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ORIGINAL RESEARCH

Half the Genetic Variance in Vitamin D Concentration is Shared with Skin Colour and Sun Exposure Genes

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Abstract

This study assessed the heritability of 25 hydroxyvitamin D_3 (25(OH)D3) in a large twin cohort and the shared effect of sun exposure and skin colour on 25(OH)D3 variance. Study participants included 1604 twin pairs and their siblings (n = 4020). Twin correlations for 25(OH)D3 concentration were r_{MZ}=0.79 (584 pairs) and r_{DZ} = 0.52 (1020 pairs) consistent with an average $h^2 = 0.50$ throughout the year. Significant phenotypic and genetic seasonal fluctuation was observed in 25(OH)D3 concentrations with heritability decreasing during the winter ($h^2 = 0.37$) compared to summer ($h^2 = 0.62$). Skin colour (measured both ordinally and quantitatively) and self-reported sun exposure were found to significantly affect 25(OH)D3 concentration. Twins with olive/dark skin had significantly lower 25(OH)D3 concentrations than those with fair/pale skin and multivariate genetic analysis showed that approximately half of the total additive genetic variation in 25(OH)D3 results from genes whose primary influence is on skin colour and sun exposure. Additionally, 37% of the total variance was attributed to shared environmental effects on vitamin D, skin colour and sun exposure measures. These results support a moderate estimate of vitamin D heritability and suggest significant influence of season, skin colour and sun exposure on the genetic variance.

Keywords Vitamin D · Genetics · Heritability · Sun exposure · Skin colour · Twin

Introduction

Vitamin D, which plays a vital role in bone health, is synthesized through the epidermis of the skin when exposed to ultra-violet radiation (UVR) (vitamin D3) (Datta et al. 2017), or absorbed from food or supplements (vitamin D2 and, to a small extent, vitamin D3). Though there are several forms of vitamin D, 25-hydroxyvitamin D (25(OH)D) is the most widely used marker of overall vitamin D status, which results from the combination of 25(OH)D2 and 25(OH)D3 (Holick 2007).

Epidemiological studies have linked vitamin D deficiency with a wide range of non-communicable diseases such as diabetes, heart failure, hypertension, obesity, cancer as well as neuropsychiatric and autoimmune diseases (Holick 2007; Eyles et al. 2013; Muscogiuri et al. 2017a, b; Savastano et al. 2017; Vaughan-Shaw et al. 2017). However, vitamin D deficiency can result from the influence of chronic disorders that limit outdoor activity (and exposure to UVR), thus it remains unclear whether vitamin D deficiency has any causal role in these disorders. Quantifying the genetic and environmental influences on vitamin D using twin studies, helps to begin to unravel these observed comorbidities.

Several studies have examined the genetic influences behind vitamin D deficiency. Family studies have provided robust evidence that heritable (genetic) factors play a major role in vitamin D status (Madsen et al. 2014; Robinson et al. 2017). Previous twin studies support this by reporting both

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genetic and common environmental variance components as major determinants in overall vitamin D concentration (Snellman et al. 2009; Karohl et al. 2010; Mills et al. 2015). However, there is inconsistency in the range of previously reported heritability (h^2) estimates of vitamin D. Most family-based studies report a moderate to high heritability (50–80%) (Hunter et al. 2001; Wjst et al. 2007; Orton et al. 2008; Shea et al. 2009; Snellman et al. 2009; Mills et al. 2015) and three studies have reported a seasonal variance in heritability (Snellman et al. 2009; Karohl et al. 2010; Mills et al. 2015). Recent genomic advances have also enabled SNP-based heritability of vitamin D levels to be estimated. Most recently, a large genome-wide association study (GWAS) based on nearly 80,000 participants identified six genome-wide significant loci and estimated that SNP-based heritability was 7.5% (with 38% of this variance explained by the six genome-wide significant loci) (Jiang et al. 2018). Family-based h^2 estimates are traditionally higher than SNP-based estimates as they include contributions from several genetic sources including common, intermediate and rare frequency alleles, DNA sequence variants, gene–gene (G × G) interaction and gene–environment (G × E) interaction effects. In comparison, SNP h^2 is based solely on the additive genetic effects of common SNPs.

Although genetic factors have been shown to substantially affect vitamin D status, several environmental factors have also been identified as potent modifiers of circulating 25(OH)D concentration, albeit inconsistently reported. For instance 25(OH)D2 is acquired solely through the use of supplements. For 25(OH)D3, variables include season, latitude, obesity, sunlight exposure, area of uncovered skin, the use of tanning beds and, to a small extent, fortification from food, such as milk and margarine (Burgaz et al. 2007; Van Der Meer et al. 2008). Some of these variables are 'upstream' factors which limit the potential to synthesize 25(OH)D3, for example, at high latitude, UVR is not sufficient to manufacture 25(OH)D3 during winter (Hollis 2005). In fact, genome-wide association studies have identified common genetic variants in the vitamin D binding protein and key enzymes required for the synthesis and catabolism of vitamin D-related pathways (Jiang et al. 2018). To date, there has been little research that examines the extent to which such environmental covariates are due to genetic or environmental variance components. For example, skin colour has been robustly estimated to have an extremely high heritability, and has also been shown to influence both forms of 25(OH)D (Eyles 2010; Fajuyigbe and Young 2016). However, even behavioural factors such as the number of hours spent outside have been shown to be genetically influenced (Duffy et al. 2004; Rees 2004). Thus, this study aimed to (i) estimate the effect of season in circulating vitamin D in the largest twin cohort to date and (ii) investigate the extent to which circulating vitamin D concentrations show any genetic and environmental overlap with the associated phenotypic measures skin colour, skin reflectance and sun exposure.

Materials and methods

Subjects

The primary subjects in this study were adolescent twins born between 1977 and 2003 (n = 3208) recruited for the Brisbane Longitudinal Twin Study on melanoma risk factors (Wright and Martin 2004; Zhu et al. 2007). Data collected from the twin pairs at approximately age 12 and their siblings (within 5 years of the twins' age) were used in this study (n = 4020; mean age = 12.6 ± 1.3, range 9–19 years, females = 2108, males = 1913). Samples were collected between May 1992 and January 2014, majorly from South-East Queensland (latitude 27° S). At this latitude, there is sufficient UVR to allow for vitamin D synthesis throughout the year (Kimlin et al. 2014). Legal guardians gave written, informed consent prior to inclusion and testing. Studies were approved by the Human Research Ethics Committee of the QIMR Berghofer Medical Research Institute.

Zygosity was primarily determined using genome-wide genotyping (95% of participants) while a standardized questionnaire was used for the remaining 5% of participants (questionnaire accuracy shown to be approximately 98%) (Martin and Martin 1975; Heath et al. 2003). The cohort included 584 monozygotic (MZ), 1020 dizygotic (DZ) complete twin pairs and 813 singletons, which were derived either from siblings of the twin pairs, single twins or, in a few cases, trizygotic triplets (considered as 1 DZ twin pair and 1 singleton for the purpose of this analysis).

Covariates

Blood samples were collected from all twin pairs and siblings; all subjects were asked to self-report the number of hours they spent in the sun during the week and on the weekend. Self-reported *sun exposure* measures were collected by mail or telephone four times (each April and September) between the twins' 12th and 14th birthday appointments. A continuous report of sun exposure during the week and during the weekend was calculated by averaging the four biannual reports, as described in Law et al. (2017). (Green and Battistutta 1990; Law et al. 2017), (n = 3957).

During the same visit, a registered nurse rated the subject's *skin tone* on a 3 point scale (1 = fair/light, 2 = medium, 3 = dark/olive; n = 3789) and measured *skin reflectance* at a sun-exposed (dorsum of left hand) and unexposed (inner left upper arm) site at a wavelength of 650 nm (n = 3957). Initial instrument standardization using a standard white tile was

carried out for each subject so that reflectance values were relative to 100% reflectance from the tile. Thus, high reflectance values indicate fairer skin with little absorptive melanin pigment. Due to the extended time period during which data was longitudinally collected (approximately 22 years), two different spectrophotometers were used during the data collection process. The first, an EEL Model 99 Reflectance Spectrophotometer, manufactured by Diffusion Systems Ltd, Hanwell, London (hereafter referred to as instrument L) was used to take reflectance measurements on 2914 individuals from 1992 to 2008, while the second, a Konica Minolta Spectrophotometer CM-2500d, manufactured by Konica Minolta Inc. Osaka, Japan, (hereafter instrument K) was used for 1041 individuals from 2009 to 2014. These were treated as independent samples in subsequent analyses.

Vitamin D measurement

In this study, both 25-hydroxyvitamin D2 (25(OH)D2) and 25-hydroxyvitamin D3 (25(OH)D3) species were measured in blood plasma using a method previously described (Eyles et al. 2009).

Linearity of 25(OH)D2 and 25(OH)D3 concentration was assessed using matrix-matched calibration standards, with R^2 values of >0.99 across the calibration range (10–125 nmol/L). Assay accuracy was assessed at four concentration levels for 25(OH)D3 (48.3, 49.4, 76.4, 139.2 nmol/L) and a single level for 25OHD2 (32.3 nmol/L) using certified reference materials purchased from the National Institute of Standards and Technology (NIST) (NIST SRM 972a levels 1–4) and was excellent at all concentration levels tested with 10% inaccuracy across all four levels. Assay repeatability was assessed via replicate analysis of an independent reference material (NIST SRM1950, 61.9 nmol/L 25OHD3). Inter-assay imprecision was $<11\%$ ($n=343$). The method limit of quantification was 1 and 5 for 25(OH)D3 and 25(OH)D2, respectively.

To assess both longitudinal and within-year (seasonal) fluctuations in 25(OH)D3 levels, mean concentrations were examined according to the year and month of sample collection.

Statistical analysis

Preliminary analysis and data quality control was conducted using SPSS 22.0 (SPSS Inc., Chicago, IL, USA), while subsequent analyses were carried out using Mx 1.60 (Neale et al. 2003), accounting for relatedness. The raw data distribution is shown in Fig. 1. As there was only slight positive skewness, data was not transformed but outliers were Winsorized to ± 3.5 SD.

Analyses were also conducted to assess the independent effects of experimental variables on 25(OH)D3 concentration and heritability. These variables were: (i) plate (or batch) number ($n=40$), (ii) plate column number, (iii) plate row number, (iv) storage time; defined as the time from blood collection to date of assay (mean = 13.8 (5.7) years), (v) month of blood collection ($n=12$) and (vi) year of sample collection ($n=22$). Other covariates assessed were the sex and age of the participant. Those aforementioned

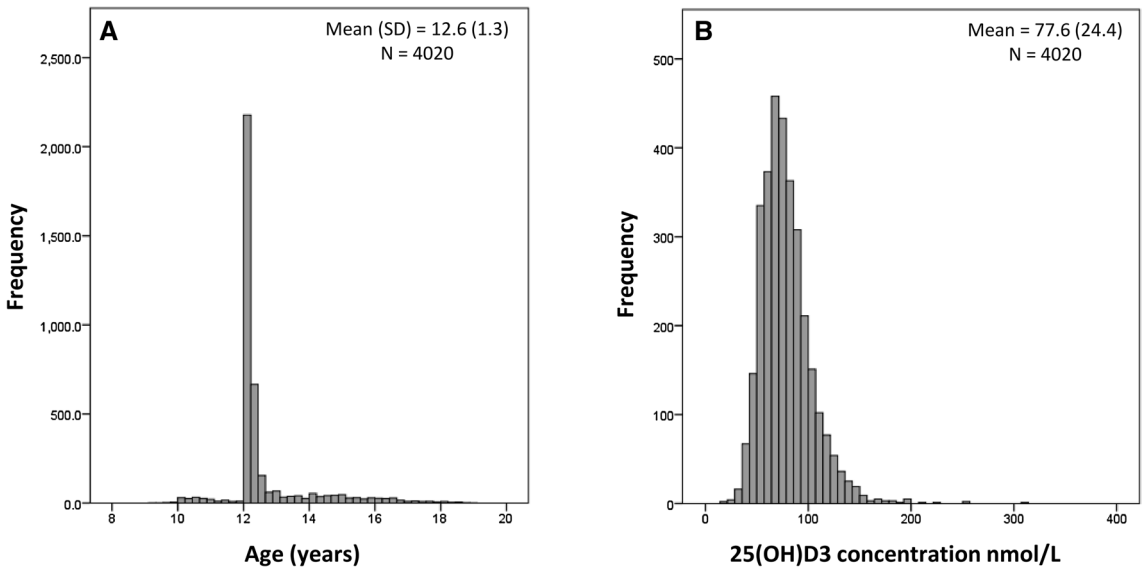

Fig. 1 Histograms depicting age (**a**) and raw 25 hydroxyvitamin D_3 (25(OH)D3) concentration (**b**) distributions in study participants at the time of first visit (modal age 12; $n=4020$)

variables that significantly affected the fit of the model were regressed out from the 25(OH)D3 measurement using a linear model that included these variables as fixed effects. Values for missing dependent variable data, (e.g. twins missing twin-pair data), were estimated using full information maximum-likelihood estimation (MLE) (n = 136).

Twin correlations, heritability of 25(OH)D3 and shared covariance with skin colour and sun exposure

Structural equation modelling was used to decompose the observed variance in 25(OH)D3 concentrations into its additive genetic (A), common environmental (C) and unique environmental (E) components. ACE models were generated examining only samples collected during winter/spring (June–November) and summer/autumn (December–May) in the southern hemisphere, to examine the effect of season on the variance components. To examine whether within-pair variability in sun exposure was affecting the seasonal ACE variance components, the variance of absolute difference in sun exposure between MZ pairs sampled in summer and winter were compared.

Multivariate analysis was performed in order to determine the sources of covariation between skin colour (both the ordinal rating and two reflectance measures [exposed and unexposed]), self-reported sun exposure and 25(OH)D3 levels. This was achieved using a Cholesky decompositions where skin colour rating was the first, sun exposed hours as the second, reflectance (exposed skin) as the third, reflectance (unexposed skin) as the fourth and vitamin D as the last latent factor. In order to account for the two different spectrophotometers used to measure skin reflectance, the two subsets (those measured with instrument L and those with instrument K) were treated as two individual variables that were loaded on to a latent factor. Phenotypic correlations between vitamin D, sun exposure, skin colour rating and reflectance were compared to genetic correlations produced using a multivariate Cholesky decomposition.

Results

Clinical characteristic of the cohort

25(OH)D2 was only detected above the lowest level of quantification in only 14 of the 4020 subjects and for all of these individuals, concentration of 25(OH)D2 was extremely low (< 24 nmol/L). Thus, for the remainder of the analysis, only 25(OH)D3 was used as a proxy for overall vitamin D concentration. 25(OH)D3 concentrations were measured for all individuals, including 1604 twin pairs and their 813 siblings (n = 4020; age range 9.0–19.0 years; see Fig. 1). The raw distribution of vitamin D concentrations is shown in Fig. 1. The mean (SD) 25(OH)D3 concentration was 77.6 (24.5) nmol/L (n = 4020). Of the 4020 individuals, 4 (0.03%) had concentrations of less than 25 nnol/L, the IOM guideline value for vitamin D deficiency (Ross et al. 2011; Paxton et al. 2013). Mean 25(OH)D3 concentrations were found to be significantly lower in subjects with olive or dark skin (73.1 (2.5) nmol/L; n = 495) than in those with medium (79.3 (1.2) nmol/L; n = 1985) or fair skin (78.6 (1.6) nmol/L; n = 1476). Distributions of covariates and examined phenotypes are shown in Table 1.

Storage effects

Samples were collected over a period of 22 years, from 1992 to 2014, and storage (at − 80 °C) time was calculated from day of sample collection to date of thawing for assay. The mean storage time for the collected samples was 12.8 (5.3) years (range 2.9–24.8 years). Storage time had only a minor effect on 25(OH)D3 concentration ($R^2 = 0.01$, $P < 0.05$) and no effect on the univariate model fit or covariate regression-coefficients (β) when included as a covariate.

Stability over time

Year of sample collection was analysed, as it is likely that attitudes towards sun exposure changed over the 22 year collection period. A slight, yet significant, downwards trend was observed in mean 25(OH)D3 levels across the 22 year period (Fig. 2a). Year of sample collection was coded as a continuous variable (n = 22) and was found to have a significant effect on the linear regression analysis ($R^2 = 0.018$, $P < 0.001$). This could be explained by public-health based changes towards sun exposure (increased awareness of sun damage, limiting sun exposure and increased use of sunscreen).

Significant sinusoidal, within year fluctuation was observed in mean 25(OH)D3 levels, and consistent with seasonal fluctuations that had lower concentrations in winter and spring (June–November) and higher in summer and autumn (December–May) (Fig. 2b). Based on these results, these months were selected to represent ‘winter’ and ‘summer’ collection times in subsequent analyses.

Twin correlations

For 25(OH)D3, an MZ correlation of r = 0.79 (0.73–0.84) and a DZ correlation of r = 0.52 (0.47–0.58) was observed. Similar differences between MZ and DZ correlations were observed in all other variables (see Table 2).

Table 1 Means (SD) for age, vitamin D concentration, skin colour rating, skin reflectance and sun exposure at first visit

	Sample size (n)	Mean ± SD (min–max)
Age (years)	4020	12.6 ± 1.3 (9–19)
Females	2108	12.6 ± 1.4 (10–18)
Males	1912	12.6 ± 1.3 (9–19)
Vitamin D (nmol/L)	4020	77.6 ± 24.4 (18.1–309.6)
Females	2108	73.5 ± 21.3 (21.5–171.6)
Males	1912	83.6 ± 26.4 (18.1–309.6)
Skin colour rating[a]	3957	1.8 ± 0.7 (1–3)
Vitamin D (nmol/L) by skin colour rating		
Rating 1 (fair)	1477	78.4 ± 23.9 (18.1–309.6)
Rating 2 (medium)	1985	79.1 ± 25.0 (18.3–255.64)
Rating 3 (dark/olive)	495	74.9 ± 23.0 (26.8–189.4)
Reflectance (exposed)[b]		
Machine L	2739	53.3 ± 5.7 (24–69)
Machine K	1043	49.6 ± 10.8 (25.8–107.7)
Reflectance (unexposed)[b]		
Machine L	2739	62.3 ± 3.8 (32–84)
Machine K	1043	76.7 ± 13.0 (21.9–152.8)
Sun exposed hours/week[c]	3948	16.3 ± 6.2 (3.5–30)
Vitamin D by sun exposed hours/week		
0–10 h/week	518	72.1 ± 22.8 (18.2–196.9)
11–20 h/week	1979	78.0 ± 24.1 (21.5–255.64)
21–30 h/week	1451	83.5 ± 25.3 (26.8–309.6)
Vitamin D by season		
Summer	1928	83.8 ± 26.6 (18.3–309.6)
Winter	2092	77.6 ± 24.4 (15.9–254.5)

Subgroups measured with EEL Model 99 Reflectance Spectrophotometer (L) or Konica Minolta Spectrophotometer CM-2500d (K)

SD standard deviation

[a]Rating by nurse on 3-point scale, 1 = fair, 2 = medium, 3 = dark

[b]Value measured by reflectometer on back of left hand (exposed), and under upper left arm (unexposed); higher values indicating fairer skin

[c]Average sun-exposed hours per week derived from Naevus questionnaire

Univariate analysis

Full univariate ACE models were fitted to estimate the heritability of each variable. Model fit was assessed, and skin colour rating was the only trait where a reduced model (AE) fit was not significantly worse than a full ACE, due to the extremely high heritability ($h^2 = 0.95$) of this trait (Table 3). Further, to assess the effect of season on 25(OH) D3 heritability, full ACE models were fitted separately for samples collected in summer and winter (see Table 4). To test the significance of these results, the full ACE models were compared to a model that equated the separate path coefficients for summer and winter. The model that equated all paths provided a significantly worse fit compared to the full ACE models $\left(\Delta X_3^2 = 80.2; p < 0.001\right)$. When season was not accounted for, additive genetic effects were found to account for approximately 50% of 25(OH)D3 variance, while common environment accounted for 32% and unique environment 18% (see Table 4). However, during summer/autumn, approximately 62% of the variance was attributed to total additive genetic factors while, interestingly, this decreased to 39% during winter/spring. This decrease in the contribution of A was compensated for by a large increase in the unique environmental variance component, which increased from only 15% in summer/autumn, to 32% in winter/spring (see Table 4). The higher E variance observed during winter was (at least) partially explained by higher within-pair variability in sun exposure. Absolute differences within MZ pairs were found to be substantially higher in winter than in summer. In winter, the mean difference between twin pairs was 0.76 ± 6.25 h, variance = 39.12 compared to summer (mean = 0.52 ± 5.61 h, variance = 31.57).

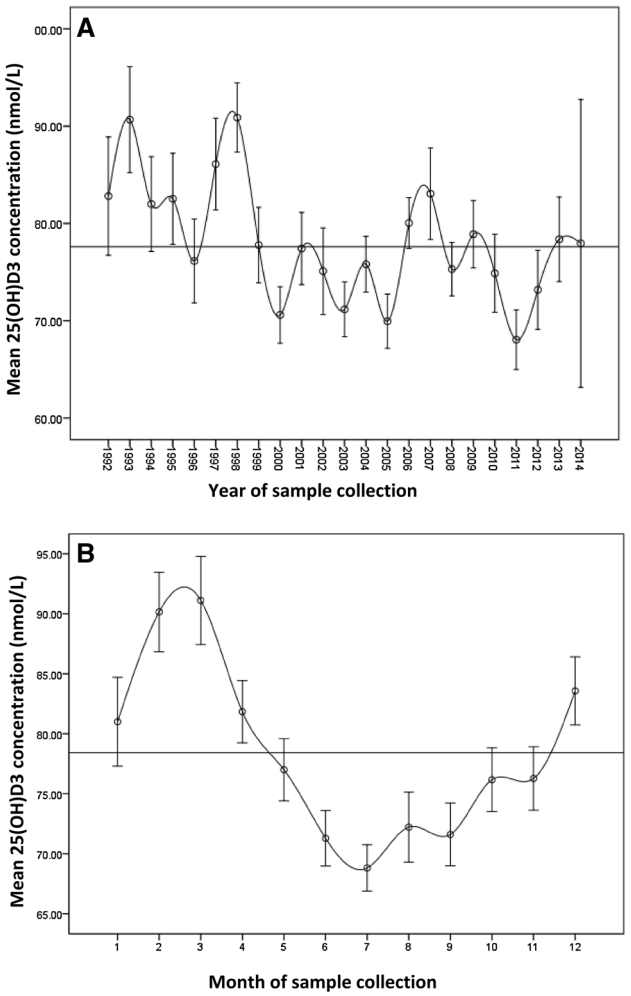

Fig. 2 Variation observed in mean 25(OH)D3 concentration (95% CI) for samples taken from twin pairs at age 12 and siblings (n = 4020) according to year of sample collection (**a**) and season of sample collection (**b**). Similar patterns were observed when examining samples taken at age 14 and 16. Bar indicates mean 25(OH)D3 concentration (77.6 nmol/L). *95% CI* 95% confidence interval

Multivariate analysis of vitamin D, skin colour, skin reflectance and sun exposure

All phenotypic and genetic correlations between the examined variables were significant ($p < 0.05$; see Table 5). The negative phenotypic correlation found between 25(OH)D3 concentration and skin colour rating, as well as 25(OH)D3 concentration and both skin reflectance measures corresponded to significant genetic correlations ($r_A = -0.17$ for skin colour, $r_A = -0.23$ for skin reflectance on exposed skin, $r_A = -0.27$ for skin reflectance on unexposed skin) (see Table 5; Fig. 3). A significant phenotypic correlation of 0.20 ($p < 0.001$) was observed between 25(OH)D3 concentration and hours of sun exposure; however, the genetic correlation between these traits was only 0.05, indicating a substantial environmental influence. In concordance, the phenotypic correlations between sun exposed hours and skin reflectance were small (−0.08 and −0.04 respectively) despite genetic correlations of −0.10 and −0.19.

The heritability of 25(OH)D3 was estimated from the univariate model to be $h^2 = 0.50$. The heritability estimates for skin colour, sun exposure, skin reflectance (exposed) and skin reflectance (unexposed) were $h^2 = 0.92$, $h^2 = 0.37$, $h^2 = 0.45$ and $h^2 = 0.48$ respectively. Of the estimated 50% total additive genetic variation (A) for 25(OH)D3, approximately half of the variation was as a result of genes also influencing skin colour and sun exposure (25% of total variation) (see Table 6). The shared environmental component (C) explained a substantial (35%) portion of the observed variance in vitamin D, of which, 25% could be explained by the skin colour and sun exposure variables, with sun exposure contributing the largest proportion (9%). This is unsurprising in that adolescent twins will likely share the vast majority of the same environment, e.g. attend the same school or play the same sport, and thus the majority of environmental variance in Vitamin D levels would be as a result of measured common environment (at the time of sample collection). The smallest effect was that of unique environment (E), where the examined variables accounted very little (~2%) to the overall 15%. Again, it is important to realise that much of the remaining 15% variance will also include experimental error.

To further analyse how the shared genetic factors between skin colour, sun exposure and vitamin D play a role in the different heritability estimates in summer and winter, a separate Cholesky decomposition was performed on those samples only collected in summer and those in winter (Table 7). These results show that the increased environmental variance observed during winter can be partly explained by a large increase in both the C and E sun exposure variance. Interestingly, those unique environmental factors specific to only vitamin D are double in winter (33%) when compared to summer (14%). In summer, variance in A is predominant for all variables.

Discussion

This study examined vitamin D concentrations in a large cohort of adolescent Australian twins and siblings (n = 4020). The average concentrations of 25(OH)D3 were consistent with previous reports of people living in Australia (Paxton et al. 2013). As expected, mean monthly concentrations collected during the Australian summer and autumn months (December–May) were numerically higher than samples collected in winter and spring (June–November), consistent with the well described seasonality in 25(OH)D levels (Hollis 2005). This was

Table 2 Monozygotic (MZ) and dizygotic (DZ) twin correlations (95% CI) for circulating vitamin D concentrations, skin colour rating, skin reflectance and sun exposure

Zygosity	n pairs	vitD	Skin colour	Ref (exposed)	Ref (unexposed)	Sun exposure
MZ female	302	0.79 (0.73–0.84)	0.94 (0.93–0.96)	L. 0.78 (0.72–0.83)	L. 0.79 (0.73–0.84)	0.59 (0.49–0.68)
				K. 0.74 (0.58–0.84)	K. 0.79 (0.65–0.88	
MZ male	282	0.70 (0.61–0.77)	0.94 (0.92–0.96)	L. 0.81 (0.75–0.86)	L. 0.85 (0.79–0.89)	0.54 (0.41–0.64)
				K. 0.91 (0.84–0.95)	K. 0.90 (0.83–0.95)	
DZ female/female	290	0.40 (0.27–0.52)	0.40 (0.25–0.50)	L. 0.65 (0.55–0.72)	L. 0.55 (0.44–0.65)	0.58 (0.47–0.67)
				K. 0.57 (0.35–0.73)	K. 0.66 (0.47–0.79)	
DZ male/male	266	0.61 (0.52–0.71)	0.51 (0.39–0.61)	L. 0.55 (0.44–0.64)	L. 0.59 (0.49–0.68)	0.34 (0.20–0.47)
				K. 0.26 (0.17–0.45)	K. 0.73 (0.53–0.86)	
DZ opposite sex	464	0.48 (0.38–0.56)	0.46 (0.37–0.55)	L. 0.56 (0.48–0.63)	L. 0.58 (0.50–0.65)	0.28 (0.18–0.38)
				K. 0.66 (0.57–0.80)	K. 0.55 (0.32–0.73)	
Total MZ	584	0.79 (0.73–0.84)	0.94 (0.93–0.96)	L. 0.78 (0.72–0.83)	L. 0.76 (0.71–0.80)	0.59 (0.49–0.68)
				K. 0.82 (0.74–0.88)	K. 0.84 (0.77–0.89)	
Total DZ	1020	0.52 (0.47–0.58)	0.46 (0.40–0.52)	L. 0.59 (0.53–0.64)	L. 0.52 (0.47–0.58)	0.39 (0.32–0.45)
				K. 0.54 (0.41–0.65)	K. 0.62 (0.50–0.72)	

Correlation for skin reflectance measures calculated separately for twins measured with instrument A and B. *CI* confidence interval, *rDZ* correlation coefficient between dizygotic twins, *rMZ* correlation coefficient between monozygotic twins

Table 3 Table summarizing total and seasonal sample size and number of twin pairs for each variable

Trait	n (total)	n (summer)	n pairs (summer)	n (winter)	n pairs (winter)
Vitamin D	4020	1928	800	2092	804
Skin colour rating[a]	3957	1986	789	1971	749
Reflectance (exposed)[b]					
Machine L	2739	1406	552	1333	564
Machine K	1043	440	182	603	264
Reflectance (unexposed)[b]					
Machine L	2739	1406	552	1333	564
Machine K	1043	440	182	603	264
Sun exposed hours/week[c]	3948	1897	613	1601	648

[a]Rating by nurse on 3-point scale, 1 = fair, 2 = medium, 3 = dark

[b]Value measured by reflectometer on back of left hand (exposed), and under upper left arm (unexposed); higher values indicating fairer skin

[c]Average sun-exposed hours per week derived from Naevus questionnaire

Table 4 Standard variance components (ACE) (95% CI) from univariate model to twin data for vitamin D concentration in samples collected during the summer (December–May), winter (June–November) and when season is not accounted for

Condition	A (%)	C (%)	E (%)	−2LL	df	ΔX^2	Δdf	p
Vitamin D								
Summer	62 (47–76)	23 (10–37)	15 (10–18)	9855.01	4003			
Winter	39 (22–54)	29 (15–42)	32 (28–38)					
Overall	50 (38–64)	32 (21–45)	18 (15–24)	10447.90				
Summer = winter	46 (32–61)	26 (17–38)	28 (20–33)	9935.16	4006	80.2	3	2.80E−17

Covariates included in the model were sex, age, plate (batch), row, column, storage time and year of sample collection

CI confidence intervals, *A* standardized genetic variance, *C* standardized common environmental variance, *E* standardized unique environmental variance. ACE represented as percentages (%)

expected and is due to the decrease of vitamin D synthesis through the skin during winter as a result of limited sun exposure. Additionally, these results indicate a slight 'lag-time' in the accumulation of vitamin D in the blood stream after UVR exposure (the UV index in Brisbane is highest between October and March, yet the mean concentrations of vitamin D are highest between December and May). This lag-time is likely a cumulative effect of the many processes involved in (and affecting) the production of pre-vitamin D and subsequent synthesis of vitamin D that moderate the accumulation in the bloodstream (Webb 2006). Further substantiating the importance of cutaneous vitamin D synthesis, significant variation was observed in the mean 25(OH)D3 concentration observed in those twins and siblings with fair or medium coloured skin (~ 77 nmol/L; n = 3461) compared to those with olive or darker coloured skin (73 nmol/L; n = 495). This was in agreement with several other studies that compared mean levels of vitamin D between Caucasians and other ethnicities (Looker et al. 2002; Rockell et al. 2006; Dix et al. 2017), one of which found that Caucasians have as much as 1.5 times higher 25(OH)D3 concentrations than African Americans and Hispanics (Looker et al. 2002). Individuals with darker natural skin colour possess higher levels of constitutive melanin pigmentation, which protects the underlying skin against damage from ultra-violet radiation (UVR). However, this decrease in UVR also reduces the vitamin D synthesis resulting in individuals with darker skin types requiring more sun exposure to synthesise the same amount of vitamin D than those with fairer skin (Farrar et al. 2011). However, it is important to note that in both the present study, as well as those previously mentioned, individuals with naturally darker skin tones have been predominantly underrepresented and that there are a multitude of ethnically-unique factors (such as genes, diet and behaviour) that contribute to vitamin D synthesis so caution should be taken when extrapolating from these results.

The overall estimated heritability of 25(OH)D3 was moderate ($h^2 = 0.50$) and in concordance with several other studies examining 25(OH)D3; though there is a large discrepancy in reported heritability estimates of vitamin D, with reported heritability's ranging from extremely low (0–4%) (Hong et al. 2018) to moderate (30–60%) (Hunter et al. 2001; Engelman et al. 2008; Shea et al. 2009) and high (70–86%) (Orton et al. 2008; Arguelles et al. 2009; Mills et al. 2015) estimates. This inconsistency can be largely attributed to differences in ancestry, geographical location, diet and culture as well as methodological differences between studies.

Surprisingly, the heritability of 25(OH)D3 increased during the summer/autumn and decrease during winter/spring months. This was also found in two other studies examining circulating vitamin D in Australian adolescents (Mills et al. 2015) and Swedish adults (Snellman et al. 2009). However, it is in disagreement with a third study in a cohort of older males in the United States that found heritability to be high in the winter months, when environmental effects were minimal, but not present in summer (Karohl et al. 2010). The differing observations could be accounted for by the small sample sizes in previous studies (all studies had n < 510) and substantial regional environmental fluctuations throughout the year. A decrease in genetic variance component (and increase in unique environment) was found during winter and is likely as a result of higher within-pair variability in sun exposure during winter, which would limit the role of vitamin D genes during winter. Alternatively, the increased genetic component during summer may also be due to unique, seasonal environmental conditions that are over-activating genetic pathways, such as those involved in vitamin D synthesis in the skin or the metabolism and storage of absorbed vitamin D. It has been shown that the UV flux between summer and winter in Brisbane is far less than that experienced is other European countries (in fact, many European countries drop to a UV index close to 0 in of winter, where Brisbane winters still have a UV index of 4.5) (Siskind et al. 2002). However, in summer the UV index in Brisbane reaches 15 (double that of London's summer) and so far less exposure time would be needed to increase vitamin D concentrations. Additionally, the Australian school calendar has its end of year break from mid-December until the end of January (the peak of summer). It is assumed that the young participants in this study would be more likely to increase the amount of time spent outdoors during this

Table 5 Table of phenotypic Pearson correlations (lower matrix) and genetic Pearson correlations (upper matrix) between skin colour, sun exposure, skin reflectance and vitamin D concentrations

	Phenotypic (lower) and genetic (upper) correlations				
	1	2	3	4	5
1. Skin colour	–	0.07**	−0.40**	−0.62**	−0.17**
2. Sun exposed hours	0.20**	–	−0.10**	−0.19*	0.05*
3. Reflectance (exposed)	−0.38**	−0.08**	–	0.61**	−0.23**
4. Reflectance (unexposed)	−0.48**	−0.04*	0.64**	–	−0.27**
5. Vitamin D	−0.03**	0.20**	−0.15**	−0.09**	–

**p < 0.01; *p < 0.05

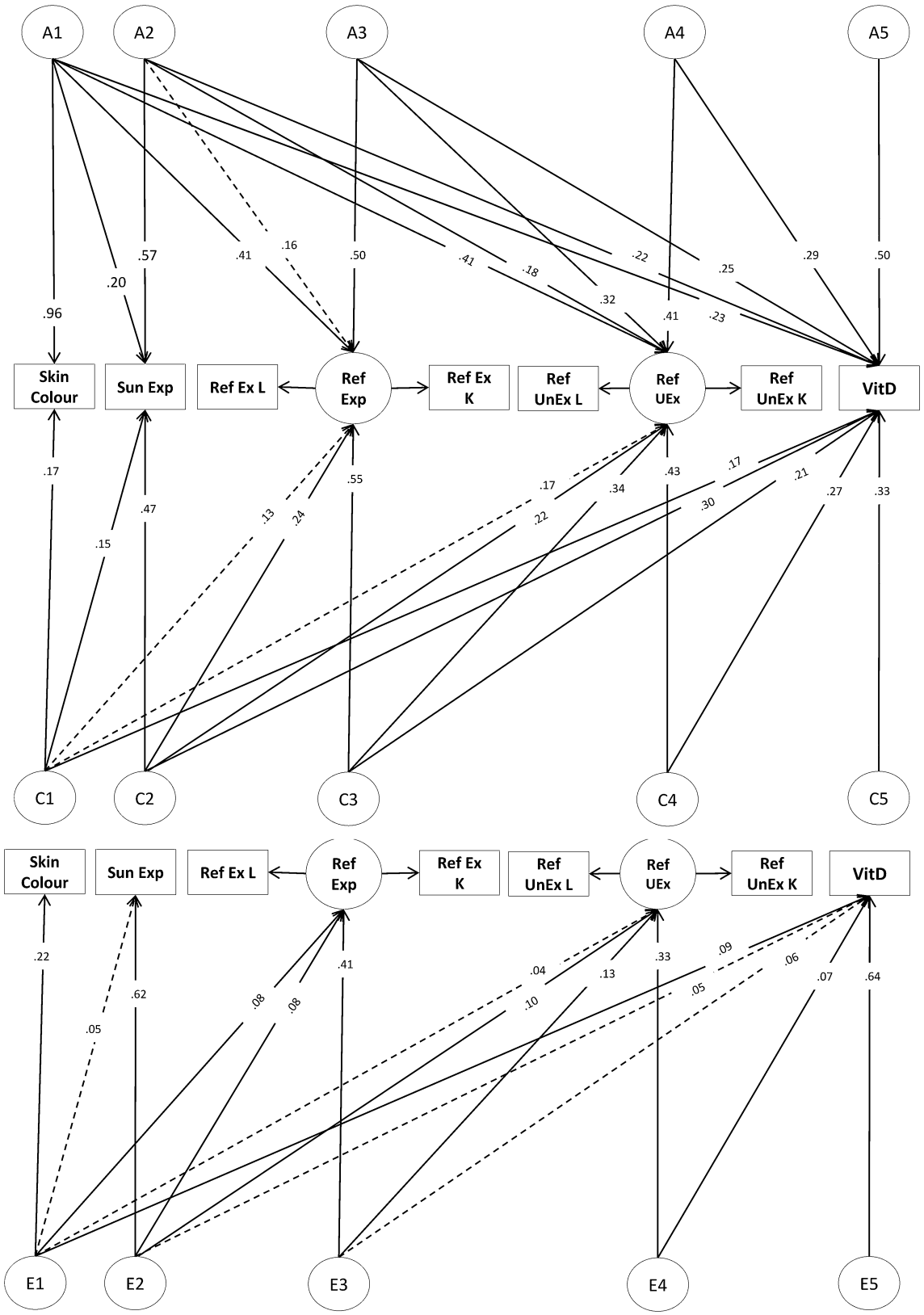

Fig. 3 Cholesky decomposition for the ACE model (E reflected below for clarity). Latent factors loadings are standardized to unit variance and must be squared to obtain standardized variance components. A1–A5: additive genetic factors; C1–C5: common environmental factors. *Sun Exp* sun exposed hours, *Ref Ex L/K* reflectance measure on exposed skin using instrument L or K, *Ref UnEx L/K* reflectance measure on unexposed skin using instrument L or K, *VitD* vitamin D concentration. Dashed lines indicate non-significance

Table 6 Results of Cholesky decomposition for the ACE model, with standardized parameters between skin colour, skin reflectance, sun exposure and vitamin D concentrations

	Standardized variance components %					Total (%)
	1	2	3	4	5	
Genetic (A)						
1. Skin colour	92.0					92.0
2. Sun exposed hours	3.9	32.8				36.7
3. Reflectance (exposed)	16.9	2.5	25.4			44.8
4. Reflectance (unexposed)	16. 9	3.1	10.6	17.0		30.7
5. Vitamin D	5.5	4.9	6.3	8.7	25.1	50.4
Common environment (C)						
1. Skin colour	3.0					3.0
2. Sun exposed hours	2.2	22.1				24.2
3. Reflectance (exposed)	1.6	5.9	29.9			37.4
4. Reflectance (unexposed)	2.9	5.2	11.6	19.2		38.9
5. Vitamin D	3.0	9.5	4.3	7.3	10.8	34.9
Unique environment (E)						
1. Skin colour	5.0					5.0
2. Sun exposed hours	0.3	38.8				39.1
3. Reflectance (exposed)	0.7	0.6	16.6			17.8
4. Reflectance (unexposed)	0.2	1.0	1.6	10.9		13.6
5. Vitamin D	0.9	0.3	0.3	0.5	12.7	14.8

Covariates included in the model were sex, age, plate (batch), row, column, storage time and year of sample collection. Total variance is calculated by summing the individual components for each trait

A standardized genetic variance, *C* standardized common environmental variance, *E* standardized unique environmental variance. ACE represented as percentages (%)

vacation period. It is also plausible that the environmental exposure to UV plateaus in summer, such that no additional variation in exposure impacts variation in vitamin D.

The multivariate results indicated that approximately half of the genetic component of vitamin D was with the same genes influencing skin colour, reflectance and sun exposure—with skin colour and reflectance accounting for approximately 40% of the total shared genetic variance with vitamin D. The remaining genetic variability (50%) in vitamin D levels is unique to vitamin D-related genetic components, likely relating to those genes involved in the hydroxylation of synthesized vitamin D into active forms in the liver and kidneys (*CYP2R1* and *CYP27B1*), genetic variants in the vitamin D binding protein (*GC*) or 25(OH)D elimination (*CYP24A1*) (Eyles et al. 2005).

Similar to the genetic component, the common environmental component is also strongly accounted for by the four other elements of the model. This is expected as it is widely assumed that twins will share most of their environment at this age through common households, diet, schools, sports etc. This would result in similar diets and sun exposure. Only 10% of the total variance was unique to vitamin D. In concordance with other studies, unique environment only represented a small amount of the overall variation might largely be due to experimental error such as differential instrumentation, nurse rating perceptions or assay batch effects (Burgaz et al. 2007). Interestingly, when examining the separate interactions between these variables during summer and winter respectively, the C and E variance components of sun exposure variance increased substantially during winter. Comparatively, overall variance in winter is as a greater result of environmental variance. This could indicate that a sun exposure threshold exists, where by below this, the amount of sun exposure one is exposed to plays a lesser role in the overall variance of vitamin D than genes. However, above this threshold, variance is accounted for more from the environment than genes.

There were significant, albeit mostly small, phenotypic and genetic correlations between all examined variables and vitamin D. The largest phenotypic correlation was between vitamin D and sun exposed hours (0.20), followed by reflectance on exposed skin (− 0.15). It is important to remember that higher reflectometer readings indicate higher reflection and thereby paler skin. It is therefore expected that this would have a negative correlation with vitamin D concentrations. Though both these variables share moderate phenotypic variations with vitamin D, the genetic correlations for sun-exposure was small (0.07)—highlighting the magnitude of environmental contribution is this interaction. The genetic correlations between vitamin D and skin colour, or vitamin D and skin reflectance, was moderate (ranging from − 0.17 to − 0.27), indicating shared genetic elements

Table 7 Results of Cholesky decomposition for the ACE model for samples collected during summer and winter respectively

Summer	Standardized variance components %					Total (%)
	1	2	3	4	5	
Genetic (A)						
1. Skin colour	91.7					91.7
2. Sun exposed hours	7.8	41.6				49.4
3. Reflectance (exposed)	18.5	8.2	24.2			50.9
4. Reflectance (unexposed)	14.1	6.0	12.5	12.5		45.1
5. Vitamin D	16.5	23.8	2.7	2.7	16.5	62.2
Common environment (C)						
1. Skin colour	4.3					4.3
2. Sun exposed hours	19.4	7.8				27.2
3. Reflectance (exposed)	9.7	3.9	10.6			24.2
4. Reflectance (unexposed)	7.3	2.9	8.0	7.9		26.1
5. Vitamin D	6.7	1.9	6.0	6.5	2.8	23.9
Unique environment (E)						
1. Skin colour	3.9					3.9
2. Sun exposed hours	1.3	22.1				23.4
3. Reflectance (exposed)	11.2	1.3	12.4			24.9
4. Reflectance (unexposed)	8.5	1.3	9.5	9.6		28.9
5. Vitamin D	1.9	0.3	2.6	2.4	6.8	14.0
Winter	Standardized variance components %					Total (%)
	1	2	3	4	5	
Genetic (A)						
1. Skin colour	87.5					87.5
2- Sun exposed hours	3.5	3.5				7.0
3- Reflectance (exposed)	0.3	0.7	1.2			2.1
4-Reflectance (unexposed)	0.2	0.5	0.8	0.8		2.3
5-Vitamin D	6.5	5.6	4.0	4.0	17.2	37.4
Common environment (C)						
1. Skin colour	2.8					2.8
2. Sun exposed hours	14.4	24.8				39.3
3. Reflectance (exposed)	25.8	23.0	31.2			80.0
4. Reflectance (unexposed)	18.8	16.7	22.7	22.6		80.7
5. Vitamin D	2.7	7.6	7.6	7.5	4.3	29.7
Unique environment (E)						
1. Skin colour	9.7					9.7
2. Sun exposed hours	28.6	25.2				53.8
3. Reflectance (exposed)	3.8	9.1	5.1			17.9
4. Reflectance (unexposed)	2.7	6.7	3.7	3.8		16.9
5. Vitamin D	2.7	16.3	1.8	1.8	10.3	33.0

Graphs show standardized parameters between skin colour, skin reflectance, sun exposure and vitamin D concentrations. Covariates included in the model were sex, age, plate (batch), row, column, storage time and year of sample collection. Total variance is calculated by summing the individual components for each trait

A standardized genetic variance, *C* standardized common environmental variance, *E* standardized unique environmental variance. ACE represented as percentages (%)

between these traits. These results are in line with previously reported heritability estimates of skin colour, reflectance and even sun exposure i.e. individuals with genes conferring red hair and pale skin spend less time in the sun (Healy et al. 2001; Duffy et al. 2004; Rees 2004; Law et al. 2017).

Although no other study to date has investigated the genetic association between skin colour and vitamin D, the present results are consistent with other studies investigating the phenotypic overlap between sun exposure and levels of circulating vitamin D.

One of the limitations of this study is that, like the majority of other studies in the field, our cohort only consisted of individuals of Caucasian/European decent. It can thus be assumed that stronger differential variance would be observed in studies examining samples with a wider range of natural skin tones where differences in skin colour are more predominant. Additionally, the Australian climate, and in particular that of South East Queensland, lends itself to warmer weather, resulting in individuals receiving more sun exposure across the year (rather than only during summer) than those in cooler climates. This would mitigate the need for excessive vitamin D storage during summer that is then used during winter, as vitamin D can continually be synthesized throughout the year.

Conclusion

This is the largest twin study to date to report heritability estimates of circulating vitamin D, and the first study examining both the genetic and phenotypic effects of skin colour and sun exposure on vitamin D status. These results highlight the need to account for ethnicity and geographical location when describing adequate concentration ranges for circulating vitamin D. As vitamin D deficiency is often asymptomatic, understanding what modifiable risk factors function in conjunction with genetic components could aid in identifying at-risk individuals and developing intervention strategies or personalized care for individuals vulnerable to vitamin D deficiency. Future GWA studies should explore the influence of season to further identify SNPS that play a role in vitamin D absorption and metabolism. This will create a platform where the genetic influence of vitamin D deficiency on various diseases can be examined using methods such as Mendelian Randomization.

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