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Vitamin D binding protein genotype is associated with plasma 25OHD concentration in West African children

V.S. Braithwaite a,⁎, K.S. Jones a, I. Schoenmakers a, M. Silver b, A. Prentice a,c, B.J. Hennig b

a MRC Human Nutrition Research, Elsie Widdowson Laboratory, Fulbourn Road, Cambridge CB1 9NL, UK
b MRC International Nutrition Group at London School of Hygiene & Tropical Medicine, Keppel St, London WC1E 7HT UK and MRC Unit, The Gambia

⁎ Corresponding author at: Vickie Braithwaite, MRC Human Nutrition Research, Elsie Widdowson Laboratory, Cambridge CB1 9NL, UK. Fax: +44 1223 437515.
E-mail address: vickie.braithwaite@mrc-hnr.cam.ac.uk (V.S. Braithwaite).

Introduction

Vitamin D plays an essential role in maintaining skeletal health. Vitamin D status is typically determined by plasma 25-hydroxyvitamin D (25OHD) concentration because it has a half-life of a couple of weeks, it is an integrated marker of different sources of vitamin D supply and it reflects the balance between supply and expenditure [1,2]. Unlike other vitamins, vitamin D is obtained both through the diet and through endogenous production from skin exposure to UVB. 25OHD status is therefore dependent on a number of non-dietary factors. These include skin pigmentation and skin exposure to UVB, i.e. factors associated with genetic, behavioural and environmental factors. Recent studies have highlighted the importance of genetic variation in vitamin D-regulating genes for vitamin D status [3,4]. Many studies have looked at multi-ethnic populations with diverse ancestry and have shown differences in 25OHD status depending on single nucleotide polymorphisms (SNPs) in vitamin D pathway-related genes. However, few studies have looked within a population, which is homogenous with respect to ancestry and to environmental influences relevant to vitamin D status.

Vitamin D binding protein (DBP) is encoded by Gc located on chromosome 4q11–q13 [5]. DBP is an albumin-like protein produced in the liver, which carries vitamin D metabolites in the circulation. DBP also has other physiological roles such as sequestering actin [6], binding fatty acids [7] and as a macrophage activating factor [8]. DBP transports vitamin D metabolites to tissues such as the liver and kidney. The major vitamin D metabolites (i.e. 25OHD and 1,25(OH)2D3) are bound to DBP (>95%) or another circulatory protein such as albumin, leaving only a small percentage circulating in its free form (for 25OHD <1%) [5,9,10]. Internalisation of DBP-bound 25OHD by
the megalin/cubulin endocytic pathway in the proximal tubule epithelium allows for hydroxylation of 25OHD by 1- hydroxylase to produce 1,25(OH)\(_2\)D, the active metabolite of vitamin D for systemic/ endocrine functions [9]. It is thought that other tissues (non-renal) which do not express megalin rely largely on the diffusion of free 25OHD through the plasma membrane for subsequent conversion to 1,25(OH)\(_2\)D for autocrine and paracrine effects. The biological relevance of the DBP-bound vitamin D metabolites versus the DBP-unbound or “free fraction” of vitamin D has not yet been established [9] and the role of DBP in the potential regulation of the free fraction is not fully understood.

The three common Gc variants, described as Gc1f, Gc1s and Gc2 are determined by two SNPs in Gc; rs7041(Asp432Glu) and rs4588 (Thr436Lys). Combinations of these two SNPs give rise to six DBP isotypes (Gc1f-1f, Gc1f-1s, Gc1f-2, Gc1s-1s, Gc1s-2, Gc2-2). These isotypes have protein configurational differences which may in turn cause differences in the binding affinity of DBP for vitamin D metabolites (see Fig. 1) [9]. However, divergent results have been published that describe either very similar [11,12], or several-fold differences in affinity between 25(OH)D and DBP isoforms [13].

Gc1f-1f has the highest and Gc2-2 the lowest binding affinity for 25OHD and therefore different Gc variants may also influence the free fraction of 25OHD; the largest free fraction being associated with Gc2-2 and the smallest with Gc1f-1f isotype (see Fig. 1) [9].

The frequency of different DBP isotypes varies by ancestry; Gc1f-1f being most common in West Africans and African Americans and least common in Caucasians [14]. Thus, studies with participants of different ancestries cannot easily distinguish between the genetic versus environmental influences on vitamin D status.

The aim of this study was to determine the Gc variant frequencies in a sample of West-Africans (n = 3129) of predominantly Mandinka origin from the isolated rural West Kiang region of The Gambia using the Infinium HumanExome BeadChip. We investigated the association of variations in DBP isotype with vitamin D metabolites (25OHD and 1,25(OH)\(_2\)D) and PTH concentration in a population of children from the same region (n = 237). This population has the opportunity for year-round endogenous vitamin D production and has a life-long low dietary calcium intake [15].

Materials & methods

Participants

The Gambia, West Africa, is a tropical country situated at latitude 13°N and has abundant sunshine, in 280–315 nm wavelength range, allowing for endogenous vitamin D production all year. The West Kiang province of The Gambia is a subsistence agricultural society where the majority of time is spent outside and customary dress does not restrict sunshine exposure to arms and face [16]. Samples and data for this study were available for West Kiang residents (collected in 2002–2003 and 2012-2014) as part of the MRC Keneba Biobank (http://www.ing.mrc.ac.uk/research_areas/the_keneba_biobank.aspx).

All study participants were self-reported as being well at the time of recruitment (n = 3129; M = 1409). Ethical approval was given by The Gambian Government/MRC Unit Joint Ethics Committee and written informed consent was obtained from participants or their parents or guardians.

Sample collection and biochemical analysis

For a subset of children biochemical data, including plasma 25OHD concentrations, were available from previous studies (n = 237; M = 87; age 11.9 (SD 4.8) years) [17–19]. Briefly, overnight-fasted venous blood samples (5–15 mL) were collected and transferred into pre-cooled lithium heparin (LiHep) and EDTA-coated tubes. The blood was separated by centrifugation at 4 °C within 45 min of collection. The plasma and the plasma depleted cell pellets were frozen at −80 °C and −20 °C respectively and were later transported on dry ice to MRC Human Nutrition Research (HNR), Cambridge, for analysis and DNA extraction. 25OHD concentration (DiaSorin, UK; assay performance monitored by DEQAS http://www.deqas.org/), parathyroid hormone (PTH; Immulite, Siemens Healthcare Diagnostics, UK; assay performance monitored by NEQAS http://www.ukneqas.org.uk), 1,25(OH)\(_2\)D (IDS, UK also DEQAS), calcium, phosphate and albumin (Kone Analyser 20i, Finland) were also measured. In a smaller number of participants (n = 172) fibroblast growth factor-23 (FGF23; C-terminal ImmunoDiagnostics Inc., CA, USA), total alkaline phosphatase (TALP) and creatinine (Cr) (Kone Analyser 20i, Finland) were also measured.

DNA extraction & genotyping

DNA was extracted from fresh or frozen plasma-depleted whole blood using standard methodology (QIAamp DNA kits, Qiagen, Manchester, UK; Nucleon Bacc2 DNA extraction kits, Scientific Laboratory Supplies Ltd, Nottingham, UK; or salting-out [20]). Samples (n = 3129) were then processed on the Infinium 240 k Human Exome Beadchip v1.0 and v1.1 (Illumina, Canada), which captures putative functional variation genome-wide. Genotypes were called using data-driven clustering (Genome Studio, Illumina, CA, USA).

The Exome Beadchip includes the two SNPs that determine DBP isotype, rs7041 and rs4588 [4,21], which were the focus of this study. The SNPs of interest were not in linkage disequilibrium (r\(^2\) = 0.004) as determined using genotype data on the whole sample (n = 3129) in Haplovew using default criteria [22]. KING kinship

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Phenotype</th>
<th>Binding Affinity</th>
<th>Free*</th>
<th>Count</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
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<td>Low</td>
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</tr>
<tr>
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<tr>
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<tr>
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<tr>
<td>rs4588-A (Lys)</td>
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<td>0.0003</td>
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</tr>
</tbody>
</table>

Fig. 1. Gc composite genotypes; DBP isotype; DBP substrate binding affinities (i.e. vitamin D metabolites ★) and relative free fraction of ★ [5] and frequency of Gc variants in this Gambian study population (n = 3129).
Biochemical analyses were compared between those homozygous for Gc1f-1f vs. other Gc variants (Gc1f-1s, Gc1s-1s, Gc1f-2 & Gc2-2) using DataDesk 6.3.1 (Data Description Inc, Ithaca, NY, USA) with Student’s 2-sample t-tests. Data are reported as mean and standard deviation (SD) for normally distributed or geometric mean (+1SD, −1SD) for negatively skewed data. Regression was used to identify relationships between vitamin D metabolites and PTH in univariate models unadjusted for age and sex, since their inclusion made no material difference to the DBP genotype effect on 25OHD concentration and so was not included for in any of the models. The inclusion of “study” as a covariate made no material difference to the DBP genotype effect on 25OHD concentration and so was not included in the analyses. The beta (β) coefficients of the In-In univariate regression analysis multiplied by 100 provide a percentage change in PTH production and secretion in the parathyroid gland [25] and/or calcium metabolism through multiple pathways. We speculate that this may be associated with differences in plasma 25OHD and PTH, potentially reflecting differences in the association between vitamin D metabolites and PTH by DBP isotypes. Gc1f homozygotes, suggested to have the highest affinity for 25OHD, had the highest plasma 25OHD concentration compared with the lower affinity DBP isotypes. There was a tendency for PTH to be lower in Gc1f homozygotes and the relationship between PTH and 25OHD or 1,25(OH)2D although this did not reach statistical significance. There were no significant Gc variant group x vitamin D metabolite interactions suggesting that the slopes were not statistically significantly different between variant groups.

Conclusions

To our knowledge this study reports the frequency of DBP isotypes in the largest cohort of rural West Africans (n = 3129) to date with frequencies of Gc1f 86.0%, Gc1s 11.0% and Gc2 3.0%.

This study adds to the body of evidence that Gc variation appears to influence vitamin D status [4] and may be associated with differences in plasma 1,25(OH)2D and PTH, potentially reflecting differences in the association between vitamin D metabolites and PTH by DBP isotypes. Gc1f homozygotes, suggested to have the highest affinity for 25OHD, had the highest plasma 25OHD concentration compared with the lower affinity DBP isotypes. There was a tendency for PTH to be lower in Gc1f homozygotes and the relationship between PTH and 25OHD or 1,25(OH)2D was stronger in Gc1f homozygotes than in other Gc variants.

These findings suggest that DBP isotype may influence vitamin D metabolism through multiple pathways. We speculate that this may be associated with a) differences in plasma 25OHD directly suppressing PTH production and secretion in the parathyroid gland [25] and/or b) differences in the free fraction of 25OHD which may modulate the availability for renal hydroxylation into 1,25(OH)2D which in turn suppresses PTH through the calcium-PTH-1,25(OH)2D axis. However, the renal internalisation of 25OHD has been reported to be dependent on the megalin/cubulin pathway [9] and may be independent of the free fraction of 25OHD. The influence of DBP isotype on free 25OHD needs further investigation.

A strength of this study is that the participants were subject to relatively uniform environmental exposures and all participants were rural Gambian of predominantly Mandinka ancestry with a dark skin type. Although we did not monitor skin exposure to the sun we anticipate that skin exposure allowing for endogenous vitamin D synthesis is likely to have been similar among participants due to
similar day to day outdoor activities in year-round tropical sunshine and similar cultural dress. Similarly, previous studies in the region have demonstrated consistently low dietary calcium intakes in otherwise healthy rural Gambian children and we do not anticipate that have demonstrated consistently low dietary calcium intakes in other- and similar cultural dress. Similarly, previous studies in the region

Fig. 2. Scatterplot of PTH and 25(OH)D and 1,25(OH)2D by Gc variant group: Gc1f-1f (n = 175) vs. other ▼ (n = 62; Gc1s-1f, Gc1s-1s, Gc1f-2, Gc1s-2). Regression lines indicate the two different genotype groups (Gc1f-1f = broken line, other = solid line). Table indicates the equations of the lines for the association between 25(OH)D and 1,25(OH)2D with PTH and 25(OH)D with 1,25(OH)2D in all participants and divided into Gc variant group.

![Graph](image)

**References**


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**Conflicts of interest**

All authors state that they have no conflicts of interest.

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