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Placental Genetic Variations in Vitamin D Metabolism and Birthweight

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Abstract

Introduction—Vitamin D has pleiotropic functions that regulate fetal growth and development. We investigated associations of common placental genetic variations in vitamin D metabolism with birthweight.

Methods—The study was conducted among participants (506 maternal-infant pairs) of a pregnancy cohort study. Data were collected using interviewer-administered questionnaires and post-delivery medical record abstraction. DNA, extracted from placental samples collected at delivery, was genotyped for eight single nucleotide polymorphisms (SNPs) in five vitamin D metabolism genes (CUBN, LRP2, VDR, GC, and CYP2R1). Linear and logistic regression models were used to evaluate associations of SNPs with birthweight and risk of low birthweight, respectively. Effect modification of associations by infant sex was examined using stratified analyses and interaction terms in regression models

Results—Mean (standard-deviation) birthweight among all, male, and female infants was 3,482.1 (549.9), 3,544.6 (579.0) and 3,419.2 (512.5) grams, respectively. Each copy of the minor allele of rs2282679 (GC) was associated with a 68.6g (95%CI:3.1,134.7g) increase in birthweight overall. Sex-specific associations were observed for SNP rs4667591 (LRP2) (p-value for interaction < 0.001). Each copy of the minor allele of rs4667591 was associated with a 124.7g (95%CI:20.1,229.0g) increase in birthweight among female infants, and a suggested 81.6g decrease in birthweight among male infants (95%CI:-183.7,20.5g).

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Conflicts of Interest

The authors have no conflicts of interest to disclose.

Discussion—Our study identified overall and sex-specific associations between placental genetic variations in vitamin D metabolism and birthweight. If confirmed by larger replication studies, observed associations may provide insight into mechanistic underpinnings of the relationships between placental vitamin D metabolism and birth size.

Keywords

Birthweight; vitamin D; placenta; genetic variation

INTRODUCTION

Vitamin D is a pleiotropic hormone that is important in fetal growth, development, and programming [1]. Maternal vitamin D deficiency is a major public health concern that has been linked with poor birth outcomes, including fetal growth restriction, [2-5] a risk factor for life course morbidity and mortality [6]. However, underlying mechanisms of these relationships have not been fully described. Findings from several studies suggest the importance of maternal or fetal genetic variations in vitamin D metabolism [7, 8]. Despite the role of the placenta in vitamin D metabolism, including conversion of 25-hydroxyvitamin D to its biologically active form, [9, 10] and presence of functional vitamin D receptors in the placenta, whether placental genetic variations in vitamin D metabolism are associated with birthweight remains unexplored.

The placenta is a highly complex organ that plays an active role in fetal growth, development, and programming in addition to serving as the maternal-fetal interface [11]. Vitamin D acts through VDR and cAMP/protein kinase A signaling pathways to regulate human chorionic gonadotropin expression, its secretion in human synctiotrophoblasts, [12] and placental sex steroid production [13]. In addition, vitamin D plays a role in glucose availability for trans-placental transport [14]. As a regulator of calcium homeostasis and transport, it can influence fetal growth through the promotion of skeletal muscle and bone development [15]. Therefore, placental genetic variations that are involved in vitamin D metabolism may be associated with fetal growth. These associations could potentially be sex-specific, given differences in fetal growth trajectories and birthweight between male and female infants [16] as well as previous reports of differences in vitamin D-birthweight associations between male and female infants [17]. We investigated overall and infant sex-specific associations of placental genetic variations related to vitamin D metabolism with birthweight.

METHODS

Study Setting and Study Population

The setting for the current study was the Omega study, a cohort study of dietary and metabolic risk factors associated with adverse pregnancy outcomes. Briefly, Omega study participants were recruited between 1996 and 2008 at prenatal care clinics affiliated with the Swedish Medical Center in Seattle, Washington and Tacoma General Hospital, Tacoma, Washington [18]. Eligible participants included pregnant women who initiated prenatal care at or before 20 weeks gestation, were aged ≥ 18 years, able to speak and read English,

planned to carry the pregnancy to term, and to deliver at either of the two hospitals. Participants who provided placental samples (between 2004 and 2008) at delivery (N=547) were included in the current study. After excluding 28 participants with gestational diabetes mellitus, ten participants with preeclampsia, and three participants with missing infant sex, a total of 506 participants remained for analyses. The Institutional Review Boards of Swedish Medical Center and Tacoma General Hospital approved the study protocol. All participants provided informed consent.

Data Collection

Trained personnel conducted in-person interviews in early pregnancy, soon after recruitment, to obtain information on maternal socio-demographic and medical characteristics, family and medical history, dietary intake, and life style habits (e.g. smoking and physical activity). Participants provided peripheral blood samples during this visit. Serum 25-hydroxyvitamin D concentrations were measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) by ZRT Laboratory (ZRT Laboratory, Portland, OR). Both 25-hydroxyvitamin D2 and 25-hydroxyvitamin D3 concentrations were measured, with the sum giving total serum 25-hydroxyvitamin D concentration. At the end of pregnancy, medical records were abstracted to obtain information on the course and outcomes of pregnancy, including information on infant birth characteristics.

In addition, at delivery, placenta samples were collected. Placenta specimens were double-bagged, placed into coolers, and transported to a dedicated placenta-processing laboratory. Biopsy samples were taken using a systematic technique to achieve uniformity and adequate sampling as described before [19]. Biopsy samples were placed into cryotubes and stored at −80 °C until processing.

SNP Selection and Genotyping

For the current study, we used the available literature to identify eight single nucleotide polymorphisms (tag-SNPs) located in five candidate vitamin D metabolism genes that have been associated with vitamin D levels: CUBN (rs1801231, rs1801224 and rs1801222), LRP2 (rs4667591 and rs2229263), VDR (rs2228570), GC (rs2282679), and CYP2R1 (rs10741657) [20-22]. DNA was extracted from placenta tissues using the QIAamp Tissue Kit (QIAGEN Inc. Chatsworth, CA) following the manufacturer's instructions. DNA was quantified with the PicoGreen Quant-iT dsDNA Assay Kit (Thermo Fisher Scientific, Grand Island, NY) and a SpectraMAXGeminiXPS Spectrofluorometric Micro plate reader. Genotyping was conducted using Sequenom/Agenaiplex platform. Briefly, the protocol involves PCR amplification of DNA using SNP specific primers, from Integrated DNA Technologies (Coralville, IA), followed by a base extension reaction using the iPLEX Gold Chemistry (Agena, San Diego, CA). The treated extension product was spotted to the appropriate location on a 384-pad SpectroCHIP II using a SequenomMassARRAY Nanodispenser (Agena, San Diego, CA). A MassARRAY Analyzer Compact MALDI-TOF MS (Agena, San Diego, CA) was used for data acquisitions from the SpectroCHIP. All resultant genotyping calls were performed in real time by the MassARRAYTyper Analyzer v4.0.26.73 (Agena, San Diego, CA). Data was exported into a Microsoft Excel spreadsheet.

Outcome Measurement

Infant birthweight was abstracted from medical records. Birthweight was analyzed as a continuous variable in grams. In addition, < 2500 grams of birthweight was used to define low birthweight, which was analyzed as a categorical variable.

Statistical Analysis

Maternal and infant characteristics were summarized as follows. Continuous variables were described using mean ± standard deviation (SD) and categorical variables were described using counts and proportions for all participants (overall and stratified by infant sex). Minor allele frequency (MAF) of each SNP was calculated and Hardy Weinberg Equilibrium (HWE) was examined. Linear regression models were used to evaluate independent associations of each SNP with birthweight. This model assessed average birthweight difference per added copy of each minor allele among all infants, as well as separately for male and female infants. Similarly, logistic regression models were used when assessing risk of low birthweight in relation to each minor allele. Linear and logistic regression models were adjusted for infant sex (except for sex-specific models), gestational age at delivery and maternal race/ethnicity (non-Hispanic white/other).

We also conducted exploratory analyses using weighted genetic risk scores (wGRS), for all participants and male/female infants separately, computed by risk allele counting method weighted by individual effect estimates of the variants [23]. Regression models, similar to those described above, were fit to examine overall and sex-specific associations of wGRS with birthweight and risk of low birthweight, adjusted for race and gestational age. In all analyses, multiple testing corrections were not applied given the exploratory nature of the analyses, the relatively small number of SNPs evaluated, and selection of strong candidate genes and SNPs from the literature. Statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary NC) and R 3.2.3 software.

RESULTS

Selected characteristics of study participants are shown in **Table 1**. Average maternal early pregnancy serum 25-hydroxyvitamin D concentration was 29 ng/mL (SD=9.5 ng/mL). Approximately 88.1% of mothers were non-Hispanic White and 49.8% delivered female infants. Mean birthweights among all, male and female infants were all, male, and female infants was 3,482.1 (SD=549.9), 3,544.6 (SD=579.0) and 3,419.2 (SD=512.5) grams, respectively. The eight SNPs used to characterize variations in five vitamin D-metabolism genes are shown in **Table 2**. Genotype frequencies of all SNPs were in Hardy-Weinberg equilibrium and the SNPs minor-allele frequencies ranged from 0.03 to 0.14.

In the multivariable adjusted model (**Table 3)**, each copy of the minor allele of rs2282679 (GC) was associated with a 78.6 g (95%CI:11.1,146.2g) increase in birthweight overall (p-value=0.04). Sex-specific associations were observed for SNP rs4667591 (LRP2) (p for interactions <0.001). Each copy of the minor allele of rs4667591 was associated with 124.6 g (95%CI:20.1,229.0g) increase in birthweight among female infants, but suggests a −81.6g (95%CI:−183.7,−20.5g) decrease in birthweight among male infants. Similar, but less

pronounced sex-specific differences were observed for SNP rs1801231 (CUBN) (p-value for interactions=0.03). Each copy of the minor allele of rs1801231 was associated with a suggested 62.3g (95%CI:−54.1,178.7g) increase in birthweight among female infants, but a suggested 67.6g (95%CI:−188.2,53.0g) decrease in birthweight among male infants. wGRS of evaluated genetic variations was positively associated with the continuous measure of birthweight in overall models (1.4g, 95%CI:0.4,2.3g), but not in sex-stratified models or in models where low birthweight was the outcome.

DISCUSSION

In this study, we found evidence for associations of placental genetic variants in vitamin D metabolism with birthweight overall (in GC gene) and by infant sex (in LRP2 and CUBN genes). We also observed sex-specific differences in these associations where risk variants in the LRP2 and CUBN genes were associated with higher birthweight among females but suggesting lower birthweight among males. wGRS of the examined candidate SNPs was significantly associated with increased birthweight in overall models, but not sex-stratified models.

To our knowledge, this is the first study investigating placental genetic variations in vitamin D metabolism with birth size. Recently, Nguyen et al demonstrated that placental 25-hydroxyvitamin D levels and placental VDR mRNA expression are significantly decreased in pregnancies affected by idiopathic fetal growth retardation (defined as a birthweight of less than the 10th percentile for gestation) compared with uncomplicated gestation-matched control pregnancies [24]. Bodnar et al and Swamy et al showed that maternal SNPs in VDR influence birthweight and risk of small-for-gestational age (SGA) (defined as live-born infants at <10th percentile) among white mothers and black mothers, respectively [8, 25]. In their fully adjusted model, Bodnar et al found that risk of SGA was increased by 1.7-fold (95% CI:1.1,2.8) per each copy of the rs11168292 allele. Their study did not find associations of SGA with rs2228570, the VDR SNP we included in our study. It is interesting to note that we observed potential sex-specific differences in associations of rs2228570, which did not reach statistical significance. Our study did not evaluate the rs11168292 SNP. A recent Mendelian randomization study demonstrated that vitamin D deficiency related GRS characterized using rs10741657 (CYP2R1, also included in our study) and rs12785878 (DHCR7) maternal SNPs was associated with lower birthweight [26]. Birthweight was lower by 6g (95%CI:−12, 0g) per unit change in their adjusted GRS, suggesting evidence for a possible causal association of maternal vitamin D deficiency with offspring birthweight. In our study, we did not evaluate the rs12785878 (DHCR7) SNP, while placental variation in the rs10741657 (CYP2R1) was not associated with birthweight.

Associations between birth size and genetic variants, either maternal or fetal, in GC, CUBN, or LRP2 have not been reported before. Two copies of the minor allele of fetal rs2282679 SNP in GC was associated with risk of vitamin D insufficiency (OR = 2.76, 95% CI: 1.34-5.69) among 12 month old Caucasian infants (N=491) [27]. The role of vitamin D binding protein, the protein encoded by GC, on placental trophoblast growth and development, course of pregnancy and offspring has been well documented [9]. This protein is important in the transport of vitamin D in the circulatory system. Thus, it is hypothesized

that variation in GC could influence the facilitated transport of vitamin D into the cell. The expression of CUBN (cublin or intrinsic factor cobalamin receptor) in the placenta has been shown to increase with gestation [28]. This increase implied that cubilin may be important in placental functions, related to maternal-fetal nutrient transfer [28]. Gene expression analysis by Sabri et al revealed that LRP2 was downregulated in four placentas from fetal growth restricted pregnancies but not among 11 placentas from macrosomic or normal-term pregnancies [29]. LRP2 (low density lipoprotein-related protein 2) or megalin, is a multi-ligand endocytic receptor. It is critical for the reuptake of numerous ligands, including vitamin-binding proteins. This protein also has a role in cell-signaling. Overall, these reports support the argument for potential roles of placental genetic variants of these genes in fetal growth and development.

Our study also demonstrated potential sex-specific differences in associations of placental genetic variants related to vitamin D metabolism with birthweight. Available literature suggests that the beneficial effect of maternal vitamin D may differ among male and female offspring [17]. Findings by Gernand et al highlight infant sex-specific protective effect of maternal vitamin D in low birthweight risk [17]. Only female fetuses exhibited reduced birthweight in the setting of placental vascular pathology and maternal vitamin D deficiency. A reduced birthweight z-score with lower (<30 nmol/L) maternal 25(OH)D concentration was observed ($\beta = -0.73$; 95%CI:−1.17,−0.30) in females. We evaluated associations between maternal serum vitamin D and infant birthweight in the current study. In sex-specific models a 10 ng/ml increase in maternal serum vitamin D was associated with −7.7 g decrease in birthweight (95% CI: −92.6, 77.2 g) among female infants while it was associated with an 8.2 g increase in birthweight (95% CI: −104.8, 121.3 g) among male infants. Although not statistically significant, directions of association were different between males and females, similar to our observations for several of the vitamin D related SNPs. Differences in genetic/epigenetic markers of placental development and function in male and female fetuses, and their downstream effects in relation to vitamin D metabolism may contribute to observed sex-specific differences. This research area is underdeveloped and needs further investigations in large study populations.

Only few studies have assessed vitamin D-related genotypes and their relationships with birthweight. Our study extends this work by demonstrating associations of vitamin D related genetic variations with birthweight in a well-characterized study population. The current study is unique in its assessment of placental genetic variations, as compared to maternal genetic variation in previous studies, and evaluation of potential sex-specific differences in associations. Placental genotyping is a key strength because several vitamin D-related genes, including the ones reported here, are highly expressed in the placenta and decidual tissues in early gestation and placenta is an important component of the intrauterine environment [30, 31]. It also highlights the potential merit of evaluating both maternal and placental genetic variations, and their interactions, in this research area. There are also notable limitations of our study. First, our study may be underpowered to detect significant associations between several of our SNPs and birthweight. In our study population, several SNPs were observed to have low MAF. The individual SNP association findings were not adjusted for multiple testing, although strong priors for our candidate genes exist in other studies. Placental features, including morphology and gene expression were not evaluated in our study. Our

observations that minor alleles in rs2282679 (GC) and rs4667591 (LRP2) are associated with increased birthweight overall or in sex-specific models need careful considerations. In our study population, the C allele of rs2282679 (GC) was associated with −6.0 ng/mL (95% CI:−10.5,−1.6 ng/mL; p-value=0.01) lower maternal vitamin D level among all participants and male infants (β=−6.4 ng/ml; 95% CI:−12.5,−0.2 ng/mL; p-value=0.04), but not among female infants (β=−5.4 ng/ml; 95% CI:−12.1,1.3 ng/mL; p-value=0.11). Overall or sex-specific associations of rs4467591 (LRP2) and maternal vitamin D level was not observed. The observation for inverse association of rs2282679 (GC) with maternal vitamin D status is not consistent with our positive rs2282670-birthweight association but similar observation of inverse association of vitamin D with birthweight is not new to the literature. In a systematic review, Harvey et al. reported that six studies demonstrated significant positive associations between maternal vitamin D and offspring birthweight while seven studies reported non-significant positive associations [32]. On the other hand, one study reported significant negative association while three others found non-significant negative associations [32]. The observed increased in birthweight related to the minor alleles of the SNPs, may not be an indicator of fetal health, and it cannot exclude other adverse fetal effects (e.g. reduced bone mass) [33,34]. Therefore, associations between vitamin D and birthweight, and, the role of genetic risk factors (such as the variants we report in the current study) and modifiers are important future areas of research.

One concern in the current study is potential contamination of placental DNA with maternal DNA. During sample collection, we minimized contamination of placental specimen by maternal blood. Specimen for genotyping reported in this study was obtained from placental tissue. Our quality control procedures that involved Hardy Weinberg Equilibrium assessment minimize potential concerns of contamination. We also had maternal genotype information for a small subset (N=40) participants in our current study. Our assessment of this data was reassuring with respect to potential contamination of maternal and placental DNA. Lastly, our study may not be generalizable to other populations, since our sample consists of 88% Non-Hispanic Whites.

In conclusion, selected vitamin D-related genetic variations in the placenta are associated with birthweight. Our results also suggest that these associations may be infant sex-specific. Further research in this area may enhance mechanistic understanding of the relationships between vitamin D metabolism (e.g. synthesis and transport) and birth size. It may also help identify susceptible populations for targeted preventative efforts. Future investigations involving large number of study samples, characterization of additional vitamin D-related genotypes (maternal and placental), functional assessment of identified genotypes (e.g. gene and protein expression), and maternal-fetal genetic interactions on maternal and fetal outcomes are warranted.

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Highlights

- Vitamin D-related genetic variations in the placenta are associated with birthweight.
- Suggestive evidence supports potential infant sex-specific differences in associations of vitamin D-related genetic variations with birthweight.
- Findings may improve mechanistic understanding of the relationships between maternal vitamin D, placental vitamin D metabolism, and birth size.

Table 1

Selected characteristics of study participants

	All Infants [§]	Females[§]	Males [§]
N	506	252	254
Maternal age, y	33.6 ± 4.3	33.7 ± 4.4	33.5 ± 4.2
Gestational age, y	38.8 ± 1.9	38.9 ± 1.4	38.9 ± 1.6
Gestational age at blood draw, wk	16.4 ± 1.9	16.5 ± 2.0	16.3 ± 1.8
Pre-pregnancy BMI, kg/m2	23.7 ± 4.6	23.6 ± 4.7	23.8 ± 4.6
25-hydroxyvitamin D, ng/mL	29.1 ± 9.5	29.4 ± 10.5	28.8 ± 8.5
Infant birthweight, g	3482.1 ± 549.9	3419.2 ± 512.5	3544.6 ± 579.0
Infant low birthweight, n (%)			
Infant head circumference, cm	35.1 ± 2.6	34.8 ± 2.4	35.4 ± 2.7
Married, n (%)	469 (92.1)	235 (93.3)	231 (90.9)
≥ High school education, n (%)	452 (97.4)	226 (97.8)	225 (97.0)
Physically active during pregnancy, n (%)	427 (93.4)	211 (92.1)	215 (94.7)
Smoking status, n (%)			
Never	349 (76.5)	173 (75.9)	175 (77.1)
Quit before pregnancy	92 (20.1)	44 (19.3)	48 (21.2)
Smoked during pregnancy	15 (3.3)	11 (4.8)	4 (1.8)
Non-Hispanic White, n (%)	451 (88.6)	214 (84.9)	235 (92.5)

[§] values are means ± standard deviation, unless otherwise stated

Table 2

Placental Vitamin D Metabolism and Fetal Growth Related Single Nucleotide Polymorphisms

Gene	Name	Location	SNP	Chr[a]	Minor Allele	MAF[b]	HWE[c]
Vitamin D Metabolism Related Genotypes							
CUBN	intrinsic factor-cobalamin receptor	Coding	rs1801231	10	C	0.03	0.130
CUBN	intrinsic factor-cobalamin receptor	Coding	rs1801224	10	C	0.10	0.620
CUBN	intrinsic factor-cobalamin receptor	Coding	rs1801222	10	T	0.11	0.480
LRP2	low density lipoprotein receptor-related protein	Coding	rs4667591	2	T	0.06	0.410
LRP2	low density lipoprotein receptor-related protein	Coding	rs2229263	2	G	0.10	0.410
VDR	vitamin D (1,25-dihydroxyvitamin D3) receptor	Coding	rs2228570	12	T	0.14	0.100
GC	vitamin D binding protein	Intronic	rs2282679	4	C	0.08	0.590
CYP2R1	cytochrome P450, family 2, subfamily R, polypeptide	5’ upstream	rs10741657	11	A	0.14	0.930

[a]Chromosome

[b]Minor allele frequency

[c]Hardy Weinberg Equilibrium p-value

Table 3

Multi-variable adjusted association between birthweight and placental vitamin D metabolism-related genotypes

Locus (Index SNP, Effect allele/ Other allele)	Birthweight in grams [a]			Low birthweight [b] (< 2500 grams)
	All infants	Female Infants	Male Infants	
	N=506	N=252	N=254	N=24 (4.7%)
CUBN (rs1801231,C/T)	−13.1 (−96.7, 70.5)	62.3 (−54.1, 178.7)	−67.6 (−188.2, 53.0)	1.26 (0.39 - 4.05)
CUBN (rs1801224,C/A)	0.65 (−70.1, 71.4)	−33.5 (−131.3, 64.2)	27.3 (−74.1, 128.6)	1.04 (0.33 - 3.28)
CUBN (rs1801222,T/C)	−18.6 (−85.9, 48.8)	−31.3 (−121.3, 58.8)	−31.3 (−132.7, 69.6)	2.06 (0.76 - 5.57)
LRP2 (rs4667591,T/G)	7.6 (−65.2, 80.3)	124.6** (20.1, 229.0)	−81.6 (−183.7, 20.5)	1.04 (0.34 - 3.22)
LRP2 (rs2229263,G/A)	17.2 (−47.2, 81.7)	22.4 (−65.0, 109.7)	−2.7 (−100.8, 95.3)	1.16 (0.47 - 2.88)
VDR (rs2228570,T/G)	−4.8 (−69.1, 59.6)	−35.1 (−123.4, 53.2)	50.7 (−42.9, 144.3)	1.92 (0.71 - 5.19)
GC (rs2282679,C/A)	78.6** (11.1, 146.2)	57.7 (−36.1, 151.4)	94.6* (−3.3, 192.4)	0.53 (0.18 – 1.62)
CYP2R1 (rs10741657,A/G)	−3.8 (−66.4, 58.8)	−2.6 (−88.4, 83.3)	−16.7 (−107.9, 74.5)	1.89 (0.75 - 4.75)
Genetic risk Score (GRS)	1.36 (0.41, 2.30)	0.29 (−0.22, 0.80)	0.41 (−0.09, 0.90)	0.98 (0.99 - 1.01)

Note: P-values for sex interaction: rs4667591 (0.001), rs1801231 (0.03)

[a] β (95% CI), adjusted for sex, race and gestational age (only race and gestational age included for sex-specific models)

[b] OR (95% CI), adjusted for sex, race and gestational age

** Statistically significant estimates