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Maternal Vitamin D Receptor Genetic Variation Contributes to Infant Birthweight among Black Mothers

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Abstract

Racial disparity in pregnancy outcomes is one of the most striking and poorly understood inequalities in American health. Genetic variability may be an important host factor influencing disparate birth outcomes between non-Hispanic black (NHB) and non-Hispanic white (NHW) women. Race-specific allelic frequencies in the *vitamin D receptor (VDR)* gene suggest its potential as a gene involved in health disparities. The Healthy Pregnancy, Healthy Baby Study is a prospective cohort of pregnant women aimed at identifying genetic, social, and environmental contributors to disparities in pregnancy outcomes in Durham, NC. *VDR* haplotype tagging SNPs were genotyped via Taqman assays for 615 women. Analysis of variance was used to examine the association between maternal genotype and infant birthweight. Eight of 38 SNPs examined showed nominal significance among NHB women, with one *VDR* SNP (rs7975232) surpassing the multiple testing significance threshold. rs7975232, an anonymous polymorphism, is part of a *VDR* gene haplotype associated with variation in mRNA stability. mRNA stability can affect the amount of protein produced, thus directly affecting vitamin D levels and calcium homeostasis. In contrast to NHBs, there was no association between any *VDR* SNP and birthweight for NHWs. Genetic factors contributing to disparities in birth outcomes are not expected to be explained entirely by variation in a single gene. Nevertheless, our results suggest that maternal *VDR* gene polymorphisms do influence birthweight with differential effects accruing across racial groups. Further research identifying the functionality of *VDR* gene polymorphisms in pregnant women will improve our understanding of the underlying mechanisms influencing birthweight.

Keywords

Infant birthweight; Vitamin D receptor gene; Racial disparity

INTRODUCTION

One of the most striking inequalities in American health is the pronounced difference in birth outcomes between non-Hispanic black (NHB) and non-Hispanic white (NHW) women. In 2007, rates of preterm birth and low birthweight were 18.3% and 13.8% for NHB, compared to rates of 11.5% and 7.2% for NHW [Hamilton et al., 2009]. While many studies have examined the contribution of differences in socioeconomic status to the disparity in pregnancy outcomes [Finch, 2003; Lynch et al., 2004], the complex etiology of disparities in

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pregnancy outcomes remains unexplained. Our research group, the Southern Center on Environmentally Driven Disparities in Birth Outcomes (SCEDDBO), is committed to determining how environmental, social, and host factors jointly contribute to disparities in pregnancy outcomes. Among host factors, we are particularly interested in how genetic variability may contribute to the differences in birth outcomes between NHB and NHW women.

Based on the disproportionately high rates of vitamin D insufficiency among NHBs in the US [Adami et al., 2009; Aloia, 2008; Scragg and Camargo, 2008; Scragg et al., 2007], one potential candidate gene that may contribute to racial disparities in birth outcomes is the vitamin D receptor (*VDR*) gene. The vitamin D endocrine system, primarily responsible for calcium homeostasis, exerts its biological activity through binding to a high-affinity receptor (*VDR*) that acts as a ligand-activated gene transcription factor. Race-specific differences in allelic frequencies of *VDR* polymorphisms have been confirmed in multiple studies [Cooper and Umbach, 1996; Lee et al., 2001; Nakamura et al., 2000; Nelson et al., 2000; Valdivielso and Fernandez, 2006]. Lower levels of circulating calcitriol or 25-hydroxyvitamin D levels have in turn been linked to hyperparathyroidism [Fraser, 2009], chronic obstructive pulmonary disease [Janssens et al., 2009], lower bone mineral density [Adami et al., 2009], cardiovascular diseases, diabetes, and renal failure [Scragg et al., 2007; Zittermann et al., 2009], obesity [Foss, 2009], cancers, auto-immune diseases, and infections [Cavalier et al., 2009]. In turn, many of the health endpoints associated with lower levels of circulating calcitriol or 25-hydroxyvitamin D are characterized by racial disparities [Giles et al., 2007; Kirk et al., 2006], and several, such as cardiovascular disease [Zhang et al., 2009], obesity [Khashan and Kenny, 2009], and infection [Gomez et al., 1995; Mercer, 2005], are associated with poor pregnancy outcomes.

To date, studies examining the contributions of the *VDR* gene to infant birthweight have been focused on the association between adult women's *VDR* genotype and their own weight at birth [Jordan et al., 2005; Keen et al., 1997]. Presumably, this is due to the primary role of *VDR* in calcium homeostasis and bone density. However, the vitamin D system has been associated with a broad range of complex conditions, many of which occur disproportionately in NHBs. In contrast to previous studies, we sought to examine how variation in maternal *VDR* genotype affects infant birthweight and whether there are differential effects among racial groups.

MATERIALS AND METHODS

The Healthy Pregnancy, Healthy Baby study is an ongoing prospective cohort study within SCEDDBO of the effects of environmental, social, and genetic factors on racial disparities in pregnancy outcomes. Duke University Medical Center (DUMC) Institutional Review Board approval was obtained to enroll pregnant women from the DUMC Obstetrics Clinic and the Durham County Health Department Prenatal Clinic. Women were excluded from participation if they were less than 18 years old, were not English-literate, were less than 18 or greater than 28 weeks gestation at the time of enrollment, lived outside of Durham County, North Carolina, had a multiple gestation, had a known fetal genetic or congenital abnormality, or were not planning to deliver at DUMC. Women with medical conditions such as chronic hypertension and diabetes mellitus were included in the study.

Socio-demographic characteristics, health behaviors, pre-existing medical conditions, and pregnancy history were obtained by direct patient interview at the time of enrollment and through electronic medical record review. Psychosocial factors were measured using self-reported surveys covering depression, perceived stress, self-efficacy, active coping, social support, racism, perceived community standing, and personality. Maternal race was based

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on self-reported race and Hispanic ethnicity as recorded in the medical record at the time of prenatal care initiation. Pregnancy complications, as well as maternal and neonatal outcomes, were obtained from electronic medical record review. Maternal blood samples obtained at 24–28 weeks gestation or during inpatient admission for delivery of the pregnancy were used for candidate gene analysis. Environmental exposure studies for nicotine and cotinine metabolites and metals (lead, mercury, arsenic, cadmium, and manganese) were performed on samples collected during inpatient admission for delivery.

Haplotype tagging single nucleotide polymorphisms (SNPs) within the *VDR* gene were identified using LD Select [Carlson et al. 2004] using data from the Yoruban (YRI) and Caucasian (CEU) populations of the HapMap project (www.hapmap.org) to minimize repetitive data coming from SNPs within the same haplotype block. We chose to type the minimal number of haplotype tagging SNPs to capture the genetic diversity in both groups as they represent the ancestral populations for the NHB and NHW women in our study. All SNPs were genotyped in both racial groups. SNP genotyping was performed by TaqMan, using ‘Assays-on-Demand’ or ‘Assays-by-Design’ SNP genotyping products (Applied Biosystems, Foster City, CA, USA). For all genotype assays, quality control measures were applied, including genotyping a series of blinded duplicate samples and Centre d’Etude du Polymorphism Humain (CEPH) controls. We also required that each assay achieve 95% efficiency (i.e., the genotypes of at least 95% of the samples could be called with certainty) prior to statistical analysis. Hardy–Weinberg Equilibrium (HWE) and linkage disequilibrium (LD) were assessed separately for each race to control for ethnic differences in allele frequencies. Thirty-eight independent haplotype tagging SNPs were tested in the *VDR* gene.

Baseline socio-demographic characteristics, environmental exposures, and outcome measures were summarized and compared by maternal racial group (PROC GLM and PROC LOGISTIC). Minor allele frequencies were computed for all SNPs tested (PROC ALLELE). Analysis of variance (ANOVA, PROC GLM) was performed to examine the association between maternal genotype and infant birthweight (grams). Model covariates included known contributors to infant birthweight: maternal age (trichotomized into 18–20 yrs, 21–34 yrs, and ≥ 35 yrs categories), infant sex (male, yes/no), race (NHB vs. NHW), education (high school or less vs. any post-secondary education), insurance status (private, yes/no), parity (first born, yes/no), and tobacco exposure based on plasma cotinine level. We considered marital status as an important covariate; however, because it was highly correlated with insurance status, we chose to exclude it from adjusted analyses. Due to the seasonal effects of UVB radiation on the vitamin D endocrine system, we included season of conception as a model covariate: spring (March–May), summer (June–August), fall (September–November), and winter (December–February, reference group). Analyses were performed for the NHB and NHW groups separately in order to better identify racially-driven differences in outcomes. Statistical analyses were performed using Statistical Analysis Systems 9.1 (SAS Institute, Cary, NC). To control for multiple testing, SNP spectral decomposition [Li and Ji, 2005] was used to determine the effective number of independent statistical tests (M_{eff}) based on the linkage disequilibrium among the *VDR* SNPs. A Bonferonni correction using the effective number of SNPs resulted in a critical p-value of 0.0018 for the NHB subset (M_{eff} = 29.0626) and p=0.0024 for the NHW subset (M_{eff} = 21.1167.

RESULTS

From July 2005–August 2008, 615 women enrolled in the cohort had complete data available for birth outcomes, environmental exposure assays, and genetic analyses. NHB women represented 77.6% (477 of 615) of the sample population with the remaining 22.4% being NHW (see Table I). Compared to NHW women, NHB women were more likely to be

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younger, have lower educational attainment, be unmarried, and less likely to have private health insurance (p<0.0001). NHB women were more likely to have detectable cotinine levels (p=0.0173). There was not a significant difference in parity (p=0.10) or season of conception (p=0.26) between the two races. As shown in previous studies, NHB neonates had lower birthweights, with the mean birthweight being approximately 300 grams lower than that for NHWs (p<0.0001). Although not statistically significant, NHB neonates also had shorter gestational lengths than NHW neonates (p=0.15).

Table II describes the 38 tagging SNPs analyzed with base pair location and minor allele frequency by maternal race. Among the NHB women, only one SNP deviated from HWE (rs2853563, p=0.0475). Similarly, among the NHW women, only a single SNP deviated from HWE (rs11168266, p=0.0428). Both of these SNPs were re-examined for possible technical problems (i.e., poor separation of genotype clusters). Since no obvious technical problems were identified and because both SNPs deviated only slightly from the threshold for significance (p<0.05), neither SNP was excluded from analysis. Two SNPs were in linkage disequilibrium among the NHB women (rs4760733 and rs7963776, r^2=0.855) and there was extensive linkage disequilibrium among the NHW women (Figure 1). However, it is important to note that the tagging SNPs were selected based on both the Yoruban (African) and Caucasian LD patterns from the HapMap. Thus, it is to be expected that there would be some LD in our NHB and NHW study populations simply due to the differing patterns across racial groups. For example, the differences from the Yoruban LD patterns were more obvious in the NHW than the NHB women, as would be expected since the NHW women are, on average, more racially distinct from the Yoruban population than the NHB women.

As shown in Table III, 8 of the 38 *VDR* polymorphisms examined showed nominal significance with infant birthweight among the NHB women. In addition, one *VDR* SNP surpassed the multiple testing threshold: rs7975232, also referred to as an Apa I restriction fragment length polymorphism (RFLP) due to the historical detection with a restriction enzyme. None of the *VDR* SNPs were associated with birthweight in the NHW subgroup.

DISCUSSION

In our population of pregnant women in Durham, North Carolina, *VDR* genetic variability was strongly associated with infant birthweight among NHB, but not NHW women. Birthweight is affected by an extensive array of host, social, and environmental factors including a mother's age and overall health [Hamilton et al., 2009], her education and socio-economic status [Savitz et al., 2004], and behavioral factors such as exercise [Chasan-Taber et al., 2007] or tobacco smoke exposure [McFarlane et al., 1996]. However, despite extensive research on social contributors, much of the disparity in pregnancy outcomes between NHB and NHW remains unexplained [Hamilton et al., 2009]. Genetic influences likely play a role in fetal growth and subsequent infant birthweight, given the high correlation between parental and offspring birthweight for both normal and low birthweight [Agnihotri et al., 2008; Coutinho et al., 1997; Little, 1987; Magnus et al., 1993]. The association between maternal *VDR* genetic polymorphisms and birthweight represents not only a specific example of genetic influences on fetal growth and infant birthweight but also a potentially important explanation for significant differences in birthweight among infants born to NHB and NHW women.

Thus far, investigations involving *VDR* genetics and birthweight have examined the association between an adult woman's *VDR* genotype and her own birthweight. In one such analysis, among a sample of 66 women in the United Kingdom, Keen and colleagues detected significant differences in birthweight by genotype for the *VDR* SNP rs731236

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[Keen et al., 1997]. Women who were homozygous for the minor allele had a mean birthweight of 3.60 kg vs. 3.52 kg for those who were heterozygous and 3.46 kg for those who were homozygous for the major allele, representing an overall difference in birthweight of 140 grams, with such differences persisting through the first year of life. Although the role of the vitamin D endocrine system in bone metabolism and mineral homeostasis is well established, recent studies have shown that vitamin D and *VDR* are expressed and active in a wide variety of non-classic tissues [DeLuca, 2008; Dixon and Mason, 2009]. In addition to associations with numerous health outcomes as described above [Cooper and Umbach, 1996; Lee et al., 2001; Nakamura et al., 2000; Nelson et al., 2000; Valdivielso and Fernandez, 2006], vitamin D and *VDR* appear to be involved in reproductive physiology. Evans and colleagues demonstrated that vitamin D and *VDR* expression at the fetal-maternal interface is much higher in placental and decidual tissues in early gestation and appears to affect placental implantation through immunomodulatory pathways [Evans et al., 2004]. Diaz and colleagues have shown that pregnancy-related hypertension and preeclampsia are associated with not only low maternal vitamin D levels [Halhali et al., 2000], but also altered gene expression at the fetal-maternal interface [Diaz et al., 2002], demonstrating a potential role for vitamin D effects in late gestation as well. Furthermore, *VDR* knockout mice have impaired reproduction with females demonstrating decreased estradiol, increased gonadotropins, abnormal ovarian follicular development, and uterine hypoplasia [Yoshizawa et al., 1997]. Such factors are not only important contributors to infant birthweight but may represent causal pathways for effects of the vitamin D system on other pregnancy outcomes.

Further investigation of the vitamin D system with regards to the racial disparity in pregnancy outcomes is warranted, given the significant incidence of vitamin D insufficiency among NHB women in the US [Adami et al., 2009; Aloia, 2008; Scragg and Camargo, 2008; Scragg et al., 2007]. In a cohort of pregnant women, Bodnar et al. found that vitamin D deficiency (<10 ng/mL) and insufficiency (11–32 ng/mL) occurred in 29% and 54% of African American women, respectively, compared with 5% and 42% in white women in the northeastern US [Bodnar et al., 2007]. Given that 90% of their study participants reported taking prenatal vitamins, which contain 400 IU vitamin D, standard supplementation is inadequate to achieve normal vitamin D levels in pregnant women and their infants [Cockburn et al., 1980]. Findings from ongoing studies using 2000 and 4000 IU vitamin D in pregnant women in the southeastern US [Hollis and Wagner, 2009] will provide much needed information regarding the effectiveness of routine screening and supplementation. In addition to low dietary vitamin D intake contributing to deficiency, factors such as sunlight/ultraviolet B photon exposure, dark skin pigmentation, and obesity may represent important gene*environment interactions contributing to racial disparity. Season of conception was not a significant contributor to infant birthweight among NHB women in our study population. However, seasonality may be an important factor among NHW women, with varying levels of significance in independent models of infant birthweight (parameter-level p-values ranging 0.03–0.20) and merits further study.

The rs7975232 SNP, previously identified as the ApaI polymorphism based on restriction enzymes, is an anonymous polymorphism within an intronic or non-coding region of the *VDR* gene. Along with rs731236 (TaqI), this SNP is part of a *VDR* gene haplotype that has been associated with variation in mRNA stability [Uitterlinden et al., 2004]. The more or less stable an mRNA molecule is can significantly affect the amount of protein produced, thus directly affecting vitamin D levels and calcium homeostasis. To date, interpretation of association studies involving the *VDR* gene has been hindered by the fact that most of the polymorphisms examined are anonymous, or located in a non-coding sequence of the gene. Therefore, we would speculate that a statistically important “anonymous” polymorphism association is in linkage disequilibrium with an allele of a truly functional sequence variation elsewhere in the gene. Further analysis would be required to identify the specific

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functional variant involved, including analysis of additional genetic markers in the *VDR* gene and construction of functional assays.

Although we had a moderately large sample size available for analysis, our study population was not evenly distributed among racial and ethnic subpopulations. Because of our clinical enrollment sites and sampling strategy (restriction to residence within Durham County, North Carolina and intentional oversampling of NHB), the majority of women in our cohort are NHB. While we did analyze NHB and NHW women separately, we acknowledge that further population stratification may exist within each of these racial groups that was not accounted for in the current analysis. This will be a focus in future analyses of our data set. In addition, we acknowledge that the non-significant results for the NHW women may be a product of the substantially smaller sample for NHW compared to NHB.

Another limitation of this analysis is that we did not perform infant genotyping analyses. As a consequence, we cannot comment on the potential contribution of the fetal *VDR* gene on birthweight, which is expressed in placental and decidual tissues. Despite such limitations, our study is the first to examine an extensive number of independent SNPs in the *VDR* gene among pregnant women. Further, we identified multiple nominally associated SNPs within the NHB group, one of which remained statistically significant after applying a multiple testing correction. We have attempted to put our findings in the context of other pregnancy studies regarding actual vitamin D levels rather than genetic markers alone. We believe that the totality of our data, taken in the context of previous work by other investigators, suggests a plausible role for vitamin D and its receptor in pregnancy. We acknowledge that our investigation is an early step to better understanding the role of the vitamin D endocrine system in pregnancy outcomes.

The genetic factors that contribute to adverse pregnancy outcomes are certainly more complex than to be explained entirely by variation in a single gene. Nevertheless, our results suggest that variation in the maternal vitamin D receptor gene influences infant birthweight with differential effects across racial lines. Further research to identify the functionality of *VDR* gene polymorphisms and their potential interactions with innate immune function will improve our understanding of the underlying mechanisms influencing normal and low birthweight.

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References

Adami S, Bertoldo F, Braga V, Fracassi E, Gatti D, Gandolini G, Minisola S, Battista Rini G. 25-hydroxy vitamin D levels in healthy premenopausal women: association with bone turnover markers and bone mineral density. Bone. 2009; 45:423–426. [PubMed: 19465168]

Agnihotri B, Antonisamy B, Priya G, Fall CH, Raghupathy P. Trends in human birth weight across two successive generations. Indian J Pediatr. 2008; 75:111–117. [PubMed: 18334789]

Aloia JF. African Americans, 25-hydroxyvitamin D, and osteoporosis: a paradox. Am J Clin Nutr. 2008; 88:545S–550S. [PubMed: 18689399]

Bodnar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. J Nutr. 2007; 137:447–452. [PubMed: 17237325]

Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA. Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. Am J Hum Genet. 2004; 74:106–120. [PubMed: 14681826]

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Cavalier E, Delanaye P, Chapelle JP, Souberbielle JC. Vitamin D: current status and perspectives. Clin Chem Lab Med. 2009; 47:120–127. [PubMed: 19099529]

CDC. Differences in prevalence of obesity among black, white, and Hispanic adults -United States, 2006–2008. MMWR Morb Mortal Wkly Rep. 2009; 58:740–744. [PubMed: 19609247]

Chasan-Taber L, Evenson KR, Sternfeld B, Kengeri S. Assessment of recreational physical activity during pregnancy in epidemiologic studies of birthweight and length of gestation: methodologic aspects. Women Health. 2007; 45:85–107. [PubMed: 18032169]

Cockburn F, Belton NR, Purvis RJ, Giles MM, Brown JK, Turner TL, Wilkinson EM, Forfar JO, Barrie WJ, McKay GS, et al. Maternal vitamin D intake and mineral metabolism in mothers and their newborn infants. Br Med J. 1980; 281:11–14. [PubMed: 7407476]

Cooper GS, Umbach DM. Are vitamin D receptor polymorphisms associated with bone mineral density? A meta-analysis. J Bone Miner Res. 1996; 11:1841–1849. [PubMed: 8970884]

Coutinho R, David RJ, Collins JW Jr. Relation of parental birth weights to infant birth weight among African Americans and whites in Illinois: a transgenerational study. Am J Epidemiol. 1997; 146:804–809. [PubMed: 9384200]

DeLuca HF. Evolution of our understanding of vitamin D. Nutr Rev. 2008; 66:S73–87. [PubMed: 18844850]

Diaz L, Arranz C, Avila E, Halhali A, Vilchis F, Larrea F. Expression and activity of 25-hydroxyvitamin D-1 alpha-hydroxylase are restricted in cultures of human syncytiotrophoblast cells from preeclamptic pregnancies. J Clin Endocrinol Metab. 2002; 87:3876–3882. [PubMed: 12161526]

Dixon KM, Mason RS. Vitamin D. Int J Biochem Cell Biol. 2009; 41:982–985. [PubMed: 18723110]

Evans KN, Bulmer JN, Kilby MD, Hewison M. Vitamin D and placental-decidual function. J Soc Gynecol Investig. 2004; 11:263–271.

Finch BK. Early origins of the gradient: the relationship between socioeconomic status and infant mortality in the United States. Demography. 2003; 40:675–699. [PubMed: 14686137]

Foss YJ. Vitamin D deficiency is the cause of common obesity. Med Hypotheses. 2009; 72:314–321. [PubMed: 19054627]

Fraser WD. Hyperparathyroidism. Lancet. 2009; 374:145–158. [PubMed: 19595349]

Giles T, Aranda JM Jr, Suh DC, Choi IS, Preblick R, Rocha R, Frech-Tamas F. Ethnic/racial variations in blood pressure awareness, treatment, and control. J Clin Hypertens (Greenwich). 2007; 9:345–354. [PubMed: 17485970]

Gomez R, Ghezzi F, Romero R, Munoz H, Tolosa JE, Rojas I. Premature labor and intra-amniotic infection. Clinical aspects and role of the cytokines in diagnosis and pathophysiology. Clin Perinatol. 1995; 22:281–342. [PubMed: 7671540]

Halhali A, Tovar AR, Torres N, Bourges H, Garabedian M, Larrea F. Preeclampsia is associated with low circulating levels of insulin-like growth factor I and 1,25-dihydroxyvitamin D in maternal and umbilical cord compartments. J Clin Endocrinol Metab. 2000; 85:1828–1833. [PubMed: 10843160]

Hamilton BE, Martin JA, Ventura SJ. Births: Preliminary Data for 2007. National Vital Statistics Reports. 2009; 27:1–23. [PubMed: 19754006]

Hollis, BW.; Wagner, CL. Evaluation of Vitamin D Requirements During Pregnancy. Medical University of South Carolina: NIH; 2009.

Janssens W, Lehouck A, Carremans C, Bouillon R, Mathieu C, Decramer M. Vitamin D beyond bones in chronic obstructive pulmonary disease: time to act. Am J Respir Crit Care Med. 2009; 179:630–636. [PubMed: 19164701]

Jordan KM, Syddall H, Dennison EM, Cooper C, Arden NK. Birthweight, vitamin D receptor gene polymorphism, and risk of lumbar spine osteoarthritis. J Rheumatol. 2005; 32:678–683. [PubMed: 15801025]

Keen RW, Egger P, Fall C, Major PJ, Lanchbury JS, Spector TD, Cooper C. Polymorphisms of the vitamin D receptor, infant growth, and adult bone mass. Calcif Tissue Int. 1997; 60:233–235. [PubMed: 9069157]

Khashan AS, Kenny LC. The effects of maternal body mass index on pregnancy outcome. Eur J Epidemiol. 2009; 24:697–705. [PubMed: 19653107]

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Kirk JK, D'Agostino RB Jr, Bell RA, Passmore LV, Bonds DE, Karter AJ, Narayan KM. Disparities in HbA1c levels between African-American and non-Hispanic white adults with diabetes: a meta-analysis. Diabetes Care. 2006; 29:2130–2136. [PubMed: 16936167]

Lee BK, Lee GS, Stewart WF, Ahn KD, Simon D, Kelsey KT, Todd AC, Schwartz BS. Associations of blood pressure and hypertension with lead dose measures and polymorphisms in the vitamin D receptor and delta-aminolevulinic acid dehydratase genes. Environ Health Perspect. 2001; 109:383–389. [PubMed: 11335187]

Li J, Ji L. Adjusting multiple testing in multilocus analyses using the eigenvalues of a correlation matrix. Heredity. 2005; 95:221–227. [PubMed: 16077740]

Little RE. Mother's and father's birthweight as predictors of infant birthweight. Paediatr Perinat Epidemiol. 1987; 1:19–31. [PubMed: 3506187]

Lynch J, Smith GD, Harper S, Hillemeier M. Is income inequality a determinant of population health? Part 2. U.S. National and regional trends in income inequality and age- and cause-specific mortality. Milbank Q. 2004; 82:355–400. [PubMed: 15225332]

Magnus P, Bakketeig LS, Skjaerven R. Correlations of birth weight and gestational age across generations. Ann Hum Biol. 1993; 20:231–238. [PubMed: 8489198]

McFarlane J, Parker B, Soeken K. Physical abuse, smoking, and substance use during pregnancy: prevalence, interrelationships, and effects on birth weight. J Obstet Gynecol Neonatal Nurs. 1996; 25:313–320.

Mercer BM. Preterm premature rupture of the membranes: current approaches to evaluation and management. Obstet Gynecol Clin North Am. 2005; 32:411–428. [PubMed: 16125041]

Nakamura M, Morimoto S, Ogihara T, Kakudo K. The worldwide controversy about the polymorphism of the vitamin D receptor gene and bone mineral density. Med Hypotheses. 2000; 54:495–497. [PubMed: 10783495]

Nelson DA, Vande Vord PJ, Wooley PH. Polymorphism in the vitamin D receptor gene and bone mass in African-American and white mothers and children: a preliminary report. Ann Rheum Dis. 2000; 59:626–630. [PubMed: 10913060]

Savitz DA, Kaufman JS, Dole N, Siega-Riz AM, Thorp JM Jr, Kaczor DT. Poverty, education, race, and pregnancy outcome. Ethn Dis. 2004; 14:322–329. [PubMed: 15328932]

Scragg R, Camargo CA Jr. Frequency of leisure-time physical activity and serum 25-hydroxyvitamin D levels in the US population: results from the Third National Health and Nutrition Examination Survey. Am J Epidemiol. 2008; 168:577–586. [PubMed: 18579538]

Scragg R, Sowers M, Bell C. Serum 25-hydroxyvitamin D, ethnicity, and blood pressure in the Third National Health and Nutrition Examination Survey. Am J Hypertens. 2007; 20:713–719. [PubMed: 17586404]

Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. Genetics and biology of vitamin D receptor polymorphisms. Gene. 2004; 338:143–156. [PubMed: 15315818]

Valdivielso JM, Fernandez E. Vitamin D receptor polymorphisms and diseases. Clin Chim Acta. 2006; 371:1–12. [PubMed: 16563362]

Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, Kawakami T, Arioka K, Sato H, Uchiyama Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. Nat Genet. 1997; 16:391–396. [PubMed: 9241280]

Zhang L, Cox RG, Graham J, Johnson D. Association of Maternal Medical Conditions and Unfavorable Birth Outcomes: Findings from the 1996–2003 Mississippi Linked Birth and Death Data. Matern Child Health J. 2009 Epub 2009 Sep 17.

Zittermann A, Schleithoff SS, Frisch S, Gotting C, Kuhn J, Koertke H, Kleesiek K, Tenderich G, Koerfer R. Circulating calcitriol concentrations and total mortality. Clin Chem. 2009; 55:1163–1170. [PubMed: 19359534]

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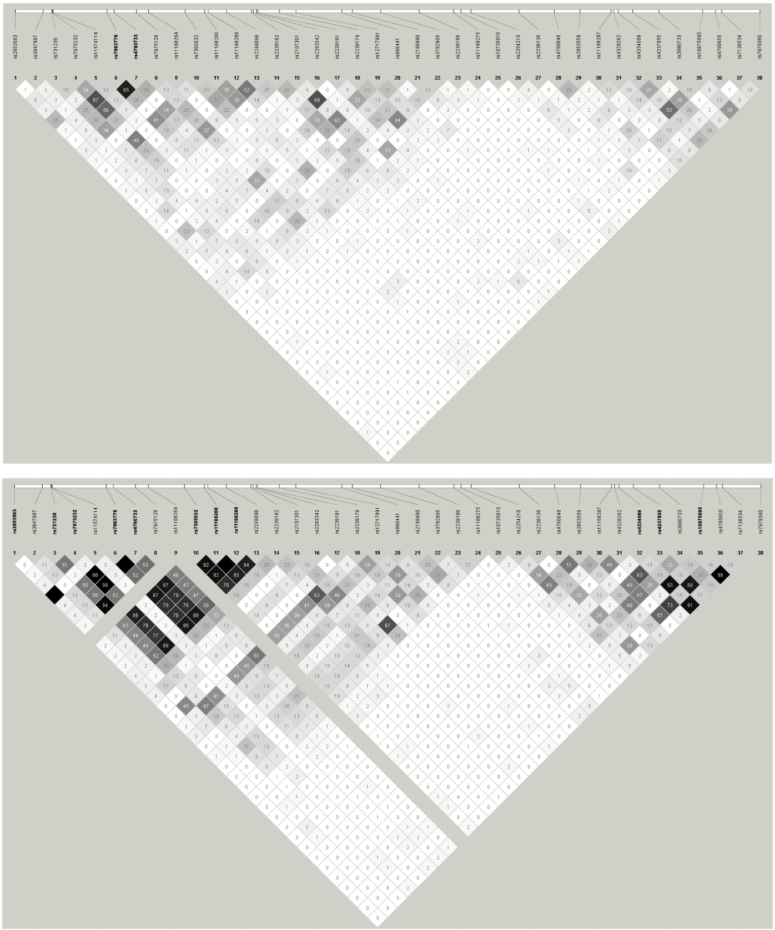

Figure 1.

Linkage Disequilibrium plots (r^2 shown) for NHB and NHW subsets. ◆ = $r^2 \geq 0.95$, diminishing shades of gray for $0.95 < r^2 < 1$, ⬩ = $r^2 \leq 0.05$

a) Non-Hispanic Black subset

b) Non-Hispanic White subset

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Table I

Demographic and Pregnancy Characteristics of Study Cohort by Race

	All n =615	Non-Hispanic White n = 138	Non-Hispanic Black n = 477	Unadjusted p-values by race group
Maternal Age mean (SD)	26.66 (6.25)	30.53 (6.04)	25.54 (5.86)	p<0.0001
18–20 years % (*n*)	19.19% (118)	6.52% (9)	22.85% (109)	
21–34 years % (*n*)	67.15% (413)	63.77% (88)	68.13% (325)	
35 years or greater % (*n*)	13.66% (84)	29.71% (41)	9.01% (43)	
Maternal education, % (n) with post-secondary education	49.76% (306)	78.99% (109)	41.3% (197)	p<0.0001
Unmarried % (*n*)	70.03% (430)	28.26% (39)	82.14% (391)	p <0.0001
Medicaid/Uninsured % (*n*)	72.68% (447)	28.26% (39)	85.53% (408)	p<0.0001
First Birth % (*n*)	37.56% (231)	43.48% (60)	35.85% (171)	not significant
Mean plasma cotinine level ng/ml (SD)	20.59 (56.12)	17.77 (51.17)	21.41 (57.50)	p=0.0173
Season of conception				not significant
Winter % (n)	23.74% (146)	22.46% (31)	24.11% (115)	
Spring % (n)	26.18% (161)	27.54% (38)	25.79% (123)	
Summer % (n)	26.18% (161)	21.01% (29)	27.67% (132)	
Fall % (n)	23.90% (147)	28.99% (40)	22.43% (107)	
Infant Sex Male % (*n*)	51.38% (316)	57.25% (79)	49.69% (237)	not significant
Mean Birthweight (grams, SD)	3102.63 (640.35)	3334.71 (552.35)	3035.49 (648.83)	p<0.0001
Mean Gestational Age (days, SD)	269.57 (17.14)	272.30 (14.32)	268.78 (17.81)	0.0335

n = number within particular cell, SD = standard deviation

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Table II

VDR Single Nucleotide Polymorphisms and Minor Allele Frequency by Race

			MAF Non-Hispanic White	MAF Caucasian Population*	MAF Non-Hispanic Black	MAF Yoruban Population*
SNP	**Base pair location**	**Minor Allele**	***n=138***	**-**	***n = 477***	**-**
rs2853563†	46522005	T	0.03	0.04	0.19	0.18
rs3847987	46524335	A	0.14	0.12	0.07	0.06
rs731236	46525024	C	0.41	0.44	0.29	0.29
rs7975232	46525104	C	0.44	0.43	0.34	0.37
rs11574114	46525150	T	0.03	0.04	0.15	0.23
rs7963776	46529644	G	0.44	0.43	0.38	0.42
rs4760733	46530230	A	0.44	0.43	0.42	0.48
rs7975128	46532095	A	0.40	0.44	0.30	0.26
rs11168264	46533130	G	0	0	0.25	0.23
rs7305032	46536127	G	0.41	0.4	0.26	0.25
rs11168266††	46537800	C	0.41	0.41	0.41	0.33
rs11168268	46538079	G	0.41	0.41	0.35	0.29
rs2248098	46539623	A	0.46	0.44	0.51	0.41
rs2239182	46541678	T	0.47	0.46	0.43	0.39
rs2107301	46541837	A	0.25	0.26	0.17	0.14
rs2283342	46542126	C	0.11	0.12	0.03	0
rs2239181	46542216	C	0.13	0.11	0.14	0.09
rs2239179	46544033	C	0.43	0.47	0.34	0.29
rs12717991	46545393	T	0.35	0.37	0.31	0.32
rs886441	46549231	G	0.21	0.18	0.40	0.39
rs2189480	46550095	T	0.33	0.36	0.35	0.39
rs3782905	46552434	C	0.32	0.30	0.22	0.22
rs2239186	46555677	G	0.15	0.20	0.05	0.03
rs11168275	46558542	G	0.18	0.24	0.37	0.42
rs10735810	46559162	A	0.42	0.41	0.23	0.19

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			MAF Non-Hispanic White	MAF Caucasian Population*	MAF Non-Hispanic Black	MAF Yoruban Population*
SNP	**Base pair location**	**Minor Allele**	***n=138***	**-**	***n = 477***	**-**
rs2254210	46559981	A	0.38	0.35	0.33	0.29
rs2238136	46563980	T	0.25	0.25	0.08	0.04
rs4760648	46566932	C	0.42 (T)	0.47 (T)	0.48	0.50
rs2853559	46569072	A	0.41	0.38	0.19	0.14
rs11168287	46571681	G	0.44	0.41	0.28	0.26
rs4328262	46571915	G	0.37	0.45	0.34	0.32
rs4334089	46572282	G	0.76	0.73	0.38	0.31
rs4237855	46573470	G	0.35	0.43	0.27	0.20
rs3890733	46575640	T	0.37	0.29	0.14	0.12
rs10875695	46579304	A	0.25	0.26	0.50	0.57
rs4760655	46580398	G	0.33	0.33	0.07	0
rs7136534	46580893	T	0.25	0.25	0.12	0.09
rs7979360	46584059	G	0	0	0.43	0.57

MAF = Minor Allele Frequency, SNP = single nucleotide polymorphism

*Population-level minor allele frequencies taken from www.hapmap.org

†SNP is not in Hardy Weinberg Equilibrium in non-Hispanic Black subset

††SNP is not in Hardy Weinberg Equilibrium in non-Hispanic White subset

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Table III

Covariate-Adjusted p-values for *VDR* SNPs that were Nominally Associated with Infant Birthweight by Race

		Non-Hispanic White	Non-Hispanic Black
SNP	**Functionality**	**n=138**	**n=477**
rs731236	Synonymous	0.4202	0.0375
rs7975232	Anonymous, previously linked to rs731236, may affect mRNA stability (Apa I RFLP)*	0.8587	0.0009†
rs11574114	Anonymous	0.8198	0.0216
rs7975128	Anonymous	0.5831	0.0031
rs7305032	Anonymous	0.6794	0.0166
rs11168266	Anonymous	0.8531	0.0189
rs11168268	Anonymous	0.5813	0.0434
rs10875695	Anonymous	0.4673	0.0151

mRNA = messenger RNA, RFLP = restriction fragment length polymorphism

† Met threshold for multiple testing correction ($p<0.0018$).

* [Uitterlinden et al.,2004]