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Genomics, Proteomics, and Metabolomics

Associations Between Prenatal Vitamin D and Placental Gene Expression

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ABSTRACT

Background: Vitamin D is a hormone that regulates gene transcription. Prenatal vitamin D has been linked to immune and vascular function in the placenta, a key organ of pregnancy. Transcriptome-wide RNA sequencing can provide a more complete representation of the placental effects of vitamin D.

Objectives: We investigated the association between prenatal vitamin D concentrations and placental gene expression in a large, prospective pregnancy cohort.

Methods: Participants were recruited from Shelby County, TN, United States, in the Conditions Affecting Neurocognitive Development and Learning in Early childhood (CANDLE) study. Vitamin D (plasma total 25-hydroxyvitatmin D, [25(OH)D]) was measured at midpregnancy (16–28 wk) and delivery. RNA was sequenced from placental samples collected at birth. We identified differentially expressed genes (DEGs) using adjusted linear regression models. We also conducted weighted gene coexpression network analysis.

Results: The median 25(OH)D of participants was 21.8 ng/mL at midpregnancy ($N = 774$; IQR: 15.4–26.5 ng/mL) and 23.6 ng/mL at delivery ($n = 753$; IQR: 16.8–29.1 ng/mL). Placental expression of 17 DEGs was associated with 25(OH)D at midpregnancy, but only 1 DEG was associated with 25(OH)D at delivery. DEGs were related to energy metabolism, cytoskeletal function, and transcriptional regulation. We identified 2 weighted gene coexpression network analysis gene modules whose expression was associated with 25(OH)D at midpregnancy and 1 module associated with 25(OH)D at delivery. These modules were enriched for genes related to mitochondrial and cytoskeletal function and were regulated by transcription factors including *ARNT2* and *FOSL2*. We also identified 12 modules associated with 25(OH)D in females and 1 module in males.

Conclusions: 25(OH)D during midpregnancy, but not at delivery, is associated with placental gene expression at birth. Future research is needed to investigate a potential role of vitamin D in modulating placental mitochondrial metabolism, intracellular transport, and transcriptional regulation during pregnancy.

Keywords: vitamin D, 25-hydroxyvitamin D, placenta, transcriptomics, developmental origins of health and disease

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Abbreviations: CANDLE, Conditions Affecting Neurocognitive Development and Learning in Early childhood; DEG, differentially expressed gene; ECHO-PATHWAYS, Environmental influences on Child Health Outcomes prenatal and early childhood pathways to health consortium; FDR, false discovery rate; HEI, Healthy Eating Index; NDI, Neighborhood Deprivation Index; TF, transcription factor; WGCNA, weighted gene coexpression network analysis.

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Introduction

The developmental origins of health and disease framework holds that adverse exposures such as malnutrition during early development influence later-life risk of disease through changes that alter the body's structure and function [1]. Vitamin D is a critical nutrient influencing development and health. During fetal development, vitamin D is involved in skeletal formation and growth, immune regulation, and placentation [2]. Maternal vitamin D deficiency during pregnancy is associated with increased risk of adverse pregnancy and birth outcomes, including low birth weight, small for gestational age, preeclampsia, and gestational diabetes [3–6]. Prenatal vitamin D might also program later-life health, including neurologic and cardiometabolic health, through its role in endocrine function [7]. In the CANDLE (Conditions Affecting Neurocognitive Development and Learning in Early childhood) Study, prenatal vitamin D concentrations have been linked to childhood neurodevelopmental and respiratory outcomes [8–10]. Vitamin D deficiency and inadequacy during pregnancy and lactation is prevalent throughout the world, reaching ~33% in the United States [11,12].

Vitamin D is a preprohormone both found in the diet and synthesized endogenously in skin by humans. Vitamin D is hydroxylated twice to activate it: first in the liver to the prohormone 25-hydroxyvitamin D [25(OH)D] and then in the kidney to the active hormone 1,25-dihydroxyvitamin D [1,25(OH)$_2$D] by CYP27B1 [13]. During pregnancy, the placenta is a key site of 1,25(OH)$_2$D activation [14]. The placenta expresses both vitamin D–activating *CYP27B1* and the vitamin D–inactivating *CYP24A1*, and the vitamin D receptor (*VDR*), which is a transcription factor (TF) activated by vitamin D [14]. This suggests that the placenta is capable of local vitamin D homeostasis and the vitamin D signaling might play an important role in the placenta [14]. Vitamin D has been linked to invasiveness, transport function, and immune and inflammatory modulation in the placenta [14–19]. Vitamin D treatment promotes trophoblast invasion accompanied by increased matrix metalloprotease expression and secretion in primary human extravillous trophoblasts isolated from first trimester placentas and cell lines [15,16]. Vitamin D also promotes amino acid transporter expression, which might link vitamin D status and fetal growth through nutrient transport via these receptors [17]. Additionally, vitamin D deficiency activates the Hippo signaling pathway, which controls organ size during development, and is linked to fetal growth restriction in a rat model [18]. Vitamin D treatment is also associated with reduced inflammatory signaling. Compared with untreated controls, 1,25(OH)$_2$D treatment dose dependently downregulated mRNA and protein expression of the inflammatory cytokines TNF-α and IL-6 in placentas from pre-eclamptic pregnancies [19]. Similarly, overrepresentation analysis identified that the inflammation and immune regulation pathway was enriched for downregulated genes associated with 25(OH)D treatment in primary villous fragments from treated term human placenta compared with that from untreated controls [14]. Accordingly, vitamin D could modulate pathways central to placental function as a conduit for fetal nutrition and a regulator of maternal–fetal immune interactions.

Candidate gene-based studies have revealed associations between placental gene expression and vitamin D supplementation or status, including positive associations with placental amino acid transporter gene expression [20], downregulation of an antiangiogenic factor associated with preeclampsia [21], and regulation of the inflammatory response after an immune challenge [22]. In 1 recent transcriptome-wide study, short-term vitamin D treatment was associated with tissue remodeling and gene transcription in both transcriptomic and proteomic analysis in primary villous tissue isolated from human placentas [14]. Additionally, vitamin D deficiency has been shown to elicit sex-specific effects on placental candidate gene expression in a mouse model, including *Vdr*, *Cyp24a1*, and *Cyp27b1* [22]. Furthermore, testosterone might influence placental vitamin D metabolism, as it has been shown to downregulate gene expression of vitamin D–activating *CYP27B1* and upregulate expression of vitamin D–inactivating *CYP24A1* [23]. However, the relationship between prenatal vitamin D concentrations and transcriptome-wide placental gene expression remains to be studied in an epidemiological context. Thus, we aimed to investigate the association between maternal vitamin D status during pregnancy and human placental gene expression, as well as the role of fetal sex as an effect modifier in a large, diverse prospective birth cohort.

Methods

Study participants and data collection

This analysis was conducted using samples collected as part of the CANDLE Study. This prospective birth cohort conducted in Shelby County, TN, United States, has been described in detail elsewhere [24]. Between December 2006 and July 2011, 1503 pregnant participants were recruited during their second trimester and were considered eligible if they were between 16 and 28 wk of gestation, had an uncomplicated singleton pregnancy, and planned to give birth at 1 of the 5 participating Shelby County health care centers. Participants were included in this analysis if they had RNA sequencing data, maternal plasma 25(OH)D concentrations measured at enrollment (midpregnancy) or delivery, and complete covariate data. All research activities for the CANDLE cohort were approved by the University of Tennessee Health Sciences Center institutional review board [24] and the Environmental influences on Child Health Outcomes prenatal and early childhood pathways to health consortium (ECHO-PATHWAYS) single institutional review board [25].

At the midpregnancy study enrollment visit, demographic data were collected, including maternal age, race/ethnicity, educational attainment, and health insurance status. Other variables such as prepregnancy BMI and Healthy Eating Index (HEI) 2010 were calculated from self-reported data as previously described [26]. Neighborhood deprivation index (NDI) was derived with principal components analysis to generate a census tract-level continuous variable incorporating levels of education, professional employment, owner occupied housing, poverty, and unemployment as previously described [27]. At the midpregnancy visit and late-pregnancy visit (during the third trimester), participant urine samples were collected, which were subsequently used to measure urinary cotinine adjusted for specific gravity as previously described [28]. At either urine collection time point, urinary cotinine of >200 ng/mL was used to classify maternal smoking status as previously described [29, 30]. At delivery, birth and fetal data were collected, including mode of delivery, labor status, and fetal sex.

Maternal plasma collection and vitamin D measurement

Maternal plasma vitamin D was measured as previously described [8,9]. At the midpregnancy visit (16–28 wk gestation) and at delivery, maternal blood samples were collected, transported on ice, and centrifuged at 4 °C. Aliquots of the resulting plasma were stored at −20 °C until further analysis. Samples were processed and frozen within 6 h of collection. Plasma 25(OH)D concentrations (a total of vitamin D-2 and D-3) were measured using a commercial enzymatic immunoassay kit (Immunodiagnostic Systems), according to the manufacturer's instructions. The analysis was performed at the University of Tennessee Health Science Center in a laboratory that participates in the College of American Pathology Quality Assessment Program for 25(OH)D assays. The minimum detection limit for this assay was 2 ng/mL. National Institute of Standards and Technology SRM972 vitamin D was used as a standard for quality assurance of 25(OH)D, with interassay variability of <6% and precision within 1 SD of mean 25(OH)D concentration.

Placental sample collection and RNA sequencing

The ECHO-PATHWAYS consortium generated placental RNA sequencing data from 794 participants in the CANDLE cohort as described by LeWinn et al [25]. Briefly, placental tissue was collected by CANDLE researchers within 15 min of delivery and a piece of placental villous tissue of ∼2 cm × 0.5 cm × 0.5 cm was dissected from the middle of the placental parenchyma [30]. The tissue was further split into 4 cubes, which were refrigerated in RNALater at 4 °C overnight, transferred to fresh RNALater, and stored at −80 °C. The tissue was manually dissected to remove maternal decidual tissue, and the remaining fetal villous tissue was used for RNA isolation as previously described [30]. Briefly, ∼30 mg tissue was homogenized using a TissueLyser LT instrument (Qiagen) and RNA was isolated using the AllPrep DNA/RNA/miRNA Universal Kit (Qiagen). Only samples with RNA integrity number of >7 as measured using a Bioanalyzer 2100 with RNA 6000 Nanochips (Agilent) were sequenced.

RNA sequencing was performed at University of Washington Northwest Genomics Center as previously described [30]. Total RNA was poly-A enriched, complementary DNA libraries were prepared using the TruSeq Stranded mRNA kit (Illumina), and each library was sequenced to an approximate depth of 30 million reads on an Illumina HiSeq 4000 instrument. RNA sequencing quality control was performed using both the FASTX-tool (version 0.0.13) and FastQC (version 0.11.2) toolkits [31]. Transcript abundances were estimated by aligning to the GRCh38 transcriptome (Gencode version 33) using Kallisto [32], then collapsed to the gene level using the Bioconductor tximport package [33], and scaled to the average transcript length.

Statistical analysis of covariate data

We used the National Academy of Sciences 25(OH)D cutoff for bone health (20 ng/mL) to classify participants as adequate or inadequate [34] and tested the relationship between vitamin D inadequacy and participant characteristics using the Wilcoxon–Mann–Whitney U test for continuous variables and χ^2 tests for categorical variables. We considered $P < 0.05$ significant in these analyses.

Differentially expressed gene identification

RNA sequencing data was filtered to include only protein-coding genes. Expression of these genes was normalized using the trimmed mean of M values followed by conversion to log counts per million (logCPM) [35]. Genes with low expression were removed by filtering for genes with a mean logCPM > 0 as previously described [30,36,37]. Filtering by expression was conducted separately for the samples with plasma 25(OH)D measured at midpregnancy and plasma 25(OH)D measured at delivery. After filtering, we tested the association between midpregnancy 25(OH)D and expression of 12,892 placental genes and the association between delivery 25(OH)D and expression of 12,893 placental genes. Differentially expressed genes (DEGs) were identified using the *edgeR* limma-voom pipeline [38]. Independent linear models were constructed for maternal plasma 25(OH)D at each time point as the exposure variable. We used a directed acyclic graph (DAG) to identify covariates, including confounding and precision variables (Supplemental Figure 1). Our independent variable, plasma 25(OH)D concentration, depends on vitamin D intake from foods and supplements, as well as endogenous synthesis. Vitamin D synthesis is influenced by factors including sun exposure due to latitude, season, clothing, and skin pigmentation [39]. Although self-identified race may be correlated with skin pigmentation, it is a poor proxy variable [40]. Our DAG encodes the assumption that self-reported race, as a social construct, is not related to the placenta transcriptome. Thus, we do not include maternal self-reported race as a confounding variable in this analysis. However, experiencing structural inequities could influence the placenta transcriptome [41]. Plasma 25(OH)D concentrations have been linked to socioeconomic advantages and higher diet quality in this cohort previously [9,10]. Thus, measures of multiple facets related to socioeconomic status and structural inequities, including NDI, education, and health insurance type were identified as confounding variables. These measures are highly correlated with self-reported race in this population. We also identified maternal prepregnancy BMI, age, and smoking status as potential confounding variables. Additionally, we identified RNA sequencing batch, delivery method, labor status, and fetal sex as precision variables. The final models were adjusted for the continuous variables NDI, maternal prepregnancy BMI, and maternal age at delivery, and the categorical variables maternal education (less than high school education, high school graduate/GRE, graduated college or technical school, or graduate work or more), maternal health insurance type (private only, Medicaid or Medicare only, both public and private, or no insurance), smoking status (no, yes), RNA sequencing batch (1, 2, 3, or 4), delivery method (vaginal or cesarean section), labor status (spontaneous, spontaneous with augmentation, induced, or no labor), and fetal sex (female or male).

In the CANDLE study, midpregnancy vitamin D concentrations have been associated with neurodevelopmental and respiratory outcomes [8–10]. Thus, our study focuses on the associations between midpregnancy 25(OH)D and placental gene expression. Since vitamin D acts as a hormone, it is possible that responses may be nonlinear [42]. For instance, vitamin D has both low-dose and high-dose effects linked to cardiovascular disease and vascular calcification [43,44]. Additionally, although the National Academy of Sciences cutoff using bone health as a main end point is available [34], there are not universally accepted cutoffs for sufficient vitamin D concentrations during pregnancy [45,46]. Thus, we evaluated 25(OH)D as a categorical variable using tertiles of plasma 25(OH)D concentrations at midpregnancy to evaluate potential nonlinear relationships between vitamin D and placental gene expression as described previously [10] in addition to evaluating 25(OH)D as a continuous variable. We used the lowest tertile (from 5.9 to <17.4 ng/mL) as the referent, compared with the middle (from 17.4 to <25.1 ng/mL) and highest (from 25.1 to 60.2 ng/mL) tertiles of 25(OH)D concentrations at midpregnancy.

Although our primary interest is related to midpregnancy 25(OH)D concentrations, we also considered that vitamin D concentrations measured at delivery could influence placental gene expression in samples collected at birth. Thus, we also evaluated the relationship between placental gene expression and plasma 25(OH)D at delivery as both a continuous and a categorical variable. We used the lowest tertile (from 5.7 to <18.9 ng/mL) as the referent, compared with the middle (from 18.9 to <27.0 ng/mL) and highest (from 27.0 to 85.0 ng/mL) tertiles of 25(OH)D concentrations at delivery. Finally, we also considered whether changes in 25(OH)D concentrations from midpregnancy to delivery [defined as the difference in 25(OH)D at delivery and 25(OH)D at midpregnancy] were associated with placental gene expression. In this model, 25(OH)D concentrations at midpregnancy was included as a covariate because we expected that, for example, a participant with a relatively low midpregnancy 25(OH)D concentration could have a greater response to a 10-ng/mL change in 25(OH)D than a participant with a relatively high or replete midpregnancy 25(OH)D concentration.

Fetal sex could also modify the relationship between vitamin D concentrations and placental gene expression, which we tested in sex-stratified models. The interaction between fetal sex and vitamin D concentrations was assessed after adjustment for the same covariates. We used the Benjamini–Hochberg procedure to control the false discovery rate (FDR) and considered FDR of <0.05 significant [47].

Weighted gene coexpression network analysis

Gene expression may covary, particularly for genes that belong to the same biological pathways or that are regulated by the same TFs, so we conducted weighted gene coexpression network analysis (WGCNA) on the entire CANDLE RNA sequencing data set ($N = 794$). RNA sequencing data were filtered as described earlier, and count data were then normalized using conditional quantile normalization (*cqn::cqn* function) to gene length and guanine-cytosine content [48]. WGCNA was conducted using the *WGCNA* package (version 1.72-1) [49] as an unsigned network constructed using Pearson correlation, hierarchical clustering based on cluster mean averages, and modules containing ≥20 genes. Modules were determined using dynamic tree cut (*WGCNA::cutreeDynamic*). We identified 39 modules and the nonspecific *gray* module, containing all unassigned genes (6180 genes) and which was excluded from subsequent analyses.

Modules were characterized by identifying hub genes, conducting Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway (release 108) overrepresentation analysis (*limma::kegga* function) on gene members, and TF overrepresentation analysis using a placenta-specific transcription regulatory network [50]. Genes assigned to a given module that were highly correlated with that module's eigengene ($|r| > 0.8$) were selected as the given module's hubgenes. In overrepresentation tests, KEGG pathways and TFs were considered significantly enriched when FDR was <0.05. KEGG pathways were restricted a priori to exclude human disease and drug development pathways. Thus, the analysis focused on pathways related to metabolism, genetic information processing, environmental information processing, cellular processes, and organismal systems. Characterization of all WGCNA modules by hubgenes, overrepresented KEGG pathways, and overrepresented TFs are presented in Supplemental Tables 1–3, respectively.

Multiple linear regression was used to identify WGCNA modules associated with maternal vitamin D concentrations after adjustment for covariates selected from the DAG. In sex-stratified analyses, the interaction between fetal sex and vitamin D concentrations was assessed after adjustment for the same covariates. We considered $P < 0.05$ significant in these analyses.

Results

This analysis included 774 CANDLE participants with complete covariate data, RNA sequencing data, and maternal plasma 25(OH)D concentrations at midpregnancy and 753 participants with complete covariate data at delivery. Compared with the complete CANDLE cohort ($N = 1503$), participants included in this analysis were older, more socioeconomically advantaged, and had higher plasma 25(OH)D concentrations at birth (but not at midpregnancy) (Supplemental Table 4). The median 25(OH)D concentration at midpregnancy was 21.8 ng/mL (IQR: 15.4–26.5 ng/mL), and 324 (41.9%) participants had inadequate concentrations (Figure 1A). At delivery, the median concentration was 23.6 ng/mL (IQR: 16.8–29.1 ng/mL), and 291(38.6%) participants had inadequate concentrations (Figure 1B). Plasma 25(OH)D concentrations between midpregnancy and delivery were highly correlated (Figure 1C) ($n = 752$, Spearman $\rho = 0.858$; $P < 0.001$). Notably, most participants' 25(OH)D were relatively stable from midpregnancy to delivery (median: 1.7 ng/mL; IQR: 0.2–4.0 ng/mL). Participants with adequate plasma 25(OH)D at midpregnancy (≥20 ng/mL) were older, had lower prepregnancy BMI, lived in neighborhoods with lower NDI scores, had higher HEI-2010 scores, and had higher plasma 25(OH)D concentrations at birth than participants with inadequate plasma 25(OH)D at midpregnancy (<20 ng/mL) (Table 1). They were also more likely to self-identify as White, have private insurance, have induced labor, and have completed additional education after high school.

We first evaluated the associations between placental gene expression and midpregnancy plasma 25(OH)D as a continuous and a categorical variable (Supplemental Figure 2). We identified no DEGs whose placental expression was associated with midpregnancy 25(OH)D as a continuous variable. However, in categorical analyses of midpregnancy 25(OH)D tertiles and placental gene expression, we identified 7 genes that differed in

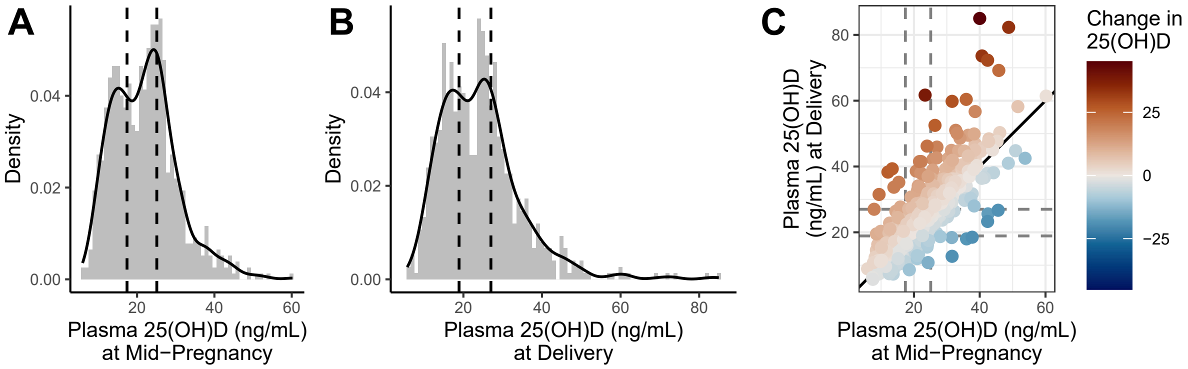

FIGURE 1. Vitamin D concentrations at midpregnancy and delivery. Density plots of vitamin D concentrations measured as plasma 25(OH)D concentrations at (A) enrollment during midpregnancy (n = 774) and (B) at delivery (n = 753). Tertile cutoffs are indicated by dotted vertical lines. (C) A scatter plot of vitamin D concentrations at midpregnancy and delivery (n = 752) shows high correlation. The identity line (black) indicates equal 25(OH)D concentrations at both time points, although some participants' 25(OH)D concentrations increased (red) or decreased (blue) from midpregnancy to delivery. 25(OH)D, 25-hydroxyvitamin D.

the middle tertile (Table 2) (n = 261, 17.4–25.0 ng/mL) and 12 genes that differed in the highest tertile (Table 3) (n = 259, 25.1–60.2 ng/mL) compared with the lowest tertile (n = 254, 5.9–17.3 ng/mL) as the reference. Notably, no DEGs were identified when comparing the middle and highest tertiles using the middle tertile as the reference. Two genes, *ARNT2* and *PKM*, were upregulated in both the middle and highest tertiles compared with the lowest tertile. We identified no DEGs whose placental expression was associated with continuous or categorical 25(OH)D in sex-stratified analyses.

We also investigated the relationship between maternal 25(OH)D concentrations and placental gene expression, both measured at delivery (Supplemental Figure 3). In our analysis of 25(OH)D as a continuous variable in all samples and in sex-stratified analysis, we identified no genes whose expression was significantly associated with maternal vitamin D concentrations at delivery. It was only when we analyzed vitamin D concentrations as a categorical variable using tertiles that we observed 1 gene, *MAGEF1*, which was significantly downregulated in the highest tertile ($\log_2$FC = −0.097, FDR = 0.012). We identified no DEGs whose placental expression was associated with 25(OH)D in sex-stratified analyses.

Given that midpregnancy and delivery 25(OH)D concentrations are highly correlated but most DEGs were associated with midpregnancy 25(OH)D concentrations, we conducted an analysis of change in 25(OH)D concentrations (Supplemental Figure 4). We found no DEGs associated with change in 25(OH)D concentrations from midpregnancy to delivery in the whole sample or in sex-stratified analyses.

The relationship between maternal 25(OH)D at midpregnancy and delivery and WGCNA modules for placental gene expression were evaluated after adjustment for covariates in all samples and in sex-stratified analyses (Figure 2A). In all samples, the *darkgreen* module was positively associated with maternal 25(OH)D at midpregnancy, and the *lightcyan* module was negatively associated with maternal 25(OH)D at both midpregnancy and delivery. The *darkgreen* module contained 77 genes, including 3 DEGs associated with the middle tertile of 25(OHD): *ARNT2*, *BTG2*, and *PKM* (Supplemental Table 1). Many WGCNA gene modules were enriched for the gene targets of placental TFs (Supplemental Table 3). Some of these TFs were associated with 25(OH)D in our analysis, suggesting that vitamin D-linked TFs could modulate gene coexpression. Of particular note, the DEGs *ARNT2* and *FOSL2* were enriched in two 25(OH)D-associated modules (*darkgreen* and *grey60*), suggesting these 25(OH)D-associated TFs modulate 25(OH)D-associated WGCNA modules (Figure 2B). The *darkgreen* module genes were not significantly overrepresented in any KEGG pathways. There were 121 genes in the *lightcyan* module, which were significantly overrepresented in pathways related to mitochondrial function (oxidative phosphorylation and thermogenesis) and neurotransmission (retrograde endocannabinoid signaling) (Figure 2C). We also considered whether change in 25(OH)D concentrations between midpregnancy and delivery was associated with WGCNA eigengene module expression. The *blue* module (781 genes) was inversely associated with the change in 25(OH)D from midpregnancy to delivery. The *blue* module was enriched for mRNA surveillance and the spliceosome pathway (Figure 2C). In males, the *lightcyan* module was negatively associated with maternal 25(OH)D at midpregnancy, while the *grey60* (113 genes) as positively associated with maternal 25(OH)D at midpregnancy. The *grey60* module did not have DEGs identified as hub genes or enriched KEGG pathways, but the *grey60* module genes were enriched for *ARNT2* gene transcription targets (Figure 2B). In females, the *lightcyan* and *brown* (485 genes) modules were negatively associated with maternal 25(OH)D at midpregnancy and delivery, while the *blue* module was negatively associated with maternal 25(OH)D at delivery. Moreover, in females, the *magenta* (244 genes), *purple* (232 genes), and *violet* (48 genes) modules were positively associated with maternal 25(OH)D at midpregnancy and delivery, while the *black* (280 genes), *darkgrey* (70 genes), *lightyellow* (98 genes), *royalblue* (94 genes), and *tan* (203 genes) modules were positively associated with maternal 25(OH)D at delivery (Figure 3A) [51–57]. Finally, in females, the *black* module was positively associated with the change in 25(OH)D from midpregnancy to delivery (Figure 2A). The *black* module was enriched for pathways related to vascular function, including angiogenesis (Apelin, Notch, and Wnt signaling) and vascular smooth muscle contraction. The *magenta* module was enriched for vascular smooth muscle contraction and axon guidance. The *purple* module was enriched for focal adhesion and axon guidance. The

TABLE 1
Characteristics and sociodemographic factors by maternal vitamin D status at midpregnancy.

	Inadequate (*n* = 324)	Adequate (*n* = 450)	*P* value
Maternal age at birth (y)			<0.001
Median	26	28	
IQR	22–30	24–32	
Maternal prepregnancy BMI			0.002
Median	27	25	
IQR	23–33	22–31	
Maternal education			<0.001
<High school	44 (13.6)	20 (4.4)	
High school graduate/GRE	168 (51.9)	176 (39.1)	
Graduated college or technical school	80 (24.7)	179 (39.8)	
Some graduate work or more	32 (9.9)	75 (16.7)	
Income ($)			<0.001
Median	22,500	50,000	
IQR	7500–50,000	22,500–80,000	
Missing	31	9	
Maternal race			<0.001
White	72 (22.2)	222 (49.3)	
Black/African American	225 (69.4)	208 (46.2)	
Asian	<10	<10	
Multiple race	24 (7.4)	14 (3.1)	
Other race	<10	<10	
Maternal ethnicity			0.242
Not Hispanic/Latino	316 (97.5)	444 (98.7)	
Hispanic/Latino	<10	<10	
Fetal sex			0.778
Female	163 (50.3)	231 (51.3)	
Male	161 (49.7)	219 (48.7)	
Labor type			0.048
Spontaneous	67 (20.7)	82 (18.2)	
Spontaneous, augmented	104 (32.1)	113 (25.1)	
Induced	92 (28.4)	165 (36.7)	
No labor	61 (18.8)	90 (20.0)	
Delivery method			0.157
Vaginal	205 (63.3)	262 (58.2)	
C-section	119 (36.7)	188 (41.8)	
Cotinine ≥200 ng/mL at prenatal visits			0.011
No	290 (89.5)	425 (94.4)	
Yes	34 (10.5)	25 (5.6)	
Gestational age at midpregnancy visit (d)			0.060
Median	158	161	
IQR	141–177	145–182	
Missing	13	13	
Gestational age at birth (d)			0.123
Median	274	275	
IQR	269–279	271–279	
Missing	2	2	
Preterm birth (<37 wk gestation)			<0.001
No	288 (89.4)	430 (96.0)	
Yes	34 (10.6)	18 (4.0)	
Missing	2	2	
Neighborhood deprivation index			<0.001
Median	0.548	−0.156	
IQR	−0.344 to 1.031	−0.592 to 0.674	
Health insurance type			<0.001
Private only	107 (33.0)	276 (61.3)	
No insurance	<10	<10	
Medicaid (TennCare) or Medicare only	206 (63.6)	161 (35.8)	
TennCare and private	10 (3.1)	13 (2.9)	
25(OH)D (ng/mL) at midpregnancy			<0.001
Median	14.5	25.8	
IQR	12.1–16.9	23.1–29.7	
25(OH) D (ng/mL) at delivery			< 0.001
Median	16.2	28.1	
IQR	13.3–19.3	24.6–33.7	
Missing	7	16	
HEI-2010, total score			< 0.001
Median	59.1	63.3	
IQR	50.8–67.4	55.3–71.3	
Missing	41	32	

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.
Continuous variables are reported as median and interquartile range (IQR), and associations were tested using the Wilcoxon–Mann–Whitney test. Categorical variables are reported as *n* (%), and associations were tested using χ^2 test. Maternal vitamin D status at midpregnancy was defined as adequate if plasma 25(OH)D $\geq$ 20 ng/mL or inadequate if plasma 25(OH)D $>$ 20 ng/mL.

TABLE 2
Significant associations between the middle tertile of midpregnancy 25(OH)D concentrations and placental gene expression [false discovery rate (FDR) < 0.05] using the lowest tertile as a reference.

Gene symbol	Gene description	log$_2$(FC)	FDR
AHNAK	AHNAK nucleoprotein [Source:HGNC Symbol;Acc:HGNC:347]	0.181	0.013
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2 [Source:HGNC Symbol;Acc:HGNC:16876]	0.641	0.012
BTG2	BTG anti-proliferation factor 2 [Source:HGNC Symbol;Acc:HGNC:1131]	−0.249	0.012
FOSL2	FOS like 2, AP-1 transcription factor subunit [Source:HGNC Symbol;Acc:HGNC:3798]	0.270	0.019
PKM	Pyruvate kinase M1/2 [Source:HGNC Symbol;Acc:HGNC:9021]	0.165	0.024
RABIF	RAB interacting factor [Source:HGNC Symbol;Acc:HGNC:9797]	−0.071	0.031
TANC2	Tetratricopeptide repeat, ankyrin repeat and coiled-coil containing 2 [Source:HGNC Symbol;Acc:HGNC:30212]	0.226	0.026

violet module was enriched for cytokine–cytokine receptor interaction. The *tan* module was enriched for 20 pathways related to immune responses, including chemokine signaling, natural killer cell mediated cytotoxicity, and platelet activation (Figure 2C). The *black*, *blue*, *magenta*, *purple*, and *violet* modules

TABLE 3

Significant associations between the highest tertile of midpregnancy 25(OH)D concentrations and placental gene expression [false discovery rate (FDR) < 0.05] using the lowest tertile as a reference.

Gene symbol	Gene description	$\log_2$(FC)	FDR
ANAPC10	Anaphase promoting complex subunit 10 [Source:HGNC Symbol;Acc:HGNC:24077]	−0.092	0.042
ARNT2	Aryl hydrocarbon receptor nuclear translocator 2 [Source:HGNC Symbol;Acc:HGNC:16876]	0.614	0.019
COX17	Cytochrome c oxidase copper chaperone COX17 [Source:HGNC Symbol;Acc:HGNC:2264]	−0.141	0.033
DYNC1H1	Dynein cytoplasmic 1 heavy chain 1 [Source:HGNC Symbol;Acc:HGNC:2961]	0.086	0.019
MAGEF1	MAGE family member F1 [Source:HGNC Symbol;Acc:HGNC:29639]	−0.082	0.042
MAZ	MYC-associated zinc finger protein [Source:HGNC Symbol;Acc:HGNC:6914]	0.099	0.031
MRPS14	Mitochondrial ribosomal protein S14 [Source:HGNC Symbol;Acc:HGNC:14049]	−0.083	0.019
MYH9	Myosin heavy chain 9 [Source:HGNC Symbol;Acc:HGNC:7579]	0.103	0.034
NDUFC1	NADH:ubiquinone oxidoreductase subunit C1 [Source:HGNC Symbol;Acc:HGNC:7705]	−0.118	0.025
PKM	Pyruvate kinase M1/2 [Source:HGNC Symbol;Acc:HGNC:9021]	0.186	0.019
RPL21	Ribosomal protein L21 [Source:HGNC Symbol;Acc:HGNC:10313]	−0.098	0.044
SEC11C	SEC11 homolog C, signal peptidase complex subunit [Source:HGNC Symbol;Acc:HGNC:23400]	−0.135	0.031

Abbreviation: 25(OH)D, 25-hydroxyvitamin D.

did not have DEGs identified in Table 2 and Table 3 among their hubgenes or enriched TFs.

Discussion

In this investigation of the relationship between prenatal vitamin D concentrations quantified at different time points in pregnancy and the placental transcriptome in a large, diverse, prospective pregnancy cohort, we identified DEGs and coexpressed gene modules associated with plasma 25(OH)D. We identified 17 genes whose expression was altered in the middle and/or highest tertiles of maternal midpregnancy vitamin D concentrations compared with the lowest tertile, including *ARNT2* and *PKM*, which were significantly different from the reference lowest tertile in both the middle and highest tertiles. These DEGs encode proteins related to energy metabolism, cytoskeletal dynamics, and placental transcriptional regulation. Notably, only 1 of these DEGs was associated with 25(OH)D concentrations at delivery. This suggests that maternal 25(OH)D concentrations during pregnancy might influence placental biology at delivery beyond the direct effects of vitamin D on VDR-dependent gene expression. We also conducted WGCNA to investigate associations between coexpressed gene modules and 25(OH)D concentrations. We identified 7 modules related to maternal vitamin D concentrations at midpregnancy,12 modules related to maternal vitamin D concentrations at delivery, and 2 modules related to change in 25(OH)D from midpregnancy to delivery, which provided further support for associations between vitamin D concentrations and mitochondrial function, cytoskeletal function, and key TFs.

Although vitamin D can exert direct effects on VDR-dependent gene expression, it is notable that the majority of DEGs were associated with midpregnancy 25(OH)D concentrations. This suggests that there might be long-term effects of midpregnancy vitamin D concentrations on placental function at delivery, but delivery 25(OH)D concentrations might not affect placental function (Figure 3). The differential gene expression reported in this study could be explained indirectly by vitamin D-dependent changes in placental development, which continues to grow with the fetus and build increasingly complex vasculature throughout gestation [51,52]. Placental gene regulation must be dynamic during pregnancy to respond to elevated oxygen tension when maternal circulation is invaded in early pregnancy and an increasingly proinflammatory state as pregnancy advances toward parturition [53,54]. Low prenatal vitamin D has been linked to reduced placental weight and vascular development in human studies [55,56]. Mechanistic studies indicate that vitamin D promotes placental angiogenesis and nutrient transport [17,57]. Thus, low prenatal vitamin D is linked to reduced nutrient availability for fetal growth and placental metabolism. Additionally, vitamin D has been linked to altered placental DNA methylation, which in turn impact vitamin D-dependent gene expression [14,58]. Thus, nutrient availability, vascular development, and epigenetic modifications represent indirect routes by which midpregnancy 25(OH)D could influence placental gene expression at delivery.

As the placenta grows throughout pregnancy, its energetic needs increase [59]. From an evolutionary perspective, one of the first roles of $1,25(OH)_2D$ mediated by the VDR was regulating energy metabolism [60]. Indeed, in this study, vitamin D concentrations were positively associated with expression of genes encoding glycolytic enzymes (*PKM*) but negatively associated with mitochondrial proteins (*MRPS14*) and specifically components of the electron transport chain (*COX17* and *NDUFC1*). *PKM* encodes pyruvate kinase, a regulator of trophoblast invasion in the placenta. $1,25(OH)_2D$ has been shown to increase pyruvate kinase activity in human fibroblast and neuroblastoma cell lines [61–63]. In vitro studies suggest that $1,25(OH)_2D$ and VDR regulate mitochondrial function in skeletal muscle [64–66] and vitamin D supplementation improved strength and muscle mass in older adults [66]. In human skeletal muscle cells, $1,25(OH)_2D$ treatment resulted in extensive changes in expression of genes related not only to mitochondrial function (including downregulated *COX17* expression, as we report in this study) but also to cytoskeletal and intracellular membrane trafficking [64].

The cytoskeleton is crucial in key placental processes, including trophoblast invasion, cellular division and proliferation, autophagy, and intracellular transport [67]. We also report associations between maternal vitamin D concentrations and placental expression of genes involved in cytoskeletal function: motor proteins (*DYNC1H1* and *MYH9*) and other genes related to cellular transport (*AHNAK*, *RABIF*, and *TANC2*). Some of these genes are

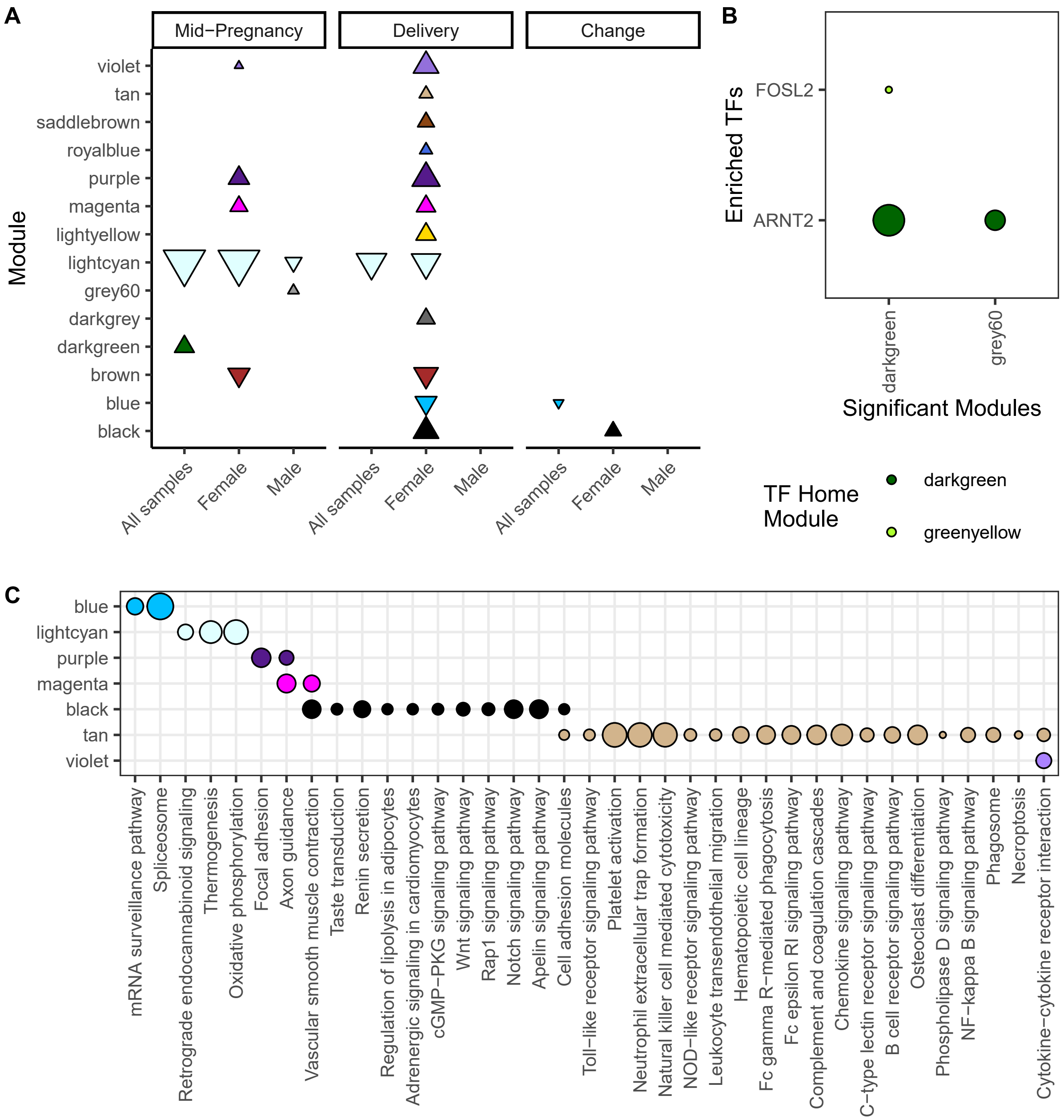

FIGURE 2. Weighted gene coexpression network analysis (WGCNA) modules associated with measures of 25(OH)D during pregnancy. The point size corresponds to $-\log(p)$ in each panel. (A) Associations between modules and maternal 25(OH)D concentrations at midpregnancy and delivery, as well as the change in 25(OH)D from midpregnancy to delivery were assessed with multiple linear regression adjusted for covariates ($P < 0.05$). Negative associations are indicated with downward-pointing triangles and positive associations are indicated with upward-pointing triangles. (B) Gene coexpression modules can be coregulated by transcription factors (TFs). TFs that were differentially expressed with 25(OH)D and whose gene targets were significantly enriched in the modules from panel A were identified using overrepresentation analysis (false discovery rate < 0.05). (C) KEGG pathways that were significantly enriched for module genes for modules in panel A were identified through an overrepresentation analysis (false discovery rate < 0.05). 25(OH)D, 25-hydroxyvitamin D.

essential for placental development, such as *MYH9*, which is required for placental vascular development and knockouts lead to embryonic death [68,69]. Vitamin D regulates cytoskeletal reorganization in other tissues [70,71]. In trophoblasts, vitamin D treatment promotes cellular migration and invasion in vitro, processes that are dependent on cytoskeletal reorganization [15,16,72,73]. Cytoskeletal proteins and intracellular membrane trafficking pathways are also necessary for placental nutrient transport, including trafficking amino acid and glucose transporters, as well as fusion into the multinucleate syncytium [74,75]. Thus, cytoskeletal-related and trafficking-related DEGs support the importance of prenatal vitamin D across domains of placental function.

As a ligand for the VDR, 1,25(OH)$_2$D is involved in regulating transcription of VDR response genes, and we observed associations between vitamin D concentrations and other TFs (*ARNT2* and *FOSL2*) [76,77]. Indeed, the VDR is also thought to influence the transcription of other TFs, leading to a 2-phase response to vitamin D [78]. Thus, we also investigated gene coexpression using WGCNA to identify modules of coexpressed genes

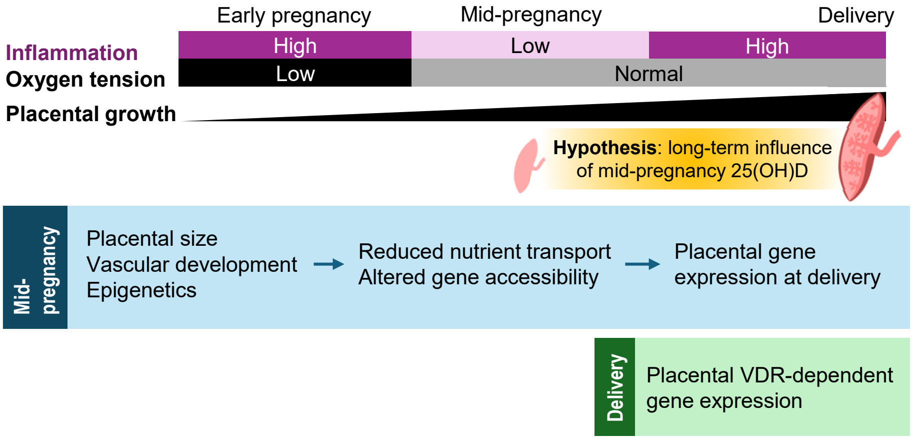

FIGURE 3. Proposed mechanisms of differential gene expression associated with midpregnancy and delivery 25(OH)D. The placenta and conceptus develop and grow throughout gestation, as the placental builds increasingly complex vasculature [51,52]. Gene regulation and expression is dynamic to respond to changing conditions, including an increase in oxygen tension after the placenta invades maternal circulation and proinflammatory states both at implantation and in late gestation in preparation for labor [53,54]. Thus, midpregnancy exposures, like circulating 25(OH)D, could influence placental development and growth, leading to indirect impacts on placental gene expression at birth. Low vitamin D has been linked to reduced placental weight, impaired vascular development, and changes in the epigenetic landscape [14,17,55–57]. In turn, these changes influence placental functions like nutrient transport and alter gene accessibility, which can influence placental gene expression measured at delivery. At delivery, 25(OH)D concentrations are more relevant to concentrations of the active hormone, 1,25$(OH)_2$D, which directly affects gene expression by activating the VDR. 25(OH)D, 25-hydroxyvitamin D; VDR, vitamin D receptor.

associated with 25(OH)D concentrations. A benefit of coexpression methods like WGCNA is that they can identify coordinated changes in gene coexpression, even when effect sizes for individual genes are relatively small, as reported in this study. We identified 2 modules associated with 25(OH)D concentrations: the *lightcyan* modules that was inversely associated with 25(OH)D concentrations at midpregnancy and delivery and the *darkgreen* modules that was positively associated with 25(OH)D concentrations at midpregnancy. In this analysis, *ARNT2* and *FOSL2* were positively associated with vitamin D concentrations, and their targets were enriched in the *darkgreen* WGCNA module. In a placenta-specific transcription regulatory network, *ARNT2* is regulated by and regulated the *VDR* [50]. *ARNT2* and its paralog *ARNT* dimerize with *HIF1A* to regulate the hypoxic response and dimerize with *AHR* to regulate placental vascular development through pregnancy [76,79]. *FOSL2* is a subunit of the activator protein (AP) 1 TF, which plays an essential role in development as a regulator of cellular differentiation, proliferation, and apoptosis [80]. AP-1 is made up of protein subunits from the proteins including the Fos family, and the specific composition of the AP-1 dimer influences its function [80]. Although other members of the Fos family are necessary for trophoblast invasion, migration, and development and *FOSL2* is highly expressed in extravillous trophoblasts, the role of *FOSL2* in the placenta is less clear. However, *FOSL2* is crucial in bone formation and activated by 1,25$(OH)_2$D in osteoclasts [81]. Further investigation of the roles of placental *ARNT2* and *FOSL2* in response to prenatal vitamin D is warranted.

In this study, we also investigated fetal sex as an effect modifier for the relationship between maternal 25(OH)D concentrations and placental gene expression. At the level of individual genes, we did not find evidence of sex-specific effects on the association between vitamin D concentrations and placental gene expression. However, when investigating associations between vitamin D concentrations and patterns of gene coexpression, we identified 4 modules that were associated with vitamin D concentrations at midpregnancy only in females and 1 module that was associated with vitamin D concentrations at midpregnancy only in males. The *magenta* and *purple* modules were related to aspects of cytoskeletal function, and the *violet* module was related to cellular signaling, in keeping with roles of vitamin D we have previously discussed. At delivery, the *lightcyan* module was significantly negatively associated with vitamin D concentrations in all samples and in females but not in males. Additionally, vitamin D concentrations at delivery were associated with 11 other modules only in females, including the *tan* (immune function) and *black* (vascular function) modules. Some female-specific modules are only associated with delivery 25(OH)D, which could indicate that these modules are related to the direct, VDR-dependent effects of vitamin D. Additionally, these patterns only observed in females might be related to sex differences in vitamin D metabolism [82]. Evidence from mice shows male placentas express higher concentrations of vitamin D–inactivating *Cyp24a1* in vitamin D sufficiency and lower concentrations of vitamin D–activating *Cyp27b1* in vitamin D deficiency compared with equivalent female placentas [22]. In human trophoblasts, testosterone treatment downregulated *CYP27B1* expression and upregulated *CYP24A1* expression in a time-dependent and dose-dependent manner and in term placentas, males had lower *CYP27B1* mRNA expression [23]. Thus, the differences in gene coexpression observed at delivery in females call for further study to investigate associations between sex differences in vitamin D homeostasis and placental gene expression.

Our findings align with the handful of studies that have investigated the effect of vitamin D on the placental transcriptome using RNA sequencing [14,83]. In an in vitro study using cultured primary trophoblasts treated with 20 μM 25(OH)

D for 8 h and reported upregulation of *TANC2* [14] in agreement with our findings reported here. Additionally, downregulation of immune, inflammatory, and cytokine-binding pathways in conjunction with upregulation of transcriptional regulatory pathways were reported [14]. Combining transcriptomic and proteomic analysis also identified cytoskeletal binding as an enriched pathway following 25(OH)D treatment [14]. Recently, a randomized controlled trial providing the recommended dose of 10 μg (400 IU) vitamin D/d or the high-dose of 90 μg (3600 IU) vitamin D/d from the late first trimester through delivery used RNA sequencing to investigate placental gene expression in a random subset of the study ($n = 70$) using a threshold of FDR of <0.1 [83]. This study identified DEGs associated with maternal 25(OH)D at baseline in early pregnancy, suggesting that prenatal vitamin D status during early pregnancy influences placental function at term. Additionally, this study reported that high-dose vitamin D supplementation was associated with enrichment in the cell adhesion pathway [83]. Additionally, we report that *ARNT2*, which is positively regulated by the VDR [50], is positively associated with midpregnancy 25(OH)D. The DEGs associated with 25(OH)D in this analysis should be experimentally validated using a range of doses in future studies.

Our results should be interpreted in the context of limitations. First, we conducted RNA sequencing on placental samples collected at birth, which provides a snapshot of a highly coordinated temporal process. Thus, variation in gene expression at birth may not reflect placental gene expression during gestation. Second, expression was quantified in bulk samples and could mask cell type-specific changes in gene expression related to vitamin D concentrations [84]. The placenta is a complex organ made up of multiple cell types, including trophoblasts, fibroblasts, endothelial cells, and immune cells, which may respond to vitamin D in a cell type-specific manner. Third, there are limitations of 25(OH)D measurement by immunoassay, such as higher sensitivity to the 25(OH)D-3 form of 25(OH)D form and potential for high concentrations of vitamin D–binding protein observed in pregnancy to negatively bias 25(OH)D measurements. Finally, given the observational study design, we cannot draw causal links between midpregnancy vitamin D concentrations and placental gene expression, and there is still the possibility of residual confounding, despite adjustment for a variety of covariates. This study has several strengths. First, because all participants were recruited from the same county, potential confounding by geographical differences in sunlight exposure was reduced. Second, this richly characterized and socioeconomically and racially diverse cohort allowed us to conduct a well powered, rigorous analysis of prenatal vitamin D concentrations and placental gene expression, using a more rigorous FDR threshold than previous studies in this field. Third, this analysis used a transcriptome-wide approach, which enabled us to holistically assess how vitamin D might influence placental function.

These findings in a large, diverse, prospective cohort study identify roles for vitamin D in placental energy metabolism, cytoskeletal function, and transcriptional regulation. Notably, prenatal vitamin D concentrations during midpregnancy were related to placental expression of 17 DEGs, while vitamin D concentrations measured at delivery were associated with only 1 DEG. This suggests that midpregnancy vitamin D could play a role in programing placental gene expression. Among these DEGs, we identified important transcriptional regulators. Our findings also suggest that sex-specific effects of vitamin D may be subtle and were only observed in the coexpression analysis. Future research is needed to investigate the potential programing effect of vitamin D during pregnancy on placental mitochondrial metabolism, intracellular transport, and transcriptional regulation.

Author contributions

The authors' responsibilities were as follows—MP, AGP, KZL, NRB, QZ, SS: designed the research; MP: performed statistical analysis; MP, MMM, SL, JM, TB, EJF, HYC, KJD, DAE, KNC, KZL, NRB, QZ, SS, AGP: wrote, critically reviewed, and edited the manuscript; MP, AGP: had primary responsibility for final content; and all authors: read and approved the final manuscript.

Conflict of interest

The authors report no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tjnut.2024.10.019.

References

[1] D.J. Barker, The fetal and infant origins of disease, Eur. J. Clin. Invest. 25 (7) (1995) 457–463.

[2] C.L. Wagner, B.W. Hollis, The implications of vitamin D status during pregnancy on mother and her developing child, Front. Endocrinol (Lausanne). 9 (2018) 500.

[3] R.L. Wilson, A.J. Leviton, S.Y. Leemaqz, P.H. Anderson, J.A. Grieger, L.E. Grzeskowiak, et al., Vitamin D levels in an Australian and New Zealand cohort and the association with pregnancy outcome, BMC Pregnancy Childbirth 18 (1) (2018) 251.

[4] S. Fogacci, F. Fogacci, M. Banach, E.D. Michos, A.V. Hernandez, G.Y.H. Lip, et al., Vitamin D supplementation and incident preeclampsia: a systematic review and meta-analysis of randomized clinical trials, Clin. Nutr. 39 (6) (2020) 1742–1752.

[5] A. Milajerdi, F. Abbasi, S.M. Mousavi, A. Esmaillzadeh, Maternal vitamin D status and risk of gestational diabetes mellitus: a systematic review and meta-analysis of prospective cohort studies, Clin. Nutr. 40 (5) (2021) 2576–2586.

[6] R. Zhao, L. Zhou, S. Wang, H. Yin, X. Yang, L. Hao, Effect of maternal vitamin D status on risk of adverse birth outcomes: a systematic review and dose–response meta-analysis of observational studies, Eur. J. Nutr. 61 (6) (2022) 2881–2907.

[7] F.Y. Ideraabdullah, A.M. Belenchia, C.S. Rosenfeld, S.W. Kullman, M. Knuth, D. Mahapatra, et al., Maternal vitamin D deficiency and developmental origins of health and disease (DOHaD), J. Endocrinol. 241 (2019) R65–R80.

[8] F.A. Tylavsky, M. Kocak, L.E. Murphy, J.C. Graff, F.B. Palmer, E. Völgyi, et al., Gestational vitamin 25(OH)D status as a risk factor for receptive language development: a 24-month, longitudinal, observational study, Nutrients 7 (12) (2015) 9918–9930.

[9] M.M. Melough, L.E. Murphy, J.C. Graff, K.J. Derefinko, K.Z. LeWinn, N.R. Bush, et al., Maternal plasma 25-hydroxyvitamin D during

gestation is positively associated with neurocognitive development in offspring at age 4-6 years, J. Nutr. 151 (1) (2021) 132–139.
[10] S.N. Adams, M.A. Adgent, T. Gebretsadik, T.J. Hartman, S. Vereen, C. Ortiz, et al., Prenatal vitamin D levels and child wheeze and asthma, J. Matern Fetal Neonatal Med. 34 (3) (2021) 323–331.
[11] L.M. Bodnar, H.N. Simhan, R.W. Powers, M.P. Frank, E. Cooperstein, J.M. Roberts, High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates, J. Nutr. 137 (2) (2007) 447–452.
[12] C. Palacios, L. Gonzalez, Is vitamin D deficiency a major global public health problem? J. Steroid Biochem. Mol. Biol. 144 (A) (2014) 138–145.
[13] D.D. Bikle, Vitamin D metabolism, mechanism of action, and clinical applications, Chem. Biol. 21 (3) (2014) 319–329.
[14] B. Ashley, C. Simner, A. Manousopoulou, C. Jenkinson, F. Hey, J.M. Frost, et al., Placental uptake and metabolism of 25(OH)vitamin D determine its activity within the fetoplacental unit, eLife 11 (2022) e71094.
[15] S.Y. Chan, R. Susarla, D. Canovas, E. Vasilopoulou, O. Ohizua, C.J. McCabe, et al., Vitamin D promotes human extravillous trophoblast invasion in vitro, Placenta 36 (4) (2015) 403–409.
[16] R.H. Kim, B.J. Ryu, K.M. Lee, J.W. Han, S.K. Lee, Vitamin D facilitates trophoblast invasion through induction of epithelial-mesenchymal transition, Am. J. Reprod. Immunol. 79 (2) (2018) e12796.
[17] Y.-Y. Chen, T.L. Powell, T. Jansson, 1,25-Dihydroxy vitamin D3 stimulates system A amino acid transport in primary human trophoblast cells, Mol. Cell. Endocrinol. 442 (2017) 90–97.
[18] J. Wang, F. Qiu, Y. Zhao, S. Gu, J. Wang, H. Zhang, Exploration of fetal growth restriction induced by vitamin D deficiency in rats via Hippo-YAP signaling pathway, Placenta 128 (2022) 91–99.
[19] N. Noyola-Martínez, L. Díaz, E. Avila, A. Halhali, F. Larrea, D. Barrera, Calcitriol downregulates TNF-α and IL-6 expression in cultured placental cells from preeclamptic women, Cytokine 61 (1) (2013) 245–250.
[20] J.K. Cleal, P.E. Day, C.L. Simner, S.J. Barton, P.A. Mahon, H.M. Inskip, et al., Placental amino acid transport may be regulated by maternal vitamin D and vitamin D-binding protein: results from the Southampton Women's Survey, Br. J. Nutr. 113 (12) (2015) 1903–1910.
[21] E.V. Schulz, L. Cruze, W. Wei, J. Gehris, C.L. Wagner, Maternal vitamin D sufficiency and reduced placental gene expression in angiogenic biomarkers related to comorbidities of pregnancy, J. Steroid Biochem. Mol. Biol. 173 (2017) 273–279.
[22] N.Q. Liu, D.P. Larner, Q. Yao, R.F. Chun, Y. Ouyang, R. Zhou, et al., Vitamin D-deficiency and sex-specific dysregulation of placental inflammation, J. Steroid Biochem. Mol. Biol. 177 (2018) 223–230.
[23] A. Olmos-Ortiz, J. García-Quiroz, R. López-Marure, I. González-Curiel, B. Rivas-Santiago, A. Olivares, et al., Evidence of sexual dimorphism in placental vitamin D metabolism: testosterone inhibits calcitriol-dependent cathelicidin expression, J. Steroid Biochem. Mol. Biol. 163 (2016) 173–182.
[24] L. Sontag-Padilla, R.M. Burns, R.A. Shih, B.A. Griffin, L.T. Martin, A. Chandra, et al., The Urban Child Institute CANDLE study: methodological overview and baseline sample description [Internet], RAND Corporation, December 2015. Available from: https://www.rand.org/pubs/research_reports/RR1336.html.
[25] K.Z. LeWinn, C.J. Karr, M. Hazlehurst, K. Carroll, C. Loftus, R. Nguyen, et al., Cohort profile: the ECHO prenatal and early childhood pathways to health consortium (ECHO-PATHWAYS), BMJ Open 12 (10) (2022) e064288.
[26] P.M. Guenther, K.O. Casavale, S.I. Kirkpatrick, J. Reedy, H.A.B. Hiza, K.J. Kuczynski, et al., Update of the Healthy Eating Index: HEI-2010, J. Acad. Nutr. Diet. 113 (4) (2013) 569–580.
[27] S.M. Quraishi, M.F. Hazlehurst, C.T. Loftus, R.H.N. Nguyen, E.S. Barrett, J.D. Kaufman, et al., Association of prenatal exposure to ambient air pollution with adverse birth outcomes and effect modification by socioeconomic factors, Environ. Res. 212 (E) (2022) 113571.
[28] Y. Ni, A.A. Szpiro, M.T. Young, C.T. Loftus, N.R. Bush, K.Z. LeWinn, et al., Associations of pre- and postnatal air pollution exposures with child blood pressure and modification by maternal nutrition: a prospective study in the CANDLE cohort, Environ. Health Perspect. 129 (4) (2021) 47004.
[29] S.F. Schick, B.C. Blount, P. Jacob, N.A. Saliba, J.T. Bernert, A. El Hellani, et al., Biomarkers of exposure to new and emerging tobacco delivery products, Am. J. Physiol. Lung Cell. Mol. Physiol. 313 (3) (2017) L425–L452.
[30] A.G. Paquette, J. MacDonald, S. Lapehn, T. Bammler, L. Kruger, D.B. Day, et al., A comprehensive assessment of associations between prenatal phthalate exposure and the placental transcriptomic landscape, Environ. Health Perspect. 129 (9) (2021) 97003.
[31] J. Brown, M. Pirrung, L.A. McCue, FQC Dashboard: integrates FastQC results into a web-based, interactive, and extensible FASTQ quality control tool, Bioinformatics 33 (19) (2017) 3137–3139.
[32] N.L. Bray, H. Pimentel, P. Melsted, L. Pachter, Near-optimal probabilistic RNA-seq quantification, Nat. Biotechnol. 34 (5) (2016) 525–527.
[33] C. Soneson, M.I. Love, M.D. Robinson, Differential analyses for RNA-seq: transcript-level estimates improve gene-level inferences, F1000Res 4 (2015) 1521.
[34] [Internet]A.C. Ross, C.L. Taylor, A.L. Yaktine, H.B. Del Valle (Eds.), Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Dietary reference intakes for calcium and vitamin D, National Academies Press, Washington (DC), 2011. Available from: http://www.ncbi.nlm.nih.gov/books/NBK56070/ (cited 2024 Mar 6).
[35] M.D. Robinson, A. Oshlack, A scaling normalization method for differential expression analysis of RNA-seq data, Genome Biol. 11 (3) (2010) R25.
[36] A.G. Paquette, J. MacDonald, T. Bammler, D.B. Day, C.T. Loftus, E. Buth, et al., Placental transcriptomic signatures of spontaneous preterm birth, Am. J. Obstet. Gynecol. 228 (1) (2023) 73.e1–73.e18.
[37] A.G. Paquette, S. Lapehn, S. Freije, J. MacDonald, T. Bammler, D.B. Day, et al., Placental transcriptomic signatures of prenatal exposure to hydroxy-polycyclic aromatic hydrocarbons, Environ. Int. 172 (2023) 107763.
[38] C.W. Law, Y. Chen, W. Shi, G.K. Smyth, voom: precision weights unlock linear model analysis tools for RNA-seq read counts, Genome Biol. 15 (2) (2014) R29.
[39] J.J. Neville, T. Palmieri, A.R. Young, Physical determinants of vitamin D photosynthesis: a review, JBMR Plus 5 (1) (2021) e10460.
[40] J.L. Chan, A. Ehrlich, R.C. Lawrence, A.N. Moshell, M.L. Turner, A.B. Kimball, Assessing the role of race in quantitative measures of skin pigmentation and clinical assessments of photosensitivity, J. Am. Acad. Dermatol. 52 (4) (2005) 609–615.
[41] G.E. Miller, A.E. Borders, A.H. Crockett, K.M. Ross, S. Qadir, L. Keenan-Devlin, et al., Maternal socioeconomic disadvantage is associated with transcriptional indications of greater immune activation and slower tissue maturation in placental biopsies and newborn cord blood, Brain Behav. Immun. 64 (2017) 276–284.
[42] U. Querfeld, R.H. Mak, Vitamin D deficiency and toxicity in chronic kidney disease: in search of the therapeutic window, Pediatr. Nephrol. 25 (12) (2010) 2413–2430.
[43] T.B. Drüeke, Z.A. Massy, Role of vitamin D in vascular calcification: bad guy or good guy? Nephrol. Dial. Transplant. 27 (5) (2012) 1704–1707.
[44] A. Zittermann, Vitamin D and cardiovascular disease: an update, Anticancer Res. 34 (9) (2014) 4641–4648.
[45] M. Kiely, A. Hemmingway, K.M. O'Callaghan, Vitamin D in pregnancy: current perspectives and future directions, Ther. Adv. Musculoskelet. Dis. 9 (6) (2017) 145–154.
[46] M.E. Kiely, C.L. Wagner, D.E. Roth, Vitamin D in pregnancy: where we are and where we should go, J. Steroid Biochem. Mol. Biol. 201 (2020) 105669.
[47] Y. Benjamini, Y. Hochberg, Controlling the false discovery rate: a practical and powerful approach to multiple testing, J. R. Stat. Soc. Ser. B. Methodol. 57 (1) (1995) 289–300.
[48] K.D. Hansen, R.A. Irizarry, Z. Wu, Removing technical variability in RNA-seq data using conditional quantile normalization, Biostatistics 13 (2) (2012) 204–216.
[49] P. Langfelder, S. Horvath, WGCNA: an R package for weighted correlation network analysis, BMC Bioinform 9 (2008) 559.
[50] A. Paquette, K. Ahuna, Y.M. Hwang, J. Pearl, H. Liao, P. Shannon, et al., A genome scale transcriptional regulatory model of the human placenta, Sci. Adv. 10 (26) (2024) eadf3411.
[51] M. Castellucci, M. Schepe, I. Scheffen, A. Celona, P. Kaufmann, The development of the human placental villous tree, Anat. Embryol. (Berl) 181 (2) (1990) 117–128.
[52] H. Pinar, C.J. Sung, C.E. Oyer, D.B. Singer, Reference values for singleton and twin placental weights, Pediatr. Pathol. Lab. Med. 16 (6) (1996) 901–907.
[53] G. Mor, P. Aldo, A.B. Alvero, The unique immunological and microbial aspects of pregnancy, Nat. Rev. Immunol. 17 (8) (2017) 469–482.
[54] G.J. Burton, T. Cindrova-Davies, H.W. Yung, E. Jauniaux, Hypoxia and reproductive health: oxygen and development of the human placenta, Reproduction 161 (1) (2021) F53–F65.

[55] M.J. Mead, C.A. McWhorter, M.D. Rodgers, M.D. Ebeling, J.R. Shary, M.J. Gregoski, et al., Does maternal vitamin D status influence placental weight or vascular and inflammatory pathology? Secondary analysis from the Kellogg Pregnancy Study, J. Steroid Biochem. Mol. Biol. 233 (2023) 106358.

[56] M. He, H. Mirzakhani, L. Chen, R. Wu, A.A. Litonjua, L. Bacharier, et al., Vitamin D sufficiency has a limited effect on placental structure and pathology: placental phenotypes in the VDAART trial, Endocrinology 161 (6) (2020) bqaa057.

[57] M. Grundmann, M. Haidar, S. Placzko, R. Niendorf, N. Darashchonak, C.A. Hubel, et al., Vitamin D improves the angiogenic properties of endothelial progenitor cells, Am. J Physiol, Cell Physiol. 303 (9) (2012) C954–C962.

[58] L. Chen, C.L. Wagner, Y. Dong, X. Wang, J.R. Shary, Y. Huang, et al., Effects of maternal vitamin D3 supplementation on offspring epigenetic clock of gestational age at birth: a post-hoc analysis of a randomized controlled trial, Epigenetics 15 (8) (2020) 830–840.

[59] O.R. Vaughan, A.L. Fowden, Placental metabolism: substrate requirements and the response to stress, Reprod. Domest. Anim. 51 (Suppl 2) (2016) 25–35.

[60] A. Hanel, C. Carlberg, Vitamin D and evolution: pharmacologic implications, Biochem. Pharmacol. 173 (2020) 113595.

[61] K.Y.F. Tsai, B. Tullis, J. Mejia, P.R. Reynolds, J.A. Arroyo, Regulation of trophoblast cell invasion by pyruvate kinase isozyme M2 (PKM2), Placenta 103 (2021) 24–32.

[62] B. Lunghi, E. Meacci, M. Stio, A. Celli, P. Bruni, P. Nassi, et al., 1,25-Dihydroxyvitamin D3 inhibits proliferation of IMR-90 human fibroblasts and stimulates pyruvate kinase activity in confluent-phase cells, Mol. Cell. Endocrinol. 115 (2) (1995) 141–148.

[63] A. Celli, C. Treves, P. Nassi, M. Stio, Role of 1,25-dihydroxyvitamin D3 and extracellular calcium in the regulation of proliferation in cultured SH-SY5Y human neuroblastoma cells, Neurochem. Res. 24 (5) (1999) 691–698.

[64] Z.C. Ryan, T.A. Craig, C.D. Folmes, X. Wang, I.R. Lanza, N.S. Schaible, et al., 1α,25-dihydroxyvitamin D3 regulates mitochondrial oxygen consumption and dynamics in human skeletal muscle cells, J. Biol. Chem. 291 (3) (2016) 1514–1528.

[65] S.P. Ashcroft, J.J. Bass, A.A. Kazi, P.J. Atherton, A. Philp, The vitamin D receptor regulates mitochondrial function in C2C12 myoblasts, Am. J. Physiol. Cell Physiol. 318 (3) (2020) C536–C541.

[66] J. Salles, A. Chanet, C. Guillet, A.M. Vaes, E.M. Brouwer-Brolsma, C. Rocher, et al., Vitamin D status modulates mitochondrial oxidative capacities in skeletal muscle: role in sarcopenia, Commun, Biol. 5 (1) (2022) 1288.

[67] E. Maltepe, S.J. Fisher, Placenta: the forgotten organ, Ann. Rev. Cell Dev. Biol. 31 (2015) 523–552.

[68] J. Crish, M.A. Conti, T. Sakai, R.S. Adelstein, T.T. Egelhoff, Keratin 5-Cre-driven excision of nonmuscle myosin IIA in early embryo trophectoderm leads to placenta defects and embryonic lethality, Dev. Biol. 382 (1) (2013) 136–148.

[69] Y. Zhang, C. Liu, R.S. Adelstein, X. Ma, Replacing nonmuscle myosin 2A with myosin 2C1 permits gastrulation but not placenta vascular development in mice, Mol Biol. Cell. 29 (19) (2018) 2326–2335.

[70] A. Fajol, S. Honisch, B. Zhang, S. Schmidt, S. Alkahtani, S. Alarifi, et al., Fibroblast growth factor (Fgf) 23 gene transcription depends on actin cytoskeleton reorganization, FEBS Lett 590 (6) (2016) 705–715.

[71] D.X. Tishkoff, K.A. Nibbelink, K.H. Holmberg, L. Dandu, R.U. Simpson, Functional vitamin D receptor (VDR) in the T-tubules of cardiac myocytes: VDR knockout cardiomyocyte contractility, Endocrinology 149 (2) (2008) 558–564.

[72] W. Peng, Y. Liu, H. Qi, Q. Li, Alpha-actinin-4 is essential for maintaining normal trophoblast proliferation and differentiation during early pregnancy, Reprod. Biol. Endocrinol. 19 (1) (2021) 48.

[73] L. Zheng, A. Lindsay, K. McSweeney, J. Aplin, K. Forbes, S. Smith, et al., Ryanodine receptor calcium release channels in trophoblasts and their role in cell migration, Biochim. Biophys. Acta Mol. Cell Res. 1869 (1) (2022) 119139.

[74] S.J. Renaud, M.J. Jeyarajah, How trophoblasts fuse: an in-depth look into placental syncytiotrophoblast formation, Cell Mol. Life Sci. 79 (8) (2022) 433.

[75] R. Fuchs, I. Ellinger, Endocytic and transcytotic processes in villous syncytiotrophoblast: role in nutrient transport to the human fetus, Traffic 5 (10) (2004) 725–738.

[76] B. Keith, D.M. Adelman, M.C. Simon, Targeted mutation of the murine arylhydrocarbon receptor nuclear translocator 2 (Arnt2) gene reveals partial redundancy with Arnt, Proc. Natl. Acad. Sci. U.S.A. 98 (12) (2001) 6692–6697.

[77] S.K. Hansen, C. Nerlov, U. Zabel, P. Verde, M. Johnsen, P.A. Baeuerle, et al., A novel complex between the p65 subunit of NF-kappa B and c-Rel binds to a DNA element involved in the phorbol ester induction of the human urokinase gene, EMBO J 11 (1) (1992) 205–213.

[78] C. Carlberg, Vitamin D and its target genes, Nutrients 14 (7) (2022) 1354.

[79] Y. Li, C. Zhou, W. Lei, K. Wang, J. Zheng, Roles of aryl hydrocarbon receptor in endothelial angiogenic responses, Biol. Reprod. 103 (5) (2020) 927–937.

[80] J. Hess, P. Angel, M. Schorpp-Kistner, AP-1 subunits: quarrel and harmony among siblings, J. Cell Sci. 117 (25) (2004) 5965–5973.

[81] J.-P. David, M. Rincon, L. Neff, W.C. Horne, R. Baron, Carbonic anhydrase II is an AP-1 target gene in osteoclasts, J. Cell. Physiol. 188 (1) (2001) 89–97.

[82] A. Wierzbicka, M. Oczkowicz, Sex differences in vitamin D metabolism, serum levels and action, Br. J. Nutr. 128 (11) (2022) 2115–2130.

[83] A.L. Vestergaard, M.K. Andersen, R.V. Olesen, P. Bor, A. Larsen, High-dose vitamin D supplementation significantly affects the placental transcriptome, Nutrients 15 (24) (2023) 5032.

[84] E.R. Elkin, K.A. Campbell, S. Lapehn, S.M. Harris, V. Padmanabhan, K.M. Bakulski, et al., Placental single cell transcriptomics: opportunities for endocrine disrupting chemical toxicology, Mol. Cell. Endocrinol. 578 (2023) 112066.