sputum adjusted for sex, age, BMI, and FEV<sub>1</sub>/FVC ratio. All adjusted p values are for trend.

<sup>[5]</sup> 1µg beclometasone assumed equivalent to 1µg budesonide, 0.5 mcg fluticasone dipropionate and 0.75 mcg ciclesonide

Definitions: CI, confidence interval; IQR, inter-quartile range; SD, standard deviation; ppb, parts per billion