Published in final edited form as:
Am J Perinatol. 2014 April ; 31(4): 327–334. doi:10.1055/s-0033-1349345.

Adrenomedullin Signaling Pathway Polymorphisms and Adverse Pregnancy Outcomes

Patricia M. Lenhart, BS[1] **[Graduate Student]**,
Dept. of Cell Biology and Physiology, University of North Carolina School of Medicine, 111 Mason Farm Road, 6330 MBRB, Campus Box 7545, Chapel Hill, NC 27599, Phone: 919-966-5193, Fax: 919-966-5230

Alison Wise, BS[2] **[Graduate Student]**,
Department of Biostatistics, Gillings School of Global Public Health, 3104-D McGavran-Greenberg Hall, Campus Box 7420, Chapel Hill, NC 27599

Amy H. Herring, ScD[2,6] **[Professor]**,
Department of Biostatistics, Gillings School of Global Public Health, 3104-D McGavran-Greenberg Hall, Campus Box 7420, Chapel Hill, NC 27599, Phone: 919-843-6368, Fax: 919-966-3804

Thutrang Nguyen, BA[3] **[Supervisor]**,
Sequenom SNP Genotyping Facility, Boston Children's Hospital, 221 Massachusetts Ave, Apt. 708, Boston MA 02115, Phone: 617-645-1915

Kathleen M. Caron, PhD[1] **[Associate Professor]**, and
Dept. of Cell Biology and Physiology, University of North Carolina School of Medicine, 111 Mason Farm Road, 6340B MBRB, Campus Box 7545, Phone: 919-966-5215, Fax: 919-966-5230

Alison M. Stuebe, MD, MSc[4,5] **[Assistant Professor of Obstetrics and Gynecology]**
Division of Maternal-Fetal Medicine, 3010 Old Clinic Building, Campus Box 7516, Chapel Hill, NC 27599, Phone: 919-966-1601, Fax: 919-966-6377,

Patricia M. Lenhart: patricia_lenhart@med.unc.edu; Alison Wise: aswise@email.unc.edu; Amy H. Herring: amy_herring@unc.edu; Thutrang Nguyen: thutrang@alum.wellesley.edu; Kathleen M. Caron: kathleen_caron@med.unc.edu; Alison M. Stuebe: astuebe@med.unc.edu

Abstract

Objective—Reduced maternal plasma levels of the peptide vasodilator adrenomedullin have been associated with adverse pregnancy outcomes. We measured the extent to which genetic

Correspondence and reprint requests to: Alison Stuebe, MD, MSc, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, School of Medicine, University of North Carolina at Chapel Hill, 3010 Old Clinic Building, CD# 7516, Chapel Hill, NC 27599-7516. Phone: 919-966-1601. Fax: 919-966-6377, astuebe@med.unc.edu, and Kathleen M. Caron, PhD, Department of Cell Biology & Physiology, CB # 7545, 6340B MBRB, 111 Mason Farm Road, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27599. Tel: 919-966-5215, Fax: 919-966-5230, kathleen_caron@med.unc.edu.
[1]Department of Cell Biology & Physiology, The University of North Carolina, Chapel Hill, North Carolina, USA 27599
[2]Department of Biostatistics, Gillings School of Global Public Health, The University of North Carolina, Chapel Hill, North Carolina, USA 27599
[3]Division of Genetics and Endocrinology, Children's Hospital of Boston, Harvard Medical School, Boston, Massachusetts
[4]Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, North Carolina, USA 27599
[5]Department of Maternal and Child Health, Gillings School of Global Public Health, The University of North Carolina, Chapel Hill, North Carolina, USA 27599
[6]Carolina Population Center, Chapel Hill, North Carolina, USA 27599

Disclosure: The authors report no conflicts of interest.

Prior presentation: Preliminary findings were presented at the 33rd Annual Meeting of the Society for Maternal-Fetal Medicine, February 11–16, 2013, in San Francisco, CA.

polymorphisms in the adrenomedullin signaling pathway are associated with birth weight, glycemic regulation, and preeclampsia risk.

Study Design—We genotyped 1353 women in the Pregnancy, Infection, and Nutrition Postpartum Study for 37 ancestry-informative markers and for single-nucleotide polymorphisms (SNPs) in adrenomedullin (*ADM*), complement factor H variant (*CFH*), and calcitonin receptor-like receptor (*CALCRL*). We used linear and logistic regression to model the association between genotype and birth weight, glucose loading test (GLT) results, preeclampsia, and gestational diabetes (GDM). All models were adjusted for pregravid BMI, maternal age, and probability of Yoruban ancestry. P values of <0.05 were considered statistically significant.

Results—Among Caucasian women, *ADM* rs57153895, a proxy for rs11042725, was associated with reduced birth weight z-score. Among African-American women, *ADM* rs57153895 was associated with increased birth weight z-score. Two *CALCRL* variants were associated with GDM risk. *CFH* rs1061170 was associated with higher GLT results and increased preeclampsia risk.

Conclusion—Consistent with studies of plasma adrenomedullin and adverse pregnancy outcomes, we found associations between variants in the adrenomedullin signaling pathway and birth weight, glycemic regulation, and preeclampsia.

Keywords

adrenomedullin; genetics; gestational diabetes; preeclampsia; single nucleotide polymorphisms

Introduction

Adrenomedullin (ADM), a peptide hormone vasodilator, plays critical roles in female reproductive biology. Evidence from a wide range of *in vitro* experiments, rat and mouse models, as well as human studies strongly supports the fact that ADM is essential in the establishment and maintenance of a healthy pregnancy.[1] Throughout the course of a normal human pregnancy, ADM in the maternal plasma increases, peaking at levels three- to five-fold higher than in the nonpregnant state[2–7] by the third trimester. Precise regulation of maternal ADM levels may be necessary in healthy pregnancies, as complications including gestational diabetes,[8] preeclampsia,[7,9–13] and preterm labor[14–15] have all been associated with perturbations in ADM protein levels.

ADM mediates its effects via several signaling components, including its receptor, calcitonin receptor-like receptor (CALCRL), and complement factor H (CFH), a protein that binds directly to ADM and enhances its activity.[16] Several single-nucleotide polymorphisms (SNPs) in *ADM*, *CALCRL*, and *CFH* have been associated with health complications including dysglycemia[17] (*ADM* rs11042725), gestational hypertension[13] and decreased urinary sodium excretion[18] (*ADM* rs3814700), glaucoma[19] (*CALCRL* rs1157699, rs6759535, and rs840617), essential hypertension in women[20] (*CALCRL* rs696574), reduced birth weight[21] (*CALCRL* rs698576), age-related macular degeneration[22] (*CFH* rs1061170), and susceptibility to meningococcal disease[23] (*CFH* rs1065489). However, despite what is known about the important role of ADM in reproduction, few studies have addressed the association of genetic polymorphisms in *ADM* and its signaling partners with adverse pregnancy outcomes. Therefore, we sought to measure the extent to which SNPs in *ADM*, *CALCRL*, and *CFH* are associated with birth weight, glycemic regulation in pregnancy, and preeclampsia risk in a secondary analysis of a prospective cohort study among Caucasian and African-American women in central North Carolina.

Materials and Methods

The Pregnancy, Infection, and Nutrition (PIN) Cohort study comprises three prospective cohorts of more than 5000 women enrolled in early to mid-pregnancy. The study's primary goal was to identify risk factors for preterm birth in a prospective fashion. Participants enrolled in PIN1 and PIN2 were 24–29 weeks gestation at study entry, and were recruited from University of North Carolina Resident and Private Physician Obstetrics Clinic and the Wake County Department of Human Services and Wake Area Health Education Center prenatal care clinics from August 1995 through June 2000. Subjects enrolled in PIN3 were less than 20 weeks gestation at study entry and were recruited from the prenatal clinics at UNC hospitals from January 2001 to June 2005. The Institutional Review Board of the University of North Carolina at Chapel Hill approved the study. Women who consented for genetic studies and had extracted DNA available were eligible for inclusion in this analysis.

Maternal consent and DNA extracted from peripheral blood was available for 1480 pregnancies. We allowed for only one pregnancy during the study period. If data were available for multiple pregnancies (N=20), we included the pregnancy with the most complete SNP data (n = 1460). We further excluded participants with discordant self-reported race and ancestry estimates calculated from genotyped ancestry informative markers (n=5) or failed genotyping in >20% of the ancestry markers (N=64), leaving 1391 eligible participants. Finally, we excluded women who were missing data on pre-gravid BMI (n=38), leaving 1353 women available for analysis, of whom 940 were non-Hispanic Caucasian and 413 were non-Hispanic African-American.

Determination of pre-gravid BMI

Pre-gravid BMI was calculated based on self-reported pre-gravid weight and height at the first prenatal visit. Self-reported pre-gravid weights were examined for biological plausibility and imputed if deemed appropriate (<5% of weights were imputed) according to a previously described algorithm.[24] This imputed weight was calculated using the measured weight at the first prenatal visit (if taken prior to 15 weeks) minus the recommended amount of weight to be gained in the first and second trimesters as defined by the Institute of Medicine.[25]

Study covariates

The PIN datasets include information from telephone interviews, self-administered questionnaires, medical chart abstraction, and biological specimen collection. Information on race/ethnicity (non-Hispanic Caucasian, non-Hispanic African-American, and other) and maternal age was self-reported by the mother.

Outcome assessment

Birth weight z-score was determined using reference populations for sex and self-reported race.[26] Glucose homeostasis was evaluated using glucose loading test (GLT) screening results, which study participants underwent as part of routine clinical care at 24–29 weeks gestation. Trained abstractors ascertained gestational diabetes through prospective review of prenatal records. Participants with GLT values ≥140 mg/dL at UNC sites or ≥130 mg/dL at Wake County sites underwent a diagnostic 100g oral glucose tolerance test (OGTT). Individuals with 2 or more values above established cut points (fasting >95 mg/dL, 1 hour >180mg/dL, 2 hour >155 mg/dL, 3 hour >140mg/dL) were diagnosed with gestational diabetes.[27]

To ascertain preeclampsia and gestational hypertension, trained abstractors reviewed prenatal and intrapartum records. Prenatal records were abstracted according to the

following criteria. For PIN 1 and 2 (enrolled 1995–2000), gestational hypertension was defined by contemporary ACOG criteria as either SBP ≥ 140 mmHg, DBP ≥ 90 mmHg, or SBP increase ≥ 30 mmHg or DPB increase ≥15 mmHg over baseline measured at < 20 weeks' gestation. For PIN 3 (enrolled 2001–2005), gestational hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg with previously normal blood pressure. Preeclampsia was defined as gestational hypertension with a urine dipstick ≥ 1+ or ≥ 300 mg of protein in a 24-hour urine. Intrapartum records were reviewed for physician-recorded diagnosis of either isolated gestational hypertension or preeclampsia. In some cases, prenatal records and clinical intrapartum diagnoses were discordant. Among participants with discordant results for prenatal record and intrapartum physician diagnosis of preeclampsia or gestational hypertension (N = 125), we further reviewed the full intrapartum record. Gestational hypertension was defined as SBP ≥ 140 mmHg or DBP ≥ 90 mmHg, and preeclampsia was defined as gestational hypertension with concurrent proteinuria (≥ 300 mg protein in a 24 hour urine, dipstick ≥ 1+, or urine protein to creatinine ratio ≥ .14). Isolated gestational hypertension was defined as gestational hypertension in the absence of proteinuria.

Genotyping

Genotyping for 8 SNPs was performed at the Children's Hospital of Boston using the Sequenom iPLEX platform.[28] We genotyped SNPs in *ADM*, *CALCRL*, and *CFH* previously reported to be associated with adverse health outcomes.[13,17–23] All SNPs were tested for Hardy-Weinberg equilibrium among self-identified Caucasian participants using a threshold of $p < 0.001$.

Population stratification

In genetic association studies, differences in allele frequency among ethnic groups can confound relationships between genotype and disease outcome. To address population stratification in this cohort, genotyping was performed for 37 ancestry-informative markers that have been used successfully in other genetic association studies.[29] STRUCTURE was used to infer population substructure and assign individuals to populations using probabilistic clustering methods.[30] We analyzed self-identified Caucasian and African-American participants separately, and we included probability of Yoruban ancestry as a covariate among self-identified African-American women.

Statistical analysis

We used linear regression to model associations between maternal genotype and birth weight z-score and glucose loading test (GLT) results, adjusting for maternal age, pregravid BMI, and pregravid BMI squared. We included a quadratic term to allow for non-linear associations between pregravid BMI and outcomes of interest. We used maximum likelihood logistic regression to model associations between maternal genotype and diagnosis of gestational diabetes or preeclampsia, adjusting for maternal pregravid BMI and age. In both models, we considered the association of the risk allele to be additive.

We excluded from logistic regression analysis SNPs for which there were fewer than 5 cases among minor allele carriers. Because the purpose of our study was exploratory rather than confirmatory, adjustment for multiple comparisons was not performed. Thus in this pilot study, p values of <.05 were considered statistically significant.

Results

A total of 940 Caucasian and 413 African-American women were included in our analysis. African-American participants were younger, had a slightly higher pregravid BMI, and

delivered earlier than Caucasian participants (Table 1). Infants born to African-American mothers had lower birth weights and mean birth weight z-scores than infants born to Caucasian mothers (t-test $p < .01$). GLT results, GDM, and preeclampsia rates were similar in the two groups (Table 2).

Table 3 shows the associations between the ADM signaling pathway genetic variants of interest and pregnancy outcomes. Significant ($p<0.05$) associations are highlighted in bold in the table. Among Caucasian women, *ADM* rs57153895, a proxy for rs11042725, was associated with reduced birth weight z-score (−0.10 per G allele, 95% CI −0.19 to −0.01). Among African-American women, *ADM* rs57153895 was associated with increased birth weight z-score (0.18 per G allele, 95% CI 0.03 to 0.34). Two variants in *CALCRL*, rs69696574 and rs840617, were associated with increased GDM risk (rs69696574: OR 2.35 per C allele, 95% CI 1.04–5.27 and rs840617: OR per T allele, 2.66, 95% CI 1.13–6.25). *CFH* rs1061170 was associated with higher GLT results (4.39 mg/dL per T allele, 95% CI 0.22 to 8.57) and with increased preeclampsia risk (OR 2.02 per T allele, 95% CI 1.11–3.68). Table 4 summarizes these SNPs, the human diseases they have been previously associated with, and their significant associations with pregnancy outcomes in the current study.

Comment

Consistent with studies of plasma ADM and pregnancy complications, we found associations between variants in ADM signaling pathway components and birth weight, glycemic regulation, gestational diabetes, and preeclampsia. These findings support our hypothesis that genetic variants in *ADM*, its receptor, *CALCRL*, and its binding protein *CFH*, that have previously been shown to be associated with disease conditions are also associated with adverse pregnancy outcomes.

We found that an *ADM* variant previously associated with dysglycemia[17] in non-pregnant populations, rs57153895 (a proxy for rs11042725), was associated with birth weight. This variant had opposing effects depending on race, as it was associated with lower birth weight in Caucasian women but higher birth weight among African-American women, which may reflect differences in linkage disequilibrium patterns in Caucasian versus African-American women. The association between ADM and birth weight described here is consistent with previous studies that have shown a relationship between ADM and fetal growth. Antagonism of ADM has been shown to cause intrauterine growth restriction in the rat,[31,32] and female mice that are haploinsufficient for *Adm* have pregnancies with increased rates of fetal growth restriction.[33] Most recently, using *Adm* knockout mice, we have shown that fetal-derived ADM is required for the maternal vascular adaptation to pregnancy.[34] Alterations in ADM levels have also been associated with fetal growth in studies in human populations.[35–38] Our finding of a genetic variant in *ADM* associated with birth weight provides further evidence for the importance of maternal ADM in fetal growth.

Complement factor H, another important component of the ADM signaling pathway, binds to ADM and enhances its activity.[16] Here, we found that the *CFH* variant rs1061170, previously associated with age-related macular degeneration,[22] was associated with higher GLT results and increased preeclampsia risk among African-American women. Circulating levels of CFH have been shown to negatively correlate with insulin sensitivity in human subjects.[39] Consistent with this previous finding, our results suggest a role for CFH in glucose tolerance, which may be especially important during pregnancy. Furthermore, this *CFH* variant was also associated with increased preeclampsia risk, which is of particular interest given that dysregulated activation of complement is known to play a role in the pathogenesis of preeclampsia.[40]

CALCRL, as the G-protein coupled receptor for ADM, is an essential mediator of ADM's effects. A prior study showed an association between a *CALCRL* SNP and reduced birth weight in an African-American population,[21] demonstrating the importance of *CALCRL* variants in human pregnancy. Two of the *CALCRL* variants tested here, rs696574 and rs840617, were previously shown to be associated with essential hypertension in women[20] and glaucoma,[19] respectively. In the present study, we found evidence that CALCRL also plays a role in glycemic regulation during pregnancy, as both of *CALCRL* variant rs696574 and rs840617 were associated with increased gestational diabetes risk in African-American women.

Our study has several strengths, including our use of ancestry informative markers to control for population stratification and prospective ascertainment of outcomes within a pregnancy cohort. However, our results must be interpreted in the context of the study design. The small number of cases of GDM and preeclampsia led to large confidence intervals around estimates of association. In addition, pre-pregnancy weight was self-reported. Furthermore, multiple testing is a concern. We limited our analysis to variants known to be associated with disease outcomes in other studies, but it is possible that our findings are due to chance, and further studies will be needed to validate observed associations. Furthermore, future studies should be directed at determining the mechanisms by which these *ADM*, *CALCRL*, and *CFH* polymorphisms may alter ADM activity. An *ADM* SNP that was not part of our analysis has been shown to associate with lower plasma ADM levels.[41] Thus, it is biologically plausible that the SNPs included in our study may similarly affect ADM levels or alternatively, ADM activity through its signaling partners, CALCRL and CFH.

Taken together, these results support a central role for the ADM signaling pathway in human pregnancy and pregnancy complications. Future studies are needed to determine whether screening for maternal carriage of these SNPs could be used to identify women at risk for adverse pregnancy outcomes.

Acknowledgments

This study received support from the Eunice Kennedy Shriver National Institute of Child Health and Human Development (HD28684, HD28684A, HD37584, HD39373, HD060860), the National Institute of Diabetes and Digestive and Kidney Diseases (DK61981, DK56350), the National Cancer Institute (CA109804-01), National Institute of Health General Clinical Research Center (RR00046), the Carolina Population Center, the UNC University Research Council and the Nutrition and Obesity Research Council (DK056350). Dr. Stuebe receives support from the WRHR Career Development Center at UNC (5K12HD050113). Dr. Caron receives support from The Burroughs Wellcome Fund and the March of Dimes Foundation. Ms. Lenhart received support from the National Heart, Lung, and Blood Institute (F30HL104778) and Ms. Wise received support from the National Institute of Environmental Health Sciences (T32ES007018).

We would also like to thank all of the PIN investigators that are not coauthors (David Savitz, John Thorp, Kelly Evenson, and Nancy Dole) for their help in obtaining funding and designing the study, and we would like to thank Kathryn Carrier for technical assistance. Additionally, we would like to thank the field collection staff for their dedication and hard work, and we would like to thank all the families involved in the PIN studies for their participation.

Abbreviations

SNP	single nucleotide polymorphism
ADM	adrenomedullin
CALCRL	calcitonin receptor-like receptor
CFH	complement factor H

GDM	gestational diabetes mellitus
PIN	Pregnancy, Infection, and Nutrition
BMI	body mass index
GLT	glucose loading test
OGTT	oral glucose tolerance test
SBP	systolic blood pressure
DBP	diastolic blood pressure

References

1. Lenhart PM, Caron KM. Adrenomedullin and pregnancy: perspectives from animal models to humans. Trends Endocrinol Metab. 2012; 23(10):524–32. [PubMed: 22425034]
2. Minegishi T, Nakamura M, Abe K, et al. Adrenomedullin and atrial natriuretic peptide concentrations in normal pregnancy and pre-eclampsia. Mol Hum Reprod. 1999; 5(8):767–70. [PubMed: 10421805]
3. Hayashi Y, Ueyama H, Mashimo T, Kangawa K, Minamino N. Circulating mature adrenomedullin is related to blood volume in full-term pregnancy. Anesth Analg. 2005; 101(6):1816–20. [PubMed: 16301265]
4. Di Iorio R, Marinoni E, Scavo D, Letizia C, Cosmi EV. Adrenomedullin in pregnancy. Lancet. 1997; 1;349(9048):328.
5. Kobayashi K, Kubota T, Aso T, Hirata Y, Imai T, Marumo F. Immunoreactive adrenomedullin (AM) concentration in maternal plasma during human pregnancy and AM expression in placenta. Eur J Endocrinol. 2000; 142(6):683–7. [PubMed: 10822233]
6. Hoshimoto K, Hayashi M, Ohkura T. Mature adrenomedullin concentrations in plasma during pregnancy. J Matern Fetal Neonatal Med. 2002; 11(2):126–9. [PubMed: 12375542]
7. Senna AA, Zedan M, el-Salam GE, el-Mashad AI. Study of plasma adrenomedullin level in normal pregnancy and preclampsia. Medscape J Med. 2008; 6;10(2):29.
8. Di Iorio R, Marinoni E, Urban G, Costantini A, Cosmi EV, Letizia C. Fetomaternal adrenomedullin levels in diabetic pregnancy. Horm Metab Res. 2001; 33(8):486–90. [PubMed: 11544563]
9. Hata T, Miyazaki K, Matsui K. Decreased circulating adrenomedullin in pre-eclampsia. Lancet. 1997; 29;350(9091):1600.
10. Di Iorio R, Marinoni E, Letizia C, Alò P, Villaccio B, Cosmi EV. Adrenomedullin, a new vasoactive peptide, is increased in preeclampsia. Hypertension. 1998; 32(4):758–63. [PubMed: 9774376]
11. Lauria MR, Standley CA, Sorokin Y, Yelian FD, Cotton DB. Adrenomedullin levels in normal and preeclamptic pregnancy at term. J Soc Gynecol Investig. 1999; 6(6):318–21.
12. Dikensoy E, Balat O, Pence S, Balat A, Cekmen M, Yurekli M. The changes of plasma malondialdehyde, nitric oxide, and adrenomedullin levels in patients with preeclampsia. Hypertens Pregnancy. 2009; 28(4):383–9. [PubMed: 19843001]
13. Boć-Zalewska A, Seremak-Mrozikiewicz A, Barlik M, Kurzawińska G, Drews K. The possible role of adrenomedullin in the etiology of gestational hypertension and preeclampsia. Ginekol Pol. 2011; 82(3):178–84. [PubMed: 21721459]
14. Marinoni E, Di Iorio R, Letizia C, Villaccio B, Alberini A, Cosmi EV. Amniotic fluid concentrations of adrenomedullin in preterm labor. Obstet Gynecol. 1999; 93(6):964–7. [PubMed: 10362163]
15. Di Iorio R, Marinoni E, Letizia C, et al. Influence of labor on fetoplacental adrenomedullin concentrations. Am J Obstet Gynecol. 2001; 185(3):697–702. [PubMed: 11568800]
16. Pio R, Martinez A, Unsworth EJ, et al. Complement factor H is a serum-binding protein for adrenomedullin, and the resulting complex modulates the bioactivities of both partners. J Biol Chem. 2001; 13;276(15):12292–300.

17. Ong KL, Tso AW, Leung RY, et al. A genetic variant in the gene encoding adrenomedullin predicts the development of dysglycemia over 6. 4 years in Chinese. Clin Chim Acta. 2011; 30;412(3–4):353–7.

18. Li Y, Staessen JA, Li LH, et al. Blood pressure and urinary sodium excretion in relation to the A-1984G adrenomedullin polymorphism in a Chinese population. Kidney Int. 2006; 69(7):1153–8. [PubMed: 16609682]

19. Cao D, Liu X, Guo X, Cong Y, Huang J, Mao Z. Investigation of the association between CALCRL polymorphisms and primary angle closure glaucoma. Mol Vis. 2009; 27;15:2202–8.

20. Sano M, Kuroi N, Nakayama T, et al. Association study of calcitonin-receptor-like receptor gene in essential hypertension. Am J Hypertens. 2005; 18(3):403–8. [PubMed: 15797661]

21. Wang L, Wang X, Laird N, Zuckerman B, Stubblefield P, Xu X. Polymorphism in maternal LRP8 gene is associated with fetal growth. Am J Hum Genet. 2006; 78(5):770–7. [PubMed: 16642433]

22. Haines JL, Hauser MA, Schmidt S, et al. Complement factor H variant increases the risk of age-related macular degeneration. Science. 2005; 15;308(5720):419–21.

23. Davila S, Wright VJ, Khor CC, et al. Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease. Nat Genet. 2010; 42(9): 772–6. [PubMed: 20694013]

24. Saldana TM, Siega-Riz AM, Adair LS. Effect of macronutrient intake on the development of glucose intolerance during pregnancy. Am J Clin Nutr. 2004; 79(3):479–486. [PubMed: 14985225]

25. Institute of Medicine. Nutrition during pregnancy. Washington, DC: National Academies Press; 1990.

26. Oken E, Kleinman KP, Rich-Edwards J, Gillman MW. A nearly continuous measure of birth weight for gestational age using a United States national reference. BMC Pediatr. 2003; 8;3:6.

27. Carpenter MW, Coustan DR. Criteria for screening tests for gestational diabetes. Am J Obstet Gynecol. 1982; 144(7):768–773. [PubMed: 7148898]

28. Campbell CD, Lyon HN, Nemesh J, et al. Association studies of BMIand type II diabetes in the neuropeptide Y pathway: a possible role for NPY2R as a candidate gene for type 2 diabetes in men. Diabetes. 2007; 56:1460–1467. [PubMed: 17325259]

29. Gajdos ZK, Butler JL, Henderson KD, et al. Association studies of common variants in 10 hypogonadotropic hypogonadism genes with age at menarche. J Clin Endocrinol Metab. 2008; 93(11):4290–4298. [PubMed: 18728166]

30. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155(2):945–959. [PubMed: 10835412]

31. Witlin AG, Li ZY, Wimalawansa SJ, Grady JJ, Grafe MR, Yallampalli C. Placental and fetal growth and development in late rat gestation is dependent on adrenomedullin. Biol Reprod. 2002; 67(3):1025–31. [PubMed: 12193417]

32. Penchalaneni J, Wimalawansa SJ, Yallampalli C. Adrenomedullin antagonist treatment during early gestation in rats causes fetoplacental growth restriction through apoptosis. Biol Reprod. 2004; 71(5):1475–83. [PubMed: 15229133]

33. Li M, Yee D, Magnuson TR, Smithies O, Caron KM. Reduced maternal expression of adrenomedullin disrupts fertility, placentation, and fetal growth in mice. J Clin Invest. 2006; 116(10):2653–62. [PubMed: 16981008]

34. Li M, Schwerbrock NMJ, Lenhart PM, et al. Fetal-derived adrenomedullin mediates the innate immune milieu of the placenta. J Clin Invest. 2013 In Press.

35. Di Iorio R, Marinoni E, Letizia C, Gazzolo D, Lucchini C, Cosmi EV. Adrenomedullin is increased in the fetoplacental circulation in intrauterine growth restriction with abnormal umbilical artery waveforms. Am J Obstet Gynecol. 2000; 182:650–654 50. [PubMed: 10739524]

36. Yamashiro C, Kanenishi K, Akiyama M, Tanaka H, Shiota A, Hata T. Adrenomedullin concentrations in early 2nd-trimester amniotic fluid: relation to preterm delivery and fetal growth at birth. Gynecol Obstet Invest. 2002; 54:99–104 51. [PubMed: 12566752]

37. Akturk A, Onal EE, Atalay Y, et al. Maternal and umbilical venous adrenomedullin and nitric oxide levels in intrauterine growth restriction. J Matern Fetal Neonatal Med. 2007; 20:521–525 52. [PubMed: 17674265]

38. Yamashiro C, Hayashi K, Yanagihara T, Hata T. Plasma adrenomedullin levels in pregnancies with appropriate for gestational age and small for gestational age infants. J Perinat Med. 2001; 29:513–518. [PubMed: 11776682]

39. Moreno-Navarrete JM, Martínez-Barricarte R, Catalán V, et al. Complement factor H is expressed in adipose tissue in association with insulin resistance. Diabetes. 2010; 59(1):200–9. [PubMed: 19833879]

40. Lynch AM, Salmon JE. Dysregulated complement activation as a common pathway of injury in preeclampsia and other pregnancy complications. Placenta. 2010; 31(7):561–7. [PubMed: 20427084]

41. Cheung BM, Ong KL, Tso AW, et al. Plasma adrenomedullin level is related to a single nucleotide polymorphism in the adrenomedullin gene. Eur J Endocrinol. 2011; 165:571–577. [PubMed: 21798961]

Table 1

Study population

	Non-Hispanic Caucasian	Non-Hispanic African-American
N	940	413
Maternal age (mean (SD))	28.3 (6.1)	24.0 (5.4)
Pregravid BMI (mean (SD))	25.1 (6.5)	27.8 (8.3)
Gestational age at birth, median (IQR)	39.3 (37.7, 40.3)	38.9 (36.7,40.1)

Table 2

Outcomes of interest

	N*	Non-Hispanic Caucasian	N*	Non-Hispanic African-American
		Mean (SD)		**Mean (SD)**
Birth weight, g	933	3275 (651)	402	2953 (733)
Birth weight z score	933	0.002 (1.00)	398	−0.460 (1.04)
GLT, mg/dL	842	108.5 (26.3)	366	105.9 (31.7)
		N (%)		N (%)
GDM	899	56 (6.2)	386	24 (6.2)
Preeclampsia	940	47 (5.0)	413	30 (7.3)

*Number of participants with outcome data available

Table 3

Associations between genetic variants in the adrenomedullin pathway and pregnancy outcomes

Gene	SNP_ID	Ref	Other	Other allele fraction	Birth weight z-score[1]	GLT (mg/dL)*	GDM OR†	Preeclampsia OR†
Caucasian								
ADM §	rs57153895	A	G	0.48	**−0.10 (−0.19, −0.01)**	−0.64 (−3.10, 1.81)	1.05 (0.72–1.54)	1.5 (0.69–2.59)
ADM §	rs7935242	T	C	0.52	0.08 (−0.01, 0.17)	0.81 (−1.68, 3.30)	0.98 (0.67–1.45)	0.95 (0.62–1.44)
CALCRL	rs1157699	C	T	0.32	−0.03 (−0.12, 0.07)	−1.95 (−4.54, 0.64)	0.79 (0.51–1.22)	1.39 (0.91–2.13)
CALCRL	rs6759535	T	C	0.54	−0.07 (−0.16, 0.02)	−0.50 (−2.97, 1.97)	0.80 (0.54–1.17)	1.09 (0.72–1.67)
CALCRL	rs696574	T	C	0.85	0.04 (−0.08, 0.17)	1.38 (−2.11, 4.87)	1.45 (0.79–2.66)	0.78 (0.46–1.35)
CALCRL	rs840617	A	T	0.85	0.04 (−0.09, 0.16)	1.49 (−1.98, 4.95)	1.42 (0.78–2.61)	0.89 (0.50–1.57)
CFH	rs1061170	C	T	0.59	−0.02 (−0.11, 0.08)	0.37 (−2.17, 2.90)	1.14 (0.77–1.71)	1.29 (0.83–2.00)
CFH	rs1065489	G	T	0.17	−0.06 (−0.18, 0.06)	−0.19 (−3.47, 3.09)	1.37 (0.85–2.21)	1.30 (0.77–2.20)
African-American‡								
ADM §	rs57153895	A	G	0.29	**0.18 (0.03, 0.34)**	3.83 (−0.9, 8.57)	1.42 (0.78–2.59)	0.70 (0.38–1.32)
ADM §	rs7935242	T	C	0.77	−0.16 (−0.33, 0)	−4.18 (−9.2, 0.84)	0.77 (0.40–1.50)	1.11 (0.58–2.10)
CALCRL	rs1157699	C	T	0.16	0.03 (−0.15, 0.22)	−3.53 (−8.96, 1.89)	1.26 (0.65–2.44)	0.42 (0.16–1.11)
CALCRL	rs6759535	T	C	0.42	−0.06 (−0.2, 0.09)	−3.96 (−8.27, 0.35)	0.70 (0.38–1.27)	0.68 (0.39–1.18)
CALCRL	rs696574	T	C	0.71	0.00 (−0.16, 0.16)	0.16 (−4.87, 5.19)	**2.35 (1.04–5.27)**	0.66 (0.38–1.17)
CALCRL	rs840617	A	T	0.71	−0.01 (−0.17, 0.15)	0.41 (−4.52, 5.34)	**2.66 (1.13–6.25)**	0.68 (0.39–1.21)
CFH	rs1061170	C	T	0.61	−0.08 (−0.22, 0.06)	**4.39 (0.22, 8.57)**	1.80 (0.95–3.42)	**2.02 (1.11–3.68)**
CFH	rs1065489	G	T	0.05	−0.03 (−0.34, 0.28)	6.17 (−3.43, 15.77)	--	--

Bold indicates $p < 0.05$

*Linear regression model, effect estimate (95% CI) per other allele, adjusting for pregravid BMI, BMI squared, and maternal age

†Logistic regression model, OR (95% CI) per other allele, adjusting for pregravid BMI and maternal age

‡Adjusted for probability of Yoruban ancestry

§Proxies for *ADM* rs11042725

--Excluded from logistic regression due to < 5 cases among women carrying one or two copies of the minor allele

Table 4

Summary of *ADM*, *CALCRL*, and *CFH* SNPs, their previously reported disease associations, and significantly associated pregnancy complications in the current study

SNP	Previously Reported Disease Association	Association with Pregnancy Outcomes
ADM rs57153895	Dysglycemia[17]	Decreased birth weight (Caucasian) Increased birth weight (African-American)
CLR rs696574	Essential hypertension in women[20]	Increased gestational diabetes mellitus risk (African-American)
CLR rs840617	Glaucoma[19]	Increased gestational diabetes mellitus risk (African-American)
CFH rs1061170	Age-related macular degeneration[22]	Higher glucose loading test result (African-American) Increased preeclampsia risk (African-American)