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Environmental and genetic determinants of two vitamin D metabolites in healthy Australian children

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Abstract

Background: Vitamin D deficiency has been associated with adverse health outcomes. We examined genetic and environmental determinants of serum 25(OH)D₃ and 1,25(OH)₂D₃ in childhood.

Methods: The study sample consisted of 322 healthy Australian children (predominantly Caucasians) who provided a venous blood sample. A parental interview

was conducted and skin phototype and anthropometry measures were assessed. Concentrations of 25(OH)D₃ and 1,25(OH)₂D₃ were measured by selective solid-phase extraction-capillary liquid chromatography-tandem mass spectrometry. These concentrations were deseasonalised where relevant to remove the effect of month of sampling.

Results: Deseasonalised log 25(OH)D₃ and 1,25(OH)₂D₃ concentrations were only moderately correlated ($r=0.42$, $p<0.001$). The following predicted both 25(OH)D₃ and 1,25(OH)₂D₃: UVR 6 weeks before the interview, natural skin and eye colour, height and vitamin D allelic metabolism score. The following predicted 25(OH)D₃ only: lifetime sunburns and vitamin D allelic synthesis score. Overall, 43.5% and 25.6% of variation in 25(OH)D₃ and 1,25(OH)₂D₃ could be explained. After accounting for 25(OH)D₃ concentrations, higher UVR 6 weeks before the interview and vitamin D allelic metabolism score further predicted 1,25(OH)₂D₃ concentrations.

Conclusions: Environmental factors and genetic factors contributed to both vitamin D metabolite concentrations. The intriguing finding that the higher ambient UVR contributed to higher 1,25(OH)₂D₃ after accounting for 25(OH)D₃ concentrations requires further evaluation.

Keywords: 1,25-dihydroxyvitamin D₃; allelic score; determinants; ultraviolet radiation; vitamin D 25-hydroxyvitamin D₃.

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Introduction

Vitamin D deficiency, serum level less than 20 ng/mL (50 nmol/L) or insufficiency, serum level 20 to 29 ng/mL (50–75 nmol/L) is common with an estimated 1 billion people having either condition worldwide [1]. In Australia about 30% of adults have vitamin D insufficiency [2] and a recent report showed that over 16% of Australian infants were vitamin D insufficient [3]. In addition to its metabolic role in skeletal health and calcium homeostasis, vitamin D insufficiency may be associated with an increased risk of several other adverse health outcomes such as multiple sclerosis [4], cancer, type 1 and type 2 diabetes [5].

Vitamin D₃ is synthesised in the epidermis from 7-dehydrocholesterol (7-DHC) in a reaction catalysed by ultraviolet B (UVB). About 85%–90% of vitamin D is carried in blood circulation by the vitamin D binding protein (VDBP) which has common genetic polymorphisms that produce variant proteins that differ in their affinity for vitamin D and affect its bioavailable levels [6]. New polyclonal and proteomic methods to measure vitamin D-binding protein will enhance our ability to understand its biological role [7]. Vitamin D₃ is then rapidly hydroxylated in the liver to 25-hydroxyvitamin D₃, then hydroxylated into its biologically active form, 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) mainly in the kidneys. While the latter is the biologically active form, it has extremely low circulating levels, (partly related to a relatively short circulating half-life) resulting in challenging analytical detectability, using most currently available assays. Furthermore, whilst 25 hydroxylases are relatively unregulated, renal 1 α -hydroxylase is tightly regulated in an endocrine fashion, by hormones including parathyroid hormone and fibroblast growth factor 23 (FGF23). Consequently, 25(OH)D₃ is the current recommended biomarker for vitamin D status [8] as it is the substrate for conversion to the active 1,25(OH)₂D₃ and its concentration reflects hepatic vitamin D stores.

Many studies have shown that environmental factors such as sun exposure, life style factors [9], ultraviolet radiation (UVR) [10] and skin pigmentation [11] are associated with 25(OH)D₃ concentrations. Genetic factors also influence circulating vitamin D concentrations [12–16]. Genome-wide association studies (GWAS) have shown that multiple single nucleotide polymorphisms (SNPs) located at or near vitamin D metabolic pathway genes, such as 24-hydroxylase (*CYP24A1*) and vitamin D binding protein (*GC*) are associated with serum 25(OH)D₃ concentrations [14–16], accounting for 1%–4% of its overall variance [16].

Few studies have comprehensively examined both environmental and genetic factors in parallel. Previously we reported that sun exposure-related factors account for 36%, genetic factors account for 14% and skin phenotypes accounts for 3.5% of 25(OH)D₃ variation in an Australian adult population [17]. In a separate study of Australian infants, we found that ambient UVR levels 6 weeks before blood collection can predict 10% of 25(OH)D₃ variation [3]. In addition, past studies have focused only on 25(OH)D₃; however, new advances in the measurements of 1,25(OH)₂D₃ now provide the opportunity to investigate the determinants of this metabolite. No study has examined the genetic and environmental factors of both 25(OH)D₃ and 1,25(OH)₂D₃ simultaneously in children. In this current study we examined both environmental and

genetic determinants of two major vitamin D-circulating metabolites, 25(OH)D₃ and 1,25(OH)₂D₃, in a population of healthy children in Melbourne, Australia.

Materials and methods

Participants

This study is based on a sample of 322 unselected control children from the Early Environment and Type 1 Diabetes Prevention Project. They were all born and residing in Victoria, and were recruited between March 2008 and November 2011 at the Royal Children's Hospital Day Surgery Unit Melbourne, Australia (latitude 38° South, 145° East).

Children under 18 years admitted for a 1-day surgical procedure, such as plastic surgery, eye surgery, circumcision, hernia repair and others, were eligible to participate in the study. Children who had severe congenital abnormalities, prolonged physical or mental illness, which may affect their activity such as playing, walking, or attending normal school, were excluded. The median age at recruitment was 8.42 years (IQR 6.26–11.06 years) and they were divided into three age groups <5, 5–10 and >10 years (see Table 1), 55% were males and the majority (62.4%) were Caucasians.

Children and their guardians (usually parents) were interviewed by trained research nurses who conducted clinical examinations and helped parents to complete the questionnaires on the day of admission. The questionnaires collected information on direct sun exposure behaviours of both children and parents, including how much time they usually spent in the sun during summer and winter, on weekends and weekdays, and freckling by the end of summer using previously validated instruments [18].

Ethics approval was obtained from the Human Research Ethics Committee of the Royal Children's Hospital, Melbourne, Australia. Informed, written consent for the study was obtained from parents/guardians, and from children 12 years of age and older.

Examination data

The research nurses conducted the examination and noted the natural skin, hair and eye colour using standardized coloured photographs which we have previously shown to explain 39.7% ($p < 0.001$) of the variation in constitutive skin pigmentation measured by spectrophotometer [19]. Child ethnicity (Caucasian, Asian, African, Aboriginal or Torres Strait Islander, Middle Eastern or other) was determined by parental report. The number of naevi on the left arm was counted using a standardised protocol [20]. Height and weight was measured and body mass index [$\text{BMI} = \text{weight (kg)}/\text{height (m)}^2$] was calculated. Gender- and age-specific BMI cut off points were used to classify children as normal, overweight or obese [21].

Monthly average of the total daily ambient UVR dose was obtained in Standard Erythral Dose units for Melbourne from 2008 to 2011 [22]. The interview months' UVR was calculated as the average of daily total UVR assessed on the year and month of the interview. Likewise, the UVR for 1 month, 6 weeks and 2 months before the interview were determined [19].

Table 1: Child characteristics by serum 25-hydroxyvitamin D₃ [25(OH)D₃] and 1,25 dihydroxyvitamin D₃ [1,25(OH)₂D₃] concentrations.

Factor	n (%)	Log _e deseasonalised 25(OH)D ₃ , mean ^a (SD)	Log _e deseasonalised 1,25(OH) ₂ D ₃ , mean ^b (SD)
Age, years			
(0–4.9)	42 (13.0%)	2.74 (0.39)	3.60 (0.48)
(5–9.9)	172 (53.4%)	2.83 (0.38)	3.69 (0.6)
(10–16.9)	108 (33.5%)	2.92 (0.35)	3.86 (0.61)
Gender			
Male	177 (55.0%)	2.84 (0.36)	3.70 (0.61)
Female	145 (45.0%)	2.86 (0.39)	3.78 (0.58)
Season of blood collection			
Summer	38 (11.8%)	3.08 (0.27) ^c	4.00 (0.69) ^c
Autumn	80 (24.8%)	2.9 (0.37) ^c	3.85 (0.62) ^c
Winter	113 (35.1%)	2.62 (0.41) ^c	3.33 (0.56) ^c
Spring	91 (28.3%)	2.78 (0.42) ^c	3.69 (0.64) ^c
Ethnicity			
Caucasian	201 (62.4%)	2.93 (0.3)	3.76 (0.56)
Non-Caucasian	64 (19.9%)	2.62 (0.46)	3.64 (0.59)
Missing	57 (17.7%)		
Body mass index (BMI) category			
Normal	166 (51.6%)	2.90 (0.33)	3.76 (0.58)
Overweight	44 (13.7%)	2.70 (0.47)	3.66 (0.57)
Obese	45 (14.0%)	2.77 (0.37)	3.7 (0.6)
Missing	67 (20.8%)		
Eye colour			
Brown	140 (43.5%)	2.74 (0.39)	
Hazel	38 (11.8%)	2.86 (0.38)	3.83 (0.71)
Green	22 (06.8%)	3.05 (0.26)	3.79 (0.47)
Blue/grey	68 (21.1%)	3.00 (0.31)	3.85 (0.63)
Missing	54 (16.8%)		
Natural skin colour (on examination)			
Dark	13 (4.0%)	2.28 (0.35)	3.57 (0.37)
Olive	47 (14.6%)	2.76 (0.47)	3.67 (0.65)
Olive/medium	75 (23.3%)	2.83 (0.32)	3.73 (0.56)
Medium/fair	90 (28.0%)	2.93 (0.29)	3.76 (0.58)
Fair	43 (13.4%)	3.00 (0.29)	3.81 (0.55)
Missing	54 (16.8%)		

^aMean of the natural-log-transformed deseasonalised 25(OH)D₃ concentration. ^bMean of the natural-log-transformed deseasonalised 1,25(OH)₂D₃ concentration. ^cThe mean was obtained from the natural-log-transformed non-deseasonalised 25(OH)D₃ and 1,25(OH)₂D₃.

Laboratory data

All 322 children provided a peripheral blood sample, which was separated to isolate plasma from the cellular component within 2 h of collection. DNA was extracted from the white blood cell component using the Qiagen Flexigene Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

Up to 10 mL peripheral blood samples were collected in EDTA tubes for each child prior to anaesthesia which were centrifuged within 2 h. Plasma and plasma-depleted whole blood were stored at –70°C until analysis. Plasma 25(OH)D₃ and 1,25(OH)₂D₃ were measured with selective solid-phase extraction capillary liquid chromatography-tandem mass spectrometry at the Qu Lab, University at Buffalo, New York. This central laboratory has achieved a limit of quantification of 5.04 pg/mL (12 pmol/L) and 0.1 ng/mL (0.25 nmol/L), interday accuracy of 9.5%–13.3% and 2.7%–14% and interday precision of 6.7%–16.6% and 3.1%–8.0% for 1,25(OH)₂D₃

and 25(OH)D₃, respectively [23]. In DEQAS report the deviations were all <18.4% from two batches of samples. The nine-point calibration curve showed excellent linearity with correlation coefficient greater than 0.99 for both 25(OH)D₃ and 1,25(OH)₂D₃. The method separated the two targeted metabolites from other vitamin D₃ metabolites such as 25(OH)D₃ epimer and 24,25dihydroxyvitamin D₃ [24]. Thus, the possible interfering effect of these metabolites in 25(OH)D₃ and 1,25(OH)₂D₃ measurements has been removed.

Based on the evidence of their association with circulating vitamin D from past genetic association studies, we selected 12 SNPs located at or near genes related to the vitamin D metabolic pathway (*CYP2R1* (rs10741657), *DHCR7* (rs12785878), *CYP24A1* (rs6013897, rs2181874, rs2762941, rs4809960), *GC* (rs4588, rs2282679) *VDR* (rs11168275, rs4760655), and skin pigmentation genes (rs8045560, rs3936623) [16, 25, 26]. Genotyping was performed using the Sequenom Mass Array system, of which a detailed description of the assay has been published elsewhere [27]. Sequenom iPLEX chemistry was

used according to manufacturer instructions. Cluster plots for all 'non-conservative' genotype calls were manually inspected and re-genotyping was done if necessary. After the exclusion of samples not meeting the quality control threshold (departure from the Hardy-Weinberg equilibrium $p < 0.01$ and $< 90\%$ call rate), genotype data for 311 participants were generated.

Allele scores and SNPs selection

We constructed two vitamin D allele scores, synthesis score (Synscore) and metabolic score (Metscore), using four out of the 12 examined vitamin D-related SNPs. *CYP2R1* (rs10741657), *DHCR7* (rs12785878), *CYP24A1* (rs6013897) and *GC* (rs2282679), were selected based on findings from the GWAS of the SUNLIGHT Consortium and our univariate results (data not shown) [16, 28]. *DHCR7* (rs12785878) and *CYP2R1* (rs10741657) are in genes located upstream, and *CYP24A1* (rs6013897) and *GC* (rs2282679) are in genes located downstream of 25(OH) D_3 production (metabolism). Synscore is the sum of the vitamin D-increasing alleles in the upstream genes (*CYP2R1* and *DHCR7* with a score range 0–4) and Metscore is the sum of vitamin D-increasing alleles in the gene located downstream (*GC* and *CYP24A1* with a score range 0–4) of 25(OH) D_3 [29].

These allelic scores can be used as instrumental variables for 25(OH) D_3 and 1,25(OH) $_2D_3$ concentration when testing, in Mendelian randomisation analysis, for likely causal association as it represents variants with direct effect on substrate availability for vitamin D metabolites' synthesis.

Statistical analysis

In the univariate analysis, linear regression models were used to examine the environmental, and genetic determinants of 25(OH) D_3 and 1,25(OH) $_2D_3$ concentrations. The distribution of both vitamin D metabolites was skewed and thus log-transformed prior to analysis to improve normality and allow parametric statistical analysis. Log-scale summary values were presented and regression coefficients were interpreted as percent change in outcome per 1 unit change in exposure variable (% change = $1 - \exp(\beta)$ for negative β or $\exp(\beta) - 1$ for positive β). As 25(OH) D_3 and 1,25(OH) $_2D_3$ had very different blood levels and units (measured in nmol/L and pmol/L for 25(OH) D_3 and 1,25(OH) $_2D_3$, respectively), the log values and their relative change allowed easier comparison and interpretation of the determinants' effect on these metabolites.

For models not including UVR as a potential confounder, deseasonalised vitamin D concentrations were used as the outcome measure to correct for seasonal variation. We calculated deseasonalised 25(OH) D_3 and 1,25(OH) $_2D_3$ by removing the seasonal variation. We fitted sinusoidal model to data on 25(OH) D_3 and 1,25(OH) $_2D_3$ levels and month (t) of sample collection to model seasonal variation [30, 31]:

$$\text{Concentration level 25(OH)}D_3 \text{ or } 1,25(\text{OH})_2D_3 = \beta_0 + \beta_1 \sin(2\pi/12) + \beta_2 \cos(2\pi/12)$$

The amplitude was calculated using the formula $\sqrt{\beta_1^2 + \beta_2^2}$. This predicted value was subtracted from the actual value and added to the overall mean. For some analyses it is of interest to remove the

natural seasonal fluctuation in vitamin D levels by deseasonalisation which has been used in previous studies [30, 31].

To construct the final predictive model, we used forward step-wise regression starting with one variable in the model, testing the addition of each variable, based on its R-square from the univariate analysis and its impact on model improvement, until there was no improvement of the model using the likelihood ratio test. Interaction between predictors for both 25(OH) D_3 and 1,25(OH) $_2D_3$ concentrations in the final models was assessed by the P-value associated with the Wald test of a product term for the relevant factors in categorical terms.

We used Stata version 14 (StataCorp, College Station, TX, USA) for all analyses.

Results

Demographic and anthropometric measures of the participants (n=322) with the corresponding mean of natural-log-transformed serum 25(OH) D_3 and 1,25(OH) $_2D_3$ concentrations are presented in Table 1. Serum 25(OH) D_3 concentrations was only moderately correlated with 1,25(OH) $_2D_3$ concentrations with or without deseasonalisation (Figure 1). There was seasonal variation for both 25(OH) D_3 and 1,25(OH) $_2D_3$ concentrations; participants sampled in summer months (December to February) had higher concentrations of both metabolites, compared with those sampled in winter months (June to August) (Figure 2). We examined how 25(OH) D_3 concentrations predicted 1,25(OH) $_2D_3$ concentrations and found that using 25(OH) D_3 categories in logistic regression did not fit the data better than a simple linear regression model ($p = 1.00$).

Higher ambient UVR, in various time intervals prior to sample collection were strongly associated with both 25(OH) D_3 and 1,25(OH) $_2D_3$ blood concentrations. We examined the association of ambient UVR in the month of blood collection and 1 month, 6 weeks and 2 months before blood collection, with both metabolites. The ambient UVR in 6 weeks before sample collection had the strongest magnitude of effect on both 25(OH) D_3 and 1,25(OH) $_2D_3$ (Table 2).

Phenotypic and environmental factors

The associations between selected factors, based on their statistical significance and r^2 , and vitamin D concentration (25(OH) D_3 and 1,25(OH) $_2D_3$) in univariate analysis are presented in Table 2. We examined age, gender and anthropometrics measures as predictors of deseasonalised 25(OH) D_3 and 1,25(OH) $_2D_3$ concentrations. While

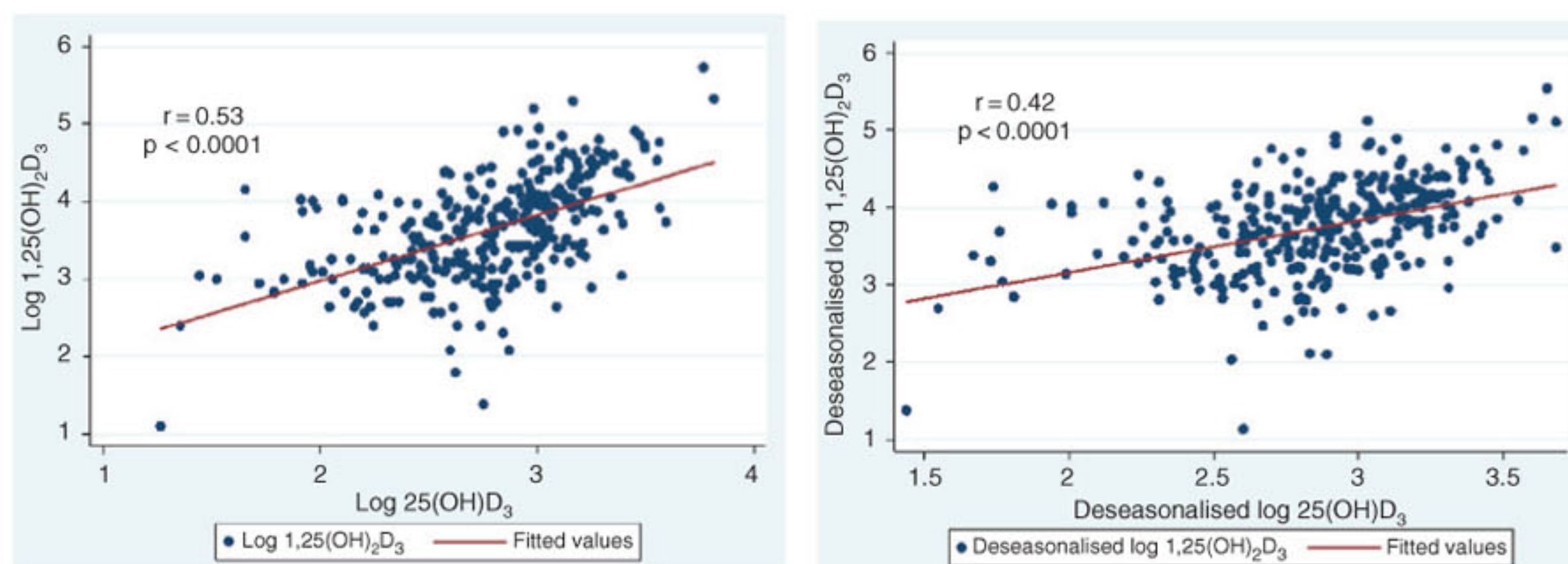


Figure 1: Comparison of serum deseasonalised 25(OH)D₃ and deseasonalised 1,25(OH)₂D₃ concentrations. The correlation coefficient for the raw data $r = 0.58$, $p < 0.0001$.

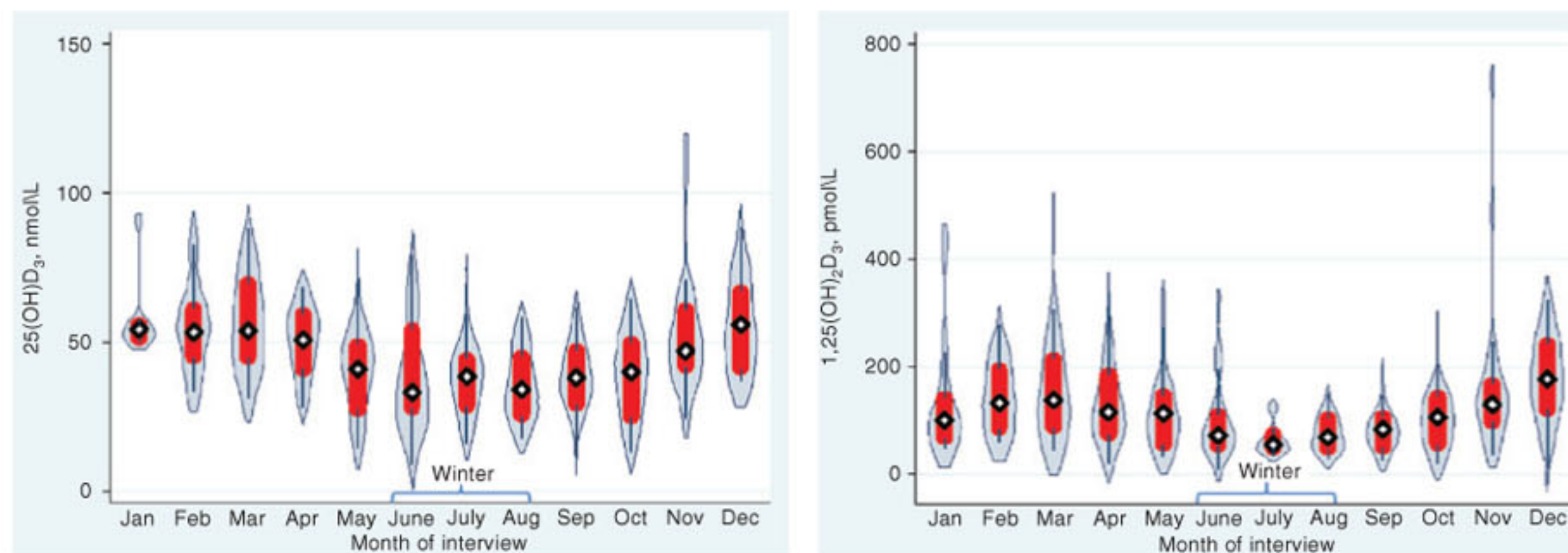


Figure 2: Month to month variation of serum 25(OH)D₃ and 1,25(OH)₂D₃ concentrations. Summer (Dec to Feb), Autumn (Mar to May), Winter (Jun to Aug) and Spring (Sep to Nov).

there was a positive association between age and both deseasonalised 25(OH)D₃ and 1,25(OH)₂D₃ concentrations ($p < 0.01$ and $p = 0.002$, respectively), gender was not associated with them ($p = 0.72$ and $p = 0.21$, respectively). For anthropometric measures, height was the only factor associated with both 25(OH)D₃ and 1,25(OH)₂D₃ concentrations; mean increase of 0.03 units, $p < 0.01$ and 0.04 units, $p = 0.01$ per 10 cm increase, respectively. Weight was positively associated with 1,25(OH)₂D₃ ($p = 0.04$), and in contrast, age- and gender-adjusted BMI was inversely associated with 25(OH)D₃ ($p < 0.01$).

Caucasians had higher deseasonalised 25(OH)D₃ concentrations (mean difference 0.3, $p < 0.001$) but no difference in deseasonalised 1,25(OH)₂D₃ ($p = 0.12$) when compared to non-Caucasian. Lighter natural hair colour and skin colour were associated with higher deseasonalised 25(OH)D₃ but not with deseasonalised 1,25(OH)₂D₃. Lighter eye colour was associated

with both deseasonalised 25(OH)D₃ and 1,25(OH)₂D₃ concentrations.

Child skin reaction during sun exposure, lifetime sun burns, freckling by the end of last summer and time in the sun during last summer and winter were associated with higher deseasonalised 25(OH)D₃ concentrations (Table 2). Only lifetime sun burns were associated with higher deseasonalised 1,25(OH)₂D₃ concentrations.

Variation in 25(OH)D₃ and 1,25(OH)₂D₃ concentrations with genetic factors

Of the 12 selected SNPs, rs2282679, rs4588 (*GC*), rs6013897 (*CYP24A1*), rs12785878 (*DHCR7*), and rs10741657 (*CYP2R1*) were associated with higher concentrations of both deseasonalised 25(OH)D₃ and 1,25(OH)₂D₃ (data not shown). Complete genotyping data to calculate Metscore and

Table 2: The relationship of environmental and genetic factors with 25(OH)D₃ and 1,25(OH)₂D₃ in univariate analysis.

Determinant	Log _e deseasonalised 25(OH)D ₃			Log _e deseasonalised 1,25(OH) ₂ D ₃		
	Mean change ^a (95% CI)	p-Value	(r ²) ^c	Mean change ^a (95% CI)	p-Value	(r ²) ^c
Gender F (M)	-0.02 (-0.1, 0.07)	0.72	0	-0.08 (-0.22, 0.05)	0.21	0
Ethnicity						
Caucasians/non-Caucasians	0.30 (0.21, 0.40)	<0.001	13	0.13 (-0.03, 0.29)	0.12	0
Eye colour		<0.001	11		0.07	3
Brown	Reference			Reference		
Hazel	0.12 (-0.01, 0.25)	0.06		0.18 (-0.03, 0.38)	0.09	
Green	0.31 (0.15, 0.47)	<0.001		0.14 (-0.11, 0.40)	0.28	
Blue/grey	0.26 (0.15, 0.36)	<0.001		0.20 (0.04, 0.37)	0.02	
Test of trend		<0.01	96		0.02	2.2
Natural skin colour on examination		<0.001	17		0.61	1
Dark	Reference			Reference		
Olive	0.48 (0.27, 0.69)	<0.001		0.11 (-0.25, 0.46)	0.56	
Olive/medium	0.55 (0.35, 0.75)	<0.001		0.16 (-0.17, 0.50)	0.34	
Medium/fair	0.65 (0.45, 0.85)	<0.001		0.20 (-0.14, 0.53)	0.25	
Fair	0.72 (0.50, 0.93)	<0.001		0.24 (-0.11, 0.60)	0.18	
Test of trend		<0.01	12.8		0.11	1
Weight (kg)	0.001 (-0.01, 0.004)	0.36	0.3	0.004 (0.00, 0.008)	0.04	2
Height (cm)	0.003 (0.02, 0.89)	0.002	4	0.004 (0.001, 0.01)	0.01	2.3
BMI (unit)	-0.01 (-0.01, 0.003)	0.27	0.5	0.004 (-0.01, 0.02)	0.61	0.1
BMI category		0.002	4.2		0.55	0.01
Normal weight	Reference			Reference		
Overweight	-0.20 (-0.32, -0.08)	0.001		-0.10 (-0.30, 0.09)	0.301	
Obese	-0.14 (-0.26, -0.01)	0.03		-0.06 (-0.25, 0.14)	0.56	
Test of trend		<0.01	3.2		0.42	0.3
End of last summer-freckles		<0.001	8		0.05	2
No freckles	Reference	<0.001		Reference	0.14	2
Few freckles	0.20 (0.12, 0.29)	<0.001		0.06 (-0.08, 0.20)	0.40	
Some freckles	0.18 (0.03, 0.33)	0.02		0.27 (0.02, 0.52)	0.04	
Many freckles	0.29 (0.03, 0.55)	0.03		0.26 (-0.16, 0.69)	0.23	
(Test of trend)		<0.001	5.9		0.03	1.5
Lifetime sunburns		<0.001	10		0.05	2
Never	Reference			Reference		
Once	0.22 (0.13, 0.32)	<0.001		0.14 (-0.02, 0.30)	0.08	
2-4 times	0.26 (0.17, 0.36)	<0.001		0.22 (0.06, 0.38)	0.01	
5 or more times	0.29 (0.00, 0.59)	0.05		0.17 (-0.31, 0.65)	0.49	
Test of trend		<0.01	9		<0.01	2
Time in sun in recent summer	0.001 (0.00, 0.002)	0.002	2.9	0.001 (-0.01, 0.002)	0.26	0.4
Time in sun in recent winter	0.001 (0.0, 0.0014)	<0.01	2.2	0.001 (-0.01, 0.002)	0.25	0.4
Season of blood collection ^d		<0.0001	13		<0.0001	0.14
Winter	Reference			Reference		
Summer	0.46 (0.32, 0.61)	<0.001		0.67 (0.44, 0.89)	<0.001	
Autumn	0.28 (0.16, 0.39)	<0.001		0.52 (0.35, 0.70)	<0.001	
Spring	0.16 (0.05, 0.26)	<0.001		0.36 (0.19, 0.53)	<0.001	
Test of trend		<0.01	5		0.002	3
UVR at month of interview ^d	0.01 (0.008, 0.014)	<0.001	14	0.016 (0.01, 0.02)	<0.0001	12
UVR 6 weeks before interview ^d	0.01 (0.009, 0.02)	<0.001	18	0.017 (0.013, 0.02)	<0.0001	15
Genetic factors						
Metabolism score (rs2282679 + rs6013897)						
0	Reference			Reference		
1	-0.10 (-0.84, 0.65)	0.80		-0.59 (-1.75, 0.57)	0.32	
2	-0.01 (-0.74, 0.72)	0.99		-0.01 (-1.14, 1.13)	0.99	
3	0.12 (-0.61, 0.85)	0.75		0.15 (-0.98, 1.29)	0.79	
4	0.17 (-0.56, 0.90)	0.64		0.18 (-0.96, 1.32)	0.76	
Test of trend		<0.001	4.4		<0.001	6.03

Table 2 (continued)

Determinant	Log _e deseasonalised 25(OH)D ₃			Log _e deseasonalised 1,25(OH) ₂ D ₃		
	Mean change ^a (95% CI)	p-Value	(r ²) ^c	Mean change ^a (95% CI)	p-Value	(r ²) ^c
Synthesis score (rs12785878 + rs10741657)						
0	Reference			Reference		
1	0.14 (−0.03, 0.31)	0.10		0.1 (−0.18, 0.38)	0.48	
2	0.23 (0.07, 0.39)	0.005		0.15 (−0.12, 0.41)	0.28	
3	0.38 (0.22, 0.55)	<0.001		0.27 (0.00, 0.55)	0.05	
4	0.33 (0.11, 0.56)	0.004		0.14 (−0.24, 0.51)	0.46	
Test of trend		<0.001	7.79		0.06	1

^aMean change of the natural-log-transformed deseasonalised 25(OH)D₃ concentration per 1 unit change in exposure. ^bMean change of the natural-log-transformed deseasonalised 1,25(OH)₂D₃ concentration per 1 unit change in exposure. ^cPercentage of relative contribution.

^dDeseasonalised levels are not appropriate. This represents the mean change of the natural-log-transformed 25(OH)D₃ and 1,25(OH)₂D₃ for seasons per 1 category/unit change in exposure.

Synscore were available for 309 (96%) and 307 (95%) participants, respectively. While higher Metscore showed positive associations with both deseasonalised 25(OH)D₃ and 1,25(OH)₂D₃ ($p_{\text{trend}} < 0.001$; $r^2 = 0.04$ and $p_{\text{trend}} < 0.001$; $r^2 = 0.06$), Synscore showed a positive association with deseasonalised 25(OH)D₃ ($p_{\text{trend}} < 0.001$; $r^2 = 0.08$) and a marginal association with deseasonalised 1,25(OH)₂D₃ ($p_{\text{trend}} = 0.06$). Table 3 shows that in the ‘genetic only’ model, Synscore and Metscore were retained as major predictors for 25(OH)D₃ with a contribution of 11.2% to its variance. Metscore was the only retained genetic predictor for 1,25(OH)₂D₃ concentrations, with a contribution of 6.0% to its variance. No individual SNPs were predictive.

Final predictive models for 25(OH)D₃ and 1,25(OH)₂D₃ concentrations

The final predictive model for 25(OH)D₃ retained seven important factors: UVR 6 weeks before interview, ethnicity, skin colour, height, history of sun burn during life-time, Synscore and Metscore. Overall, these factors explained more than 43% of the variation in serum 25(OH)D₃ concentrations. In contrast, the final predictive model for 1,25(OH)₂D₃ retained four factors: UVR 6 weeks before interview, eye colour, height and Metscore. These factors explained over 25% of the variation of this biological active metabolite (Table 4).

Concentration 25(OH)D₃ alone accounted for 18% of the variation in 1,25(OH)₂D₃ concentrations. Interestingly, UVR 6 weeks before interview and Metscore remained positively associated with 1,25(OH)₂D₃ even after further adjustment for 25(OH)D₃ in the final model. No interaction was detected between the predictors of 1,25(OH)₂D₃ in the final model. However, for 25(OH)D₃ increasing Synscore

had a greater impact on 25(OH)D₃ concentrations among Caucasians than non-Caucasians.

Discussion

This study demonstrates for the first time a multivariable model, which incorporates comprehensive environmental and genetic factors, for childhood circulating 25(OH)D₃ and 1,25(OH)₂D₃ concentrations. It shows the seasonal variation of 25(OH)D₃ and vitamin D deficiency rate of approximately 80% in winter, based on 25(OH)D₃ <20 ng/mL (<50 nmol/L), among this Australian paediatric population. Importantly, we are the first to report on the distribution of 1,25(OH)₂D₃ among healthy children and its relation to a range of environmental and genetic factors. We found that the ambient UVR in 6 weeks before blood collection was the main factor influencing both child serum 25(OH)D₃ and 1,25(OH)₂D₃ concentrations. Ambient UVR 6 weeks before blood collection accounted for approximately 15% of 25(OH)D₃ variance and 18% for 1,25(OH)₂D₃ variance and contributed to a clear seasonal distribution for both with a late winter nadir. Not surprisingly in view of the endocrine regulation of 1 α -hydroxylase, the correlation between 25(OH)D₃ and 1,25(OH)₂D₃ concentrations was only moderate and we identified for the first time that selected gene variants related to vitamin D metabolism predicted higher 1,25(OH)₂D₃ concentrations in children for a given 25(OH)D₃ concentrations.

Strengths of this study include the detailed childhood sun exposure behaviours as well as extensive environmental data that enabled us to explore the interplay between genetic and environmental determinants. The availability of both 25(OH)D₃ and 1,25(OH)₂D₃ concentrations which was measured by a recommended assay in

Table 3: Multivariable linear regression models: environmental and genetic determinants of 25(OH)D₃ and 1,25(OH)₂D₃ concentrations.

Determinant	Log _e 25(OH)D ₃			Log _e 1,25 (OH) ₂ D ₃		
	Mean change ^a (95%CI)	p-Value	(r ²) ^c	Mean change ^b (95%CI)	p-Value	(r ²) ^c
Environmental factors only						
UVR 6 weeks before interview (per unit increase)	0.011 (0.008, 0.014)	<0.001		0.016 (0.01, 0.02)	<0.001	
Lifetime sunburns ^d	0.1 (0.05, 0.15)	<0.001		0.11 (0.03, 0.2)	0.01	
End of summer freckles ^d	0.07 (0.01, 0.13)	0.02				
Time in sun during last winter ^d	0.0007 (0.00, 0.001)	0.02				
Environmental factors total contribution		<0.001	27.89		<0.001	16.53
Phenotypic factors only						
Eye colour ^d	0.043 (0.01, 0.08)	<0.03		0.05 (0.001, 0.11)	0.05	
Natural skin colour ^d	0.10 (0.06, 0.14)	<0.001				
Height (cm)	0.003 (0.001, 0.005)	0.002				
BMI Category ^d	-0.06 (-0.12, -0.01)	0.03				
Phenotypic factors total contribution		<0.001	20.95		<0.001	6.11
Genetic factors only						
Metabolism score ^d	0.08 (0.04, 0.13)	<0.001		0.17 (0.09, 0.24)	<0.001	
Synthesis score ^d	0.1 (0.06, 0.14)	<0.001		0.06 (0.00, 0.13)	0.06	
Genetic factors total contribution		<0.001	11.22		<0.001	6.03

^aMean change of the natural-log-transformed deseasonalised 25(OH)D₃ concentration per 1 unit change in exposure. ^bMean change of the natural-log-transformed deseasonalised 1,25(OH)₂D₃ concentration per 1 unit change in exposure. ^cPercentage of relative contribution, ^dper category increase, see Table 2.

Table 4: Final multifactorial examination of 25(OH)D₃ and 1,25(OH)₂D₃ predictors in healthy Australian children.

Determinant	Log _e 25(OH)D ₃			Log _e 1,25 (OH) ₂ D ₃		
	Mean change ^a (95%CI)	p-Value	(r ²)	Mean change ^b (95%CI)	p-Value	(r ²)
UVR 6 weeks before interview	0.012 (0.009, 0.014)	<0.001		0.02 (0.013, 0.023)	<0.001	
Natural skin colour	0.08 (0.04, 0.13)	<0.001				
Eye colour				0.07 (0.010, 0.13)	0.02	
Caucasian	0.14 (0.04, 0.25)	0.01				
Lifetime sunburns	0.07 (0.02, 0.13)	<0.001				
Height (cm)	0.002 (0.00, 0.004)	0.03		0.01 (0.001, 0.10)	0.007	
Metabolism score	0.06 (0.01, 0.11)	0.03		0.13 (0.04, 0.21)	0.005	
Synthesis score	0.07 (0.03, 0.12)	0.002				
Final predictive model		<0.001	43.54		<0.001	25.59

^aMean change of the natural-log-transformed deseasonalised 25(OH)D₃ concentration per 1 unit change in exposure. ^bMean change of the natural-log-transformed deseasonalised 1,25(OH)₂D₃ concentration per 1 unit change in exposure.

a central laboratory [24], allowed us to conduct a comparison of their determinants which were rarely assessed in this age group. Because recent work in children has shown that 25(OH)D₃ concentration may contain D₃ epimer (which has uncertain bioactivity), we provided fractionated 25(OH)D₃ with D₃ epimer and 24,25(OH)₂D₃ removed. Another strength is the use of the genetic data to construct a genetic score and its inclusion into the predictive models for both metabolites. Melbourne, Australia is well suited to this type of investigation as it is a city where vitamin D food fortification is uncommon and child supplementation is not routinely given. Despite a relatively large population, some of our findings were limited in power

due to small numbers for particular genotypes and ethnic groups. Limitations of our study also include ethnicity was limited to parental report. However, it was consistent with the finding from the objective skin type assessment. Only a very basic dietary assessment was carried out and the use of vitamin D supplements in the last year was not recorded, (would be very low in our studied population as the major source of vitamin D was assumed to be sunlight exposure) [32]. In addition, our data did not include information about serum calcium, phosphate and parathyroid hormone concentrations which may contribute to the tight regulation of 1,25(OH)₂D₃ concentrations [33, 34] via renal 1 α -hydroxylation.

The finding that UVR levels at 6 weeks before interview had the strongest association with $25(\text{OH})\text{D}_3$ was consistent with recent findings from Australia [17, 35] and Canada [36]. Previous studies have identified seasonal variation in $1,25(\text{OH})_2\text{D}_3$ in geriatric participants and linked this to the seasonal variation in its substrate [$25(\text{OH})\text{D}_3$] [37]. We are the first to show that UVR levels 6 weeks before interview remained a predictor of $1,25(\text{OH})_2\text{D}_3$ even after adjustment for $25(\text{OH})\text{D}_3$ concentrations. This finding, if replicated in future studies, could help explain why observational studies, often based on UVR-derived $25(\text{OH})\text{D}_3$ concentrations in general populations, have shown that higher $25(\text{OH})\text{D}_3$ concentrations were associated with reduced disease risk compared with randomised control trials, using dietary vitamin D_3 supplementation have not [38], i.e. in the former, $25(\text{OH})\text{D}_3$ concentrations are a marker of past UVR exposure and our data indicate this would have been with a hidden additional benefit on $1,25(\text{OH})_2\text{D}_3$ concentrations and thus presumably active vitamin D activity. In contrast, higher $25(\text{OH})\text{D}_3$ concentrations following vitamin D_3 dietary supplementation would not be accompanied by any UVR-induced boost to $1,25(\text{OH})_2\text{D}_3$ [39].

Most previous studies showed a consistent effect of *CYP2R1* (rs10741657) [16, 40, 41], *DHCR7* (rs12785878) [16, 42], *CYP24A1* (rs6013897) [16, 40] and *GC* (rs2282679) [16, 40, 41] which was found to be associated with vitamin D concentrations in our study. *GC*-globulin is the major VDBP which acts as a transporter for both $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ leaving $<0.1\%$ and $<1\%$, respectively, to circulate in free form [43, 44]. Lower VDBP, which is linked to *GC* variants, were previously reported to be associated with low $25(\text{OH})\text{D}_3$ concentrations [16]. Combining *GC* variants with *CYP24A1* (rs6013897) on Metscore provided a more powerful instrument for the prediction of $25(\text{OH})\text{D}_3$ concentrations in our study which is consistent with a previous report [29]. Interestingly, we extend this concept to the association of Metscore with $1,25(\text{OH})_2\text{D}_3$ concentrations and showed the same pattern. This suggests that bioavailability of both metabolites are affected by these gene variants.

Although the final predictive models demonstrated here were only able to account for 43% and 25% of $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ concentrations, respectively. This compares well with the 1%–4% accounted for by genetic factors in a vitamin D GWAS study [16]. Previously, a vitamin D synthesis or metabolism score has been successfully used as an instrumental variable in a Mendelian randomisation study [28]. Here we confirm that such scores have substantially improved the predictive model for $25(\text{OH})\text{D}_3$ concentrations and we extended this approach by showing that the Metscore predicted

$1,25(\text{OH})_2\text{D}_3$ concentrations, beyond the blood concentrations of $25(\text{OH})\text{D}_3$.

Conclusions

Here, we have developed predictive models for both $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ concentrations in a population of Australian children. These findings are based on a multiethnic population and can be generalised to healthy child populations. The findings highlight the importance of both environmental factors, in particular ambient UVR levels 6 weeks before blood collection, and genetic factors in determining both $25(\text{OH})\text{D}_3$ and $1,25(\text{OH})_2\text{D}_3$ concentrations in children.

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References

1. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011;96:1911–30.

2. Nowson CA, McGrath JJ, Ebeling PR, Haikerwal A, Daly RM, et al. Vitamin D and health in adults in Australia and New Zealand: a position statement. *Med J Aust* 2012;196:686–7.
3. Allen KJ, Koplin JJ, Ponsonby A-L, Gurrin LC, Wake M, et al. Vitamin D insufficiency is associated with challenge-proven food allergy in infants. *J Allergy Clin Immunol* 2013;131:1109–16. e6.
4. Van der Mei I, Ponsonby A-L, Dwyer T, Blizzard L, Taylor B, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. *J Neurol* 2007;254:581–90.
5. Rosen CJ, Adams JS, Bikle DD, Black DM, Demay MB, et al. The nonskeletal effects of vitamin D: an Endocrine Society scientific statement. *Endocr Rev* 2012;33:456–92.
6. Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. *N Engl J Med* 2013;369:1991–2000.
7. Nielson CM, Jones KS, Chun RF, Jacobs JM, Wang Y, et al. Free 25-hydroxyvitamin D: impact of vitamin D binding protein assays on racial-genotypic associations. *J Clin Endocrinol Metab* 2016;101:2226–34.
8. Glendenning P, Chew GT. Controversies and consensus regarding vitamin D deficiency in 2015: whom to test and whom to treat? *Med J Aust* 2015;202:470–1.
9. Thieden E, Philipsen PA, Heydenreich J, Wulf HC. Vitamin D level in summer and winter related to measured UVR exposure and behavior. *Photochem Photobiol* 2009;85:1480–4.
10. Armas LA, Dowell S, Akhter M, Duthuluru S, Huerter C, et al. Ultraviolet-B radiation increases serum 25-hydroxyvitamin D levels: the effect of UVB dose and skin color. *J Am Acad Dermatol* 2007;57:588–93.
11. Hagenau T, Vest R, Gissel T, Poulsen C, Erlandsen M, et al. Global vitamin D levels in relation to age, gender, skin pigmentation and latitude: an ecologic meta-regression analysis. *Osteoporos Int* 2009;20:133–40.
12. Bouillon R. Genetic and environmental determinants of vitamin D status. *Lancet* 2010;376:148–9.
13. Berry D, Hyppönen E. Determinants of vitamin D status: focus on genetic variations. *Curr Opin Nephrol Hypertens.* 2011;20:331–6.
14. Engelman CD, Fingerlin TE, Langefeld CD, Hicks PJ, Rich SS, et al. Genetic and environmental determinants of 25-hydroxyvitamin D and 1, 25-dihydroxyvitamin D levels in Hispanic and African Americans. *J Clin Endocrinol Metab* 2008;93:3381–8.
15. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, et al. Genome-wide association study of circulating vitamin D levels. *Hum Mol Genet* 2010;19:2739–45.
16. Wang TJ, Zhang F, Richards JB, Kestenbaum B, Van Meurs JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. *Lancet* 2010;376:180–8.
17. Lucas RM, Ponsonby A-L, Dear K, Valery PC, Taylor B, et al. Vitamin D status: multifactorial contribution of environment, genes and other factors in healthy Australian adults across a latitude gradient. *J Steroid Biochem Mol Biol* 2013;136:300–8.
18. Van der Mei IA, Blizzard L, Ponsonby AL, Dwyer T. Validity and reliability of adult recall of past sun exposure in a case-control study of multiple sclerosis. *Cancer Epidemiol Biomarkers Prev* 2006;15:1538–44.
19. Pezic A, Ponsonby AL, Cameron FJ, Rodda C, Ellis JA, et al. Constitutive and relative facultative skin pigmentation among victorian children including comparison of two visual skin charts for determining constitutive melanin density. *Photochem Photobiol* 2013;89:714–23.
20. English D, MacLennan R, Rivers J, Kelly J, Armstrong B. Epidemiological studies of melanocytic naevi: protocol for identifying and recording naevi. *Int Agency Res Cancer, Lyon, France, IARC Internal Rep* 1990;90:1–22.
21. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br Med J* 2000;320:1240–3.
22. Ponsonby A-L, Pezic A, Cameron FJ, Rodda C, Ellis JA, et al. Phenotypic and environmental factors associated with elevated autoantibodies at clinical onset of paediatric type 1 diabetes mellitus. *Results Immunol* 2012;2:125–31.
23. Weinstock-Guttman B, Zivadinov R, Qu J, Cookfair D, Duan X, et al. Vitamin D metabolites are associated with clinical and MRI outcomes in multiple sclerosis patients. *J Neurol Neurosurg Psychiatry* 2011;82:189–95.
24. Duan X, Weinstock-Guttman B, Wang H, Bang E, Li J, et al. Ultra-sensitive quantification of serum vitamin D metabolites using selective solid-phase extraction coupled to microflow liquid chromatography and isotope-dilution mass spectrometry. *Anal Chem* 2010;82:2488–97.
25. Frederiksen B, Liu E, Romanos J, Steck A, Yin X, et al. Investigation of the vitamin D receptor gene (VDR) and its interaction with protein tyrosine phosphatase, non-receptor type 2 gene (PTPN2) on risk of islet autoimmunity and type 1 diabetes: the Diabetes Autoimmunity Study in the Young (DAISY). *J Steroid Biochem Mol Biol* 2013;133:51–7.
26. Dastani Z, Li R, Richards B. Genetic regulation of vitamin D levels. *Calcif Tissue Int* 2013;92:106–17.
27. Ellis JA, Scurrah KJ, Li YR, Ponsonby A-L, Chavez RA, et al. Epistasis amongst PTPN2 and genes of the vitamin D pathway contributes to risk of juvenile idiopathic arthritis. *J Steroid Biochem Mol Biol* 2015;145:113–20.
28. Vimalaswaran KS, Cavadino A, Berry DJ, Jorde R, Dieffenbach AK, et al. Association of vitamin D status with arterial blood pressure and hypertension risk: a mendelian randomisation study. *Lancet Diabetes Endocrinol* 2014;2:719–29.
29. Berry DJ, Vimalaswaran KS, Whittaker JC, Hingorani AD, Hyppönen E. Evaluation of genetic markers as instruments for mendelian randomization studies on vitamin D. *PLoS One* 2012;7:e37465.
30. Lucas R, Ponsonby A-L, Dear K, Valery P, Pender M, et al. Sun exposure and vitamin D are independent risk factors for CNS demyelination. *Neurology* 2011;76:540–8.
31. Van der Mei IA, Ponsonby A-L, Dwyer T, Blizzard L, Taylor BV, et al. Vitamin D levels in people with multiple sclerosis and community controls in Tasmania, Australia. *J Neurol* 2007;254:581–90.
32. Munns C, Zacharin MR, Rodda CP, Batch JA, Morley R, et al. Prevention and treatment of infant and childhood vitamin D deficiency in Australia and New Zealand: a consensus statement. *Med J Aust* 2006;185:268.
33. Farrow EG, Davis SI, Summers LJ, White KE. Initial FGF23-mediated signaling occurs in the distal convoluted tubule. *J Am Soc Nephrol* 2009;20:955–60.
34. Hill KM, Martin BR, Wastney ME, McCabe GP, Moe SM, et al. Oral calcium carbonate affects calcium but not phosphorus balance in stage 3–4 chronic kidney disease. *Kidney Int* 2013;83:959–66.
35. Nair-Shalliker V, Clements M, Fenech M, Armstrong BK. Personal Sun Exposure and Serum 25-hydroxy Vitamin D Concentrations. *Photochem Photobiol* 2013;89:208–14.

36. Greenfield JA, Park PS, Farahani E, Malik S, Vieth R, et al. Solar ultraviolet-B radiation and vitamin D: a cross-sectional population-based study using data from the 2007 to 2009 Canadian Health Measures Survey. *BMC Public Health* 2012;12:660.
37. Bouillon RA, Auwerx JH, Lissens WD, Pelemans WK. Vitamin D status in the elderly: seasonal substrate deficiency causes 1, 25-dihydroxycholecalciferol deficiency. *Am J Clin Nutr* 1987;45:755–63.
38. Theodoratou E, Tzoulaki I, Zgaga L, Ioannidis JP. Vitamin D and multiple health outcomes: umbrella review of systematic reviews and meta-analyses of observational studies and randomised trials. *BMJ* 2014;348:g2035.
39. Hart PH, Gorman S, Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? *Nat Rev Immunol* 2011;11:584–96.
40. Cooper JD, Smyth DJ, Walker NM, Stevens H, Burren OS, et al. Inherited variation in vitamin D genes is associated with predisposition to autoimmune disease type 1 diabetes. *Diabetes* 2011;60:1624–31.
41. Ramos-Lopez E, Brück P, Jansen T, Herwig J, Badenhoop K. CYP2R1 (vitamin D 25-hydroxylase) gene is associated with susceptibility to type 1 diabetes and vitamin D levels in Germans. *Diabetes Metab Res Rev* 2007;23:631–6.
42. Wehr E, Trummer O, Giuliani A, Gruber H-J, Pieber TR, et al. Vitamin D-associated polymorphisms are related to insulin resistance and vitamin D deficiency in polycystic ovary syndrome. *Eur J Endocrinol* 2011;164:741–9.
43. Gozdzik A, Zhu J, Wong BY-L, Fu L, Cole DE, et al. Association of vitamin D binding protein (VDBP) polymorphisms and serum 25 (OH) D concentrations in a sample of young Canadian adults of different ancestry. *J Steroid Biochem Mol Biol* 2011;127: 405–12.
44. Lauridsen AL, Vestergaard P, Hermann A, Brot C, Heickendorff L, et al. Plasma concentrations of 25-hydroxy-vitamin D and 1, 25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. *Calcif Tissue Int* 2005;77: 15–22.