PATHWAYS TO SHORTENED GESTATION AMONG AFRICAN AMERICAN WOMEN

DISSERTATION

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ABSTRACT

**Background.** Preterm birth (PTB) is a devastating syndrome impacting 1 in 7.5 births to African American women. Improved methods to predict and prevent PTB are sorely needed and require a mechanistic understanding of PTB not yet achieved. Gestation may be shortened through premature initiation of an inflammatory cascade. Impaired interleukin(IL)-1β regulation may play a role among African American women and can be considered according to IL-1β and IL-1 receptor antagonist(Ra) production, cortisol levels, and glucocorticoid sensitivity. Chapter 2 reviews how a bio-panel of these mediators may predict early birth.

Several single nucleotide polymorphisms (SNPs) have been linked to lower IL-1Ra and psychosocial stress/depressive symptoms to enhanced IL-1β production, cortisol elevations, and decreased glucocorticoid sensitivity. Therefore, Chapters 3 and 4 address the following aims: evaluate if 1) allele status predicts shortened gestation and whether relationships are mediated by: a) IL-1Ra production, and/or b) modification of IL-1β and IL-1Ra production relationship, and 2) psychosocial stress/depressive symptoms predict shortened gestation and whether relationships are related to: a) IL-1β production, b) cortisol levels, and/or c) glucocorticoid sensitivity.

**Methods.** This prospective cohort study enrolled 96 African American women at 28-32 weeks gestation. Stress and depressive symptom inventories were administered.
Blood was drawn. Allelic discrimination was performed. IL-1β and IL-1Ra production were measured following *ex vivo* stimulation. Plasma cortisol levels and the neutrophil:lymphocyte ratio were measured (loss of expected positive association suggests decreased glucocorticoid sensitivity). Medical records were reviewed. Associations were examined.

**Results.** Women lacking minor allele A at IL1RN SNP rs2637988 had 3.09 times higher odds of delivery before 39 weeks, 95% CI=1.07-8.92. The relationship between IL-1Ra production and timing of delivery depended upon allele status. One ng/ml greater IL-1Ra production was associated with 29% lower odds of delivery before 38 weeks among women possessing minor allele A, 95% CI=.41-1.2, but 2.78 times greater odds among women lacking minor allele A, 95% CI=1.06-7.27. The SNP moderated the association between IL-1β and IL-1Ra production, β=-1.23, t=-3.00, p=.004. Though, influential data points were identified. Higher IL-1β production predicted delivery before 38 weeks, OR=1.45, 95% CI=1.10-1.91.

Women with significant depressive symptoms delivered 5.59 days earlier than women without significant depressive symptoms, β=-.26, t=-2.35, p=.021. One μg/dl greater cortisol was associated with delivery .61 days earlier, β=-.25, t=-2.26, p=.026. The relationship between cortisol and the neutrophil:lymphocyte ratio did not differ by depressive symptom status. However, one μg/dl greater cortisol was associated with 1.41 times greater odds of delivery before 39 weeks among women with, 95% CI=1.01-1.96, but not without significant depressive symptoms, OR=.98, 95% CI=.83-1.14. A one unit positive difference in the neutrophil:lymphocyte ratio trended toward greater odds of
delivery before 39 weeks among women with, \( OR=2.28, 95\% CI=0.95-5.57 \), but not without significant depressive symptoms, \( OR=0.72, 95\% CI=0.45-1.16 \).

**Conclusions.** Results support an inflammatory etiology to early birth among African Americans. The IL1RN SNP may relate to timing of delivery by modifying the IL-1\( \beta \):IL-1Ra relationship. Results also suggest that elevated cortisol may play a role in early delivery. Decreased glucocorticoid sensitivity may be important among women without depressive symptoms. Continued work in this area is warranted.
DEDICATION

This document is dedicated to my family. You taught me that anything is possible with hard work and perseverance. You are my inspiration and my strength, every day.

Thank you.
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CHAPTER 1: INTRODUCTION
Preterm Birth

In 2014, nearly 10% of 3,985,924 U.S. births occurred preterm (i.e., before 37 completed weeks gestation; Hamilton, Martin, Osterman, & Curtain, 2015). Many of these infants and children go on to experience significant illness, both acute (e.g., respiratory distress, hemorrhage) and long term (e.g., vision impairment, cognitive delays; Behrman & Stith Butler, 2007; Iacovidou, Varsami, & Syggellou, 2010). PTB is also the #1 and #2 cause of neonatal and infant death, respectively (Heron, 2015). PTB’s economic impact is also substantial. In the U.S., preterm babies account for 57% of all newborn hospital costs and an average $51,600 per preterm infant per year considering total lifetime costs (i.e., ~$20 billion annually per 2014 PTB rates alone; Behrman & Stith Butler, 2007; Kowlessar, Jiang, & Steiner, 2013).

African American women are significantly more likely to deliver prior to 37 weeks (13.23% versus 8.9%, respectively) and 32 weeks (4.1% versus 1.6%, respectively) than white women (Hamilton BE Ph et al., 2015; MacDorman & Mathews, 2011). Further, the overall mortality rate among African American infants is more than twice that of white infants, with 55% of this racial disparity attributed to preterm-related causes (MacDorman & Mathews, 2011; Murphy, Kochanek, Xu, & Heron, 2015). In fact, decreasing this racial gap in PTB by 1%, for one year, in a single state would prevent 232 PTBs, save 39 lives, and preserve $64.5 million (Xu, Grigorescu, Siefert, Lori, & Ransom, 2009).

Improving birth outcomes and eliminating health disparities are national priorities; however, progress has been slow (National Institute of Nursing Research, 2011; US Department of Health and Human Services, 2010; US Department of Health and Human Services, 2014). Predictors of impending PTB are sorely lacking. For instance, preventive progesterone therapy and cervical cerclage, while effective, can be
recommended only when risk is deemed high according to history of PTB or incidental finding of short cervix, which occur in only ~7% of gestations (Berghella, Rafael, Szychowski, Rust, & Owen, 2011; Conde-Agudelo et al., 2013; Facco & Simhan, 2013; Petrini et al., 2005; Romero et al., 2012; The American College of Obstetricians and Gynecologists, 2012). PTB risk, then, commonly becomes apparent only upon labor onset; treatment administered at this time may delay delivery for several days but does not prevent PTB (Haas, Caldwell, Kirkpatrick, McIntosh, & Welton, 2012; Kenyon, Boulvain, & Neilson, 2010). Improved methods to predict and prevent PTB are badly needed and require an understanding of pathways to PTB not yet achieved.

**The Inflammatory Pathway to Preterm Birth**

A key pathway by which gestation may be shortened is through premature initiation of an inflammatory cascade. At the time of labor, rising cytokine levels are consistently noted within the blood and maternal tissues (Osman et al., 2003; Osman, Young, Jordan, Greer, & Norman, 2006; Skrablin et al., 2007; Torbe et al., 2007; von Minckwitz et al., 2000). Importantly, distinct cytokine profiles are noted in conjunction with PTB among African American versus white women (Brou et al., 2012; Menon, Williams, & Fortunato, 2007; Velez et al., 2008). Interleukin(IL)-1β is elevated in peripheral blood and amniotic fluid of African American women delivering preterm versus African American women delivering at term (Brou et al., 2012; Menon et al., 2007; Velez et al., 2008). Among white women, IL-1β levels are similar in comparing those delivering preterm versus term; IL-6 and IL-8 levels differ (Brou et al., 2012; Menon et al., 2007; Menon, Camargo, Thorsen, Lombardi, & Fortunato, 2008; Velez et al., 2008). IL-1 receptor antagonist(Ra), a competitive inhibitor of IL-1β, is slightly lower among African American women delivering preterm versus African American women delivery at term.
White women experiencing PTB show significant elevations in IL-1Ra compared to white women delivering at term (Brou et al., 2012; Gabay, Lamacchia, & Palmer, 2010).

Rising IL-1β levels propagate inflammatory events, directly and indirectly promote pro-labor mediator production and activity, and ultimately provide a prime environment for uterine contraction, cervical ripening, and membrane rupture (Blanks, Shmygol, & Thornton, 2007; Chwalisz et al., 1994; Fortunato & Menon, 2003; Friebel-Hoffmann, Chiao, & Rauk, 2001; Hua et al., 2012; Kumar et al., 2006; Rauk & Chiao, 2000; Sadowsky, Adams, Gravett, Witkin, & Novy, 2006). Therefore, impaired IL-1β regulation may play an important role in pathways to shortened gestation among African American women specifically. Importantly, one’s ability to regulate IL-1β may be appreciated prior to notable IL-1β elevations by measuring IL-1β production upon immune challenge, ability to inhibit IL-1β activity through IL-1Ra production, levels of cortisol (thought to have downstream proinflammatory effects), and immune cell sensitivity to glucocorticoids (glucocorticoids exert anti-inflammatory effects at the cellular level). In other words, ex vivo techniques may provide a glimpse into future in vivo events; here, women showing impaired IL-1β regulation during pregnancy may be at risk for premature initiation of the labor-associated inflammatory cascade.

Genetic Variation

The heritability of PTB has been estimated at about 15-35% (i.e., percent of variation in gestational age at delivery attributed to maternal genetic variation; Kistka et al., 2008; Wu et al., 2015). Notably, genes flagged for their potential role in PTB most commonly associate with pathways pertinent to the inflammatory response (Uzun et al., 2012). However, in meta-analysis, only several variants have been consistently implicated in PTB and show only modest associations (Dolan et al., 2010). Therefore, candidate gene studies remain important in identifying variants of interest, determining if
a variant plays a particularly important role among specific populations, and investigating the pathway by which the identified variant may influence length of gestation.

Three single nucleotide polymorphisms (SNPs) located within an intron of the IL1RN gene on chromosome 2 (rs2637988), an intergenic region 11158 base pairs from the CHAT gene on chromosome 10 (rs1917805), and an intron of the SLC26A11 gene on chromosome 17 (rs12452028) appear to be associated with peripheral IL-1Ra levels (i.e., lower IL-1Ra levels associated with possession (IL1RN) and lack of (CHAT/SLC26A11) the minor alleles among non-pregnant populations; Korthagen, van Moorsel, Kazemier, Ruven, & Grutters, 2012; Tekola Ayele et al., 2012). Therefore, these variants may be associated with shortened gestation by decreasing IL-1Ra bioavailability or modifying the relationship between IL-1β and IL-1Ra production such that, among women with a particular allele status, IL-1β production predominates.

Variants of the IL1RN gene have been implicated in PTB but there is a paucity of studies evaluating the biological mechanisms underlying these associations (Dolan et al., 2010; Jones et al., 2012). To my knowledge, variants of the CHAT and SLC26A11 genes have not been evaluated in the context of pregnancy.

**Psychosocial Stress**

African American women are exposed to higher levels of psychosocial stress than white women (Albert et al., 2008; Boardman & Alexander, 2011; Schulz et al., 2000; Turner & Lloyd, 2004). Women reporting heightened psychosocial stress are more likely to report significant depressive symptoms and both stress and depressive symptoms have been linked to shortened gestation (Grote et al., 2010; Holden et al., 2012; Jesse, Walcott-McQuigg, Mariella, & Swanson, 2005; Lobel et al., 2008; Mustillo et al., 2004). Stress and depressive symptoms are also associated with dysregulation of endocrine and immune systems among some populations: production of IL-1β can be enhanced,
cortisol levels elevated, and glucocorticoid sensitivity diminished (Avitsur et al., 2003; Cohen et al., 2012; Cole, Mendoza, & Capitanio, 2009; Coussons-Read, Okun, & Nettles, 2007; Davis et al., 2008; Katz et al., 2011; Lisi et al., 2013; Miller, Cohen, & Ritchey, 2002; Miller, Chen, & Zhou, 2007; Miller, Rohleder, & Cole, 2009; Stieglitz et al., 2015). Thus, psychosocial stress and depressive symptomatology may be associated with shortened gestation via these mechanisms. Pregnancy is certainly a time of significant physiologic change and there is a need to determine whether psychoneuroimmunologic findings hold true among this population.

Specific Aims

Based on the reviewed literature and guided by the psychoneuroimmunologic framework, which holds that genetics and psychosocial factors influence immune-related health, this dissertation addressed the following aims among African American women sampled at 28 weeks – 32 weeks 6 days gestation (Figure 1.1; Ader, 1980).

Aim 1. Evaluate if allele status at three IL-1Ra-related candidate SNPs predicts shortened gestation and whether this relationship is mediated by: A) lower IL-1Ra production, and/or B) modification of the relationship between IL-1β and IL-1Ra production such that, among women with a particular allele status, IL-1β production predominates.

Aim 2. Evaluate if greater psychosocial stress and/or significant depressive symptoms predict shortened gestation and whether relationships are related to the variables’ associations with: A) greater IL-1β production, B) cortisol elevations, and/or C) decreased glucocorticoid sensitivity.

Approach

To address these specific aims, a prospective cohort design was employed for a final sample of 96 U.S.-born non-Hispanic African American women. 28 weeks – 32
weeks 6 days gestation, three types of psychosocial stress were measured using the Prenatal Life Events Scale, Revised Prenatal Distress Questionnaire, and Experiences of Discrimination Scale. Depressive symptoms were assessed according to the Center for Epidemiologic Studies Depression Scale. Whole blood was collected between the hours of 11am and 4pm.

Genetic variation was determined by whole blood deoxyribonucleic acid purification and probe-based allelic discrimination. Whole blood IL-1β and IL-1Ra production was measured in supernatants by multiplex electrochemiluminescence following ex vivo stimulation with lipopolysaccharide. Plasma cortisol levels were determined by enzyme-linked immunosorbent assay. Neutrophil and lymphocyte counts were measured by complete blood count with differential and glucocorticoid sensitivity was determined by assessing the relationship between cortisol levels and the neutrophil:lymphocyte ratio (positive association expected when immune cells are sensitive to glucocorticoids; Cohen et al., 2012). Length of gestation was determined per ultrasound-determined or -confirmed estimated date of delivery and actual date of delivery per medical record review. Logistic and linear regression models were fit to address the study aims (STATA 12.0, College Station, TX).

**Conclusion**

PTB is a devastating syndrome that disproportionately affects African American women. Ability to predict who will experience PTB and prevent PTB is greatly limited; advanced mechanistic understanding is required to improve upon available methods. The presented dissertation aims to address these deficits by evaluating proposed pathways to shortened gestation among African American women. Specifically, chapter 2 reviews the importance of bio-panel development and provides support for testing a panel of inflammatory regulation among African American women. Several possible
extensions of the current dissertation for future work are also discussed, including employing a longitudinal design and incorporating additional measures such as stimulated production of tumor necrosis factor-α and its competitive inhibitors. Chapter 3 presents background, methods, results, and conclusions related to Aim 1, which evaluates the proposed genetic pathway to shortened gestation. Chapter 4 presents background, methods, results, and conclusions related to Aim 2, which evaluates the proposed psychosocial pathway to shortened gestation. Finally, Chapter 5 provides a synopsis of lessons learned and future directions in this important line of research aiming to promote optimal outcomes for moms and babies through the prevention of PTB.
References


Figure 1.1. Dissertation Specific Aims. Aim 1 evaluates if allele status at three IL-1Ra-related candidate SNPs predicts shortened gestation and whether this relationship is mediated by: A) lower IL-1Ra production, and/or B) modification of the relationship between IL-1β and IL-1Ra production such that, among women with a particular allele status, IL-1β production predominates. Aim 2 evaluates if greater psychosocial stress and/or significant depressive symptoms predict shortened gestation and whether relationships are related to the variables' associations with: A) greater IL-1β production, B) cortisol elevations, and/or C) decreased glucocorticoid sensitivity. IL-1Ra = interleukin-1 receptor antagonist; IL-1β = interleukin-1β.
CHAPTER 2: A PROPOSED BIO-PANEL TO PREDICT RISK FOR SPONTANEOUS PRETERM BIRTH AMONG AFRICAN AMERICAN WOMEN*

Abstract

Preterm birth (PTB), or birth prior to 37 weeks gestation, impacts 11.5% of U.S. deliveries. PTB results in significant morbidity and mortality among affected children and imposes a large societal financial burden. Racial disparities in PTB are alarming. African American women are at more than 1.5 times the risk for PTB than white women. Unfortunately, the medical community’s ability to predict who is at risk for PTB is extremely limited. History of a prior PTB remains the strongest predictor during a singleton gestation. Cervical length and fetal fibronectin measurement are helpful tools. However, usefulness is limited, particularly among the 95% of U.S. women currently pregnant and lacking a history of PTB. Therefore, preventive therapies do not reach a great number of women who may benefit from them. This manuscript, in response to the pressing need for predictors of PTB risk and elimination of racial disparities in PTB, presents a proposed bio-panel for use in predicting risk for spontaneous PTB among African American women. This bio-panel, measured each trimester, includes stimulated production of interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist (Ra), soluble(s) TNF receptor(R) 1, and sTNFR2, and cortisol responsiveness. We hypothesize that greater IL-1β and TNF-α production, decreased IL-1Ra, sTNFR1, and sTNFR2 production, and decreased cortisol responsiveness at each time point as well as a more expedient alignment with this unfavorable profile over time will be associated with PTB. The choice to focus on inflammatory parameters is supported by data highlighting a crucial role for inflammation in labor. Specific inflammatory mediators have been chosen due to their potential importance in preterm labor among African American women. The bio-panel also focuses on inflammatory regulation (i.e., cytokine production upon ex vivo stimulation), which is hypothesized to provide insight into potential in vivo leukocyte responses and potential for initiation of a preterm inflammatory cascade.
Production of receptors antagonists is also considered, as pro-inflammatory mediator effects can be greatly influenced by their balance with respective antagonists. Finally, leukocyte responsiveness to cortisol is included as a measure of cortisol’s ability to convey anti-inflammatory signals. The development of a bio-panel predictive of risk for spontaneous PTB among African American women would represent a significant advancement. Available preventive therapies, namely progesterone supplementation, could be delivered to women deemed at risk. Further, the identification of biological predictors of PTB may uncover novel targets for preventive therapies.
Preterm Birth

Preterm birth (PTB), or birth occurring at less than 37 weeks gestation, accounts for 11.5% of all U.S. deliveries (Martin et al., 2013). Children born preterm are significantly more prone to serious acute and chronic illness, including bronchopulmonary dysplasia, necrotizing enterocolitis, vision/hearing impairment, and neurodevelopmental disability, as well as death (Behrman & Stith Butler, 2007). In the U.S., newborn hospital stays average 3.4 days and $3,200 but reach 14.3 days and $21,500 for preterm infants (Kowlessar, Jiang, & Steiner, 2013). Therefore, the acute care of preterm infants alone accounts for about 57% of the $12.2 billion in total annual newborn hospital costs (Kowlessar et al., 2013).

Racial disparities in the U.S. are striking, with significantly more African American infants being born preterm than white infants (16.26% vs. 10.17%, respectively; Martin et al., 2013). Of additional concern, infant mortality among African Americans is more than twice that of whites (Matthews & MacDorman, 2013). Improving birth outcomes and achieving health equity are national priorities (US Department of Health and Human Services, 2014); however, progress toward these goals has been slow. Our current ability to predict who will experience PTB is remarkably limited. In fact, the American College of Obstetricians and Gynecologists (ACOG) recommends against universal biological screening for PTB risk, as no test has proven sufficiently beneficial (The American College of Obstetricians and Gynecologists, 2012). In part due to lack of predictive ability, PTB prevention is often not feasible.

Prediction and Prevention

The strongest predictor of PTB in a current singleton pregnancy is PTB in a prior pregnancy (Behrman & Stith Butler, 2007). Women with a history of PTB exhibit 1.5-2 fold higher risk of subsequent PTB compared to women lacking this history, with earlier
and greater numbers of previous PTBs conveying greater risk (Iams, 2014). When the first PTB is spontaneous, risk for subsequent spontaneous PTB reaches more than 5 fold (Laughon, Albert, Leishear, & Mendola, 2014). Importantly, knowledge of this risk factor allows for early preventive measures. Progesterone supplementation is a proven preventive pharmacotherapy for women with a singleton pregnancy and prior PTB, lowering recurrent PTB risk by as much as 45% compared to women receiving placebo (Dodd, Jones, Flenady, Cincotta, & Crowther, 2013).

Shorter cervical length (CL) in early- to mid-pregnancy is also associated with earlier labor onset. Among pregnant women with a prior spontaneous PTB, those who subsequently deliver prior to 35 weeks gestation are >10 times more likely to have a short cervix (CL <25mm) in early pregnancy than women who deliver at term (Crane & Hutchens, 2008). Unfortunately, CL is less predictive of PTB among nulliparas and multiparas without a PTB history, among whom short cervix is associated with 14% probability of delivering prior to 35 weeks (Iams et al., 2001). Further, prevalence of the more conservative < 15mm CL cut-off is so low that evaluation of an estimated 238 nulliparas or 1075 multiparas without previous PTB would be required to prevent one PTB (Facco & Simhan, 2013). Adding measurement of cervicovaginal fetal fibronectin, a glycoprotein typically limited to the maternal-fetal interface, to CL improves PTB prediction; however, a positive test is still only associated with a 50% probability of PTB (Iams et al., 2001). Nevertheless, prophylactic progesterone and cervical cerclage benefit women with a short cervix (Berghella, Rafael, Szychowski, Rust, & Owen, 2011; Conde-Agudelo et al., 2013; Romero et al., 2012), highlighting the usefulness of even an imperfect measure of risk in directing clinical decision-making.

Although history of PTB and short cervix are useful predictors, the ability to identify women at risk for PTB is incomplete. For example, only ~5% of U.S. births are to
multiparous women with singleton gestation and a history of PTB (Petrini et al., 2005), a key clinical population for which PTB prevention is considered. If all women falling into this category received prophylactic progesterone, the national PTB rate would drop by only 0.3% (Petrini et al., 2005). Approximately 40% of U.S. births occur to nulliparous women (Martin et al., 2013), among whom about 8% deliver preterm (Garn, Nagulesapillai, Metcalfe, Tough, & Kramer, 2014). In addition, approximately 6% of multiparous women with no history of PTB will deliver preterm (Garn et al., 2014). As both groups lack a history of PTB, the likelihood of preventive action is greatly reduced. Here, an incidental finding of short CL may be the only indicator of altered prenatal physiology. Similarly, women known to be at higher risk, such as African Americans (Martin et al., 2013), the socioeconomically disadvantaged (Blumenshine, Egerter, Barclay, Cubbin, & Braveman, 2010), or women with high psychosocial stress exposure (Shapiro, Fraser, Frasch, & Seguin, 2013), cannot routinely be provided with such preventive therapies since the clinical value for these groups has not been established.

Early identification of women at risk for PTB and who may benefit from preventive strategies is key. Current intervention strategies prompted by signs and symptoms of preterm labor, such as regular uterine contractions and/or ruptured membranes, are of limited clinical value. Anti-contractile, such as nifedipine and terbutaline, delay birth by only several days (Haas, Caldwell, Kirkpatrick, McIntosh, & Welton, 2012). Similar delays result from the administration of antibiotics to women experiencing preterm premature rupture of membranes (PPROM; Kenyon, Boulvain, & Neilson, 2010). While these approaches may provide time for the administration of glucocorticoids, which do reduce risk for respiratory distress, intraventricular hemorrhage, and necrotizing enterocolitis (Bonanno & Wapner, 2012), they are ultimately not effective strategies to prevent PTB.
There is a clear need for accurate and reliable identification of women at greatest risk for PTB, ideally well in advance of symptom onset. Identifying early deviations from healthy gestational physiology is crucial in achieving this goal. Here, as part of a line of work aiming to close this critical gap in obstetric knowledge, we present a biological panel potentially predictive of impending PTB among African American women. Novel components of this inflammatory bio-panel include biomarkers hypothesized to play the most important role in preterm labor among African American women specifically, assessment of inflammatory regulation as opposed to inflammation, and analysis of change over time.

**The Hypothesis**

We hypothesize that a biological panel measured each trimester consisting of stimulated interleukin (IL)-1β, tumor necrosis factor (TNF)-α, IL-1 receptor antagonist (Ra), soluble(s) TNF receptor(R) 1, and sTNFR2 production, and cortisol responsiveness will predict risk for spontaneous PTB among African American women (see Figure 1). Specifically, we posit that greater IL-1β and TNF-α production, decreased IL-1Ra, sTNFR1, and sTNFR2 production, and decreased cortisol responsiveness at each time point as well as a more expedient alignment with this unfavorable profile over time will be associated with PTB.

We suggest prospective testing of the proposed bio-panel in a lower risk cohort of African American women, excluding those who are likely candidates for preventive therapies (e.g., women with a history of PTB) or to undergo early induction or cesarean section (e.g., women with gestational hypertension or diabetes). This approach better enables evaluation of the natural progression of pregnancy and labor. IL-1β, TNF-α, IL-1Ra, sTNFR1, and sTNFR2 production will be quantified using a minimally invasive ex vivo assay in which whole blood is incubated with lipopolysaccharide (LPS), a non-
specific innate immune stimulant, and levels compared to control values (see Table 1). Leukocyte responsiveness to cortisol can be quantified as the correlation between plasma cortisol levels and the neutrophil:lymphocyte ratio.

Why an Inflammatory Profile? Inflammation is a consistently noted component of labor, whether preterm or term (Gomez-Lopez, StLouis, Lehr, Sanchez-Rodriguez, & Arenas-Hernandez, 2014). This includes labors without evidence of infectious etiology (e.g., uterine overdistension; Romero, Dey, & Fisher, 2014). Production of pro-inflammatory cytokines, namely IL-1β, TNF-α, IL-6, and IL-8, is enhanced among laboring women. For example, levels of leukocyte IL-1β and IL-8 mRNA in the maternal circulation are higher among term laboring versus non-laboring women (Yuan, Jordan, McInnes, Harnett, & Norman, 2009). Serum IL-1β and IL-6 levels are elevated during preterm labor versus quiescent pregnancy (Torbe et al., 2007). Elevated serum IL-1β and IL-6 has been reported among women with PPROM who birth within two days versus those who do not (Skrablin et al., 2007). Further, TNF-α, IL-6, and IL-8 levels are elevated in the uterus, cervix, and fetal membranes at term cesarean following trial of labor versus quiescent pregnancy (Osman et al., 2003; Young et al., 2002). Such findings support a role for inflammation in labor.

Inflammation is known to beget greater inflammation. For example, infusion of IL-1β into the amniotic compartment of rhesus monkeys results in rapid release of TNF-α (Baggia, Gravett, Witkin, Haluska, & Novy, 1996). Likewise, culturing of human uterine cells with IL-1β or TNF-α further propagates inflammation. For instance, a 6-hour incubation with 1ng/ml IL-1β or TNF-α induces dramatic increases in IL-8 (Hua et al., 2012). An 8-hour incubation with 5ng/ml IL-1β results in a 50,000-fold increase in IL-6 (Friebe-Hoffmann, Chiao, & Rauk, 2001). Therefore, subtle changes in the inflammatory profile may indicate that an inflammatory cascade is poised to ensue.
A progressing inflammatory cascade is certainly capable of promoting labor-associated cellular and molecular processes (Christiaens et al., 2008; Farina & Winkelman, 2005). For example, culturing human uterine cells with IL-1β or TNF-α induces production of cyclooxygenase-2 and prostaglandins (Jiang et al., 2012; Rauk & Chiao, 2000), and increases in oxytocin (Friebe-Hoffmann et al., 2001), oxytocin receptor mRNA expression, and oxytocin receptor binding potential (Rauk, Friebe-Hoffmann, Winebrenner, & Chiao, 2001). Similarly, culturing human decidual cells with TNF-α induces changes in progesterone receptor (PR) ratios consistent with labor-associated functional progesterone withdraw (35). PR-A, an inhibitive receptor, rises in a dose dependent manner, while PR-B, the active receptor responsible for communicating anti-contractile signals, remains unaffected (Jiang et al., 2012). Intracervical application of IL-1β, TNF-α, and IL-8 among guinea pigs results in marked dissolution of the fibrous cervical tissue (Chwalisz et al., 1994). Exposure to IL-1β and TNF-α (through amniotic infusion among rhesus monkeys or human fetal membrane culture) induces matrix metalloproteinase (MMP) production and caspase activity, thereby promoting collagen remodeling and apoptosis (Fortunato & Menon, 2003; Kumar et al., 2006; Sadowsky, Adams, Gravett, Witkin, & Novy, 2006). Once such processes are underway, uterine contraction, cervical ripening, dilation, and effacement, rupture of fetal membranes, and likely birth are soon to follow (Blanks, Shmygol, & Thornton, 2007; Chwalisz et al., 1994; Kumar et al., 2006; Sadowsky et al., 2006; Zakar & Hertelendy, 2007). Each of these critical events of labor can be theoretically and temporally tied back to enhanced inflammation.

Inflammatory markers have been evaluated as predictors of PTB among asymptomatic women. Recently, Conde-Agudelo and colleagues (2011) and Hee (2011) systematically reviewed the prediction of PTB by various biomarkers, including
peripheral, cervicovaginal, and amniotic fluid levels of C-reactive protein (CRP), IL-6, IL-8, TNF-α, IL-2, IL-10, and MMP-8. Only elevated amniotic fluid MMP-8 (likelihood ratio [LR]+ 40; Conde-Agudelo et al., 2011) and serum TNF-α (LR+ 10; Hee, 2011) were associated with substantial increases in the likelihood of PTB. While these findings lend support to the theory that an inflammatory cascade is involved in the initiation of spontaneous preterm labor, several factors prohibit the use of these biomarkers among general clinical populations. First, these findings must be replicated. Second, biomarkers indicating a substantial increase in likelihood of PTB following a positive result failed to indicate more than a minimal decrease in likelihood of PTB following a negative result. Third, the testing conditions make widespread use difficult. MMP-8 levels were determined through invasive amniocentesis (Yoon et al., 2001) and serum TNF-α was predictive among women with a history of PTB, a group already known to be at high risk (Vogel et al., 2007). There is much work to be done in learning how to transform this knowledge into the development of clinically meaningful screening tools.

**Why these Biomarkers among African American women?** There is a growing body of evidence that meaningful differences may exist between races in the predictive value of given biomarkers. In a multivariate adaptive regression splines (MARS) analysis, maternal race influenced which amniotic fluid and plasma mediators differentiated between women in term versus preterm labor (Menon, Bhat, Saade, & Spratt, 2014). Here, predictive mediators among African American women included IL-1β, TNF-α, IL-1Ra, sTNFR1, and sTNFR2 (Menon et al., 2014). Predictive mediators among white women included IL-1Ra and sTNFR1 but not IL-1β, TNF-α, or sTNFR2 (Menon et al., 2014). Additional reports provide the levels of these biological mediators among African American and white women (see Table 2). For example, African American women experiencing preterm labor exhibited increased amniotic fluid and
plasma IL-1β and TNF-α as compared to African American women experiencing term labor (Brou et al., 2012; Menon, Williams, & Fortunato, 2007; Velez et al., 2008). This pattern was not present among white women, who were more likely to display differing IL-6 and IL-8 profiles (Menon et al., 2007; Menon, Camargo, Thorsen, Lombardi, & Fortunato, 2008). Culture media from 16 LPS-stimulated fetal membranes collected during elective repeat cesarean contained significantly greater IL-1 among African American women versus white and significantly greater IL-6 among white women versus African American (Menon, Merialdi, Lombardi, & Fortunato, 2006).

Brou et al. found that African American women (n = 191) in preterm labor did not display elevated amniotic fluid or plasma IL-1Ra versus those in term labor, which could help balance increases in IL-1β (Brou et al., 2012). Among white women, plasma IL-1Ra did significantly rise during preterm versus term labor (Brou et al., 2012). Considering the TNF receptors, comparisons of amniotic fluid/maternal plasma levels during preterm and term labor revealed rather unpredictable patterns (Brou et al., 2012). However, ex vivo LPS stimulation of fetal membranes uncovered an imbalance among African American women similar to that of IL-1β:IL-1Ra. Here, stimulation of fetal membranes collected during term cesarean resulted in significant TNF-α production among both African American (n = 9) and white (n = 14) women; however, sTNFR1 and 2 did not rise among African American women as was witnessed among white (Menon et al., 2007).

These data suggest that biological pathways to PTB may, at least partially, differ according to maternal race. Therefore, we propose that bio-panel development be tailored to inflammatory alterations most salient within racially specified cohorts. This is a critical consideration when attempting to narrow the racial gap in PTB. Indeed, personalized medicine has offered the promise of improved prevention, diagnosis, and
treatment of a number of diseases and a more personalized approach to PTB bio-panel
development may also play a large role in moving the work forward.

**Why Inflammatory Regulation?** Regulation of the production and activity of
potentially labor-stimulating inflammatory mediators, or lack thereof, may be
fundamentally different between women who deliver at term versus preterm. The first
component of our proposed regulatory profile includes IL-1β and TNF-α production upon
*ex vivo* LPS immune challenge. As described, researchers have focused on steady state
peripheral or localized levels of inflammatory markers in the prediction of PTB. Several
limitations to these approaches decrease their clinical usefulness. First, cytokines are
rapidly degraded and quantities often approach or exceed the lower limit of detection
(House, 2001). Therefore, significant elevations may not be appreciable until rising
levels are actively propagating labor events. Second, elevations may be an artifact of
acute (e.g., exercise) or chronic (e.g., adipose) non-immune influences (Conceicao et
al., 2012; Farhangi, Keshavarz, Eshraghian, Ostadrahimi, & Saboor-Yaraghi, 2013). As
a result, serum or plasma cytokine levels may not reliably reflect immune activation or
impaired ability to regulate excessive inflammation.

During labor, circulating leukocytes release cytokines peripherally (Yuan et al.,
2009), rapidly influx the maternal tissues (Osman et al., 2003; Thomson et al., 1999),
and are the primary source of cytokines within the cervix, myometrium, and fetal
membranes (Young et al., 2002). As such, there may be opportunity to gain critical
insights into a woman’s ability to regulate pro-inflammatory activity by examining how
peripherally obtained lymphocytes respond to *ex vivo* immune challenge during
quiescent pregnancy. Peripherally obtained leukocytes that respond to *ex vivo* challenge
by producing large quantities of pro-inflammatory cytokines may very well respond
equally robustly to an *in vivo* challenge, instigate an inflammatory cascade, and initiate
labor. It may also be that even a seemingly mild challenge, such as subclinical choriodecidual infection, results in labor among women whose immune systems respond particularly vigorously. An increased propensity for peripheral leukocytes to produce pro-inflammatory cytokines may be detected prior to notable steady state elevations of the peripheral or localized levels of cytokines themselves; i.e., ex vivo immune challenge may allow a glimpse into future events.

As the second component of the regulatory profile, we suggest quantification of naturally occurring pro-inflammatory receptor antagonists, specifically IL-1Ra, sTNFR1, and sTNFR2, following LPS immune challenge. An important means by which IL-1β and TNF-α carry out their action is by binding with their respective receptors and triggering transcription of additional pro-inflammatory mediators. Their ability to promote this feed-forward loop appears to be crucial to propagating labor events. For example, double knock-out of IL-1 and TNF receptors decreased the incidence of PTB from 69% to 8% among mice undergoing intrauterine inoculation with killed Escherichia coli (Hirsch, Filipovich, & Mahendroo, 2006). Ultimately, in the context of inflammatory health conditions, the effects of pro-inflammatory mediators can be greatly influenced by the balance with their respective receptor antagonists (Arend, 2002).

Another novel component of the proposed bio-panel includes measurement of maternal leukocyte responsiveness to the anti-inflammatory actions of the glucocorticoid cortisol. Cortisol carries out multiple biological functions, including dampening leukocyte pro-inflammatory responses and controlling leukocyte trafficking, and synthetic forms have been widely used as immunosuppressive drugs (Nicolaides, Charmandari, Chrousos, & Kino, 2014). Cortisol’s ability to serve as an endogenous anti-inflammatory agent depends upon both bioavailability and responsiveness of leukocytes to conveyed anti-inflammatory signals. Decreased cortisol responsiveness may be particularly
detrimental to pregnant women, among whom careful regulation of inflammatory activity is important.

There are multiple ways to assess leukocyte responsiveness to cortisol, including completing rather involved mRNA transcriptional analyses or ex vivo multi-condition cultures. In otherwise healthy individuals, high cortisol levels induce neutrophil leukocytosis as well as lymphocytopenia in an attempt to control leukocyte influx into sites of inflammation (Fauci, Dale, & Balow, 1976). Therefore, we propose that the correlation between plasma cortisol levels and the neutrophil:lymphocyte ratio be measured, with high or low correlations serving as indicators of high or low cortisol responsiveness, respectively. This approach, which requires only two common laboratory procedures, has greater potential for widespread use and has been applied successfully to psychoneuroimmunologic studies of stress-induced impaired cortisol responsiveness (Cohen et al., 2012; Cole, 2008; Cole, Mendoza, & Capitanio, 2009). In sum, this continuous variable is hypothesized to provide important additional information regarding a woman’s ability to regulate pro-inflammatory activity during the course of pregnancy.

**Why Longitudinal Assessment?** It is plausible that the extent to which a woman’s functional profile deviates from that seen during healthy gestation over time will offer improved prediction over cross-sectional data. At the time of labor, specific subsets of leukocytes appear to be attracted to the maternal-fetal interface. When challenged ex vivo, these leukocytes produce significant amounts of cytokines such as IL-1β and TNF-α (Vega-Sanchez et al., 2010). This ‘activated’ cellular state speculatively propagates labor events. Whether leukocytes are primed in the maternal peripheral blood leading up to the events of labor remains unclear. Cytokine production upon innate immune challenge is attenuated during quiescent pregnancy versus non-pregnancy (Aguilar-
Valles, Poole, Mistry, Williams, & Luheshi, 2007; Fofie, Fewell, & Moore, 2005). Also, while Denney and colleagues found that ex vivo LPS stimulated IL-1β and TNF-α production remained relatively constant during healthy gestation, as measured in each trimester (Denney et al., 2011), Daher et al. report progressive increases in LPS-stimulated TNF-α production in normal pregnancy, with the highest values at the time of labor (Daher, Fonseca, Ribeiro, Musatti, & Gerbase-DeLima, 1999). If leukocytes are primed in the peripheral circulation in preparation for labor prior to recruitment to maternal tissues, subtle phenotypic changes may be best appreciated through examination of their rate of change over time as opposed to a snapshot of their function in a given trimester. Further, it may be that the balance between the propensity to produce pro-inflammatory cytokines, produce their respective receptor antagonists, and communicate the signals of critical anti-inflammatory agents such as cortisol is what is altered in preparation for labor. The answer to this question is certainly worth exploring.

**Significance of the Hypothesis**

There is much work to be done if birth outcomes in the U.S. and worldwide are to be optimized. An important focus of this work is eliminating racial disparities in PTB. In the current manuscript, we have presented a bio-panel proposed to predict risk for spontaneous PTB among African American women. Our hypothesis is predicated on recent advancements in reproductive immunology and our approach aims to traverse some of the hurdles encountered during previous efforts to biologically predict PTB. Achieving the goal of accurate, reliable, and sufficiently early prediction would represent a major advancement in the field of obstetrics. Women deemed at risk could be provided with progesterone, the most promising preventive approach available to date, or other future preventive therapies. Further, the identification of biological predictors of PTB may uncover novel targets for preventive therapies.
References


<table>
<thead>
<tr>
<th>Variable</th>
<th>Measurement Approach</th>
<th>Information Gained</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β Production</td>
<td>Whole blood production following <em>ex vivo</em> LPS stimulation</td>
<td>Production of the pro-inflammatory cytokine upon innate immune challenge</td>
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<tr>
<td>TNF-α Production</td>
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<tr>
<td>IL-1Ra Production</td>
<td>Whole blood production following <em>ex vivo</em> LPS stimulation</td>
<td>Availability of endogenous receptor antagonists to counteract pro-inflammatory cytokine activity</td>
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<tr>
<td>sTNFR1 Production</td>
<td></td>
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<tr>
<td>sTNFR2 Production</td>
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<tr>
<td>Cortisol Responsiveness</td>
<td>Correlation between plasma cortisol levels and neutrophil:lymphocyte ratio</td>
<td>Ability of cortisol to convey anti-inflammatory signals to leukocytes</td>
</tr>
</tbody>
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Table 2.2
Labor-associated Profiles among African American and White Women

<table>
<thead>
<tr>
<th>Comparison (Race)</th>
<th>Analyte (Median pg/ml)</th>
<th>p Value</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td><strong>Amniotic Fluid Concentrations</strong></td>
<td></td>
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<tr>
<td></td>
<td>IL-1β (80.0 v. 23.7)</td>
<td>&lt;0.001</td>
<td>(Menon et al., 2007)</td>
</tr>
<tr>
<td></td>
<td>TNF-α (1009.34 v. 67.91)</td>
<td>&lt;0.001</td>
<td>(Velez et al., 2008)</td>
</tr>
<tr>
<td>Preterm Laboring v. Term Laboring</td>
<td>IL-6 (2042 v. 2366)</td>
<td>0.60</td>
<td>(Menon et al., 2008)</td>
</tr>
<tr>
<td>Women (African American)</td>
<td>IL-8 (237.7 v. 23.74)</td>
<td>0.90</td>
<td>(Menon et al., 2007)</td>
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<td></td>
<td>IL-1Ra (2399.1 v. 2243.6)</td>
<td>0.20</td>
<td>(Brou et al., 2012)</td>
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<td></td>
<td>TNFR1 (285 v. 690)</td>
<td>0.01</td>
<td>(Brou et al., 2012)</td>
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<td>TNFR2 (2824 v. 2099)</td>
<td>0.20</td>
<td>(Brou et al., 2012)</td>
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<td></td>
<td>IL-1β (25.5 v. 21.3)</td>
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<td>TNF-α (138.39 v. 67.62)</td>
<td>0.075</td>
<td>(Velez et al., 2008)</td>
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<td>Preterm Laboring v. Term Laboring</td>
<td>IL-6 (3773 v. 1682)</td>
<td>0.0003</td>
<td>(Menon et al., 2008)</td>
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<td>Women (white)</td>
<td>IL-8 (25.64 v. 22.64)</td>
<td>&lt;0.001</td>
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<td></td>
<td>IL-1Ra (1132.1 v. 1526.2)</td>
<td>0.97</td>
<td>(Brou et al., 2012)</td>
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<td></td>
<td>TNFR1 (448 v. 233.8)</td>
<td>0.11</td>
<td>(Brou et al., 2012)</td>
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<td></td>
<td>TNFR2 (2070 v. 1622.5)</td>
<td>0.25</td>
<td>(Brou et al., 2012)</td>
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<tr>
<td><strong>Maternal Plasma Concentrations</strong></td>
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<tr>
<td>Preterm Laboring v. Term Laboring</td>
<td>IL-1β (119.5 v. 52)</td>
<td>0.03</td>
<td>(Brou et al., 2012)</td>
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<tr>
<td>Women (African American)</td>
<td>TNF-α (50.4 v. 18)</td>
<td>0.03</td>
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<td></td>
<td>IL-8 (435 v. 178.7)</td>
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<td>IL-1Ra (81.6 v. 91.2)</td>
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<td>(Brou et al., 2012)</td>
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<td>TNFR1 (1462.6 v. 777)</td>
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<td>TNFR2 (27153.5 v. 24252.4)</td>
<td>0.21</td>
<td>(Brou et al., 2012)</td>
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<td>Preterm Laboring v. Term Laboring</td>
<td>IL-1β (51.2 v. 44.1)</td>
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<td>(Brou et al., 2012)</td>
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<td>Women (white)</td>
<td>TNF-α (18.6 v. 10)</td>
<td>0.79</td>
<td>(Brou et al., 2012)</td>
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<td></td>
<td>IL-8 (311.5 v. 104)</td>
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<td></td>
<td>IL-1Ra (152.6 v. 89.9)</td>
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<tr>
<td></td>
<td>TNFR1 (1729.7 v. 873)</td>
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<td></td>
<td>TNFR2 (26679 v. 26993)</td>
<td>0.98</td>
<td>(Brou et al., 2012)</td>
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<tr>
<th></th>
<th>Unstimulated v. LPS-Stimulated at Term Elective Cesarean (African American)</th>
<th>Unstimulated v. LPS-Stimulated at Term Elective Cesarean (white)</th>
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<tr>
<td></td>
<td>IL-1 (21.6 v. 179.8)</td>
<td>IL-1 (13.1 v. 23.05)</td>
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<td></td>
<td>TNF-α (51.3 v. 1062.4)</td>
<td>TNF-α (28 v. 531.8)</td>
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<td>IL-6 (270 v. 343.5)</td>
<td>IL-6 (200 v. 867)</td>
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<td>sTNFR1 (132.5 v. 93.4)</td>
<td>sTNFR1 (92.1 v. 165.7)</td>
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<td></td>
<td>sTNFR2 (331 v. 174.2)</td>
<td>sTNFR2 (168.1 v. 223.7)</td>
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<td>(Menon et al., 2006)</td>
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<td>0.0002</td>
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<td>0.006</td>
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<td>(Menon et al., 2007)</td>
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Figure 2.1. Theoretical Framework of Bio-panel Proposed to Predict Risk for Spontaneous PTB among African American Women. Three components of inflammatory regulation are measured during the first, second, and third trimesters of pregnancy. We hypothesize that this bio-panel will identify women at risk for PTB by their tendency to produce higher levels of IL-1β and TNF-α, and lower levels of IL-1Ra, sTNFR1, and TNFR2, and exhibit a diminished response to cortisol earlier in pregnancy. As such, a threshold will be reached which allows for a feed-forward inflammatory cascade, labor-associated cellular and molecular processes, the signs and symptoms of labor, and, ultimately, preterm delivery of the neonate.
CHAPTER 3: AN IL1RN POLYMORPHISM AND IL-1β PRODUCTION EACH PREDICT SHORTENED GESTATION AMONG AFRICAN AMERICAN WOMEN: WHAT ROLE DOES IL-1RA PRODUCTION PLAY?
Abstract

Background. Preterm birth (PTB) disproportionately impacts African Americans. Etiology remains elusive but PTB may be triggered by elevated interleukin (IL)-1β and/or diminished IL-1 receptor antagonist (Ra). Therefore, we examined relationships among three IL-1Ra-related single nucleotide polymorphisms (SNPs), IL-1β and IL-1Ra production, and timing of delivery among African Americans. Methods. Ninety-six participants were enrolled at 28-32 weeks gestation. Blood samples were collected. Allelic discrimination was performed. IL-1β and IL-1Ra production were measured following *ex vivo* stimulation. Timing of delivery was determined by medical record review. Mediation was assessed per the joint significance test of *a* and *b*. Covariates included PTB history, pre-labor induction/cesarean, education, and insurance status.

Results. Women lacking minor allele A at IL1RN SNP rs2637988 had 3.09 times higher odds of delivery before 39 weeks, 95% *confidence interval (CI)* = 1.07-8.92. The relationship between IL-1Ra production and odds of delivery before 38 weeks depended upon IL1RN SNP rs2637988 allele status, odds ratio (*OR*) = .25, 95% *CI* = .09-.75. One ng/ml greater IL-1Ra production was associated with 29% lower odds of delivery before 38 weeks among women possessing minor allele A, 95% *CI* = .41-1.2, but 2.78 times greater odds of delivery before 38 weeks among women lacking minor allele A, 95% *CI* = 1.06-7.27. The SNP moderated the association between IL-1β and IL-1Ra production, β = -1.23, *t* = -3.00, *p* = .004. Though, influential data points were identified. Higher IL-1β production predicted earlier delivery, with no influential points identified, *OR*₃₉ *weeks* = 1.18, 95% *CI* = 1.01-1.38, *OR*₃₈ *weeks* = 1.45, 95% *CI* = 1.10-1.91. Conclusions. Results support an inflammatory etiology to early birth among African Americans. IL1RN SNP rs2637988 and IL-1β production predicted earlier delivery. IL-1Ra production predicted timing of delivery but depended upon allele status. The SNP may relate to
timing of delivery by modifying the IL-1β:IL-1Ra relationship (i.e., IL-1β predominates among women lacking minor allele A). Larger samples are needed but this may be a promising area for future work.
Background

One in ten U.S. births are preterm (i.e., prior to 37 weeks gestation), with African American women 1.5 times more likely to experience preterm birth (PTB) than white women (Hamilton, Martin, Osterman, & Curtain, 2015). Babies born preterm are less likely to survive, those surviving face significant short- and long-term morbidities, and society at large incurs average excess costs of $51,600 per preterm infant (Behrman & Stith Butler, 2007; Heron, 2015; Stoll et al., 2015; Tan, Poon, Lian, & Ho, 2014; Vieira & Linhares, 2011). Improved methods to determine risk for and prevent the occurrence of PTB, particularly among African American women, are needed.

To achieve these goals, a deeper understanding of the mechanistic pathways leading to PTB is required. Specifically, an advancing inflammatory cascade is consistently noted during spontaneous labor, and promotes uterine contraction, cervical ripening, and membrane rupture (Fortunato & Menon, 2003; Kumar et al., 2006; Osman, Young, Jordan, Greer, & Norman, 2006; Sadowsky, Adams, Gravett, Witkin, & Novy, 2006; Torbe et al., 2007). In fact, onset of the inflammatory cascade may be among the first events of both preterm and term labor; however, what instigates this inflammatory cascade remains unknown.

The proinflammatory cytokine interleukin (IL)-1β is significantly elevated in blood and amniotic fluid of African American women in preterm labor versus African American women in term labor (this pattern does not hold among white women; Brou et al., 2012; Menon, Williams, & Fortunato, 2007; Velez et al., 2008). IL-1 receptor antagonist (Ra), IL-1β’s competitive inhibitor, is not elevated among African American women in preterm labor versus African American women in term labor (Brou et al., 2012; Gabay, Lamacchia, & Palmer, 2010). Therefore, enhanced IL-1β activity through pregnancy may play an important role in pathways to PTB among African American women. IL-1Ra
production may also be associated with timing of delivery if levels are low or fail to rise adequately for a given rise in IL-1β.

IL-1β and IL-1Ra profiles appear to be influenced by a complex combination of exposures, including those that are genetically determined. For example, tagging single nucleotide polymorphism (SNP) rs2637988 of the IL1RN (i.e., IL-1Ra) gene on the long arm of chromosome 2 has been linked to IL-1Ra levels among healthy adults and outcomes with an inflammatory component (e.g., osteoarthritis progression, cognitive deterioration, idiopathic pulmonary fibrosis, non-Hodgkin lymphoma; Barlo et al., 2011; Benke et al., 2011; Hosgood et al., 2011; Korthagen, van Moorsel, Kazemier, Ruven, & Grutters, 2012; Wu et al., 2013). Variants of the IL1RN gene have also been implicated in PTB (Dolan et al., 2010; Jones et al., 2012).

Further, tagging SNPs rs1917805 within 11200 base pairs of the CHAT gene and rs12452028 of the SLC26A11 gene suggested association with IL-1Ra levels in a genome-wide study of African Americans (Tekola Ayele et al., 2012). The CHAT or choline O-acetyltransferase gene is most commonly studied in relation to Alzheimer’s disease. The SLC26A11 gene, which encodes a sulfate transporter, is most commonly studied in relation to renal physiology. Each has also been tied to inflammatory processes (Vijayaraghavan et al., 2013; Vincourt, Jullien, Amalric, & Girard, 2003). The relationships among these tagging SNPs and immune function during pregnancy, as well as pregnancy outcomes, has not been assessed.

To address these critical gaps and move toward improved biological prediction and targeted prevention of early birth, the current study sought to test two pathways regarding the associations among the three candidate polymorphisms, IL-1β and IL-1Ra production during pregnancy, and timing of delivery among African American women: A) first, I evaluated whether the SNPs were associated with timing of delivery and whether
lower IL-1Ra production mediated this relationship; B) next, I evaluated whether the SNPs modified the relationship between IL-1β and IL-1Ra production such that, among women with a particular allele status, IL-1β production would predominate. In terms of delivery outcomes, I examined whether the predictors were associated with delivery before 39 weeks as well as delivery before 38 weeks gestation. These cut points were chosen in accordance with the 39 week U.S. definition of full term pregnancy and data supporting 38 weeks as the point of lowest neonatal morbidity among African American women specifically (Loftin, Chen, Evans, & DeFranco, 2012; Spong, 2013).

Methods

Study Design and Participants. Potential participants were recruited from The Ohio State University and Riverside Methodist Hospital OB/GYN Clinics, and the greater Columbus, Ohio community. Inclusion criteria included African American race, non-Hispanic ethnicity, age 18-35, singleton gestation, and ultrasound dating. Exclusion criteria included self-reported smoking, alcohol use, or illicit drug use beyond the first trimester, chronic conditions or regular use of medications with immune implications (e.g., diabetes, rheumatoid arthritis, corticosteroids, progesterone), fetal anomaly, or complication of pregnancy at the time of enrollment (e.g., gestational diabetes, gestational hypertension, preeclampsia, oligohydramnios). Development of a complication following enrollment was not grounds for exclusion.

Ninety-six eligible women attended a single study visit at 28 weeks – 32 weeks 6 days gestation. Participants were asked to report cold- or flu-like illness or antibiotic use to allow for scheduling/rescheduling of study visits at least seven days removed. During the study visit, background interviews were performed and blood was drawn. Each venipuncture was performed between the hours of 1100 and 1600. After delivery, medical records were reviewed. Of those enrolled, two participants were excluded due to
unsuccessful venipuncture, one due to inaccurately provided estimated date of delivery (and therefore sampling), and one due to loss to follow-up. Therefore, final analyses included 92 women.

The Ohio State University Biomedical and OhioHealth Institutional Review Boards approved the study. Informed consent and HIPAA authorization was obtained from all study participants. At the time of enrollment, compensation was provided in the form of a $50 Babies’R’Us gift card.

**DNA Purification and Allelic Discrimination.** K$_2$EDTA-treated whole blood was stored in aliquots at -80°C until processed in batches. At this time, blood was quick-thawed in a 37°C water bath and deoxyribonucleic acid (DNA) extracted and purified using 5 Prime PCR DNA Column Kits according to manufacturer instructions (5 Prime, Gaithersburg, MD). DNA concentration and purity were determined using the Nanodrop 2000 (Thermo Scientific, Wilmington, DE) and samples diluted to reach a final concentration of 20ng of DNA per well. Allelic discrimination was performed using probe-based TaqMan SNP Genotyping Assays and Master Mix (Applied Biosystems, Foster City, CA), amplification by polymerase chain reaction, and the CFX96 Touch Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Allele calls were made according to relative fluorescence units and required tight duplicate agreement.

**IL-1β and IL-1Ra Production.** IL-1β and IL-1Ra production were determined using a highly standardized stimulation protocol. Within one hour of collection, 50μl of heparinized whole blood was aliquoted in sterile fashion, in hood, into 500μl LPS solution; i.e., 500pg LPS from *Salmonella abortus equi* S-form (Enzo Life Sciences, Ann Arbor, MI) per ml sterile RPMI medium (MP Biomedicals, Santa Ana, CA). This procedure was also repeated using sterile RPMI medium alone to produce a control condition. Cultures were incubated at 37°C for 4 hours and centrifuged at 3000 RPM for
5 minutes. Supernatants were aspirated and stored in aliquots at -80°C until thawed in batches. IL-1β levels were determined using the MSD Pro-inflammatory II 4-plex Immunoassay and Sector Imager 2400 per manufacturer instructions (Meso Scale Discovery, Gaithersburg, MD). Intra- and inter-assay coefficients of variation were 6.1% and 4.5%, respectively. IL-1Ra levels were determined using the Human IL-1Ra Platinum ELISA (ebiosciences, San Diego, CA) and PowerWave Spectrophotometer (BioTek, Winooski, VT) per manufacturer instructions. Intra- and inter-assay coefficients of variation were 9.4% and 17.2%, respectively. For each analyte, production was calculated by subtracting the quantity produced in the control condition from the quantity produced in the LPS-stimulated condition.

**Pregnancy Outcomes.** Gestational age at delivery was determined per provider-confirmed estimated date of delivery and actual date of delivery by medical record review. The binary outcomes of delivery before 39 weeks versus at 39+ weeks gestation and before 38 weeks versus 38+ weeks gestation were coded. The 39-week cut point was chosen in accordance with the definition of full term pregnancy per the defining term pregnancy workgroup as guided by U.S. neonatal morbidity and mortality data (i.e., preterm: < 37 weeks; early term: 37 weeks – 38 weeks 6 days; full term: 39 weeks – 40 weeks 6 days; late term: 41 weeks – 41 weeks 6 days; Spong, 2013). Data also suggests that fetal maturation is accelerated among African Americans and when the pregnancy reaches 38 weeks, neonatal outcomes are similar to or possibly better than when pregnancy progresses beyond 39 weeks (Allen, Alexander, Tompkins, & Hulsey, 2000; Balchin, Whittaker, Lamont, & Steer, 2008; Balchin, Whittaker, Lamont, & Steer, 2011; Loftin et al., 2012; Patel, Steer, Doyle, Little, & Elliott, 2004). Therefore, the 38-week cut point was also evaluated. Variables related to obstetric history, complications
of pregnancy, presentation to labor and delivery, and infant health were also collected through review of prenatal, labor and delivery, and newborn medical records.

**Statistical Analysis.** Sample characteristics were examined according to mean/standard deviation and count/frequency. Factors with potential for confounding were identified by examining bivariate relationships among demographic and pregnancy characteristics and the primary variables of interest using $\chi^2$ tests, *Fisher's exact* tests when a cell contained < 5 observations, and *t* tests with $\alpha$ at .05. Hardy-Weinberg equilibrium of the genetic data was also assessed using $\chi^2$ tests at $\alpha = .05$.

Primary Aims A and B were tested by building logistic and linear regression models using STATA 12.0 (College Station, TX; $\alpha = .05$). Each model is described below using the following symbols: $\ln (p/p-1) =$ binary outcome variable; $i =$ intercept; $c =$ total effect of the predictor ($X$, $Z$, or $XZ$) on the outcome; $a =$ effect of the predictor ($X$, $Z$, or $XZ$) on the mediator; $b =$ effect of the mediator ($M$) on the outcome adjusted for the predictor ($X$, $Z$, or $XZ$); $c' =$ effect of the predictor ($X$, $Z$, or $XZ$) on the outcome adjusted for the mediator ($M$); and $e =$ the residual.

For Aim A, a single-mediator pathway was tested by building three consecutive models. For Model A1, allele status served as the predictor and delivery before 39 weeks as the outcome ($\ln (p/p-1) = i_1 + cX + e_1$). For Model A2, allele status served as the predictor and IL-1Ra production as the outcome ($M = i_2 + aX + e_2$). For Model A3, allele status and IL-1Ra production served as predictors and delivery before 39 weeks as the outcome ($\ln (p/p-1) = i_3 + c'X + bM + e_3$).

For Aim B, a mediated moderation pathway was tested by building three consecutive models. For Model B1, an allele status by IL-1Ra production interaction term, allele status, and IL-1Ra production served as predictors and delivery before 39 weeks as the outcome ($\ln (p/p-1) = i_4 + c_1XZ + c_2X + c_3Z + e_4$). For Model B2, an allele
status by IL-1Ra production interaction term, allele status, and IL-1Ra production served as predictors and IL-1β production as the outcome \( M = i_6 + a_1XZ + a_2X + a_3Z + e_5 \). For Model B3, an allele status by IL-1Ra production interaction term, allele status, IL-1Ra production, and IL-1β production served as predictors and delivery before 39 weeks as the outcome \( \ln \left( \frac{p}{p-1} \right) = i_6 + c_1'XZ + c_2'X + c_3'Z + bM + e_6 \). When moderation was detected, the sample was stratified by allele status to further assess relationships.

The presence of mediation and/or mediated moderation was determined using the joint significance test of \( a \) and \( b \), which requires significance of both the \( a \) and \( b \) coefficients within a given pathway and performs well in terms of power and control of Type I Error (Cohen & Cohen, 1983; MacKinnon, Lockwood, Hoffman, West, & Sheets, 2002). The above processes were repeated predicting delivery before 38 weeks. In each model, covariates were also included as appropriate. Further, linear regression models were examined to assure assumptions were met. In the event non-normality of the outcome variable resulted in non-normal error terms and/or heteroskedasticity, the outcome variable was transformed and used to verify findings. Each model was also examined for the presence of influential data points using residual, leverage, and influence diagnostics followed by sensitivity analyses as needed.

**Results**

**Sample Characteristics.** Demographic characteristics of the final sample of 92 are summarized in Table 3.1. All participants described themselves as African American and 10.9% identified with one or more additional race, including American Indian or Alaskan Native, Asian, White, and Puerto Rican. Most women were unmarried or separated (76.1%), had not completed a bachelor’s degree (73.9%), and did not report private insurance (67.4%). Women were, on average, 26.4 years old at the time of delivery (\( SD = 4.5, \text{ range} = 19-35 \)). As shown in Table 3.2, most participants had
previously experienced pregnancy (82.6%) and delivery (68.5%). Few women had previously delivered preterm (9.8%). No women reported gestational hypertension or preeclampsia at enrollment; however, 12 women (13.1%) developed one of these conditions by the time of delivery. Of note, 41.3% were induced or underwent cesarean section prior to the initiation of labor. Women delivered their babies at an average 39 weeks gestation (range 34 weeks 1 day – 41 weeks 1 day) and babies weighed an average 7#1oz (range 3#14.2oz – 9#4.2oz).

As shown in Tables 3.1 and 3.2, only gestational age at delivery and birth weight significantly differed among women delivering before 38 weeks versus at 38+ weeks gestation, $t = -13.36, p < .001; t = -6.54, p < .001$, respectively. A history of PTB also showed marginal association with delivery before 38 weeks, Fisher’s exact, $p = .06$. Results were similar in comparing women delivering before 39 weeks versus at 39+ weeks gestation, $t = -12.03, p < .001; t = -4.57, p < .001; Fisher’s exact, p = .07$, respectively. Women delivering before 39 weeks were also less likely to undergo pre-labor induction/cesarean section, $\chi^2 = 6.18, p = .013$. No demographic or pregnancy variables were associated with the SNPs under study. Higher educational attainment was significantly associated with higher IL-1β production and private insurance status was marginally associated with higher IL-1β production, $t = 2.38, p = .019; t = -1.87, p < .07$, respectively. Also, women with higher educational attainment and private insurance had higher IL-1Ra production, $t = 2.47, p = .015; t = 2.94, p = .004$, respectively. Therefore, history of preterm birth, pre-labor induction/cesarean, educational attainment, and insurance status were included as covariates in the primary analyses.

The allele frequencies observed in this sample and comparisons with expected frequencies are shown in Table 3.3. Observed and expected values did not significantly differ; however, CHAT SNP rs1917805 trended toward higher representation of the GG
genotype/lower representation of the AA genotype than was expected, \( p = .051 \). CHAT SNP rs1917805 allele frequencies did fall intermediary to those reported in global estimates and among African Americans through the 1000 genomes project (1000 Genomes Project Consortium et al., 2012). Descriptive statistics associated with IL-1β production (ng/dl) and IL-1Ra production (ng/ml) are also as follows: \( M = 4.12, SD = 3.09, \) range = \( .21 – 17.98 \); \( M = 4.82, SD = 1.43, \) range = \( 1.47 – 8.85 \), respectively.

**Single-Mediator Pathway: IL-1Ra Production.** Results from the single-mediator pathway and the 39-week outcome are depicted in Figure 3.1. Unadjusted and adjusted estimates from each model are also presented in Table 3.4. Results reported below are adjusted for history of PTB, pre-labor induction/cesarean section, educational attainment, and insurance status.

In Model A1 (estimating \( c \)), women possessing minor allele A for IL1RN SNP rs2637988 displayed a 68% lower odds of delivery before 39 weeks, odds ratio (\( OR \)) = .32, 95% confidence interval (\( CI \)) = .11-.93. Put otherwise, women lacking minor allele A at IL1RN SNP rs2637988 had 3.09 times higher odds of delivery before 39 weeks, 95% \( CI \) = 1.07-8.92. CHAT SNP rs1917805 and SLC26A11 SNP rs12452028 did not predict delivery before 39 weeks, \( ps > .185 \). In Model A2 (estimating \( a \)), women possessing minor allele C for SLC26A11 SNP rs12452028 had \( .70 \) ng/ml higher IL-1Ra production than women lacking minor allele C, \( \beta = .23, t = 2.26, p = .026 \). IL1RN SNP rs2637988 and CHAT SNP rs1917805 were not significantly associated with IL-1Ra production, \( ps > .086 \). In Model A3 (estimating \( b \), controlling for \( c \)), IL-1Ra production did not predict odds of delivery before 39 weeks, \( OR = 1.13, 95\% CI = .78-1.64 \).

Therefore, while IL1RN SNP rs2637988 was associated with odds of delivery before 39 weeks and SLC26A11 SNP rs12452028 was associated with IL-1Ra production, the full single-mediator pathway fails to meet criteria for joint significance of \( a \)
and \( b \) for any SNP and the 39-week outcome. Also of note, no significant associations emerged with odds of delivery before 38 weeks serving as the outcome, \( ps > .128 \).

**Mediated Moderation Pathway: IL-1Ra and IL-1β Production.** No significant associations emerged with CHAT SNP rs1917805 or SLC26A11 SNP rs12452028 serving as predictors, \( ps > .191 \). Therefore, these variables were removed from subsequent models. Results from the mediated moderation pathway, focusing on IL1RN SNP rs2637988 and the 38-week outcome, are depicted in Figure 3.2. Unadjusted and adjusted estimates from each model are also presented in Table 3.5. Results reported below are adjusted for history of PTB, pre-labor induction/cesarean section, educational attainment, and insurance status.

In Model B1 (estimating \( c \)), there was a significant IL1RN SNP rs2637988 by IL-1Ra production interaction in predicting odds of delivery before 38 weeks; i.e., the relationship between IL-1Ra production and odds of delivery before 38 weeks depended upon IL1RN SNP rs2637988 allele status, \( OR = .25, 95\% CI = .09-.75, \) Figure 3.3. Among women possessing minor allele A, one ng/ml greater IL-1Ra production was associated with 29% lower odds of delivery before 38 weeks, \( 95\% CI = .41-1.2. \) Among women lacking minor allele A, one ng/ml greater IL-1Ra production was associated with 2.78 times greater odds of delivery before 38 weeks, \( 95\% CI = 1.06-7.27. \)

In Model B2 (estimating \( a_i \)), the relationship between IL-1β production and IL-1Ra production depended upon IL1RN SNP rs2637988 allele status, \( \beta = -1.23, t = -3.00, \) \( p = .004. \) Among women possessing minor allele A, .39 ng/dl higher IL-1β production was associated with one ng/ml higher IL-1Ra production, \( \beta = .18, t = 1.62, \) \( p = .109. \) Among women lacking minor allele A, 1.92 ng/dl higher IL-1β production was associated with one ng/ml higher IL-1Ra production, \( \beta = .89, t = 4.17, \) \( p < .001. \) Three observations, however, were particularly influential. Case A strongly influenced the full model's
estimates (Cook’s distance = .62) and interaction coefficient (DFBETA = -1.89). Cases B and C showed mild influence (Cook’s distance of 0.08 and 0.05; DFBETA = .37 and .23, respectively). As depicted in Figure 3.4, omission of these cases removes the ability to detect a significant interaction.

In Model B3 (estimating \( b \) controlling for \( c' \)), one ng/dl greater IL-1\( \beta \) production was associated with 1.39 times greater odds of delivery before 38 weeks, 95% CI = 1.11-1.74 (Figure 3.5). Therefore, the mediated moderation pathway meets criteria of joint significance of \( a \) and \( b \); however, the significance of the \( a_1 \) pathway relies on several influential observations. The significant interaction between IL1RN SNP rs2637988 and IL-1Ra production (identified in Model B1, estimating \( c \)) and the main effect of IL-1\( \beta \) production (identified in Model B3, estimating \( b \), controlling for \( c' \)) in predicting odds of delivery before 38 weeks were not impacted by influential data points.

To follow-up, I also stratified the sample by IL1RN SNP rs2637988 allele status and evaluated relationships among IL-1\( \beta \) production, IL-1Ra production, and odds of delivery before 38 weeks. Among women possessing minor allele A (n = 66), a significant positive association between IL-1\( \beta \) and IL-1Ra production was noted, \( r_s = .28, p = .022 \). This relationship was stronger among women lacking minor allele A (n = 26), \( r_s = .42, p = .034 \). In logistic regression models, among women possessing minor allele A, one ng/ml greater IL-1Ra production was associated with 51% lower odds of delivery before 38 weeks, \( OR = .49, 95\% CI = .26-1.91 \), and one ng/dl greater IL-1\( \beta \) production was associated with 1.45 times greater odds of delivery before 38 weeks, 95% CI = 1.10-1.91. Among women lacking minor allele A (n = 26), associations between IL-1Ra and IL-1\( \beta \) production and odds of delivery before 38 weeks were not statistically significant, \( OR = 1.64, 95\% CI = .58-4.66 \), \( OR = 1.16, 95\% CI = .84-1.59 \). However, as expected per the results from the interacting model, the association between IL-1Ra
production and odds of delivery before 38 weeks was in the opposite direction of that detected among women possessing minor allele A. Also of note, there were no significant allele status by IL-1Ra production interactions in predicting odds of delivery before 39 weeks, $p \geq .13$. However, after removing the IL1RN SNP rs2637988 and IL-1Ra production variables, IL-1β production was significantly associated with odds of delivery before 39 weeks: one ng/dl greater IL-1β production was associated with 1.18 times greater odds of delivery before 39 weeks, 95% CI = 1.01-1.38 (Figure 3.5).

**Discussion**

This study first assessed whether the IL-1Ra-related SNPs predicted timing of delivery and whether this relationship was mediated by lower IL-1Ra production. In short, the answer appears to be no. Women lacking minor allele C for SLC26A11 SNP rs12452028 did demonstrate significantly lower IL-1Ra production but the polymorphism did not show any signs of association with timing of delivery. Only IL1RN SNP rs2637988 was significantly associated with delivery before 38 weeks; women lacking minor allele A had greater odds of early delivery. Women lacking minor allele A also had slightly higher IL-1Ra production than those possessing minor allele A. This finding differs from those in a study of healthy adults; here, minor allele A was associated with lower mononuclear cell IL-1Ra mRNA (Korthagen et al., 2012). This may be explained by differences in the population under study (i.e., pregnant African American women) or measurement techniques (i.e., whole blood production). Nonetheless, IL-1Ra production alone proved to be a poor predictor of timing of delivery among the full sample.

I also investigated a more complex pathway. I hypothesized that the SNPs may modify the relationship between IL-1Ra production and timing of delivery as mediated by the SNP’s modifying effect on the relationship between IL-1β and IL-Ra production.
Study findings support this mediated moderation model. The ability of IL-1Ra production to predict timing of delivery depended upon allele status at the IL1RN SNP rs2637988. Allele status also modified the relationship between IL-1β and IL-1Ra production. Among women possessing minor allele A, there is a good amount of variability in IL-1β production for a given level of IL-1Ra production, \( r_s = .28, p = .022 \). Among these women, the combination of IL-1β and IL-1Ra production must be considered. Indeed, following stratification by allele status, greater IL-1Ra production was protective and greater IL-1β production was harmful. Both pieces of information are important in predicting timing of delivery.

Among women lacking minor allele A, IL-1β production and IL-1Ra production were highly correlated, \( r_s = .42, p = .034 \). With the positive difference in IL-1β production considerably greater for a unit difference in IL-1Ra production, these women may be particularly predisposed to a profile dominated by IL-1β production. Indeed, it was this group that had significantly higher odds of early delivery. IL-1β production, then, may go on to play the major role in predicting timing of delivery, as IL-1Ra availability is insufficient to counteract its effects. Among these women, high levels of IL-1Ra production may merely be important in that they are a marker of high IL-1β production.

Importantly, this pathway must be considered with caution, as the significance of the IL1RN SNP rs2637988 by IL-1Ra production interaction in predicting IL-1β production relied on influential data points, particularly Case A. Case A was an outlier on IL-1β production but not IL-1Ra production. She did not appear to be experiencing an acute inflammatory event, as neither her white blood cell count nor temperature was particularly elevated. Further, unstimulated production of IL-1β was below the lower limit of detection. Therefore, it was the stimulated production alone that was quite large. She went on to experience spontaneous labor and delivery just over 37 weeks gestation.
It may be that the detected interaction in predicting IL-1β production was spurious and is not reproducible. Or it may be that our sample size was too small to adequately replicate the true risk profile. Indeed, among 92 participants, only 26, including Case A, lacked minor allele A. Further, the sample was healthy overall, with a PTB rate of 7.6% as opposed to the national rate of 13.23% (Hamilton et al., 2015). This is largely the result of study design, as I aimed to assess the natural progression of pregnancy and therefore limited enrollment of women at high risk for early induction or cesarean section. However, greater variability in the distribution of gestational age at delivery would improve ability to detect relationships. Moreover, 41.3% of our sample did not have the opportunity to go into labor due to pre-labor induction or cesarean, which is consistent with typical US patterns (Laughon et al., 2012; Zhang et al., 2010). This likely reduced ability to observe patterns in the natural progression of pregnancy and labor.

This being said, it is prudent to consider the results of the current study assuming that the IL1RN SNP rs2637988 did not moderate the relationship between IL-1β production and IL-1Ra production. So let us assume that IL-1β production does not predominate among women possessing a particular allele. Therefore, estimates for \( c^1 \), \( c^2 \), and \( c^3 \) and \( b \) should be considered independently: the relationship between IL-1Ra production and timing of delivery depend on IL1RN SNP rs2637988 allele status and IL-1β production predicts timing of delivery.

Notably, all of the relationships in conjunction with Aim B were identified using the 38-week outcome. While the direction of the relationships did not change in using the 39-week outcome, these relationships were not statistically significant. There may be meaning to this finding. Specifically, it was noted that the full term definition of 39 weeks – 40 weeks 6 days put forth by the workgroup of experts and stakeholders stems from U.S. vital statistics indicating that babies born within this timeframe experience optimal
outcomes in terms of morbidity and mortality (Spong, 2013). This window may, however, be shifted forward one week due to accelerated fetal maturation among African Americans (Allen et al., 2000; Balchin et al., 2008; Balchin et al., 2011; Patel et al., 2004). For example, Loftin and colleagues (2012) published a paper in which composite adverse neonatal outcomes were calculated according to gestational age at delivery. White and African American women significantly differed at the 38- and 39-week time points. Morbidity was lowest at 39 weeks for babies born to white mothers but 38 weeks for babies born to African American mothers. This pattern may suggest a maternal gestational physiology striving to reach 38 as opposed to 39 weeks among African American women. Therefore, this cut point may better separate altered versus appropriate physiology, particularly for mechanistic pathways common to African American women. This is an noteworthy topic for future work.

It is interesting to consider these findings and potential interpretations in relation to the few studies that have evaluated the relationship between IL-1Ra and timing of delivery. In one, higher second trimester plasma IL-1Ra predicted PTB among a cohort of 470 Hispanic women; when plasma IL-6 also reached the 80th percentile, the relationship between IL-1Ra and timing of birth became much stronger (Ruiz et al., 2012). Similarly, white women experiencing preterm labor demonstrated elevated plasma IL-1Ra versus white women in term labor; IL-1β levels, on the other hand, were quite similar (Brou et al., 2012). In combination with our findings, these data suggest that high IL-1Ra may be serving as a marker of enhanced inflammation; but may also be of significance in and of itself among certain groups (i.e., if IL-1Ra is sufficient to dampen IL-1β activity). It must also be considered that peripheral levels of IL-1Ra and stimulated levels of IL-1Ra are not measuring the same construct. The present work can only be interpreted in regards to whole blood response to LPS, a potent innate immune
stimulant. Impaired innate immune function has certainly been implicated in many inflammatory conditions and may be particularly important during pregnancy (Muralidharan & Mandrekar, 2013). It would be interesting to assess whether identified relationships were stable if, for example, adaptive immune function was evaluated.

In conclusion, what remains constant in the interpretation of the results of the current study is as follows: 1) IL1RN SNP rs2637988 allele status is associated with delivery before 39 weeks, 2) IL-1β production appears to be a good predictor of timing of delivery among our full sample of African American women for both delivery before 39 and 38 weeks, and 3) IL-1Ra production serves as a useful predictor of delivery before 38 weeks only when allele status at SNP rs2637988 of the IL1RN gene is known. The implication of these findings is significant and, importantly, can be applied to the study of early birth among African American women specifically. First, this work confirms IL1RN as a noteworthy gene, with polymorphisms potentially contributing to early birth. Further, IL-1β production is identified as a candidate for inclusion in bio-panels prospectively predicting risk for early birth, which adds to the literature regarding biological prediction (Conde-Agudelo, Papageorghiou, Kennedy, & Villar, 2011; Hee, 2011). Further, these findings support the notion that knowledge of allele status at specific chromosomal positions may significantly facilitate bio-panel design. This certainly falls in line with the concept of personalized medicine, in which genetic testing enables improved care and treatment plans (Hamburg & Collins, 2010). These findings provide exciting opportunities for continued work in this area.
References


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doi:10.1007/s00251-010-0596-7


Table 3.1
Demographic Characteristics

<table>
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<th></th>
<th>Full Sample (n = 92)</th>
<th>Delivery Before 38 Weeks (n = 17)</th>
<th>Delivery 38+ Weeks (n = 75)</th>
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<td></td>
<td>n (%)</td>
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<td>67 (89.3)</td>
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<tr>
<td>+ Additional Race(s)</td>
<td>10 (10.9)</td>
<td>2 (11.8)</td>
<td>8 (10.7)</td>
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<td>55 (73.3)</td>
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<td>30 (32.6)</td>
<td>5 (29.4)</td>
<td>25 (33.3)</td>
<td></td>
</tr>
<tr>
<td>Mean Maternal Age (SD)</td>
<td>26.4 (4.5)</td>
<td>25.6 (4.5)</td>
<td>26.5 (4.5)</td>
<td>t = -.79, p = .43</td>
</tr>
</tbody>
</table>

Note. Test statistics shown are from comparisons between women delivering before 38 weeks gestation and at 38+ weeks gestation using \( \chi^2 \) tests, Fisher’s exact tests when cells contained < 5 observations, and \( t \) tests.
Table 3.2

Pregnancy Characteristics and Delivery Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Full Sample (n=92)</th>
<th>Delivery Before 38 Weeks (n=17)</th>
<th>Delivery 38+ Weeks (n=75)</th>
<th>Test Statistic, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>16 (17.4)</td>
<td>3 (17.7)</td>
<td>13 (17.3)</td>
<td>Fisher's exact, p = 1.00</td>
</tr>
<tr>
<td>≥2</td>
<td>76 (82.6)</td>
<td>14 (82.4)</td>
<td>62 (82.7)</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>29 (31.5)</td>
<td>7 (41.2)</td>
<td>22 (29.3)</td>
<td>χ² = .90, p = .34</td>
</tr>
<tr>
<td>≥1</td>
<td>63 (68.5)</td>
<td>10 (58.8)</td>
<td>53 (70.7)</td>
<td></td>
</tr>
<tr>
<td>Previous Preterm Birth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>9 (9.8)</td>
<td>4 (23.5)</td>
<td>5 (6.7)</td>
<td>Fisher's exact, p = .06</td>
</tr>
<tr>
<td>No</td>
<td>83 (90.2)</td>
<td>13 (76.5)</td>
<td>70 (93.3)</td>
<td></td>
</tr>
<tr>
<td>Current Gestational Diabetes Mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>Fisher's exact, p = 1.00</td>
</tr>
<tr>
<td>No</td>
<td>92 (100)</td>
<td>17 (100)</td>
<td>75 (100)</td>
<td></td>
</tr>
<tr>
<td>Current Gestational Hypertension/Preeclampsia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>12 (13.0)</td>
<td>4 (23.5)</td>
<td>8 (10.7)</td>
<td>Fisher's exact, p = .22</td>
</tr>
<tr>
<td>No</td>
<td>80 (87.0)</td>
<td>13 (76.5)</td>
<td>67 (89.3)</td>
<td></td>
</tr>
<tr>
<td>Presentation for Delivery</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Labor</td>
<td>54 (58.7)</td>
<td>12 (70.6)</td>
<td>42 (56.0)</td>
<td>χ² = 1.22, p = .27</td>
</tr>
<tr>
<td>For Induction/Cesarean</td>
<td>38 (41.3)</td>
<td>5 (29.4)</td>
<td>33 (44.0)</td>
<td></td>
</tr>
<tr>
<td>Mean Gestational Age at Delivery (SD)</td>
<td>39 Weeks 0 Days (1 Week 1 Day)</td>
<td>36 Weeks 5 Days (6 Days)</td>
<td>39 Weeks 4 Days (5 Days)</td>
<td>t = -13.36, p &lt; .001</td>
</tr>
<tr>
<td>Mean Birth weight in Grams (SD)</td>
<td>3204.2 (482.8)</td>
<td>2631.9 (466.5)</td>
<td>3333.9 (383.8)</td>
<td>t = -6.54, p &lt; .001</td>
</tr>
</tbody>
</table>

Note. Test statistics shown are from comparisons between women delivering before 38 weeks gestation and at 38+ weeks gestation using χ² tests, Fisher's exact tests when cells contained < 5 observations, and t tests.
Table 3.3

Hardy-Weinberg Test of Equilibrium

<table>
<thead>
<tr>
<th>Gene</th>
<th>SNP</th>
<th>Chromosome (Position)</th>
<th>Major Allele (Frequency)a</th>
<th>Minor Allele (Frequency)a</th>
<th>Possess Minor Allele Genotype (n)</th>
<th>Lack Minor Allele Genotype (n)</th>
<th>HWE p Valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL1RN</td>
<td>rs2637988</td>
<td>Chr.2 (113876779)</td>
<td>G (0.522)</td>
<td>A (0.478)</td>
<td>AA or AG (66)</td>
<td>GG (26)</td>
<td>0.689</td>
</tr>
<tr>
<td>11158 bps from CHAT</td>
<td>rs1917805</td>
<td>Chr.10 (50805983)</td>
<td>A (0.837)</td>
<td>G (0.163)</td>
<td>GG or AG (25)</td>
<td>AA (67)</td>
<td>0.051</td>
</tr>
<tr>
<td>SLC26A11</td>
<td>rs12452028</td>
<td>Chr.17 (78212231)</td>
<td>G (0.832)</td>
<td>C (0.168)</td>
<td>CC or CG (29)</td>
<td>GG (63)</td>
<td>0.650</td>
</tr>
</tbody>
</table>

aCalculated according to chromosome pairs for each participant.

bHWE χ² comparisons of observed versus expected allele frequencies for the current sample.

bps = base pairs; SNP = Single Nucleotide Polymorphism; HWE = Hardy-Weinberg Equilibrium.
Table 3.4

Logistic and Linear Regression Models for the Aim A Single-Mediator Pathway

<table>
<thead>
<tr>
<th></th>
<th>Model A1 Predicting Delivery Before 38 Weeks</th>
<th>Model A2 Predicting IL-1Ra Production</th>
<th>Model A3 Predicting Delivery Before 38 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OR (95% CI)</strong></td>
<td><strong>OR^a (95% CI)</strong></td>
<td><strong>β, t</strong></td>
<td><strong>β, t^a</strong></td>
</tr>
<tr>
<td><strong>IL1RN rs2637988</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack Minor Allele A</td>
<td>Reference*</td>
<td>Reference**</td>
<td>Reference*</td>
</tr>
<tr>
<td>Possess Minor Allele A</td>
<td>.40 (.15-1.04)</td>
<td>.32 (.11-.93)</td>
<td>-.17, -1.68</td>
</tr>
<tr>
<td><strong>CHAT rs1917805</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack Minor Allele G</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference*</td>
</tr>
<tr>
<td>Possess Minor Allele G</td>
<td>1.51 (.57-3.97)</td>
<td>1.74 (.59-5.13)</td>
<td>-.13, -1.25</td>
</tr>
<tr>
<td><strong>SLC26A11 rs12452028</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lack Minor Allele C</td>
<td>Reference</td>
<td>Reference</td>
<td>Reference*</td>
</tr>
<tr>
<td>Possess Minor Allele C</td>
<td>1.96 (.76-5.02)</td>
<td>2.01 (.72-5.63)</td>
<td>.19, 1.86</td>
</tr>
<tr>
<td><strong>IL-1Ra Production</strong></td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>History of Preterm Birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No History</td>
<td>---</td>
<td>Reference*</td>
<td>Reference*</td>
</tr>
<tr>
<td>History</td>
<td>---</td>
<td>4.66 (.87-24.85)</td>
<td>-.11, -1.03</td>
</tr>
<tr>
<td><strong>Presentation for Delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In Labor</td>
<td>---</td>
<td>Reference</td>
<td>Reference**</td>
</tr>
<tr>
<td>For Induction/Cesarean</td>
<td>---</td>
<td>.35 (.12-.97)**</td>
<td>.07, .70</td>
</tr>
<tr>
<td><strong>Educational Attainment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Bachelor’s Degree</td>
<td>---</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>&gt;Bachelor’s Degree</td>
<td>---</td>
<td>1.46 (.27-7.78)</td>
<td>.14, .93</td>
</tr>
<tr>
<td><strong>Insurance Status</strong></td>
<td>---</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Private</td>
<td>---</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>Did Not Report Private</td>
<td>---</td>
<td>2.44 (.49-12.00)</td>
<td>-.23, -1.51</td>
</tr>
</tbody>
</table>

*Note. OR = odds ratio, CI = confidence interval, IL-1Ra = interleukin 1 receptor antagonist.
aadjusted for history of preterm birth, pre-labor induction/cesarean, educational attainment, and insurance status
*p < .1, **p < .05, ***p < .01
Table 3.5

Logistic and Linear Regression Models for the Aim B Mediated Moderation Pathway

<table>
<thead>
<tr>
<th></th>
<th>Model B1 Predicting Delivery Before 38 Weeks</th>
<th>Model B2 Predicting IL-1β Production</th>
<th>Model B3 Predicting Delivery Before 38 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>OR^a (95% CI)</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>IL1RN rs2637988 by IL-1Ra Production</td>
<td>.31(.12-.83)**</td>
<td>.26(.09-.75)**</td>
<td>.39(.12-1.21)</td>
</tr>
<tr>
<td>Lack Minor Allele A</td>
<td>Reference*</td>
<td>Reference**</td>
<td>Reference</td>
</tr>
<tr>
<td>Possess Minor Allele A</td>
<td>166(.91-30145)</td>
<td>414(1-121795)</td>
<td>45(.13-16280)</td>
</tr>
<tr>
<td></td>
<td>1.19, 3.11</td>
<td>1.14, 2.92</td>
<td>147(.2-146202)</td>
</tr>
<tr>
<td>IL-1Ra Production</td>
<td>2.13(.01-5.03)*</td>
<td>2.8(1.1-7.26)**</td>
<td>1.38(.49-3.92)</td>
</tr>
<tr>
<td></td>
<td>.96, 4.65***</td>
<td>.89, 4.17***</td>
<td>1.93(.58-6.48)</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>---</td>
<td>1.3(1.1-1.63)**</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>---</td>
<td>1.4(1.1-1.74)**</td>
</tr>
<tr>
<td>History of Preterm Birth</td>
<td>No History</td>
<td>Reference**</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>.68(.18-2.53)</td>
<td>.58(.13-2.53)</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>.01, .08</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>.04, -.45</td>
<td>---</td>
<td>.58(.05-6.48)</td>
</tr>
<tr>
<td>Presentation for Delivery</td>
<td>In Labor</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>.62(.06-5.94)</td>
<td>.58(.05-6.48)</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>.15, 1.07</td>
<td>---</td>
</tr>
<tr>
<td>Educational Attainment</td>
<td>&lt;Bachelor's Degree</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>.62(.06-5.94)</td>
<td>.58(.05-6.48)</td>
</tr>
<tr>
<td></td>
<td>Reference</td>
<td>.15, 1.07</td>
<td>---</td>
</tr>
<tr>
<td>Insurance Status</td>
<td>Private</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>1.27(16-10.26)</td>
<td>1.68(.18-16.11)</td>
</tr>
<tr>
<td></td>
<td>Did not Report Private</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td></td>
<td>---</td>
<td>.02, .11</td>
<td>---</td>
</tr>
</tbody>
</table>

Note. OR = odds ratio, CI = confidence interval, IL-1Ra = interleukin 1 receptor antagonist.

^a adjusted for history of preterm birth, pre-labor induction/cesarean, educational attainment, and insurance status

*p < .1, **p < .05, ***p < .01
Figure 3.1. Aim A Single-Mediator Pathway. This pathway fails to meet criteria for mediation. IL1RN SNP rs2637988 predicts delivery before 39 weeks, OR = .32, 95% CI .11-.93; this relationship is not mediated by IL-1Ra production. SLC26A11 SNP rs12452028 predicts IL-1Ra production, β = .23, t = 2.26, p = .026. However, IL-1Ra production does not predict delivery before 39 weeks, OR = 1.13, 95% CI 1.78-1.64. Reported estimates are adjusted for PTB history, pre-labor induction/cesarean section, educational attainment, and insurance status. IL-1Ra = interleukin-1 receptor antagonist, SNP = single nucleotide polymorphism. *p < .1, **p < .05
Figure 3.2. Aim B Mediated Moderation Pathway. This pathway meets criteria for mediated moderation ($a_1: \beta = -1.23$, $t = -3.00$, $p = .004$; $b: OR = 1.39$, 95% CI = 1.11-1.74). Estimates are adjusted for PTB history, pre-labor induction/cesarean, education, and insurance status. IL-1Ra = interleukin-1 receptor antagonist, SNP = single nucleotide polymorphism. 

* $p < .10$; ** $p < .05$; *** $p < .01$
Figure 3.3. IL-1Ra Production and Timing of Delivery according to IL1RN SNP rs2637988 Minor Allele A Status. The relationship between IL-1Ra production and odds of delivery before 38 weeks depends upon IL1RN SNP rs2637988 allele status, \( OR = 0.25, \) 95% CI = 0.09-0.75. Among women possessing minor allele A, greater IL-1Ra production predicts lower odds of delivery before 38 weeks, \( OR = 0.71, \) 95% CI = 0.41-1.2. Among women lacking minor allele A, greater IL-1Ra production predicts higher odds of delivery before 38 weeks, \( OR = 2.78, \) 95% CI = 1.06-7.27. Reported estimates are adjusted for PTB history, pre-labor induction/cesarean section, educational attainment, and insurance status. IL-1Ra = interleukin-1 receptor antagonist, ng = nanogram, ml = milliliter, SNP = single nucleotide polymorphism.
Figure 3.4. IL-1β and IL-1Ra Production according to IL1RN SNP rs2637988 Minor Allele A Status. The relationship between IL-1β production and IL-1Ra production depend upon IL1RN SNP rs2637988 allele status, $\beta = -1.23$, $t = -3.00$, $p = .004$. However, Cases A, B, and C influenced the interaction coefficient (DFBETA = -1.89, .37 and .23, respectively). Omission of these cases removes the ability to detect the interaction. Reported estimates are adjusted for PTB history, pre-labor induction/cesarean section, educational attainment, and insurance status. IL-1b = interleukin-1β, dl = deciliter, IL-1Ra = interleukin-1 receptor antagonist, ng = nanogram, ml = milliliter.
Figure 3.5. IL-1β Production and Timing of Delivery. One ng/dl greater IL-1β production predicts 1.39 times the odds of delivery before 38 weeks, 95% CI = 1.11-1.74 (model holds IL1RN SNP rs2637988 by IL-1Ra production interaction, IL1RN SNP rs2637988, and IL-1Ra production constant). One ng/dl greater IL-1β production predicts 1.18 times the odds of delivery before 39 weeks, 95% CI = 1.01-1.38 (IL1RN SNP rs2637988 by IL-1Ra production interaction, IL1RN SNP rs2637988, and IL-1Ra production removed from model). Reported estimates are adjusted for PTB history, pre-labor induction/cesarean section, educational attainment, and insurance status.
CHAPTER 4: DEPRESSIVE SYMPTOMS AND CORTISOL LEVELS PREDICT SHORTENED GESTATION AMONG AFRICAN AMERICAN WOMEN: WHERE DOES GLUCOCORTICOID SENSITIVITY FIT IN?
Abstract

Background. One in 7.5 African American mothers deliver preterm. Biological pathways remain poorly understood. Depression-associated cortisol elevations and decreased glucocorticoid sensitivity may promote early labor via hormonal and immune pathways. Therefore, we evaluated whether prenatal depressive symptoms were associated with: A) cortisol elevations and earlier delivery, and/or B) decreased glucocorticoid sensitivity (operationalized as loss of expected positive association between cortisol and the neutrophil:lymphocyte ratio and greater neutrophil:lymphocyte ratio and earlier delivery).

Methods. This prospective cohort study enrolled 96 participants at 28-32 weeks gestation. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale. Plasma cortisol levels were determined by ELISA, neutrophil:lymphocyte ratio by complete blood count with differential, and timing of delivery by medical record review. Associations were examined using linear/logistic regression, adjusting for sleep quality, pre-labor induction/cesarean, time of sampling, and preterm birth history. Results. Women with significant depressive symptoms had marginally higher cortisol (i.e., 1.69 μg/dl), \( \beta = .19, t = 1.78, p = .078 \), and delivered 5.59 days earlier than women without significant depressive symptoms, \( \beta = -.26, t = -2.35, p = .021 \). One μg/dl greater cortisol was associated with delivery .61 days earlier, \( \beta = -.25, t = -2.26, p = .026 \). The relationship between cortisol and the neutrophil:lymphocyte ratio did not differ by depressive symptom status, \( \beta = .45, t = 1.09, p = .280 \). There were significant interactions in predicting earlier delivery. One μg/dl greater cortisol was associated with 1.41 times greater odds of delivery before 39 weeks among women with significant depressive symptoms, 95% CI=1.01-1.96, but not women without significant depressive symptoms, OR=.98, 95% CI=.83-1.14. A one unit positive difference in the neutrophil:lymphocyte ratio trended toward greater odds of delivery before 39 weeks.
among women with significant depressive symptoms, \( OR=2.28, \ 95\% \ CI=.95-5.57, \) but not women without significant depressive symptoms, \( OR=.72, \ 95\% \ CI=.45-1.16. \)

Conclusions. These results suggest that elevated cortisol but not decreased glucocorticoid sensitivity may play a role in early delivery among women with depressive symptoms. Interestingly, women without significant depressive symptoms delivering before 39 weeks displayed a profile most consistent with decreased glucocorticoid sensitivity. These pathways may be important targets in predicting/preventing early birth; longitudinal data among larger samples is needed.
Background

One in 10 births to U.S. women and 1 in 7.5 births to U.S. African American women are preterm (i.e., prior to 37 weeks gestation; Hamilton, Martin, Osterman, & Curtain, 2015). Preterm babies and their families face significant challenges. Short- and long-term morbidity is more prevalent and death more likely among babies born preterm (Heron, 2015; Stoll et al., 2015; Tan, Poon, Lian, & Ho, 2014; Vieira & Linhares, 2011). Parents of preterm babies experience higher levels of depressive symptoms and greater traumatization than parents of full term babies (Mehler et al., 2014). Additionally, excess average societal costs reach $51,600 per preterm infant (Behrman & Stith Butler, 2007). Improved methods to prevent preterm birth (PTB), particularly among African American women, are sorely needed.

Identification of women at risk and development of preventive therapies requires characterization of underlying biological processes leading to early birth. The current literature supports a role for hypothalamic-pituitary-adrenal (HPA) activity in directing the gestational ‘clock.’ Cortisol levels increase through pregnancy and promote placental corticotropin-releasing hormone (CRH) production (D'Anna-Hernandez, Ross, Natvig, & Laudenslager, 2011; Kirschbaum, Tietze, Skoluda, & Dettenborn, 2009; Sandman et al., 2006). Cortisol and CRH stimulate uterine contractions and increased levels of each during pregnancy have been linked to earlier birth (Giurgescu, 2009; Guendelman, Kosa, Pearl, Graham, & Kharrazi, 2008; Sun, Brockman, Campos, Pitzer, & Myatt, 2006; Wadhwa et al., 2004; Wang et al., 2012).

Gestation may be cut short through premature initiation of an inflammatory cascade. Proinflammatory cytokine levels rise during labor and this inflammatory milieu promotes many critical processes of parturition (Fortunato & Menon, 2003; Kumar et al., 2006; Osman, Young, Jordan, Greer, & Norman, 2006; Sadowsky, Adams, Gravett,
Inflammation and infection have been repeatedly linked to PTB (Conde-Agudelo, Papageorghiou, Kennedy, & Villar, 2011; Kramer et al., 2010; Lee et al., 2013; Ruiz et al., 2012; Sorokin et al., 2010). What remains to be determined is how an inflammatory cascade ensues during pregnancy.

African American women are at heightened risk for depression, which, in turn, is consistently linked to early birth (Grigoriadis et al., 2013; Paradies et al., 2015). Elevated cortisol is also often noted in the context of depression (Stetler & Miller, 2011). The few studies examining relationships among depressive symptoms, cortisol levels, and early birth together have failed to tie both the psychological risk factor and its biological correlate to timing of delivery (Field et al., 2009; O'Keane et al., 2011). Depressive symptoms, and related psychosocial stress, also decrease glucocorticoid sensitivity in some populations (Cole, 2008; Katz et al., 2011; Miller, Cohen, & Ritchey, 2002). In this case, cells become less sensitive to cortisol, including its immunosuppressive effects. For example, cortisol directs leukocyte trafficking and demonstrates positive associations with the neutrophil:lymphocyte ratio (Steer et al., 1998). Individuals experiencing a major long-term stressor lose this expected association and their immune cells produce greater amounts of proinflammatory cytokines (Cohen et al., 2012). The enhanced inflammation associated with decreased glucocorticoid sensitivity may be detrimental to pregnancy. It is unknown whether decreased glucocorticoid sensitivity plays a role in early birth among depressed women or increases risk for early birth at all.

To address these gaps and work toward better prediction and prevention of PTB through identification of biological mechanisms underlying shortened gestation, the current study evaluated: A) whether significant prenatal depressive symptoms were associated with elevated cortisol levels and if both depressive symptoms and cortisol levels were associated with shorter gestation, and B) whether significant prenatal
depressive symptoms were associated with decreased glucocorticoid sensitivity, operationalized as a decrease in the expected positive association between cortisol levels and the neutrophil:lymphocyte ratio. The study also evaluated whether the relationships among cortisol level, the neutrophil:lymphocyte ratio, and length of gestation depended upon depressive symptom status. It was hypothesized that: 1) among women with significant depressive symptoms, greater cortisol levels but a lower neutrophil:lymphocyte ratio would predict shorter gestation (i.e., this profile would indicate a disconnect between cortisol levels and the neutrophil:lymphocyte ratio and therefore suggest a decrease in glucocorticoid sensitivity); and 2) among women without significant depressive symptoms, greater cortisol levels and a higher neutrophil:lymphocyte ratio would predict earlier delivery (i.e., values for each should vary together when immune cells are sensitive to glucocorticoid signaling and therefore if greater cortisol predicts earlier delivery, a greater neutrophil:lymphocyte ratio should also predict earlier delivery).

**Methods**

**Study Design and Participants.** Women were recruited from The Ohio State University and Riverside Methodist Hospital Obstetric and Gynecology Clinics and the central Ohio community. Eligible participants were African American, non-Hispanic, 18-35 years old, and pregnant with one baby. An ultrasound-confirmed or -determined estimated date of delivery was also required. Women smoking, consuming alcohol, or using illicit drugs beyond the first trimester per self-report were not eligible. Additional exclusion criteria included regular use of medications or chronic conditions with implications for endocrine/immune-related physiology (e.g., corticosteroids, progesterone, diabetes, rheumatoid arthritis). Women with diagnosed fetal anomaly or
complications of pregnancy such as gestational diabetes, gestational hypertension, preeclampsia, or oligohydramnios at the time of enrollment were also not eligible.

Ninety-six eligible women attended a single study visit at 28 weeks – 32 weeks 6 days of pregnancy. Staff asked participants to report symptoms of cold/flu or use of antibiotics prior to the study visit; if reported, visits were scheduled/rescheduled at least seven days removed. Participants were asked to awaken at least 2.5 hours prior to the visit start time and refrain from exercise or caffeine intake on the day of the visit. During the study visit, demographic information was collected, health histories obtained, and the Center for Epidemiologic Studies Depression Scale (CES-D) and Pittsburgh Sleep Quality Index (PSQI) administered. Blood was drawn between the hours of 11am and 4pm. Following delivery, prenatal, labor and delivery, and newborn medical records were reviewed. Two participants were excluded from analyses due to unsuccessful venipuncture, one due to inaccurate due date (and, therefore, time of sampling), and one due to loss to follow-up. One participant displayed extremely elevated cortisol levels (6 standard deviations above the mean), which significantly impacted all related estimates. This data was also excluded. Therefore, final analyses included 91 participants.

The Ohio State University Biomedical and OhioHealth Institutional Review Boards approved the study. Informed consent and HIPAA authorization was obtained from all women at the time of enrollment. At enrollment, participants were also provided with modest compensation in the form of a $50 gift card.

**Demographic, Clinical, and Pregnancy Characteristics.** Demographic information was provided by self-report. Maternal pre-pregnancy body mass index (kg/m²) was calculated using self-reported pre-pregnancy weight and height measured by stadiometer at the study visit. Sleep quality over the past month was assessed using the Pittsburgh Sleep Quality Index (PSQI), an 19-item self-report measure (Buysse,
Reynolds, Monk, Berman, & Kupfer, 1989). Questions include, for example, how often the respondent had trouble sleeping because they could not “get to sleep within 30 minutes” and how they rated their “sleep quality overall.” Seven component scores, including subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbance, use of sleep medication, and daytime dysfunction, were calculated. Scores could range from 0-21, with higher scores indicative of worse sleep quality and scores > 5 indicative of poor sleep quality. Among pregnant women, internal consistency reaches .7-.76, test-retest correlations reach .56, and the index functions independent of age and education (Skouteris, Wertheim, Germano, Paxton, & Milgrom, 2009). For analyses, the sum of the component scores, also termed the global score, was used.

Pregnancy characteristics were determined by medical record review.

**Depressive Symptoms.** Prenatal depressive symptoms were assessed using the Center for Epidemiologic Studies Depression Scale (CES-D), a 20-item self-report measure (Radloff, 1977). Respondents were asked to indicate whether each item (e.g., “I felt lonely”, “I felt that everything I did was an effort”) was experienced rarely or none of the time, some or a little of the time, occasionally or a moderate amount of time, or most or all of the time during the past week. Scores could range from 0-60, with higher scores indicative of greater depressive symptomatology and scores > 16 considered clinically significant (Thomas, Jones, Scarinci, Mehan, & Brantley, 2001). The scale was designed for the general population but has been validated among African American women and in the context of generally healthy and high-risk pregnancy (internal consistency reliability = .83-.92, test-retest correlations = .63-.78, convergent validity correlations = .62-.79; Canady, Stommel, & Holzman, 2009; Maloni, Park, Anthony, & Musil, 2005; Rozario & Menon, 2010). For analyses, participants were categorized as with (CES-D ≥ 16) or without (CES-D < 16) significant depressive symptoms.
**Cortisol Levels.** Heparinized whole blood was centrifuged at 3000 RPMI for 10 minutes. Plasma was aspirated and stored at -80°C until assayed in batches. At this time, plasma was thawed and cortisol quantified in duplicate using Cortisol ELISA kits (Calbiotech, Spring Valley, CA) and the PowerWave Microplate Spectrophotometer (BioTek, Winooski, VT) per manufacturer instructions. Intra- and inter-assay coefficients of variation were 3.7% and 9.7%, respectively. Plasma cortisol level in μg/dl was used for analyses.

**Neutrophil:Lymphocyte Ratio.** K²EDTA-preserved whole blood was transported to The Ohio State University Wexner Medical Center Core Laboratory. Complete blood counts with differential were performed using volume, conductivity, and scatter technology using standard laboratory techniques. Neutrophil percentage was divided by lymphocyte percentage to produce the neutrophil:lymphocyte ratio, which was used for analyses.

**Delivery Outcomes.** Timing of delivery was determined per provider-confirmed estimated date of delivery and actual date of delivery by medical record review. Timing of delivery was considered in two ways: 1) according to the continuous measure of gestational age at delivery (i.e., days gestation), and 2) according to the binary outcome of delivery before 39 weeks versus at 39+ weeks. Thirty-nine weeks is considered full term as defined by the US workgroup on defining term pregnancy. At this time, neonatal morbidity and mortality is at its lowest according to U.S. data (Spong, 2013). The events of labor and delivery and infant birth weight were also determined by medical record review.

**Statistical Analysis.** Sample demographic, clinical, and pregnancy characteristics, and the primary study variables were examined according to count/frequency and mean/standard deviation. Bivariate relationships among sample
characteristics, predictors, and outcomes using $\chi^2$ tests, Fisher’s exact tests (if < 5 observations/cell), t tests, and Spearman’s rank correlations as appropriate with $\alpha$ at .05 were examined to identify variables with potential for confounding. Bivariate Spearman’s rank correlations were also examined among the main study variables.

Linear and logistic regression models, as appropriate for continuous and binary outcome variables, were fit to test the primary aims (STATA 12.0, College Station, TX; $\alpha = 0.05$). Each model is described below using the following symbols: $Y$ = continuous outcome; $ln \ (p/p-1)$ = binary outcome; $\alpha$ = intercept; $\beta$ = total effect of a predictor ($X$, $Z$, or $XZ$) on an outcome; and $e$ = the residual.

For Aim A, first, depressive symptom status (i.e., women with versus without significant depressive symptoms) served as the predictor and cortisol level as the outcome ($Y_{cortisol} = \alpha + \beta X_{depressive \ symptom \ status} + e$). Next, two separate models were fit in which each depressive symptom status and cortisol level served as the predictor and gestational age at delivery as the outcome ($Y_{gestational \ age} = \alpha + \beta X_{depressive \ symptom \ status} + e$; $Y_{gestational \ age} = \alpha + \beta X_{cortisol} + e$). Lastly, one model was fit in which both depressive symptom status and cortisol level served as predictors and gestational age at delivery as the outcome ($Y_{gestational \ age} = \alpha + \beta X_{depressive \ symptom \ status} + \beta X_{cortisol} + e$). Linear regression models predicting gestational age at delivery were repeated using logistic regression and delivery before 39 weeks serving as the outcome, i.e., $ln \ (p/p-1)_{Delivery \ Before \ 39 \ Weeks}$.

For Aim B, first, a depressive symptom status by cortisol level interaction term, depressive symptom status, and cortisol level served as predictors and the neutrophil:lymphocyte ratio as the outcome ($Y_{neutrophil:lymphocyte} = \alpha + \beta X_{depressive \ symptom \ status} + e + \beta X_{cortisol} + \beta X_{depressive \ symptom \ status} + \beta X_{cortisol} + e$). Next, a depressive symptom status by cortisol level interaction term, depressive symptom status, and cortisol level served as predictors and gestational age at delivery as the outcome ($Y_{gestational \ age} = \alpha + \beta X_{depressive \ symptom \ status} + e$).
symptom status*cortisol + \( \beta X \) depressive symptom status + \( \beta Z \) cortisol + e). Lastly, a depressive symptom status by neutrophil:lymphocyte ratio interaction term, depressive symptom status, and the neutrophil:lymphocyte ratio served as predictors and gestational age at delivery as the outcome (\( Y_{gestational \ age} = \alpha + \beta X Z \) depressive symptom status*neutrophil:lymphocyte + \( \beta X \) depressive symptom status + \( \beta Z \) neutrophil:lymphocyte + e). Linear regression models predicting gestational age at delivery were repeated using logistic regression and delivery before 39 weeks serving as the outcome, i.e., \( \ln \left( \frac{p}{p-1} \right) \) Delivery Before 39 Weeks. The sample was also stratified according to depressive symptom status and delivery before or at 39+ weeks and relationships examined for each group separately to follow-up on significant interactions.

Each model was also run adjusting for covariates as appropriate. Linear regression model assumptions were also assessed. When non-normal error terms and/or heteroskedasticity resulted from non-normality of the outcome variable, this variable was transformed and the model was reassessed to confirm findings. Models were also examined for influential data using residual, leverage, and influence diagnostics and sensitivity analyses as appropriate.

Results

Sample Characteristics. As shown in Table 4.1, participants were primarily unmarried/separated (75.8%), completed less than a bachelor’s degree (73.6%), and did not report private insurance (67%). Participants ranged from 19-35 years old at the time of delivery and had an average pre-pregnancy body mass index of 28.2 (SD = 5.7), which falls within the World Health Organization categorization of overweight (i.e., 25-29.9; 2000). Average PSQI global score was 7.5 (SD = 3.5), which is indicative of generally poor sleep quality. As shown in Table 4.2, most participants had been pregnant before (82.4%) and delivered at least one baby (68.1%). Few women experienced PTB in a previous pregnancy (9.9%) or gestational hypertension or
preeclampsia during the assessed pregnancy (12.1%). Almost half of participants (40.7%) were induced or underwent cesarean section before going into labor and newborns weighed an average 7#1oz (range 3#14.2oz – 9#4.2oz).

Women with significant depressive symptoms reported worse sleep quality than women without significant depressive symptoms, \( t = 3.58, p = .001 \). Worse sleep quality was also associated with shorter gestation, \( r_s = -.22, p = .035 \), and women delivering before 39 weeks had marginally worse sleep quality than women delivering at 39+ weeks, \( t = 1.79, p = .077 \). Women undergoing pre-labor induction/cesarean section were more likely to report significant depressive symptoms and deliver at 39+ weeks than women presenting in labor, \( \chi^2 = 4.38, p = .036; \chi^2 = 5.78, p = .016 \), respectively. No other demographic, clinical, or pregnancy characteristics were associated with both a predictor and outcome variable for the main analyses. However, cortisol levels demonstrate a known diurnal pattern and rise as pregnancy progresses (D'Anna-Hernandez et al., 2011). Further, the literature supports that a history of PTB strongly predicts repeat PTB (Laughon, Albert, Leishear, & Mendola, 2014). In this sample, women with a history of PTB (n = 9) showed a trend toward shorter gestation and greater frequency of delivery before 39 weeks versus women without a history of PTB (n = 82), \( t = -1.84, p = .070; \text{Fisher's exact, } p = .067 \), respectively. Therefore, sleep quality, pre-labor induction/cesarean, time of awakening on the day of the blood draw (\( M = 7:30\text{am, } SD = 1.5\text{ hours} \)), time of blood draw (\( M = 1:00\text{pm, } SD = 1.5\text{ hours} \)), gestational age at blood draw (\( M = 30\text{ Weeks 3 Days, } SD = 1\text{ week 3 days} \)), and PTB history were included as covariates in the primary analyses.

Bivariate associations and univariate descriptions of the main study variables are presented in Table 4.3. Average CES-D score was 13.4 (SD = 10.6), with 26 (28.6%) participants scoring at or above the cut-off for clinically significant depressive symptoms.
CES-D score was negatively associated with gestational age at delivery and, among the full sample, the rank order of cortisol levels was positively associated with neutrophil percentages and the neutrophil:lymphocyte ratio and negatively associated with lymphocyte percentages, \( p_s \leq .05 \). Average gestational age at delivery was 39 weeks 0 days (\( SD = 1 \) week 3 days).

**Aim A: Depressive Symptoms, Cortisol Levels, and Timing of Delivery.**

Unadjusted and adjusted estimates for Aim A Models using the continuous gestational age outcome are shown in Table 4.4. Results reported below are adjusted for sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and PTB history. Women with significant depressive symptoms had marginally higher cortisol levels (i.e., 1.69 \( \mu g/dl \)) than women without significant depressive symptoms, \( \beta = .19, t = 1.78, p = .078 \). Women with significant depressive symptoms also delivered 5.59 days earlier than women without significant depressive symptoms, \( \beta = -.26, t = -2.35, p = .021 \); Figure 4.1. Further, one \( \mu g/dl \) greater cortisol was associated with delivery .61 days earlier, \( \beta = -.25, t = -2.26, p = .026 \); Figure 4.2. When depressive symptoms and cortisol levels were considered simultaneously as predictors of gestational age at delivery, each relationship was slightly attenuated but the trend remained, \( \beta = -.22, t = -1.98, p = .051 \); \( \beta = -.21, t = -1.88, p = .064 \), respectively. When models were repeated using the 39-week cut point, no significant relationships emerged, \( ps \geq .14 \).

**Aim B: Depressive Symptoms, Glucocorticoid Sensitivity, and Timing of Delivery.** Unadjusted and adjusted estimates for Aim B Models using the 39-week outcome are shown in Table 4.5 (no significant relationships were identified using the continuous gestational age outcome, \( ps \geq .16 \)). Results reported below are adjusted for
sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and PTB history.

The relationship between cortisol level and the neutrophil:lymphocyte ratio did not significantly differ according to depressive symptom status (i.e., there was no depressive symptom status by cortisol level interaction in predicting the neutrophil:lymphocyte ratio), $\beta = .45, t = 1.09, p = .280$. However, there was a significant depressive symptom status by cortisol level interaction in predicting delivery before 39 weeks, \textit{odds ratio (OR)} = 1.44, 95\% \textit{confidence interval (CI)} = 1.00-2.08; Figure 4.3. Among women with significant depressive symptoms, one μg/dl greater cortisol was associated with 1.41 times greater odds of delivery before 39 weeks, 95\% CI = 1.01-1.96. Among women without significant depressive symptoms, cortisol level was not associated with delivery before 39 weeks, $\text{OR} = .98, 95\% \text{CI} = .83-1.14$. There was also a significant depressive symptom status by neutrophil:lymphocyte ratio interaction in predicting delivery before 39 weeks, $\text{OR} = 3.16, 95\% \text{CI} = 1.16-8.66$; Figure 4.4. Among women with significant depressive symptoms, a one unit positive difference in the neutrophil:lymphocyte ratio trended toward greater odds of delivery before 39 weeks, $\text{OR} = 2.28, 95\% \text{CI} = .95-5.57$. Again, among women without significant depressive symptoms, the neutrophil:lymphocyte ratio did not predict delivery before 39 weeks, $\text{OR} = .72, 95\% \text{CI} = .45-1.16$.

In stratifying the sample according to depressive symptom status and timing of delivery, Pearson correlations for log-transformed cortisol levels and the neutrophil:lymphocyte ratio were as follows among: the full sample (n = 91), $r = .21, p = .049$; women with significant depressive symptoms delivering before 39 weeks (n = 12), $r = .28, p = .379$; women with significant depressive symptoms delivering at 39+ weeks (n = 14), $r = .30, p = .297$; women without significant depressive symptoms delivering
before 39 weeks (n = 21), $r = .12$, $p = .593$; and women without significant depressive symptoms delivering at 39+ weeks (n = 44), $r = .17$, $p = .276$; Figure 4.5.

**Discussion**

These results support a role for both depressive symptoms and cortisol elevations in the development of shortened gestation among African American women. These findings are certainly in line with a substantial literature connecting maternal depression with PTB (e.g., OR = 1.37, 95% CI 1.04-1.81 in a meta-analysis of 30 studies)(Grigoriadis et al., 2013). Presented results also confirm this relationship among African American women, specifically. Some previous studies have failed to link both depressive symptoms and cortisol levels to early birth. For example, among 27 depressed and 38 non-depressed women primarily of Caucasian race, O'Keane and colleagues (2011) report associations among depression and second trimester salivary cortisol and depression and shortened gestation. However, salivary cortisol itself did not predict earlier birth. Field et al. (2009) also reported associations among depression and cortisol and depression and shortened gestation among 336 African American women but did not appear to evaluate the relationship between cortisol and length of gestation. Given cortisol’s ability to both promote additional placental CRH release and directly influence pro-labor processes, elevated cortisol is certainly theoretically capable of promoting early labor and delivery in the right context (for review, see Li, Zhu, Myatt, & Sun, 2014).

A statistically significant relationship between depressive symptoms and cortisol levels was not detected; however, a trend was indeed present. Inadequate sample size is of course a usual suspect, as a good number of studies have identified a relationship between depression and elevated cortisol, including during pregnancy (Field et al., 2009; Lopez-Duran, Kovacs, & George, 2009; O'Keane et al., 2011; Stetler & Miller, 2011).
Other studies, particularly those sampling at a single time point, have not found this relationship (Shelton, Schminkey, & Groer, 2015). Indeed, cortisol’s diurnal pattern may make it more difficult to identify differences using this method. For example, among pregnant women, those diagnosed with depression display lower awakening cortisol levels but a decrease in cortisol’s expected decline throughout the day (O’Connor et al., 2014). Overall, cortisol production appears to be higher among depressed women; however, time of awakening and time of sampling for each participant would certainly impact results if a single time point were considered. In the current study, measures were taken to standardize the timing of the blood draw and models included time of awakening, time of blood draw, and gestational age at sampling as covariates. Future work using alternate techniques that reflect cortisol levels over an extended period, such as hair cortisol concentration, could improve ability to detect differences.

Also important to consider, the cross-sectional nature of the depressive symptom and cortisol data in the present study prohibits any conclusions regarding whether depressive symptoms lead to cortisol elevations, cortisol elevations promote the development of depressive symptoms, or perhaps the two simply vary together in response to additional unmeasured variables. The fact that depressive symptoms and cortisol elevations continued to show a trend toward prediction of shortened gestation when the other variable was held constant is interesting. There is likely more to the story and this is an interesting topic for future work.

It was also hypothesized that women with significant depressive symptoms may exhibit decreased glucocorticoid sensitivity based on a building body of psychoneuroimmunologic literature primarily assessing non-pregnant populations (for review, see Horowitz & Zunszain, 2015). In addition, glucocorticoid sensitivity has been found to decrease as pregnancy progresses, with higher prenatal depressive symptoms,
and among minority/low income versus non-minority/high income pregnant women (Corwin et al., 2013; Katz et al., 2011). In the current study, this was not the case. In fact, cortisol and the neutrophil:lymphocyte ratio were strongly correlated among women with significant depressive symptoms. Two recent studies have reported similar results. Prenatal depressive symptoms and plasma cortisol levels were shown to negatively correlate with second trimester proinflammatory cytokine levels, suggesting appropriate immune cell response to cortisol signaling (Shelton et al., 2015). Women with significant depressive symptoms, using the ≥16 cut off on the CES-D, were also reported to display higher placental glucocorticoid receptor mRNA levels during the third trimester than women without significant depressive symptoms (Reynolds et al., 2015).

These findings are interesting to consider in light of the interactions identified in the current study. The relationship between both cortisol levels and the neutrophil:lymphocyte ratio and odds of delivery before 39 weeks depended upon depressive symptom status. Among women with significant depressive symptoms, both higher cortisol levels and a higher neutrophil:lymphocyte ratio were associated with earlier delivery. This is what would be expected if cortisol levels are indeed important to timing of parturition and glucocorticoid sensitivity is maintained (i.e., both variables should rise together). Among women without significant depressive symptoms, cortisol levels were not predictive and a lower neutrophil:lymphocyte ratio increased odds of birth before 39 weeks, although this relationship was not significant. While the current study is not powered to assess this possibility, this disconnect between cortisol levels and the neutrophil:lymphocyte ratio may be indicative of decreased glucocorticoid sensitivity among women without significant depressive symptoms who go on to deliver early. Therefore, glucocorticoid sensitivity, or lack thereof, may play an important role in early birth among this group. Future work would certainly benefit from following patterns
of glucocorticoid sensitivity throughout pregnancy and postpartum among larger samples of both depressed and non-depressed women using multiple methods (e.g., cellular response to glucocorticoids, glucocorticoid receptor profiles, methylation patterns of related genes).

In sum, findings support that significant depressive symptoms and cortisol elevations are each associated with shortened gestation among African American women. Depressive symptoms and cortisol levels also showed a trend toward association with one another. This is significant, as few prior studies have assessed the psychological and biological predictors together in the context of pregnancy. An important next step is to better characterize the time course and determine whether cortisol elevation is, in fact, a biological mediator inducing shortened gestation. Further, relationships among cortisol and the neutrophil:lymphocyte ratio and delivery before 39 weeks depended upon depressive symptom status. The profile most consistent with decreased glucocorticoid sensitivity actually belonged to women without significant depressive symptoms delivering before 39 weeks. It may be that women strongly sensitive to glucocorticoids (i.e., depressed) are particularly susceptible to shortened gestation in response to cortisol elevations and women less sensitive to glucocorticoids (i.e., non-depressed) are particularly susceptible to shortened gestation in response to the enhanced inflammation present in the context of decreased glucocorticoid sensitivity. These questions are certainly worth exploring.
References


Table 4.1
Demographic and Clinical Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Full Sample (n = 91)</th>
<th>Delivery Before 39 Weeks (n = 33)</th>
<th>Delivery 39+ Weeks (n = 58)</th>
<th>Test Statistic, p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Relationship Status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unmarried/Separated</td>
<td>69 (75.8)</td>
<td>27 (81.8)</td>
<td>42 (72.4)</td>
<td>$\chi^2 = 1.01, p = .31$</td>
</tr>
<tr>
<td>Married</td>
<td>22 (24.2)</td>
<td>6 (18.2)</td>
<td>16 (27.6)</td>
<td></td>
</tr>
<tr>
<td>Educational Attainment</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>&lt;Bachelor’s Degree</td>
<td>67 (73.6)</td>
<td>25 (75.8)</td>
<td>42 (72.4)</td>
<td>$\chi^2 = .12, p = .73$</td>
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<tr>
<td>&gt;Bachelor’s Degree</td>
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<td>8 (24.2)</td>
<td>16 (27.6)</td>
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<tr>
<td>Insurance Status</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did Not Report Private</td>
<td>61 (67.0)</td>
<td>24 (72.7)</td>
<td>37 (63.8)</td>
<td>$\chi^2 = .76, p = .38$</td>
</tr>
<tr>
<td>Private</td>
<td>30 (33.0)</td>
<td>9 (27.3)</td>
<td>21 (36.2)</td>
<td></td>
</tr>
<tr>
<td>Mean Maternal Age (SD)</td>
<td>26.4 (4.5)</td>
<td>26.5 (4.9)</td>
<td>26.3 (4.3)</td>
<td>$t = .29, p = .77$</td>
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<tr>
<td>Mean Pre-Pregnancy Body Mass Index (SD)</td>
<td>28.2 (5.7)</td>
<td>28.5 (5.2)</td>
<td>28.1 (6.0)</td>
<td>$t = .30, p = .77$</td>
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<td>Mean Sleep Quality (SD)</td>
<td>7.5 (3.5)</td>
<td>8.3 (3.9)</td>
<td>6.7 (3.1)</td>
<td>$t = 1.79, p = .08$</td>
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</table>

Note. Test statistics shown are from comparisons between women delivering before 39 weeks gestation and at 39+ weeks gestation using $\chi^2$ tests and $t$ tests.
Table 4.2
Pregnancy Characteristics and Delivery Outcomes

<table>
<thead>
<tr>
<th></th>
<th>Full Sample (n = 91)</th>
<th>Delivery Before 39 Weeks (n = 33)</th>
<th>Delivery 39+ Weeks (n = 58)</th>
<th>Test Statistic, p value</th>
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</thead>
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<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
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<tr>
<td>Gravidity</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>16 (17.6)</td>
<td>6 (18.2)</td>
<td>10 (17.2)</td>
<td>Fisher’s exact, p = .91</td>
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<tr>
<td>≥2</td>
<td>75 (82.4)</td>
<td>27 (81.8)</td>
<td>48 (82.8)</td>
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<td>Parity</td>
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<tr>
<td>0</td>
<td>29 (31.9)</td>
<td>12 (36.4)</td>
<td>17 (29.3)</td>
<td>χ² = .48, p = .49</td>
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<tr>
<td>≥1</td>
<td>62 (68.1)</td>
<td>21 (63.6)</td>
<td>41 (70.7)</td>
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<td>Previous Preterm Birth</td>
<td></td>
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<td>Yes</td>
<td>9 (9.9)</td>
<td>6 (18.2)</td>
<td>3 (5.2)</td>
<td>Fisher’s exact, p = .07</td>
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<tr>
<td>No</td>
<td>82 (90.1)</td>
<td>27 (81.8)</td>
<td>55 (94.8)</td>
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<td>Current Gestational Diabetes Mellitus</td>
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<td>Yes</td>
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<td>0 (0)</td>
<td>Fisher’s exact, p = 1.00</td>
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<td>91 (100)</td>
<td>33 (100)</td>
<td>58 (100)</td>
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<td>Current Gestational Hypertension/Preeclampsia</td>
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<td>Yes</td>
<td>11 (12.1)</td>
<td>7 (21.2)</td>
<td>4 (6.9)</td>
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<tr>
<td>No</td>
<td>80 (87.7)</td>
<td>26 (78.8)</td>
<td>54 (93.1)</td>
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<td>Presentation for Delivery</td>
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<tr>
<td>In Labor</td>
<td>54 (59.3)</td>
<td>25 (75.8)</td>
<td>29 (50.0)</td>
<td>χ² = 5.78, p = .02</td>
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<td>For Induction/Cesarean</td>
<td>37 (40.7)</td>
<td>8 (24.2)</td>
<td>29 (50.0)</td>
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<tr>
<td>Mean Birth weight in Grams (SD)</td>
<td></td>
<td></td>
<td></td>
<td>t = -4.54, p &lt; .001</td>
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<tr>
<td></td>
<td>3203.2 (485.4)</td>
<td>2925.6 (536.8)</td>
<td>3361.0 (374.5)</td>
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</tbody>
</table>

Note. Test statistics shown are from comparisons between women delivering before 39 weeks gestation and at 39+ weeks gestation using χ² tests, Fisher’s exact tests when cells contained < 5 observations, and t tests.
Table 4.3

Bivariate Associations and Univariate Descriptive Statistics for Main Study Variables

<table>
<thead>
<tr>
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<th>(4)</th>
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<th>(6)</th>
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<tbody>
<tr>
<td>(1) Depressive Symptoms (CES-D Total Score)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>(2) Cortisol Level (µg/dl)</td>
<td>.15</td>
<td>1</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>(3) Neutrophil Percentage</td>
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<td>.23**</td>
<td>1</td>
<td></td>
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</tr>
<tr>
<td>(4) Lymphocyte Percentage</td>
<td>.07</td>
<td>-.23**</td>
<td>-.92***</td>
<td>1</td>
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<tr>
<td>(5) Neutrophil:Lymphocyte Ratio</td>
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<td>.95***</td>
<td>-.99***</td>
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<tr>
<td>(6) Gestational Age at Delivery (Days)</td>
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<td>.02</td>
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<tr>
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<td>12.1</td>
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<td>5.4</td>
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Note. Spearman’s rank correlations provided.

*p < .1, **p < .05, ***p < .01
Table 4.4
Linear Regression Models for Aim A

<table>
<thead>
<tr>
<th>Model A1 Predicting Cortisol Level</th>
<th>Model A2 Predicting Gestational Age at Delivery</th>
<th>Model A3 Predicting Gestational Age at Delivery</th>
</tr>
</thead>
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<tr>
<td>$\beta$, $t$</td>
<td>$\beta$, $t^a$</td>
<td>$\beta$, $t$</td>
</tr>
<tr>
<td>Depressive Symptoms</td>
<td></td>
<td></td>
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<tr>
<td>Without Significant</td>
<td>Reference</td>
<td>Reference*</td>
</tr>
<tr>
<td>With Significant</td>
<td>.10, .97</td>
<td>.19, 1.78</td>
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<tr>
<td>Cortisol Level (µg/dl)</td>
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</tr>
<tr>
<td>Sleep Quality</td>
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<td>-.07, -.63</td>
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<td>Presentation for Delivery</td>
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<td></td>
</tr>
<tr>
<td>In Labor</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>For Induction/Cesarean</td>
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<td>History of Preterm Birth</td>
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<tr>
<td>History</td>
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<td>Time of Awakening</td>
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<td>Time of Blood Draw</td>
<td>---</td>
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<tr>
<td>Gestational Age at Blood Draw</td>
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<td>-.05, -.5</td>
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</table>

*a adjusted for sleep quality, pre-labor induction/cesarean, preterm birth history, time of awakening, time of blood draw, and gestational age at blood draw.

*p < .1, **p < .05, ***p < .01
Table 4.5  
Linear and Logistic Regression Models for Aim B

<table>
<thead>
<tr>
<th>Model B1 Predicting the Neutrophil:Lymphocyte Ratio</th>
<th>Model B2 Predicting Delivery Before 39 Weeks</th>
<th>Model B3 Predicting Delivery Before 39 Weeks</th>
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<tr>
<td>( \beta, t )</td>
<td>( \beta, t^a )</td>
<td>OR (95% CI)</td>
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<tr>
<td>Depressive Symptoms by Cortisol Level (ng/ml)</td>
<td>.56, 1.45</td>
<td>.45, 1.09</td>
</tr>
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<td>Depressive Symptoms by Neutrophil:Lymphocyte Ratio</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Cortisol Level (( \mu)g/dl)</td>
<td>.12, 1.01</td>
<td>.12, .92</td>
</tr>
<tr>
<td>Neutrophil:Lymphocyte Ratio</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Sleep Quality</td>
<td>---</td>
<td>-.04, -.30</td>
</tr>
<tr>
<td>Presentation for Delivery In Labor</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>For Induction/Cesarean</td>
<td>---</td>
<td>.01, .12</td>
</tr>
<tr>
<td>History of Preterm Birth</td>
<td>No History</td>
<td>---</td>
</tr>
<tr>
<td>History</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Time of Awakening</td>
<td>---</td>
<td>-.13, -.105</td>
</tr>
<tr>
<td>Time of Blood Draw</td>
<td>---</td>
<td>-.12, -.106</td>
</tr>
<tr>
<td>Gestational Age at Blood Draw</td>
<td>---</td>
<td>.03,.25</td>
</tr>
</tbody>
</table>

\(^a\)adjusted for sleep quality, pre-labor induction/cesarean, preterm birth history, time of sampling.  
\(*p < .1, **p < .05, ***p < .01\)
Figure 4.1. Depressive Symptom Status and Gestational Age at Delivery. Women with significant depressive symptoms delivered their babies 5.59 days earlier than women without significant depressive symptoms, $\beta = -0.26$, $t = -2.35$, $p = 0.021$ (estimates adjusted for sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and preterm birth history).
Figure 4.2. Cortisol Levels and Gestational Age at Delivery. One μg/dl greater cortisol was associated with delivery .61 days earlier, $\beta = -0.25$, $t = -2.26$, $p = .026$ (estimates adjusted for sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and preterm birth history).
Figure 4.3. Cortisol Levels and Gestational Age at Delivery according to Depressive Symptom Status. Among women with significant depressive symptoms, one μg/dl greater cortisol was associated with 1.41 times greater odds of delivery before 39 weeks, 95% CI = 1.01-1.96. There was no association among women without significant depressive symptoms, OR = .98, 95% CI = .83-1.14 (estimates adjusted for sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and preterm birth history).
Among women with significant depressive symptoms, a one unit positive difference in the neutrophil:lymphocyte ratio trended toward greater odds of delivery before 39 weeks, $OR = 2.28$, 95% CI = .95-5.57. There was no association among women without significant depressive symptoms, $OR = .72$, 95% CI = .45-1.16. (estimates adjusted for sleep quality, pre-labor induction/cesarean, time of awakening, time of blood draw, gestational age at blood draw, and preterm birth history).
Figure 4.5. Cortisol Levels and Neutrophil:Lymphocyte Ratio according to Depressive Symptom Status and Gestational Age at Delivery. Pearson correlation coefficients among: the full sample (n = 91), r = .21, p = .049; women with significant depressive symptoms delivering before 39 weeks (n = 12), r = .28, p = .379; women with significant depressive symptoms delivering at 39+ weeks (n = 14), r = .30, p = .297; women without significant depressive symptoms delivering before 39 weeks (n = 21), r = .12, p = .593; and women without significant depressive symptoms delivering at 39+ weeks (n = 44), r = .17, p = .276. Of note, cortisol levels are depicted in μg/dl; however, levels were log transformed to better meet normality assumptions before estimating correlation coefficients.
CHAPTER 5: CONCLUSIONS
It is not difficult to make the argument that preterm birth (PTB) is worth studying. In the U.S., a baby is born every 8 seconds (Hamilton, Martin, Osterman, & Curtain, 2015). One can definitively state that the process of childbearing touches us all: as sons and daughters, mothers and fathers, brothers and sisters. The human body adapts in miraculous ways to support a growing fetus and bring the baby into the world at the appropriate time. When this process goes awry, the consequences are devastating: babies die, children face illness, families are severely impacted (Heron, 2015; Vieira & Linhares, 2011). Sadly, PTB is not rare. In the U.S., a preterm baby is born every 90 seconds. Further, 14.8% of U.S. births are to African American moms but these same women bare the burden of more than 20% of PTBs (Hamilton et al., 2015). These are problems that must be addressed.

In Chapter 2 of this dissertation, I put forth the case that early identification of women at risk for spontaneous PTB, which accounts for 70% of all PTBs, is critical (Goldenberg, Culhane, Iams, & Romero, 2008). These women could be provided with proven preventive therapies, such as progesterone. If the pathway by which PTB is likely to ensue is also identified, novel preventive therapies targeting the specific cellular/molecular processes may be developed. Why, then, are screening tests lacking? Why is the research community unable to develop targeted treatments? In short, pathways leading to early birth are extremely complicated. In fact, spontaneous PTB is increasingly accepted as "a syndrome attributable to multiple pathologic processes", with "genetic and environmental factors" likely contributing to each (Romero, Dey, & Fisher, 2014, pp. 761-762). Therefore, pathway studies serve as the foundation upon which prediction and prevention work must rely.

This dissertation takes the first steps in a line of pathway work aiming to address these critical gaps in knowledge. Here, I have focused on impairments in inflammatory
regulation as a mechanism possibly linking genetic and psychosocial exposures with early birth. In Chapter 3, I evaluated if genetic variation was associated with length of gestation via altered interleukin(IL)-1Ra production and/or by altering the relationship between IL-1β and IL-1Ra production. Women lacking the minor allele at a tagging single nucleotide polymorphism (SNP) of the IL1RN gene had decreased odds of reaching 39 weeks. Greater IL-1β production was associated with decreased odds of reaching 38 weeks and the predictive value of IL-1Ra depended upon IL1RN allele status. Women lacking the minor allele appear to display a profile dominated by IL-1β production. Therefore, greater IL-1β production may be sufficient to predict earlier delivery because levels of the antagonist are insufficient to halt its activity. Among women possessing the minor allele, earlier delivery was associated with both higher IL-1β production and lower IL-1Ra production; i.e., both variables became important in predicting timing of delivery.

There are important implications to these findings. First, in terms of bio-panel development, we present a case in which prediction of timing of delivery is increasingly improved when knowledge of clinical history is supplemented with physiological data and then when physiological data is supplemented with genetic data. For example, let us examine three logistic regression models using the Chapter 3 dataset with the following variables serving as predictors and odds of reaching 38 weeks serving as the outcome: 1) history of PTB (i.e., the only information generally available to practitioners), 2) model 1 plus ex vivo IL-1β and IL-1Ra production, and 3) model 2 plus IL1RN tagging SNP allele status and its interaction with IL-1Ra production. As shown in Table 5.1, increasing model performance is evidenced by the increasing proportion of variability in the dependent variable explained by each successive model (pseudo R-squared) as well as likelihood ratio tests indicating that the added variables significantly improve the fit of the
model. This supports further bio-panel development focused on aspects of inflammatory regulation, including how proinflammatory mediators and their respective antagonists work together. Further, the fact that a biological predictor performed differently dependent upon a specific genetic variant suggests that personalization of bio-panels may be possible through genetic testing. In other words, allele status at particular genetic loci may direct clinicians to the appropriate bio-panel for their patient. This is certainly a possibility worth testing, particularly in terms of groups of genes known to influence different aspects of inflammatory regulation.

Further, if greater availability of IL-1Ra curbs IL-1β activity, preventive treatment with synthetic IL-1Ra may be beneficial. Indeed, others have considered this possibility. IL-1Ra infusion has prevented IL-1β-induced but not lipopolysaccharide(LPS)-induced PTB in murine models (Fidel et al., 1997; Romero & Tartakovsky, 1992). This difference is likely due to the fact that LPS infusion induces heightened production of a number of proinflammatory mediators, allowing the inflammatory cascade to proceed despite reduced IL-1 signaling. Therefore, additional antagonists will likely need to be considered. In Chapter 2, we discussed tumor necrosis factor (TNF)-α and its soluble receptors as a proinflammatory/antagonist dyad worth evaluating in the context of PTB. Notably, double knockout of both the IL-1 and TNF Type 1 receptors does significantly reduce risk for bacterially induced PTB in the murine model (Hirsch, Filipovich, & Mahendroo, 2006). Therefore, combined treatment with synthetic IL-1Ra and soluble TNF receptor compounds may be particularly beneficial. However, great care must be taken to ensure that the inflammatory response is not overly reduced, as minor infections could become significant problems. Much work remains to be done but this may be an avenue with great potential.
I also evaluated if greater psychosocial stress and/or significant depressive symptoms predicted shortened gestation and whether relationships were related to the variables’ associations with: A) greater IL-1β production, B) cortisol elevations, and/or C) decreased glucocorticoid sensitivity. Of note, we do not report results for IL-1β production or the psychosocial stress variables in Chapter 4. IL-1β production showed a trend toward positive association with prenatal distress; however, no associations with any of the stress variables or depressive symptoms were significant. This may be an interesting topic for future work among larger samples. Further, scores on the psychosocial measures were significantly positively associated with depressive symptoms but only depressive symptoms seemed to play a role in timing of delivery (Table 5.2). It is important to consider that psychosocial stress may be conceptualized in many ways. For this dissertation, I chose to evaluate psychosocial stress related to life events encountered during the prenatal period, pregnancy-related experiences, and discrimination-related experiences.

Specifically, the Prenatal Life Events Scale (PLES) assesses acute stress by asking subjects to indicate if they or a close family member or friend have experienced 28 events during pregnancy (e.g., death of loved one; Lobel, 1996b). For items endorsed, subjects are asked how negative or undesirable the event was from 0 (not at all) to 3 (very much). Total life event stress was used for analyses and therefore captures the exposure and subjective rating of its impact. The Revised Prenatal Distress Questionnaire (NUPDQ) assesses stress experienced during windows of pregnancy (Lobel, 1996a; Yali & Lobel, 1999). Subjects were asked to indicate if they were feeling not at all (0), somewhat (1), or very much (2) bothered, upset, or worried about factors pertinent to pregnancy in general (e.g., working or caring for their family) and the latter months of pregnancy (e.g., whether the baby might come too early; Lobel et al., 2008).
Total score was used for analyses and therefore reflects subjective reports of stress. The Experiences of Discrimination Scale (EOD) measures cumulative experience of discrimination over the lifetime (Krieger, 1990; Krieger & Sidney, 1996). Subjects are asked if they have experienced race-, ethnicity-, or color-based discrimination in nine situations (e.g., at work). Each situation is scored 0 (no experience), 1 (once), 2 (2-3 times), or 3 (>4 times). Total frequency score was used for analyses.

The lack of association between scores on these measures and timing of delivery may be related to several issues. First, both the PLES and NUPDQ focus on acutely experienced stress. While some have linked stress experienced during the current pregnancy to birth outcomes, others have proposed that chronically endured stress may be particularly detrimental (Latendresse, 2009; Lobel et al., 2008; Zhu, Tao, Hao, Sun, & Jiang, 2010). Indeed, chronic stress, such as prolonged childhood adversity, is considered a potential cause of a myriad of health problems throughout the lifespan (for review, see Nusslock & Miller, 2015). Others have proposed that patterns of stress across pregnancy are more important in predicting timing of delivery than cross-sectional assessments. For example, Glynn, Dunkel Schetter, Hobel, and Sandman (2008) found that women reporting increasing levels of stress from second to third trimester were more likely to deliver preterm. The cross-sectional stress data, however, did not predict risk for PTB. Therefore, measurement of change over time may be necessary. Further, the EOD assesses exposure to discrimination but fails to capture an individual’s subjective appraisal of the stress related to this exposure. The psychoneuroimmunologic framework is consistent in its supposition that the psychological stress response is important – measurement of appraisal of the stress related to exposures to discrimination may be key (Ader, 2000; Christian, 2011; Glaser & Kiecolt-Glaser, 2005).
It may also be that there is something unique about the physiologic profile associated with depression. Psychoneuroimmunologic researchers have increasingly prescribed to the notion that psychosocial stress-induced inflammation may actually be causative in the development of future depressed mood (Irwin & Miller, 2007; O’Connor, Moynihan, & Caserta, 2014; Slavich & Irwin, 2014). If this is the case, significant depressive symptoms may actually serve as an indirect measure of profound and/or prolonged stress-induced pathophysiology. Subjective measures of psychosocial stress may not adequately capture this variation in physiologic response to stress. It may be prudent, then, to compare the physiologic profiles of women with similar reports of psychosocial stress that do and do not go on to develop depression. Changes specific to the depressed profile may be particularly important in pathways to PTB.

Chapter 4 did report that depressive symptoms were significantly associated with earlier delivery and marginally associated with greater cortisol levels. Greater cortisol levels were associated with earlier delivery. However, relationships between cortisol levels and the neutrophil:lymphocyte ratio and the 39-week outcome were impacted by depressive symptom status. Among women with significant depressive symptoms, higher cortisol and a higher neutrophil:lymphocyte ratio increased odds of delivery before 39 weeks. Among women without significant depressive symptoms, cortisol did not predict earlier delivery and a lower neutrophil:lymphocyte ratio trended toward increased odds of delivery before 39 weeks. We also noted the strongest correlations between cortisol and the neutrophil:lymphocyte ratio among women with significant depressive symptoms. Women without significant depressive symptoms delivering before 39 weeks showed the greatest disconnect between cortisol levels and the neutrophil:lymphocyte ratio, which may suggest decreased glucocorticoid sensitivity.
In considering these results, the dissertation’s overarching hypothesis that impaired inflammatory regulation may predispose women to a premature inflammatory cascade and early birth must be revisited. Notably, cortisol may both promote and dampen proinflammatory activity. For example, cortisol and related placental corticotropin-releasing hormone (CRH) elevations promote production of cortisol by the fetal adrenal gland, surfactant protein-A by the fetal lungs, and ultimately proinflammatory cytokine production within maternal tissues (for review, see Vrachnis, Malamas, Sifakis, Tsikouras, & Iliodromiti, 2012). In contrast, cortisol exerts anti-inflammatory effects at the cellular level. If immune cells are sensitive to the cortisol, the glucocorticoid reduces inflammatory activity (for review, see Silverman & Sternberg, 2012). Therefore, it may be that cortisol serves a proinflammatory and pro-labor role among women with significant depressive symptoms, a group that demonstrates both cortisol elevations and appears to be quite sensitive to cortisol signaling. Among women without significant depressive symptoms, it may be that decreased glucocorticoid sensitivity increases risk for shortened gestation. Therefore, the presence or absence of significant depressive symptoms may put women at risk for particular pathways to early birth. More detailed assessment of both immune cell and placental responsiveness to cortisol across pregnancy is warranted. This can be accomplished by, for example, determining the degree to which dexamethasone inhibits cytokine production upon ex vivo stimulation or measuring glucocorticoid-induced transcriptional activation.

In addition to the possibilities for future assessment already discussed, the work initiated in this dissertation may be furthered in several ways. First, a larger sample size may be needed to detect associations that were marginal among our current sample. In addition, longitudinal assessment of psychosocial and physiological data will likely improve ability to detect differences. This would allow for within-person assessment of
associations between proinflammatory mediators and their respective antagonists as well as cortisol levels and their expected correlates (e.g., neutrophil:lymphocyte ratio, CRH) over time. These measures of change may serve as better predictors of timing of delivery. More detailed examination of immune cell function must also be considered. For example, Yuan and colleagues (2009) report that peripheral leukocytes display a ‘primed’ phenotype during preterm and term labor – e.g., migratory ability increases, receptors are upregulated. To my knowledge, peripheral leukocyte phenotype has not been followed longitudinally across pregnancy to determine if subtle changes over time can predict risk for PTB.

In sum, this dissertation reports several important associations among genetic variants, depressive symptoms, IL-1β regulation during pregnancy, and shortened gestation. There is no doubt that many questions remain to be answered in the goal of understanding the pathways leading to PTB. But each question may be viewed as an exciting opportunity. Specifically, each pathway finding has the potential to feed directly into continued work on bio-panel development and preventive treatment design. Development of a sensitive and specific screening tool for the prediction of early birth would be a major advancement in the field of obstetrics; women could be provided with progesterone, a proven preventive therapy that currently reaches few women. The ability to block pathologic processes at the molecular level, long before contractions begin, the cervix dilates, and fetal membranes degrade, could reduce PTB to a rare syndrome. These are certainly noble goals worthy of considerable and enthusiastic research effort.
References


Table 5.1
Comparisons of Restricted and Unrestricted Logistic Regression Models predicting Delivery Before 38 Weeks

<table>
<thead>
<tr>
<th>Model</th>
<th>Predictors</th>
<th>Model Likelihood Ratio Chi-Square</th>
<th>Model $p$ Value</th>
<th>Model Pseudo R-Squared</th>
<th>Likelihood Ratio Test $p$ Value</th>
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</thead>
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<td>A</td>
<td>1. History of PTB</td>
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<tr>
<td>B</td>
<td>1. History of PTB 2. IL-1β Production 3. IL-1Ra Production</td>
<td>15.4</td>
<td>0.0015</td>
<td>0.175</td>
<td>0.0028$^1$</td>
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<td>C</td>
<td>1. History of PTB 2. IL-1β Production 3. IL-1Ra Production 4. Allele Status 5. Allele Status*IL-1Ra Production</td>
<td>21.64</td>
<td>0.0006</td>
<td>0.246</td>
<td>0.0012$^1$ 0.0441$^2$</td>
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</tbody>
</table>

Note. PTB = preterm birth; IL = interleukin.

$^1$Likelihood Ratio Test Comparing Model to Model A

$^2$Likelihood Ratio Test Comparing Model to Model B
Table 5.2

**Spearman’s Rank Correlations**

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<th>(2)</th>
<th>(3)</th>
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<th>(5)</th>
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<td></td>
</tr>
<tr>
<td>(2) IL-1β Production (ng/dl)</td>
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<tr>
<td>(3) Prenatal Life Events Scale Score</td>
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<tr>
<td>(4) Revised Prenatal Distress Questionnaire Score</td>
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<td>0.18*</td>
<td>0.51***</td>
<td>1.00</td>
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<tr>
<td>(5) Experiences of Discrimination Frequency Count</td>
<td>0.02</td>
<td>-0.01</td>
<td>0.28***</td>
<td>0.17</td>
<td>1.00</td>
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<tr>
<td>(6) Center for Epidemiologic Studies Depression Scale Score</td>
<td>-0.21**</td>
<td>0.90</td>
<td>0.58***</td>
<td>0.65***</td>
<td>0.25**</td>
<td>1.00</td>
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</tbody>
</table>

*p < .1, **p < .05; ***p < .01
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Schulz, A., Israel, B., Williams, D., Parker, E., Becker, A., & James, S. (2000). Social inequalities, stressors and self reported health status among african american and


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