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VDR gene methylation as a molecular adaption to light exposure: Historic, recent and genetic influences

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Abstract

Objectives: The vitamin D receptor (VDR) is a member of the nuclear receptor family of transcription factors. We examined whether degree of VDR gene methylation acts as a molecular adaptation to light exposure. We explored this in the context of photoperiod at conception, recent UV irradiance at 305 nm, and gene-latitude effects.

Methods: Eighty subjects were examined for VDR gene-CpG island methylation density. VDR gene variants were also examined by PCR-RFLP.

Results: Photoperiod at conception was significantly positively related to *VDR* methylation density, explaining 17% of the variance in methylation ($r^2 = 0.17$; $P = .001$). Within this model, photoperiod at conception and plasma 25(OH)D independently predicted methylation density at the *VDR*-CpG island. Recent UV exposure at 305 nm led to a fivefold increase in mean methylation density ($P = .02$). Again, UV exposure and plasma 25(OH)D independently predicted methylation density at the *VDR*-CpG island. In the presence of the BsmI mutant allele, methylation density was increased ($P = .01$), and in the presence of the TaqI or FokI mutant allele, methylation density was decreased ($P = .007$ and .04 respectively). Multivariate modelling suggests plasma 25(OH)D, photoperiod at conception, recent solar irradiance, and *VDR* genotype combine as independent predictors of methylation at the *VDR*-CpG island, explaining 34% of the variance in methylation ($R^2 = 0.34$, $P < .0001$).

Conclusions: Duration of early-life light exposure and strength of recent irradiance, along with latitudinal genetic factors, influence degree of VDR gene methylation consistent with this epigenetic phenomenon being a molecular adaptation to variation in ambient light exposure. Findings contribute to our understanding of human biology.

1 | INTRODUCTION

The vitamin D receptor (VDR) is a transcription factor belonging to the steroid nuclear receptor superfamily. Its activity is regulated by 1,25-dihydroxycholecalciferol (1,25 (OH)D3; calcitriol), a ligand that is derived from both diet and UV-mediated skin photosynthesis (Beckett, Duesing, et al., 2016). Calcitriol liganded VDR action modulates gene expression including that of chromatin modifiers and remodelers, and may influence DNA methylation (Fetahu, Hobaus, & Kallay, 2014). Additionally, the VDR gene is itself potentially regulated by DNA methylation at CpG islands (cytosine-guanine dense regions). Differential methylation in promotor regions of genes modulates gene expression, with hypermethylation generally linked to decreased expression, and hypomethylation associated with increased expression (Deaton & Bird, 2010).

Biological responses to the activation/stimulation of this transcription factor are tissue-specific and generally complex. They range from control of mineral homeostasis to regulation of growth, differentiation and patency of several cell types including those of the immune system, bone, skin, pancreas as well as other target tissues (Bouillon et al., 2008). Vitamin D-related diet, environment, genetic and epigenetic mechanisms may therefore conspire to influence gene expression, with wide pleiotropic effects (Pike & Meyer, 2010). Indeed, it is now recognized that calcitriol liganded VDR action regulates gene expression at the level of single gene loci as well as at the level of gene networks (Pike & Meyer, 2010), making the level of complexity involved particularly challenging to understand.

Vitamin D is a steroid hormone, and like many hormone actions is central to phenotypic plasticity, modifying gene expression and phenotypic outcomes in response to environmentally originated cues. With this in mind, the main focus of the present study is to examine whether *VDR* methylation can act as a "plastic" regulatory mechanism that is sensitive to light exposure. For example, if a seasonal reduction in UV-B led to VDR gene hypomethylation, increased levels of *VDR* expression might help compensate for a reduced capacity to photosynthesize vitamin D in the skin.

The ability of all organisms, including humans, to modulate phenotype as a response to environmental challenge is central to the life sciences. Some environments and associated selection pressures are extremely dynamic. For example, light exposure shifts according to season and latitude, with variable exposures possible at key stages in the lifecycle such as during early gestation and during the adolescent growth spurt. It is therefore important that humans do not retain overly rigid phenotypes, but maintain a degree of phenotypic plasticity to allow responses that are appropriate to key periods of sensitivity, such as these early life events as well as ensuring a flexible response over the entire human life course. In this respect, vitamin D nutrigenetics have been linked to several clinically relevant conditions (Martin, Veysey, Yates, & Lucock, 2014) as well as developmental phenomena, including evidence linking early life nutrition to reduced lumbar spine bone mineral density in later life, a finding consistent with the developmental origins of adult disease (DOAD) paradigm (Dennison et al., 2001).

Additionally, vitamin D status has been linked to human skin pigmentation phenotype, whereby ancestral *Homo* migrations from equatorial to northerly latitudes required the evolution of skin depigmentation in regions where UV-B exposure is highly seasonal in order to better facilitate vitamin D photosynthesis (Jablonski & Chaplin, 2000). This will have been further driven at these northerly latitudes by the move from a hunter-gatherer lifestyle with ample preformed dietary vitamin D, to one based on the domestication of crops which would have been a poorer dietary source of the vitamin (Chaplin & Jablonski, 2013; Gibbons, 2007). Pigmentation/depigmentation as an evolved trait is polygenic, with several genes involved (Lucock et al., 2015). Given the pleiotropic effect of *VDR*, and methylation controlling expression, the question arises as to whether UV-B related *VDR* methylation could coordinate several genes and ultimately contribute to adaptive phenotypes such as human integumentary pigmentation. It is clear *VDR* genotype is important, and we recently demonstrated that VDR gene polymorphisms correlate with latitude as a surrogate for UV-B exposure (Lucock et al., 2015), a finding consistent with related observations (Hochberg & Templeton, 2010).

The present study investigates whether the degree of VDR gene methylation acts as a molecular adaptation to light exposure. We examine this to see if historic (photoperiod at conception), recent (total UV irradiance at 305 nm in previous 6 weeks), and latitudinal (polymorphism related methylation occurrence) effects can influence/infer *VDR* epigenetic modification. We examine this in an elderly Australian study population that is relatively homogeneous, being primarily white and of northern and western European descent.

2 | METHODS

2.1 | Subjects and sample collection

Eighty subjects were drawn from a completed cross-sectional study of 831 elderly participants (aged $\geq$65 years, 58.5% female) living on the Central Coast of NSW, Australia (The Retirement Health and Lifestyle Study, RHLS). Details of the selection and randomization process used are described previously (Beckett, Duesing, et al., 2016).

Blood was collected and stored at −20°C (whole blood) and −80°C (plasma). DNA was isolated from peripheral

blood cells using Qiagen QIAmp DNA mini-kit as per the manufacturer's protocol for blood, including RNAse treatment. Age, sex and self-reported history of smoking were recorded. Written informed consent was obtained from all participants as per the approval obtained from the University of Newcastle Human Research Ethics Committee (H-2008-0431).

2.2 | DNA methylation

Percentage DNA methylation (methylation density) was assessed at the CpG island of the VDR gene using Qiagen EpiTect II methylation enzyme kits and PCR assays at the concentrations and cycle conditions recommended by the manufacturer (Holemon et al., 2007), and as reported by Beckett, Duesing et al. (2016). The *VDR* CpG island (ID no: 103069) spans the promotor region, transcription start site and part of the gene body (Chr 12:48298645-48299537), covering 892 base pairs including 72 CpG sites.

2.3 | Plasma 25(OH)D

Plasma 25(OH)D levels were measured using a 25(OH)D vitamin D ELISA kit for serum and plasma (Enzo Life Sciences, NY, USA) as previously described (Beckett et al., 2014).

2.4 | Sun exposure: Cumulative irradiance

A time lag of approximately 6 weeks between seasonal solar UVB exposure and serum calcidiol response would be expected (Lucas et al., 2005). Information on the cumulative solar irradiance (305 nm) over the 6 weeks prior to sample collection was collected as previously described (Beckett, Duesing, et al., 2016) using NASA's Total Ozone Mapping Spectrometer (TOMS) program via NASA's Aura OMI level 3 atmospheric portal (http://gdata1.sci.gsfc.nasa.gov/daac bin/G3/gui.cgi?instance_id = omi). Total cumulative UV irradiance was treated as a categorical variable by stratifying exposure into tertiles.

2.5 | Photoperiod at conception

The photoperiod at conception, in minutes, was calculated via the Online- Photoperiod Calculator V 1.94 L [Lammi, © 1996–2008 (Lammi, 2008)]. An assumption built into our model is that a pregnancy was full term and lasted exactly 9 months.

2.6 | VDR genotyping

Genotyping for polymorphisms in the VDR gene was achieved using RFLP-PCR as previously described (Beckett, Le Gras, et al., 2016). Due to the small cohort size, genotype was coded as a binary variable (presence of the mutant allele versus absence of the mutant allele; see Supporting Information Table S1 for details).

2.7 | Statistics

Statistical analyses were performed using JMP (Version 11, SAS Institute Inc., Cary, NC, USA). Where necessary, variables were transformed to normalize distributions prior to regression analysis. Log(x) was calculated for percentage methylation (expressed as proportions) and plasma calcidiol (25(OH)D). The remaining variables were approximately normally distributed. Standard least squares regression was used to assess the relationships between multiple parameters and variables of interest, and pairwise comparisons of least-squares means were made using Tukey's HSD tests. Where appropriate, mixed direction stepwise regression was performed using significance levels of $P \leq .250$ or $P > .250$ to enter (forward step) or remove (backward step) variables from the model, respectively. Adjusted R^2 values and P-values are reported for final models, and standardized parameter estimates (β) and P-values reported for individual variables. Descriptive statistics (means, 95% confidence intervals) were calculated and presented with back transformation where appropriate. Outcomes were considered to be statistically significant at $P \leq .05$. P-values for multiplicative interaction of terms ($p_{\text{interaction}}$) were calculated where appropriate.

3 | RESULTS

3.1 | Photoperiod at conception correlates with VDR methylation density in later life

When assessed as a simple univariate analysis, photoperiod at conception was significantly positively related to *VDR* methylation density (Figure 1), with photoperiod potentially explaining 7% of the variance in methylation at the *VDR* CpG island ($r^2 = 0.07$; $\beta = .27$, $P = .02$).

As DNA methylation can potentially vary by age and sex, and as we have previously demonstrated an association between *VDR* CpG island methylation density and plasma 25(OH)D levels (Beckett, Duesing, et al., 2016), we assessed the relationship between photoperiod at conception and *VDR* methylation density with adjustment for these variables (Model 1; Table 1). This model explains 17% of the variance in methylation at the *VDR* CpG island ($R^2 = 0.17$, $P = .001$), and photoperiod at conception is a significant independent predictor of methylation density ($\beta = 0.22$, $P = .04$). Plasma 25(OH)D is also a significant independent predictor in this model ($\beta = 0.34$, $P = .0021$), which is not unexpected as we have previously reported that plasma 25(OH)D alone potentially explains 12% of the variance in methylation at the *VDR* CpG island (Beckett, Duesing, et al., 2016). However,

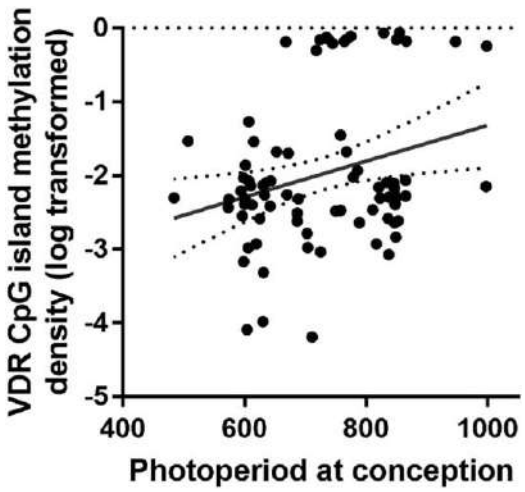

FIGURE 1 The correlation between photoperiod at conception and methylation density at the *VDR* CpG island, $r^2 = 0.07$, $P = .02$. Linear regression line and 95% confidence intervals are plotted

the effects of photoperiod and plasma 25(OH)D in predicting methylation density at the *VDR* CpG island appear to be additive, not interactive ($p_{\text{interaction}} = 0.2$).

The model predicting methylation density at the *VDR* remained significant when additional adjustments were applied for BMI and cigarette smoking history (Model 2; Table 1) and cumulative UV irradiance in the 6 weeks prior to sample collections (Model 3; Table 1).

3.2 | Cumulative UV irradiance prior to sample collection correlates with VDR methylation density, when adjusted for plasma 25(OH)D

When methylation density at the *VDR* CpG island was assessed by tertile of cumulative UV exposure in the 6 weeks prior to sample collection, there was no significant difference between methylation density in each tertile (Figure 2A). However, when analysis was adjusted for age, sex and plasma 25(OH)D levels a significant increase in methylation was found from the first to second tertile (Figure 2B), with a greater than fivefold increase from 0.45% mean methylation density in the first tertile to 2.56% in the second tertile. Results remain similar when additional adjustments for BMI and smoking were applied. However, the effects of cumulative UV irradiance and plasma 25(OH)D in predicting methylation density at the *VDR* CpG island appear to be additive, not interactive ($p_{\text{interaction}} = 0.8$).

3.3 | VDR methylation density varies by common VDR polymorphisms

Stepwise regression analysis was used to assess the relationship between *VDR* polymorphisms and methylation density at the *VDR* CpG island. *VDR* polymorphisms for BsmI, ApaI, TaqI, FokI, and Tru91 were entered into the analysis and following stepwise regression, BsmI, TaqI, FokI, and Tru91 were selected for inclusion into the model, and BsmI, TaqI, and FokI were identified as significant independent predictors of *VDR* CpG island methylation density (Table 2). In the presence of the BsmI mutant allele, methylation density was increased, and in the presence of the TaqI or FokI mutant allele, methylation density was decreased. When additional adjustments were applied for age, sex and plasma 25(OH)D, BMI, cigarette smoking history and cumulative solar irradiance, results remained similar (Table 2). Pairwise comparisons of back transformed adjusted least-squares means for BsmI, TaqI, and FokI genotypes are shown in Figure 3. The genotypic effects did not interact with plasma 25(OH)D in the prediction of methylation density at the *VDR* CpG island (BsmI $p_{\text{interaction}} = 0.6$; TaqI $p_{\text{interaction}} = 0.2$; FokI $p_{\text{interaction}} = 0.09$).

3.4 | Multivariate modelling suggests plasma 25(OH)D, photoperiod at conception, cumulative solar irradiance, and VDR genotype combine as independent predictors of methylation density at the VDR CpG island

A complete regression model including all potential predictors (plasma 25(OH)D, cumulative UV irradiance, day length at conception, carriage of the BsmI, TaqI and FokI mutant alleles, age, sex, smoking, and BMI) of methylation density at the *VDR* CpG island potentially explains 34% of variance in methylation density at the CpG island ($R^2 = 0.34$, $P < .0001$). Plasma 25(OH)D, photoperiod at conception, cumulative UV irradiance, BMI, TaqI and FokI genotype were each independent significant predictors of methylation density (Table 3).

TABLE 1 The relationship between photoperiod at conception and methylation density at the VDR CpG island

	Univariate analysis	Model 1[a]	Model 2[b]	Model 3[c]
Model R^2 (*P*-value)	0.07 (0.02)	0.17 (0.001)	0.20 (0.001)	0.22 (0.0009)
β photoperiod at conception (*P*-value)	0.27 (0.02)	0.22 (0.04)	0.26 (0.02)	0.24 (0.03)

[a]Adjusted for age, sex and plasma 25(OH)D.
[b]Adjusted for age, sex and plasma 25(OH)D, BMI and cigarette smoking history.
[c]Adjusted for age, sex and plasma 25(OH)D, BMI, cigarette smoking history and cumulative solar irradiance.

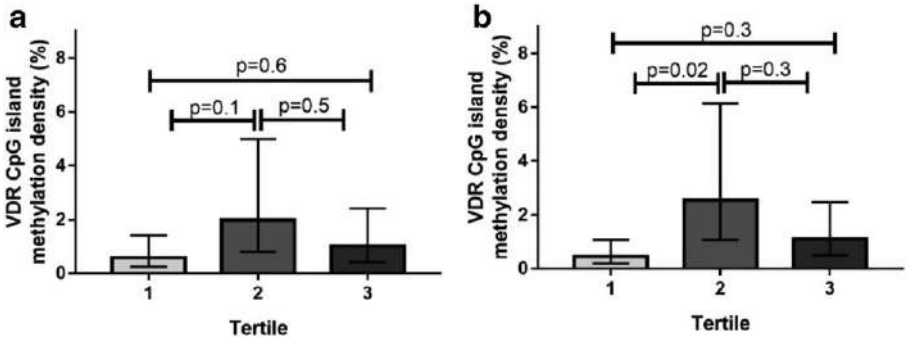

FIGURE 2 *VDR* CpG island methylation density (%) by cumulative UV exposure tertile (A) Univariate analysis (B) Adjusted for age, sex and plasma 25(OH)D levels. Least-squares means and confidence intervals are plotted. *P*-values are the results from Tukey HSD tests

4 | DISCUSSION

4.1 | Overview

The data presented suggest that duration of early life exposure and strength of recent UV irradiance, along with geographic factors related to several *VDR* polymorphisms, influence the degree of VDR gene methylation in a predictable way. As such, it is consistent with this epigenetic phenomenon being a molecular adaptation to variation in ambient light exposure.

A longer photoperiod at conception is related to *VDR* CpG island methylation, as is elevated solar exposure during the 6 weeks prior to blood sampling. Interestingly, *VDR* polymorphisms BsmI and TaqI, which demonstrate a significant increase in mutant B and ancestral T alleles, respectively, as one moves from low UV to high UV latitudes (Lucock et al., 2015), also exhibit statistically significant higher levels of CpG island methylation in the presence of these mutant B, and ancestral T alleles (Table 2). Overall, irradiance increases *VDR* methylation, likely reducing gene expression.

4.2 | Evolution/adaptation

It has been suggested that the variant VDR gene forms part of an evolutionary complex capable of adapting humans to an altering UV exposome, and as such raises the question, "Is *VDR* an agent of short term adaptation, or is it a component within a cassette of genes that are known to be altered in the longer term to adapt the human phenome to the prevailing conditions?"(Hochberg & Templeton, 2010; Lucock et al., 2015). This study provides evidence to support both views, suggesting it has an important, and differential, influence on human biology. It seems remarkable that it reflects a methylation profile spanning the lifecycle, as well as being adaptive in the short term. Despite being an elderly population (≥65 years), it was still possible to detect a methylation signature related to day length at the presumptive time of conception. This period, particularly early embryogenesis, is when ossification of the skeleton begins, a physiological process closely linked to vitamin D (Lucock et al., 2014). It should be noted that the presumptive time of conception assumes pregnancy lasts precisely 9 months, although clearly this measure of conception will represent a narrow periconceptional window that includes implantation and very early embryogenesis. Furthermore, the average duration of pregnancy is 280 days, meaning a substantial number of pregnancies last longer than 280 days.

This longer term, historic correlation suggests that it may fit a DOAD framework, with potentially wide ranging implications given the pleiotropic nature of the VDR gene. In support of this, a low birth weight reflecting compromised gestational nutrition has been shown to reduce lumbar spine bone mineral density in later life in a VDR gene polymorphism specific fashion (Dennison et al., 2001). It is interesting to speculate whether this early-life adaptive phenomenon in our subjects is still visible later in life because it exhibits little postnatal flexibility. The short term (cumulative 6 week) response to UV-B at 305 nm, is, by its very character, a flexible/plastic adaptive response to contemporaneous environmental (UV-B) cues.

TABLE 2 The relationships between common VDR polymorphisms and methylation density at the VDR CpG Island

Polymorphism	Unadjusted model β (*P*-value)	Adjusted[a] β (*P*-value)
BsmI	***0.50 (.003)***	***0.38 (.02)***
Taq1	***–0.51 (.003)***	***–0.45 (.006)***
FokI	***–0.22 (.05)***	***–0.29 (.04)***
Tru91	0.20 (.07)	0.11 (.3)
Model (R^2, p)	***0.14 (.006)***	***0.25 (.001)***

[a]Adjusted for age, sex and plasma 25(OH)D, BMI, cigarette smoking history and cumulative solar irradiance. Positive associations indicate increased gene methylation in the presence of the mutant allele. Statistically significant values are highlighted with italic and bold text.

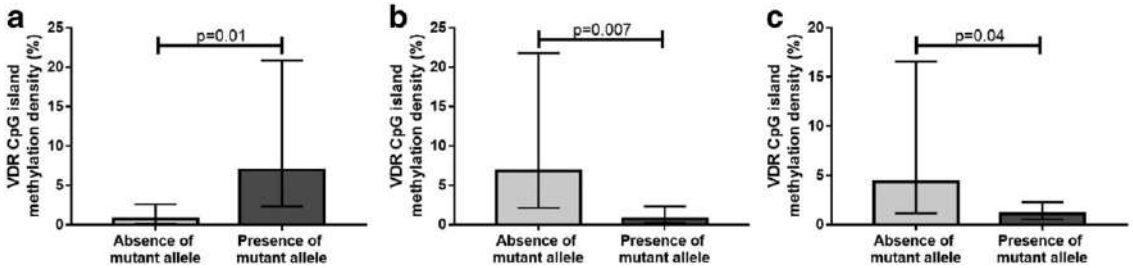

FIGURE 3 *VDR* CpG island methylation density (%) by (A) BsmI (B) TaqI, and (C) FokI genotype. Least-squares means and confidence intervals are plotted, adjusted for age, sex and plasma 25(OH)D, BMI, cigarette smoking history and cumulative solar irradiance. *P*-values are the results from students *t*-test

It would be interesting to examine whether postnatal *VDR* CpG island methylation was a mechanism to condition neonates to prevailing early-life nutritional or solar regimes based on maternal gestational exposures (diet and UV-B). Indeed, it is noteworthy that in our analyses, photoperiod and plasma calcidiol appear to be additive, not interactive in predicting methylation density at the *VDR* CpG island. Although temporally distinct, the recent short term cumulative UV-B related *VDR* CpG island methylation signature similarly shows that UV irradiance and plasma calcidiol appear to be additive, not interactive in predicting this outcome. However, we recognize that effect sizes are small.

4.3 | Pleiotropy

Much discussion exists on the pleiotropic effects of calcitriol liganded VDR, with a large number of target genes identified. Examples of major transcriptionally regulated genes include those related to the calcium binding proteins calbindin-D_{28k}, found in the kidney, and calbindin-D_{9k}, found in the intestine. Additionally, VDR transcriptional targets include the bone matrix proteins osteopontin (also known as bone sialoprotein I) and osteocalcin, which are produced by the osteoblasts and which help to elaborate the bone matrix structure (Bortman, Folgueira, Katayama, Snitcovsky, & Brentani, 2002). These calcium binding proteins in kidney, intestine and bone are critical for maintaining calcium homeostasis. Other genes related to binding proteins, calcium channels, early response genes like c-fos, and growth factors, are also directly regulated by the VDR (Bortman et al., 2002). Indeed, recent research has shown 2276 genomic positions occupied by the VDR and 229 genes with altered expression profiles in response to vitamin D (Ramagopalan et al., 2010).

4.4 | Skeletal biology

Given that DOAD and VDR genetics may play a role in adult bone mineral density (Dennison et al., 2001), and knowing the role of light in vitamin D synthesis along with the vitamin's subsequent role in calcium homeostasis via its interaction with the VDR (heterodimerises with the vitamin A dependent RXR nuclear receptor to activate vitamin D response elements on target genes, including those for calcium binding proteins), it is perhaps not surprising that studies have shown maternal UV-B exposure in pregnancy is related to bone size in infancy, independent of height and lean mass (Sayers & Tobias, 2009). While similarly, prenatal sunlight has been shown to be one of the most significant determinants of height (Waldie, Poulton, Kirk, & Silva, 2000). These studies provide a direct link between an early-life environmental cue and an obvious vitamin D/*VDR*/calcium mediated end point.

Although these data suggest early gestational light exposure is important in vitamin D-related genomic processes, so too are nutrigenetic events associated with the adolescent growth spurt, with the skeletal epiphyses finally fusing by the age of 20 years. It is worth mentioning that among the

TABLE 3 Independent predictors of VDR CpG island methylation density in a multivariate model

Variable	β	*P*-value
Plasma 25(OH)D	***0.33***	***0.003***
Photoperiod at conception	***0.26***	***0.02***
Cumulative UV irradiance[a]		
Second Tertile	***0.31***	***0.02***
Third Tertile	−0.06	0.6
BMI	***0.27***	***0.01***
TaqI[b]	***–0.40***	***0.009***
FokI[b]	***–0.23***	***0.03***
BsmI[b]	0.25	0.1
Age	−0.11	0.3
Sex	−0.04	0.7
Smoking[c]	0.05	0.7

Reference groups for categorical variables: [a]First tertile; [b]Absence of mutant allele; [c]Never smokers.

various hypotheses put forward to explain the pygmy phenotype, a shortage of vitamin D and hence altered bone mineral metabolism has been a popular postulate (Gluckman, Beedle, & Hanson, 2011). The pygmy phenotype is common in dense equatorial jungle environments globally, where solar exposure would be limited and nutritional stress high. Mechanisms such as vitamin D photosynthesis and *VDR* methylation may therefore be components in this adaptive phenotype, which has been suggested to improve movement through forests and confers an improved ability to climb. Small body size (<155 cm) also helps in heat dispersal and is associated with early sexual maturation—a useful trait in difficult environments where mortality is high (average life expectancy around 19 years). Pygmies cease growth early at around 12 years and exhibit no adolescent growth spurt. Other theories exist, and suggest the GHBP/GHR/IGF1 system may be altered (Becker et al., 2013), and that heritable epigenetics is involved by linking environment to modification in gene expression, with geographically distant pygmy groups likely evolving their short stature independently (Migliano et al., 2013).

Although entirely hypothetical, it is interesting to consider the present findings within this contemporary, real world anthropological context. The relationship we describe between light and *VDR* methylation would be consistent with the pygmy adaptive phenotype. That is to say, while gestational photoperiod/sunlight exposure is positively associated with height and *VDR* methylation, limited light exposure and dietary restriction would, conversely, be negatively associated with height and lead to *VDR* hypomethylation, a potentially flexible response in genes to the restricted light exposure encountered in dense jungle. Others have shown that height as a trait is associated with a 1.7-fold increase in enriched genomic VDR binding (Ramagopalan et al., 2010).

4.5 | Light as an exposomal cue and VDR variation

In a broader context, altered *VDR* methylation and hence gene expression becomes an adaptive response to major exposomal cues that may influence both an individual or species-wide flexible response to a shifting environmental context. In the case of light, it might relate to either season or latitude, and might also change according to critical phases of the lifecycle; embryogenesis, infancy, adolescence, pregnancy and senescence. While others have shown that personal sun exposure can influence global DNA methylation (Nair-Shalliker, Dhillon, Clements, Armstrong, & Fenech, 2014), this is the first study to show a clear gene specific methylation response to various light-related phenomena.

As described earlier, latitudinal factors related to the occurrence of *VDR* polymorphisms BsmI and TaqI potentially influence the degree of VDR gene methylation in a predictable way. As such, it is consistent with epigenetic adaptation to variation in ambient light exposure. However, while the *VDR* FokI genotype did show a significant relationship with CpG island methylation, the effect was the apparent opposite of what was seen for BsmI and TaqI when considered in a latitudinal context. However, this may not be entirely surprising since BsmI and TaqI form components within an important *VDR* BsmI/ApaI/TaqI haplotype block. The frequencies for the most common halotypes (baT, BAt, bAT and BAT) in this population have been reported previously by the present authors (Beckett, Le Gras, et al., 2016), and exist in high linkage disequilibrium. Indeed, FokI plays a different role as it generates a modified protein (Kostner et al., 2009). The polymorphic "F" allele (major allele) has a later start codon than the ancestral "f" allele (minor allele), generating a shorter more transcriptionally active protein (Kostner et al., 2009; Jurutka, Whitfield, Hsieh, Thompson, & Haussler, 2001; Whitfield et al., 2001). When this function of the F allele is considered in a geographic context against previously published data on latitudinal occurrence of FokI alleles (Lucock et al., 2015), it suggests that an increasing UV exposure with reduced latitude enhances the prevalence of an allele conferring increased VDR activity. This apparent latitudinal contrast between BsmI, TaqI and FokI may be of interest in considering future light/vitamin D related molecular equilibria.

4.6 | Relevance to disease phenotype

Certainly the *VDR* genotypes we found to be influenced by light have been well linked to clinical phenotype, particularly cancer, although results are mixed and potentially gender specific. There are many cancers where it can be argued that the FokI "F" allele is protective compared to the "f" allele, which generally fits our narrative. Kostner et al. (2009) provide data indicating an association of *VDR* polymorphisms and cancer risk; the strongest relationships exist for breast cancer (Bsm1, Fok1), prostate cancer (Fok1) and malignant melanoma (Fok1). Data also indicate an association of *VDR* polymorphisms and cancer prognosis being strongest for prostate cancer (Fok1), breast cancer (Bsm1, Taq1), melanoma (Bsm1) and renal cell carcinoma (Taq1). Additionally, in the context of bone mineralization, FokI FF genotype has been shown to be associated with high lumbar spine bone mineral density compared to the other FokI genotypes (Kanan, 2013). Others have also shown that FokI influences bone mineralization (Zajícková, Zofková, Bahbouh, & Krepelová, 2002), although here, FF individuals had intermediate effect. Although it is not possible to infer causality, the prevalence of *VDR* genotype with latitude, the role of *VDR* genotype in disease, and the occurrence of certain diseases

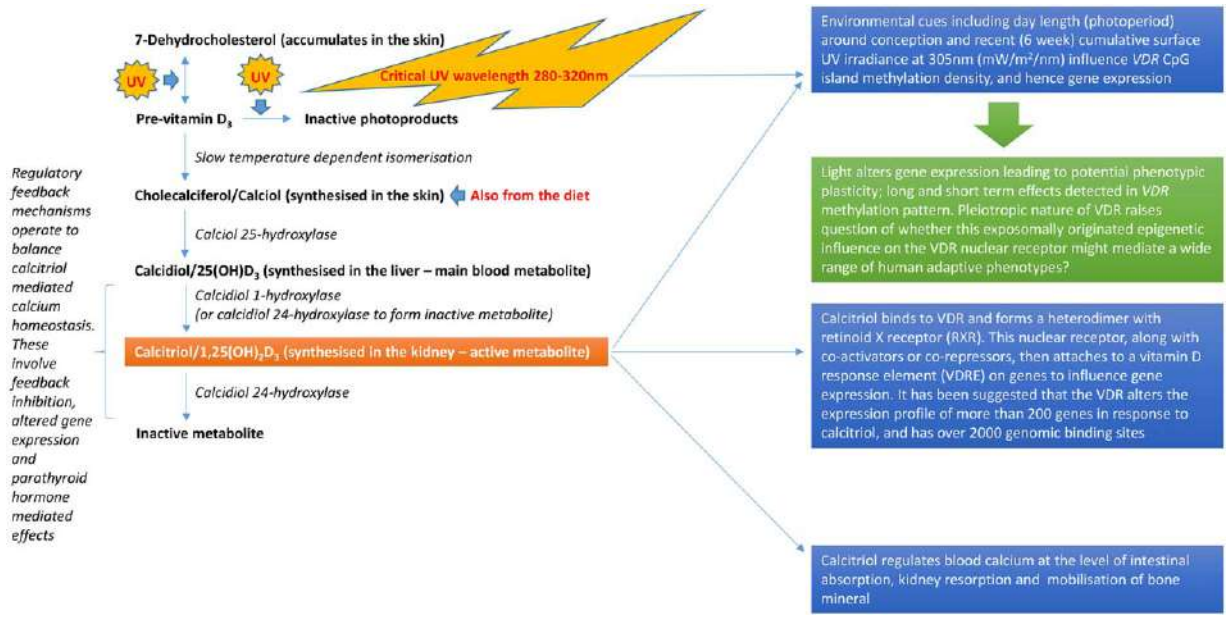

FIGURE 4 Schematic showing how light might affect VDR gene methylation, and hence potentially influence phenotypic plasticity. This is examined within a framework of vitamin D metabolism

by latitude or season warrant further investigation to see whether firm links can be established, including any importance of *VDR* CpG island methylation. Seasonality at conception has already been linked to multiple sclerosis (Bayes, Weir, & O'leary, 2010; Templer et al., 1992), and month of birth influences immune-related disease implicating UV-B and vitamin D as potential risk factors (Disanto et al., 2012). In this study, risk of Crohn's disease, rheumatoid arthritis, ulcerative colitis, and lupus were inversely correlated to second trimester UV-B exposure and third trimester vitamin D status. Additionally, seasonality at conception has also been correlated with brain tumors in adults (Brenner et al., 2004), the expression of neurotransmitter-related genes in psychiatric patients (Chotai, Serretti, Lattuada, Lorenzi, & Lilli, 2003), and in the development of specific behavioral traits (Eisenberg, Campbell, Mackillop, Lum, & Wilson, 2007). Latitude has also been linked to pancreatic cancer mortality in Australia, with the suggestion that vitamin D and UV exposure might be involved in disease etiology (Altieri et al., 2016; Neale, Youlden, Krnjacki, Kimlin, & van der Pols, 2009). Furthermore, a relationship is already well established between type II diabetes and vitamin D status (Khan, Kunutsor, Franco, & Chowdhury, 2013).

4.7 | Study limitations

The data we present have been adjusted for age, sex and serum calcidiol, along with other key environmental and/or lifestyle factors such as smoking, BMI and cumulative solar irradiance at 305 nm in the 6 weeks prior to blood sampling. When adjusted, the data maintained significance, indicating that the degree of VDR gene methylation is likely related to light exposure. While cause and effect are not proven, the authors suggest this might act as a molecular adaptation to varying levels of light exposure within and across the lifecycle. What we do not have is expression data, and future studies should focus on examining mRNA transcription to strengthen support for this paradigm. The other limitation of the present study is power. Future work would benefit from examining a larger cohort, although the present cohort is still very well characterized.

Although, logically, light and blood vitamin D levels might be expected to be related, the present study suggests that these two variables are additive, not interactive in predicting VDR gene CpG island methylation. This might stem from a lag phase between solar exposure and assimilation of calcidiol in the blood (Lucas et al., 2005). A more comprehensive assessment of this and information on daily activity, clothing and skin pigmentation might help overcome some of the more obvious study limitations; however, unfortunately it was not available to us in this study. It should also be noted that a reduced skin synthesis of vitamin D occurs in the elderly, and it is quite possible that in young people you might see an interaction. Another limitation relates to whether satellite data represent the dose of radiation at ground level. However, the authors believe that studies like this with estimated exposure are necessary to justify future, more expensive methods of measuring personal UV exposure.

4.8 | Concluding statement

In summary, duration of early-life exposure and strength of recent irradiance, along with latitudinal-related genetic

factors, influence the degree of VDR gene methylation in a generally predictable manner. We suggest that this is consistent with this epigenetic phenomenon being a molecular adaptation to variation in ambient light exposure. Figure 4 shows how this idea fits into the broader context of vitamin D metabolism. This is a novel finding, and may help explain why, in a recent study, 55 diseases were significantly linked to birth month (Boland, Shahn, Madigan, Hripcsak, & Tatonetti, 2015). The authors' findings were discussed in the context of environmental exposure to light and hence vitamin D synthesis. Others have also demonstrated that ancestry and maternal calcidiol jointly influence DNA methylation in infants, but found that vitamin D differences do not explain lower overall methylation in African ancestral groups (Mozhui, Smith, & Tylavsky, 2015). Perhaps this relates to the filtering effect of pigmentation on light exposure, but in any event generally supports the findings presented in the current article. While the obvious correlate is with disease, it is also worth considering the phenomenon in the context of adaptive phenotypes. The most obvious ones being a potential role in adaptive skeletal changes and in contemporary models related to the evolution of skin depigmentation. However, given the pleiotropic effects of vitamin D, there are likely to be many other possible effects that ultimately shape the human phenome (Ramagopalan et al., 2010). One only has to consider that more than 4,000 protein-coding mRNAs in white blood cells and adipose tissue have seasonal expression profiles, with inverted patterns observed between Europe and Oceania (Dopico et al., 2015), reinforcing the idea that mechanisms to regulate seasonal response clearly do exist and play a significant role in human biology and our ability to adapt to a changing environment.

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COMPETING INTERESTS

We have no competing interests.

AUTHOR CONTRIBUTIONS

Idea researched and developed by ML and EB; Genetics examined by EB and CM; Statistical input EB and ML; Physical science aspects carried out by JF and PJ; Vitamin analysis EB and PJ; Overall chief investigator and clinician with ultimate oversight MV; Anthropological aspects led by NJ, GC and ML; Article crafted in final form by ML, EB, MV, NJ, GC, KD and ZY; all authors contributed to final manuscript.

ETHICS

Informed consent was obtained prior to participation under University of Newcastle Human Research Ethics Committee approval number H-2008-0431.

DATA ACCESSIBILITY

Access to the ARC RHLS database is via the RHLS Steering Committee (Chair—A/Prof Martin Veysey).

REFERENCES

Altieri, B., Grant, W. B., Casa, S. D., Orio, F., Pontecorvi, A., Colao, A., … Muscogiuri, G. (2016). Vitamin D and pancreas: the role of sunshine vitamin in the pathogenesis of Diabetes Mellitus and Pancreatic Cancer. *Critical Reviews in Food Science and Nutrition*, *31*, 0. [Epub ahead of print].

Bayes, H. K., Weir, C. J., & O'leary, C. (2010). Timing of birth and risk of multiple sclerosis in the Scottish population. *European Neurology*, *63*, 36–40.

Becker, N. S., Verdu, P., Georges, M., Duquesnoy, P., Froment, A., Amselem, S., … Heyer, E. (2013). The role of GHR and IGF1 genes in the genetic determination of African pygmies' short stature. *European Journal of Human Genetics*, *21*, 653–658.

Beckett, E., Martin, C., Duesing, K., Yates, Z., Veysey, M., & Lucock, M. (2014). Vitamin D receptor genotype modulates the correlation between circulating levels of miR-7a/b and Vitamin D intake in an elderly cohort. *Journal of Nutrigenetics and Nutrigenomics*, *7*, 264–273.

Beckett, E. L., Duesing, K., Martin, C., Jones, P., Furst, J., King, K., … Lucock, M. (2016). Relationship between methylation status of vitamin D-related genes, vitamin D levels, and methyl-donor biochemistry. *Journal of Nutrition & Intermediary Metabolism*, *6*, 8–15.

Beckett, E. L., Le Gras, K., Martin, C., Boyd, L., Ng, X., Duesing, K., … Lucock, M. (2016). Vitamin D receptor polymorphisms

relate to risk of adenomatous polyps in a sex-specific manner. *Nutrition and Cancer*, *68*, 193–200.

Boland, M. R., Shahn, Z., Madigan, D., Hripcsak, G., & Tatonetti, N. P. (2015). Birth month affects lifetime disease risk: a phenome-wide method. *Journal of the American Medical Informatics Association*, *22*, 1042–1053.

Bortman, B., Folgueira, M. A., Katayama, M. L., Snitcovsky, I. M., & Brentani, M. M. (2002). Antiproliferative effects of 1,25-dihydroxy vitamin D3 on breast cells: A mini review. *Brazilian Journal of Medical and Biological Research*, *35*, 1–9.

Bouillon, R., Carmeliet, G., Verlinden, L., van Etten, E., Verstuyf, A., Luderer, H. F., … Demay, M. (2008). Vitamin D and human health: Lessons from vitamin D receptor null mice. *Endocrine Reviews*, *29*, 726–776.

Brenner, A. V., Linet, M. S., Shapiro, W. R., Selker, R. G., Fine, H. A., Black, P. M., & Inskip, P. D. (2004). Season of birth and risk of brain tumors in adults. *Neurology*, *63*, 276–281.

Chaplin, G., & Jablonski, N. G. (2013). The human environment and the vitamin D compromise: Scotland as a case study in human biocultural adaptation and disease susceptibility. *Hum Biology*, *85*, 529–552.

Chotai, J., Serretti, A., Lattuada, E., Lorenzi, C., & Lilli, R. (2003). Gene environment interaction in psychiatric disorders as indicated by season of birth variations in tryptophan hydroxylase (TPH), serotonin transporter (5-HTTLPR) and dopamine receptor (DRD4) gene polymorphisms. *Psychiatry Research*, *119*, 99–111.

Deaton, A. M., & Bird, A. (2010). CpG islands and the regulation of transcription. *Genes & Development*, *25*, 1010–e1022.

Dennison, E. M., Arden, N. K., Keen, R. W., Syddall, H., Day, I. N., Spector, T. D., & Cooper, C. (2001). Birthweight, vitamin D receptor genotype and the programming of osteoporosis. *Paediatric and Perinatal Epidemiology*, *15*, 211–219.

Disanto, G., Chaplin, G., Morahan, J. M., Giovannoni, G., Hyppönen, E., Ebers, G. C., & Ramagopalan, S. V. (2012). Month of birth, vitamin D and risk of immune-mediated disease: a case control study. *BMC Medicine*, *10*, 69.

Dopico, X. C., Evangelou, M., Ferreira, R. C., Guo, H., Pekalski, M. L., Smyth, D. J., … Todd, J. A. (2015). Widespread seasonal gene expression reveals annual differences in human immunity and physiology. *Nature Communications*, *6*, 7000. DOI: 10.1038/ncomms8000

Eisenberg, D. T., Campbell, B., Mackillop, J., Lum, J. K., & Wilson, D. S. (2007). Season of birth and dopamine receptor gene associations with impulsivity, sensation seeking and reproductive behaviors. *PLoS One*, *2*, e1216.

Fetahu, I. S., Hobaus, J., & Kallay, E. (2014). Vitamin D and the epigenome. *Frontiers in Physiology*, *5*, 164.

Gibbons, A. (2007). European skin turned pale only recently, gene suggests. *Science*, *316*, 364.

Gluckman, P., Beedle, A., & Hanson, M. (2011). *Principle of evolutionary medicine*. Oxford, UK: Oxford University Press.

Hochberg, Z., & Templeton, A. (2010). Evolutionary perspective in skin color, vitamin D and its receptor. *Hormones*, *9*, 307–311.

Holemon, H., Korshunova, Y., Ordway, J. M., Bedell, J. A., Citek, R. W., Lakey, N., … Jeddeloh, J. A. (2007). MethylScreen: DNA methylation density monitoring using quantitative PCR. *Biotechniques*, *43*, 683–693.

Jablonski, N. G., & Chaplin, G. (2000). The evolution of human skin coloration. *Journal of Human Evolution*, *39*, 57–106.

Jurutka, P. W., Whitfield, G. K., Hsieh, J. C., Thompson, P. D., & Haussler, C. A. (2001). Molecular nature of the vitamin D receptor and its role in regulation of gene expression. *Reviews in Endocrine and Metabolic Disorders*, *2*, 203–216.

Kanan, R. M. (2013). The effect of FokI vitamin D receptor polymorphism on bone mineral density in Jordanian perimenopausal women. *Indian Journal of Human Genetics*, *19*, 233–238.

Khan, H., Kunutsor, S., Franco, O. H., & Chowdhury, R. (2013). Vitamin D, type 2 diabetes and other metabolic outcomes: A systematic review and meta-analysis of prospective studies. *Proceedings of the Nutrition Society*, *72*, 89–97.

Kostner, K., Denzer, N., Müller, C. S., Klein, R., Tilgen, W., & Reichrath, J. (2009). The relevance of vitamin D receptor (*VDR*) gene polymorphisms for cancer: A review of the literature. *Anticancer Research*, *29*, 3511–3536.

Lammi, J. (1996–2008). Online-Photoperiod Calculator V 1.94 L; Retrieved from http://www.sci.fi/~benefon/sol.html.

Lucas, J. A., Bolland, M. J., Grey, A. B., Ames, R. W., Mason, B. H., Horne, A. M., … Reid, I. R. (2005). Determinants of vitamin D status in older women living in a subtropical climate. *Osteoporosis International*, *16*, 1641–1648.

Lucock, M., Yates, Z., Martin, C., Choi, J. H., Boyd, L., Tang, S., … Veysey, M. (2014). Vitamin D, folate, and potential early lifecycle environmental origin of significant adult phenotypes. *Evolution, Medicine, and Public Health*, *1*, 69–91.

Lucock, M., Jones, P., Martin, C., Beckett, E., Yates, Z., Furst, J., & Veysey, M. (2015). Vitamin D: Beyond Metabolism. *Journal of Evidence-Based Complementary & Alternative Medicine*, *20*, 310–322.

Martin, C. E., Veysey, M., Yates, Z. R., & Lucock, M. D. (2014). Vitamin D: genetics, environment & health. *Journal of Food and Nutritional Disorders*, *3*, 5. Doi: 10.4172/2324-9323.1000155.)

Migliano, A. B., Romero, I. G., Metspalu, M., Leavesley, M., Pagani, L., Antao, T., … Kivisild, T. (2013). Evolution of the pygmy phenotype: evidence of positive selection from genome-wide scans in African, Asian, and Melanesian pygmies. *Human Biology*, *85*, 251–284.

Mozhui, K., Smith, A. K., & Tylavsky, F. A. (2015). Ancestry dependent DNA methylation and influence of maternal nutrition. *PLos One.* Doi: 10.1371/journal.pone.0118466

Nair-Shalliker, V., Dhillon, V., Clements, M., Armstrong, B. K., & Fenech, M. (2014). The association between personal sun exposure, serum vitamin D and global methylation in human lymphocytes in a population of healthy adults in South Australia. *Mutation Research*, *765*, 6–10.

Neale, R. E., Youlden, D. R., Krnjacki, L., Kimlin, M. G., & van der Pols, J. C. (2009). Latitude variation in pancreatic cancer mortality in Australia. *Pancreas*, *38*, 387–390.

Pike, J. W., & Meyer, M. B. (2010). The vitamin D receptor: New paradigms for the regulation of gene expression by 1,25-dihydroxyvitamin D3. *Endocrinology Metabolism Clinics of North America*, *39*, 255–269.

Ramagopalan, S. V., Heger, A., Berlanga, A. J., Maugeri, N. J., Lincoln, M. R., Burrell, A., … Knight, J. C. (2010). A ChIP-seq defined genome-wide map of vitamin D receptor binding: associations with disease and evolution. *Genome Research*, *20*, 1352–1360.

Sayers, A., & Tobias, J. H. (2009). Estimated maternal ultraviolet B exposure levels in pregnancy influence skeletal development of the child. *The Journal of Clinical Endocrinology & Metabolism*, *94*, 765–771.

Templer, D. I., Trent, N. H., Spencer, D. A., Trent, A., Corgiat, M. D., Mortensen, P. B., & Gorton, M. (1992). Season of birth in multiple sclerosis. *Acta Neurologica Scandinavica*, *85*, 107–109.

Waldie, K. E., Poulton, R., Kirk, I. J., & Silva, P. A. (2000). The effects of pre- and post-natal sunlight exposure on human growth: Evidence from the Southern Hemisphere. *Early Human Development*, *60*, 35–42.

Whitfield, G. K., Remus, L. S., Jurutka, P. W., Zitzer, H., Oza, A. K., Dang, H. T., . . . Haussler, M. R. (2001). Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Molecular and Cellular Endocrinology*, *177*, 145–159.

Zajícková, K., Zofková, I., Bahbouh, R., & Krepelová, A. (2002). Vitamin D receptor gene polymorphisms, bone mineral density and bone turnover: FokI genotype is related to postmenopausal bone mass. *Physiological Research*, *51*, 501–509.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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