Maternal Interleukin Genotypes Are Associated With NICU Outcomes Among Low-Birth-Weight Infants

Kelley L. Baumgartel, PhD, Maureen W. Groer, PhD, Susan M. Cohen, DNS, FAAN, Dianxu Ren, MD, PhD, Diane L. Spatz, PhD, and Yvette P. Conley, PhD

1Health Promotion & Development, School of Nursing, University of Pittsburgh, Pittsburgh, PA, USA
2College of Nursing, University of South Florida, Tampa, FL, USA
3College of Medicine Internal Medicine, University of South Florida, Tampa, FL, USA
4School of Nursing, University of Pittsburgh, Pittsburgh, PA, USA
5School of Nursing, University of Pennsylvania, Philadelphia, PA, USA

Abstract

Background—Maternal interleukin (IL) single nucleotide polymorphisms (SNPs) are associated with obstetrical outcomes. Conversely, infant SNPs are associated with subsequent neonatal intensive care unit (NICU) outcomes. Little is known about relationships between maternal SNPs and neonatal outcomes.

Purpose—To examine the relationships between maternal IL genotypes and neonatal outcomes.

Methods—An ancillary study was conducted among mothers (N = 63) who delivered very low-birth-weight infants (N = 74). Maternal DNA was extracted from breast milk and genotyped. Outcomes included fecal calprotectin, length of stay, scores for neonatal acute physiology with perinatal extension (SNAPPE-II), weight gain, oxygen needs, necrotizing enterocolitis, intraventricular hemorrhage, sepsis, retinopathy of prematurity, blood transfusions, and feeding intolerance. Multivariate analyses examined the relationships between maternal IL SNPs and outcomes, controlling for gestational age and the ratio of maternal milk to total milk.
Results—Absence of a minor allele in 2 IL6 SNPs was associated with fecal calprotectin ($p = .0222$, $p = .0429$), length of stay ($p = .0158$), SNAPPE-II ($p = .0497$), weight gain ($p = .0272$), and days on oxygen ($p = .0316$). IL6 genotype GG (rs1800795) was associated with length of stay ($p = .0034$) and calprotectin ($p = .0213$). Minor-allele absence in 2 IL10 SNPs was associated with days on oxygen ($p = .0320$). There were associations between IL10 genotype TT (rs1800871) and calprotectin ($p = .0270$) and between IL10 genotypes AA (rs1800872 and rs1800896) and calprotectin ($p = .0158$, $p = .0045$).

Conclusion—Maternal IL SNPs are associated with NICU outcomes. A potential clinical application includes an antenatal risk profile to identify neonatal needs.

Keywords

prematurity; single nucleotide polymorphism; interleukin; calprotectin; SNAPPE-II; human milk

Prematurity-related complications are the leading cause of death for children under 5 years old, and preterm births continue to increase worldwide (World Health Organization, n.d.). Complications from prematurity include, but are not limited to, retinopathy, necrotizing enterocolitis (NEC), and respiratory distress, and the associated stays in the neonatal intensive care unit (NICU) cost the U.S. health-care system more than US $26 billion annually (Behrman & Butler, 2007).

Particular maternal cytokines have been implicated in obstetrical complications including preterm birth (Neta et al., 2010; Sorokin et al., 2010) and small-for-gestational-age (SGA) infant (Neta et al., 2010). Cytokines are an integral part of the inflammatory cascade. Interleukin (IL)-4, a proinflammatory cytokine, induces both antibody and immunoglobulin E production. Single nucleotide polymorphisms (SNPs) in the promoter region of the IL4 gene have been associated with varying levels of IL-4 (Cabantous et al., 2009; Cabantous et al., 2015; Nguyen et al., 2004); furthermore, particular maternal IL4 genotypes are associated with obstetrical complications, including preterm birth (Harmon et al., 2013) and infant SGA (Engel et al., 2005). IL-6 is a pleiotropic cytokine that is poorly regulated in preterm infants (Currie et al., 2011) and induces monoclonal antibody growth. SNPs in the promoter region of the IL6 gene influence IL-6 levels (Malarstig, Lindahl, Wallentin, & Siegbahn, 2006), and altered maternal IL-6 production is associated with obstetrical complications, including preterm labor (Chaemsaiithong et al., 2016; Lee et al., 2007). Particular maternal genetic variations of IL6 are also associated with spontaneous preterm birth (Moura et al., 2009) and SGA among term infants (Harmon et al., 2014). IL-10 is a regulatory cytokine, and SNPs in the promoter region of the IL10 gene are associated with IL-10 levels (Capasso et al., 2007; Lowe, Galley, Abdel-Fattah, & Webster, 2003; Qaddourah et al., 2014; Yilmaz, Yentur, & Saruhan-Direskeneli, 2005). Maternal IL10 genetic variation has been associated with pregnancy loss (Cochery-Nouvellon et al., 2009; Qaddourah et al., 2014), perhaps due to the role of IL-10 in maintaining maternal–fetal tolerance (Denney et al., 2011).

Much of the work surrounding maternal IL polymorphisms and/or IL levels focuses on obstetrical outcomes. To our knowledge, there has never been a study that examined the relationship between maternal IL4, IL6, and/or IL10 genotypes and subsequent NICU
complications. The purpose of this study was to examine the relationships between maternal IL4, IL6, and IL10 genotypes and NICU outcomes. The potential utility of this research includes infant prognoses and precision care for high-risk infants.

**Material and Method**

**Subjects**

This ancillary study included women (N = 64) who delivered infants (N = 73, including multiples) with a birth weight <1,500 g at Tampa General Hospital (Tampa, FL; Groer et al., 2014). The purpose of the parent study was to examine health outcomes of preterm infants in relationship to the volume of maternal milk and exposure to cytokines, chemokines, and growth factors. Mothers with HIV and infants with major congenital anomalies were excluded from enrollment. Also excluded were infants whom the neonatologist determined to be too critical to survive. The University of South Florida’s Institutional Review Board (IRB) approved all aspects of the parent study. We obtained material transfer permission from the University of Pittsburgh prior to shipping samples from the University of South Florida College of Nursing to the University of Pittsburgh and obtained separate IRB approval from the University of Pittsburgh, which added genomic data collection to the parent project.

The following maternal variables had been collected by the parent-study research staff and were available for analyses: maternal age, parity, income, education, ethnicity, race, marital status, working status, and pregnancy history. Medical records provided information about the labor and delivery of the infant(s), and the following information about infants were collected from the NICU medical record: sex, ethnicity, gestational age at birth, birth weight, Apgar scores, scores for neonatal acute physiology with perinatal extension-II (SNAPPE-Is), ratio of mother’s own milk to total milk administered, length of stay, weight gain at 6 weeks, and days on oxygen. Dichotomous NICU outcome variables included sepsis, retinopathy of prematurity, NEC, intraventricular hemorrhage, blood transfusions, and feeding intolerance. Infants were enrolled in the study as soon as possible after NICU admission. Weekly maternal milk and infant stool samples were collected in tandem longitudinally, and we included 3 weeks of data in this ancillary study.

**DNA Extraction and Genotyping**

Maternal genomic DNA was extracted from breast milk whey using Qiagen DNA Extraction Mini Kit (Baumgartel, 2016). We collected genotype data using TaqMan allele discrimination assays to genotype seven functional promoter polymorphisms of IL4 (rs2070874, rs2243250), IL6 (rs1800795, rs1800796), and IL10 (rs1800871, rs1800872, rs1800896). We performed TaqMan allelic discrimination with the ABI Prism 7000 Sequence Detection System and SDS software v1.2.3 (Applied Biosystems Inc., Carlsbad, CA). We included negative controls and repeated a portion of the samples to confirm that they repeatedly discriminated into the same genotype. We also included duplicates and performed independent blinded double calls. For each SNP, we evaluated Hardy–Weinberg equilibrium (HWE), reexamining blinded raw data for the SNPs where HWE was violated to rule out genotyping error.
Infant Fecal Calprotectin

Researchers have used fecal calprotectin as a biomarker of inflammation within the preterm population, as calprotectin is an accurate indicator of neutrophil migration toward the gastrointestinal (GI) tract (Kapel et al., 2005). Weekly stool samples were stored at room temperature until they were transported to the laboratory for processing and frozen at −80°C until analysis (Groer, Ashmeade, Louis-Jacques, Beckstead, & Ming, 2016). Investigators weighed out 100 mg of stool, placed it in a 15-ml conical tube, and agitated it with a wooden stirrer. Extraction buffer was added and the sample vortexed to form a fine slurry, then placed on a shaker for 25 min. From this slurry, 1 ml was removed and centrifuged at 10,000 g for 20 min. The supernatant was removed for analysis by ELISA (Calprest, Eurospital, Trieste, Italy). Calprotectin was expressed as microgram/gram of stool. Every assay included a standard curve and quality controls, and all samples were done in duplicate. Intra-assay coefficient of variation was 7.7%.

SNAPPE-II

SNAPPE-II is a physiology-based admission score that uses neonatal vital signs and blood results to characterize neonatal mortality risk within the first 12 hr of life (Richardson, Cocoran, Escobar, & Lee, 2001). SNAPPE-II is an extension of the score for neonatal acute physiology-II and has a higher discrimination than that score. University of South Florida College of Nursing research staff calculated the SNAPPE-II for each participating infant from data available in the medical record.

Statistical Analyses

We performed all statistical analyses using SAS (v. 9.4). We assessed univariate outliers using frequency tables and graphical methods including histograms and normal probability plots and multivariate outliers using scatterplots. We assessed missing data for both amount (percentage) and pattern (random vs. nonrandom). In addition to the Shapiro–Wilk test, we evaluated normality graphically and at each weekly time point with frequency histograms and normal probability plots. To assess linearity, independence, and homoscedasticity, we evaluated bivariate scatterplots.

We first performed univariate analyses for each association and included any relationship with a p value ≤ .20 in multivariate regression models, where we considered p ≤ .05 significant. To examine the relationships between maternal SNPs and categorical infant outcomes, we used Fisher’s exact test. Due to the small sample size and the need for subset analyses based on race/ethnicity, we included analyses for minor-allele absence. We examined multivariate models that included the total population using both minor-allele absence and genotype as an independent variable. We chose this method because including the total population increased our sample size, giving more power to examine genotype-specific relationships. Multivariate analyses controlled for both gestational age and the ratio of maternal milk to total milk administered. We examined continuous outcomes using multiple linear regression and binary outcomes with multiple logistical regression.
Results

The average maternal age of participants was 28.3 years, and more of the mothers identified themselves as African American (39.7%) than any other race or ethnicity (see Table 1 for maternal demographic and delivery characteristics). The average gestational age of infants at delivery was 28 weeks, and average birth weight was 1,069 g (see Table 2 for infant characteristics and NICU outcomes). The cesarean section rate was approximately 76%. Three SNPs violated HWE: rs2070874, rs2243250, rs1800796 (see Table 3 for genotype frequencies and HWE). The mean day of life for entry into Week 1 of data collection was 5.4 ± 0.04 days and the mean corrected gestational age at that time was 29 ± 0.36 weeks.

When controlling for gestational age at delivery and ratio of maternal milk to total milk received, we found a significant association between IL6 SNP (rs1800795) minor-allele absence and the number of days on oxygen (p = .0316) in the total population (see Table 4). Minor-allele absence for IL6 (rs1800795) was also associated with fecal calprotectin at Week 2 (p = .0222), though only among Caucasians. Additionally, among the total population, there were significant inverse relationships between IL6 SNP (rs1800796) genotype GG and length of stay (p = .0034) and fecal calprotectin at Week 3 (p = .0213). Among Caucasians, there was a significant association between IL6 (rs1800796) minor-allele absence and calprotectin at Week 3 (p = .0429). Among African Americans, there were significant relationships between IL6 (rs1800796) minor-allele absence and length of stay (p = .0158) and SNAPPE-II (p = .0497). There was a significant relationship among Hispanics between IL6 (rs1800796) minor-allele absence and weight at 6 weeks of life (p = .0272).

When examining the relationships between maternal IL10 and infant outcomes, we found a significant relationship between rs1800871 genotype TT and Week 3 calprotectin in the total population (p = .0270). We also found a significant association between IL10 (rs1800872) minor-allele absence among Caucasians and Week 1 calprotectin (p = .0196). We observed a similar relationship in the total population but with genotype AA and Week 3 calprotectin (p = .0158). There was a significant association between IL10 (rs1800896) minor-allele absence and days on oxygen (p = .0320) among African Americans. Within the total population, there were significant associations between both IL10 (rs1800896) genotype AA and minor-allele absence and Week 2 calprotectin (p = .0045 and p = .0057, respectively).

Discussion

Our findings in the present study suggest that maternal IL SNPs may predict NICU outcomes in very low-birth-weight (VLBW) infants. Previous work has examined the relationships between maternal IL SNPs and obstetrical complications (Engel et al., 2005; Harmon et al., 2014; Moura et al., 2009; Qaddourah et al., 2014; Sowmya et al., 2014). Other studies have focused on the NICU infant’s IL genotype/s and subsequent outcomes, including respiratory distress syndrome (RDS; Capasso et al., 2007; Li et al., 2015; Shen, Du, Wang, & Zeng, 2014), abnormal periventricular ultrasound findings (Dordelmann et al., 2006), and bronchopulmonary dysplasia (Yanamandra, Boggs, Loggins, & Baier, 2005). Similar to our work, which examined the relationship between maternal genotype and infant...
outcome, Pogliani, Muggiasca, Arrigoni, Rossi, and Zuccotti (2010) examined maternal methylenetetrahydrofolate reductase (MTHFR) genotypes and subsequent neonatal cerebral lesions. The authors reported that perinatal prothrombotic disorders are different in their presentation and suggested that maternal genotyping of MTHFR may predict outcomes. Chaemsaithong et al. (2016) suggested that a bedside test that measures IL-6 in amniotic fluid from an amniocentesis may identify inflammation among women who subsequently experience preterm labor. Both of these studies are exemplars of antenatal identification of complications, and the present study further supports this approach.

Infection and inflammation are probable biological determinants of preterm birth (Brown, Speechley, Macnab, Natale, & Campbell, 2015; Romero et al., 2006), suggesting that infants born preterm were in an inflammatory-enhanced environment in utero. This presumption is especially relevant when examining the relationship between IL6 SNPs and SNAPPE-II scores. The infant's vital signs and lab results used to calculate SNAPPE-II scores are collected within hours of birth, thus this score may reflect the immediately preceding in utero environment. Previous work has identified relationships between IL6 SNPs and SGA infants (Harmon et al., 2014) and preterm birth (Moura et al., 2009; Sugita et al., 2012; Velez, Fortunato, Williams, & Menon, 2008). Interestingly, both Velez, Fortunato, Williams, and Menon (2008) and Harmon et al. (2014) uncovered these relationships only among African Americans, an observation that our findings share. IL-6 is a pleiotropic cytokine that may influence the ability of the placenta to become adequately implanted (Conde-Agudelo, Romero, Kusanovic, & Hassan, 2011), and higher levels of IL-6 are associated with preterm labor (Chaemsaithong et al., 2016). The maternal IL6 SNP associated with SNAPPE-II scores is a functional SNP, with CG genotypes associated with higher IL-6 levels (Malarstig, Wallentin, & Siegbahn, 2007). IL-6 production during pregnancy may influence neonatal outcomes, and mortality risk may be reflected in the SNAPPE-II score, as we observed in the African American subset. NICU length of stay was also associated with this SNP in both African Americans and the total study population.

Maternal IL SNPs were also associated with infant oxygen requirements in the present study, and recent evidence suggests that elevated umbilical cord IL-6 levels are positively associated with RDS (Sorokin et al., 2014). Tracheal aspirate IL-6 levels are used as a biomarker for lung inflammation in ventilated preterm infants (Lista et al., 2008). The underlying pathophysiology of respiratory deterioration in the NICU may implicate IL-6, and our findings suggest that antenatal identification of infant risk may inform postnatal care plans. For example, delayed cord clamping (45 s) is associated with lower rates of RDS in preterm infants (Chiruvolu et al., 2015). The NICU team is usually present for preterm infant births, and this simple and promising intervention can be prioritized for women with genotypes associated with high-acuity NICU respiratory needs.

Infant fecal calprotectin was frequently associated with maternal IL alleles, with significant relationships found at all 3 weeks and in all but the Hispanic subset. Preterm infants with NEC symptoms experience a transient rise in fecal calprotectin when compared with preterm infants of the same gestational age without NEC (Campeotto et al., 2007). The identification of infants who are at risk for a hyperinflamed GI environment may inform the NICU nurse's care plan to minimize this inflammation, including an exclusive human milk diet.
Maternal milk provides an extrauterine immunological link to preterm infants and contains each of the three ILs examined in this study. Variable exogenous IL levels from human milk could help explain the disparity we observed in infant outcomes, particularly the calprotectin findings. The relationship between maternal IL SNPs and longitudinal NICU outcomes among the present cohort of VLBW infants receiving human milk suggests that milk composition may mediate the relationship between maternal SNPs and neonatal outcomes. This possibility is especially relevant now because more donor milk is being used in the NICU setting, and one feeding represents milk from multiple mothers. It may be that the relationships we uncovered in this study are a result of the effects of maternal SNPs on IL production that influenced the milk's immunological profile. Future studies should examine appropriate pathways of bioactive milk components and their influence on neonatal outcomes. The unique symbiotic nature of the mother and the human milk–fed preterm infant suggests that we examine both as a dyad rather than as separate entities.

**Limitations**

There were several limitations to this study, including a small sample size. Due to differences in allele frequencies across races, we further decreased our power by doing subgroup analyses of Caucasians, Hispanics, and African Americans, although for an exploratory study, we believe this subgroup analysis was necessary. Much of this study is based on self-reported variables, including ethnicity. Self-reported ethnicity does not adequately capture inherent biological differences, and ancestral markers are a more reliable way of obtaining biologically relevant information that accounts for admixture (Yaeger et al., 2008). Additionally, the cesarean section rates were high (nearly 76%), though this finding is similar to that in a recent study of a nearly 71% cesarean section rate among VLBW infants (Griffin, Lee, Profit, & Tancedi, 2015).

HWE was violated for three SNPs (rs2070874, rs2243250, and rs1800796). We were able to eliminate genotyping error; therefore, we believe HWE violation was due to a biased sample of women who delivered preterm infants, which enriched for the alleles under investigation. The SNPs included in this study have been implicated in a variety of obstetrical complications including SGA (rs2070874 and rs2243250; Engel et al., 2005), spontaneous preterm birth (rs1800795; Wu et al., 2013), and pregnancy loss (rs1800871 and rs1800872; Cochery-Nouvellon et al., 2009).

**Relevance to Nursing Practice**

Nurses are well positioned to implement precision evidence-based bedside techniques. This study suggests that maternal IL genotypes play a role in clinical outcomes among VLBW infants. Findings from this study may contribute to the development of a specific antenatal risk profile of high-risk obstetrical patients that informs the neonatal nurse’s care plan. In addition, this study provides further evidence that nurses and other health professionals should have an understanding of how human milk protects infants. This research also has implications for considering the use of donor milk in the NICU. Additionally, since breast milk may be influenced by maternal genotypes, findings from this study may impact how donor milk is batched at milk banks for pasteurization. Perhaps more global to the impact of...
this study’s findings on nursing is the importance of preparedness in nurse-scientists in genomics (Conley et al., 2015), particularly as to how genetics/genomics informs screening and prognoses (Calzone & Jenkins, 2011).

Acknowledgments

Funding: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Targeted Research and Academic Training of Nurses in Genomics (NR00975909); Sigma Theta Tau Research Award, University of Pittsburgh; Judith A. Erlen Research Award; International Society of Nurses in Genetics Research Award; Corrine M. Barnes Award; NINR, R21 NR01309401A1.

References

Baumgartel KL, Groer MW, Cohen SM, Ren D, Spatz DL, Conley YP. Effect of Promoter Polymorphisms on Cytokine Concentration in Preterm Breast Milk and Subsequent Infant Outcomes. Journal of Human Lactation. 2016; 32:425–437. DOI: 10.1177/0890334416646725 [PubMed: 27250867]

Behrman RE, Butler AS, editors. Preterm birth: Causes, consequences, and prevention. Washington, DC: National Academies Press; 2007. Retrieved from http://www.ncbi.nlm.nih.gov/books/NBK11362/

Brown HK, Speechley KN, Macnab J, Natale R, Campbell MK. Biological determinants of spontaneous late preterm and early term birth: A retrospective cohort study. British Journal of Obstetrics and Gynaecology. 2015; 122:491–499. DOI: 10.1111/1471-0528.13191 [PubMed: 25414127]

Cabantous S, Poudiougbou G, Oumar AA, Traore A, Barry A, Vitte J, et al. Dessein AJ. Genetic evidence for the aggravation of Plasmodium falciparum malaria by interleukin 4. Journal of Infectious Diseases. 2009; 200:1530–1539. DOI: 10.1086/644600 [PubMed: 19835477]

Cabantous S, Ranque S, Poudiougbou G, Traore A, Berbache S, Vitte J, et al. Marquet S. Genotype combinations of two IL4 polymorphisms influencing IL-4 plasma levels are associated with different risks of severe malaria in the Malian population. Immunogenetics. 2015; 67:283–288. DOI: 10.1007/s00251-015-0836-3 [PubMed: 25935236]

Calzone KA, Jenkins J. Genomics education in nursing in the United States. Annual Review of Nursing Research. 2011; 29:151–172.

Campeotto F, Kalach N, Lapillonne A, Butel MJ, Dupont C, Kapel N. Time course of faecal calprotectin in preterm newborns during the first month of life. Acta Paediatrica. 2007; 96:1531–1533. DOI: 10.1111/j.1651-2227.2007.00457.x [PubMed: 17714537]

Capasso M, Avvisati RA, Piscopo C, Laforgia N, Raimondi F, de Angelis F, Iolascon A. Cytokine gene polymorphisms in Italian preterm infants: Association between interleukin-10 -1082 G/A polymorphism and respiratory distress syndrome. Pediatric Research. 2007; 61:313–317. DOI: 10.1203/pdr.0b013e318030d108 [PubMed: 17314689]

Chaemsaithong P, Romero R, Korzeniewski SJ, Martinez-Varea A, Dong Z, Yoon BH, et al. Yeo L. A rapid interleukin-6 bedside test for the identification of intra-amniotic inflammation in preterm labor with intact membranes. Journal of Maternal-Fetal & Neonatal Medicine. 2016; 29:349–359. DOI: 10.3109/14767058.2015.1006620 [PubMed: 25758618]

Chiruvolu A, Tolia VN, Qin H, Stone GL, Rich D, Conant RJ, Inzer RW. Effect of delayed cord clamping on very preterm infants. American Journal of Obstetrics and Gynecology. 2015; 213:e671–e677. DOI: 10.1016/j.ajog.2015.07.016

Cochery-Nouvellon E, Nguyen P, Attaoua R, Cornillet-Lefebvre P, Mercier E, Vitry F, Gris JC. Interleukin 10 gene promoter polymorphisms in women with pregnancy loss: Preferential association with embryonic wastage. Biology of Reproduction. 2009; 80:1115–1120. DOI: 10.1095/biolreprod.108.072215 [PubMed: 19208551]

Conde-Agudelo A, Romero R, Kusanovic JP, Hassan SS. Supplementation with vitamins C and E during pregnancy for the prevention of preeclampsia and other adverse maternal and perinatal
outcomes: A systematic review and metaanalysis. American Journal of Obstetrics and Gynecology. 2011; 204:e501–e512. DOI: 10.1016/j.ajog.2011.02.020

Conley YP, Heitkemper M, McCarthy D, Anderson CM, Corwin EJ, Daack-Hirsch S, et al. Voss J. Educating future nursing scientists: Recommendations for integrating omics content in PhD programs. Nursing Outlook. 2015; 63:417–427. DOI: 10.1016/j.outlook.2015.06.006 [PubMed: 26123776]

Currie AJ, Curtis S, Strunk T, Riley K, Liyanage K, Prescott S, et al. Burgner D. Preterm infants have deficient monocyte and lymphocyte cytokine responses to group B streptococcus. Infection and Immunity. 2011; 79:1588–1596. DOI: 10.1128/IAI.00535-10 [PubMed: 21300777]

Denney JM, Nelson EL, Wadhwa PD, Waters TP, Mathew L, Chung EK, et al. Culhane JF. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine. 2011; 53:170–177. DOI: 10.1016/j.cytto.2010.11.005 [PubMed: 21123081]

Dordelmann M, Kerk J, Dressler F, Brinkhaus MJ, Bartels DB, Dammann CE, et al. Dammann O. Interleukin-10 high producer allele and ultrasound-defined periventricular white matter abnormalities in preterm infants: A preliminary study. Neuropediatrics. 2006; 37:130–136. DOI: 10.1055/s-2006-924554 [PubMed: 16967363]

Engel SA, Olshan AF, Savitz DA, Thorp J, Erichsen HC, Chanock SJ. Risk of small-for-gestational age is associated with common anti-inflammatory cytokine polymorphisms. Epidemiology. 2005; 16:478–486. [PubMed: 15951665]

Griffin IJ, Lee HC, Profit J, Tancedi DJ. The smallest of the small: Short-term outcomes of profoundly growth restricted and profoundly low birth weight preterm infants. Journal of Perinatology. 2015; 35:503–510. DOI: 10.1038/jp.2014.233 [PubMed: 25590218]

Groer M, Duffy A, Morse S, Kane B, Zaritt J, Roberts S, Ashmeade T. Cytokines, chemokines, and growth factors in banked human donor milk for preterm infants. Journal of Human Lactation. 2014; 30:317–323. DOI: 10.1177/0890344414527795 [PubMed: 24663954]

Groer M, Ashmeade T, Louis-Jacques A, Beckstead J, Ming J. Relationships of feeding and mother’s own milk with fecal calprotectin levels in preterm infants. Breastfeeding Medicine. 2016; 11:207–212. DOI: 10.1089/bfm.2015.0115. [PubMed: 27002351]

Harmon QE, Engel SM, Olshan AF, Moran T, Stuebe AM, Luo J, et al. Avery CL. Association of polymorphisms in natural killer cell-related genes with preterm birth. American Journal of Epidemiology. 2013; 178:1208–1218. DOI: 10.1093/aje/kwt108 [PubMed: 23982189]

Harmon QE, Engel SM, Wu MC, Moran TM, Luo J, Stuebe AM, et al. Olshan AF. Polymorphisms in inflammatory genes are associated with term small for gestational age and preeclampsia. American Journal of Reproductive Immunology. 2014; 71:472–484. DOI: 10.1111/aji.12241 [PubMed: 24702779]

Kantorowska A, Wei JC, Cohen RS, Lawrence RA, Gould JB, Lee HC. Impact of donor milk availability on breast milk use and necrotizing enterocolitis rates. Pediatrics. 2016; 137:1–8. DOI: 10.1542/peds.2015-3123

Kapel N, Roman C, Caldari D, Sieprath F, Canioni D, Khal-foun Y, et al. Ruemmele FM. Fecal tumor necrosis factor-alpha and calprotectin as differential diagnostic markers for severe diarrhea of small infants. Journal of Pediatric Gastroenterology and Nutrition. 2005; 41:396–400. [PubMed: 16205505]

Lee SE, Romero R, Jung H, Park CW, Park JS, Yoon BH. The intensity of the fetal inflammatory response in intraamniotic inflammation with and without microbial invasion of the amniotic cavity. American Journal of Obstetrics & Gynecology. 2007; 197:294 e291–e296. DOI: 10.1016/j.ajog.2007.07.006 [PubMed: 17826426]

Li W, Long C, Renjun L, Zhangxue H, Yin H, Wanwei L, et al. Yuan S. Association of SCNN1A single nucleotide polymorphisms with neonatal respiratory distress syndrome. Scientific Reports. 2015; 5:17317.doi: 10.1038/srep17317 [PubMed: 26611714]

Lista G, Castoldi F, Bianchi S, Battaglioli M, Cavigioli F, Bosoni MA. Volume guarantee versus high-frequency ventilation: Lung inflammation in preterm infants. Archives of Disease in Childhood Fetal and Neonatal Edition. 2008; 93:F252–F256. DOI: 10.1136/adc.2006.112102 [PubMed: 17405870]
Lowe PR, Galley HF, Abdel-Fattah A, Webster NR. Influence of interleukin-10 polymorphisms on interleukin-10 expression and survival in critically ill patients. Critical Care Medicine. 2003; 31:34–38. DOI: 10.1097/01.CCM.0000038211.80952.F3 [PubMed: 12544990]

Malarstig A, Lindahl B, Wallentin L, Siegbahn A. Soluble CD40L levels are regulated by the -3459 A>G polymorphism and predict myocardial infarction and the efficacy of antithrombotic treatment in non-ST elevation acute coronary syndrome. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006; 26:1667–1673. DOI: 10.1161/01.ATV.0000229087.8873.36

Malarstig A, Wallentin L, Siegbahn A. Genetic variation in the interleukin-6 gene in relation to risk and outcomes in acute coronary syndrome. Thrombosis Research. 2007; 119:467–473. DOI: 10.1016/j.thromres.2006.05.001 [PubMed: 16782174]

Moura E, Mattar R, de Souza E, Torloni MR, Goncalves-Primo A, Daher S. Inflammatory cytokine gene polymorphisms and spontaneous preterm birth. Journal of Reproductive Immunology. 2009; 80:115–121. DOI: 10.1016/j.jri.2008.11.007 [PubMed: 19375805]

Olsen R, Greisen G, Schroder M, Brok J. Prophylactic probiotics for preterm infants: A systematic review and meta-analysis of observational Studies. Neonatology. 2016; 109:105–112. DOI: 10.1159/0004414274 [PubMed: 26624488]

Pogliani L, Muggiasca L, Arrigoni L, Rossi E, Zuccotti G. Maternal methylenetetrahydrofolate reductase (MTHFR) homozygosity and neonatal outcome: Follow-up of 42 pregnancies at risk. Journal of Child Neurology. 2010; 25:701–704. DOI: 10.1177/0883073809344622 [PubMed: 20357240]

Richardson DK, Cocoran JD, Escobar GJ, Lee SK. SNAP-II and SNAPPE-II: Simplified newborn illness severity and mortality risk scores. Journal of Pediatrics. 2001; 138:92–100. [PubMed: 11148519]

Romero R, Espinosa J, Kusanovic JP, Gotsch F, Hassan S, Erez O, et al. Mazor M. The preterm parturition syndrome. British Journal of Obstetrics and Gynaecology. 2006; 113:17–42. DOI: 10.1111/j.1471-0528.2006.0120.x

Sorokin Y, Romero R, Mele L, Iams JD, Peaceman AM, Leveno KJ, et al. Sibai B. Umbilical cord serum interleukin-6, C-reactive protein, and myeloperoxidase concentrations at birth and association with neonatal morbidities and long-term neurodevelopmental outcomes. American Journal of Perinatology. 2014; 31:717–726. DOI: 10.1055/s-0033-1359723 [PubMed: 24338120]

Sugita N, Kobayashi T, Kikuchi A, Shimada Y, Hirano E, Sasahara J, et al. Yoshie H. Immunoregulatory gene polymorphisms in Japanese women with preterm births and periodontitis. Journal of Reproductive Immunology. 2012; 93:94–101. DOI: 10.1016/j.jri.2012.01.005 [PubMed: 22382006]
Velez DR, Fortunato SJ, Williams SM, Menon R. Interleukin-6 (IL-6) and receptor (IL6-R) gene haplotypes associate with amniotic fluid protein concentrations in preterm birth. Human Molecular Genetics. 2008; 17:1619–1630. DOI: 10.1093/hmg/ddn049 [PubMed: 18276608]

World Health Organization. Preterm birth. n.d. Retrieved November 18, 2015, from http://www.who.int/mediacentre/factsheets/fs363/en/

Wu W, Clark EA, Stoddard GJ, Watkins WS, Esplin MS, Manuck TA, et al. Jorde LB. Effect of interleukin-6 polymorphism on risk of preterm birth within population strata: A meta-analysis. BioMed Central Genetics. 2013; 14:30. doi: 10.1186/1471-2156-14-30 [PubMed: 23617681]

Yaeger R, Avila-Bront A, Abdul K, Nolan PC, Grann VR, Birchette MG, et al. Joe AK. Comparing genetic ancestry and self-described race in African Americans born in the United States and in Africa. Cancer Epidemiology, Biomarkers, & Prevention. 2008; 17:1329–1338. DOI: 10.1158/1055-9965.EPI-07-2505

Yanamandra K, Boggs P, Loggins J, Baier RJ. Interleukin-10 -1082 G/A polymorphism and risk of death or bronchopulmonary dysplasia in ventilated very low birth weight infants. Pediatric Pulmonology. 2005; 39:426–432. DOI: 10.1002/ppul.20182 [PubMed: 15678510]

Yilmaz V, Yentur SP, Saruhan-Direskeneli G. IL-12 and IL-10 polymorphisms and their effects on cytokine production. Cytokine. 2005; 30:188–194. DOI: 10.1016/j.cyto.2005.01.006 [PubMed: 15863393]
Table 1

Maternal Demographic and Delivery Characteristics.

| Characteristic                        | n (%), or Mean ± SD |
|---------------------------------------|---------------------|
| Age, years                            | 28.3 ± 6.8          |
| Total pregnancies                     | 3.1 ± 2.4           |
| Prepregnancy BMI, kg/m²               | 27.8 ± 7.3          |
| Ethnicity                             |                     |
| Caucasian                             | 21 (32.8)           |
| African American                      | 25 (39.1)           |
| Hispanic                              | 13 (20.3)           |
| Asian                                 | 2 (3.1)             |
| Other                                 | 1 (1.6)             |
| Missing                               | 2 (3.1)             |
| Highest educational level completed   |                     |
| Grammar/elementary school             | 4 (6.3)             |
| Middle school                         | 6 (9.4)             |
| High school                           | 36 (56.5)           |
| College                               | 14 (21.9)           |
| Postgraduate degree                   | 4 (6.5)             |
| Delivery method                       |                     |
| Vaginal                               | 15 (23.4)           |
| Cesarean section                      | 49 (76.6)           |

Note. N = 64. BMI = body mass index.
Table 2

Infant Characteristics and NICU Outcomes.

| Characteristic or Outcome | Mean ± SD          |
|---------------------------|--------------------|
| Gestational age at delivery, weeks | 28.3 ± 2.4         |
| Birth weight, g          | 1,069.6 ± 216.8    |
| Apgar 1 min              | 6.0 ± 1.9          |
| Apgar 5 min              | 7.4 ± 1.5          |
| Days on oxygen           | 15.2 ± 21.3        |
| Length of NICU stay, days | 70.5 ± 37.0        |

|        | n (%)       |
|--------|-------------|
| Sex, male | 38 (52.0) |
| ROP, yes  | 13 (19.1)  |
| BPD, yes  | 4 (5.6)    |
| Sepsis, yes | 10 (14.1) |
| NEC, yes  | 3 (4.2)    |
| IVH, yes  | 9 (12.9)   |
| Blood transfusion, yes | 33 (45.2) |
| Feeding intolerance, yes | 15 (21.1) |

Note. N = 73. BPD = bronchopulmonary dysplasia; IVH = intraventricular hemorrhage; NEC = necrotizing enterocolitis; ROP = retinopathy of prematurity; NICU = neonatal intensive care unit.
Table 3
Genotype Frequency and Hardy–Weinberg Equilibrium (HWE), Total Population.

| SNP         | n (%)    | Study MAF | HWE       |
|-------------|----------|-----------|-----------|
| rs2070874   |          |           |           |
| CC          | 45 (70.31) | T = 0.148 | p = .001 * |
| TT          | 8 (12.5)   |           |           |
| CT          | 11 (17.19) |           |           |
| rs2243250   | n/a      |           | p = .00001 * |
| CC          | 28 (43.75) |           |           |
| TT          | 20 (31.25) |           |           |
| CT          | 16 (25)    |           |           |
| rs1800795   |          | C = 0.195 | p = .3011 |
| CC          | 5 (7.81)   |           |           |
| GG          | 39 (60.94) |           |           |
| CG          | 20 (31.25) |           |           |
| rs1800796   |          | C = 0.109 | p = .0423 * |
| CC          | 3 (4.69)   |           |           |
| GG          | 50 (78.13) |           |           |
| CG          | 11 (17.19) |           |           |
| rs1800871   |          | T = 0.227 | p = .252 |
| TT          | 7 (11.11)  |           |           |
| CC          | 34 (53.97) |           |           |
| CT          | 22 (34.92) |           |           |
| rs1800872   |          | A = 0.242 | p = .1232 |
| CC          | 32 (50.79) |           |           |
| AA          | 9 (14.29)  |           |           |
| AC          | 22 (34.92) |           |           |
| rs1800896   |          | G = 0.313 | p = .7227 |
| GG          | 11 (17.46) |           |           |
| AA          | 23 (36.51) |           |           |
| AG          | 29 (46.03) |           |           |

Note. N = 64. MAF = minor-allele frequency; SNP = single nucleotide polymorphism.

* Significant at p < .05.
Table 4
Multivariate Model for Continuous Infant Outcomes With Maternal Minor-Allele Presence and/or Genotype.

| Gene/SNP/Population | Infant Outcome | Predictor          | Estimate | p Value |
|---------------------|----------------|--------------------|----------|---------|
| **IL6**             |                |                    |          |         |
| rs1800795           |                |                    |          |         |
| Caucasian           | Calprotectin Week 2<sup>a</sup> | MAP—no            | -0.743   | 0.022*  |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | 0.011    | 0.9038  |
|                     |                | Ratio of MOM to total milk | -0.535 | 0.3383  |
| Total population    | Days on oxygen | MAP—no            | -9.588   | 0.0316* |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | -5.048   | <0.01*  |
|                     |                | Ratio of MOM to total milk | 1.02   | 0.9076  |
| rs1800796           |                |                    |          |         |
| Caucasian           | Calprotectin Week 3<sup>a</sup> | MAP—no            | 0.815    | 0.0429* |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | 0.167    | 0.0530  |
|                     |                | Ratio of MOM to total milk | 0.252 | 0.5708  |
| African American    | Length of stay | MAP—no            | -32.318  | 0.0158* |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | -9.142   | <0.001  |
|                     |                | Ratio of MOM to total milk | -10.227 | 0.5371  |
| SNAPPE-II<sup>a</sup> |                | MAP—no            | -0.668   | 0.0497* |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | -0.059   | 0.1350  |
|                     |                | Ratio of MOM to total milk | 0.579 | 0.1310  |
| Hispanic            | Weight at 6 weeks<sup>a</sup> | MAP—no            | -0.195   | 0.0272* |
|                     |                | MAP—yes (ref)      |          |         |
|                     |                | Gestational age    | 0.089    | 0.0027* |
|                     |                | Ratio of MOM to total milk | 0.519 | 0.0822  |
| Total population    | Length of stay | Genotype GG        | 69.376   | 0.0034* |
|                     |                | Genotype AA        | -7.257   | 0.4626  |
|                     |                | Genotype AG (ref)  |          |         |
|                     |                | Gestational age    | -8.411   | <0.0001*|
|                     |                | Ratio of MOM to total milk | -12.07 | 0.4194  |
| rs1800795           | Calprotectin Week 3<sup>a</sup> | Genotype GG        | 0.974    | 0.0213* |
|                     |                | Genotype AA        | -0.063   | 0.7730  |
|                     |                | Genotype AG (ref)  |          |         |
|                     |                | Gestational age    | 0.065    | 0.0968  |
|                     |                | Ratio of MOM to total milk | 0.257 | 0.4826  |

*Biol Res Nurs. Author manuscript; available in PMC 2018 March 06.
| Gene/SNP/Population | Infant Outcome                  | Predictor                           | Estimate | p Value   |
|---------------------|---------------------------------|-------------------------------------|----------|-----------|
|                      | IL10                            |                                     |          |           |
| rs1800871           | Total population                 | Calprotectin Week 3<sup>a</sup>     |          |           |
|                     | Genotype TT                      |                                     | 0.732    | .0270<sup>*</sup> |
|                     | Genotype CC                      |                                     | 0.143    | .4381     |
|                     | Genotype CT (ref)                |                                     |          |           |
|                     | Gestational age                  |                                     | 0.059    | .1299     |
|                     | Ratio of MOM to total milk       |                                     | 0.174    | .6452     |
| rs1800872           | Caucasian                        | Calprotectin Week 1<sup>a</sup>     |          |           |
|                     | MAP—no                           |                                     | -0.997   | .0196<sup>*</sup> |
|                     | MAP—yes (ref)                    |                                     | -0.202   | .0987     |
|                     | Gestational age                  |                                     |          |           |
|                     | Ratio of MOM to total milk       |                                     | -0.044   | .9474     |
|                     | Total population                 | Calprotectin Week 3<sup>a</sup>     |          |           |
|                     | Genotype CC                      |                                     | 0.203    | .2892     |
|                     | Genotype AA                      |                                     | 0.768    | .0158<sup>*</sup> |
|                     | Genotype AC (ref)                |                                     |          |           |
|                     | Gestational age                  |                                     | 0.063    | .1466     |
|                     | Ratio of MOM to total milk       |                                     | 0.074    | .8523     |
| rs1800896           | African American                 | Days on oxygen                      |          |           |
|                     | MAP—no                           |                                     | 21.589   | .0320<sup>*</sup> |
|                     | MAP—yes (ref)                    |                                     |          |           |
|                     | Gestational age                  |                                     | -4.699   | .0016<sup>*</sup> |
|                     | Ratio of MOM to total milk       |                                     | -5.597   | .7021     |
|                     | Total population                 | Calprotectin Week 2<sup>a</sup>     |          |           |
|                     | MAP—no                           |                                     | 0.644    | .0057<sup>*</sup> |
|                     | MAP—yes (ref) Gestational age    |                                     | 0.058    | .2023     |
|                     | Ratio of MOM to total milk       |                                     | -0.251   | .5478     |
|                     | Calprotectin Week 2<sup>a</sup>  |                                     |          |           |
|                     | Genotype GG                      |                                     | 0.220    | .3795     |
|                     | Genotype AA                      |                                     | 0.741    | .0045<sup>*</sup> |
|                     | Genotype AG (ref)                |                                     |          |           |
|                     | Gestational age                  |                                     | 0.055    | .2297     |
|                     | Ratio of MOM to total milk       |                                     | -0.379   | .3928     |

Note. MAP = minor-allele presence; MOM = mom’s own milk; ref = reference; SNP = single nucleotide polymorphism; SNAPPE-II = scores for neonatal acute physiology with perinatal extension.

<sup>a</sup>Natural log transformed.

<sup>*</sup>Significant association at \( p < .05 \).