THE GENETICS AND EPIDEMIOLOGY OF REPRODUCTIVE DISORDERS

By

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To my mom, Linda, for her strength, love and courage

and

To my husband, Brad, for his love, kindness and never ending support

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LIST OF ABBREVIATIONS

AA	African Americans
ANOVA	Analysis of variance
BV	Bacterial vaginosis
COL1A2	Collagen, type 1, alpha-2
CRH	Corticotropin releasing hormone
CRHBP	Corticotropin releasing hormone binding protein
EA	European Americans
EGF	Epidermal growth factor
EOTAXIN	Chemokine, CC motif, ligand 11
FDR	False discovery rate
FGF2	Fibroblast growth factor 2
GMCSF	Granulocyte macrophage colony stimulating factor
HHC	Hyperhomocysteinaemia
HPA	Hypothalamic-pituitary-adrenal axis
HWE	Hardy-Weinberg equilibrium
IL-10	Interleukin 10
IL10RA	Interleukin 10 receptor alpha
IL-12p40	Interleukin 12 subunit p40
IL-12p70	Interleukin 12 subunit p70
IL-13	Interleukin 13
IL-15	Interleukin 15

IL-1α	Interleukin 1 alpha
IL-1β	Interleukin 1 beta
IL-1R1	Interleukin 1 receptor 1
IL-1R2	Interleukin 1 receptor 2
IL1RAP	Interleukin 1 receptor accessory protein
IL-1RN	Interleukin 1 receptor antagonist
IL-2	Interleukin 2
IL-3	Interleukin 3
IL-4	Interleukin 4
IL-4R	Interleukin 4 receptor
IL-5	Interleukin 5
IL-6	Interleukin 6
IL-6R	Interleukin 6 receptor
IL-7	Interleukin 7
IL8RA	Interleukin 8 receptor alpha
IP10	Interferon gamma inducible protein 10
IUGR	Intrauterine growth restriction
MCP1	Monocyte chemotactic protein 1
MIP1α	Macrophage inflammatory protein 1 alpha
mL	Milliliters
MTHFR	Methylenetetrahydrofolate reductase
MTRR	Methionine synthase reductase
ng	Nanograms

p	p-value
PDGF-AA	Platelet derived growth factor, alpha
PDGF-BB	Platelet derived growth factor, beta
PON1	Paraoxonase 1
PROM	Premature rupture of the membranes
PPROM	Preterm premature rupture of the membranes
PTB	Preterm birth
PTGER3	Prostaglandin E receptor 3
PTL	Preterm labor
RANTES	Regulated upon activation, normally T-expressed and presumably secreted
SNP	Single nucleotide polymorphism
TFPI	Tissue factor pathway inhibitor
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
TNF-R1	Tumor necrosis factor receptor 1
TNF-R2	Tumor necrosis factor receptor 2
VEGF	Vascular endothelial growth factor
YRI	Yoruban

CHAPTER I

OVERVIEW

There are approximately 6 million pregnancies in the United States each year and about 1 million (~17%) of these have complications which result in fetal loss (Ventura et al., 2008). Multiple maternal environmental risk factors including smoking, infection and nutrition have been identified. Studies have examined genetic variants for association with many of these disorders and while there has been some success, this field remains unstudied or poorly understood. The focus of this dissertation is on examining the epidemiology and genetic mechanisms of two very common reproductive disorders: bacterial vaginosis (BV) and preterm birth (PTB).

The introduction and background of both BV and PTB are presented in Chapter II, part A. Clinical diagnosis, treatment, epidemiology and genetic associations for both disorders are discussed. Additionally, strategies for studying these disorders are presented. Part B lays out the hypothesis and specific aims of this dissertation.

To understand the epidemiology and immunology of BV, cervical cytokine levels are examined in Chapter III. Part A identifies differences in cervical cytokine concentrations by BV status and race. Part B examines cervical cytokine network differences by BV status and race. These studies show that BV positive (BV^+) European Americans (EA) have significantly lower levels of the pro-inflammatory cytokines interferon gamma inducible protein (IP10) and monocyte chemotactic protein (MCP1) compared to BV negative (BV^-) women; while BV^+ African Americans (AA) have

significantly higher levels of the pro-inflammatory cytokine interleukin 1 alpha (IL-1α). Additionally, correlation analysis reveals that AA have a stronger correlated response to infection than EA.

Chapter IV examines the genetic regulation of some of the pro- and anti-inflammatory cytokines examined in Chapter III. Part A examines single nucleotide polymorphisms (SNPs) in anti-inflammatory cytokine genes and receptors for associations with cervical anti-inflammatory cytokine concentrations. In Part B, SNPs in pro-inflammatory cytokine genes and receptors that associate with cervical pro-inflammatory cytokine concentrations are identified. Part C determines if SNPs in toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4) are associated with pro- and anti-inflammatory cytokine concentrations. These studies indicate that in EA SNPs in interleukin 6 receptor (IL-6R) and interleukin 10 receptor alpha (IL-10RA) are associated with cervical concentrations of interleukin 6 (IL-6) and interleukin 10 (IL-10), respectively and these associations are influenced by BV status. In AA, variants in tumor necrosis factor alpha receptor 2 (TNFR2), interleukin 8 receptor alpha (IL-8RA) and interleukin 1 receptor accessory protein (IL-1RAP) are associated with cervical concentrations of tumor necrosis factor alpha (TNF-α), interleukin 8 (IL-8) and interleukin 1 beta (IL-1β), respectively; these associations are also influenced by BV status. Additionally, in EA but not AA, SNPs in TLR4 are associated with levels of IL-6, IL-1β and TNF-α and are influenced by BV status. These studies reveal that the gene receptors, not necessarily the genes themselves are important in regulating cervical cytokine levels and that genetic regulation differs by BV status and race.

Chapter V identifies maternal and fetal SNPs associated with PTB. Part A focuses on single locus associations with PTB, and part B highlights associations that overlap with an independent analysis of PTB in an EA population. These studies indicate that the strongest single locus maternal and fetal associations, including those that are strongly associated in a pooled population of two independent EA populations, are involved in the following pathways: extracellular matrix strength (COL1A2), inflammation and infection (IL1RAP), complement and coagulation (TFPI and PON1) and prostaglandin synthesis (PTGER3).

CHAPTER II

INTRODUCTION

A. Introduction and Background

Bacterial Vaginosis

Epidemiology

Prevalence. BV is one of the most prevalent vaginal disorders in adult women. It is currently estimated that approximately 30-50% of AA and 10-20% of EA are affected (Eschenbach, 1993; Hillier et al., 1995; McGregor and French, 2000; Cauci et al., 2002). Additionally, BV occurs in approximately 15-50% of pregnant women and is the most common type of lower genital tract infection in pregnancy (Eschenbach, 1993; Hillier et al., 1995; McGregor and French, 2000; Wadhwa et al., 2001; Cauci et al., 2002). However, because nearly 50% of affected women do not experience symptoms, and consequently do not seek diagnosis or treatment, the true prevalence of BV may be higher (Mitchell, 2004).

BV is associated with endometritis, nonchlamydial pelvic inflammatory disease, an increase in risk of contracting and transmitting HIV and urinary tract infections (Taha et al., 1998; Sweet, 2000; Ness et al., 2004b). Pregnant women with BV are at an increased risk for adverse reproductive outcomes such as miscarriage, PTB, premature rupture of the membranes (PROM), amniotic fluid infection, chorioamnionitis and

postpartum endometritis (Koumans and Kendrick, 2001; CDC, 2002; O'Brien, 2005). Infants born to women with BV have an increased risk for localized infectious complications, bacteremia, culture-negative sepsis syndrome and necrotizing enterocolitis (Dammann and Leviton, 1997; Amaya et al., 2002; Stelmach et al., 2004).

Table 2-1. Risk factors for BV

Risk Factor	*Referent Group*	*OR*	[1]*Reference*
AA	EA	2.5-2.8	(Culhane et al., 2001; Koumans et al., 2007)
Mexican American	EA	1.6	(Koumans et al., 2007)
Douching between 1-5 times per month	No douching in past 6 months	2.1-2.5	(Culhane et al., 2001; Koumans et al., 2007)
Lifetime number of sex partners (1-5)	0	1.4-2.4	(Culhane et al., 2001; Koumans et al., 2007)
Birth control pill use	No use	0.65	(Koumans et al., 2007)
High school education or less	Continued education after high school	1.4	(Koumans et al., 2007)
Moderate to high stress	Low-stress	2.2-2	(Culhane et al., 2001)

[1]Culhane *et al* 2001 included 454 pregnant women at ~14 weeks gestation and Koumans *et al* 2007 included 2,906 women from the National Health and Nutrition Examination Survey (NHANES)

Risk Factors. Common risk factors for BV include race, stress, education, sexual activity, contraceptive use, and frequent douching (Table 2-1) (Culhane et al., 2001; O'Brien, 2005). AA women are at a two times greater risk for developing BV than EA after adjusting for socio-demographic variables such as education and income (Ness et al., 2003). The reasons why AAs are more susceptible to BV than EAs are poorly understood and very few studies have directly addressed this issue.

Diagnosis and treatment

Clinical Diagnosis. BV is a serious reproductive disorder that is most often asymptomatic and therefore difficult to diagnose. Women with symptoms are clinically diagnosed by the Amsel criteria characterized by three of the four symptoms: 1) increased vaginal pH (>4.5), 2) presence of an amine fishy odor after adding 10% potassium hydroxide to the vaginal secretion, 3) a white-grayish adherent discharge and 4) presence of "clue-cells", which are epithelial cells covered by adherent anaerobic bacteria (Amsel et al., 1983).

Microbiology. BV is characterized by the presence of *Gardnerella vaginalis, Bacteroides* and *Mobiluncus* and the absence of lactobacilli. *G. vaginalis* and *Bacteroides* are facultative anaerobic gram-negative rods. However, *G. vaginalis* is often referred to as gram-variable because the cell wall of the micro-organism sometimes stains gram-positive. *Mobiluncus* species are curved anaerobic gram variable rods that generally stain gram-positive. Lactobacilli are facultative anaerobic gram-positive bacteria that are components of the healthy vaginal microflora.

The Spiegel method demonstrated that gram staining is an acceptable procedure for identifying the various bacteria associated with BV (Spiegel et al., 1983). The Nugent score expands this method by quantifying the micro-organisms in the vaginal tract and is the gold standard for diagnosing BV (Nugent et al., 1991). The presence of *Lactobacillus* species, *Gardnerella* species (such as *G. vaginalis*, *Prevotella*, *Bacteroides* and *Porphyromonas* species) and *Mobiluncus* species are detected by gram stain. An individual gram stain score ranging from 0 to 4 for *Lactobacillus* and *Gardnerella* and 0 to 2 for *Mobiluncus* is determined based on the number of morphotypes or colonies

present per oil-immersion field under a microscope (Table 2-2). This score is totaled with 0-3 indicating normal flora, 4-6 indicating intermediate flora and 7-10 indicating BV. The Nugent score not only has a high degree of interobserver reproducibility but also correlates well with clinical signs and symptoms of the disorder (Joesoef et al., 1991; Hillier et al., 1992)

Table 2-2. Nugent scoring system

Score	*Lactobacillus species*	*Gardnerella and Bacteroides*	*Mobiluncus species*
0	>30	0	0
1	5-30	1	1-4
2	2-4	2-4	>5
3	1	5-30	
4	0	>30	

Average number of colonies corresponds to gram stain score

Treatment. Treatment generally includes the administration of the antibiotics metronidazole or clindamycin (CDC, 2006). Metronidazole inhibits DNA replication of anaerobic bacteria; while clindamycin inhibits protein synthesis of gram-positive bacteria and other anaerobes. However, studies have shown that several microbes associated with BV develop resistance to these antibiotics after treatment; therefore, the chance of re-occurrence is high (Beigi et al., 2004; Ferris et al., 2004). Additionally, the effectiveness of these treatments in reducing adverse pregnancy outcomes associated with BV such as PTB is inconsistent and unclear (McGregor et al., 1994; Joesoef et al., 1995; Vermeulen and Bruinse, 1999; Hay et al., 2001; Lamont et al., 2003).

Cervical and vaginal immunity

Local innate immunity plays a critical role in regulating the response to micro-organisms in the genital tract (Fidel, 2003; Russell et al., 2004). Pro-inflammatory cytokines such as IL-1 alpha (IL-1α), IL-1β, IL-6, IL-8 and TNF-α are critical mediators of inflammation and the response to infection; while anti-inflammatory cytokines such as interleukins -4 (IL-4), -10 (IL-10) and -13 (IL-13) keep the pro-inflammatory response in check. Toll-like receptors are important factors of the innate immune system and are essential for initiating pro- and anti-inflammatory responses (Janssens and Beyaert, 2003; O'Connell et al., 2005). The regulation of the immune response to infection is a delicate network of pro- and anti- inflammatory cytokines. Any disruptions, genetic or environmental, may alter cytokine gene expression and predispose individuals to an increased risk of developing infections such as BV.

Vaginal and cervical cytokine expression. Elevated vaginal, cervical and amniotic fluid pro-inflammatory levels are associated with a wide range of reproductive disorders including PTB, PROM and recurrent miscarriage (Poniedzialek-Czajkowska et al., 2000; Kurkinen-Raty et al., 2001; Holst et al., 2005; Menon et al., 2007; Hattori et al., 2007; Menon et al., 2008a; Menon et al., 2008b). In general, these levels do not fluctuate throughout pregnancy; however, BV^+ women have significantly higher cervical and vaginal pro-inflammatory cytokine levels, particularly IL-1β, which has been replicated in multiple studies (Imseis et al., 1997; Mattsby-Baltzer et al., 1998; Donders et al., 2003; Cauci et al., 2003; Wasiela et al., 2005; Basso et al., 2005; Hedges et al., 2006; St John E. et al., 2007). Furthermore, studies have shown that vaginal levels of IL-1α, and IL-8 are higher in BV^+ compared to BV^- women; however, these results have failed to replicate

(Imseis et al., 1997; Cauci et al., 2003; Wasiela et al., 2005; Basso et al., 2005; Hedges et al., 2006). Generally, vaginal levels of IL-6 and TNF-α are not significantly different between BV^+ and BV^- women (Imseis et al., 1997; Mattsby-Baltzer et al., 1998; Alvarez-Olmos et al., 2004; Wasiela et al., 2005; Basso et al., 2005; Hedges et al., 2006). Few studies have examined anti-inflammatory cytokine levels between BV^+ and BV^- women. However, one study showed levels of IL-4 are higher in BV^+ compared to BV^- women and another study showed concentrations of IL-10 are lower in BV^+ compared to BV^- women (Cherpes et al., 2007; Anton et al., 2008).

Genetics. Polymorphisms in pro-inflammatory, anti-inflammatory and toll-like receptor genes have been tested for associations with BV; however, many of these studies are either not significant or fail to replicate (Table 2-3). Additionally, the studies that do detect association with BV only detect moderate effect sizes (risk genotype OR 2.2 or less uncorrected). Serum cytokine concentration heritability for IL-6, IL-10 and TNF-α range from 53-72% and IL-1β has the highest heritability of the cytokines at 83% (Reuss et al., 2002; de Craen et al., 2005; Worns et al., 2006). However, few studies have examined polymorphisms for associations with vaginal or cervical cytokine concentrations even though several polymorphisms in innate immunity genes have been associated with serum cytokine levels (Nakashima et al., 2002; Reuss et al., 2002; Rafiq et al., 2007).

Table 2-3. Abbreviated list of genes studied for associations with BV

Gene	Gene Name	Result	Reference
IL-1β	Interleukin-1 beta	Association	(Goepfert et al., 2005; Cauci et al., 2007)
IL-1RN	Interleukin-1 receptor antagonist	Association No Association	(Genc et al., 2004a) (Annells et al., 2004; Genc et al., 2004a; Cauci et al., 2007)
IL-6	Interleukin-6	Association	(Goepfert et al., 2005)
IL-8	Interleukin-8	No Association	(Goepfert et al., 2005)
IL-10	Interleukin-10	Association	(Goepfert et al., 2005)
TLR2	Toll-like receptor 2	No Association	(Hartel et al., 2004)
TLR4	Toll-like receptor 4	Association	(Goepfert et al., 2005)
TNF	Tumor necrosis factor alpha	No Association	(Goepfert et al., 2005)

Of the few studies that have tested for genetic association with vaginal cytokine concentrations, one described a significant association between TNF-α-308 (rs1800629), Nugent score and vaginal TNF-α levels (Genc et al., 2006). Women with the AA genotype at this SNP and a Nugent score of greater than 7 have significantly higher vaginal TNF-α levels compared to those with the GG genotype and a Nugent score greater than 7 or the AA genotype and a Nugent score less than 7 (Genc et al., 2006). Another study found that TLR4+896A (rs4986790) homozygotes infected with *Prevotella*, *Bacteroides*, *Porphyromonas* species and/or *G. vaginalis* have significantly higher vaginal levels of IL-1β and interleukin 1 receptor antagonist (IL-1RN) compared to those not infected (Genc et al., 2004b). The lack of additional significant associations and replicated studies illustrates the importance of not only examining intermediate phenotypes such as vaginal, cervical or amniotic fluid cytokine concentrations but also examining other genes in the biological pathway such as cytokine receptors (Annells et al., 2004; Genc et al., 2004a; Genc et al., 2004b; Goepfert et al., 2005; Cauci et al., 2007).

Spontaneous Preterm Birth

Epidemiology

Prevalence. There are 5 million infants born each year, approximately 12% of which are born preterm (defined as less than 37 weeks of gestation) (Martin et al., 2005). Prematurity-related conditions are responsible for approximately one-third of all infant deaths, making this one of the leading causes of infant mortality in the United States (MacDorman et al., 2008). PTB can be divided into two categories: indicated PTB, which accounts for about 25% of all PTBs and spontaneous PTB (sPTB), which accounts for about 75% of all PTBs (Meis et al., 1987; Meis et al., 1995a; Meis et al., 1998). Preeclampsia, fetal distress, intrauterine growth restriction, placental abruption and fetal demise account for the majority of indicated PTBs; while infection and preterm PROM (PPROM) are responsible for two thirds of all sPTBs (Figure 2-1) (Meis et al., 1987; Tucker et al., 1991; Meis et al., 1995a; Meis et al., 1998). The remaining third of sPTBs are idiopathic meaning there are no clear mechanistic reasons for the early delivery.

Preterm infants are at a higher risk to suffer from necrotizing enterocolitis, hyaline membrane disease, intraventricular hemorrhage, respiratory distress, jaundice and sepsis (Creasy and Resnik, 2004). Also, since preterm infants generally have very low birth weight, they have an increased risk of cerebral palsy and mental retardation (Creasy and Resnik, 2004). It is important that the mechanisms of PTB, especially sPTB, are fully examined to develop better diagnostic and treatment methods that could lead to prevention of PTB.

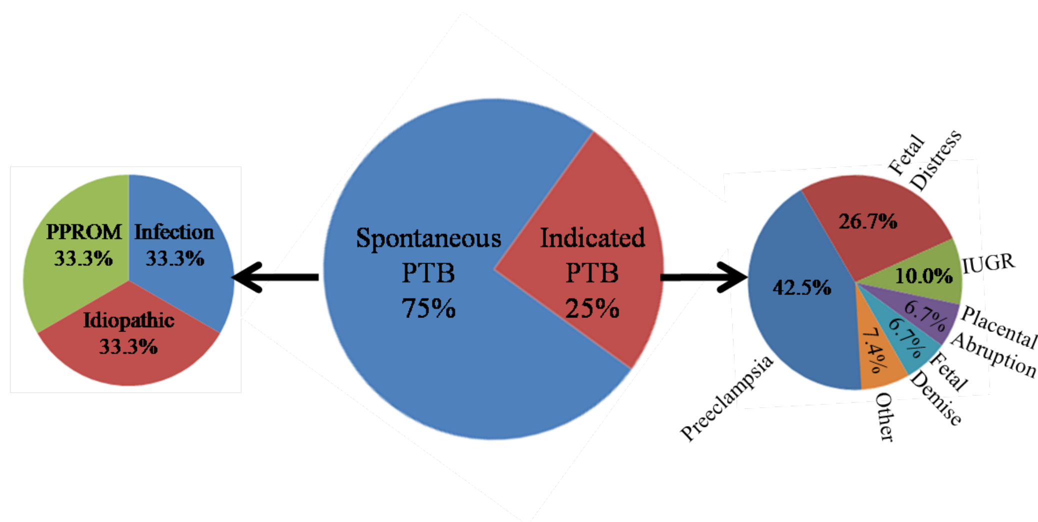


Figure 2-1. Main types and causes of PTB. The prevalence of the two types of PTB, spontaneous and indicated, are presented. Also described is the breakdown of common factors causing each type of PTB.

Risk factors. Maternal risk factors for PTB include multiple gestations, African ancestry, stress, maternal age less than19 or greater than 35, smoking, poor nutrition and low body mass index (Table 2-4) (Von Der Pool, 1998). AA are at an increased risk for PTB compared to EA (Creasy and Resnik, 2004). The rate of PTB for AA is 18% compared with 12% in EA (Martin et al., 2007). This disparity remains after accounting for socio-demographic differences such as income, age, marital status, smoking and health insurance coverage (Giscombe and Lobel, 2005). Additionally, history of preterm delivery and second-trimester abortion are also risk factors for PTB (Von Der Pool, 1998). Obesity has been associated with an increased risk for PTB; however, one study found obesity was protective against PTB (Hendler et al., 2005; Driul et al., 2008; Salihu et al., 2008; Hacini et al., 2008). Also, all of these factors combined only explain a fraction of PTBs, especially sPTBs.

Table 2-4. Risk factors for PTB a) sPTB b) indicated PTB

a)

Risk Factor	*Referent Group*	*OR*	[1]*Reference*
African Ancestry	Non-African	1.5	(Goldenberg et al., 1998)
Maternal age <19 years	Maternal age >19 years	1.8-2.4	(Meis et al., 1995b; Goldenberg et al., 1998)
Body mass index <19.8	Body mass index >19.8	2.5	(Goldenberg et al., 1998)
Maternal smoking (≥10 cigarettes/day)	No maternal smoking	1.5	(Meis et al., 1995b)
Parity ≥3	Parity = 1	1.6	(Meis et al., 1995b)
Previous sPTB	No previous sPTB	2.7	(Goldenberg et al., 1998)
Vaginal bleeding	Absence of vaginal bleeding	1.5-2	(Meis et al., 1995b; Goldenberg et al., 1998)
Pelvic infection	Absence of pelvic infection	1.3	(Goldenberg et al., 1998)
Positive fetal fibronectin	Negative fetal fibronectin	3.3	(Goldenberg et al., 1998)
Cervical length ≤25mm	Cervical length >25mm	3.5	(Goldenberg et al., 1998)

b)

Risk Factor	*Referent Group*	*OR*	[1]*Reference*
Maternal age ≥35 years	Maternal age 20-34	1.9	(Meis et al., 1995b)
Parity ≥3	Parity = 1	1.8	(Meis et al., 1995b)
Previous stillbirth	No history of stillbirth	3.3	(Meis et al., 1995b)
Maternal smoking (≥10 cigarettes/day)	No maternal smoking	1.3	(Meis et al., 1995b)
Vaginal bleeding	Absence of vaginal bleeding	2.0	(Meis et al., 1995b)
Bacteriuria	No bacteriuria	2.1	(Meis et al., 1995b)

[1]Goldenberg *et al* 1998 included 2,929 pregnancies collected by the National Institute of Child Health and Human Development Maternal Fetal Medicine Network and sPTB was defined as gestation <37 weeks and Meis *et al* 1995 included 26,205 pregnancies collected by the Cardiff Births Survey and sPTB was defined as gestation ≤257 days (36.7 weeks).

Diagnosis and treatment

Clinical Diagnosis. Diagnosis of preterm labor (PTL) is difficult because the symptoms, such as pelvic pressure, vaginal discharge, backache and cramps are hard to define (Creasy and Resnik, 2004). Diagnosis of PTL is made when persistent uterine contractions are followed by dilation of the cervix greater than 2 cm, greater than 50% effacement, or PROM (Von Der Pool, 1998). Digital examination of the cervix is not always accurate until significant dilation and effacement have occurred (Creasy and Resnik, 2004). Presence of four or more contractions per hour can also be used for diagnosis; however using these criteria alone is a poor predictor of PTL (Iams et al., 1995). Therefore, diagnosing women who have symptoms of PTL but have a cervical dilation of <2 cm and/or have effacement <50% remains very difficult.

Other methods of PTL diagnosis include measurement of cervical length and molecular biomarkers such as corticotrophin-releasing hormone (CRH), fetal fibronectin, and cytokines. Both a cervical length of less than 25 mm and increased levels of CRH can independently be diagnostic measures of PTL (Vogel et al., 2005; Goldenberg et al., 2005). However, results are inconsistent on the clinical effectiveness of these methods (Honest et al., 2003; Vogel et al., 2005). The most utilized biomarker for PTL is fetal fibronectin (fFN) (Von Der Pool, 1998). When found, it may indicate microruptures in the fetal membranes or disruption of the choriodecidual interface (Lockwood et al., 1991). Cytokines can also be used as biochemical markers of PTL (Vogel et al., 2005). IL-6 was the best predictor of PTL in symptomatic women; however, due to the short half-life of cytokines, accurate testing is difficult (Vogel et al., 2005).

Treatment. There are currently many methods of treating PTL including administration of corticosteroids, tocolytic agents and antibiotics. The most commonly used treatment is tocolytic agents, such as magnesium sulfate, prostaglandin synthesis inhibitors, calcium channel blockers and beta-adrenergic agonists (Creasy and Resnik, 2004; Simhan and Caritis, 2007).

Magnesium sulfate suppresses myometrial contractions and has minimal maternal and fetal side effects (Creasy and Resnik, 2004). The most common maternal side effects include headache, nausea, dizziness, dry mouth, lethargy and blurred vision (Hollander et al., 1987; Cox et al., 1990; Dirge et al., 1998).

Prostaglandin synthesis inhibitors are the most commonly used tocolytic agents. They inhibit the cyclooxygenase enzyme, which is required for synthesis of prostaglandin G, a precursor of prostaglandins E and F, both of which are important in stimulating labor (Creasy and Resnik, 2004). Maternal side effects are uncommon; however, possible serious fetal side effects include pulmonary hypertension, intraventricular hemorrhage and closure of the utero ductus arteriosus, which allows oxygenated blood to be delivered from the placenta to the fetus (Creasy and Resnik, 2004). These side effects are unlikely if the treatment is only administered for a short duration (<48 hours) (Creasy and Resnik, 2004).

Calcium channel blockers act on voltage-dependent calcium channels to block the influx of calcium ions through the cell membrane and thus suppress contractions (Fleckenstein, 1977; McDonald et al., 1994). There are minimal maternal and fetal side effects, the most common of which are vasodilation, transient tachycardia and mild decrease in blood pressure (Ferguson et al., 1989).

Beta-adrenergic agonists suppress uterine contractions by inhibiting myosin light-chain kinase; however, these are not commonly administered due to the high rate of serious cardiovascular and metabolic maternal side effects (Creasy and Resnik, 2004). Cardiovascular side effects include hypotension, cardiac arrhythmia, myocardial ischemia and pulmonary edema (Creasy and Resnik, 2004). The most common metabolic side effect is hyperglycemia (Creasy and Resnik, 2004).

These treatment options have proven ineffective in reducing PTB prevalence; in fact, the prevalence of PTB has increased from 10.7% in 1992 to 12.8% in 2006 (Hamilton et al., 2007). This may be due to the fact that most of these treatments are not effective in prolonging labor for more than 48-72 hours. Additionally, many of these treatments have harmful side effects for the mother and fetus after prolonged treatment (Creasy and Resnik, 2004; Simhan and Caritis, 2007). It is critical that a better understanding of the etiology of PTB is gained in order to develop better diagnostic and treatment options for those at risk of PTB.

Biological pathways

There are four main proposed pathways that lead to sPTB (Figure 2-2): 1) inflammation and infection, 2) activation of maternal or fetal hypothalamic pituitary-adrenal axis, 3) decidual hemorrhage and 4) pathological uterine distension (Lockwood and Kuczynski, 2001). The individual factors involved in these pathways and how they affect sPTB are poorly understood.

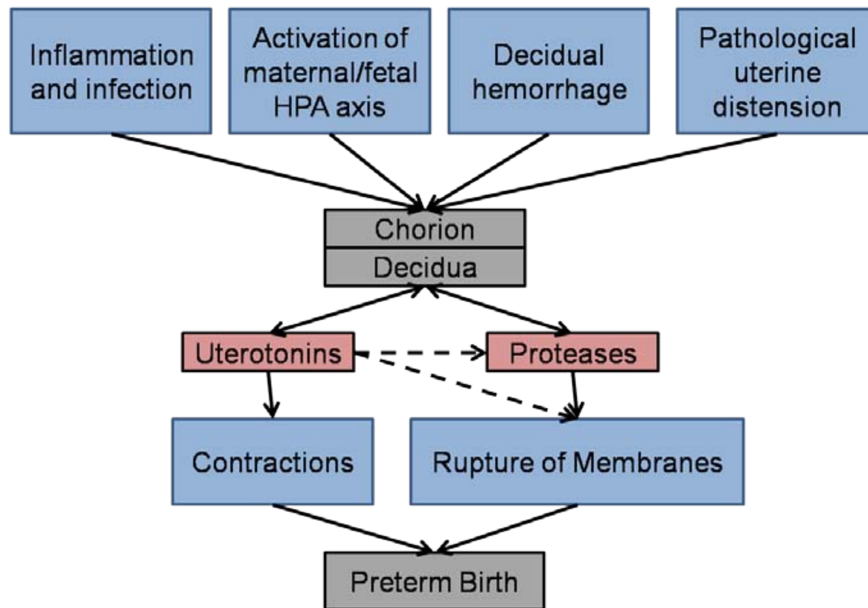


Figure 2-2. Pathways of PTB. Adapted from Lockwood et al (2001) *Paediatr. Perinat. Epidemiol.* 2, 560-567. The four proposed PTB pathways include inflammation/infection, activation of the maternal fetal HPA axis, decidual hemorrhage and uterine distention.

Inflammation/Infection Pathway. As discussed previously, infection accounts for about 25% of all sPTBs (Vogel et al., 2005). The most common type of lower genital tract infection in adult women is BV, which is associated with adverse pregnancy outcomes such as sPTB and PPROM (Wadhwa et al., 2001). Infection occurs through four main pathways: 1) ascension from the vagina and cervix, 2) transmission from the placenta, 3) dissemination from the peritoneal cavity through the fallopian tubes and 4) introduction from amniocentesis, fetal blood sampling or chorionic villous sampling (Romero et al., 2002). Infection can lead to PTB through several mechanisms (Goldenberg et al., 2000). Bacterial colonization of the chorion, amnion, placenta and decidua leads to an increase of cytokines and chemokines such as IL-1α, IL-1β, IL-6, IL-8, TNF-α and granulocyte

colony stimulating factor (GMCSF) (Goldenberg et al., 2000). This causes an increase of prostaglandin production that can trigger myometrial contractions.

Hypothalamic-Pituitary-Adrenal (HPA) Axis Pathway. During pregnancy the fetal-placental-decidual component produces hormones, neuropeptides, growth factors and cytokines. Changes in concentrations of these proteins, particularly fetal adrenal dehydroepiandrosterone sulfate, causes the system to shift from progesterone-dominant to estrogen-dominant (Wadhwa et al., 2001). This shift causes the formation of gap junctions, expression of oxytocin receptors and the production of prostaglandins that induce labor (Wadhwa et al., 2001).

The major factor that regulates HPA axis activity and response to stress is corticotropin-releasing hormone (CRH) (Vale et al., 1981; Challis et al., 1995). As pregnancy progresses, CRH increases and activates the pituitary-adrenal axis to drive cortisol secretion (Chrousos et al., 1998). CRH binding protein (CRH-BP) neutralizes most of the free CRH; however, in the third trimester, CRH-BP levels drastically decline resulting in an increase of CRH (Linton et al., 1993). Therefore, as mentioned above, placental secretion of CRH can be used to determine labor progression (McLean et al., 1995). Maternal stress can interrupt this mechanism causing cortisol to be overproduced and this may result in sPTB (Challis et al., 1995; Lockwood and Kuczynski, 1999). CRH also increases prostaglandins and oxytocins, both of which induce contractions (Jones et al., 1989; Jones and Challis, 1990).

Decidual Bleeding Pathway. Decidual hemorrhaging due to vaginal bleeding is associated with a three-fold increased relative risk for sPTB (Williams et al., 1991). This increases production of coagulation factors, such as factor 7 (F7) and factor 10 (F10) that

are important for the production of thrombin (F2) which is critical in clot formation. An overproduction of thrombin causes contractions and activates matrix metalloproteinases (MMPs) which lead to the degradation of the extracellular membrane (ECM). ECM degradation initiates changes in the cervix and rupture of the membranes which may result in PTB.

Uterine Distension Pathway. Multifetal gestation or hydramnios cause an increase in uterine volume resulting in excessive stretching and uterine distension. Mechanical signals such as contraction-associated proteins (CAPs) including connexin-43, oxytocin receptor and prostaglandin F receptor originate from the fetal genome and regulate the growth and stimulation of the uterus (Creasy and Resnik, 2004). Excessive uterine distension induces CAP gene expression and myometrial activation (Creasy and Resnik, 2004). This results in uterine contractions and PTB.

Genetics

There is evidence to suggest that sPTB has a genetic component. For example, mothers who were preterm or have a sister who delivered prematurely are at a higher risk for PTB compared to those with no history or family history of PTB (Carr-Hill and Hall, 1985; Porter et al., 1997). Furthermore, history of previous PTB is one of the best predictors for PTB (Bakketeig et al., 1979; Carr-Hill and Hall, 1985; Porter et al., 1997). Twin studies estimate the heritability of sPTB at 20-40% (Treloar et al., 2000; Clausson et al., 2000). There has been an extensive amount of research in the last ten years examining genetic variants for association with PTB. Many studies have found

significant associations particularly in the complement coagulation (decidual hemorrhage) and inflammation/infection pathways (Table 2-5).

Table 2-5. Significant genetic associations with PTB

Gene	Gene Name	Population	Reference
Genes Involved In Coagulation			
F2	Coagulation factor II	Fetal	(Gopel et al., 1999)
F5	Coagulation factor V	Fetal	(Gopel et al., 1999)
		Maternal	(Erhardt et al., 2000; Hao et al., 2004; Velez et al., 2008a)
F7	Coagulation factor VII	Fetal	(Hartel et al., 2005)
		Maternal	(Hartel et al., 2005)
MMP9	Matrix Metalloproteinase 9	Fetal	(Ferrand et al., 2002)
MTHFR	5,10-methylenetetrahydrofolate reductase	Fetal	(Chen et al., 2004b)
MTRR	Methionine synthase reductase	Maternal	(Engel et al., 2006)
PAI1	Plasminogen activator inhibitor (SERPINE1)	Fetal	(Chen et al., 2007)
PLAT	Tissue plasminogen activator	Maternal	(Velez et al., 2008a)
Genes Involved In Inflammation/Infection			
IL-1β	Interleukin 1, beta	Fetal	(Genc et al., 2002)
IL-1R2	Interleukin 1 receptor, type II	Maternal	(Hao et al., 2004)
IL-1RN	Interleukin 1 receptor antagonist	Fetal	(Genc et al., 2002; Bessler et al., 2004)
		Maternal	(Murtha et al., 2006)
IL-4	Interleukin 4	Fetal	(Kalish et al., 2004)
		Maternal	(Annells et al., 2004; Kalish et al., 2004)
IL-6	Interleukin 6	Maternal	(Simhan et al., 2003b; Hartel et al., 2004)
IL-6R	Interleukin 6 receptor	Maternal	(Velez et al., 2007; Velez et al., 2008b)
IL-10	Interleukin 10	Maternal	(Annells et al., 2004)
TLR2	Toll-like Receptor 2	Fetal	(Krediet et al., 2007)
TLR4	Toll-like Receptor 4	Fetal	(Lorenz et al., 2002)
TNF	Tumor necrosis factor alpha	Fetal	(Aidoo et al., 2001; Menon et al., 2006d)
		Maternal	(Roberts et al., 1999; Chen et al., 2003; Macones et al., 2004; Moore et al., 2004; Amory et al., 2004; Annells et al., 2004; Engel et al., 2005)
TNFRI	Tumor necrosis factor receptor 1A	Maternal	(Menon et al., 2006c; Menon et al., 2006d)
TNFRII	Tumor necrosis factor receptor 1B	Maternal	(Menon et al., 2006c; Menon et al., 2006d)

Coagulation Factors. Variants in the coagulation genes F2, F5 and F7 are associated with PTB; a result that has been replicated (Table 2-5). In particular, the F5 Leiden mutation is found more often in infant (~16%) and mother (~18%) PTB cases compared to infant (~5%) and mother (~7%) controls (Gopel et al., 1999; Erhardt et al., 2000). F5 is important for conversion of prothrombin to thrombin, which is critical in blood clotting (Rosing and Tans, 1997a; Rosing and Tans, 1997b). Other factors such as PLAT, which is essential for the conversion of plasminogen to plasmin, is associated with PTB in EA (Velez et al., 2008a). Plasmin is a critical factor in the degradation of the ECM which can lead to PROM and PTB. Also, problems in the coagulation pathway may result in placental thrombosis, infarction or hypoperfusion; all of which can lead to PTB (Dizon-Townson et al., 1997b).

Homocysteine metabolism. During pregnancy, changes in the coagulation system occur that increase the risk of thromboembolic events, such as hyperhomocysteinaemia (HHC) (Gerhardt et al., 2000). HHC occurs when there is a disruption in homocysteine metabolism and is commonly caused by vitamin B12 or folate deficiency and mutations in the methylenetetrahydrofolate reductase (MTHFR) gene (Wang et al., 2001). HHC is associated with an increased risk of thromboembolism, coronary heart disease, pre-eclampsia, recurrent miscarriage and placental abruption; all of which can lead to PTB (Wang et al., 2001). One study examining MTHFR C677T (rs1801133) in 250 Chinese PTB families and 250 controls found that fetal CT and TT genotypes were associated with PTB (OR 2.01, 95% CI 1.21-3.32 and OR 1.82, 95% CI 1.02-3.26, respectively) (Chen et al., 2004b). Another study examining 136 case and 573 control EA and AA maternal samples in total, found no association between MTHFR C677T and PTB (Engel

et al., 2006). However in this study, methionine synthase reductase (MTRR), an important factor in the conversion of homocysteine to methionine, was associated with an increased risk of PTB in EA (OR 2.0, 95% CI 1.1-3.6), but not in AA (Engel et al., 2006).

Cytokines and Chemokines. A few studies have shown IL-1RN, IL-4, IL-6, IL-10 and TNF-α are associated with PTB in different populations; however, many of these studies have failed to replicate (Dizon-Townson et al., 1997a; Amory et al., 2001; Simhan et al., 2003b; Amory et al., 2004; Bessler et al., 2004; Annells et al., 2004; Menon et al., 2006c). The most studied cytokine mutation with PTB is TNF-α -308 (rs1800629). Several studies have associated TNF-α -308 with PTB and PPROM (Roberts et al., 1999; Moore et al., 2004). However, these results have not consistently replicated and a recent meta-analysis found no significant evidence for association between TNF-α -308 and PTB (Dizon-Townson et al., 1997a; Menon et al., 2006b). Additionally, TNF-α leads to a greater risk for chorioamnionitis, defined as inflammation of the membrane surrounding the fetus (Simhan et al., 2003c). There is evidence that the presence of both a polymorphism in TNF-α and BV can lead to increased risk for sPTB (Macones et al., 2004).

Strategies for Studying Complex Disorders

Genetic associations of BV and sPTB often fail to replicate. These diseases are complex and therefore it is probable that more than one genetic variant contributes to the mechanisms of these disorders. Additionally, many of these studies are constrained by poorly defined phenotypes. The studies on BV presented in the following chapters address these issues by examining intermediate phenotypes defined as cervical pro- and

anti-inflammatory cytokine concentrations. These intermediate phenotypes have more power to detect association with BV during pregnancy as well as provide a bigger picture of the genetics and immunology involved in the pathogenesis of BV. Also, variants in cytokine receptors along with the cytokine genes themselves are examined with each cervical cytokine concentration, to provide a broader understanding of the complex regulation of cervical immunity. To address issues involved in the intricacy of sPTB, a large candidate gene association study is performed. This allows for the examination of multiple genes involved in the four main PTB pathways. Also, approximately 1,300 SNPs in this study overlap with a previous candidate gene association study of sPTB by Velez *et al* 2008a. This allows for a detailed comparison of significant associations in these two studies.

Another major problem contributing to the lack of replication of genetic association studies is ethnic heterogeneity. It is known that AA are at about a two times greater risk of BV and PTB compared to EA (Ness et al., 2003; Creasy and Resnik, 2004). Also EA and AA have different patterns of genetic variation across the genome, particularly in genes involved in immune function such as IL-1, IL-4, IL-6, IL-8, IL-10, IL-13, TLR2 and TNF-α (Hoffmann et al., 2002; Rovin et al., 2002; Nakashima et al., 2002; Pyo et al., 2003; Nguyen et al., 2004; Yim et al., 2004; Sakagami et al., 2004; Ness et al., 2004a; Tarazona-Santos and Tishkoff, 2005; Zabaleta et al., 2007; Velez et al., 2007; Fujihara et al., 2007). Additionally, amniotic fluid levels of pro-inflammatory cytokines such as TNF-α differ between EA and AA (Menon et al., 2008b; Velez et al., 2008c). However, few studies examining associations with BV and PTB account for ethnic heterogeneity. For the studies on BV, ethnic heterogeneity will be addressed by

analyzing AA and EA separately to allow for identification of differing mechanisms that may lead to vaginal colonization of harmful micro-organisms during pregnancy. The study sample on PTB is from Norway and is ethnically homogeneous; additionally, the cases and controls were carefully selected to ensure a well defined phenotype. Carefully designed studies may lead to a greater understanding of not only the genetic underpinnings of complex diseases but also the higher prevalence of BV and PTB observed in AA.

B. Hypotheses and Specific Aims

Hypothesis: Genetic polymorphisms within biologically relevant pathways, including infection and neuroendocrine pathways, are associated with complex reproductive disorders such as BV and PTB.

Specific Aim I: Identify cervical cytokine concentration and correlation differences by BV status and race.

a. Identify cervical cytokine concentration differences by BV status and race.

b. Identify cervical cytokine correlation differences by BV status and race.

A nested case control analysis will be performed from a prospective cohort study conducted at Magee-Womens Hospital in Pittsburgh, PA to identify cervical cytokine concentrations and correlations associated with BV in EA and AA women, separately. Differences in cytokine concentrations and correlations by race will also be evaluated in BV^+ and BV^- women, separately. To determine differences in cytokine concentrations, two-sample t-tests or Kruskal-Wallis non-parametric tests will be used depending on normality of the cytokine concentrations. To determine cervical cytokine expression patterns, pair-wise correlations will be calculated with Spearman's Rho. Differences in correlation coefficients will be identified using t-tests.

Specific Aim II: Identify SNPs associated with cervical pro- and anti-inflammatory cytokine concentrations in the presence and absence of BV.

a. Perform analysis of variance (ANOVA) in EA and AA separately using pro-inflammatory cytokine concentrations with the corresponding genes and receptors.

b. Perform ANOVA in EA and AA separately using anti-inflammatory cytokine concentrations with the corresponding genes and receptors.

c. Perform ANOVA in EA and AA separately using pro- and anti-inflammatory cytokine concentrations with toll-like receptor genes.

The Magee Womens cohort will be used to identify SNPs associated with cervical cytokine concentrations in EA and AA women, separately. BV status will be included in all ANOVA models. SNPs will be tested for deviations from Hardy-Weinberg equilibrium in the entire dataset.

Specific Aim III: Identify SNPs associated with spontaneous preterm birth (sPTB).

a. Perform single locus association and haplotype analysis in maternal and fetal samples separately by testing SNPs in candidate genes for allele and genotype frequency differences between PTB cases and controls.

b. Determine the effect size of significantly associated SNPs in a pooled sample of two independent EA populations.

The Norwegian Mother and Child Cohort Study (MoBa) will be used to study the genetic associations of PTB. Approximately 160 genes biologically relevant to the pathways of PTB will be examined. Fisher's exact test or chi-square tests for association

will be performed for mother and fetal genotypes, separately. Tests for deviations from HWE will be performed in cases and controls separately. Haplotype analysis using a 2, 3 and 4 SNP sliding window will also be performed on all genes. Samples from the Centinneal study (Cenn) collected in Nashville, TN will be used to determine SNPs that have are associated in both studies. These SNPs will be tested for association with logistic regression in the Cenn and MoBa samples combined to determine the effect size.

CHAPTER III

EXAMINING CERVICAL CYTOKINE CONCENTRATIONS AND CORRELATIONS BY BV STATUS AND RACE

Overview parts A and B

BV is a serious vaginal disorder that affects AA more often than EA. This disparity cannot be explained by socio-economic variables alone and is likely, in part, due to differing inflammatory responses to harmful micro-organisms. To explore these differences, cytokine concentrations and correlation patterns are examined in Chapter III. Part A investigates differences in twenty-eight cervical cytokine, chemokine and growth factor concentrations by BV status and race. BV^+ EA had significantly lower pro-inflammatory levels of IP10 ($p = 0.001$) and MCP1 ($p = 0.006$) and BV^+ AA had significantly higher pro-inflammatory levels of IL-1α ($p < 0.001$) compared to BV^- women. BV^- EA had significantly higher levels of IL-1α ($p = 0.047$), IL-6 ($p = 0.010$), IL-10 ($p = 0.016$) and PDGF-BB ($p = 0.010$) than AA; however, there were no significant concentration differences between BV^+ EA and AA women.

Part B examines the coordinated regulation of the extensive network of cytokines, chemokines and growth factors involved in the immune response to BV. In EA there were significantly more correlations involving immuno-regulatory cytokines in BV^- compared to BV^+ women; conversely, for AA there were no differences in the correlation patterns between BV^+ and BV^- women. Overall, BV^+ AA had a stronger correlated response compared to EA; alternatively, there were no differences between the races in

BV^- women. These studies elucidated the immunological differences in response to BV, and demonstrated baseline differences as well as differences by race in the coordinated expression of inflammatory factors in the presence of BV. This may, at least partially, explain the disparity observed in the prevalence of this disorder.

A. Cervical Cytokine Concentrations Differ by BV Status and Race

Introduction

BV is one of the most prevalent vaginal disorders in adult women, affecting 15-20% of pregnant women (Eschenbach, 1993; Hillier et al., 1995; McGregor and French, 2000; Cauci et al., 2002). AA women are at a 2 times greater risk for BV than EA, and this disparity remains after controlling for common risk factors, many of which occur more frequently in AA women (Koumans and Kendrick, 2001; Ness et al., 2003). Furthermore, the risk of PTB attributable to BV and vaginal inflammation is greater among AA than their EA counterparts (Hillier et al., 1995; Simhan et al., 2005b). The reasons for this racial disparity are unclear, and few studies have directly addressed this issue.

Vaginal immunity and cervical cytokine expression are clearly important in the pathogenesis of BV. Higher vaginal concentrations of IL-1α, IL-1β and IL-6 have been observed in BV^+ compared to BV^- women (Platz-Christensen et al., 1993; Mattsby-Baltzer et al., 1998; Cauci et al., 2003; Wasiela et al., 2005; St John E. et al., 2007). However, these results are not consistent and little is known about population differences with respect to lower genital tract immunity, particularly in the presence of BV. Our

purpose was to compare the cervical inflammatory milieu, as represented by a panel of 28 cytokines, chemokines and growth factors, by both BV status and race.

Materials and Methods

Subjects

A nested case control analysis was performed from a prospective cohort study conducted at Magee-Womens Hospital in Pittsburgh, PA. This study was approved by the University of Pittsburgh and Vanderbilt University Institutional Review Boards. All women provided demographic, medical, and clinical information through standardized, closed question interviews administered by research personnel.

Inclusion criteria for the cohort study were singleton intrauterine gestation prior to 13 weeks. Exclusion criteria included vaginal bleeding, fetal anomalies, known thrombophilias, pre-gestational diabetes mellitus, chronic hypertension requiring medication, current or planned cervical cerclage, immune compromise (HIV positive, use of systemic steroids within six months, use of post-transplant immunosuppressive medication), and autoimmune disease (inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, scleroderma). These exclusions were developed prior to study enrollment because they are believed to associate with an altered immune response that could bias the associations we propose to examine.

Additional exclusion criteria were chosen after enrollment to eliminate characteristics that could confound associations with cytokine concentrations. These exclusions included: presence of *Trichomonas vaginalis*, *Neisseria gonorrhea* and

Chlamydia trachomatis, antibiotic use three months prior to pregnancy and race other than self-identified EA or AA. Women with an intermediate BV score (Nugent score of 4-6) were also excluded because of small numbers.

Table 3-1. Socio-demographic characteristics

	EA Women			AA Women			AA vs EA
	BV⁻	*BV⁺*	*p value*	*BV⁻*	*BV⁺*	*p value*	*p value*
Age, years[1]	25.6 (5.2)	24.7 (5.3)	0.496	26.3 (6.4)	25.8 (5.0)	0.685	0.308
Body Mass Index[1]	26.4 (5.9)	26.1 (4.6)	0.797	29.7 (7.5)	28.4 (7.9)	0.444	0.008
Gestational age at enrollment (weeks)[1]	9.4 (2.9)	9.2 (3.8)	0.839	9.5 (3.2)	9.0 (3.2)	0.485	0.978
Cigarettes/day 3 months prior to pregnancy[2]			0.215			0.031	0.001
None	20 (36.4)	5 (17.9)		23 (59.0)	12 (28.6)		
1-5	2 (3.6)	2 (7.1)		5 (12.8)	9 (21.4)		
6-10	15 (27.3)	7 (25.0)		5 (12.8)	14 (33.3)		
>10	18 (32.7)	14 (50.0)		6 (15.4)	7 (16.7)		
Cigarettes/day during pregnancy[2]			0.145			0.138	0.038
None	24 (43.6)	6 (21.4)		24 (61.5)	17 (40.5)		
1-5	12 (21.8)	6 (21.4)		6 (15.4)	15 (35.7)		
6-10	15 (27.3)	11 (39.3)		6 (15.4)	8 (19.1)		
>10	4 (7.3)	5 (17.9)		3 (7.7)	2 (4.8)		

[1]Mean is presented with standard deviation in parentheses and the p-value was calculated with t-tests
[2]Number of events is reported with percentage in parentheses and the p-value is calculated with Fisher exact tests

Demographic and clinical characteristics

This study consisted of 164 women: 28 EA BV^+, 42 AA BV^+, 55 EA BV^- and 39 AA BV^-. Socio-demographic differences by BV status and race are presented in Table 3-1. There were no statistically significant differences between BV^+ and BV^- EA women; however, AA BV^+ women were more likely to have smoked prior to pregnancy ($p = 0.031$). Overall, AA had a higher BMI ($p = 0.008$) and were less likely to smoke both prior to ($p = 0.001$) and after pregnancy ($p = 0.038$) than EA.

Microbiologic assessment

Two vaginal swabs were collected for culture and identification of vaginal flora. BV was diagnosed by vaginal pH ≥ 4.7 and a score of 7 through 10 from a Gram-stained vaginal smear interpreted using the Nugent method (Nugent et al., 1991). The identification of *T. vaginalis* was by culture using Diamonds media, incubated at 37° C in 5% CO_2 for up to 5 days. Each day, microscopic identification by direct observation of motile forms was performed. If the culture media was negative for five days, the results were considered negative. *C. trachomatis* and *N. gonorrhoeae* were identified using nucleic acid amplification tests and culture, respectively.

Cytokine measurements

At a first trimester study visit (median gestation 6.5 weeks), in accordance with a standardized protocol, a pelvic examination was performed using a clean, non-lubricated speculum. Two Dacron swabs were placed in the cervix and left there for 10 seconds to achieve saturation for the assay of cytokines. These swabs were placed in a plastic tube

containing 4 ml of Purified Bovine Serum (final dilution of 1:5), and stored at -80°C until assayed. The sample was thawed at room temperature, placed in a spin-X centrifuge filter unit and centrifuged at 12,000 rpm for 20 minutes.

The Luminex LabMAP™ and a Beadlyte® analyte kit (Upstate, Charlottesville, Virginia) were used to assay the following 28 cytokines from a single aliquot of 50μL of specimen: EOTAXIN (chemokine CC motif ligand 11), GMCSF, interferon gamma (IFN-γ), IP10, interleukins (IL-) 1α, 1β, 2, 3, 4, 5, 6, 7, 8, 10, 12 subunit p40, 12 subunit p70, 13, 15, MCP1, platelet derived growth factor (PDGF-AA and PDGF-BB), fms-related tyrosine kinase 3 (FLT3), macrophage inflammatory protein 1-alpha (MIP1α), regulated upon activation, normally T-expressed and presumably secreted (RANTES), TNF-α, vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and fibroblast growth factor 2 (FGF2). Multiplex-based platforms quantify cytokine concentrations in plasma with a high degree of agreement and correlation to ELISA, while providing improved sensitivity (Prabhakar et al., 2002; Pickering et al., 2002; Nelson et al., 2003; Biagini et al., 2004)

These factors were chosen because they are early response cytokines and molecules important in the downstream cascade of inflammatory events. A monoclonal antibody specific for a cytokine is covalently linked to a fluorescent bead set that captures the cytokine. A complementary biotinylated monoclonal cytokine antibody then completes the immunological “sandwich” and the reaction is detected with streptavidin-phycoerythrin. Each sample was frozen at -80°C after initial collection and was never thawed until this study. After thawing, multiplex assays were performed. These samples were previously analyzed in duplicate using ELISA for IL-1β, IL-6, and IL-8. We found

a very high degree of correlation between concentrations determined by ELISA and by multiplex assay (r^2 = 0.95 to 0.97) for each of the cytokines.

The cervical fluid collection protocol used in this study has served as the validated methodology for several large multi-center trials (Carey et al., 1993; Goldenberg et al., 1996; Carey et al., 2000). The inter-swab change in weight (pre vs post-collection) is ±1.2% and the inter-assay variation for cervical cytokines has been consistently <8% (Simhan et al., 2003a; Simhan et al., 2005a). Microbiologic and cytokine assays were performed in the Research Microbiology Laboratory at the Magee-Womens Research Institute by lab technologists who were blinded to any clinical or identifying data from the study subjects.

Statistical analysis

Statistical analyses were performed using Stata version 9 (StataCorp, 2007). Demographic and clinical differences between BV^+ and BV^- women were determined by Fisher's exact test for dichotomous variables or t-tests for continuous variables. Shapiro-Wilks tests were performed to evaluate the normality of each cytokine distribution. Mann-Whitney U tests were used to evaluate concentration differences by BV status for EA and AA separately. A total of 56 comparisons were made and false discovery rate (FDR) (Benjamini and Hochberg, 1995) was used to correct for multiple testing. A q value of 0.2 was used to determine the FDR threshold value.

To examine the possibility of concentration differences between AA women and EA women, Mann-Whitney U tests were performed in BV^+ and BV^- women, separately. The results of these 56 comparisons were also corrected for multiple tests with FDR.

Results

Differences in cytokine concentrations by BV status

In EA, IL-10 (medians 37.5 and 73.3 ng/mL), IP10 (medians 1807.8 and 3972.8 ng/mL), MCP1 (medians of 348.0 and 859.0 ng/mL), MIP-1α (medians 417.3 and 833.2 ng/mL), PDGF-AA (medians 499.1 and 1093.5 ng/mL) and PDGF-BB (1445.1 and 2362.2 ng/mL) were decreased in BV^+ compared to BV^- women (Table 3-2a, Appendix Table 1). IL-1α was increased in BV^+ EA women. Only IP10 and MCP1 were significant after correction for multiple testing. In AA, IL-1α (medians of 1165.2 and 558.4 ng/mL) and FLT3 (medians 353.5 and 245.0 ng/mL) concentrations were elevated among BV^+ compared to BV^- women; however, only IL-1α was significant after correction for multiple testing (Table 3-2b, Appendix Table 1).

Cytokine concentrations by race

EA BV^- women had higher levels of IL-1α (medians of 904.9 and 558.4 ng/mL), IL-6 (3967.1 and 2162.2 ng/mL), IL-10 (medians of 73.3 and 43.0 ng/mL) and PDGF-BB (medians of 2362.2 and 1204.7 ng/mL) compared to AA BV^- women (Table 3-3, Appendix Table 1). However, these results did not hold up to correction for multiple testing. There were no significant differences between races for BV^+ women (Appendix Table 1).

Table 3-2. Significant cytokine differences between BV^- and BV^+ women a) EA, b) AA

a)

Cytokine	*BV^-*	*BV^+*	*nominal p*
IL-1α	904.9 (166.0 – 11098.1)	1973.1 (82.4 – 25581.0)	0.030
IL-10	73.3 (0.0** – 497.4)	37.5 (0.0** – 443.0)	0.016
IP10	3972.8 (5.0 – 30181.9)	1807.8 (146.6 – 25000.0)	0.001*
MCP1	859.0 (40.0 – 30631.9)	348.0 (48.0 – 22146.0)	0.006*
MIP-1 α	833.2 (122.1 – 12604.3)	417.3 (97.0 – 15810.0)	0.043
PDGF-AA	1093.5 (78.4 – 10122.0)	499.1 (99.3 – 10916.0)	0.048
PDGF-BB	2362.2 (236.2 – 33201.0)	1445.1 (40.0 – 14663.7)	0.015

b)

Cytokine	*BV^-*	*BV^+*	*nominal p*
FLT3	245.0 (12.0 – 1170.0)	353.5 (12.0 – 15139.1)	0.046
IL-1α	558.4 (104.0 – 5554.6)	1165.2 (163.8 – 33876.5)	<0.001*

Median cytokine concentrations (ng/mL) and ranges in parentheses are presented for BV^+ and BV^- women.
* Result significant after correction for multiple testing using FDR (q=0.2)
** - Below sensitivity of laboratory assay.

Table 3-3. Significant cytokine differences between BV^- EA and AA

Cytokine	*EA*	*AA*	*p*
IL-1α	904.9 (166.0 – 11098.1)	558.4 (104.0 – 5554.6)	0.047
IL-6	3967.1 (62.2 – 25000.0)	2162.2 (5.0 – 14744.0)	0.010
IL-10	73.3 (0.0** – 497.4)	43.0 (1.3 – 391.0)	0.016
PDGF-BB	2362.2 (236.2 – 33201.0)	1204.7 (12.0 – 9651.0)	0.010

Median cytokine concentrations (ng/mL) and ranges in parentheses are presented for BV^+ and BV^- women.
* Result significant after correction for multiple testing using FDR (q=0.2)
** - Below sensitivity of laboratory assay.

Cytokine concentrations excluding women with C. albicans

A study examining cytokine output in vaginal epithelial cells found that in response to *Candida albicans* high levels of IL-1α are produced while there is little to no production of IL-6, IL-10 and MCP-1 (Steele and Fidel, Jr., 2002). After removing the 38 individuals infected with *C. albicans* from our study, EA BV^+ women still had significantly lower concentrations of IP10 ($p = 0.003$) compared to BV^- women, and AA BV^+ women still had significantly higher levels of IL-1α ($p = 0.002$) than BV^- women. Additionally, EA BV^- women had significantly higher levels of IL-10, IL-12(p40) and PDGF-BB than AA BV^- women. There were still no significant differences in cytokine concentrations between EA and AA BV^+ women.

Discussion

Our findings support the notion that the nature of the inflammatory milieu in the cervix is different between BV^+ and BV^- women. We have also identified several cytokines that differ by race in BV^- but not BV^+ women. Genc and colleagues have previously demonstrated that activation of the IL-1 system accompanies disruption of vaginal flora, particularly among women who go on to have a PTB (Genc et al., 2004c). Our data are in agreement with this concept; in AA, IL-1α concentrations were significantly higher in BV^+ compared to BV^- women, and this result was almost significant after FDR corrections in EA women.

Also in EA, a number of cervical cytokine concentrations including the anti-inflammatory cytokine IL-10, pro-inflammatory chemokines IP10, MCP1 and MIP-1α, and growth factors PDGF-AA and PDGF-BB were found to be decreased in BV^+

compared to BV$^-$ women. Low serum levels of IL-10 were associated with a greater risk of developing a variety of infections (Opal and DePalo, 2000). Additionally, it has been hypothesized that a decrease in concentrations of pro-inflammatory cytokines in the lower genital tract may indicate an immune “hyporesponsiveness” that predisposes to subsequent ascending microbial invasion (Simhan et al., 2003a). It is unclear whether lower levels of pro-inflammatory and anti-inflammatory cytokines and chemokines predispose to BV, or are caused by BV; however, reduced production of these critical factors may be a mechanism for the development and propagation of BV.

In BV$^-$ women, our data demonstrate lower cervical concentrations of IL-1α, IL-6, IL-10 and PDGF-BB in AA compared to EA women. Recent research examining the distribution of cytokine gene polymorphisms between EA and AA have found that these two populations differ significantly in allelic distribution at commonly assayed sites in IL-1α, IL-6 and IL-10 that could give rise to a differential inflammatory response (Ness et al., 2004a; Zabaleta et al., 2007). Therefore, this could alter the production of the cytokines, possibly explaining why lower levels of these cytokines were observed in AA compared to EA women. It is also recognized that EA and AA women have differing immune responses to infection (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004). While we did not observe differing cytokine concentrations in BV$^+$ women, it is possible that because EA women have higher levels of both pro- and anti-inflammatory cytokines they are less susceptible to infection with BV. This mechanism could help to explain why AA women are at a greater risk for developing BV compared to EA women even after accounting for socio-demographic risk factors.

However, our findings should be taken as exploratory as these results did not hold up after correcting for multiple tests.

Additionally, a study examining cytokine output in vaginal epithelial cells found that in response to *Candida albicans* high levels of IL-1α are produced, while there is little to no production of IL-6, IL-10 and MCP-1 (Steele and Fidel, Jr., 2002). After removing women with *C. albicans* from our analysis we found that our results, in general, remained the same. This demonstrates that while *C. albicans* does affect the vaginal cytokine milieu, strong cytokine concentration differences between BV^+ and BV^- women still exist after accounting for infection with *C. albicans*. Also, it is important to note that after removing women with *C. albicans* infection the differences in cytokine levels between EA and AA BV^- women were even stronger and remained significant after correction for multiple testing. However, these results should be interpreted with caution as the sample size is small (EA: 44 BV^-, 24 BV^+ and AA: 26 BV^-, 32 BV^+) and power may be limited to detect smaller effects.

It remains unclear based on our, or any other, data whether BV infection induces an altered cytokine response or if altered vaginal immunity predisposes an individual to BV. Future research will need to be conducted to determine this relationship. In conclusion we were able to demonstrate that BV^+ and BV^- women differed in a number of cytokines that are related to inflammation and that these differences appear to be population specific. In addition, our data support the conclusion that cytokine levels are similar in AA and EA women in the presence of BV, but that there may be differences between these populations in the absence of BV. If this is true, it would suggest that BV

either fosters or is fostered by a common set of cytokine and chemokine changes, regardless of race or ethnicity.

B. Patterns of Cervical Cytokine Concentrations Differ by BV Status and Race

Introduction

The study of inflammation in the setting of lower genital tract infection has focused on a handful of inflammatory molecules, most often considered individually. Studies in part A of this chapter identified individual cytokines such as IP10, MCP1 and IL-1α that differ by BV status (Ryckman et al., 2008d). However, investigating cytokines individually does not provide insight into their *holistic* function as members of a system or network, and it is probable that structured correlations among key cytokines, chemokines and growth factors that represent networked responses are more important in understanding both susceptibility and response to BV. The coordination of this expression remains unclear, particularly with respect to BV.

Some studies have examined correlations among a few vaginal pro-inflammatory cytokine concentrations (IL-1α, IL-1β, IL-6 and IL-8) and found stronger correlations in BV^+ compared to BV^- women (Cauci et al., 2003; Wasiela et al., 2005; Ryckman et al., 2008c). These studies suggest that there is a strong correlated response to BV, particularly among pro-inflammatory cytokines.

Additionally, it is well documented that AA and EA can exhibit different inflammatory responses to similar stimuli (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004). In previous studies, described in part A, 28 cytokine, chemokine and growth factor concentrations were evaluated for differences by BV status and race (Ryckman et al., 2008d). No statistically significant differences in cervical cytokine concentration between AA and EA BV^+ women were observed.

However, in BV^- women, IL-1α, IL-6, IL-10, and PDGF-BB concentrations were higher in EA compared to AA. This may be a plausible mechanism to explain why AA are at a greater risk for BV than EA even after adjusting for socio-demographic differences such as education and income (Ness et al., 2003). Additionally, it is possible that the interrelationships of the components of the cytokine network may differ by BV status or race. If this is true, then such a difference may contribute to the disparity observed in BV prevalence between AA and EA women.

To more completely examine the network of cervical cytokine expression, we measured cervical levels of pro-inflammatory cytokines, immuno-regulatory cytokines and growth factors. We assessed the patterns of correlations among these immune factors by BV status. We also compared EA to AA to determine if the correlation patterns of cervical immune factors differed by race in women with healthy vaginal flora or in those with BV.

Materials and Methods

Subjects

A detailed description of subject recruitment, socio-demographic characteristics and microbiological assessment can be found in Chapter III, part A. However, in this study, an additional AA BV^+ woman was excluded due to a positive *C. trachomatis* culture at the second visit. Therefore, the total sample size was 83 EA (28 BV^+ and 55 BV^-) and 80 AA (41 BV^+ and 39 BV^-) women.

Cytokine characterization

A complete description of cytokine measurement and assays are described in Chapter III, part A. The cytokines were classified based on the literature as follows: pro-inflammatory (EOTAXIN, IL-1α, IL-1β, IL-6, IL-8, IP10, MIP1α, MCP1, RANTES and TNF-α), immuno-regulatory (IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p40, IL-12p70, IL-13 and IL-15) and growth factors (EGF, FGF2, FLT3, GMCSF, IL-3, IL-7, PDGF-AA, PDGF-BB and VEGF) (Stein, 1998; Parslow et al., 2001; Brunicardi and ed, 2005; Federman et al., 2007).

Statistical analysis

Spearman's rank correlation was calculated to determine correlations for all pair-wise combinations of the 28 cytokines, chemokines and growth factors using JMP-IN® (Sall et al., 2005). Overall correlation structure was assessed with two analyses: 1) testing for differences in the number of correlations present in each group and 2) testing for differences (heterogeneity) in correlation coefficients between each pair of cytokines among groups. The first method determines if the global pattern of cytokine correlations is different between BV statuses or races for any group of cytokines or for an individual cytokine. The second method examines individual cytokine pairs to determine exactly what cytokine correlations differ between groups.

McNemar's chi-square test was performed with Stata version 9 to determine if the number of correlations for each cytokine and group of cytokines differed by BV status or race (StataCorp, 2007). McNemar's exact test was used if there were less than 5 observations per group. McNemar's test determines the dependence of categorical data

that are matched or paired. For this study, the categorical data is defined as the number of correlations that were significant in both groups, neither group, or one group but not the other. A $p < 0.05$ was considered significant.

To test for heterogeneity of correlation coefficients between BV^+ and BV^- women and between ancestral groups, a t-test on the Fisher *r*-to-*z* transformations of the Spearman correlation coefficients was performed. This analysis is similar to previous work by both Ryckman *et al.* (2008c) and Velez *et al* (2008c).

Correction for multiple testing

The results for differences in the number of correlations between BV^+ and BV^- women and between races were corrected for multiple testing separately, using FDR with a significance of 0.2 (Benjamini and Hochberg, 1995). Due to the large number of factors being studied, only the cytokines significant after FDR correction are presented in the results and discussion. The results for heterogeneity between correlation coefficients were not corrected for multiple testing due to the exploratory nature of this analysis.

Results

Significant correlations by race and BV status

Three-hundred seventy eight correlations were examined for each of the following four groups: EA, BV^+ and BV^-; AA, BV^+ and BV^- (Appendix Table 2). In EA there were 180 significant correlations in BV^+ and 199 in BV^- women (Appendix Figure 1a and b).

In AA, 225 correlations in BV^+ and 206 correlations in BV^- women were significant (Appendix Figure 1c and d).

Correlation and heterogeneity patterns by BV status

In EA, there were significantly more correlations involving at least one immuno-regulatory cytokine in BV^- than BV^+ women (Table 3-4a). This is especially true for correlations with IL-4. Twenty-four correlations were heterogeneous between BV^+ and BV^- women and twenty of these are more correlated in BV^+ women (Figure 3-1a, Appendix Table 3). This heterogeneity was driven by correlations involving pro-inflammatory cytokines, particularly MIP-1α.

In contrast, in AA there were no significant differences in correlation patterns between BV^+ and BV^- women for any functional group or individual cytokine (Table 3-4b). Also only 13 out of 23 heterogeneous correlations between BV^+ and BV^- women were more correlated in BV^+ women (Figure 3-1b, Appendix Table 3). This heterogeneity was driven by correlations involving pro-inflammatory cytokines, particularly IL-1α that had eight heterogeneous correlations.

Table 3-4. Differences in the number of significant correlations between BV^+ and BV^- women a) EA, b) AA

a)

First Factor	Second Factor	P<0.05 In Both Groups	P<0.05 In BV^+ Only	P<0.05 In BV^- Only	P>0.05 In Both Groups	[1]nominal p
Pooled Comparisons						
ALL	ALL	128	52	71	127	0.10
PI	ALL	52	39	30	104	0.28
IR	ALL	79	19	44	65	<0.01*
GF	ALL	75	31	39	62	0.34
Individual						
PI	PI	12	8	9	16	1.00
PI	IR	21	11	13	45	0.84
PI	GF	19	20	8	43	0.04
IR	IR	20	2	10	4	0.04
IR	GF	38	6	21	16	0.01*
GF	GF	18	5	10	3	0.30
Individual Cytokine Comparisons						
MIP-1α	ALL	9	12	3	3	0.04
IL-4	ALL	2	0	10	15	<0.01*
PDGF-AA	ALL	13	0	8	6	<0.01*
VEGF	ALL	2	12	2	11	<0.01*

b)

First Factor	Second Factor	P<0.05 In Both Groups	P<0.05 In BV^+ Only	P<0.05 In BV^- Only	P>0.05 In Both Groups	[1]nominal p
Pooled Comparisons						
ALL	ALL	159	66	47	106	0.09
PI	ALL	65	51	34	75	0.06
IR	ALL	100	31	19	57	0.12
GF	ALL	93	36	26	52	0.20
Individual						
PI	PI	11	12	8	14	0.50
PI	IR	28	18	9	35	0.12
PI	GF	26	21	17	26	0.63
IR	IR	27	0	4	5	0.13
IR	GF	45	13	6	17	0.17
GF	GF	22	2	3	9	1.00
Individual Cytokine Comparisons						
RANTES	ALL	8	12	3	4	0.04
IL-12 (p40)	ALL	9	6	0	12	0.03

The first factor denotes the group (GF = Growth Factors, IR = Immuno-regulatory cytokines, PI = Pro-inflammatory or ALL= All 28 cytokines) of one of the cytokines in the pairwise correlation. The second factor represents the group of the other cytokine in the pairwise correlation. For example the correlation between IL-1α and EOTAXIN would be counted in three groups in the above table: 1) ALL-ALL 2) PI - ALL and 3) PI - PI. Each value represents the number of correlations that were either significant in both BV^+ and BV^-, significant in BV^+ but not BV^-, significant in BV^- but not BV^+ or not significant in BV^+ and BV^-. The p-value tests for the differences between these groups.

* Significant after correction for multiple testing using FDR (q=0.2)

[1]P-value calculated with McNemar's chi-square test

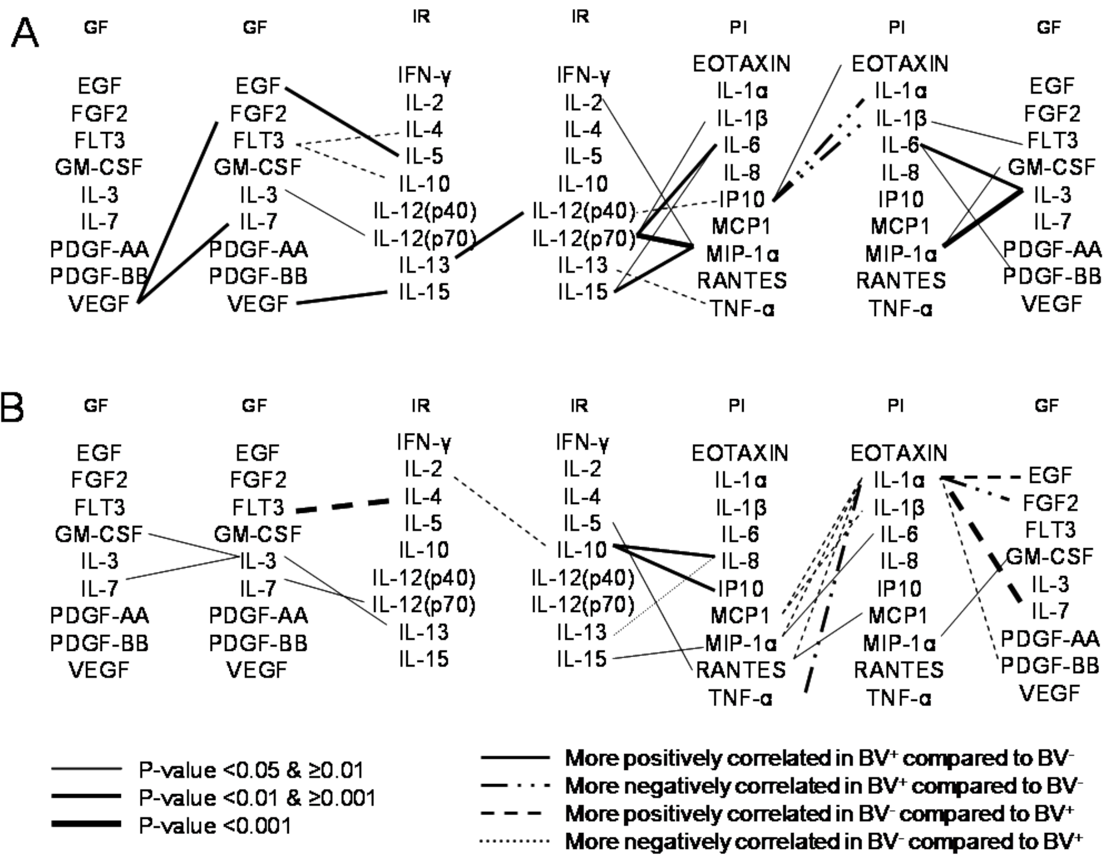


Figure 3-1: Significant heterogeneity between BV^+ and BV^- women a) EA, b) AA.
GF = Growth Factors, IR = Immuno-regulatory cytokines, PI = Pro-inflammatory cytokines. Significant heterogeneity by BV status is represented by the presence of a line with the thickness of the line indicating the significance of heterogeneity defined by p-value < 0.05, not corrected for multiple testing. The type of line (solid (—) dotted (…), dashed (⁻⁻) or solid and dotted (⁻..⁻)) indicates the direction of the heterogeneity.

Correlation and heterogeneity patterns by race

In BV^+ women, AA had significantly more correlations compared to EA (Table 3-5a). This was not due to any one set of factors but involves correlations with pro-inflammatory cytokines, immuno-regulatory cytokines, and growth factors. Specifically, the pro-inflammatory cytokine RANTES, the immuno-regulatory cytokines IL-4, IL-5 and IL-10 and the growth factors FGF2, FLT3 and PDGF-BB were driving this significance; however, only some immuno-regulatory cytokines were significant after

correction for multiple testing (Table 3-5a). Eleven out of seventeen correlations that showed heterogeneity between EA and AA were more correlated in EA compared to AA (Figure 3-2a, Appendix Table 3). However, this pattern does not appear to be driven by any particular group of cytokines.

In contrast, the only significant difference observed between EA and AA in BV^- women was between pro-inflammatory cytokines and growth factors; however, no individual cytokine drives this effect (Table 3-5b). The majority of heterogeneous correlations between EA and AA were more significant in AA than EA; but again, no particular group of cytokines drives this effect (Figure 3-2b, Appendix Table 3).

Correlations excluding women with C. albicans

Due to reports that cytokine production in vaginal epithelial cells is affected by the presence of *C. albicans* (Steele and Fidel, Jr., 2002) a secondary correlation structure analysis excluding 38 individuals with *C. albicans* was performed (EA: 24 BV^+ and 44 BV^- AA: 31 BV^+ and 26 BV^-). The results were generally very similar to the previous ones (data not shown). However, caution needs to be taken in the interpretation of these results as the sample size is small.

Table 3-5. Differences in the number of significant correlations between AA and EA
a) BV^+, b) BV^-

a)

First Factor	Second Factor	P<0.05 In Both Groups	P<0.05 In BV^+ Only	P<0.05 In BV^- Only	P>0.05 In Both Groups	[1]nominal p
Pooled Comparisons						
ALL	ALL	140	40	85	113	<0.01*
PI	ALL	68	23	48	86	<0.01*
IR	ALL	81	17	50	59	<0.01*
GF	ALL	81	25	48	53	0.01*
Individual						
PI	PI	15	5	8	17	0.58
PI	IR	25	7	21	37	0.01*
PI	GF	28	11	19	32	0.20
IR	IR	19	3	8	6	0.23
IR	GF	37	7	21	16	0.01*
GF	GF	16	7	8	5	1.00
Individual Cytokine Comparisons						
RANTES	ALL	9	2	11	5	0.02
IL-4	ALL	1	1	10	15	0.01*
IL-5	ALL	7	0	10	10	<0.01*
IL-10	ALL	16	0	8	3	0.01*
FGF2	ALL	14	1	8	4	0.04
FLT3	ALL	9	3	12	3	0.04
PDGF-BB	ALL	13	1	8	5	0.04

b)

First Factor	Second Factor	P<0.05 In Both Groups	P<0.05 In BV^+ Only	P<0.05 In BV^- Only	P>0.05 In Both Groups	[1]nominal p
Pooled Comparisons						
ALL	ALL	154	45	52	127	0.54
PI	ALL	56	26	43	100	0.04
IR	ALL	100	23	19	65	0.54
GF	ALL	89	25	30	63	0.50
Individual						
PI	PI	11	10	8	16	0.81
PI	IR	24	10	13	43	0.68
PI	GF	21	6	22	41	<0.01*
IR	IR	30	0	1	5	1.00
IR	GF	46	13	5	17	0.10
GF	GF	22	6	3	5	0.51
Individual Cytokine Comparisons						
PDGF-	ALL	11	10	2	4	0.04

The first factor denotes the group (GF = Growth Factors, IR = Immuno-regulatory cytokines, PI = Pro-inflammatory or ALL= All 28 cytokines) of one of the cytokines in the pairwise correlation. The second factor represents the group of the other cytokine in the pairwise correlation. For example the correlation between IL-1α and EOTAXIN would be counted in three groups in the above table: 1) ALL-ALL 2) PI - ALL and 3) PI - PI. Each value represents the number of correlations that were either significant in both EA and AA, significant in EA but not AA, significant in AA but not EA or not significant in AA and EA. The p-value tests for the differences between these groups.
* indicates significant after correction for multiple testing using FDR (q=0.2)[1]P-value calculated with McNemar's chi-square test

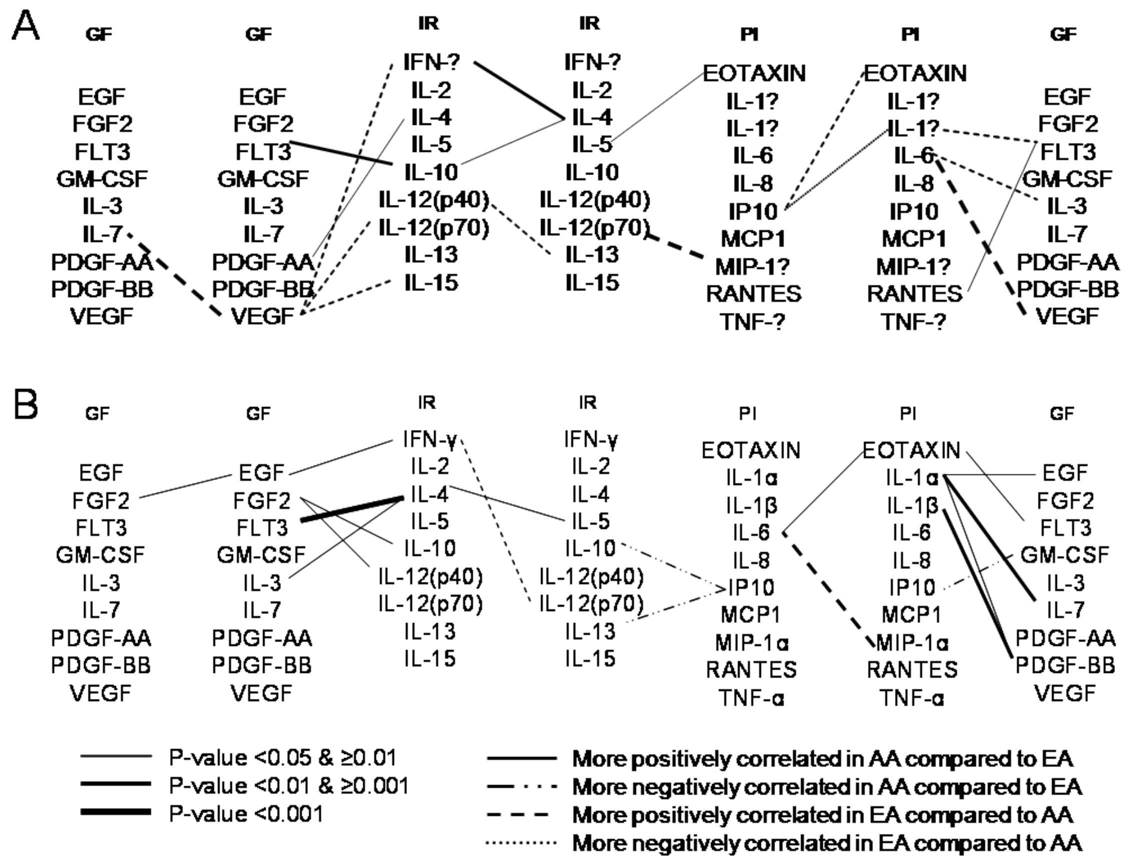


Figure 3-2. Significant heterogeneity between EA and AA a) BV$^+$ women, B) BV$^-$ women.

GF = Growth Factors, IR = Immuno-regulatory cytokines, PI = Pro-inflammatory cytokines. Significant heterogeneity by races is represented by the presence of a line with the thickness of the line indicating the significance of heterogeneity defined by p-value < 0.05, not corrected for multiple testing. The type of line (solid (—) dotted (…), dashed (⁻⁻) or solid and dotted (⁻..⁻)) indicates the direction of the heterogeneity.

Discussion

The response to infection requires a delicate balance between pro-inflammatory cytokines, immuno-regulatory cytokines and growth factors. It is likely that the expression of these factors is tightly coordinated, and this coordination can be detected using correlation analyses. Yet, few studies have examined networks of correlations with respect to BV. To fully evaluate the pattern of coordination in different groups of

cytokines, chemokines and growth factors, we determined if the number of significant correlations differs between BV^+ and BV^- women and between AA and EA women. Additionally, we examined individual correlation coefficients to determine if there was heterogeneity between BV^+ and BV^- women or between EA and AA women.

In comparisons of BV^+ to BV^- EA women, there were fewer correlations involving immuno-regulatory cytokines, specifically IL-4. It is important to note that in chapter III part A we did not find any significant differences in cervical concentrations between BV^+ and BV^- women for these immuno-regulatory cytokines (Ryckman et al., 2008d). This indicates that while immuno-regulatory cytokine levels were not individually suppressed in BV^+ women, the functional networks involving these cytokines was less prominent in BV^+ than in BV^- women. The absence of strong correlations among immuno-regulatory cytokines in BV^+ women suggests that these cytokines are not being regulated in a coordinated fashion; if these cytokines, specifically IL-4, IL-10 and IL-13, are not being produced at sufficient levels, an overproduction of pro-inflammatory cytokines may occur, which has been observed in EA women with BV (Platz-Christensen et al., 1993; Sturm-Ramirez et al., 2000; Cauci et al., 2003; Ryckman et al., 2008c). This trend was not observed in AA; in fact, there were no significant differences in the number of correlations between BV^+ and BV^- women for any group of cytokines, indicating a disparity in the correlated response of cervical cytokines to infection between AA and EA.

In EA, the number of significant correlations between BV^+ and BV^- women differs only for immuno-regulatory cytokines and the differences in specific correlation coefficients was driven by pro-inflammatory cytokines, specifically MIP-1α. All of the

correlations with MIP-1α were more significant in BV^+ compared with BV^- women. In chapter III part A we found that in EA, cervical concentrations of several pro-inflammatory cytokines, including MIP-1α, were lower in BV^+ compared to BV^- women (Ryckman et al., 2008d). This suggests a hypo-responsiveness to infection that appears to be strongly coordinated.

In AA differences in specific correlation coefficients between BV^+ and BV^- women were driven by the pro-inflammatory cytokine IL-1α. The majority of these were more significantly correlated in BV^- compared with BV^+ women. In chapter III part A, we found that in AA, cervical levels of IL-1α were elevated in BV^+ compared to BV^- women (Ryckman et al., 2008d). While there was an increase in cervical levels of IL-1α in BV^+ women, this was not correlated with other cytokine levels, which is particularly interesting since IL-1 induces and is induced by several other cytokines and growth factors (Dinarello, 1996). It is possible that strong correlations were absent in BV^+ women because the median cervical IL-1α levels were two times greater than levels in BV^- women and other cytokines do not vary to that same degree.

As with all correlation analyses, it is unclear if this effect causes BV or is caused by BV. If one assumes that the baseline state is the absence of infection, it is reasonable to argue that the changes in correlation patterns that we observed are in fact the result of infections and not the cause of it as suggested by others (Cauci and Culhane, 2007).

It is evident that EA and AA have different immune responses to infection and have differing susceptibilities to BV (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004); yet, few studies have focused on differences between these groups with respect to the cervical immune response to BV. The number of

significant correlations between EA and AA BV^- women did not differ significantly in most cases. The one exception was that AA had more significant correlations between growth factors and pro-inflammatory cytokines compared with EA. In contrast, in BV^+ women, AA had more significant correlations than EA for pro-inflammatory cytokines, immuno-regulatory cytokines and growth factors. In particular, the immuno-regulatory cytokines IL-4, IL-5 and IL-10 exhibit more significant correlations in AA than EA. In chapter III part A, IL-1α, IL-6, IL-10 and PDGF-BB were elevated in EA compared to AA BV^- women (Ryckman et al., 2008d). In contrast, there were no cervical cytokine concentration differences between AA and EA BV^+ women (Ryckman et al., 2008d). In BV^+ women, individual cytokine levels did not significantly differ between AA and EA, and the correlated response to local infection was stronger in AA. This hyper-responsive immunity, particularly involving immuno-regulatory cytokines, could be a plausible explanation for why AA with BV are at greater risk for developing adverse reproductive outcomes, such as PTB, than their EA counterparts.

Although several cytokine differences between groups were detected, studies in this chapter were limited by small sample sizes. The strongest cervical concentration difference between BV^- and BV^+ women was for IL-1α in AA and based on the log transformed means and standard deviations the power to detect this effect was 95.6% (calculated on Stata version 10). However, the power to detect a difference in IL-1β in EA, a cytokine consistently found at higher concentrations in BV^+ EA women, was only 35.2% and 114 individuals in each group would be needed reach 80% power. Addtionally, the differences by race in BV^- women were not significant after correction for multiple testing; however, the power for the strongest association (IL-6) was only

42.1%. Also, there were no differences by race BV^+ women; however, for the most significant result (IL-12p70), the power to detect an effect was 18.2% and 233 individuals in each group would be needed to achieve 80% power. This illustrates that our studies were underpowered to detect many effects that may have been missed, however, for the effects we did see there was either adequate power or the effect was strong enough to be detected regardless of power.

Few studies have examined the coordinated regulation of an extensive network of cytokines, chemokines and growth factors involved in the immune response to BV. Our results have identified key factors involved in the coordinated immune response to BV in EA and AA. These coordinated responses did not differ much between AA and EA BV^- women; however, the coordinated regulation is very different between EA and AA BV^+ women, an aspect very few studies have addressed. Based on our data, we hypothesize that the coordination of inflammatory factors in the cervix is important in understanding the immune response to BV, as well as differences between AA and EA women with respect to the host consequences of bacterial vaginosis during pregnancy.

CHAPTER IV

GENETIC ASSOCIATIONS OF PRO- AND ANTI-INFLAMMATORY CERVICAL CYTOKINES

Overview parts A, B and C

Local innate immunity is critical in regulating the response to bacteria in the genital tract (Fidel, 2003; Russell et al., 2004). Several studies have demonstrated that elevated amniotic fluid concentrations of pro-inflammatory cytokines leads to a hyper-responsive immunity that is associated with adverse birth outcomes such as PTB (Menon et al., 2007; Menon et al., 2008a; Menon et al., 2008b). Conversely, low concentrations of multiple cytokines create a hypo-responsiveness that predisposes women to infection and inflammation such as chorioamnionitis (Simhan et al., 2003). Studies in chapter III demonstrated that individual cytokines as well as cytokine networks differ by not only BV status but also race (Ryckman et al., 2008d). Additionally, several studies show polymorphisms in pro-inflammatory genes are associated with protein concentrations; however, only a few focus on vaginal, cervical or amniotic fluid cytokine expression (Genc et al., 2006; Rafiq et al., 2007; Velez et al., 2007).

Chapter IV examines genetic associations with cervical anti-inflammatory and pro-inflammatory cytokines. In part A three anti-inflammatory cytokines (IL-4, IL-10 and IL-13) are examined for associations with SNPs in their respective genes and receptors. These associations are explored in the context of BV status and race. In EA, two SNPs in IL10RA (rs4936414 and rs2512143) were significantly associated with IL-10

concentration after correcting for multiple testing and adjusting for BV status. In AA, there were no significant results that held up to correction for multiple testing.

Part B assesses the impact of genetic variation on cervical levels of IL-1α, IL-1β, IL-6, IL-8 and TNF-α and determines if this relationship is influenced by BV. In AA, but not EA, higher cervical levels of IL-1β, IL-8 and TNF-α were observed in BV^+ women with a particular genotype in IL-1RAP (rs6765375), IL-8RA (rs1008562) and TNFR2 (rs1201157), respectively. In EA, but not AA, cervical IL-6 concentrations were higher in BV^+ women with a particular genotype in IL-6R (rs4075015). However, none of these results held up to a global correction for multiple tests.

Part C examines the impact of genetic variation in TLR genes on cervical concentrations of pro- and anti- inflammatory cytokines, and determines if this relationship is influenced by BV. In EA BV^- women, higher cervical concentrations of IL-1β were observed with a particular genotype in TLR4 (rs1554973), a result that remained significant after correction for multiple testing. Additionally, in EA, rs1927911 and rs2149356 (TLR4) were associated with cervical IL-1β, IL-6 and TNF-α concentrations. Importantly, this effect is modified by BV status. For example, women with the CC genotype at rs1927911 or rs2149356 have higher cervical concentrations of IL-1β and IL-6 than women with the CT/TT or AA/AC genotypes, but only among BV^- women. However, this was not significant after correction for multiple testing. These studies demonstrate a significant relationship between cervical cytokine concentrations and cytokine receptor genes. Additionally, this relationship is affected by both BV status and race.

A. Genetic Associations of Cervical Anti-inflammatory Cytokines Differs by BV Status and Race

Introduction

The balance of pro- and anti-inflammatory components of the cervical immune system is critical in regulating the response to micro-organisms and infections of the lower genital tract. Anti-inflammatory cytokines are important in keeping inflammation in check; however, by attenuating the host immune response, one might speculate that these cytokines contribute to a state of "impaired" defense. Decreased concentrations of several pro-inflammatory cytokines in the lower genital tract, which may occur because of an increase in anti-inflammatory cytokines, indicate an immune "hyporesponsiveness" that increases the risk for subsequent ascending microbial invasion (Simhan et al., 2003a).

Previous studies in chapter III found fewer correlations involving immuno-regulatory cytokines in EA BV^+ compared to BV^- women (Ryckman et al., 2008b). This may indicate a disruption in anti-inflammatory cytokines leading to impaired immunity. Additionally, there are likely to be a number of factors that influence or affect cervical immunity including demographic and environment exposures. The relationship between alterations in the vaginal ecology that occur with BV and disruption of the vaginal inflammatory milieu, which might lead to a predisposition for adverse pregnancy outcomes, is of particular interest. However, genetic contributors to this cervical immunity are poorly understood. This study examines the contribution of maternal genotype to the concentration of ILs-4, -10, and -13 in the cervix during the first trimester

of pregnancy. Also, the contribution of genotype in the context of BV status and race is examined.

Material and Methods

Subjects

Cervical IL-4, IL-10 and IL-13 were examined in this study. A detailed description of subject recruitment, socio-demographic characteristics, microbiologic assessment and cytokine measurement is in Chapter III, part A.

DNA genotyping

A total of 72 SNPs in 6 genes were examined: IL-4 (8 SNPs), IL-4 receptor (IL-4R, 27 SNPs), IL-10 (5 SNPs), IL-10 receptor alpha (IL-10RA, 11 SNPs), IL-10 receptor beta (IL-10RB, 15 SNPs) and IL-13 (6 SNPs) (Appendix Table 4). SNPs were selected based on their ability to tag surrounding variants in the Caucasian (CEPH) and Yoruban (YRI) populations of the HapMap database (http://www.hapmap.org). A minor allele frequency of 0.20 in CEPH and 0.07 in YRI and an r^2 of 0.80 was used to determine tagSNPs. Genotyping was performed on the Illumina GoldenGate platform (Illumina, San Diego, CA). Genotyping efficiency was >95% for all SNPs and individuals analyzed. There were 64 EA (20 BV^+, 44 BV^-) and 52 AA (32 BV^+, 20 BV^-) women with genotype information in this study.

Quality control

A total of 5 SNPs in AA and 30 SNPs in EA were excluded from subsequent analyses due to low minor allele frequency such that one or more genotype/BV status groups contained fewer than 5 individuals, thus preventing the analysis of dominant, recessive or additive models. Therefore, 67 SNPs were analyzed in AA and 42 SNPs were analyzed in EA. Cytokine concentrations were assessed for normality with the Shapiro-Wilk test; however, none of the cytokines were normally distributed ($p < 0.01$). Cytokine concentrations were transformed using the Box Cox method.

Statistical analysis (Chapter IV part A, B and C)

The relationship between each cytokine and its respective gene and receptors was analyzed with two-way analysis of variance (ANOVA). Analyses were stratified by race and included the interaction between BV status and SNP. The full model was: transformed cytokine concentration = $\mu + \alpha$(SNP) + β(BV status) + γ(BV status*SNP) + ε. Additive, dominant and recessive models were analyzed for all cytokine concentrations; however, models were excluded if there were fewer than 5 individuals in any genotype per BV status in the model. Only the best model (the model with the most significant p-value) was considered for subsequent analysis. Significant SNP or interaction effects ($p < 5 \times 10^{-3}$) were analyzed further with the Sidak test to determine which group (BV^+ or BV^-) was driving the significance. The Sidak test calculates p-values that are corrected for multiple testing. Calculations were performed using Stata version 9 (StataCorp, College Station, TX). A total of 524 tests were performed and results were adjusted for this number of tests with FDR (q=0.2). This number included

tests for all evaluated genetic models, cytokine concentrations, both races and interaction and single SNP main effects.

To determine if significant results were dependent on race, ANOVA was used to examine SNP and race main effects as well as a SNP and race interactions in BV^+ and BV^- women, separately. Allelic and genotypic distributions were compared between ethnic groups using Fisher's exact tests. Also, using Powermarker software, HWE was calculated for each marker with Fisher's exact tests (Liu and Muse, 2005). The analyses examining effects of race were not corrected for multiple testing.

Cytokine associations excluding women with C. albicans

To investigate if the presence of *C. albicans* affected these associations, the analyses were performed excluding individuals with *C. albicans* as described in part A of this chapter. There were negligible differences for the results that were statistically significant after correction for multiple testing (data not shown).

Results

Genetic associations with cytokine concentrations

Among EA, 4 SNPs in IL10RA were associated with IL-10 concentration (Table 4-1, Appendix Table 5). Two of these, rs2512143 and rs4936414, were significant after correction for multiple testing ($p = 6x10^{-4}$ and $p = 1x10^{-4}$, respectively). Women with the GG genotype at rs2512143 had significantly higher cervical IL-10 concentrations than women with the AA/AG genotypes (medians 79.7 ng/ml vs 43.0 ng/ml). Women with the

TT genotype at rs4936414 had significantly higher cervical IL-10 concentrations than women with the CC/CT genotypes (medians 86.2 ng/ml vs 39.8 ng/ml) (Figure 4-1). The effects were stronger in BV^+ women ($p = 0.045$ for rs2512143 and $p = 0.006$ for rs4936414). The other two SNPs (rs2508445 and rs2229113) were not significant after correction for multiple testing ($p = 0.002$ for both), and BV status did not influence this effect. None of the interaction terms between genotype and BV status were significant for these models. All four of the SNPs in IL10RA were in strong LD with one another (Figure 4-1). Additionally, there were no SNPs in AA or in IL-4 or IL-13 genes for EA that were significantly ($p<5x10^{-3}$) associated with the concentration of these cytokines (Appendix Table 5).

Ethnic heterogeneity and quality control

The SNPs analyzed show large differences in their ethnic distribution (Appendix Table 4). Of 75 SNPs genotyped, 50 had significant differences in allele frequencies between ethnic groups, and, of those 50 SNPs, 32 were significant at $p < 0.001$. Similarly, 45 out of 75 SNPs had significant differences in genotype frequencies between ethnic groups, and, of those 45 SNPs, 28 were significant at $p < 0.001$. There were 3 SNPs that deviated from HWE in AA, none of which had $p < 0.01$. In EA, 5 SNPs deviated from HWE, only one of which had a $p < 0.01$.

To determine if significant associations with cytokine concentration were affected by race, race main effects and race/SNP interactions were examined with each cytokine in BV^+ and BV^- women separately. However, these results were not corrected for multiple testing. In BV^+ women there were significant race/SNP interactions at

rs4936414 ($p = 0.03$) and rs2508445 ($p = 0.03$) with IL-10 concentrations. In BV^- women there was a significant race main effect at rs2508445 ($p = 0.04$) with IL-10 concentrations.

Table 4-1. Significant associations between SNPs in IL-10RA and cervical IL-10 concentrations in EA

	Model	*nominal p-values for ANOVA model*				*[1]Genotype 1 vs 2*		*[2]BV^+ vs BV^-*	
RS#	*(Genotype 1 vs 2)*	*Model*	*SNP*	*BV*	*Intxn*	*BV^+*	*BV^-*	*Genotype 1*	*Genotype 2*
rs2229113	AA/AG vs GG	0.014	0.002	0.509	0.228	0.052	0.357	1.000	0.604
rs2508445	GG vs GT/TT	0.014	0.002	0.509	0.228	0.052	0.357	0.717	0.153
rs2512143	AA/AG vs GG	0.005	$6x10^{-4}$*	0.428	0.364	0.045	0.103	0.693	1.000
rs4936414	CC/CT vs TT	$8x10^{-4}$*	$1x10^{-4}$*	0.688	0.138	0.006	0.085	0.529	0.983

[1]p-values for pairwise comparisons between the two genotype groups in BV^+ and BV^- women
[2]p-values for pairwise comparisons between BV^+ and BV^- women in each genotype group
* significant after correction for multiple testing using FDR (q = 0.2)

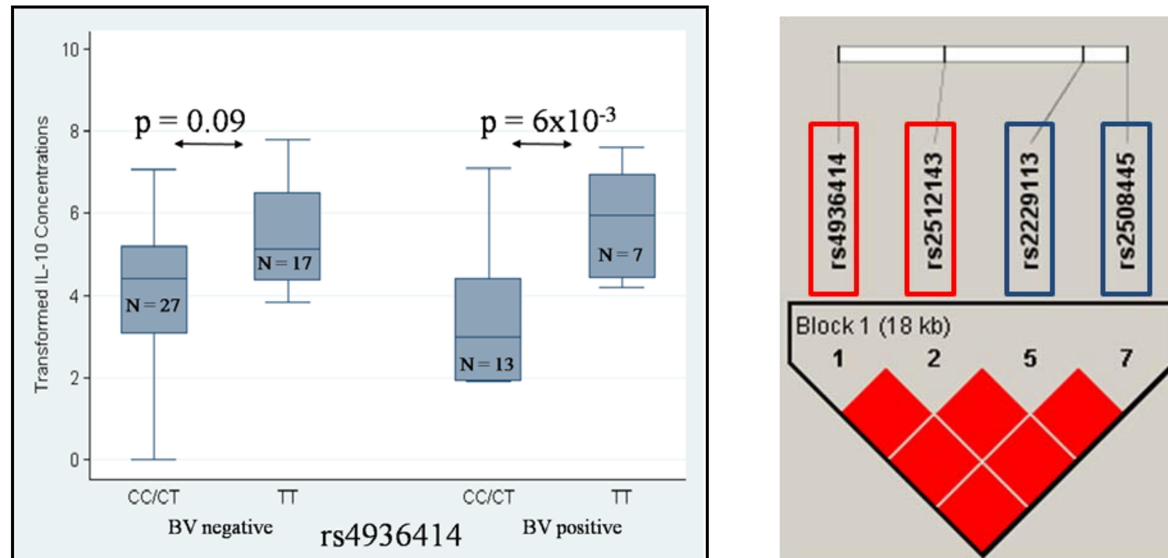


Figure 4-1 Significant associations between IL-10RA and cervical IL-10 concentrations Association between IL-10RA at rs4936414 with cervical IL-10 concentration is depicted (left graph). LD pattern in IL-10RA (right graph). SNPs boxed in red represent significant associations after correction for multiple testing. SNPs boxed in blue represent associations that were not significant after correction for multiple testing. LD is measured as D′. All r^2 values were reflective of D′ ($r^2 > 0.84$).

Discussion

Our data demonstrate a significant relationship between cervical concentrations of IL-10 and common polymorphic variation in the gene that encodes for this cytokine receptor (IL-10RA), particularly in EA. Importantly, the contribution of genotype to IL-10 concentration is significant, even when considered in the context of BV. These data are the first report of maternal genotype exerting an influence on cervical immunity during pregnancy.

There is previous literature supporting a contribution of genetic variation to the production of inflammatory molecules, including IL-10. Recently, it was found that peritoneal fluid concentrations of IL-10 among women with endometriosis differed by IL-10 genotype (Zhang et al., 2007). Also, placental IL-10 mRNA and protein expression varies by IL-10 -1082 (rs1800896) polymorphism status (Makris et al., 2006). Genetic regulation of IL-10 may also be linked with disorders of infection-mediated or inflammatory nature. Maternal carriage of the IL-10 -1082 G allele in the presence of chorioamnionitis was associated with early sPTB (Kerk et al., 2006). Our work adds to the extant literature by evaluating the cervical inflammatory milieu during pregnancy and by more broadly considering the genes that influence the production and function of IL-10 along with the two other major anti-inflammatory cytokines.

Of the four SNPs in IL-10RA associated with cervical IL-10 concentration, one is located in the promoter (rs4936414), one in an intron (rs2512143), another is the coding SNP for amino acid 351 (R→G) (rs2229113), and the last SNP (rs2508445) is located in the 3' UTR. These SNPs were in strong LD with one another, particularly the coding SNP (rs2229113) that is in strong LD (D' = 1, $r^2 > 0.84$) with the other three associated

SNPs. Therefore, it is possible that these changes, in particular rs2229113, are directly involved in affecting IL-10 concentration. Rs2229113 is a nonsynonomous missense mutation where an arginine is converted to glycine. Arginine is a polar amino acid with a positive (basic charge), whereas glycine is a nonpolar amino acid. This mutation may be functional; however, there is no known research on this particular mutation to date.

Additionally, all of these SNPs, except rs2508445, differ significantly in either genotype or allele frequencies between AA and EA. The LD patterns are also very different between the Caucasian and Yoruban Hapmap samples. There were significant race/SNP interactions with IL-10 concentration in BV^+ women at rs4936414 and rs2508445. These associations may explain racial differences observed not only in the prevalence of conditions such as BV, but also in cervical cytokine differences observed in chapter III.

It is interesting to note that, in our study, the polymorphisms most strongly related to IL-10 concentration are in the genes encoding the IL-10 receptor, not the IL-10 gene itself. To more fully appreciate the genetic regulation of the expression and function of cytokines, it is important to consider not only the gene encoding that cytokine, but also those that encode for critical receptors or cofactors. While such an approach increases analytic complexity, it may provide more accurate insight into these biological processes.

B. Genetic Associations of Cervical Pro-inflammatory Cytokines Differ by BV Status and Race

Introduction

Pro-inflammatory cytokines are important mediators of the inflammatory response and are critical factors in cell-mediated immunity, which constitutes the main defense against invading pathogens. Previous studies, described in part A, demonstrate that polymorphisms in receptor genes were associated with cervical anti-inflammatory cytokine concentrations, and this effect remained after adjusting for the presence of BV (Simhan et al., 2008). However, while several studies have found associations between genetic factors, pro-inflammatory serum cytokine levels and cytokine expression levels, few have focused on pro-inflammatory cervical immunity (Reuss et al., 2002; McDaniel et al., 2003; Rafiq et al., 2007). For example, vaginal IL-1β concentrations are consistently increased in women with BV, while IL-1α and IL-8 are sometimes increased in women with BV. Furthermore, IL-6 and TNF-α concentrations are rarely different between women with and without BV (Platz-Christensen et al., 1993; Imseis et al., 1997; Mattsby-Baltzer et al., 1998; Sturm-Ramirez et al., 2000; Cauci et al., 2003; Alvarez-Olmos et al., 2004; Wasiela et al., 2005; Basso et al., 2005; Hedges et al., 2006; Ryckman et al., 2008c). Additionally, several genetic polymorphisms, particularly those in IL-1β, IL-6 and IL-8, are associated with BV (Genc et al., 2004a; Goepfert et al., 2005; Cauci et al., 2007). However, few studies have examined the connection between genetic polymorphisms and cervical cytokine concentrations with respect to BV. Additionally, it

is unclear if the genetic regulation of cervical cytokine concentrations is impacted by the presence of BV infection.

AA are at a 2 times greater risk for developing BV than EA, even after controlling for socio-demographic variables such as education and income (Ness et al., 2003). Additionally, AA and EA have differing immune responses to infection as well as genetic differences in immunity-related factors (Myslobodsky, 2001; Hoffmann et al., 2002; Blake and Ridker, 2003; Ness, 2004). Few studies have examined the differences between these groups with respect to the genetic regulation of cervical immunity, particularly in the presence of BV.

In this investigation, we determined if SNPs in pro-inflammatory genes and receptors were associated with pro-inflammatory cervical concentrations of IL-1α, IL-1β, IL-6, IL-8 and TNF-α. Additionally we determined if these associations were affected by BV status and race.

Material and Methods

Subjects

Cervical IL-1α, IL-1β, IL-6, IL-8 and TNF-α were examined in this study. A detailed description of subject recruitment, socio-demographic characteristics, microbiologic assessment and cytokine measurement is in Chapter III, part A.

DNA genotyping

A total of 200 SNPs in 13 genes were examined: IL-1α (3 SNPs), IL-1β (7 SNPs), IL-1R1 (16 SNPs), IL-1R2 (23 SNPs), IL-1RN (14 SNPs), IL-1RAP (65 SNPs), IL-6 (8 SNPs), IL-6R (22 SNPs), IL-8 (3 SNPs), IL-8RA (4 SNPs), TNF-α (6 SNPs), TNFR1 (10 SNPs) and TNFR2 (19 SNPs) (Appendix Table 6). A description of TagSNP selection and genotyping is provided in part A of this chapter.

Quality control

A total of 58 SNPs in AA and 51 SNPs in EA were excluded from subsequent analyses due to low minor allele frequency such that one or more genotype/BV status groups contained fewer than 5 individuals, thus preventing the analysis of dominant, recessive and additive models. Therefore, 142 SNPs in AA and 149 SNPs in EA remained for analysis. Cytokine concentrations were assessed for normality with the Shapiro-Wilk test; however, none of the cytokines were normally distributed ($p < 0.01$). Cytokine concentrations were transformed with natural log and analyzed with ANOVA and Sidak tests. IL-8 and TNF-α were not normally distributed after transformation with the natural log or Box Cox methods; therefore, non-parametric tests including Kruskal-Wallis and the Mann-Whitney U test were used to analyze these cytokines. Further details on the statistical methods are discussed in part A of this chapter.

Correction for multiple testing

A total of 996 tests were performed and results were adjusted for this number of tests with FDR (q=0.2). This number included tests for all evaluated genetic models,

cytokine concentrations, both races and interaction and single SNP main effects. However, using this method, there were no significant results after correction. Therefore, due to the exploratory nature of this study, corrections for multiple testing were performed individually for each race, cytokine and test (either SNP or interaction), using FDR ($q = 0.2$).

Cytokine associations excluding women with C. albicans

To investigate if the presence of *C. albicans* affected these associations, the analyses were performed excluding individuals with *C. albicans*. There were negligible differences for the results that were statistically significant after correction for multiple testing (data not shown).

Results

Genetic associations with cytokine concentrations

In AA women, 2 SNPs (rs6765375 and rs1469007) in IL-1RAP had significant BV status/SNP interactions with cervical IL-1β concentrations ($p = 0.003$ and 0.004, respectively) (Table 4-2a, Appendix Table 7). These SNPs were in LD with one another ($D' = 0.73$, $r^2 = 0.18$). BV^- women with the AA/AC genotypes at rs6765375 had significantly lower cervical IL-1β levels than BV^- women with the CC genotype (medians 17.5 ng/ml vs 165.7 ng/ml, $p = 0.042$) (Figure 4-2). Additionally, BV^- women with the AA/AC genotypes had significantly lower cervical IL-1β levels compared to BV^+ women with the same genotypes (medians 17.5 ng/ml vs 264.3 ng/ml, $p = 0.013$). At rs1469007,

BV^+ women with the GT/TT genotypes had significantly higher IL-1β than BV^+ women with the GG genotype (medians 233.2 ng/ml vs 57.5 ng/ml, p = 0.043). None of the above results were significant after correction for multiple testing with FDR (q = 0.2).

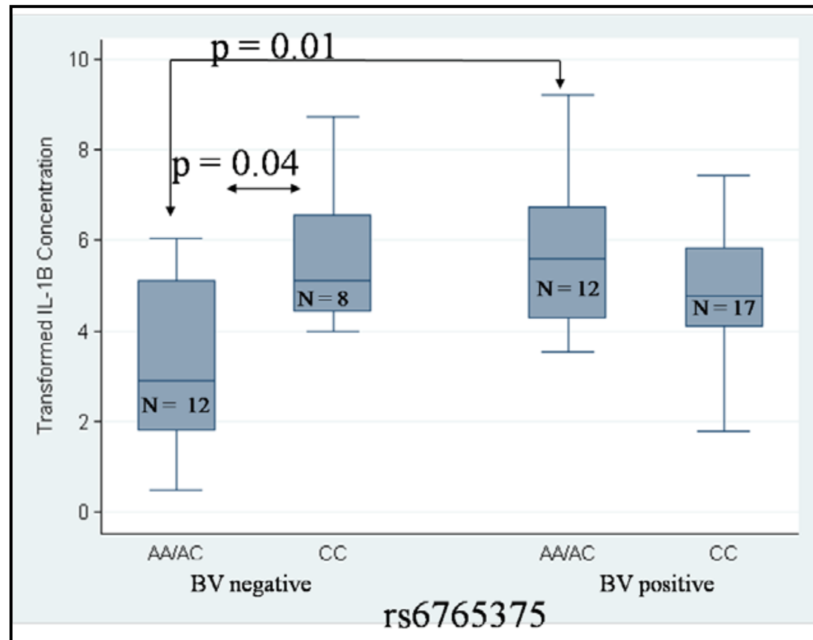


Figure 4-2. Transformed concentration of IL-1β by rs6765375 (IL-1RAP) genotype in AA

Table 4-2. Significant associations between SNPs and cervical cytokine concentrations a) AA, b) EA

a)

			Model	*nominal p-values for ANOVA model*				*[1]Genotype 1 vs 2*		*[2]BV⁺ vs BV⁻*	
Cytokine	*Gene*	*RS#*	*(Genotype 1 vs 2)*	*Model*	*SNP*	*BV*	*Intxn*	*BV^+*	*BV^-*	*Genotype 1*	*Genotype 2*
IL-1β	IL-1RAP	rs1469007	GG vs GT/TT	0.014	0.915	0.639	0.004	0.204	0.186	0.275	0.043
IL-1β	IL-1RAP	rs6765375	AA/AC vs CC	0.009	0.249	0.197	0.003	0.540	0.042	0.013	0.744
IL-8[3]	IL-8RA	rs1008562	CC vs CG/GG	-	-	-	0.012*	0.003	0.934	1.000	0.009
IL-8[3]	IL-8RA	rs1008563	CC vs CT/TT	-	-	-	0.047*	0.077	0.262	0.022	0.354
TNF-α[3]	TNFR2	rs1201157	CC vs CT/TT	-	-	-	0.006*	0.007	0.091	0.105	0.003

b)

			Model	*nominal p-values for ANOVA model*				*[1]Genotype 1 vs 2*		*[2]BV⁺ vs BV⁻*	
Cytokine	*Gene*	*RS#*	*(Genotype 1 vs 2)*	*Model*	*SNP*	*BV*	*Intxn*	*BV^+*	*BV^-*	*Genotype 1*	*Genotype 2*
IL-6	IL-6R	rs4075015	AA/AT vs TT	0.029	0.048	0.083	0.004*	0.033	0.923	0.874	0.033

[1]p-values for pairwise comparisons between the two genotype groups in BV^+ and BV^- women
[2]p-values for pairwise comparisons between BV^+ and BV^- women in each genotype group
[3]indicates Kruskal-Wallis test was performed, therefore only the interaction p-value is calculated
* significant after correction for multiple testing using FDR (q = 0.2)

In AA women 2 SNPs (rs1008562 and rs1008563) in IL-8RA had significant BV/SNP interactions with cervical IL-8 levels (p = 0.012 and 0.047, respectively) (Table 4-2a, Appendix Table 7). These SNPs were in strong LD with one another (D′ = 1.0, r^2 = 0.31). These results were significant after correction for multiple testing. BV^+ women with the CG/GG genotypes at rs1008562 had significantly higher cervical IL-8 levels than BV^+ women with the CC genotype (medians 25000.0 ng/ml vs 7884.7 ng/ml, p = 0.003) (Figure 4-3). Additionally, BV^+ women with the CG/GG genotypes had significantly higher cervical IL-8 levels than BV^- women with the same genotypes (medians 25000.0 ng/ml vs 5623.3 ng/ml, p = 0.009). At rs1008563, BV^+ women with the CT/TT genotypes had significantly higher IL-8 levels than BV^- women with the same genotype (medians 18514.6 ng/ml vs 9959.3 ng/ml, p = 0.022).

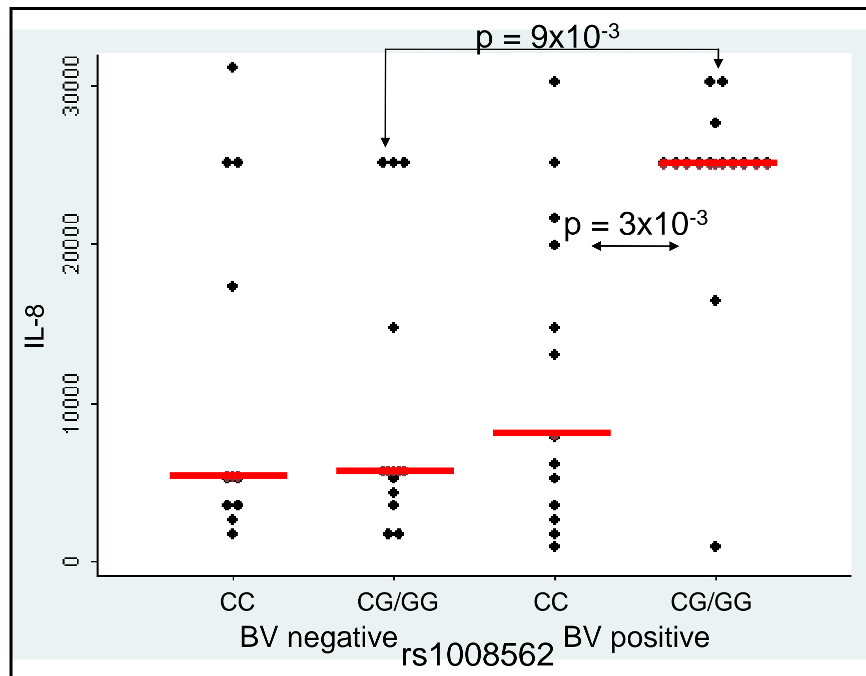


Figure 4-3. Median IL-8 concentrations by rs1008562 (IL-8RA) genotype in AA

One SNP (rs1201157) in TNFR2 had a significant BV/SNP interaction with TNF-α concentrations in AA women (p = 0.006) (Table 4-2a, Appendix Table 7). This result was significant after correction for multiple testing. BV^+ women with the CT/TT genotypes had significantly higher TNF-α cervical levels than those with the CC genotype (medians 48.5 ng/ml vs 5.0 ng/ml, p = 0.007) (Figure 4-4). Additionally, BV^+ women with the CT/TT genotypes had significantly higher TNF-α cervical levels than BV^- women with the same genotypes (medians 48.5 ng/ml vs 5.0 ng/ml, p = 0.003).

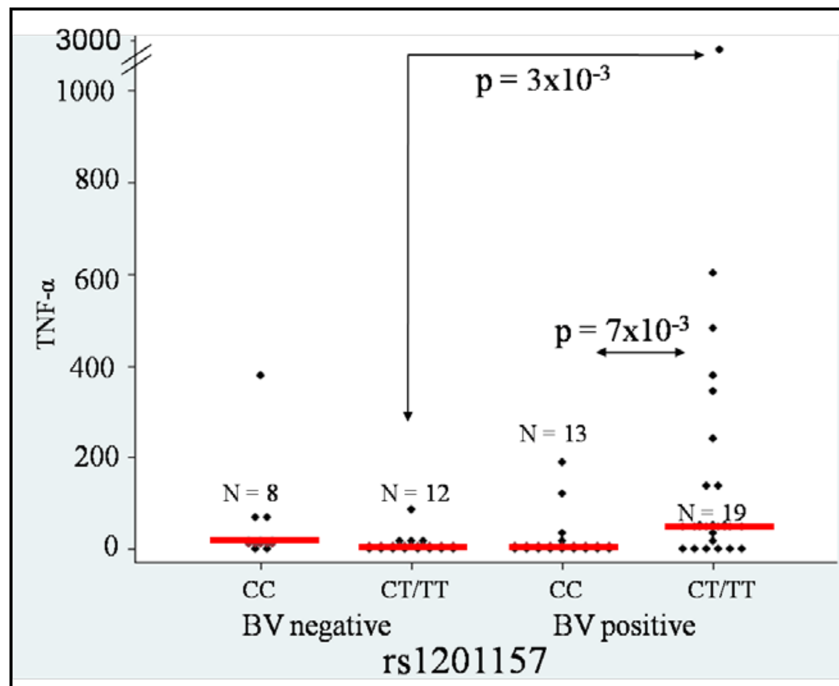


Figure 4-4. Median TNF-α concentrations by rs1201157 (TNFR2) genotype in AA

In EA women, one SNP (rs4075015) in IL-6R had a significant BV/SNP interaction with cervical IL-6 concentrations (p = 0.004) (Table 4-2b, Appendix Table 7).

This result was significant after correction for multiple testing. BV^{+} women with the TT genotype at rs4075015 had significantly lower cervical IL-6 concentrations than BV^{+} women with the AA/AT genotypes (medians 147.0 ng/ml vs 2151.3 ng/ml, p = 0.033) (Figure 4-5). Additionally, BV^{+} women with the TT genotype had significantly lower cervical IL-6 concentrations than BV^{-} women with the same genotype (medians 147.0 ng/ml vs 3444.5 ng/ml, p = 0.033).

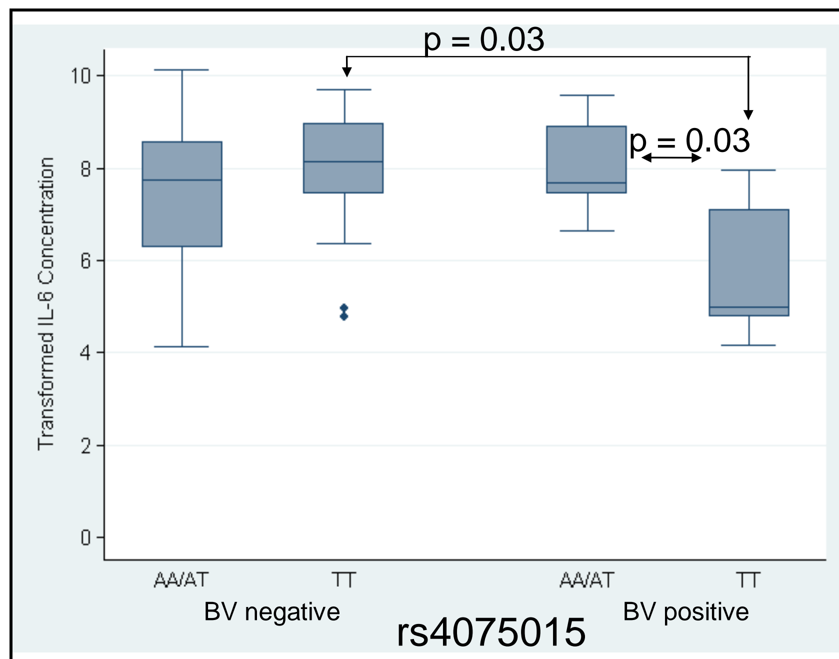


Figure 4-5. Transformed concentration of IL-6 by rs4075015 (IL-6R) genotype in EA

Ethnic heterogeneity and quality control

The SNPs analyzed show large differences in their ethnic distribution (Appendix Table 6). Of 200 SNPs genotyped, 125 had significant differences in allele frequencies between ethnic groups, and, of these, 75 SNPs were significant at p < 0.001. Similarly,

120 out of 200 SNPs had significant differences in genotype frequencies between ethnic groups, and, of these, 62 were significant at $p < 0.001$. There were 10 SNPs that deviated from HWE in AA, only 3 of which have $p < 0.01$. In EA, 14 SNPs deviated from HWE, only 3 of which had $p < 0.01$.

To determine if significant associations with cytokine concentration were affected by race, race main effects and race/SNP interactions were examined with each cytokine in BV^+ women and BV^- women, separately (Table 4-3). In BV^+ women, there were significant race main effects, race/SNP interactions or both at rs1469007 for IL-1β concentrations, rs1008562 and rs1008563 for IL-8, rs1201157 for TNF-α and rs4075015 for IL-6. However, in BV^- women, none of these associations were significant.

Table 4-3. Race main affects and SNP/race interaction p-values in BV^+ and BV^- women a) AA, b) EA

a)

				BV^+ (p-values)		*BV^- (p-values)*	
Cytokine	*Gene*	*RS#*	*Model*	*race*	*intxn*	*race*	*intxn*
IL-8	IL-8RA	rs1008562	CC vs CG/GG	-	0.003	-	0.837
IL-8	IL-8RA	rs1008563	CC vs CT/TT	-	0.007	-	0.262
TNF-α	TNFR2	rs1201157	CC vs CT/TT	-	0.047	-	0.121
IL-1β	IL-1RAP	rs6765375	AA/AC vs CC	0.106	0.879	0.068	0.598
IL-1β	IL-1RAP	rs1469007	GG vs GT/TT	0.023	0.497	0.183	0.752
IL-1β	IL-1RAP	rs9821002	AA/AC vs CC	0.258	0.726	0.055	0.575

b)

				BV^+ (p-values)		*BV^- (p-values)*	
Cytokine	*Gene*	*RS#*	*Model*	*race*	*intxn*	*race*	*intxn*
IL-6	IL-6R	rs4075015	AA/AT vs TT	<0.001	0.003	0.746	0.170

* significant after correction for multiple testing

Discussion

It remains unclear as to why some women with BV experience adverse pregnancy outcomes, such as PTB, while others do not. Several studies have observed higher serum, amniotic fluid or vaginal concentrations of IL-1β, IL-6, IL-8 and TNF-α in women with infection or in those experiencing PTB (Romero et al., 1990; El-Bastawissi et al., 2000; Velez et al., 2007; Menon et al., 2008a; Ryckman et al., 2008c; Ryckman et al., 2008d). Additionally, several studies have observed that genetic polymorphisms in pro-inflammatory genes were associated with BV and PTB; however, few of these studies have assessed the impact of genetic variation in these pro-inflammatory genes or their receptors on vaginal cytokine levels (Macones et al., 2004; Engel et al., 2005; Wang et al., 2006). In this study we examined genetic polymorphisms in IL-1α, IL-1β, IL-6, IL-8 and TNF-α and their receptors for association with cervical cytokine concentrations and determined if BV status interacted with these polymorphisms to affect cytokine levels.

One study demonstrated that amniotic fluid levels of IL-1β were elevated in AA but not EA women who delivered prematurely (Menon et al, 2007). In our study, two intronic SNPs in IL-1AP were associated with higher cervical IL-1β concentrations in AA BV^+ women with a particular genotype compared to BV^- women with the same genotype. Binding of IL-1RAP is necessary for signal transduction of IL-1β. These SNPs were in moderate LD with one another ($D' = 0.73$, $r^2 = 0.18$) in our data. While EA and AA women differed significantly in allele and genotype frequencies at rs6765375, there were no race main effects or race/SNP interactions with IL-1β levels in BV^+ or BV^- women. This indicates that although IL-1RAP may be important in the regulation of

cervical IL-1β levels, these SNPs do not necessarily help to explain the ethnic disparity observed in BV.

Furthermore, in AA BV^+ women, two SNPs (rs1008563 and rs1008562) located in the 3'UTR of IL-8RA and one intronic SNP (rs1201157) in TNFR2 were associated with elevated IL-8 and TNF-α levels, respectively. In the Hapmap Yoruban (YRI) samples, rs1201157 was in strong LD ($D' = 1.0$, $r^2 = 0.09$) with a synonymous SNP at amino acid position 56, which was not genotyped in our population. While the allele and genotype frequencies of these SNPs did not differ significantly between EA and AA, there were significant race/SNP interactions in BV^+, but not BV^-, women. These results indicate that polymorphisms in receptor genes can be extremely important in the regulation of pro-inflammatory cervical cytokine concentrations and these associations were affected by both BV status and race.

Cervical and vaginal levels of IL-6 are not consistently higher in women with BV; however, elevated cervical and amniotic fluid levels of IL-6 are frequently observed in women with microbial invasion of the intra-amniotic cavity who have also delivered prematurely (Romero et al., 1990; El-Bastawissi et al., 2000). In addition, a SNP in IL-6 (rs1800797) was associated with elevated IL-6 concentrations in EA, but not AA, women who delivered prematurely. This effect was primarily driven by microbial invasion of the amniotic cavity (Velez et al., 2007). While we did not replicate this finding with cervical concentrations of IL-6, we did find that in BV^+ women the TT genotype at rs4075015 in IL-6R was associated with lower IL-6 concentrations compared to the AA/AT genotype and BV^- women with the TT genotype. While the allele and genotype frequencies of these SNPs did not differ significantly between EA and AA, there were significant

race/SNP interactions in BV^+, but not BV^-, women. These results indicate an important underlying mechanism in the genetic control of cervical IL-6 concentrations which may be race specific.

Studies in chapter III part B, which examined cervical cytokine correlations in AA and EA BV^+ and BV^- women, found that AA had a very strong correlated cytokine network in women with BV but not in those without the condition (Ryckman et al., 2008b). Our study supports this finding in showing that genetic associations with pro-inflammatory cytokine concentrations were more prominent in AA and race/SNP interactions were not present in BV^- women. It is important to note that all of the associations observed in this study were with receptor genes or co-factors and not with the cytokine genes themselves. This result emphasizes the importance of not only examining genes that encode cytokines but also those that encode for critical receptors or cofactors. This approach, while analytically complex, will provide greater insight into the biological processes involved in cytokine regulation and the mechanism of BV as well as PTB.

C. Genetic Association of Cervical Pro- and Anti-inflammatory Cytokines by Toll-like Receptors

Introduction

Previous studies, described in parts A and B of this chapter, demonstrated that genetic polymorphisms in receptor genes are associated with IL-6 and IL-10 levels in EA but not AA; while polymorphisms in receptor genes are associated with levels of IL-8 and TNF-α in AA but not EA (Simhan et al., 2008). Many of these effects were influenced by BV status. It is probable that other genes or receptors, not examined in the previous studies, are also associated with these cervical cytokine levels. Examining these genes may help to elucidate the differing mechanisms of cervical cytokine regulation observed between AA and EA.

TLRs are important components of the innate immune system and are essential in recognizing bacterial micro-organisms and initiating the appropriate immune response (Janssens et al., 2003; O'Connell et al., 2005). In particular, TLR2 and TLR4 have been characterized in the female reproductive tract and in decidual cells at the maternal-fetal interface (Fazeli et al., 2005; Canavan et al., 2007). Therefore, these genes may be important in the regulation of cervical immunity. However, little research has examined the genetic regulation of these cytokines in the cervical milieu.

Additionally, it is likely that TLRs are involved in the pathogenesis and response to BV. BV is characterized by a mixture of gram-positive and gram-negative bacteria; TLR2 recognizes gram-positive bacteria, while TLR4 recognizes gram-negative bacteria. Therefore, these genes are plausible candidates to study in relation to BV. Genetic

polymorphisms in TLR genes may affect downstream factors such as pro- and anti-inflammatory cytokines depending on the presence of BV. A previous study identified a variant in TLR4 associated with BV-related bacteria and increased concentrations of vaginal IL-1β. Identifying factors that may lead to an altered immune response to various bacteria is important for elucidating the risk for not only BV, but also for adverse pregnancy outcomes such as PTB.

To determine if cervical cytokines are associated with common variation in TLRs, five anti-inflammatory cytokines (IL-4, -10, IL-12(p40), IL-12(p70) and -13) and six pro-inflammatory cytokines (IL-1α, -1β, -6, -8, IP10 and TNF- α) in AA and EA women were examined for associations with polymorphisms in TLR2 and TLR4. Associations were also examined for the interaction between these polymorphisms and BV.

Material and Methods

Subjects

Cervical levels of IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, IL-12p70, IL-13 and IP10 were examined in this study. A detailed description of subject recruitment, socio-demographic characteristics, microbiologic assessment and cytokine measurement is in Chapter III, part A.

DNA genotyping

Four SNPs in TLR2 and thirteen SNPs in TLR4 were examined with all eleven cytokine concentrations for this analysis (Appendix Table 8). Details on TagSNP selection and genotyping are provided in part A of this chapter.

Quality control

A total of 4 SNPs in AA and 9 SNPs in EA were excluded from subsequent analyses due to low minor allele frequency such that one or more genotype/BV status groups contained fewer than 5 individuals, thus preventing the analysis of dominant, recessive and additive models. Therefore, 13 SNPs in AA and 8 SNPs in EA were included in the analysis. Levels of IL-1α, IL-1β, IL-6, IL-10 and IL-12p70 were normally distributed (Shapiro Wilk $p > 0.01$) after transformation with natural log and were analyzed with ANOVA. Levels of IP10, IL-12p40 and IL-13 were normally distributed after implementing the Box Cox transformation and were analyzed with ANOVA. IL-4, IL-8 and TNF-α were not normally distributed after transformation by natural log or Box Cox; therefore, the Kruskal-Wallis non-parametric test was used to evaluate these cytokines. Details on the statistical analyses are discussed in part A of this chapter.

Correction for multiple testing

A total of 433 tests were performed and results were adjusted for this number of tests with FDR (q=0.2). This number included tests for all evaluated genetic models, cytokine concentrations, both races and interaction and single SNP main effects.

Cytokine associations excluding women with C. albicans

To investigate if the presence of *C. albicans* affects these associations, the analyses were performed excluding individuals with *C. albicans*. There were negligible differences for the results that were statistically significant after correction for multiple testing (data not shown).

Results

Genetic associations with cytokine concentrations

After correction for multiple testing, one SNP in TLR4 (rs1554973) was associated with IL-1β concentrations ($p = 4x10^{-4}$) in EA women (Table 4-4, Appendix Table 9). Women with the TT genotype had significantly higher IL-1β concentrations than women with the CC/CT genotypes (medians 296.2 ng/mL vs 62.0 ng/mL). There was no significant interaction between BV status and genotype; however, the SNP association was stronger in BV^- women (Figure 4-6). For example, BV^- women with the TT genotype had significantly higher concentrations of IL-1β than those with the CC/CT genotype (medians 238.7 ng/mL vs 52.8 ng/mL, $p = 0.003$), while cervical IL-1β concentrations did not differ by genotype in BV^+ women. There were no significant SNP associations after correction for multiple testing in AA women.

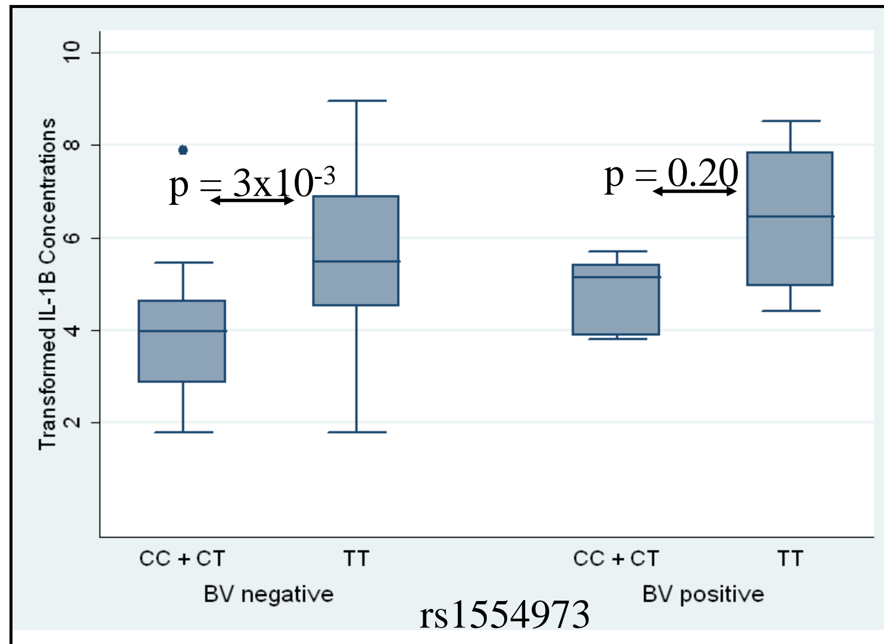


Figure 4-6. Transformed concentration of IL-1β by rs1554973 (TLR4) genotype in EA

Although not significant after initial correction for multiple testing with FDR, rs1927911 and rs2149356, both in TLR4, were the only polymorphisms associated with five or more cytokine levels in EA women (Table 4-4). These SNPs were in strong LD with one another (D′ = 1.0, $r^2 = 0.82$) as well as with rs1554973 (D′ > 0.78, $r^2 > 0.46$). At rs1927911, BV^- women with the CT/TT genotype had significantly lower cervical concentrations of IL-1β and IL-6 than those with the CC genotype (IL-1β: medians 73.6 ng/mL vs 215.0 ng/mL; IL-6: medians 1190.9 ng/mL vs 4120.0 ng/mL) (Figure 4-7a and b). In BV^+ women with the CT/TT genotypes, TNF-α concentration was significantly higher than in women with the CC genotype (medians 5.0 ng/mL vs 28.5 ng/mL) (Figure 4-7c). At rs2149356, BV^- women with the AA/AC genotypes had significantly lower cervical concentrations of IL-6 than those with the CC genotype (medians 1095.0 ng/ml vs 4778.0 ng/mL). In BV^+ women with the AA/AC genotypes, TNF-α concentration was

significantly higher than in women with the CC genotype (medians 5.0 ng/mL vs 43.0 ng/mL). There were no SNPs in AA women significantly ($p < 5x10^{-3}$) associated with any single cytokines or more than two cervical cytokine concentrations (Appendix Table 9).

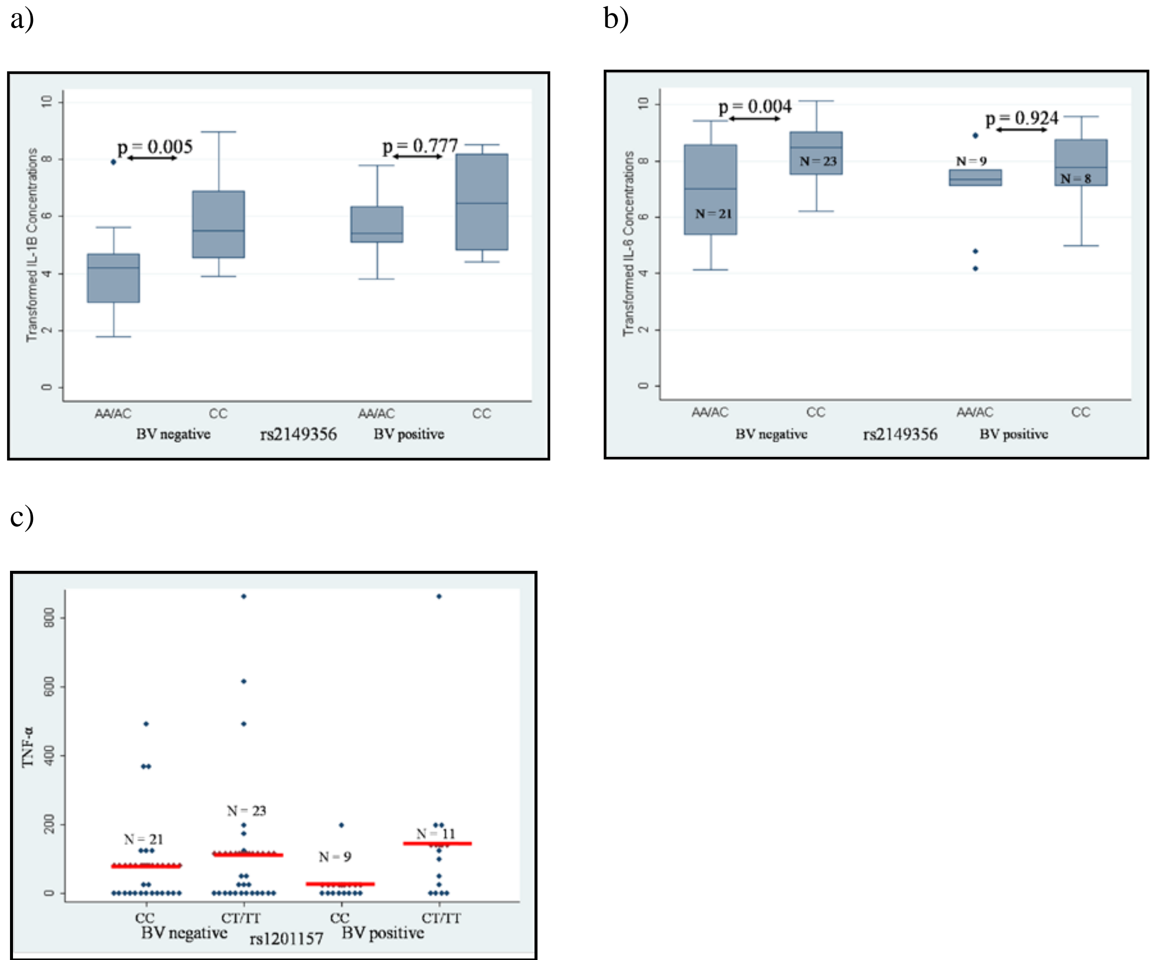


Figure 4-7. Genetic associations of rs2149356 (TLR4) genotype with multiple cervical cytokines in EA a) IL-1β, b) IL-6, c) TNF-α

Table 4-4. Significant associations between SNPs in TLR4 and cervical cytokine concentrations in EA

RS#	*Cytokine*	*Model (Genotype 1 vs 2)*	*nominal p-values for ANOVA model* Model	*SNP*	*BV*	*Intxn*	[1]*Genotype 1 vs 2* BV^+	BV^-	[2]BV^+ *vs* BV^- Genotype 1	*Genotype 2*
rs1554973	IL-1β	CC/CT vs TT	$2x10^{-4}$*	$4x10^{-4}$*	0.090	0.940	0.198	0.003	0.874	0.557
rs1927911	IL-1β	CC vs CT/TT	0.006	0.013	0.028	0.664	0.742	0.056	0.649	0.420
	IL-6	CC vs CT/TT	0.023	0.018	0.648	0.470	0.897	0.026	0.944	1.000
	IL-12p40	CC vs CT/TT	0.205	0.045	0.770	0.752	0.663	0.515	0.997	1.000
	IL-12p70	CC vs CT/TT	0.117	0.016	0.781	0.297	0.213	0.743	0.992	0.947
	TNF-α[3]	CC vs CT/TT	-	-	-	0.048	0.019	0.192	0.634	0.099
rs2149356	IL-1β	AA/AC vs CC	$8x10^{-4}$	0.004	0.027	0.352	0.777	0.005	0.180	0.910
	IL-6	AA/AC vs CC	0.005	0.009	0.559	0.280	0.924	0.004	1.000	0.818
	IL-10	AA/AC vs CC	0.113	0.033	0.269	0.133	0.164	0.992	0.386	1.000
	IL-12p40	AA/AC vs CC	0.193	0.040	0.762	0.690	0.600	0.538	1.000	0.996
	IL-12p70	AA/AC vs CC	0.071	0.009	0.848	0.316	0.164	0.551	0.958	0.992
	TNF-α[3]	AA/AC vs CC	-	-	-	0.038	0.008	0.545	0.038	0.315

[1]p-values for pairwise comparisons between the two genotype groups in BV^+ and BV^- women
[2]p-values for pairwise comparisons between BV^+ and BV^- women in each genotype group
[3]indicates Kruskal-Wallis test was performed, therefore only the interaction p-value is calculated
* significant after correction for multiple testing using FDR (q = 0.2)

Ethnic heterogeneity and quality control

The SNPs analyzed show large differences in their ethnic distribution (Appendix Table 8). All of the SNPs in TLR2 and TLR4 had significant differences in either allele or genotype frequencies between ethnic groups. Of these, 3 SNPs in TLR2 and 8 SNPs in TLR4 had significant allelic or genotypic ($p < 0.001$) differences between the two groups. There were 2 SNPs that deviated from HWE in EA, neither of which had $p < 0.01$. In AA women there were no SNPs that deviated from HWE at $p < 0.05$.

To determine if significant associations with cytokine concentration were affected by race, race main effects and race/SNP interactions were examined with each cytokine in BV^+ women and BV^- women, separately. At rs1469007 there was a significant race main effect and race/SNP interaction with IL-1β concentrations in BV^- women ($p = 0.04$ and $p = 0.003$, respectively); however, these effects were not significant in BV^+ women. Additionally, in BV^- women, for both rs1927911 and rs2149356, there were significant race main effects ($p = 0.03$ and $p = 0.009$, respectively) and race/SNP interactions ($p = 0.01$ and $p = 0.002$, respectively) with cervical IL-1β concentration, whereas in BV^+ women, these associations were not significant. Also in BV^- women, there were significant race effects with IL-6 ($p = 0.047$) and significant interaction effects with TNF-α ($p = 0.025$) at rs2149358; these effects were not present in BV^+ women. However, these associations were only marginally significant for rs1927911 ($p = 0.078$ for IL-6 and 0.058 for TNF-α).

Discussion

Previous studies in this chapter identified variants in IL-1RAP, IL-8RA and TNFR2 associated with cervical pro-inflammatory IL-1β, IL-8 and TNF-α concentrations in AA but not EA. In EA, SNPs in IL10RA were associated with cervical anti-inflammatory IL-10 concentrations (Simhan et al., 2008). Additionally, in EA, a SNP in IL-6R was associated with IL-6 concentrations, which is classically defined as a pro-inflammatory cytokine but exhibits many anti-inflammatory properties. This suggests that, particularly in EA, associations with pro-inflammatory cytokines involve genes not previously examined. We therefore examined genetic polymorphisms in TLR2 and TLR4 for associations with both cervical pro- and anti-inflammatory cytokines and the associations were also examined in the context of BV and race.

Previously, a polymorphism in TLR4 (rs4986790) was found to be associated with an increase in vaginal pH, *G. vaginalis*, anaerobic gram-negative rods, *Prevotella*, *Bacteroides* and *Porphyromonas* (Genc et al., 2004). Additionally, women with the AA genotype at rs4986790 and colonization of *G. vaginalis* and/or gram-negative rods had significantly higher vaginal IL-1β concentrations than women with the AG/GG genotype (Genc et al., 2004). While this SNP was not genotyped in our data, in the Caucasian samples from the Hapmap it is in strong LD with rs1554973 ($D' = 1.0$, $r^2 = 0.13$), which is located in the 3'UTR of TLR4 and significantly associates with cervical IL-1β concentrations in BV^- women. A similar trend in BV^+ women, albeit not significant after correction for multiple testing, was observed where the TT genotype was associated with higher cervical IL-1β concentrations than the AG/GG genotypes. This suggests that either

one of these SNPs is the causative variant associated with cervical IL-1β concentrations or these SNPs are in LD with the causative variant.

Additionally, we found several SNPs in TLR4 that were associated with multiple pro-inflammatory cytokine concentrations in EA but not AA. This indicates that in EA, TLR4 may be a key master regulator for cervical pro-inflammatory cytokine production. TLR4 generally favors the production of pro-inflammatory cytokines, and polymorphisms in this gene have been associated with several pro-inflammatory cytokine levels, including vaginal IL-1β.

We have demonstrated that TLR4 is an important regulator of cervical pro-inflammatory concentrations and that this regulation differs not only by BV status but also between AA and EA. We have identified factors that may predispose EA women to a cervical milieu characterized by an increased concentration of several pro-inflammatory cytokines. Additionally, we have identified associations in TLR genes that give greater insight into the biological processes involved in cytokine regulation, the mechanism of BV, and possibly racial disparity in clinical phenotypes such as PTB.

BV is a complex disorder and there are likely many genetic factors involved in cervical immunity. Studies in this chapter were focused on candidate genes and therefore limited in the ability to detect a wide range of factors that may previously have not been examined in association with cervical immunity and BV. Additionally, our studies consisted of small sample sizes and had limited power to detect small effect sizes as well as examine gene-gene interactions. However, few studies have examined genetic variants in relation to BV and these studies lend insight to some of the biological underpinnings involved in not only disease susceptibility but also cervical immunity.

CHAPTER V

GENETIC ANALYSIS OF PRETERM BIRTH

Overview parts A and B

PTB (gestational age <37 weeks) is a major public health problem in modern perinatal medicine and accounts for 75% of perinatal mortality and 50% of perinatal morbidity. PTB is a common condition and in some parts of the world, e.g. the US, more than 10% of pregnancies end preterm. PTB is related to long term respiratory problems and neurological morbidity. The majority of PTB (~75%) is not indicated by the presence of pregnancy complications such as preeclampsia or IUGR. sPTB can result from spontaneous contractions caused by infection, PPROM and unknown causes (Morken et al 2005). Based on human and animal studies, four main pathways have been hypothesized to lead to PTB: 1) activation of maternal or fetal hypothalamic pituitary-adrenal axis, 2) inflammation/infection, 3) decidual hemorrhage, and 4) uterine distension (Lockwood and Kuczynski, 2001). These pathways converge on a final terminal pathway where uterotonins, proteases and prostaglandins are released, leading to contractions, rupture of the membranes and eventually PTB. The individual factors involved in these putative PTB pathways and how they affect pregnancy length and outcome are poorly understood.

Chapter V examines maternal and fetal genetic associations with PTB in a Norwegian cohort. In part A, single locus associations with PTB are examined in an extensive candidate gene study encompassing approximately 160 genes involved in the

proposed PTB pathways. These studies indicate that the strongest single locus maternal and fetal associations are involved in the following pathways: extracellular matrix strength (COL1A2), inflammation and infection (IL1RAP and IL4R), complement and coagulation (TFPI), homocysteine metabolism (MTHFR), activation of the HPA axis (CRH) and serotonin transport (SLC6A4). In fetal samples, the most significant single locus association is an additive model with a SNP in the tissue factor pathway inhibitor (TFPI) gene (OR = 2.46, 95% CI = 1.57-3.86, $p = 8x10^{-5}$). The most significant single locus association in maternal samples is a SNP in COL1A2, where the AG/GG genotypes are protective against PTB when compared to the AA genotype (OR = 0.31, 95% CI = 0.15-0.64, p = 0.001).

Part B compares the associations found in a previous study by Velez *et al.*, 2008 to the results from Norway to identify associations that demonstrate significance in both studies and to estimate effect sizes. The data are analyzed together to increase sample size and to quantify the effects of SNPs that were significant in both studies. The majority of significant maternal and fetal associations in the pooled sample are involved in ECM degradation (COL1A2), prostaglandin synthesis (PTGER3) and complement/coagulation (PLAT and PON1). The most significant result in maternal pooled samples is a SNP in the prostaglandin E receptor 3 gene (PTGER3) (genotypic $p = 2x10^{-4}$). The most significant result in fetal pooled samples is in the paraoxonase 1 gene (PON1) (genotypic $p = 8x10^{-4}$). These studies identify single locus polymorphisms associated with PTB in two independent EA populations.

A. Maternal and Fetal Single Locus Associations with PTB

Introduction

PTB, defined as gestation less than 37 weeks, is one of the largest unsolved obstetrical problems worldwide. For example, in the US alone, more than 12% of the approximately five million births each year are preterm, accounting for more than $26 billion per year in excess health care costs (Martin et al., 2005; Anonymous, 2006). Preterm-related conditions are responsible for as much as 75% of perinatal mortality and a disproportionately large percentage of infant morbidities (McCormick, 1985; Hack and Fanaroff, 1999; Raju et al., 2006; MacDorman et al., 2008). The majority of PTB (~75%) results from spontaneous contractions caused by infection, PPROM and unknown causes (Morken et al., 2005). Based on human and animal studies, four main pathways have been hypothesized to lead to PTB: 1) activation of maternal or fetal hypothalamic pituitary-adrenal axis, 2) inflammation/infection, 3) decidual hemorrhage, and 4) uterine distension (Lockwood and Kuczynski, 2001). These pathways converge on a final terminal pathway where uterotonins, proteases and prostaglandins are released, leading to contractions, rupture of the membranes and eventually PTB. The individual factors involved in these putative PTB pathways and how they affect pregnancy length and outcome are poorly understood.

Among the types of risk factors that have been hypothesized to lead to PTB, there is increasing evidence that PTB has a significant genetic component. First, family history of PTB and previous PTB are among the largest known risk factors for PTB (Bakketeig et al., 1979; Carr-Hill and Hall, 1985; Porter et al., 1997; Esplin et al., 2008). Second,

twin studies estimate the heritability of PTB at 20-40% (Treloar et al., 2000; Clausson et al., 2000). In addition, there is substantial evidence to suggest geographic ancestry is associated with risk for PTB; therefore, it is presumable that genetic differences affect risk (Menon et al., 2006d; Menon et al., 2007; Menon et al., 2008a). Finally, in the last ten years many studies have found significant genetic associations with PTB, particularly with genes in the inflammation/infection pathway (Varner and Esplin, 2005; Menon et al., 2006a; Pennell et al., 2007). However, often these studies fail to replicate (Menon et al., 2006b). Therefore, to demonstrate true genetic effects, multiple studies of carefully defined cohorts are required. Due to the large number of false positive results that are generated in association studies, true associations and findings of biological significance will only be identified when well-defined studies that are consistently replicated in independent populations are performed. To elucidate the genetic etiology of PTB, approximately 160 candidate genes were investigated for single locus and haplotypic associations with PTB.

Materials and Methods

Subjects

The Norwegian Mother and Child Cohort (MoBa) is a Norwegian national pregnancy study that provides opportunities to explore the causes of complex phenomena. The main objective of this cohort is to develop a long term resource to test hypotheses relating to pregnancy outcomes and childhood health. Epidemiological data and biological material were collected from 100,000 pregnancies during the period of

1999-2008 by the Norwegian Institute of Public Health. The target population consisted of all pregnant women in Norway. Women were invited to participate during their routine ultrasound scan at gestational week 17-18. All hospitals and maternity units with more than 100 deliveries annually were invited to participate, and 50 of 52 such units participated. The total participation rate was 42.7% (Magnus et al., 2006). The MoBa study collected data from three questionnaires focused on nutrition, overall health status, and child outcome. In addition to the questionnaire variables, data were added from the Medical Birth Registry of Norway (MBRN). The MoBa study has been described in detail elsewhere (Magnus et al., 2006). The present study used samples derived from a subset of the MoBa cohort available at its outset (53,711 pregnancies).

Data from the MBRN on gestational age, type of delivery onset, intrauterine fetal death (IUFD), congenital malformations and plurality were used in this study. Gestational age was determined based on expected date of parturition according to ultrasound and/or the date of last menstrual period. Ultrasound was the main method of determining gestational age as most pregnancies in Norway are dated by ultrasound. Type of delivery onset was recorded as spontaneous labor, induced labor, or prelabor caesarean section. The two latter groups were regarded as iatrogenic preterm deliveries and were excluded from our study. IUFD and plurality were also registered in the MBRN, making it possible to identify and exclude these cases. Congenital malformations, registered as ICD-10 codes in the MBRN were excluded. These definitions have been previously described (Morken et al., 2005).

The cases in this study were defined as singletons with spontaneous onset of PTB between 22 gestational weeks and 0 out of 7 days ($22^{0/7}$) and $37^{0/7}$ gestational weeks in

women aged 20 – 35 years. This age range was chosen to ensure that women at high risk for PTB due to age (young women less than 20 years and older women greater than 35 years) were not included in the study. The exclusion criteria were pre-existing medical conditions such as diabetes, hypertension, autoimmune diseases, inflammatory bowel diseases, systemic lupus erythematosus, rheumatoid arthritis, scleroderma or any immune-compromised condition. Women were also excluded if they had pregnancy complications such as preeclampsia, hypertension, diabetes, small for gestational age (according to intrauterine growth curves), abruption of the placenta, placenta previa, cervical cerclage or fetal malformations. The controls were selected according to the same criteria as above except for spontaneous onset of term delivery with gestational age between $39^{0/7}$ and $40^{6/7}$ weeks. The Ethics Committee of the southern healthcare region of Norway approved the MoBa study. There were 214 case mother-baby-father trios that will be further examined in an independent family analysis and 220 control mother-baby pairs selected for this study.

DNA genotyping and quality control

A total of 1,536 tag SNPs were selected from 167 PTB candidate genes (Appendix Table 10). SNPs were selected based on their ability to tag surrounding variants in the Caucasian (CEPH) and Yoruban (YRI) populations of the HapMap database (http://www.hapmap.org). A minor allele frequency of 0.07 in CEPH and 0.20 in YRI and an r^2 of 0.80 was used to determine tagSNPs. Genotyping was performed on the Illumina GoldenGate Assay system. 1,430 SNPs had genotyping efficiency greater than 95% and were used for subsequent analyses.

Demographic and clinical characteristics

Both maternal and fetal samples were excluded at onset if the last menstrual period and birthweight were not consistent with case-control status. Additionally, families (mother-baby-father case trios and mother-baby controls) were excluded if greater than 0.01% of the SNPs had Mendelian errors. This was done to ensure high quality genotyping efficiency. Additionally, individuals were excluded for low genotyping efficiency (<95%). The final sample size included 419 fetal samples (203 cases, 216 controls) and 424 maternal samples (207 cases, 217 controls).

Clinical differences between cases and controls are presented in Table 5-1. There were no statistically significant differences between cases and controls for APGAR score at 1 minute or 5 minutes, maternal age or smoking; however, as expected, cases had babies with lower birthweight and gestational age (p <0.001 for both). Additionally, cases and controls significantly differed in parity (p <0.001).

Table 5-1. Clinical characteristics

Variable	[1]Cases (n=207)	Controls (n=217)	[2]p
Parity	0 [0-4]	1 [0-4]	<0.001
Gestational Age (days)	253 [182-258]	280 [273-286]	<0.001
Birthweight (grams)	2810 [950-4000]	3650 [2610-4970]	<0.001
APGAR at 1 minute (% <7)	16 (8%)	12 (6%)	0.355
APGAR at 5 minutes (% <7)	3 (1%)	5 (2%)	0.522
Maternal Age (yrs)	29 [20-34]	30 [21-34]	0.203
Smoking (%)	22 (12%)	21 (11%)	0.770

Medians are reported with the range in brackets
[1]Cases are defined as < 37 weeks gestation compared with the women delivering at term between 39-40 weeks (controls).
[2]P-values are calculated by Mann Whitney U- test for continuous variables and chi-square test or Fisher's exact test for dichotomous variables.

Statistical analysis

Chi-square tests were used to examine differences between cases and controls for APGAR at 1 minute (<7 versus ≥7), APGAR at 5 minutes (<7 versus ≥7) and smoking (non-smokers versus smokers). Parity (number of times a woman has given birth defined by birth registry), gestational age (days), birthweight (grams) and maternal age were tested for normality using Shapiro-Wilks tests. None of these variables were normally distributed therefore, these variables were analyzed with nonparametric Mann-Whitney two-sample ranksum tests. Stata version 10 statistical software was used for all analyses.

Gene names, marker positions (base pair – bp) and marker function were identified using the SNPper database (http://snpper.chip.org). Allele and genotype differences between cases and controls, deviations in HWE and inbreeding coefficients were calculated with PLINK (http://pngu.mgh.harvard.edu/purcell/plink/), the Whole-genome Association Study Pipeline (WASP) and Powermarker software programs (http://chgr.mc.vanderbilt.edu/wasp/) (Liu and Muse, 2005; Purcell et al., 2007). Statistical significance for these analyses was determined using chi-square tests or Fisher's exact tests (if there were less than five individuals for any allele or genotype). These results were corroborated using a prevalence based association test (PRAT) (Ryckman et al., 2008a). This test calculates a global allele frequency based on the prevalence of the disease in order to calculate expected frequencies of each genotype in cases and controls separately, and a permutation test is used to determine the significance. The global allele frequency was determined using a prevalence of 5%. Using prevalences of 10% and 20% yielded similar results (data not shown). FDR (q = 0.2) was used to correct for multiple testing in maternal and fetal samples, separately.

Significant ($p < 5x10^{-3}$) allelic or genotypic associations were followed up with additive, dominant and recessive models, using logistic regression. This cutoff, although arbitrary, was used to reduce Type I error but still maintain a large enough set of findings for follow-up analyses, hence also reducing Type II error. Significant ($p < 0.05$) logistic regression models were adjusted for parity because this was the only risk factor that was significantly different between cases and controls. These analyses were performed with Stata version 10. Haplotype analysis using a 2, 3 or 4 marker sliding window was performed in genes with significant ($p < 5x10^{-3}$) allelic or genotypic associations using Unphased (Dudbridge, 2008). This software uses a quasi-Newton algorithm to maximize the likelihood of each haplotype when an individual's phase is unknown. The overall test of association is a likelihood ratio test based on the null hypothesis that all of the haplotypes have equal odds ratios. Graphical representation of significant results and haplotypes was performed with Haploview (Barrett et al., 2005).

Results

Maternal single locus association

There were 125 significant allelic and/or genotypic single locus results out of 1430 SNPs analyzed. Eleven SNPs from ten different genes were significantly associated with PTB at allelic or genotypic $p < 5x10^{-3}$ (Figure 5-1, Table 5-2 and Appendix Table 11). None of these results were significant after correction for multiple testing with FDR. Of the top 11 SNPs, 5 deviated from HWE, 2 of which were in cases (PGRMC2 rs11726595, $p = 0.004$; TFPI rs3213739, $p = 0.01$) and 3 in controls (CBS rs6586282, p =

0.008; F7 rs1475931, p = 0.002; PON1 rs854569, p = $1.7x10^{-5}$). For all 5 SNPs, the inbreeding coefficients in cases and controls were in opposite directions, indicating that deviations were not likely caused by genotyping error, since cases and controls were randomly placed on each plate for genotyping.

These associations were corroborated using the method PRAT (Table 5-2). All of the PRAT results were significant in cases but not controls or controls but not cases with the exception of COL1A2 rs2472 which was significant in both cases and controls. As demonstrated previously (Ryckman *et al.* 2008a), significance in either cases or controls or both, indicates an association with PTB.

Table 5-2. Maternal single locus association results a) allelic and genotypic association b) logistic regression adjusting for parity

a)

Gene	Gene Name	SNP rs#	Role	Allele	Case Freq.	Control Freq.	Case v Control p: Allele	Case v Control p: Genotype	PRAT p: Case	PRAT p: Control
CBS	Cystathionine beta-synthase	rs6586282	Intron	A	0.18	0.15[2]	0.22	4.7×10^{-3}	0.19	1×10^{-3}
COL1A2	Collagen Type 1, alpha-2	rs2472	Intron	G	0.03	0.07	1.6×10^{-3}	1.2×10^{-3}	0.01	$<1\times10^{-3}$
CRHR2	Corticotropin-releasing hormone receptor 2	rs4722999	Intron	G	0.35	0.44	5.0×10^{-3}	0.02	6×10^{-3}	0.67
F7	Coagulation factor 7	rs1475931	Intron	A	0.20	0.22[2]	0.46	2.3×10^{-3}	0.37	$<1\times10^{-3}$
IL1RAP	Interleukin 1 receptor accessory protein	rs7628333	Intron	A	0.22	0.32	1.8×10^{-3}	4.7×10^{-3}	4×10^{-3}	0.16
		rs3821744	Intron	A	0.28	0.37	4.7×10^{-3}	0.01	0.01	0.49
IL1RN	Interleukin 1 receptor antagonist	rs315920	Promoter	A	0.15	0.23	4.9×10^{-3}	0.01	0.01	0.47
PGRMC2	Progesterone receptor membrane component 2	rs11726595	Downstream	G	0.23[1]	0.19	0.11	4.7×10^{-3}	4×10^{-3}	0.51
PON1	Paraoxonase 1	rs854569	Intron	A	0.17	0.23[2]	0.04	4.4×10^{-3}	0.69	1×10^{-3}
TFPI	Tissue factor pathway inhibitor	rs3213739	Intron	A	0.38[1]	0.46	0.03	2.4×10^{-3}	8×10^{-3}	0.11
TREM1	Triggering receptor expressed on myeloid cells 1	rs4711668	Intron	A	0.36	0.27	2.9×10^{-3}	9.0×10^{-3}	7×10^{-3}	0.24

[1]cases deviated from HWE at rs11726595 (p = 0.004) and rs3213739 (p = 0.01)

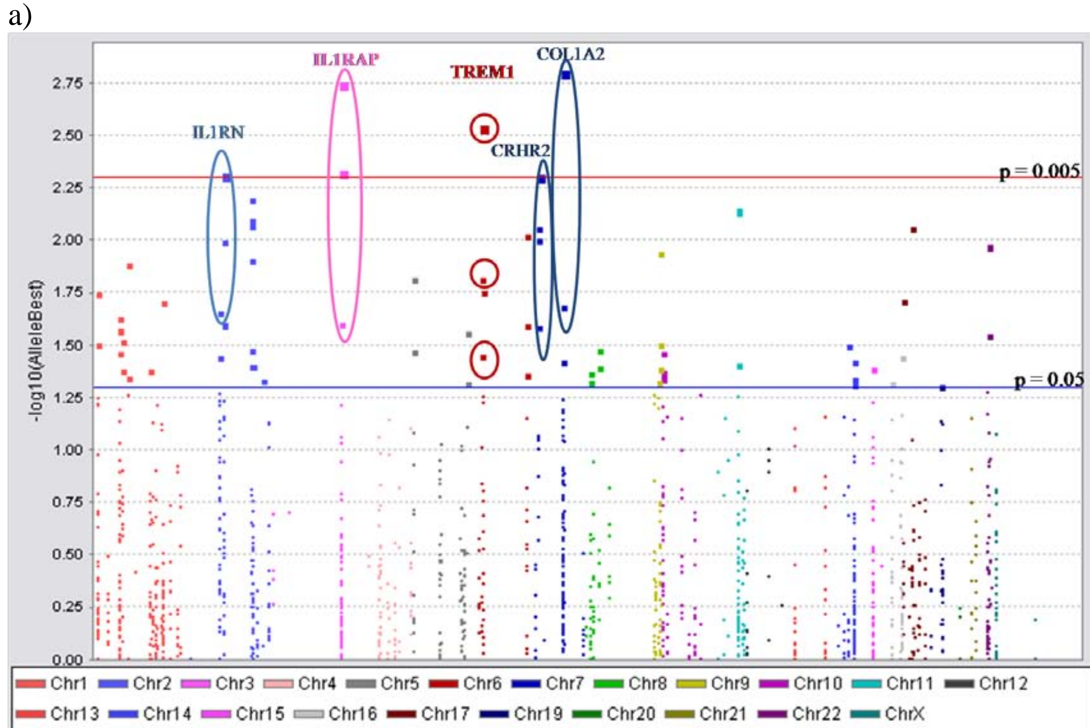
[2]controls deviated from HWE at rs6586282 (p = 0.008), rs1475931 (p = 0.002) and rs854569 (p = 1.7×10^{-5})

b)

Gene	SNP rs#	Model	OR	95% CI	Model p	Adjusted Model p[1]
CBS	rs6586282	Additive	0.79	0.54-1.14	0.209	0.215
COL1A2	rs2472	AAvsAG/GG	0.32	0.16-0.66	0.002	0.002
CRHR2	rs4722999	Additive	0.67	0.51-0.89	0.006	0.007
F7	rs1475931	AAvsAC/CC	0.23	0.06-0.82	0.023	0.008
IL1RAP	rs7628333	AA/AGvsGG	1.88	1.28-2.77	0.001	0.003
	rs3821744	Additive	1.58	1.16-2.14	0.004	0.006
IL1RN	rs315920	AA/AGvsGG	1.83	1.22-2.75	0.004	0.004
PGRMC2	rs11726595	AAvsAG/GG	1.64	1.11-2.44	0.013	0.016
PON1	rs854569	AA/ACvsCC	1.68	1.13-2.50	0.010	0.008
TFPI	rs3213739	AA/ACvsCC	1.94	1.29-2.92	0.001	0.001
TREM1	rs4711668	AA/AGvsGG	0.55	0.37-0.81	0.002	0.002

[1] Models adjusted for parity

a)



b)

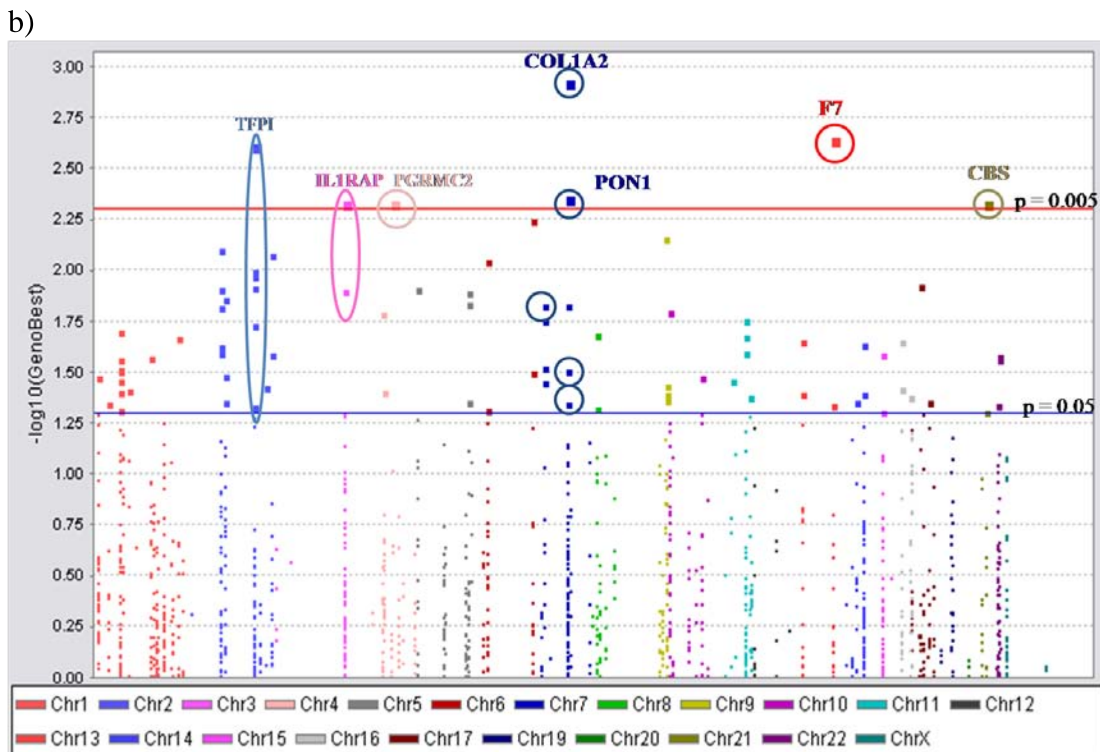


Figure 5-1. Maternal single locus association results. Each point on the graph represents an association test either allelic (a) or genotypic (b). The x axis is SNP position in chromosomal order and the y axis is the inverse negative log of the p-value. Significant associations are highlighted in genes with at least one association at $p < 0.005$.

Fetal single locus association

There were 142 significant allelic and/or genotypic single locus results out of 1,430 SNPs. Eighteen SNPs from ten different genes were significantly associated with PTB at allelic or genotypic $p < 5x10^{-3}$ (Figure 5-2, Table 5-3 and Appendix Table 11). Only rs6434222 in TFPI was significant after correction for multiple testing with FDR. Of the top 18 SNPs, 3 deviated from HWE, one of which deviated in cases (HSD17B7 rs4656381, p = 0.01) and two in controls (C6orf48 rs2471980, p = 0.03; HSD11B1 rs3753519, p = 0.01). For the SNPs that deviated from HWE the inbreeding coefficients in cases and controls were in opposite directions; this is similar to what was observed in the maternal data, indicating that deviations are not likely caused by genotyping error.

These results were further corroborated using PRAT (Table 5-3a). All of the PRAT results were significant in cases but not controls or controls but not cases with the exception of G8 protein (C6orf48) rs2471980, COL1A2 rs420257, CRH rs6472257 and 11-beta-hydroxysteroid dehydrogenase, type 1 (HSD11B1) rs3753519, which were significant in both cases and controls, indicating association (Ryckman et al 2008).

Table 5-3. Fetal single locus association results a) allelic and genotypic association b) logistic regression adjusting for parity

a)

Gene	*Gene Name*	*SNP rs#*	*Role*	*Allele*	*Case Freq.*	*Control Freq.*	*Case vs Control p*		*PRAT p*	
							Allele	*Genotype*	*Case*	*Control*
C6orf48	c6 open reading frame 48	rs2471980	Promoter	G	0.28	0.36[2]	0.02	$3.8x10^{-3}$	0.03	0.02
COL1A2	Collagen, type 1 alpha-2	rs420257	Intron	G	0.24	0.34	$1.4x10^{-3}$	$9.6x10^{-4}$	$1x10^{-3}$	0.03
		rs441051	Intron	A	0.17	0.25	$2.8x10^{-3}$	0.01	$5x10^{-3}$	0.54
CRH	Corticotropin-releasing hormone	rs6472257	Promoter	A	0.09	0.14	0.02	$4.2x10^{-3}$	0.03	0.02
HSD11B1	11-beta-hydroxysteroid dehydrogenase, type 1	rs3753519	Intron	A	0.09	0.15[2]	0.01	$1.5x10^{-3}$	0.03	$<1x10^{-3}$
HSD17B7	17-Beta-hydroxysteroid dehydrogenase 7	rs4656381	Intron	A	0.35[1]	0.28	0.03	$2.9x10^{-3}$	$1x10^{-3}$	0.09
IL4R	Interleukin 4 receptor	rs2239347	Intron	C	0.52	0.41	$1.4x10^{-3}$	$3.8x10^{-3}$	$1x10^{-3}$	0.69
MTHFR	Methylenetetrahydrofolate reductase	rs1994798	Intron	G	0.37	0.47	$3.9x10^{-3}$	0.01	$4x10^{-3}$	0.48
		rs9651118	Intron	G	0.31	0.21	$2.5x10^{-3}$	$8.7x10^{-3}$	$2x10^{-3}$	0.39
PON1	Paraoxonase 1	rs854548	Downstream	A	0.20	0.29	$2.5x10^{-3}$	$9.6x10^{-3}$	0.01	0.77
		rs854552	3′ UTR	G	0.21	0.30	$1.8x10^{-3}$	$6.0x10^{-3}$	$3x10^{-3}$	0.62
SLC6A4	Solute carrier family 6	rs4251417	Intron	A	0.07	0.14	$8.0x10^{-4}$	$2.7x10^{-3}$	0.01	0.76
TFPI	Tissue factor pathway inhibitor	rs12693471	Downstream	G	0.26	0.36	$4.0x10^{-3}$	0.01	$1x10^{-3}$	0.67
		rs3213739	Intron	A	0.38	0.48	$4.4x10^{-3}$	0.01	$2x10^{-3}$	0.68
		rs6434222	Intron	T	0.17	0.08	$*5.8x10^{-5}$	$*1.0x10^{-4}$	$<1x10^{-3}$	0.57
		rs7586970	Intron	G	0.26	0.36	$3.7x10^{-3}$	0.01	$1x10^{-3}$	0.63
		rs8176508	Intron	A	0.41	0.32	$3.7x10^{-3}$	0.01	$2x10^{-3}$	0.34
		rs8176541	Intron	A	0.26	0.36	$4.3x10^{-3}$	0.01	$1x10^{-3}$	0.65

[1]cases deviated from HWE at rs4656381 (p = 0.01)

[2]controls deviated from HWE at rs2471980 (p = 0.03) and rs3753519 (p = 0.01)

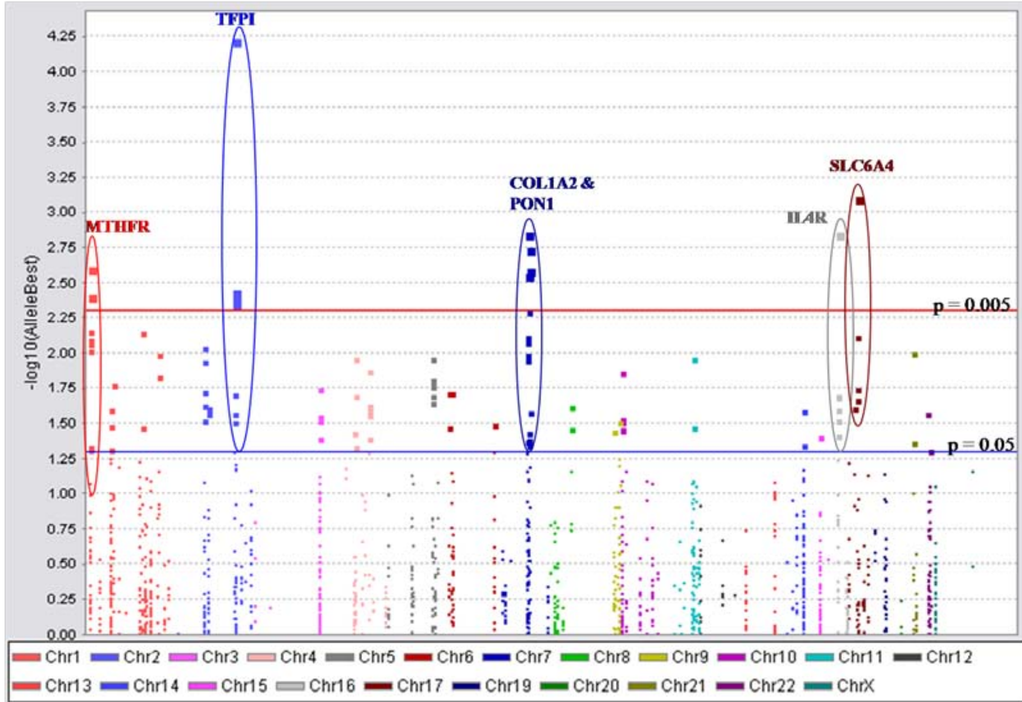
*significant after correction for multiple testing using FDR (q = 0.20)

b)

Gene	*SNP rs#*	*Model*	*OR*	*95% CI*	*Model p*	*Adjusted Model p*[1]
C6orf48	rs2471980	CC/CGvsGG	0.32	0.16-0.65	0.001	0.002
COL1A2	rs420257	AAvsAG/GG	0.48	0.32-0.71	$2.24x10^{-4}$	$1.65x10^{-4}$
	rs441051	Additive	1.68	1.19-2.37	0.003	0.004
CRH	rs6472257	AA/AGvsGG	1.91	1.19-3.07	0.007	0.003
HSD11B1	rs3753519	AA/AGvsGG	1.98	1.24-3.15	0.004	0.004
HSD17B7	rs4656381	AAvsAG/GG	0.33	0.17-0.64	0.001	0.003
IL4R	rs2239347	Additive	1.58	1.19-2.09	0.001	0.003
MTHFR	rs1994798	Additive	0.67	0.50-0.88	0.004	0.003
	rs9651118	Additive	1.65	1.20-2.29	0.002	0.002
PON1	rs854548	AA/AGvsGG	1.83	1.24-2.71	0.003	0.002
	rs854552	AAvsAG/GG	0.53	0.36-0.79	0.002	$8.77x10^{-4}$
SLC6A4	rs4251417	Additive	2.16	1.35-3.44	0.001	0.001
TFPI	rs12693471	Additive	0.63	0.47-0.86	0.004	0.004
	rs3213739	Additive	1.54	1.15-2.05	0.003	0.004
	rs6434222	Additive	2.49	1.59-3.91	$7.02x10^{-5}$	$7.72x10^{-5}$
	rs7586970	Additive	0.63	0.46-0.86	0.003	0.003
	rs8176508	AA/ATvsTT	0.55	0.37-0.82	0.003	0.003
	rs8176541	Additive	1.57	1.16-2.14	0.004	0.004

[1] Models adjusted for parity

a)



b)

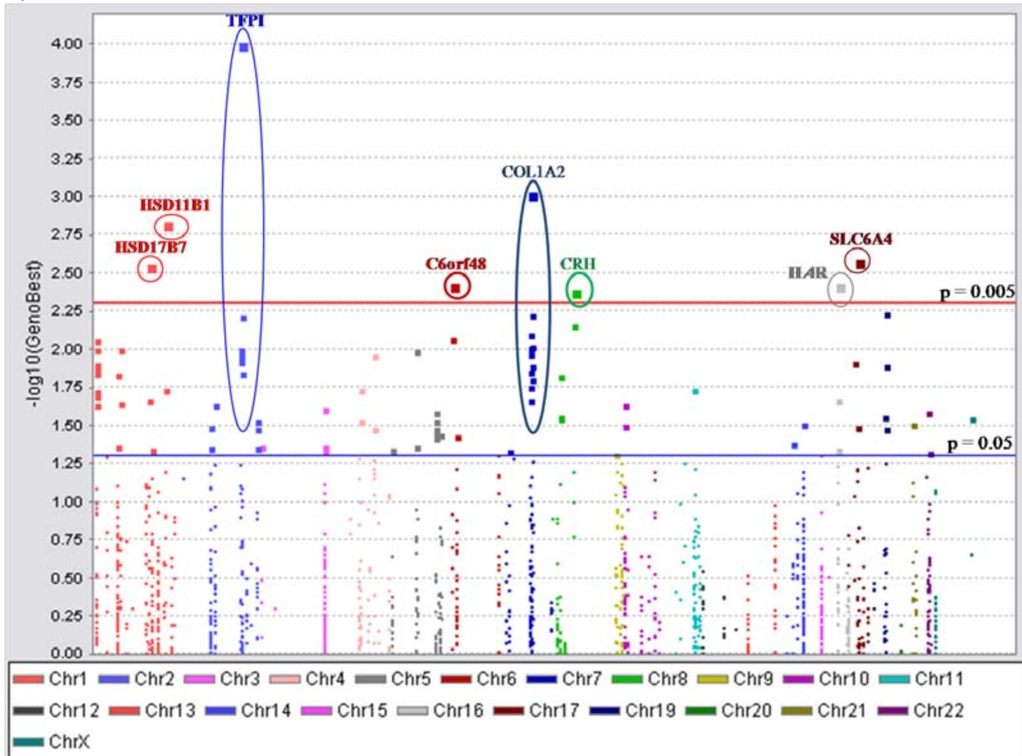


Figure 5-2. Fetal single locus association results. Each point on the graph represents an association test either allelic (a) or genotypic (b). The x axis is SNP position in chromosomal order and the y axis is the inverse negative log of the p-value. Significant associations are highlighted in genes with at least one association at $p < 0.005$.

COL1A2

Thirty-four SNPs in COL1A2 were examined for association with PTB in maternal and fetal samples and ten of these associated with PTB (Figure 5-3a and Appendix Table 10). Seven SNPs were associated with PTB only in fetal samples, one SNP was associated only in maternal samples and two SNPs were associated in both maternal and fetal samples. The most significant maternal single locus result was rs2472 (allelic $p = 1.6 \times 10^{-3}$, genotypic $p = 1.2 \times 10^{-3}$) (Table 5-2a). This SNP had an OR of 0.32 (95% CI = 0.16-0.66, p = 0.002) when comparing the AG/GG genotypes to the AA genotype (Table 5-2b). This SNP was also significantly associated in fetal samples (allelic p = 0.01, genotypic p = 0.01) (Appendix Table 11).

In fetal samples there were significant haplotype associations spanning two areas of the gene (Figure 5-3a). One appears to be driven by the fetal single locus effect at rs420257 and the other appears to be driven by the fetal single locus effect at rs2472. In maternal samples haplotype associations center around the maternal single locus effect at rs2472. The LD patterns between maternal and fetal cases and maternal and fetal controls were very similar (Appendix Figure 2a, b, i, j).

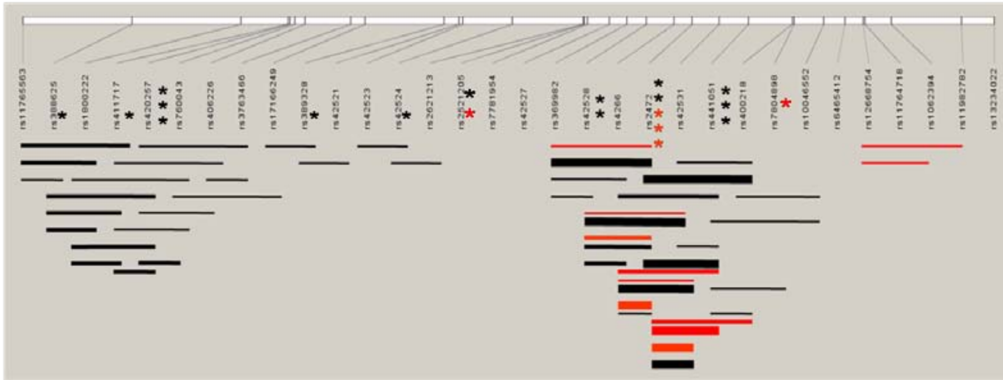
TFPI

Seventeen SNPs in TFPI were examined for association with PTB in maternal and fetal samples and nine of these associated with PTB (Figure 5-3b and Appendix Table 11). Eight SNPs were significantly associated with PTB in both maternal and fetal samples and one SNP (rs6434222) was associated only in fetal samples. Rs6434222, was the most significant fetal single locus association (allelic $p = 5.8 \times 10^{-5}$, genotypic p =

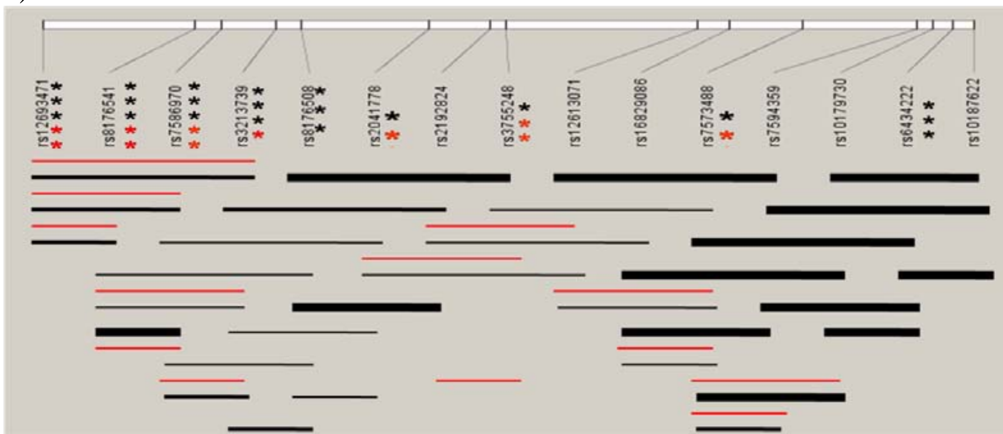
$1.0x10^{-4}$) (Table 5-3a). The best logistic regression model was additive with an OR of 2.49 (95% CI = 1.59-3.91, p = $7.02x10^{-5}$) (Table 5-3b).

In fetal samples there were significant haplotype associations spanning two areas of the gene (Figure 5-3b). One appears to be driven by five significant fetal single locus associations (rs12693471, rs8176541, rs7586970, rs3213739 and rs8176508). Three of these SNPs (rs12693471, rs8176541 and rs7586970) had both significant single locus and haplotype associations in maternal samples. The other significant haplotype effects in fetal samples appear to be driven by rs6434222. Maternal single locus and haplotype associations were not observed for this SNP. In maternal controls there was very weak LD between the 3-SNP block (defined by rs12693471, rs8176541and rs7586970) and rs6434222 (D′ = 0.41) compared to fetal control samples where there is strong LD between rs6434222 and the SNPs in the 3-SNP block (D′ = 0.83) (Appendix Figure 2h and l). The LD patterns between maternal and fetal cases were very similar with strong LD between the 3-SNP block and rs6434222 (D′ > 0.84) (Appendix Figure 2g and k).

a)



b)



* $0.01 \leq$ p-value < 0.05

** $5\text{x}10^{-3} \leq$ p-value < 0.01

*** p-value $< 5\text{x}10^{-3}$

Figure 5-3. Maternal and fetal significant single locus and haplotype associations. a) COL1A2, b) TFPI Asterisks to the right of a SNP indicates significant single locus allelic associations with PTB, red indicates significance in maternal samples and black indicates significance in fetal samples. The number of asterisks denotes the strength of significance. Lines denote a significant haplotype, red is for maternal samples and black is in fetal samples. The thickness of the line denotes the strength of significance. Only SNPs with MAF > 0.05 in maternal or fetal samples are presented in graph.

Discussion

There are four main pathways hypothesized to lead to PTB: 1) activation of maternal or fetal hypothalamic pituitary-adrenal axis, 2) inflammation/infection, 3) decidual hemorrhage, and 4) uterine distension (Lockwood and Kuczynski, 2001). Our study explored candidate genes in these pathways for associations with PTB. Recent studies based on epidemiological data, including one involving the MoBa, suggest that paternal factors have very little contribution on the risk of PTB (Basso et al., 1999; Wilcox et al., 2008). These studies suggest that paternal genes and therefore fetal genes will not contribute the risk of PTB. Of the 167 genes studied, 29 associated in maternal but not fetal samples, 29 associated in fetal but not maternal samples, and 26 associated in both maternal and fetal samples (Figure 5-4). Interestingly, the only association significant after correction for multiple testing was in fetal samples at rs6434222 in TFPI. This SNP was not significantly associated with PTB in maternal samples and there was only weak LD in maternal controls with this SNP, whereas in fetal controls there was strong LD between this SNP and other SNPs with significant associations. This indicates that this SNP may exhibit an independent fetal single locus association that is not driven entirely by the maternal genome. However, careful interpretation is needed, as there may not be enough power to detect significantly different LD patterns between maternal and fetal samples. Our study suggests that there is, in fact, a fetal and, therefore, paternal genetic contribution to the risk of PTB.

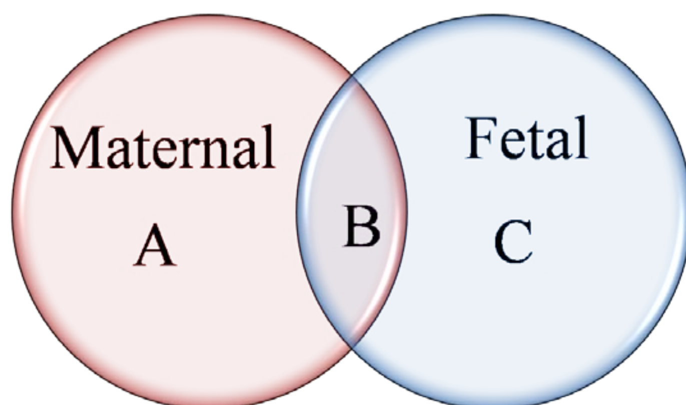


Figure 5-4. Significant allelic or genotypic maternal and fetal results. Bolded genes indicate that the same SNP in this gene was significant in both maternal and fetal samples for either allele or genotype tests. **A:** ADRB2, AP3M2, CARD15, CBS, COL1A1, EDN2, EPHX1, F3, F7, F10, FAS, GSTP1, IL18, IL1β, IL1R2, IL1RN, IL2RB, IL6R, IL8RA, KL, MMP2, MTHFD1, NAT1, PAFAH1B1, PGRMC2, PLA2G4A, PLAT, PTGES, SLC35B2 **B:** ADH1B**, COL1A2**, COL3A1, COL5A1, CRHR2, CYP19A1, **IL1R1**, **IL1RAP**, **IL2RA**, **IL4R**, **MTHFR**, MTRR, NFKB1, NR3C1, PGR, PLG, PON1, PON2, **PTGER3**, **PTGFR**, **SLC6A4**, **TFPI**, TIMP3, TREM1, TSHR, UGT1A1 **C:** C6orf48, COL5A2, CRH, CRHBP, EPHX2, F2R, F5, G0S2, HSD11B1, HSD17B7, HSPA1A, HSPA6, IGSF4C, IL1α, IL4, IL10RB, IL13, IL15, MMP8, NFKBIB, PGRMC1, PLAUR, PTGER2, SERPINE1, SMCR8, TCN2, TIMP4, TLR4, TOMM22

Based on the most significant associations in maternal and fetal samples, several pathways can be highlighted as being strongly associated with PTB. The strongest associations in maternal samples were in genes involved in collagen synthesis related to ECM stability; while the strongest fetal effects were from genes involved in the complement/coagulation pathway. Of the five collagen genes studied, all were associated with PTB in maternal or fetal samples. Three of these genes, including COL1A2, which was the single strongest maternal effect, were associated in both maternal and fetal samples. Two of the SNPs examined in COL1A2 for associations with PTB were located in exons, one is a synonymous change (rs1800222) and the other is a missense mutation (rs42524) where alanine is changed to a proline. Alanine and proline are both nonpolar amino acids, making this a conservative change; however, proline is hydrophilic and

alanine is hydrophobic, therefore this change may affect the protein structure and may be an important variant in the association with PTB. These variants were not associated with PTB in maternal samples; however, rs42524 was associated with PTB in fetal samples (allelic $p = 0.01$, genotypic $p = 0.02$). Additionally, rs42524 is in strong LD with rs2521205 in both maternal and fetal samples ($D' = 1.0$). SNP rs2521205 was associated with PTB in both maternal and fetal samples. This may indicate that this missense mutation (rs42524) is responsible for changes that may alter the functionality of this gene. Collagen, particularly types I and III, is an important component of the ECM that makes up most of the cervix. Perturbations in these genes may contribute to cervical ripening and ECM degradation that is a possible mechanism for PTL and eventually PTB. These genes have not been previously associated with PTB; however, few studies have focused on these genes despite the fact that they are good candidate for associations with PTB.

Another important pathway identified by this study is the complement/coagulation pathway. SNPs in many genes in this pathway, particularly PLAT, associated with PTB in a recent study (Velez et al, 2008). Of the 12 genes studied in this pathway, 10 of them contained SNPs that associated with PTB in either maternal samples, fetal samples, or both (Figure 5-5). Intronic SNPs in TFPI had the strongest associations in fetal samples and several SNPs in TFPI associated with PTB in both maternal and fetal samples. There was one coding SNP genotyped in TFPI (rs5940); however, this SNP was not associated with PTB in maternal or fetal samples. Additionally, there was no detectable LD or very little LD ($D' < 0.40$) between this SNP and other associated SNPs. This may be due to the low minor allele frequency of this

SNP (≤ 0.02 for maternal and fetal samples); therefore, the power to detect an association at an odds ratio of 2.0 with this SNP is small (13%, calculated with PS power). This SNP (rs5940) is a conservative missense mutation where methonine is changed to valine. While both these amino acids are hydrophobic and nonpolar, due to the differences in the structures of the side chains, this amino acid may still represent a functional change in the protein, and therefore may be an important variant in PTB risk.

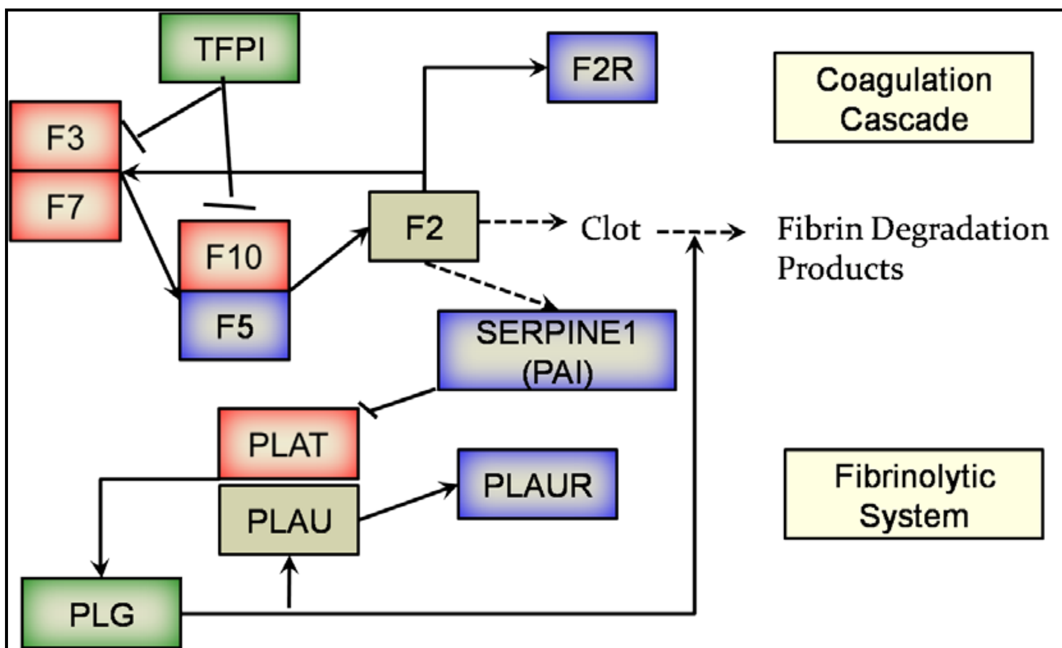


Figure 5-5. Complement/coagulation pathway. Genes shaded blue indicate SNPs were associated in fetal samples only. Genes shaded red indicate SNPs were associated in maternal samples only. Genes shaded green indicate SNPs were associated in both maternal and fetal samples. Genes shaded tan indicate there were no associations in maternal or fetal samples.

TFPI inhibits F3 and F10, both of which are important for the production of F2, a critical component in clot formation. Overproduction of F2 can cause contractions and

activate MMPs, leading to degradation of the ECM. ECM degradation causes changes in the cervix and rupture of the membranes that may result in PTB.

We demonstrated that multiple genes involved in ECM degradation and complement coagulation are associated with PTB. Many of these genes are associated in both maternal and fetal samples. Importantly, we also demonstrated that there are many genes associated with PTB in fetal samples only, supporting a paternal contribution to the risk for PTB, a result previously discounted based on epidemiological data alone.

B. Corroboration of Maternal and Fetal Single Locus Associations with PTB

Introduction

PTB is a serious reproductive disorder that accounts for a large proportion of perinatal mortality and morbidity. There has been an extensive amount of research in the last ten years looking for genetic associations with PTB. Many studies have found positive associations with this disorder, particularly in the inflammation/infection pathway; however, many of these studies fail to replicate (Menon et al., 2006b).

Recently, one such candidate gene study of PTB was performed, examining 1,536 SNPs in 130 candidate genes (Velez et al., 2008a). This study examined maternal and fetal DNA of European-descent from 172 PTBs (<36 weeks gestation) and 198 term labors (≥37 weeks gestation). The strongest maternal associations were in genes involved in the complement-coagulation pathway related to decidual hemorrhage. The genes F5, F7 and PLAT were all found to associate with PTB. The strongest association was at marker rs879293 in PLAT (allelic $p = 2.00 \times 10^{-3}$ and genotypic $p = 2.0 \times 10^{-6}$) with an odds ratio (OR) of 2.80 [CI 1.77 - 4.44] for a recessive model. The strongest fetal association was at rs17121510 in IL-10RA (allelic $p = 0.01$ and genotypic $p = 3.34 \times 10^{-4}$). The best model for the IL-10RA association was additive with an OR of 1.92 [CI 1.15-3.19]. However, this study did not include a replication dataset.

Studies in part A of this chapter demonstrated significant maternal and fetal associations with PTB. In this study we examine SNPs that were associated in both study populations and analyze the data together to determine the effect size of these replicating SNPs.

Materials and Methods

Demographic and clinical characteristics

Sample collection and results from the MoBa study are described in detail in part A of this chapter and the Cenn study is described in detail elsewhere (Velez et al. 2008). Both populations consisted of maternal and fetal EA samples. The combined data yielded a total of 764 maternal samples (353 cases and 411 controls) and 738 fetal samples (343 cases and 395 controls).

Table 5-4. Comparisons of clinical variables between MoBa and Cenn a) controls b) cases

a)

Variable	*MoBa (n=217)*	*Cenn (n = 199)*	[1]*p*
Gravidity	1 [0-7]	2 [1-8]	<0.0001
Gestational Age (days)	280 [273-286]	274 [257-296]	<0.0001
Birthweight (grams)	3650 [2610-4970]	3446 [2100-4661]	<0.0001
APGAR at 1 minute (% <7)	12 (6%)	4 (2%)	0.081
APGAR at 5 minutes (% <7)	5 (2%)	0 (0%)	0.037
Maternal Age (yrs)	30 [21-34]	28 [16-43]	0.045
Smoking (%)	21 (11%)	28 (15%)	0.284

b)

Variable	*MoBa (n=207)*	*Cenn (n = 172)*	[1]*p*
Gravidity	0 [0-5]	2 [1-9]	<0.0001
Gestational Age (days)	253 [182-258]	239 [166-255]	<0.0001
Birthweight (grams)	2810 [950-4000]	2150 [370-3790]	<0.0001
APGAR at 1 minute (% <7)	16 (8%)	42 (25%)	<0.001
APGAR at 5 minutes (% <7)	3 (1%)	10 (6%)	0.018
Maternal Age (yrs)	29 [20-34]	27 [17-40]	0.065
Smoking (%)	22 (12%)	54 (32%)	<0.001

Medians are reported with the range in brackets

[1]P-values are calculated by Mann Whitney U- test for continuous variables and chi-square test for dichotomous variables.

Case-control status definitions were different between the two studies. In the MoBa study cases were defined by a gestational age of < $37^{0/7}$ weeks and in the Cenn study cases were defined by a gestational age of < $36^{0/7}$ weeks. The controls were defined by a gestational age of ≥ $39^{0/7}$ weeks in the Moba study and ≥ $37^{0/7}$ weeks in the Cenn study. Gravidity, gestational age and birthweight differed between the two study populations in cases and controls. When data from the two studies are combined, the median gestational age was 248 days (range = 166-258) in cases and 278 days in controls (range = 257-296).

Statistical analysis

A total of 1,316 SNPs overlapped with the previous study on PTB in EA maternal and fetal samples. Sample collection and results from the Cenn study are described in detail elsewhere (Velez et al. 2008). SNPs were included for pooled analysis if the allele or genotype p-values were less than 0.05 in one study (MoBa or Cenn) and less than 0.20 in the other study. This criterion, while arbitrary, was designed to capture associations that may have been missed due to inadequate power in an individual study. However, this criterion was also designed to exclude associations that were primarily driven by only one of the two studies. The MoBa and Cenn samples were pooled together, and the SNPs that met the above criteria were analyzed for allelic and genotypic association as described in part A of this chapter. A pooled effect size was determined with logistic regression.

Results

Maternal results

Of the 58 SNPs that met the criterion for the pooled analysis, 15 had significant allelic or genotypic pooled $p < 5x10^{-3}$ (Table 5-5 and Appendix Table 13a). These 15 SNPs were from 11 different genes involved in several PTB pathways such as collagen synthesis, inflammation/infection, complement/coagulation and prostaglandin synthesis.

The most significant single locus result in the pooled sample was in prostaglandin E receptor 3 (PTGER3). There were six SNPs in this gene that met the criteria for pooled analysis and three of these (rs2072947, rs977214 and rs6665776) had significant allelic or genotypic results in the pooled samples. Two of these SNPs (rs977214 and rs6665776) strongly associated with PTB in the pooled sample (rs977214: genotypic $p = 2.6x10^{-4}$; rs6665776: genotypic $p = 4.8x10^{-4}$) (Table 5-5a). The best model for rs977214 compared the AG/GG genotypes to the AA genotype and resulted in an OR of 0.56 (95% CI = 0.38-0.82, p = 0.003), indicating a protective effect (Table 5-5b). The best model for rs6665776 compared the CC genotype to the AA/AC genotypes and had an OR of 1.75 (95% CI = 1.19-2.58, p = 0.005) (Table 5-5b). There was strong LD between these two SNPs in the pooled cases and controls (D′ = 1). Haplotype associations spanned three general regions of the gene; two of these regions were significant in the Cenn study only and one of these regions was significant in the Moba study only (Figure 5-6). Additionally, there were 13 SNPs with significant allelic or genotypic p-values less than 0.05 in both studies (Appendix Table 13). This included both SNPs with significant pooled p-values in PTGER3.

Table 5-5. Genetic associations in maternal samples. a) significant allelic and genotypic results. b) OR of significant results in pooled data.

a)

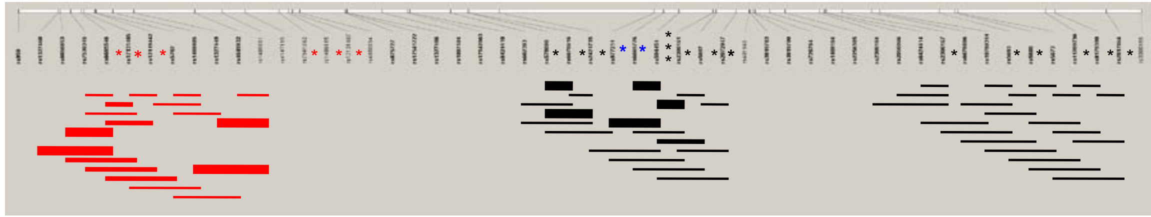
			Centennial Study				Norway MoBa Study				Pooled Data			
			Allele Freq.		p-value		Allele Freq.		p-value		Allele Freq.		p-value	
Gene	SNP	Allele	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype
AP3M2	rs4581040	G	0.32	0.24	0.042	0.131	0.30	0.24	0.041	0.112	0.31	0.24	0.004	0.017
COL1A1	rs1061237	C	0.23	0.28	0.090	0.055	0.26	0.29	0.332	0.044	0.24[1]	0.29	0.069	0.003
COL5A1	rs10745387	A	0.54	0.61	0.110	0.150	0.37	0.44	0.031	0.101	0.44	0.52	0.003	0.009
IL1R2	rs1108338	C	0.28	0.21	0.038	0.082	0.29	0.23	0.022	0.012	0.29[1]	0.22	0.002	0.001
IL1RAP	rs7628333	T	0.21	0.24	0.358	0.080	0.22	0.32	0.002	0.005	0.22	0.28	0.004	0.012
IL6R	rs4845374	T	0.87	0.82	0.139	0.110	0.12	0.17	0.042	0.027	0.43[1]	0.48[2]	0.051	0.002
	rs4329505	C	0.13	0.17	0.129	0.067	0.12	0.17	0.042	0.027	0.13	0.17	0.011	0.003
NAT1	rs7017402	A	0.15	0.10	0.038	0.088	0.13	0.09	0.043	0.048	0.14	0.09	0.004	0.013
	rs9325827	C	0.18	0.11	0.012	0.040	0.16	0.11	0.048	0.083	0.17	0.11	0.002	0.008
PLA2G4A	rs2076075	A	0.14	0.10	0.110	0.236	0.17	0.12	0.020	0.056	0.16	0.11	0.004	0.016
PLAT	rs2020922	A	0.32	0.24	0.029	0.095	0.30	0.24	0.034	0.081	0.31[1]	0.24	0.003	0.009
PTGER3	rs977214	G	0.09	0.13	0.166	0.005	0.06	0.09	0.064	0.039	0.07[1]	0.11	0.016	$2.6x10^{-4}$
	rs6665776	A	0.09	0.12	0.235	0.010	0.06	0.09	0.064	0.039	0.07[1]	0.11	0.024	$4.8x10^{-4}$
TSHR	rs17630128	C	0.33	0.25	0.031	0.023	0.36	0.30	0.072	0.192	0.35	0.28	0.004	0.010
	rs12883801	G	0.41	0.52	0.005	0.011	0.37	0.43	0.107	0.137	0.39[1]	0.47	0.001	$9.4x10^{-4}$

[1]cases deviated from HWE at rs1061237 (p = 0.01), rs1108338 (p = 0.04), rs4845374 ($p < 10^{-3}$), rs2020922 (p = 0.05), rs977214 (p = 0.02), rs6665776 (p = 0.02), rs12883801 (p = 0.04)

[2]controls deviated from HWE at rs4845374 ($p < 10^{-3}$), rs4329505 (p = 0.03), rs977214 (p = 0.05)

b)

Gene	Gene Name	SNP rs#	Role	Model	OR	95% CI	Model p
AP3M2	Adaptor-related protein complex 3	rs4581040	Downstream	Additive	1.36	1.09-1.70	0.006
COL1A1	Collagen, type 1, alpha 1	rs1061237	3′UTR	CCvsCT/TT	3.02	1.56-5.86	0.001
COL5A1	Collagen, type 5, alpha 1	rs10745387	Intron	Additive	1.35	1.11-1.65	0.003
IL1R2	Interleukin 1 receptor 2	rs1108338	Intron	AA/ACvsCC	2.89	1.58-5.29	$5.8x10^{-4}$
IL1RAP	Interleukin 1 receptor accessory protein	rs7628333	Intron	Additive	0.71	0.56-0.90	0.005
IL6R	Interleukin 6 receptor	rs4845374	Intron	AAvsAT/TT	0.65	0.49-0.88	0.004
		rs4329505	Intron	CC/CTvsTT	1.66	1.20-2.29	0.002
NAT1	N-acetyltransferase	rs7017402	Intron	Additive	0.63	0.45-0.87	0.005
		rs9325827	Intron	Additive	0.63	0.47-0.85	0.002
PLA2G4A	Phospholipase A2	rs2076075	Intron	Additive	0.65	0.48-0.87	0.004
PLAT	Tissue plasminogen activator	rs2020922	Intron	Additive	0.72	0.58-0.90	0.004
PTGER3	Prostaglandin E receptor 3	rs977214	Intron	AAvsAG/GG	0.56	0.38-0.82	0.003
		rs6665776	Intron	AA/ACvsCC	1.75	1.19-2.58	0.005
TSHR	Thyroid stimulating hormone receptor	rs17630128	3′UTR	CC/CTvsTT	0.64	0.48-0.86	0.003
		rs12883801	Downstream	AA/AGvsGG	0.49	0.33-0.72	$3.1x10^{-4}$



* 0.01 ≤ p-value < 0.05

** $5x10^{-3}$ ≤ p-value <0.01

*** p-value < $5x10^{-3}$

Figure 5-6. Significant maternal single locus and haplotype associations in MoBa and Cenn studies. PTGER3, Asterisks to the right of a SNP indicates significant single locus allelic associations with PTB, blue denotes significance in pooled samples, red indicates significance in the MoBa study only and black indicates significance in the Cenn study only. The number of asterisks denotes the strength of significance. Lines denote a significant haplotype, red is for the MoBa study and black is for the Cenn study. The thickness of the line denotes the strength of significance.

Fetal results

Of the 45 SNPs that met the criteria for the pooled analysis, 4 SNPs in 4 different genes had pooled allelic or genotypic $p < 5x10^{-3}$ (Table 5-6 and Appendix Table 13b). The most significant single locus association in pooled samples was in PON1 at rs854552 (allelic p = 0.001, genotypic $p = 7.6x10^{-4}$). The best model compared the TT genotype to the CC/CT genotypes and resulted in an OR of 1.69 (95% CI = 1.26-2.27, $p = 5.1x10^{-4}$) (Table 5-6b). There were very few haplotype or single locus associations in the Cenn study for PON1; however, there were strong haplotype associations in the MoBa data, and rs854552 was the most significant single locus association in this gene in the MoBa study (Figure 5-7). Additionally, there were 6 SNPs with allelic or genotypic p-values less than 0.05 in both studies. This did not include rs854552 in PON1; however, a different SNP in this gene (rs2272365) had significant allelic association at p < 0.05 in both studies (MoBa p = 0.041, Cenn p = 0.046). The pooled allelic p-value for this marker was 0.005.

Table 5-6. Genetic associations in fetal samples. a) significant allelic and genotypic results, b) OR of significant results in pooled data.

a)

			Centennial Study				Norway MoBa Study				Pooled Data			
			Allele Freq.		p-value		Allele Freq.		p-value		Allele Freq.		p-value	
Gene	SNP	Allele	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype
COL1A2	rs42524	G	0.77	0.79	0.440	0.055	0.19	0.26	0.010	0.022	0.43[1]	0.50[2]	0.003	0.001
COL3A1	rs3134656	A	0.50	0.39	0.005	0.026	0.47	0.41	0.067	0.098	0.48	0.40	0.001	0.004
MTHFR	rs4846052	T	0.39	0.46	0.085	0.205	0.36	0.45	0.009	0.010	0.37	0.46	0.002	0.003
PON1	rs854552	C	0.22	0.28	0.120	0.248	0.21	0.30	0.002	0.006	0.21	0.29	0.001	7.6×10^{-4}

[1]cases deviated from HWE and PRAT at rs42524 ($p < 10^{-3}$)
[2]controls deviated from HWE and PRAT at rs42524 ($p < 10^{-3}$)

b)

Gene	**Gene Name**	**SNP rs#**	**Role**	**Model**	**OR**	**95% CI**	**Model p**
COL1A2	Collagen, type 1, alpha 2	rs42524	Coding exon P549A	CCvsCG/GG	0.58	0.43-0.79	4.5×10^{-4}
COL3A1	Collagen, type 3, alpha 1	rs3134656	Intron	Additive	0.71	0.57-0.87	0.001
MTHFR	5, 10-methylenetetrahydrofolate reductase	rs4846052	Intron	CCvsCT/TT	0.59	0.43-0.80	7.4×10^{-4}
PON1	Paraoxonase 1	rs854552	3′UTR	CC/CTvsTT	1.69	1.26-2.27	5.1×10^{-4}

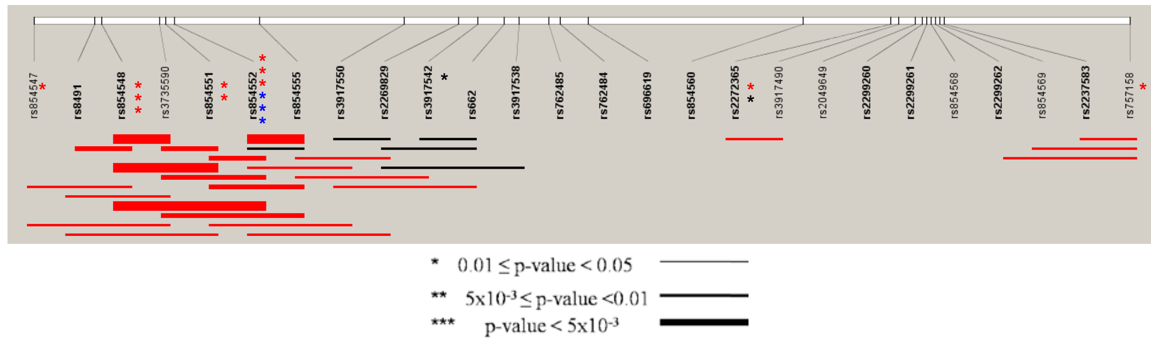


Figure 5-7. Significant fetal single locus and haplotype associations in MoBa and Cenn studies. PON1, Asterisks to the right of a SNP indicates significant single locus allelic associations with PTB, blue denotes significance in pooled samples, red indicates significance in the MoBa study only and black indicates significance in the Cenn study only. The number of asterisks denotes the strength of significance. Lines denote a significant haplotype, red is for the MoBa study and black is for the Cenn study. The thickness of the line denotes the strength of significance.

Discussion

In this study associations with several genes from multiple biological pathways had modest to strong associations with PTB in two independent studies and when these studies were pooled, several SNPs had significant associations with PTB (Figure 5-8). Previous studies in part A of this chapter identified significant maternal and fetal associations with PTB in COL1A2 in the MoBa study. In this section we demonstrated that there were significant associations in COL1A2 in maternal and fetal samples in the Cenn study as well and when these two studies were pooled, associations in COL1A2 remained significant (Figure 5-8). This was also the case for COL3A1. Additionally, COL5A1 and COL5A2 were associated with PTB in three of the four sample sets studied (Figure 5-8). Collagen plays an important role in all stages of cervical function during pregnancy (Word et al., 2007). These studies suggest that collagen genes are particularly important in the pathogenesis of PTB.

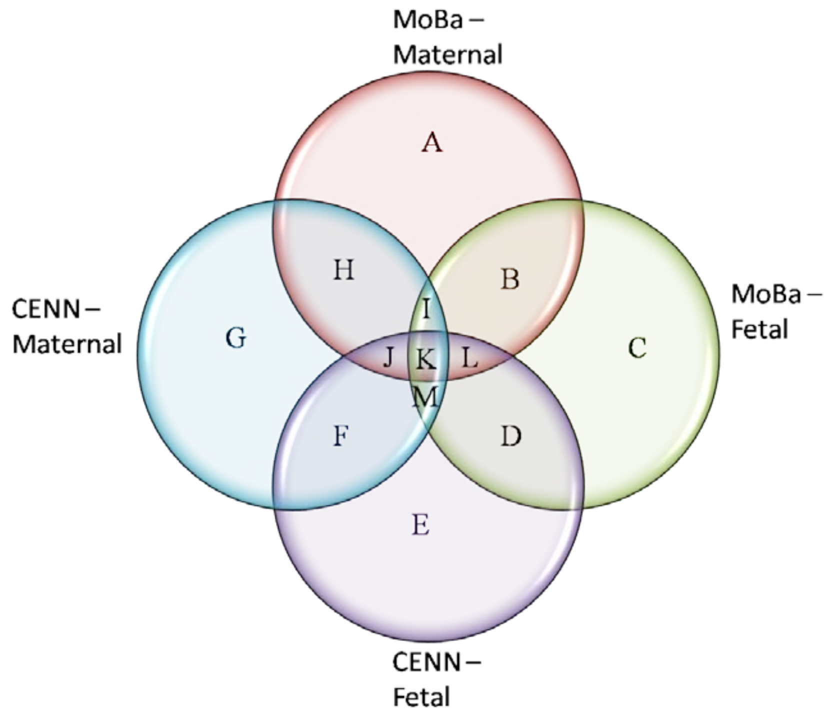


Figure 5-8. Identification of genes associated with PTB in multiple populations. A: ADRB2, CARD15, CBS, COL1A1, EDN2, EPHX1, FAS, GSTP1, IL1B, IL1RN, IL2RB, IL8RA, KL, MMP2, PAFAH1B1, PGRMC2, PTGES, SLC35B2 **B:** ADH1B, MTHFR, NFKB1, PTGFR **C:** C6orf48, CRH, F5, G0S2, HSD11B1, HSD17B7, HSPA1A, IL10RB, IL13, IL15, NFKBIB, PGRMC1, PTGER2, SERPINE1, TCN2, TIMP4, TLR4, TOMM22 **D:** HSPA6, IL1A, IL4, MMP8, TLR8 **E:** CBS, EDN2, GSTP1, HSPA14, IL1B, IL1RN, IL2RB, KIAA0664, KL, NFKBIA, PAFAH1B1, PTGS2, SLC23A1, SLC35B2 **F:** IL10RA, IL5, MMP1, MMP3 **G:** CCL2, CTLA4, DHFR, F5, IL10, NFKBIB, PGRMC1, PTGS1, SCNN1A, TEX12, TLR2, TLR3, TLR7, TNFRI, TNFRII **H:** AP3M2, F7, IL18, IL1R2, IL6R, MTHFD1, NAT1, PLA2G4A **I:** COL5A1, IL1R1, IL4R **J:** PLAT **K:** COL1A2, COL3A1, CYP19A1, IL1RAP, IL2RA, PON2, PTGER3, TIMP3, TSHR, UGT1A1 **L:** CRHR2, NR3C1, PGR, PON1, SLC6A4, TREM1 **M:** COL5A2, CRHBP, EPHX2

Additionally, the most significant associations in the pooled samples were from two major PTB pathways: 1) HPA axis activation (PTGER3) and 2) decidual hemorrhage (PON1). Activation of the HPA axis results in an increase of prostaglandins. Prostaglandins play a central role in myometrial contractions, and elevated uterine levels of prostaglandins can lead to contractions and labor. PTGER3 is a stimulatory receptor that plays a role in smooth muscle contraction. PTGER3 was significant in all four

populations studied in part A and B of this chapter (Figure 5-8). Additionally, three of the six SNPs examined in the pooled sample were associated with PTB. It is likely that polymorphisms in this gene could lead to early contractions and therefore PTB.

Another important pathway that is consistently associated with PTB is the decidual hemorrhage pathway which activates ECM degradation. PON1 is a member of the paraoxonase gene family, and mutations in this gene are associated with high-density lipoprotein (HDL) and modification of low-density lipoprotein (LDL) (Mackness et al., 1993; Mackness et al., 1996). HDL increases the production of prostaglandin E, which can lead to contractions (Chen et al., 2004a). Therefore, it is likely that the mechanisms driving the most significant association in the maternal pooled samples (PTGER3) and the most significant association in the fetal pooled samples (PON1) are functionally related. Additionally, hypothesized mutations in this gene could indirectly disrupt the balance of procoagulant and anticoagulant mechanisms in pregnancy and result in thrombosis, infarction and eventually PTB (Girling and de, 1998; Chen et al., 2004a).

The studies in this chapter were limited in that these were candidate gene studies, therefore many associations may be missed, including genes representing novel biology with respect to PTB. In addition, the case-control criteria for the MoBa and Cenn studies were different and while there were still many associations discovered, studies with more carefully defined phenotypic criteria are needed to validate these results. However, this study identified multiple genes with significant or marginally significant associations with PTB in two separate studies, which has not often been done with respect to this disorder. When these samples were combined, multiple SNPs with strong allelic or genotypic p-values were identified, including SNPs involved in inflammation and

infection, ECM degradation and decidual hemorrhage. This study has validated previously identified genetic associations with PTB and further studies can address the biological role these genes play in PTB.

CHAPTER VI

CONCLUSIONS AND FUTURE DIRECTIONS

A. Summary

Reproductive disorders, such as PTB and BV, are serious gynecological problems responsible for a large portion of perinatal morbidity and mortality. Identifying the environmental and genetic risk factors of these reproductive disorders has proven difficult, as these are complex diseases that do not follow a Mendelian pattern of inheritance. The majority of factors discovered to date either do not replicate or only explain a fraction of the risk for developing these diseases. Studies presented in the previous chapters were aimed at elucidating the genetic immunology of BV and the genetics of PTB.

Genetic Associations with BV

There are several important points that have been revealed by these studies. First, while many studies have demonstrated that vaginal and cervical cytokine concentrations differ by BV status, many of these are inconsistent. We have demonstrated that the cytokine concentrations in BV^+ and BV^- women differ between AA and EA, and the correlation structure of these cytokines is also very different between AA and EA. This may help to explain the inconsistencies of previous studies where there was ethnic heterogeneity as well as identify potential mechanisms that may explain why AA are at an increased risk for developing BV, even after accounting for socio-economic variables.

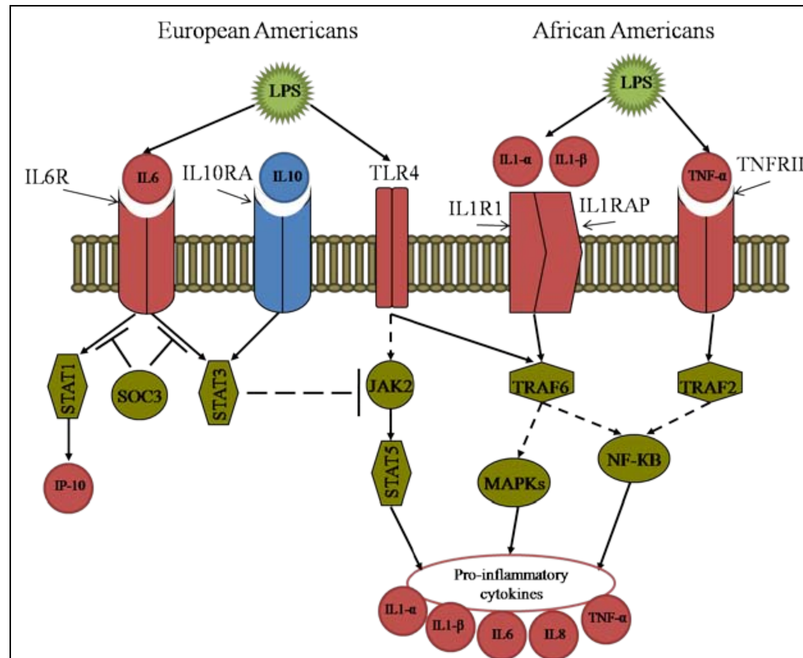


Figure 6-1. Hypothesized genetic mechanism for the regulation of cervical cytokine concentrations. Genetic associations with cytokine concentrations differs between EA (left panel) and AA (right panel). Genes and cytokines are represented as follows: blue – anti-inflammatory, pink – pro-inflammatory and green – downstream signaling molecules. Dashed lines indicate that there is an indirect connection between the two molecules, meaning that other factors lead to the inhibition or stimulation of the proceeding factor. Solid lines indicate a direct connection between the two factors, meaning that the factor shown is necessary and sufficient to stimulate the proceeding factor.

Secondly, the associations with cervical cytokine concentrations are involved with different signaling pathways between AA and EA (Figure 6-1). IL-6R, IL-10RA and TLR4, all associated with cervical cytokine concentrations in EA but not AA, act through the JAK/STAT signaling pathway to either inhibit (IL-10RA) or stimulate pro-inflammatory cytokine production (IL-6 and TLR4). IL-1RAP and TNFR2, both associated with cervical cytokine concentrations in AA but not EA, signal through the MAPK/NF-kB signaling pathway to induce production and release of pro-inflammatory cytokines. This may be another plausible mechanism for the ethnic disparity observed in the prevalence of BV. Separate downstream signaling molecules may be contributing to

not only the differences in genetic associations with cervical cytokines but also differences in the correlation structure observed between EA and AA.

Lastly, the genetic associations with cervical cytokine concentration involve the cytokine receptor genes, not the genes themselves. Most studies of the cervical milieu focus on cytokine genes, not other cofactors and receptors. This may be an indication of why many genetic association studies examining vaginal and cervical cytokines fail to detect associations or fail to replicate. Genetic associations with cervical cytokine concentrations appear to involve a complex mechanism of regulation that may contribute to the pathogenesis and possibly the progression of BV.

Genetic Associations with PTB

Four main pathways have been hypothesized to lead to PTB: 1) activation of maternal or fetal hypothalamic pituitary-adrenal axis, 2) inflammation/infection, 3) decidual hemorrhage, and 4) uterine distension (Lockwood and Kuczynski, 2001). These pathways converge on a final terminal pathway where uterotonins, proteases and prostaglandins are released, which lead to contractions, rupture of the membranes, and eventually, PTB. Our studies have identified genes associated with PTB that are involved in the previously mentioned pathways (Figure 6-2).

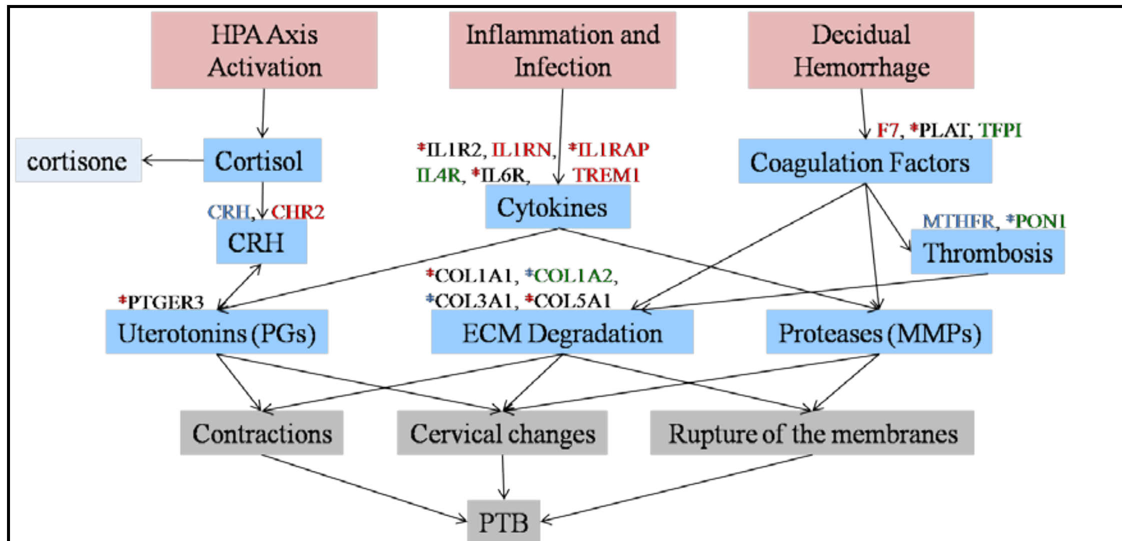


Figure 6-2. Hypothesized mechanisms for PTB. Major pathways are highlighted in pink, secondary pathways in blue and terminal pathways in grey. Genes with significant ($p < 5x10^{-3}$) associations from the MoBa study or significant pooled (MoBa and Cenn) p-values ($p < 5x10^{-3}$) are listed above the secondary pathway. Genes in red text indicate maternal associations, blue text indicates fetal associations and green text indicates maternal and fetal associations. Genes with a red asterisk indicate maternal replication, blue asterisk indicates fetal replication.

Several studies have identified SNPs in the inflammation/infection pathway that are associated with PTB; however, these studies often fail to replicate (Velez et al., 2007; Velez et al., 2008b). In this study we identified many SNPs in inflammation/infection related genes such as IL-1R2, IL-1RAP and IL-6R that consistently associated with PTB (Figure 6-2). The majority of these associations are in maternal samples only, indicating the importance of the maternal genome on infection related genetic associations with PTB. Studies in part B of chapter IV demonstrated that in a separate EA population, a SNP in IL-6R was significantly associated with cervical IL-6 concentrations. In an AA population SNPs in IL-1RAP were associated with IL-1β concentrations. Both of these associations were affected by the presence of BV. The SNPs associated with BV and PTB were not the same. Also there was no LD between these SNPs in the population

studied. Nevertheless, it is possible that there is a pleiotropic effect for these genes in influencing two related reproductive disorders. Further studies will need to be conducted to determine if one or multiple SNPs in these genes are affecting these disorders.

Another important pathway that is consistently associated with PTB is the decidual hemorrhage pathway that activates ECM degradation. Collagen genes play a central role in ECM degradation, and several SNPs from many of these genes were significant in maternal (COL1A1 and COL5A1) and fetal (COL1A2 and COL3A1) pooled samples with allelic or genotypic $p < 5x10^{-3}$. This suggests that collagen genes are particularly important in the pathogenesis of PTB. Additionally, collagen genes are critical in the uterine distension pathway, suggesting that through collagen genes multiple pathways are activated that lead to PTB.

Collagen plays an important role in all stages of cervical function during pregnancy (Word et al., 2007). During the dilation and ripening phases, collagen concentration decreases with increased collagen dispersal, solubility and degradation (Word et al., 2007). Collagen, in particular types I and types III, plays vital roles in the changes in tissue consistency associated with cervical ripening (Word et al., 2007). Perturbations in these collagen genes could cause an increased weakening of the membranes surrounding the fetus and result in rupture of the membranes, thereby leading to PTB. It is evident from significant results in two separate studies, that contributions from both the maternal and fetal genomes are involved in the associations of collagen genes with PTB.

This study identified multiple genes with significant or marginally significant associations with PTB in two separate studies. When these samples were combined,

multiple SNPs with strong allelic or genotypic p-values were identified, including SNPs involved in inflammation and infection, ECM degradation and decidual hemorrhage. This study has validated previously identified genetic associations with PTB and further studies can address the biological role these genes play in PTB.

B. Future Directions

Reproductive disorders such as BV and PTB involve complex mechanisms with built in redundancies and checkpoints. Our genetic association studies of BV, while examining an important intermediate phenotype, were limited in the ability to connect these findings directly with reproductive disorders such as BV and PTB. These limitations were due to small sizes. For this same reason other important environmental risk factors such as smoking and gene-gene interactions were not examined. Our studies of PTB were limited in the ability to identify novel biological pathways that may be associated with PTB, as our study was a candidate gene study. Additionally, our study lacked complex environmental risk factors that may be influenced by the genetic variants identified, such as serum biomarker measurements.

Therefore, while these studies have identified genes of interest, more intricate analyses must be performed to identify the true mechanisms underlying the genetic regulation of cervical immunity and PTB. Additional studies are required to validate these findings. Re-sequencing of the variants identified in the cervical cytokine studies, particularly IL-1RAP, IL-6R, IL-8RA, IL-10RA, and TLR4 and those identified in the PTB studies, particularly COL1A2, TFPI, PON1 and PTGER3 are warranted to identify functional candidate loci.

Due to the complex nature of these disorders genome wide association studies (GWAS) may be useful in identifying associations not previously examined. However, due to the large number of tests performed in these studies, large sample sizes are needed to have adequate power to detect associations. These studies may also be hard to interpret, as there will likely be thousands of significant associations. However, these

studies may identify new pathways of interest that have not previously been examined with PTB and therefore, follow-up studies can be performed to pinpoint important associations.

Many environmental factors such as nutrition, serum protein levels, smoking and race will need to be evaluated to better identify mechanisms that are influencing these disorders. In particular, studies examining multiple races are needed to determine if the genes and pathways identified are dependent on geographic ancestry or if these genes and mechanisms apply to the global population. Additional studies with intermediate phenotypes such as cervical cytokines and serum biomarkers are critical to determine important biological associations with reproductive disorders. Gene-gene and gene-environment interactions will need to be determined to realistically discern the complexity of these diseases. This will require much larger sample sizes than the current studies and will demand a well defined and characterized phenotype, as well as additional physiological measures.

Appendix Table 1. Median and ranges of all cytokines by BV status and race

Cytokine	*EA BV$^-$*	*EA BV$^+$*	*AA BV$^-$*	*AA BV$^+$*	*p value*[1]	*p value*[2]	*p value*[3]	*p value*[4]
EGF	232.3 (12.0 – 1740.6)	181.4 (12.0 – 445.0)	176.2 (12.0 – 762.2)	179.8 (0.6 – 1595.2)	0.144	0.881	0.462	0.824
EOTAXIN	184.0 (56.0 – 2165.8)	169.9 (40.0 – 4200.0)	159.3 (5.0 – 1235.0)	182.2 (28.6 – 3301.6)	0.473	0.596	0.375	0.765
FGF2	134.7 (12.0 – 7368.4)	122.5 (12.0 – 1437.4)	95.0 (12.0 – 1555.4)	65.8 (12.0 – 7716.3)	0.980	0.996	0.248	0.444
FLT3	260.5 (12.0 – 1603.8)	330.9 (12.0 – 1692.0)	245.0 (12.0 – 1170.0)	353.5 (12.0 – 15139.1)	0.339	0.046	0.649	0.555
GMCSF	113.0 (5.0 – 2524.0)	80.0 (5.0 – 2796.0)	89.9 (5.0 – 2202.0)	113.0 (5.0 – 5438.6)	0.355	0.283	0.392	0.293
IFN-γ	219.9 (5.0 – 1735.0)	172.0 (49.6 – 2021.0)	227.9 (5.0 – 1418.0)	181.0 (5.0 – 3538.1)	0.769	0.499	0.833	0.494
IL-1α	904.9 (166.0 – 11098.1)	1973.1 (82.4 – 25581.0)	558.4 (104.0 – 5554.6)	1165.2 (163.8 – 33876.5)	0.030	<0.001	0.047	0.422
IL-1β	106.6 (5.0 – 7787.5)	265.0 (5.0 – 7374.2)	116.1 (0.6 – 6170.9)	188.5 (5.0 – 10125.8)	0.106	0.085	0.405	0.287
IL-2	129.0 (5.0 – 944.0)	90.6 (5.0 – 910.7)	108.5 (5.0 – 630.0)	169.8 (5.0 – 1228.9)	0.497	0.120	0.602	0.111
IL-3	324.6 (5.0 – 2524.7)	254.7 (5.0 – 1316.8)	237.4 (5.0 – 2321.3)	281.2 (5.0 – 38028.7)	0.325	0.232	0.290	0.226
IL-4	62.9 (0.0 – 498.5)	52.5 (0.0 – 547.0)	55.0 (5.0 – 430.0)	60.9 (0.0 – 668.4)	0.211	0.909	0.464	0.571
IL-5	18.0 (5.0 – 302.0)	12.8 (5.0 – 183.0)	11.6 (3.0 – 297.0)	13.1 (5.0 – 758.4)	0.980	0.230	0.269	0.846
IL-6	3967.1 (62.2 – 25000.0)	2129.6 (117.9 – 14256.0)	2162.2 (5.0 – 14744.0)	2290.3 (288.3 – 40000.0)	0.287	0.526	0.010	0.779
IL-7	328.9 (5.0 – 1494.0)	236.3 (5.0 – 2080.0)	405.6 (5.0 – 1170.0)	392.0 (5.0 – 2365.2)	0.245	0.820	0.863	0.326
IL-8	11677.7 (260.6 – 169470.6)	6315.0 (771.0 – 30000.0)	7632.5 (1068.6 – 31552.9)	15363.8 (486.8 – 30000.0)	0.474	0.249	0.182	0.710
IL-10	73.3 (0.0 – 497.4)	37.5 (0.0 – 443.0)	43.0 (1.3 – 391.0)	49.3 (0.0 – 1381.6)	0.016	0.553	0.016	0.580
IL12P40	289.5 (8.0 – 926.0)	267.9 (38.0 – 879.3)	215.2 (8.0 – 950.0)	229.8 (8.0 – 735.5)	0.849	0.695	0.070	0.331
IL12P70	79.9 (20.0 – 1141.0)	62.6 (5.0 – 1842.0)	73.2 (5.0 – 683.0)	91.0 (5.0 – 665.2)	0.377	0.087	0.394	0.101
IL-13	100.1 (5.0 – 959.0)	81.9 (5.0 – 592.0)	83.3 (5.0 – 488.0)	133.8 (5.0 – 397.1)	0.446	0.167	0.170	0.337
IL-15	68.0 (5.0 – 564.3)	61.1 (5.0 – 302.4)	67.0 (5.0 – 396.7)	87.6 (5.0 – 706.3)	0.609	0.250	0.707	0.234
IP10	3972.8 (5.0 – 30181.9)	1807.8 (146.6 – 25000.0)	4162.0 (185.6 – 25000.0)	2932.4 (163.3 – 20303.2)	0.001	0.174	0.741	0.108
MCP1	859.0 (40.0 – 30631.9)	348.0 (48.0 – 22146.0)	773.9 (66.0 – 5500.0)	519.6 (96.1 – 4204.0)	0.006	0.313	0.191	0.171
MIP-1 α	833.2 (122.1 – 12604.3)	417.3 (97.0 – 15810.0)	911.0 (126.1 – 10194.0)	714.8 (8.0 – 6236.6)	0.043	0.315	0.507	0.149
PDGF-AA	1093.5 (78.4 – 10122.0)	499.1 (99.3 – 10916.0)	672.0 (160.3 – 7969.0)	666.2 (33.0 – 7894.8)	0.048	0.290	0.168	0.839
PDGF-BB	2362.2 (236.2 – 33201.0)	1445.1 (40.0 – 14663.7)	1204.7 (12.0 – 9651.0)	1259.6 (12.0 – 36153.1)	0.015	0.534	0.010	0.443
RANTES	545.0 (3.0 – 8000.0)	445.5 (25.0 – 8000.0)	563.0 (21.0 – 8400.0)	506.0 (3.0 – 5520.8)	0.234	0.444	0.632	0.863
TNF	6.0 (1.4 – 861.2)	6.0 (1.4 – 857.0)	14.0 (5.0 – 633.7)	6.0 (1.4 – 2887.6)	0.494	0.915	0.732	0.373
VEGF	925.3 (12.0 – 17585.4)	1163.9 (90.0 – 26310.0)	873.9 (12.0 – 12302.0)	873.9 (67.7 – 28705.8)	0.762	0.802	0.304	0.415

[1] p values compare BV$^+$ to BV$^-$ in EA
[2] p values compare BV$^+$ to BV$^-$ in AA
[3] p values compare EA to AA in BV$^-$ women
[4] p values compare EA to AA in BV$^+$ women

Appendix Table 2. Correlation coefficients and p-values in AA and EA BV$^-$ and BV$^+$ women.

		Black BV$^+$		Black BV$^-$		White BV$^+$		White BV$^-$	
		Rho	*p*	*Rho*	*p*	*Rho*	*p*	*Rho*	*p*
EOTAXIN	EGF	0.295	0.068	0.446	0.005	0.285	0.142	0.314	0.021
FGF2	EGF	0.371	0.017	0.583	<0.001	0.288	0.138	0.230	0.095
FGF2	EOTAXIN	0.550	<0.001	0.492	0.001	0.613	0.001	0.410	0.002
FLT3	EGF	0.397	0.020	0.487	0.006	0.338	0.091	0.340	0.024
FLT3	EOTAXIN	0.463	0.008	0.731	<0.001	0.018	0.931	0.422	0.004
FLT3	FGF2	0.489	0.003	0.606	<0.001	0.323	0.107	0.385	0.010
GM-CSF	EGF	0.415	0.008	0.409	0.011	0.465	0.013	0.299	0.028
GM-CSF	EOTAXIN	0.652	<0.001	0.548	<0.001	0.522	0.004	0.514	<0.001
GM-CSF	FGF2	0.621	<0.001	0.642	<0.001	0.469	0.012	0.416	0.002
GM-CSF	FLT3	0.728	<0.001	0.666	<0.001	0.400	0.043	0.645	<0.001
IFN-γ	EGF	0.358	0.022	0.666	<0.001	0.378	0.047	0.318	0.019
IFN-γ	EOTAXIN	0.664	<0.001	0.571	<0.001	0.407	0.031	0.589	<0.001
IFN-γ	FGF2	0.445	0.004	0.508	0.001	0.505	0.006	0.410	0.002
IFN-γ	FLT3	0.586	<0.001	0.648	<0.001	0.704	<0.001	0.592	<0.001
IFN-γ	GM-CSF	0.667	<0.001	0.628	<0.001	0.608	0.001	0.688	<0.001
IL-1α	EGF	0.121	0.461	0.634	<0.001	-0.012	0.953	0.233	0.091
IL-1α	EOTAXIN	-0.089	0.591	0.273	0.093	-0.349	0.069	-0.082	0.552
IL-1α	FGF2	-0.400	0.012	0.278	0.087	-0.241	0.217	-0.085	0.542
IL-1α	FLT3	0.033	0.858	0.317	0.082	0.424	0.031	0.180	0.237
IL-1α	GM-CSF	-0.039	0.816	0.231	0.156	-0.065	0.742	0.120	0.381
IL-1α	IFN-γ	0.189	0.249	0.505	0.001	0.284	0.143	0.221	0.104
IL-1β	EGF	0.367	0.025	0.311	0.057	-0.028	0.889	0.116	0.405
IL-1β	EOTAXIN	0.072	0.673	0.180	0.272	-0.251	0.199	0.030	0.830
IL-1β	FGF2	-0.036	0.834	0.106	0.521	-0.064	0.747	-0.255	0.063
IL-1β	FLT3	0.050	0.791	0.355	0.050	0.646	<0.001	0.213	0.161
IL-1β	GM-CSF	0.270	0.106	0.220	0.177	0.181	0.355	0.132	0.335
IL-1β	IFN-γ	0.188	0.266	0.318	0.048	0.574	0.001	0.193	0.157
IL-1β	IL-1α	0.564	<0.001	0.620	<0.001	0.720	<0.001	0.620	<0.001
IL-2	EGF	0.101	0.542	0.359	0.027	0.249	0.201	0.257	0.061
IL-2	EOTAXIN	0.496	0.001	0.532	<0.001	0.450	0.016	0.276	0.042
IL-2	FGF2	0.601	<0.001	0.612	<0.001	0.276	0.155	0.357	0.008
IL-2	FLT3	0.642	<0.001	0.804	<0.001	0.410	0.038	0.658	<0.001
IL-2	GM-CSF	0.791	<0.001	0.745	<0.001	0.638	<0.001	0.726	<0.001
IL-2	IFN-γ	0.373	0.019	0.579	<0.001	0.356	0.063	0.522	<0.001
IL-2	IL-1α	-0.295	0.068	0.095	0.564	-0.066	0.738	-0.114	0.409
IL-2	IL-1β	0.139	0.411	0.106	0.521	0.176	0.371	-0.006	0.963
IL-3	EGF	0.313	0.052	0.276	0.094	0.291	0.141	0.317	0.022
IL-3	EOTAXIN	0.590	<0.001	0.466	0.003	0.325	0.098	0.354	0.010
IL-3	FGF2	0.592	<0.001	0.411	0.010	0.389	0.045	0.320	0.021
IL-3	FLT3	0.769	<0.001	0.746	<0.001	0.627	0.001	0.683	<0.001
IL-3	GM-CSF	0.826	<0.001	0.571	<0.001	0.714	<0.001	0.651	<0.001
IL-3	IFN-γ	0.694	<0.001	0.454	0.004	0.737	<0.001	0.640	<0.001
IL-3	IL-1α	-0.047	0.778	0.155	0.352	0.151	0.451	0.167	0.237
IL-3	IL-1β	0.232	0.174	0.345	0.034	0.521	0.005	0.225	0.109
IL-3	IL-2	0.833	<0.001	0.672	<0.001	0.650	<0.001	0.555	<0.001
IL-4	EGF	0.303	0.064	0.295	0.072	0.064	0.747	0.111	0.427
IL-4	EOTAXIN	0.538	<0.001	0.667	<0.001	0.405	0.032	0.398	0.003
IL-4	FGF2	0.334	0.041	0.350	0.029	0.098	0.618	0.139	0.322
IL-4	FLT3	0.433	0.015	0.896	<0.001	-0.043	0.834	0.461	0.002
IL-4	GM-CSF	0.320	0.050	0.606	<0.001	0.135	0.492	0.440	0.001
IL-4	IFN-γ	0.554	<0.001	0.484	0.002	-0.061	0.756	0.323	0.017
IL-4	IL-1α	-0.221	0.182	0.163	0.321	-0.368	0.054	-0.090	0.518
IL-4	IL-1β	0.092	0.588	0.193	0.239	-0.203	0.300	-0.049	0.727
IL-4	IL-2	0.232	0.161	0.581	<0.001	0.488	0.008	0.395	0.003
IL-4	IL-3	0.368	0.025	0.642	<0.001	0.153	0.446	0.311	0.026
IL-5	EGF	0.319	0.042	0.248	0.133	0.591	0.001	0.017	0.901
IL-5	EOTAXIN	0.716	<0.001	0.663	<0.001	0.337	0.080	0.479	<0.001

IL-5	FGF2	0.444	0.004	0.152	0.355	0.317	0.100	0.197	0.154
IL-5	FLT3	0.597	<0.001	0.582	0.001	0.234	0.250	0.484	0.001
IL-5	GM-CSF	0.598	<0.001	0.631	<0.001	0.515	0.005	0.611	<0.001
IL-5	IFN-γ	0.566	<0.001	0.585	<0.001	0.365	0.056	0.580	<0.001
IL-5	IL-1α	-0.024	0.887	0.196	0.231	0.014	0.946	-0.154	0.262
IL-5	IL-1β	0.004	0.980	0.320	0.047	-0.029	0.885	0.068	0.623
IL-5	IL-2	0.476	0.002	0.445	0.004	0.128	0.516	0.394	0.003
IL-5	IL-3	0.554	<0.001	0.401	0.013	0.198	0.322	0.387	0.005
IL-5	IL-4	0.267	0.105	0.620	<0.001	0.001	0.997	0.245	0.074
IL-6	EGF	0.177	0.294	0.284	0.084	0.149	0.479	0.111	0.429
IL-6	EOTAXIN	0.314	0.062	0.588	<0.001	0.252	0.224	0.184	0.183
IL-6	FGF2	0.244	0.146	0.327	0.045	0.289	0.161	0.093	0.506
IL-6	FLT3	0.265	0.158	0.430	0.018	0.293	0.176	0.226	0.140
IL-6	GM-CSF	0.353	0.032	0.306	0.062	0.466	0.019	0.232	0.092
IL-6	IFN-γ	0.340	0.039	0.298	0.069	0.510	0.009	0.382	0.004
IL-6	IL-1α	0.069	0.689	0.286	0.082	0.112	0.596	0.405	0.002
IL-6	IL-1β	0.277	0.113	0.302	0.066	0.352	0.085	0.479	<0.001
IL-6	IL-2	0.173	0.313	0.241	0.144	0.399	0.048	0.090	0.517
IL-6	IL-3	0.409	0.013	0.224	0.176	0.778	<0.001	0.163	0.253
IL-6	IL-4	0.221	0.201	0.271	0.100	-0.011	0.958	0.050	0.722
IL-6	IL-5	0.066	0.698	0.422	0.008	-0.110	0.599	0.079	0.569
IL-7	EGF	0.304	0.054	0.578	<0.001	0.310	0.108	0.363	0.007
IL-7	EOTAXIN	0.630	<0.001	0.520	0.001	0.456	0.015	0.586	<0.001
IL-7	FGF2	0.501	0.001	0.568	<0.001	0.605	0.001	0.394	0.003
IL-7	FLT3	0.443	0.009	0.301	0.099	0.592	0.001	0.406	0.006
IL-7	GM-CSF	0.670	<0.001	0.400	0.012	0.495	0.007	0.415	0.002
IL-7	IFN-γ	0.741	<0.001	0.665	<0.001	0.716	<0.001	0.744	<0.001
IL-7	IL-1α	-0.033	0.844	0.671	<0.001	0.127	0.519	0.165	0.229
IL-7	IL-1β	-0.021	0.903	0.413	0.009	0.274	0.159	0.102	0.460
IL-7	IL-2	0.345	0.032	0.291	0.072	0.126	0.522	0.218	0.109
IL-7	IL-3	0.574	<0.001	0.147	0.379	0.476	0.012	0.450	0.001
IL-7	IL-4	0.174	0.297	0.072	0.662	-0.124	0.529	0.078	0.576
IL-7	IL-5	0.633	<0.001	0.314	0.052	0.266	0.171	0.434	0.001
IL-7	IL-6	0.130	0.442	0.444	0.005	0.396	0.050	0.206	0.134
IL-8	EGF	0.132	0.415	0.327	0.048	0.159	0.427	0.167	0.227
IL-8	EOTAXIN	0.099	0.547	0.175	0.294	-0.189	0.344	-0.066	0.634
IL-8	FGF2	0.136	0.402	0.074	0.661	-0.059	0.769	-0.129	0.351
IL-8	FLT3	0.303	0.087	0.305	0.102	0.013	0.950	0.049	0.752
IL-8	GM-CSF	0.264	0.100	-0.077	0.646	0.273	0.168	-0.165	0.230
IL-8	IFN-γ	0.199	0.218	0.205	0.218	0.211	0.290	-0.171	0.212
IL-8	IL-1α	0.337	0.036	0.281	0.088	0.244	0.219	0.495	<0.001
IL-8	IL-1β	0.527	0.001	0.339	0.038	0.338	0.084	0.464	<0.001
IL-8	IL-2	0.241	0.140	-0.084	0.614	-0.019	0.926	-0.175	0.201
IL-8	IL-3	0.384	0.016	0.058	0.733	0.447	0.022	0.007	0.961
IL-8	IL-4	0.057	0.733	0.259	0.117	-0.177	0.378	-0.077	0.579
IL-8	IL-5	0.145	0.371	0.077	0.645	0.124	0.536	-0.272	0.045
IL-8	IL-6	0.559	<0.001	0.332	0.045	0.565	0.003	0.316	0.020
IL-8	IL-7	0.051	0.756	0.086	0.608	-0.003	0.989	-0.182	0.184
IL-10	EGF	0.473	0.002	0.534	0.001	0.392	0.039	0.407	0.002
IL-10	EOTAXIN	0.504	0.001	0.628	<0.001	0.635	<0.001	0.455	<0.001
IL-10	FGF2	0.519	0.001	0.749	<0.001	0.573	0.001	0.425	0.001
IL-10	FLT3	0.703	<0.001	0.686	<0.001	0.107	0.602	0.617	<0.001
IL-10	GM-CSF	0.686	<0.001	0.795	<0.001	0.688	<0.001	0.633	<0.001
IL-10	IFN-γ	0.660	<0.001	0.646	<0.001	0.347	0.070	0.582	<0.001
IL-10	IL-1α	0.011	0.949	0.333	0.038	-0.233	0.232	-0.032	0.814
IL-10	IL-1β	0.303	0.069	0.248	0.128	-0.134	0.498	-0.116	0.401
IL-10	IL-2	0.403	0.011	0.767	<0.001	0.523	0.004	0.589	<0.001
IL-10	IL-3	0.561	<0.001	0.581	<0.001	0.365	0.061	0.589	<0.001
IL-10	IL-4	0.680	<0.001	0.550	<0.001	0.284	0.143	0.539	<0.001
IL-10	IL-5	0.444	0.004	0.522	0.001	0.520	0.005	0.375	0.005
IL-10	IL-6	0.493	0.002	0.378	0.019	0.252	0.225	0.293	0.032
IL-10	IL-7	0.461	0.002	0.603	<0.001	0.474	0.011	0.391	0.003
IL-10	IL-8	0.440	0.004	-0.138	0.407	0.082	0.684	-0.125	0.361

IL-12p40	EGF	0.357	0.038	0.289	0.082	0.319	0.105	0.331	0.016
IL-12p40	EOTAXIN	0.227	0.196	0.228	0.168	0.011	0.957	0.230	0.094
IL-12p40	FGF2	0.363	0.035	0.563	<0.001	0.233	0.241	0.152	0.278
IL-12p40	FLT3	0.587	0.001	0.624	<0.001	0.504	0.010	0.266	0.080
IL-12p40	GM-CSF	0.479	0.004	0.282	0.086	0.527	0.005	0.351	0.009
IL-12p40	IFN-γ	0.303	0.082	0.110	0.511	0.334	0.089	0.184	0.183
IL-12p40	IL-1α	0.042	0.813	0.191	0.252	0.098	0.628	0.097	0.487
IL-12p40	IL-1β	0.371	0.031	0.282	0.086	0.351	0.073	0.282	0.039
IL-12p40	IL-2	0.442	0.009	0.539	<0.001	0.502	0.008	0.412	0.002
IL-12p40	IL-3	0.569	0.001	0.641	<0.001	0.524	0.006	0.457	0.001
IL-12p40	IL-4	0.267	0.127	0.320	0.050	0.284	0.152	0.244	0.075
IL-12p40	IL-5	-0.176	0.319	-0.041	0.808	0.305	0.122	0.021	0.880
IL-12p40	IL-6	0.434	0.015	0.164	0.332	0.475	0.016	0.308	0.025
IL-12p40	IL-7	0.080	0.654	0.156	0.349	0.149	0.459	0.041	0.767
IL-12p40	IL-8	0.434	0.010	0.101	0.554	0.287	0.147	0.315	0.020
IL-12p40	IL-10	0.455	0.007	0.470	0.003	0.402	0.037	0.368	0.006
IL-12p70	EGF	0.102	0.532	0.255	0.122	0.455	0.017	0.267	0.051
IL-12p70	EOTAXIN	0.523	0.001	0.549	<0.001	0.345	0.078	0.490	<0.001
IL-12p70	FGF2	0.565	<0.001	0.442	0.005	0.535	0.004	0.420	0.002
IL-12p70	FLT3	0.601	<0.001	0.679	<0.001	0.712	<0.001	0.645	<0.001
IL-12p70	GM-CSF	0.763	<0.001	0.696	<0.001	0.681	<0.001	0.680	<0.001
IL-12p70	IFN-γ	0.674	<0.001	0.548	<0.001	0.821	<0.001	0.790	<0.001
IL-12p70	IL-1α	-0.197	0.237	0.208	0.204	0.121	0.548	0.095	0.490
IL-12p70	IL-1β	0.020	0.908	-0.060	0.715	0.448	0.019	-0.033	0.809
IL-12p70	IL-2	0.690	<0.001	0.663	<0.001	0.576	0.002	0.600	<0.001
IL-12p70	IL-3	0.739	<0.001	0.535	0.001	0.870	<0.001	0.646	<0.001
IL-12p70	IL-4	0.293	0.074	0.623	<0.001	-0.023	0.910	0.403	0.003
IL-12p70	IL-5	0.492	0.001	0.495	0.001	0.303	0.125	0.551	<0.001
IL-12p70	IL-6	0.302	0.073	0.347	0.033	0.687	<0.001	0.178	0.199
IL-12p70	IL-7	0.673	<0.001	0.292	0.071	0.639	<0.001	0.546	<0.001
IL-12p70	IL-8	0.130	0.431	-0.193	0.246	0.252	0.205	-0.091	0.507
IL-12p70	IL-10	0.502	0.001	0.690	<0.001	0.427	0.026	0.691	<0.001
IL-12p70	IL-12p40	0.325	0.061	0.298	0.069	0.418	0.030	0.220	0.110
IL-13	EGF	0.325	0.044	0.311	0.057	0.468	0.012	0.351	0.009
IL-13	EOTAXIN	0.434	0.007	0.475	0.002	0.409	0.031	0.470	<0.001
IL-13	FGF2	0.417	0.008	0.490	0.002	0.361	0.059	0.392	0.003
IL-13	FLT3	0.646	<0.001	0.496	0.005	0.340	0.089	0.532	<0.001
IL-13	GM-CSF	0.788	<0.001	0.843	<0.001	0.800	<0.001	0.794	<0.001
IL-13	IFN-γ	0.551	<0.001	0.581	<0.001	0.434	0.021	0.659	<0.001
IL-13	IL-1α	0.043	0.796	0.184	0.263	-0.057	0.774	-0.025	0.857
IL-13	IL-1β	0.334	0.044	0.097	0.558	0.097	0.623	-0.077	0.576
IL-13	IL-2	0.683	<0.001	0.704	<0.001	0.629	<0.001	0.651	<0.001
IL-13	IL-3	0.749	<0.001	0.431	0.007	0.549	0.003	0.487	<0.001
IL-13	IL-4	0.303	0.064	0.434	0.006	0.323	0.094	0.452	0.001
IL-13	IL-5	0.536	<0.001	0.587	<0.001	0.467	0.012	0.629	<0.001
IL-13	IL-6	0.230	0.184	0.272	0.098	0.412	0.040	0.094	0.500
IL-13	IL-7	0.509	0.001	0.461	0.003	0.255	0.191	0.421	0.001
IL-13	IL-8	0.244	0.140	-0.282	0.086	0.209	0.296	-0.186	0.174
IL-13	IL-10	0.587	<0.001	0.797	<0.001	0.642	<0.001	0.675	<0.001
IL-13	IL-12p40	0.298	0.087	0.137	0.412	0.721	<0.001	0.221	0.108
IL-13	IL-12p70	0.620	<0.001	0.745	<0.001	0.544	0.003	0.688	<0.001
IL-15	EGF	0.212	0.200	0.427	0.007	0.469	0.012	0.377	0.005
IL-15	EOTAXIN	0.614	<0.001	0.613	<0.001	0.500	0.007	0.422	0.001
IL-15	FGF2	0.692	<0.001	0.550	<0.001	0.621	<0.001	0.521	<0.001
IL-15	FLT3	0.688	<0.001	0.617	<0.001	0.537	0.005	0.668	<0.001
IL-15	GM-CSF	0.805	<0.001	0.666	<0.001	0.845	<0.001	0.760	<0.001
IL-15	IFN-γ	0.610	<0.001	0.465	0.003	0.740	<0.001	0.688	<0.001
IL-15	IL-1α	-0.168	0.315	0.216	0.187	0.029	0.886	0.012	0.929
IL-15	IL-1β	0.128	0.452	0.133	0.420	0.342	0.075	-0.057	0.679
IL-15	IL-2	0.769	<0.001	0.744	<0.001	0.565	0.002	0.784	<0.001
IL-15	IL-3	0.799	<0.001	0.596	<0.001	0.743	<0.001	0.618	<0.001
IL-15	IL-4	0.419	0.009	0.659	<0.001	0.141	0.473	0.391	0.003
IL-15	IL-5	0.487	0.002	0.433	0.006	0.452	0.016	0.434	0.001

IL-15	IL-6	0.372	0.028	0.443	0.005	0.666	<0.001	0.281	0.039
IL-15	IL-7	0.520	0.001	0.326	0.043	0.625	<0.001	0.430	0.001
IL-15	IL-8	0.368	0.023	0.121	0.470	0.298	0.132	-0.117	0.393
IL-15	IL-10	0.648	<0.001	0.682	<0.001	0.643	<0.001	0.785	<0.001
IL-15	IL-12p40	0.513	0.002	0.549	<0.001	0.587	0.001	0.408	0.002
IL-15	IL-12p70	0.708	<0.001	0.701	<0.001	0.831	<0.001	0.749	<0.001
IL-15	IL-13	0.682	<0.001	0.584	<0.001	0.797	<0.001	0.709	<0.001
IP10	EGF	0.274	0.083	0.076	0.651	0.225	0.250	0.125	0.367
IP10	EOTAXIN	0.036	0.826	0.030	0.858	0.577	0.001	0.064	0.644
IP10	FGF2	0.108	0.501	-0.167	0.309	0.362	0.058	-0.027	0.845
IP10	FLT3	0.228	0.196	0.106	0.570	-0.218	0.284	0.214	0.157
IP10	GM-CSF	0.115	0.481	-0.271	0.095	0.073	0.713	0.201	0.142
IP10	IFN-γ	0.206	0.195	-0.015	0.927	-0.011	0.954	0.126	0.360
IP10	IL-1α	-0.002	0.988	0.393	0.013	-0.464	0.013	0.198	0.148
IP10	IL-1β	-0.065	0.701	0.238	0.145	-0.545	0.003	0.155	0.259
IP10	IL-2	-0.197	0.230	-0.249	0.126	0.144	0.466	0.141	0.305
IP10	IL-3	0.097	0.555	-0.079	0.635	-0.060	0.767	-0.043	0.763
IP10	IL-4	0.186	0.265	-0.026	0.875	0.232	0.235	0.219	0.112
IP10	IL-5	0.094	0.561	-0.070	0.674	0.098	0.620	0.044	0.749
IP10	IL-6	0.421	0.010	0.116	0.488	0.172	0.410	0.448	0.001
IP10	IL-7	0.164	0.307	0.017	0.918	0.166	0.398	-0.078	0.569
IP10	IL-8	0.295	0.064	0.226	0.172	-0.199	0.320	0.187	0.172
IP10	IL-10	0.330	0.035	-0.306	0.058	0.319	0.098	0.229	0.093
IP10	IL-12p40	0.174	0.326	0.082	0.626	-0.231	0.246	0.253	0.064
IP10	IL-12p70	0.120	0.461	-0.096	0.562	0.054	0.790	0.077	0.576
IP10	IL-13	-0.003	0.986	-0.352	0.028	0.041	0.836	0.165	0.229
IP10	IL-15	0.190	0.253	-0.199	0.224	0.171	0.383	0.212	0.120
MCP1	EGF	0.392	0.014	0.254	0.124	0.138	0.493	0.122	0.387
MCP1	EOTAXIN	0.253	0.126	0.173	0.293	0.533	0.004	0.221	0.111
MCP1	FGF2	0.349	0.029	0.214	0.191	0.410	0.034	0.196	0.164
MCP1	FLT3	0.423	0.016	0.532	0.002	0.125	0.553	0.173	0.267
MCP1	GM-CSF	0.430	0.006	0.254	0.119	0.411	0.033	0.177	0.206
MCP1	IFN-γ	0.326	0.043	0.328	0.042	0.194	0.332	0.183	0.188
MCP1	IL-1α	-0.144	0.389	0.431	0.006	-0.341	0.081	0.063	0.652
MCP1	IL-1β	-0.008	0.961	0.478	0.002	-0.141	0.484	0.145	0.300
MCP1	IL-2	0.196	0.238	0.324	0.044	0.557	0.003	0.189	0.175
MCP1	IL-3	0.320	0.050	0.387	0.016	0.426	0.030	0.204	0.155
MCP1	IL-4	0.313	0.055	0.340	0.034	0.269	0.174	0.082	0.560
MCP1	IL-5	0.170	0.302	0.194	0.238	-0.164	0.412	0.109	0.437
MCP1	IL-6	0.611	<0.001	0.295	0.072	0.525	0.007	0.538	<0.001
MCP1	IL-7	0.318	0.048	0.176	0.284	0.316	0.108	0.080	0.570
MCP1	IL-8	0.251	0.124	0.064	0.704	-0.036	0.857	0.235	0.090
MCP1	IL-10	0.592	<0.001	0.281	0.083	0.394	0.042	0.319	0.020
MCP1	IL-12p40	0.460	0.006	0.391	0.015	0.132	0.511	0.324	0.018
MCP1	IL-12p70	0.258	0.113	0.282	0.082	0.442	0.021	0.197	0.156
MCP1	IL-13	0.404	0.012	0.218	0.182	0.346	0.077	0.169	0.226
MCP1	IL-15	0.415	0.010	0.207	0.207	0.512	0.006	0.326	0.017
MCP1	IP10	0.494	0.001	0.411	0.009	0.673	<0.001	0.455	0.001
MIP-1α	EGF	0.415	0.008	0.388	0.016	0.460	0.014	0.190	0.169
MIP-1α	EOTAXIN	0.489	0.002	0.173	0.293	0.415	0.028	0.221	0.105
MIP-1α	FGF2	0.466	0.002	0.347	0.030	0.428	0.023	0.019	0.893
MIP-1α	FLT3	0.450	0.009	0.363	0.045	0.479	0.013	0.272	0.071
MIP-1α	GM-CSF	0.741	<0.001	0.360	0.024	0.726	<0.001	0.304	0.024
MIP-1α	IFN-γ	0.502	0.001	0.552	<0.001	0.645	<0.001	0.358	0.007
MIP-1α	IL-1α	-0.086	0.604	0.402	0.011	0.033	0.866	0.314	0.020
MIP-1α	IL-1β	0.360	0.029	0.399	0.012	0.334	0.082	0.542	<0.001
MIP-1α	IL-2	0.530	0.001	0.465	0.003	0.723	<0.001	0.377	0.005
MIP-1α	IL-3	0.654	<0.001	0.456	0.004	0.839	<0.001	0.198	0.160
MIP-1α	IL-4	0.405	0.012	0.106	0.522	0.086	0.662	0.074	0.597
MIP-1α	IL-5	0.292	0.067	0.271	0.095	0.185	0.346	0.188	0.168
MIP-1α	IL-6	0.692	<0.001	0.310	0.059	0.692	<0.001	0.741	<0.001
MIP-1α	IL-7	0.375	0.017	0.447	0.004	0.421	0.026	0.253	0.062
MIP-1α	IL-8	0.435	0.005	0.082	0.624	0.407	0.035	0.230	0.092

MIP-1α	IL-10	0.675	<0.001	0.402	0.011	0.536	0.003	0.255	0.060
MIP-1α	IL-12p40	0.652	<0.001	0.416	0.009	0.411	0.033	0.437	0.001
MIP-1α	IL-12p70	0.495	0.001	0.348	0.030	0.846	<0.001	0.242	0.075
MIP-1α	IL-13	0.643	<0.001	0.350	0.029	0.596	0.001	0.217	0.111
MIP-1α	IL-15	0.676	<0.001	0.254	0.118	0.766	<0.001	0.343	0.010
MIP-1α	IP10	0.364	0.021	0.144	0.383	0.167	0.396	0.381	0.004
MIP-1α	MCP1	0.715	<0.001	0.557	<0.001	0.602	0.001	0.521	<0.001
PDGF-AA	EGF	0.536	<0.001	0.707	<0.001	0.524	0.004	0.659	<0.001
PDGF-AA	EOTAXIN	0.413	0.009	0.385	0.016	0.249	0.201	0.378	0.004
PDGF-AA	FGF2	0.316	0.044	0.536	<0.001	0.421	0.026	0.458	<0.001
PDGF-AA	FLT3	0.347	0.044	0.445	0.012	0.287	0.156	0.353	0.018
PDGF-AA	GM-CSF	0.202	0.212	0.304	0.060	0.357	0.063	0.396	0.003
PDGF-AA	IFN-γ	0.405	0.009	0.596	<0.001	0.452	0.016	0.498	<0.001
PDGF-AA	IL-1α	0.064	0.701	0.431	0.006	-0.090	0.648	0.243	0.073
PDGF-AA	IL-1β	-0.091	0.592	0.172	0.295	-0.083	0.676	-0.013	0.928
PDGF-AA	IL-2	-0.163	0.321	0.231	0.158	0.072	0.715	0.369	0.006
PDGF-AA	IL-3	0.077	0.642	0.012	0.943	0.378	0.052	0.378	0.006
PDGF-AA	IL-4	0.417	0.009	0.268	0.099	-0.109	0.580	0.191	0.166
PDGF-AA	IL-5	0.306	0.052	0.154	0.349	0.175	0.374	0.037	0.788
PDGF-AA	IL-6	0.253	0.131	0.353	0.030	0.398	0.049	0.427	0.001
PDGF-AA	IL-7	0.270	0.088	0.496	0.001	0.509	0.006	0.439	0.001
PDGF-AA	IL-8	0.085	0.603	0.399	0.013	0.256	0.197	0.129	0.347
PDGF-AA	IL-10	0.443	0.004	0.390	0.014	0.233	0.232	0.541	<0.001
PDGF-AA	IL-12p40	0.075	0.675	0.054	0.747	0.106	0.600	0.316	0.020
PDGF-AA	IL-12p70	0.085	0.604	0.199	0.225	0.488	0.010	0.502	<0.001
PDGF-AA	IL-13	0.045	0.783	0.203	0.216	0.229	0.241	0.401	0.002
PDGF-AA	IL-15	0.207	0.212	0.261	0.108	0.456	0.015	0.557	<0.001
PDGF-AA	IP10	0.387	0.012	0.121	0.464	0.506	0.006	0.281	0.038
PDGF-AA	MCP1	0.448	0.004	0.192	0.241	0.488	0.010	0.451	0.001
PDGF-AA	MIP-1α	0.299	0.061	0.238	0.145	0.464	0.013	0.328	0.014
PDGF-BB	EGF	0.583	<0.001	0.623	<0.001	0.518	0.005	0.354	0.009
PDGF-BB	EOTAXIN	0.549	<0.001	0.309	0.059	0.309	0.110	0.327	0.015
PDGF-BB	FGF2	0.649	<0.001	0.564	<0.001	0.598	0.001	0.393	0.003
PDGF-BB	FLT3	0.492	0.003	0.544	0.002	0.329	0.101	0.424	0.004
PDGF-BB	GM-CSF	0.495	0.001	0.498	0.001	0.257	0.188	0.378	0.004
PDGF-BB	IFN-γ	0.561	<0.001	0.650	<0.001	0.390	0.040	0.457	<0.001
PDGF-BB	IL-1α	-0.118	0.474	0.415	0.010	-0.098	0.620	-0.040	0.774
PDGF-BB	IL-1β	0.025	0.885	0.375	0.021	-0.054	0.785	-0.189	0.167
PDGF-BB	IL-2	0.209	0.202	0.468	0.003	0.107	0.587	0.394	0.003
PDGF-BB	IL-3	0.409	0.010	0.366	0.026	0.206	0.302	0.388	0.004
PDGF-BB	IL-4	0.405	0.012	0.385	0.017	0.025	0.901	0.396	0.003
PDGF-BB	IL-5	0.401	0.009	0.328	0.044	0.429	0.023	0.225	0.098
PDGF-BB	IL-6	0.359	0.029	0.092	0.589	0.296	0.151	0.159	0.252
PDGF-BB	IL-7	0.521	<0.001	0.404	0.012	0.472	0.011	0.396	0.003
PDGF-BB	IL-8	0.164	0.311	0.293	0.078	0.075	0.712	-0.084	0.543
PDGF-BB	IL-10	0.549	<0.001	0.565	<0.001	0.427	0.023	0.591	<0.001
PDGF-BB	IL-12p40	0.367	0.033	0.294	0.077	0.332	0.091	0.316	0.020
PDGF-BB	IL-12p70	0.358	0.023	0.294	0.073	0.400	0.038	0.532	<0.001
PDGF-BB	IL-13	0.261	0.108	0.405	0.012	0.249	0.201	0.399	0.003
PDGF-BB	IL-15	0.433	0.007	0.308	0.060	0.510	0.006	0.544	<0.001
PDGF-BB	IP10	0.438	0.004	0.024	0.886	0.513	0.005	0.240	0.077
PDGF-BB	MCP1	0.572	<0.001	0.275	0.094	0.408	0.034	0.293	0.033
PDGF-BB	MIP-1α	0.486	0.001	0.464	0.003	0.316	0.102	0.089	0.519
PDGF-BB	PDGF-AA	0.578	<0.001	0.607	<0.001	0.665	<0.001	0.596	<0.001
RANTES	EGF	0.274	0.083	0.345	0.034	0.289	0.143	0.159	0.250
RANTES	EOTAXIN	0.558	<0.001	0.243	0.136	0.469	0.014	0.271	0.045
RANTES	FGF2	0.608	<0.001	0.556	<0.001	0.469	0.014	0.443	0.001
RANTES	FLT3	0.467	0.005	0.240	0.194	-0.211	0.311	0.089	0.560
RANTES	GM-CSF	0.442	0.004	0.170	0.300	0.249	0.210	0.102	0.457
RANTES	IFN-γ	0.495	0.001	0.171	0.297	0.229	0.250	0.305	0.024
RANTES	IL-1α	-0.256	0.115	0.328	0.042	-0.113	0.575	0.189	0.168
RANTES	IL-1β	-0.087	0.608	0.118	0.474	-0.206	0.303	0.162	0.236
RANTES	IL-2	0.320	0.047	0.243	0.136	0.029	0.885	0.118	0.393

RANTES	IL-3	0.496	0.001	0.204	0.220	0.215	0.292	0.140	0.322
RANTES	IL-4	0.303	0.064	0.197	0.229	-0.025	0.900	-0.017	0.905
RANTES	IL-5	0.387	0.012	-0.098	0.552	0.110	0.584	0.016	0.910
RANTES	IL-6	0.579	<0.001	0.374	0.021	0.535	0.006	0.570	<0.001
RANTES	IL-7	0.485	0.001	0.329	0.041	0.296	0.134	0.261	0.054
RANTES	IL-8	0.268	0.095	0.450	0.005	0.406	0.036	0.361	0.007
RANTES	IL-10	0.463	0.002	0.260	0.110	0.450	0.019	0.259	0.057
RANTES	IL-12p40	0.437	0.010	0.492	0.002	0.019	0.924	0.453	0.001
RANTES	IL-12p70	0.365	0.021	0.151	0.360	0.248	0.213	0.267	0.049
RANTES	IL-13	0.246	0.130	0.050	0.764	0.197	0.325	0.074	0.593
RANTES	IL-15	0.536	0.001	0.405	0.011	0.337	0.086	0.331	0.014
RANTES	IP10	0.414	0.007	0.179	0.276	0.522	0.005	0.181	0.186
RANTES	MCP1	0.582	<0.001	0.185	0.259	0.421	0.029	0.393	0.004
RANTES	MIP-1α	0.547	<0.001	0.334	0.038	0.449	0.019	0.410	0.002
RANTES	PDGF-AA	0.397	0.010	0.398	0.012	0.551	0.003	0.484	<0.001
RANTES	PDGF-BB	0.633	<0.001	0.387	0.016	0.472	0.013	0.387	0.003
TNF-α	EGF	0.402	0.009	0.505	0.001	0.613	0.001	0.282	0.039
TNF-α	EOTAXIN	0.614	<0.001	0.502	0.001	0.582	0.001	0.561	<0.001
TNF-α	FGF2	0.765	<0.001	0.651	<0.001	0.521	0.004	0.494	<0.001
TNF-α	FLT3	0.493	0.003	0.570	0.001	0.386	0.052	0.501	<0.001
TNF-α	GM-CSF	0.724	<0.001	0.670	<0.001	0.599	0.001	0.708	<0.001
TNF-α	IFN-γ	0.686	<0.001	0.628	<0.001	0.578	0.001	0.665	<0.001
TNF-α	IL-1α	-0.356	0.026	0.254	0.118	-0.079	0.688	-0.128	0.353
TNF-α	IL-1β	-0.053	0.756	0.121	0.465	0.079	0.689	-0.055	0.692
TNF-α	IL-2	0.565	<0.001	0.638	<0.001	0.418	0.027	0.618	<0.001
TNF-α	IL-3	0.660	<0.001	0.318	0.052	0.418	0.030	0.440	0.001
TNF-α	IL-4	0.508	0.001	0.396	0.013	0.260	0.181	0.564	<0.001
TNF-α	IL-5	0.539	<0.001	0.421	0.008	0.410	0.030	0.579	<0.001
TNF-α	IL-6	0.198	0.241	0.334	0.040	0.182	0.384	0.119	0.392
TNF-α	IL-7	0.741	<0.001	0.498	0.001	0.645	<0.001	0.501	<0.001
TNF-α	IL-8	0.076	0.643	-0.178	0.284	-0.095	0.638	-0.356	0.008
TNF-α	IL-10	0.654	<0.001	0.777	<0.001	0.590	0.001	0.582	<0.001
TNF-α	IL-12p40	0.183	0.300	0.242	0.144	0.189	0.346	0.115	0.409
TNF-α	IL-12p70	0.719	<0.001	0.614	<0.001	0.507	0.007	0.548	<0.001
TNF-α	IL-13	0.539	<0.001	0.697	<0.001	0.372	0.051	0.732	<0.001
TNF-α	IL-15	0.631	<0.001	0.482	0.002	0.610	0.001	0.650	<0.001
TNF-α	IP10	0.157	0.326	-0.192	0.242	0.303	0.118	0.052	0.707
TNF-α	MCP1	0.389	0.014	0.358	0.025	0.298	0.132	0.115	0.413
TNF-α	MIP-1α	0.532	<0.001	0.369	0.021	0.469	0.012	0.222	0.103
TNF-α	PDGF-AA	0.319	0.042	0.420	0.008	0.429	0.023	0.364	0.006
TNF-α	PDGF-BB	0.596	<0.001	0.563	<0.001	0.481	0.010	0.378	0.004
TNF-α	RANTES	0.439	0.004	0.150	0.363	0.222	0.266	0.097	0.483
VEGF	EGF	0.358	0.021	0.379	0.019	0.179	0.363	0.178	0.199
VEGF	EOTAXIN	0.104	0.530	0.092	0.581	0.390	0.040	0.095	0.496
VEGF	FGF2	0.146	0.363	0.058	0.728	0.542	0.003	-0.039	0.780
VEGF	FLT3	0.246	0.161	0.236	0.209	0.436	0.026	0.238	0.120
VEGF	GM-CSF	0.170	0.295	0.270	0.101	0.389	0.041	0.221	0.108
VEGF	IFN-γ	0.173	0.280	0.340	0.037	0.643	<0.001	0.356	0.008
VEGF	IL-1α	0.257	0.114	0.521	0.001	0.320	0.097	0.367	0.006
VEGF	IL-1β	0.337	0.041	0.437	0.006	0.360	0.060	0.522	<0.001
VEGF	IL-2	0.042	0.799	0.008	0.961	0.199	0.311	0.050	0.720
VEGF	IL-3	0.216	0.186	0.143	0.393	0.512	0.006	0.290	0.037
VEGF	IL-4	-0.009	0.956	0.137	0.412	-0.129	0.514	0.048	0.732
VEGF	IL-5	0.328	0.037	0.219	0.186	0.120	0.543	0.193	0.163
VEGF	IL-6	0.097	0.568	-0.009	0.956	0.683	<0.001	0.261	0.060
VEGF	IL-7	0.197	0.218	0.252	0.128	0.734	<0.001	0.260	0.058
VEGF	IL-8	0.397	0.011	0.285	0.087	0.163	0.417	0.044	0.753
VEGF	IL-10	0.255	0.108	0.049	0.770	0.360	0.060	-0.051	0.712
VEGF	IL-12p40	-0.040	0.821	0.066	0.698	0.318	0.106	0.085	0.544
VEGF	IL-12p70	0.097	0.553	0.152	0.363	0.621	0.001	0.250	0.068
VEGF	IL-13	0.035	0.833	0.111	0.508	0.305	0.115	0.079	0.569
VEGF	IL-15	0.158	0.343	0.173	0.298	0.594	0.001	<0.00	1.000
VEGF	IP10	0.370	0.017	0.216	0.193	0.089	0.652	-0.008	0.953

VEGF	MCP1	-0.039	0.812	-0.008	0.963	0.275	0.165	-0.065	0.646
VEGF	MIP-1α	0.115	0.481	0.132	0.430	0.469	0.012	0.258	0.060
VEGF	PDGF-AA	0.115	0.475	0.201	0.225	0.354	0.064	0.162	0.241
VEGF	PDGF-BB	0.211	0.185	0.329	0.047	0.426	0.024	0.166	0.229
VEGF	RANTES	0.074	0.645	0.035	0.834	0.464	0.015	0.110	0.429
VEGF	TNF-α	0.168	0.292	-0.049	0.772	0.440	0.019	0.046	0.742

Appendix Table 3. Significant p-values for heterogeneity between correlation coefficients.

Pairwise Correlation		*Comparison*	*Group*	*p*
IL-1β	FLT3	BV^+ vs BV^-	EA	0.033
IL-4	FLT3	BV^+ vs BV^-	EA	0.037
IL-5	EGF	BV^+ vs BV^-	EA	0.007
IL-6	IL-3	BV^+ vs BV^-	EA	0.001
IL-10	FLT3	BV^+ vs BV^-	EA	0.018
IL-12p70	IL-1β	BV^+ vs BV^-	EA	0.037
IL-12p70	IL-3	BV^+ vs BV^-	EA	0.025
IL-12p70	IL-6	BV^+ vs BV^-	EA	0.009
IL-13	IL-12p40	BV^+ vs BV^-	EA	0.006
IL-15	IL-6	BV^+ vs BV^-	EA	0.044
IP10	EOTAXIN	BV^+ vs BV^-	EA	0.015
IP10	IL-1α	BV^+ vs BV^-	EA	0.004
IP10	IL-1β	BV^+ vs BV^-	EA	0.002
IP10	IL-12p40	BV^+ vs BV^-	EA	0.046
MIP-1α	GM-CSF	BV^+ vs BV^-	EA	0.013
MIP-1α	IL-2	BV^+ vs BV^-	EA	0.034
MIP-1α	IL-3	BV^+ vs BV^-	EA	<0.001
MIP-1α	IL-12p70	BV^+ vs BV^-	EA	<0.001
MIP-1α	IL-15	BV^+ vs BV^-	EA	0.007
TNF-α	IL-13	BV^+ vs BV^-	EA	0.026
VEGF	FGF2	BV^+ vs BV^-	EA	0.008
VEGF	IL-6	BV^+ vs BV^-	EA	0.026
VEGF	IL-7	BV^+ vs BV^-	EA	0.006
VEGF	IL-15	BV^+ vs BV^-	EA	0.005
IL-1α	EGF	BV^+ vs BV^-	AA	0.008
IL-1α	FGF2	BV^+ vs BV^-	AA	0.003
IL-3	GM-CSF	BV^+ vs BV^-	AA	0.026
IL-4	FLT3	BV^+ vs BV^-	AA	<0.001
IL-7	IL-1α	BV^+ vs BV^-	AA	<0.001
IL-7	IL-3	BV^+ vs BV^-	AA	0.033
IL-10	IL-2	BV^+ vs BV^-	AA	0.013
IL-10	IL-8	BV^+ vs BV^-	AA	0.009
IL-12p70	IL-7	BV^+ vs BV^-	AA	0.028
IL-13	IL-3	BV^+ vs BV^-	AA	0.035
IL-13	IL-8	BV^+ vs BV^-	AA	0.024
IP10	IL-10	BV^+ vs BV^-	AA	0.005
MCP1	IL-1α	BV^+ vs BV^-	AA	0.011
MCP1	IL-1β	BV^+ vs BV^-	AA	0.027
MIP-1α	GM-CSF	BV^+ vs BV^-	AA	0.014
MIP-1α	IL-1α	BV^+ vs BV^-	AA	0.03
MIP-1α	IL-6	BV^+ vs BV^-	AA	0.028
MIP-1α	IL-15	BV^+ vs BV^-	AA	0.018
PDGF-BB	IL-1α	BV^+ vs BV^-	AA	0.018

RANTES	IL-1α	BV^+ vs BV^-	AA	0.011
RANTES	IL-5	BV^+ vs BV^-	AA	0.029
RANTES	MCP1	BV^+ vs BV^-	AA	0.042
TNF-α	IL-1α	BV^+ vs BV^-	AA	0.007
IL-1β	FLT3	AA vs EA	BV^+	0.011
IL-4	IFN-γ	AA vs EA	BV^+	0.009
IL-5	EOTAXIN	AA vs EA	BV^+	0.035
IL-6	IL-3	AA vs EA	BV^+	0.03
IL-10	FLT3	AA vs EA	BV^+	0.005
IL-10	IL-4	AA vs EA	BV^+	0.04
IL-13	IL-12p40	AA vs EA	BV^+	0.027
IP10	EOTAXIN	AA vs EA	BV^+	0.017
IP10	IL-1β	AA vs EA	BV^+	0.038
MIP-1α	IL-12p70	AA vs EA	BV^+	0.008
PDGF-AA	IL-4	AA vs EA	BV^+	0.034
RANTES	FLT3	AA vs EA	BV^+	0.01
VEGF	IFN-γ	AA vs EA	BV^+	0.022
VEGF	IL-6	AA vs EA	BV^+	0.007
VEGF	IL-7	AA vs EA	BV^+	0.004
VEGF	IL-12p70	AA vs EA	BV^+	0.016
VEGF	IL-15	AA vs EA	BV^+	0.045
FGF2	EGF	AA vs EA	BV^-	0.049
FLT3	EOTAXIN	AA vs EA	BV^-	0.049
IFN-γ	EGF	AA vs EA	BV^-	0.031
IL-1α	EGF	AA vs EA	BV^-	0.02
IL-4	FLT3	AA vs EA	BV^-	<0.001
IL-4	IL-3	AA vs EA	BV^-	0.048
IL-5	IL-4	AA vs EA	BV^-	0.029
IL-6	EOTAXIN	AA vs EA	BV^-	0.026
IL-7	IL-1α	AA vs EA	BV^-	0.003
IL-10	FGF2	AA vs EA	BV^-	0.018
IL-12p40	FGF2	AA vs EA	BV^-	0.028
IL-12p70	IFN-γ	AA vs EA	BV^-	0.036
IP10	GM-CSF	AA vs EA	BV^-	0.026
IP10	IL-10	AA vs EA	BV^-	0.011
IP10	IL-13	AA vs EA	BV^-	0.014
MIP-1α	IL-6	AA vs EA	BV^-	0.004
PDGF-BB	IL-1α	AA vs EA	BV^-	0.028
PDGF-BB	IL-1β	AA vs EA	BV^-	0.007

Appendix Table 4. SNP positional information and ethnic comparisons for anti-inflammatory cytokine genes and receptors

				Allele Frequency		*AA vs EA*		
RS#	*Gene*	*Position*	*Allele*	*EA*	*AA*	*Allele*	*Genotype*	*Genic Region*
rs3024498	IL-10	chr1:205008152	G	0.29	0.24	0.475	0.232	Exon
rs3024496	IL-10	chr1:205008487	C	0.50	0.47	0.585	0.904	Exon
rs1800872	IL-10	chr1:205013030	A	0.16	0.38	<0.001	0.001	Promoter
rs1800896	IL-10	chr1:205013520	A	0.50	0.53	0.586	0.469	Promoter
rs1800890	IL-10	chr1:205015988	A	0.41	0.37	0.5	0.394	Promoter
rs4936414	IL-10RA	chr11:117358823	C	0.38	0.15	<0.001	<0.001	Promoter
rs2512143	IL-10RA	chr11:117365792	A	0.37	0.23	0.034	0.078	Intron
rs4252254	IL-10RA	chr11:117366265	T	0.16	0.30	0.007	0.01	Intron
rs4252270	IL-10RA	chr11:117369056	T	0.16	0.16	1	0.837	Intron
rs2229113	IL-10RA	chr11:117374880	A	0.34	0.15	0.001	0.002	Coding (exon)
rs9610	IL-10RA	chr11:117377296	A	0.37	0.71	<0.001	<0.001	Exon
rs2508445	IL-10RA	chr11:117377754	T	0.34	0.23	0.073	0.124	3' UTR
rs947889	IL-10RA	chr11:117379741	A	0.45	0.71	<0.001	0.001	3' UTR
rs4938467	IL-10RA	chr11:117380381	T	0.41*	0.54	0.017	0.055	3' UTR
rs11216666	IL-10RA	chr11:117381245	C	0.14	0.62	<0.001	<0.001	3' UTR
rs17121510	IL-10RA	chr11:117381365	G	0.09	0.32	<0.001	<0.001	3' UTR
rs2284552	IL-10RB	chr21:33565952	T	0.20	0.15	0.381	0.242	Intron
rs962859	IL-10RB	chr21:33569993	C	0.49	0.32	0.005	0.036	Intron
rs2834168	IL-10RB	chr21:33572661	G	0.41	0.19	0.001	0.002	Intron
rs2834170	IL-10RB	chr21:33574545	C	1.00	0.72	<0.001	<0.001	Intron
rs2243498	IL-10RB	chr21:33575536	A	0.46	0.60	0.046	0.117	Intron
rs2834172	IL-10RB	chr21:33577493	A	1.00	0.69	<0.001	<0.001	Intron (boundary)
rs765429	IL-10RB	chr21:33581871	A	0.49	0.67	0.005	0.018	Intron
rs2276223	IL-10RB	chr21:33582670	G	0.49	0.29	0.002	0.008	Intron
rs735299	IL-10RB	chr21:33586460	G	0.44	0.67	<0.001	0.003	Intron
rs999261	IL-10RB	chr21:33588030	C	0.17	0.06	0.004	0.007	Intron
rs999259	IL-10RB	chr21:33588362	A	0.45	0.62	0.011	0.05	Intron
rs1058867	IL-10RB	chr21:33591251	G	0.42	0.63	0.001	0.007	Exon
rs8178565	IL-10RB	chr21:33591549	G	1.00	0.77*	<0.001	<0.001	3' UTR
rs6517158	IL-10RB	chr21:33593611	C	0.17	0.72	0.037	0.071	3' UTR
rs2834175	IL-10RB	chr21:33594831	A	0.27	0.11	0.002	0.002	3' UTR
rs3091307	IL-13	chr5:132017035	G	0.24	0.56	<0.001	<0.001	Promoter
rs1295686	IL-13	chr5:132023742	A	0.24	0.59	<0.001	<0.001	Intron (boundary)
rs848	IL-13	chr5:132024399	T	0.24	0.46	0.001	0.001	Exon
rs1295683	IL-13	chr5:132026775	T	0.14*	0.06	0.054	0.166	3' UTR
rs2243204	IL-13	chr5:132027393	T	0.09	0.50	<0.001	<0.001	3' UTR
rs2243209	IL-13	chr5:132029213	C	1.00	0.86	<0.001	<0.001	3' UTR
rs2243248	IL-4	chr5:132036543	G	0.13*	0.18	0.214	0.048	Promoter
rs2070874	IL-4	chr5:132037609	T	0.09	0.40	<0.001	<0.001	Exon
rs2227284	IL-4	chr5:132040624	A	0.24	0.78	<0.001	<0.001	Intron
rs2243261	IL-4	chr5:132040705	G	1.00	0.85*	<0.001	<0.001	Intron
rs2243263	IL-4	chr5:132041198	C	0.14	0.11	0.54	0.697	Intron
rs2243268	IL-4	chr5:132041862	C	0.09	0.28	<0.001	<0.001	Intron
rs2243274	IL-4	chr5:132042731	A	0.09	0.62	<0.001	<0.001	Intron
rs2243290	IL-4	chr5:132046068	A	0.09	0.37	<0.001	<0.001	Intron (boundary)
rs4787956	IL4R	chr16:27285750	G	0.34	0.37	0.556	0.243	3' UTR
rs2057768	IL-4R	chr16:27229596	A	0.23	0.31	0.132	0.15	Promoter
rs6498012	IL-4R	chr16:27239475	C	0.30	0.32	0.888	0.833	Intron
rs4787948	IL-4R	chr16:27248560	G	0.25*	0.32	0.249	0.178	Intron
rs3024530	IL-4R	chr16:27258188	G	0.45	0.51	0.444	0.474	Intron
rs3024537	IL-4R	chr16:27260320	A	0.20	0.18	0.729	0.784	Intron
rs3024547	IL-4R	chr16:27261862	T	0.20	0.31	0.088	0.083	Intron
rs3024548	IL-4R	chr16:27262032	G	0.47	0.65	0.003	0.017	Intron

rs3024560	IL-4R	chr16:27264168	G	0.34	0.38	0.494	0.647	Intron
rs2239349	IL-4R	chr16:27266389	T	0.10	0.32	<0.001	<0.001	Intron
rs2239347	IL-4R	chr16:27266522	G	0.45	0.41	0.587	0.791	Intron
rs3024585	IL-4R	chr16:27267345	A	0.41	0.41	0.895	1	Intron
rs3024623	IL-4R	chr16:27272969	G	0.11	0.04	0.027	0.11	Intron
rs4787423	IL-4R	chr16:27274835	C	0.19	0.36	0.003	0.018	Intron
rs3024648	IL-4R	chr16:27275500	C	0.08	0.26	<0.001	0.001	Intron
rs3024658	IL-4R	chr16:27277955	A	0.08	0.33	<0.001	<0.001	Intron
rs3024668	IL-4R	chr16:27279450	A	0.09	0.44	<0.001	<0.001	Intron
rs3024670	IL-4R	chr16:27279896	G	1.00	0.87	<0.001	<0.001	Intron
rs3024675	IL-4R	chr16:27280968	C	0.03	0.25	<0.001	<0.001	Intron
rs3024676	IL-4R	chr16:27281059	A	0.24	0.42	<0.001	<0.001	Intron (boundary)
rs2234898	IL-4R	chr16:27281416	T	0.16	0.51	<0.001	<0.001	Coding (exon)
rs1805013	IL-4R	chr16:27281481	T	0.05	0.02	0.142	0.303	Coding (exon)
rs1805015	IL-4R	chr16:27281681	C	0.23	0.33	0.101	0.234	Coding (exon)
rs1801275	IL-4R	chr16:27281901	G	0.26	0.70	<0.001	<0.001	Coding (exon)
rs1805016	IL-4R	chr16:27282428	G	0.08*	0.35	<0.001	<0.001	Coding (exon)
rs8832	IL-4R	chr16:27283288	A	0.46	0.81	<0.001	<0.001	Exon
rs1029489	IL-4R	chr16:27283718	T	0.41	0.79*	<0.001	<0.001	3' UTR

* indicates deviation from HWE <0.05

Appendix Table 5. Significant SNP or interaction ANOVA models for anti-inflammatory cytokines a) AA, b) EA

a)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-4	IL-4R	rs3024537	AA/AG vs GG	0.120	0.469	0.145	0.042
IL-4	IL-4R	rs3024670	GG vs GT/TT	0.193	0.044	0.444	0.665
IL-4	IL-4R	rs2057768	AA/AG vs GG	0.041	0.239	0.192	0.021
IL-10	IL-10	rs1800872	AA/AC vs CC	0.048	0.603	0.067	0.075
IL-10	IL-10RB	rs2834170	CC vs CT/TT	0.035	0.993	0.018	0.056
IL-10	IL-10RB	rs2243498	AA vs AG/GG	0.007	0.597	0.005	0.013
IL-10	IL-10RB	rs6517158	CC/CT vs TT	0.029	0.040	0.019	0.586
IL-10	IL-10RB	rs2834172	AA vs AG/GG	0.016	0.973	0.023	0.022

b)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-10	IL-10RA	rs2508445	GG vs GT/TT	0.014	0.002*	0.509	0.228
IL-10	IL-10RA	rs2512143	AA/AG vs GG	0.005	$6x10^{-4}$*	0.428	0.364
IL-10	IL-10RA	rs2229113	AA/AG vs GG	0.014	0.002*	0.509	0.228
IL-10	IL-10RA	rs4936414	CC/CT vs TT	$8x10^{-4}$	$1x10^{-4}$*	0.688	0.138
IL-10	IL-10RB	rs2243498	AA/AG vs GG	0.165	0.441	0.143	0.041
IL-10	IL-10RB	rs765429	AA/AG vs GG	0.144	0.485	0.098	0.033
IL-10	IL-10RB	rs2276223	GG/GT vs TT	0.144	0.485	0.098	0.033
IL-10	IL-10RB	rs999261	CC/CT vs TT	0.115	0.031	0.295	0.183
IL-10	IL-10RB	rs6517158	CC/CT vs TT	0.129	0.045	0.267	0.131

[A] Full model p-value. Full model is transformed cytokine concentration = $\mu + \alpha(\text{SNP}) + \beta(\text{BV status}) + \gamma(\text{BV status*SNP}) + \varepsilon$
[B] BV status p-value adjusted for SNP and interaction
[C] SNP p-value adjusted for BV status and interaction
[D] Interaction p-value adjusted for BV status and SNP

Appendix Table 6. SNP positional information and ethnic comparisons for pro-inflammatory cytokine genes and receptors

Marker	*Gene*	*Position*	*Allele*	*Allele Frequency EA*	*AA*	*AA vs EA Allele*	*Genotype*	*Genic Region*
rs17561	IL-1α	chr2:113253454	T	0.18	0.23	0.418	0.224	Coding exon
rs2856838	IL-1α	chr2:113256203	T	0.35*	0.38	0.618	0.134	Intron
rs1878321	IL-1α	chr2:113260665	C	0.40	0.23	0.007	0.014	Promoter
rs2853550	IL-1β	chr2:113303352	T	0.28	0.04	<0.001	<0.001	3' UTR
rs1143643	IL-1β	chr2:113304533	A	0.19	0.37	0.004	0.012	Intron
rs1143634	IL-1β	chr2:113306621	T	0.11	0.19*	0.066	0.239	Coding exon
rs1143630	IL-1β	chr2:113307886	A	0.25	0.02	<0.001	<0.001	Intron
rs1143627	IL-1β	chr2:113310618	T	0.37	0.66	<0.001	<0.001	Promoter
rs1143623	IL-1β	chr2:113312060	C	0.11	0.33	<0.001	<0.001	Promoter
rs4848306	IL-1β	chr2:113314338	A	0.29	0.51	<0.001	0.008	Promoter
rs3917225	IL-1R1	chr2:102227820	G	0.10	0.41	<0.001	<0.001	Promoter
rs949963	IL-1R1	chr2:102228304	A	0.19	0.19	0.868	0.434	Promoter
rs3771202	IL-1R1	chr2:102231187	G	0.05	0.23	<0.001	<0.001	Intron
rs2287047	IL-1R1	chr2:102232572	C	0.29	0.69	<0.001	<0.001	Intron
rs3917254	IL-1R1	chr2:102235036	A	0.37	0.11	<0.001	<0.001	Intron
rs3917273	IL-1R1	chr2:102238237	A	0.20	0.52	<0.001	<0.001	Intron
rs3917292	IL-1R1	chr2:102241570	G	1.00	0.94	0.001	0.019	Intron
rs2160227	IL-1R1	chr2:102241873	C	0.47	0.71*	<0.001	<0.001	Intron
rs3917296	IL-1R1	chr2:102243351	G	0.02	0.08	0.073	0.067	Intron
rs951193	IL-1R1	chr2:102244317	G	1.00	0.97	0.040	0.127	Intron
rs3917304	IL-1R1	chr2:102246643	T	0.27	0.29*	0.775	0.849	Intron
rs3917306	IL-1R1	chr2:102247357	G	0.31	0.03*	<0.001	<0.001	Intron
rs3171845	IL-1R1	chr2:102249899	A	0.13	0.11	0.840	0.646	Intron
rs2110726	IL-1R1	chr2:102252800	T	0.07	0.27	<0.001	<0.001	Exon
rs3732131	IL-1R1	chr2:102253121	C	0.19	0.08	0.005	0.008	Exon
rs3917332	IL-1R1	chr2:102255042	A	0.13	0.23	0.040	0.138	3' UTR
rs11884283	IL-1R2	chr2:102062287	T	0.37	0.30	0.269	0.284	Promoter
rs6543105	IL-1R2	chr2:102062817	A	0.15	0.39	<0.001	0.001	Promoter
rs12467316	IL-1R2	chr2:102063167	A	0.20*	0.27	0.264	0.033	Promoter
rs4141134	IL-1R2	chr2:102065044	C	0.19	0.46	<0.001	<0.001	Promoter
rs4851520	IL-1R2	chr2:102068793	C	0.10	0.14	0.226	0.571	Intron
rs4851521	IL-1R2	chr2:102068874	T	0.42	0.42	0.895	0.740	Intron
rs4851522	IL-1R2	chr2:102069346	T	0.38	0.13	<0.001	<0.001	Intron
rs4321386	IL-1R2	chr2:102071531	G	0.13	0.11	0.538	0.772	Intron
rs1108338	IL-1R2	chr2:102072099	A	0.47	0.75	<0.001	<0.001	Intron
rs7561191	IL-1R2	chr2:102075496	A	0.41	0.41	0.900	0.486	Intron
rs4851526	IL-1R2	chr2:102076888	G	0.46	0.41	0.490	0.448	Intron
rs4851527	IL-1R2	chr2:102080894	A	0.27	0.47	0.003	0.007	Intron
rs2302589	IL-1R2	chr2:102083304	A	0.25*	0.12	0.010	0.045	Intron
rs3218861	IL-1R2	chr2:102085640	T	0.21	0.00	<0.001	<0.001	Intron
rs2160140	IL-1R2	chr2:102088048	T	0.08	0.27	<0.001	0.001	Intron
rs3218883	IL-1R2	chr2:102088791	A	0.23	0.12	0.032	0.086	Intron
rs3218927	IL-1R2	chr2:102092844	C	0.23	0.12	0.036	0.092	Intron
rs2072474	IL-1R2	chr2:102097727	C	0.41	0.16	<0.001	<0.001	Intron
rs3218979	IL-1R2	chr2:102100645	A	0.17	0.14	0.582	0.684	Intron
rs733498	IL-1R2	chr2:102102043	A	0.38	0.15	<0.001	0.001	Intron
rs3218987	IL-1R2	chr2:102103408	A	0.23	0.00	<0.001	<0.001	3' UTR
rs7589525	IL-1R2	chr2:102104909	C	0.08	0.27*	<0.001	0.002	3' UTR
rs4851531	IL-1R2	chr2:102107127	C	0.45	0.58	0.069	0.202	3' UTR
rs9817203	IL-1RAP	chr3:191711050	T	0.27	0.04*	<0.001	<0.001	Promoter
rs6800625	IL-1RAP	chr3:191717029	C	0.25	0.02	<0.001	<0.001	Intron
rs7628333	IL-1RAP	chr3:191720499	T	0.32	0.30	0.764	0.710	Intron
rs3821744	IL-1RAP	chr3:191720745	G	0.33	0.69	<0.001	<0.001	Intron

rs7615368	IL-1RAP	chr3:191723292	T	0.20	0.02	<0.001	<0.001	Intron
rs7615533	IL-1RAP	chr3:191723446	G	0.35	0.04	<0.001	<0.001	Intron
rs3773994	IL-1RAP	chr3:191726419	A	0.26	0.05	<0.001	<0.001	Intron
rs9290936	IL-1RAP	chr3:191735337	T	0.23	0.13*	0.037	0.028	Intron
rs9849030	IL-1RAP	chr3:191739881	G	0.21	0.13	0.120	0.182	Intron
rs2059020	IL-1RAP	chr3:191740326	T	0.11	0.17	0.171	0.218	Intron
rs2361832	IL-1RAP	chr3:191742739	G	0.33	0.21	0.034	0.135	Intron
rs7626071	IL-1RAP	chr3:191744368	G	0.25	0.06	<0.001	<0.001	Intron
rs3773990	IL-1RAP	chr3:191744754	T	0.12	0.08	0.387	0.465	Intron
rs2193880	IL-1RAP	chr3:191745501	A	0.10	0.49	<0.001	<0.001	Intron
rs3773989	IL-1RAP	chr3:191748131	T	0.12	0.38	<0.001	<0.001	Intron
rs1988743	IL-1RAP	chr3:191750602	C	0.35	0.70	<0.001	<0.001	Intron
rs4686554	IL-1RAP	chr3:191756520	T	0.43	0.70	<0.001	<0.001	Intron
rs16865597	IL-1RAP	chr3:191758247	C	0.24	0.10	0.004	0.020	Intron
rs2885370	IL-1RAP	chr3:191759697	G	0.12*	0.28	0.002	0.002	Intron
rs3773983	IL-1RAP	chr3:191762568	T	0.12	0.07	0.164	0.321	Intron
rs3773982	IL-1RAP	chr3:191762754	T	0.40	0.16	<0.001	<0.001	Intron
rs3773981	IL-1RAP	chr3:191762867	C	0.43	0.16	<0.001	<0.001	Intron
rs9883249	IL-1RAP	chr3:191764078	A	0.18	0.54	<0.001	<0.001	Intron
rs9845825	IL-1RAP	chr3:191764293	T	0.30	0.64	<0.001	<0.001	Intron
rs2241343	IL-1RAP	chr3:191765416	G	0.31	0.14	0.001	0.013	Intron
rs9877268	IL-1RAP	chr3:191768524	A	0.12	0.33	<0.001	<0.001	Intron
rs3773977	IL-1RAP	chr3:191769911	G	0.20	0.21	0.879	0.953	Intron
rs3773976	IL-1RAP	chr3:191769987	C	0.13	0.17	0.253	0.029	Intron
rs4687151	IL-1RAP	chr3:191773332	G	0.18	0.33	0.012	0.045	Intron
rs11929157	IL-1RAP	chr3:191776207	G	0.21	0.24	0.625	0.357	Intron
rs10937439	IL-1RAP	chr3:191776257	G	0.50	0.42	0.243	0.448	Intron
rs10937442	IL-1RAP	chr3:191776910	C	0.49	0.44	0.425	0.760	Intron
rs2161058	IL-1RAP	chr3:191776992	T	0.20	0.01	<0.001	<0.001	Intron
rs1035347	IL-1RAP	chr3:191779637	G	0.45	0.30	0.021	0.089	Intron
rs1559018	IL-1RAP	chr3:191779785	G	0.36	0.29	0.311	0.542	Intron
rs12053868	IL-1RAP	chr3:191782706	G	0.02	0.16	<0.001	<0.001	Intron
rs6444435	IL-1RAP	chr3:191786507	G	0.38	0.30	0.231	0.440	Intron
rs4687154	IL-1RAP	chr3:191786874	G	0.05	0.14	0.016	0.038	Intron
rs10513854	IL-1RAP	chr3:191788576	T	0.18	0.18	0.869	0.447	Intron
rs7628250	IL-1RAP	chr3:191791229	G	0.06	0.18	0.005	0.032	Intron
rs3773958	IL-1RAP	chr3:191792305	G	0.10	0.19	0.063	0.149	Intron
rs3773953	IL-1RAP	chr3:191797422	T	0.09	0.13	0.284	0.809	Intron
rs1469007	IL-1RAP	chr3:191807272	G	0.40	0.30	0.097	0.256	Intron
rs9875362	IL-1RAP	chr3:191808349	T	0.17	0.08	0.043	0.033	Intron
rs6781037	IL-1RAP	chr3:191810223	A	0.47	0.70	0.001	0.004	Intron
rs6765375	IL-1RAP	chr3:191810454	A	0.34	0.62	<0.001	<0.001	Intron
rs9821002	IL-1RAP	chr3:191812153	A	0.33	0.70	<0.001	<0.001	Intron
rs759783	IL-1RAP	chr3:191813479	T	0.41	0.27	0.026	0.024	Intron
rs4140711	IL-1RAP	chr3:191815356	C	0.38	0.26	0.033	0.048	Intron
rs9290939	IL-1RAP	chr3:191816578	A	0.13	0.08	0.271	0.333	Intron
rs1015704	IL-1RAP	chr3:191821114	A	0.49	0.67	0.005	0.024	Intron
rs1015705	IL-1RAP	chr3:191821361	A	0.38	0.27	0.046	0.164	Intron
rs4687163	IL-1RAP	chr3:191824141	G	0.39	0.27	0.033	0.082	Intron
rs929729	IL-1RAP	chr3:191825964	A	0.42	0.31	0.098	0.030	Intron
rs1024941	IL-1RAP	chr3:191829360	T	0.03	0.11	0.023	0.038	Intron
rs1024946	IL-1RAP	chr3:191830225	A	0.47	0.66*	0.002	0.006	Intron
rs1024949	IL-1RAP	chr3:191830434	C	0.02*	0.09	0.040	0.005	Intron
rs7626795	IL-1RAP	chr3:191833163	G	0.37	0.16	<0.001	0.001	Intron
rs4624606	IL-1RAP	chr3:191836948	A	0.35	0.26	0.152	0.149	Intron
rs9847868	IL-1RAP	chr3:191840234	C	0.43	0.27	0.008	0.013	Intron
rs9821122	IL-1RAP	chr3:191842158	G	0.46	0.73	<0.001	<0.001	Intron
rs4320092	IL-1RAP	chr3:191843717	T	0.39	0.73	<0.001	<0.001	Intron
rs9831803	IL-1RAP	chr3:191844199	T	0.48	0.18	<0.001	<0.001	Intron

rs7650510	IL-1RAP	chr3:191845818	G	0.27	0.25	0.643	0.405	Intron
rs11915384	IL-1RAP	chr3:191853466	G	0.47	0.09	<0.001	<0.001	3' UTR
rs17042917	IL-1RN	chr2:113586894	A	0.17	0.13	0.249	0.326	Promoter
rs315920	IL-1RN	chr2:113589249	T	0.13	0.23*	0.085	0.043	Promoter
rs4251961	IL-1RN	chr2:113590698	C	0.13	0.34	<0.001	0.004	Promoter
rs2637988	IL-1RN	chr2:113593010	A	0.42	0.59	0.008	0.006	Intron
rs928940	IL-1RN	chr2:113593726	G	0.34	0.13*	<0.001	<0.001	Intron
rs3213448	IL-1RN	chr2:113595528	A	0.20	0.13*	0.158	0.044	Intron
rs1794066	IL-1RN	chr2:113602581	G	0.40	0.41	0.898	0.237	Intron
rs380092	IL-1RN	chr2:113605131	A	0.33	0.68	<0.001	<0.001	Intron
rs579543	IL-1RN	chr2:113605862	T	0.15	0.31	0.003	0.009	Intron
rs315951	IL-1RN	chr2:113606817	C	0.38	0.30	0.227	0.139	Exon
rs315949	IL-1RN	chr2:113609005	T	0.38	0.37	0.889	0.344	3' UTR
rs315946	IL-1RN	chr2:113610095	T	0.03	0.15	0.001	0.015	3' UTR
rs315943	IL-1RN	chr2:113610569	C	0.40	0.37	0.558	0.152	3' UTR
rs315942	IL-1RN	chr2:113611376	A	0.31	0.32	0.886	0.865	3' UTR
rs1880243	IL-6	chr7:22532895	A	0.18	0.20	0.617	0.570	Promoter
rs12700386	IL-6	chr7:22536249	G	0.20	0.20	0.873	0.744	Promoter
rs1800797	IL-6	chr7:22539461	A	0.09	0.30	<0.001	<0.001	Promoter
rs1800796	IL-6	chr7:22539486	C	0.06	0.05	1.000	1.000	Promoter
rs1800795	IL-6	chr7:22539885	C	0.09	0.31	<0.001	<0.001	Promoter
rs2069840	IL-6	chr7:22541812	G	0.19	0.45	<0.001	0.001	Intron
rs1554606	IL-6	chr7:22541947	T	0.30	0.30	1.000	0.838	Intron
rs11766273	IL-6	chr7:22548903	A	0.03	0.09	0.027	0.145	3' UTR
rs952146	IL-6R	chr1:151182001	G	0.36*	0.38	0.598	0.008	Promoter
rs1552481	IL-6R	chr1:151189426	G	0.18	0.01	<0.001	<0.001	Promoter
rs6427641	IL-6R	chr1:151193559	A	0.38*	0.56	0.006	0.006	Intron
rs11265610	IL-6R	chr1:151193857	C	0.21	0.00	<0.001	<0.001	Intron
rs1386821	IL-6R	chr1:151195122	C	0.13	0.16*	0.567	0.163	Intron
rs4075015	IL-6R	chr1:151202269	A	0.13	0.41	<0.001	<0.001	Intron
rs4601580	IL-6R	chr1:151207490	A	0.49	0.51	0.895	0.909	Intron
rs4845618	IL-6R	chr1:151213088	T	0.43	0.54	0.121	0.227	Intron
rs6687726	IL-6R	chr1:151213393	G	0.38	0.54	0.019	0.034	Intron
rs7549338	IL-6R	chr1:151217453	C	0.39	0.45	0.428	0.601	Intron
rs4553185	IL-6R	chr1:151224028	T	0.40	0.55	0.018	0.036	Intron
rs4845622	IL-6R	chr1:151224492	C	0.13	0.33	0.001	0.002	Intron
rs4845623	IL-6R	chr1:151228850	A	0.44	0.67	<0.001	0.001	Intron
rs4537545	IL-6R	chr1:151231952	C	0.40	0.67	<0.001	<0.001	Intron
rs4845625	IL-6R	chr1:151235140	T	0.37	0.45	0.229	0.446	Intron
rs4845374	IL-6R	chr1:151240020	A	0.21	0.23	0.873	0.950	Intron
rs11265618	IL-6R	chr1:151243165	T	0.29	0.23	0.230	0.533	Intron
rs10752641	IL-6R	chr1:151245115	G	0.38	0.22	0.009	0.019	Intron
rs4329505	IL-6R	chr1:151245493	C	0.36	0.22	0.027	0.049	Intron
rs2229238	IL-6R	chr1:151250969	T	0.24*	0.19	0.360	0.077	Exon
rs4072391	IL-6R	chr1:151251953	T	0.38	0.19	0.002	0.007	Exon
rs7526293	IL-6R	chr1:151257282	T	0.47	0.19	<0.001	<0.001	3' UTR
rs2227538	IL-8	chr4:74971410	T	0.21	0.01	<0.001	<0.001	Exon
rs4694178	IL-8	chr4:74977723	A	0.25	0.48	<0.001	0.001	3' UTR
rs4694637	IL-8	chr4:74977869	A	0.27	0.48	0.001	0.003	3' UTR
rs1008563	IL-8RA	chr2:218852394	T	0.39	0.42	0.672	0.726	3' UTR
rs1008562	IL-8RA	chr2:218852478	G	0.31	0.46	0.030	0.087	3' UTR
rs2854386	IL-8RA	chr2:218853008	C	0.36	0.05	<0.001	<0.001	3' UTR
rs16858856	IL-8RA	chr2:218858287	C	0.13	0.00	<0.001	<0.001	Promoter
rs2844482	TNF-α	chr6:31647746	A	0.10	0.15	0.295	0.506	Promoter
rs1800683	TNF-α	chr6:31648050	A	0.43	0.34	0.186	0.191	Promoter
rs1799964	TNF-α	chr6:31650287	C	0.14	0.17	0.606	0.896	3' UTR
rs1800629	TNF-α	chr6:31651010	A	0.13	0.15	0.859	0.908	Promoter
rs769178	TNF-α	chr6:31655493	A	0.03	0.12	0.005	0.011	3' UTR
rs2229094	TNF-α	chr6:31648535	C	0.28	0.23	0.358	0.718	Promoter

rs740841	TNFR1	chr12:6303550	C	0.46	0.48	0.897	0.878	3' UTR
rs2302350	TNFR1	chr12:6306014	T	0.17	0.53*	<0.001	<0.001	3' UTR
rs1800693	TNFR1	chr12:6310270	G	0.30	0.44	0.019	0.083	Intron (boundary)
rs1860545	TNFR1	chr12:6317038	T	0.11	0.44	<0.001	<0.001	Intron
rs4149578	TNFR1	chr12:6317698	A	0.32	0.06	<0.001	<0.001	Intron
rs4149577	TNFR1	chr12:6317783	C	0.20	0.49	<0.001	<0.001	Intron
rs4149622	TNFR1	chr12:6320900	G	0.47	0.00	<0.001	<0.001	Intron
rs4149570	TNFR1	chr12:6321851	T	0.18	0.41	<0.001	0.001	Promoter
rs11064145	TNFR1	chr12:6325359	T	0.41	0.53	0.088	0.151	Promoter
rs3764874	TNFR1	chr12:6328535	G	0.14*	0.23	0.096	0.156	Promoter
rs590368	TNFR2	chr1:12157717	T	0.13	0.38	<0.001	<0.001	Promoter
rs522807	TNFR2	chr1:12158505	T	0.37*	0.07	<0.001	<0.001	Promoter
rs652625	TNFR2	chr1:12159617	T	0.31	0.09	<0.001	<0.001	Promoter
rs496888	TNFR2	chr1:12167072	G	0.34	0.30	0.570	0.747	Intron
rs976881	TNFR2	chr1:12168020	A	0.26	0.27	0.887	0.358	Intron
rs3766730	TNFR2	chr1:12174942	T	0.04	0.15	0.007	0.002	Intron
rs616645	TNFR2	chr1:12175090	C	0.25	0.21	0.459	0.732	Intron
rs816050	TNFR2	chr1:12176257	T	0.31	0.17	0.019	0.042	Intron
rs474247	TNFR2	chr1:12180441	A	0.12	0.26	0.005	0.014	Intron
rs1201157	TNFR2	chr1:12183297	T	0.38	0.39	0.896	0.845	Intron (boundary)
rs653667	TNFR2	chr1:12186074	C	0.31	0.34	0.680	0.167	Intron (boundary)
rs1061622	TNFR2	chr1:12187221	G	0.24	0.25*	0.765	0.577	Coding exon
rs5746051	TNFR2	chr1:12196238	G	0.08	0.19	0.019	0.019	Intron (boundary)
rs5746053	TNFR2	chr1:12196564	A	0.13	0.19	0.220	0.428	Intron (boundary)
rs235219	TNFR2	chr1:12198621	A	0.02	0.15	<0.001	0.003	Intron
rs1061624	TNFR2	chr1:12201531	G	0.31	0.51	0.003	0.014	Exon
rs1061628	TNFR2	chr1:12202265	C	0.49	0.55	0.438	0.606	Exon
rs1061631	TNFR2	chr1:12202765	A	0.10	0.28	<0.001	0.002	Exon
rs235214	TNFR2	chr1:12205769	T	0.15	0.12	0.437	0.275	3' UTR

* indicates deviation from HWE <0.05

Appendix Table 7. Significant SNP or interaction ANOVA models for pro-inflammatory cytokines a) AA, b) EA

a)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-1α	IL-1α	rs1878321	CC/CT vs TT	0.005	0.975	0.001	0.017
IL-1α	IL-1RAP	rs1469007	GG vs GT/TT	0.004	0.998	0.435	0.012
IL-1α	IL-1RAP	rs1469007	Additive	0.015	0.732	0.063	0.041
IL-1β	IL-1R2	rs4851521	CC/CT vs TT	0.032	0.025	0.061	0.155
IL-1β	IL-1R2	rs7561191	AA vs AT/TT	0.032	0.025	0.061	0.155
IL-1β	IL-1RAP	rs1015704	AA/AG vs GG	0.101	0.599	0.748	0.043
IL-1β	IL-1RAP	rs1015705	AA/AC vs CC	0.048	0.565	0.061	0.017
IL-1β	IL-1RAP	rs1469007	GG vs GT/TT	0.014	0.915	0.639	0.004
IL-1β	IL-1RAP	rs1469007	Additive	0.044	0.717	0.535	0.016
IL-1β	IL-1RAP	rs4624606	AA/AT vs TT	0.108	0.620	0.095	0.047
IL-1β	IL-1RAP	rs6765375	AA/AC vs CC	0.009	0.249	0.197	0.003
IL-1β	IL-1RAP	rs6781037	AA vs AG/GG	0.012	0.033	0.011	0.014
IL-1β	IL-1RAP	rs6781037	Additive	0.025	0.041	0.081	0.035
IL-1β	IL-1RAP	rs9821002	AA/AC vs CC	0.017	0.133	0.192	0.008
IL-6	IL-6R	rs10752641	CC vs CG/GG	0.023	0.038	0.029	0.079
IL-6	IL-6R	rs4553185	CC vs CT/TT	0.023	0.985	0.166	0.017
IL-8	IL-8RA	rs1008562	CC vs CG/GG	-	-	-	0.012
IL-8	IL-8RA	rs1008563	CC vs CT/TT	-	-	-	0.047
TNF-α[E]	TNFR2	rs1201157	CC vs CT/TT	-	-	-	0.006

b)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-1α	IL-1RAP	rs9831803	GG vs GT/TT	0.018	0.340	0.415	0.045
IL-1β	IL-1RAP	rs1015705	AA/AC vs CC	0.013	0.030	0.030	0.048
IL-1β	IL-1RAP	rs4140711	CC/CT vs TT	0.010	0.023	0.049	0.037
IL-1β	IL-1RAP	rs4687163	AA vs AG/GG	0.008	0.019	0.028	0.031
IL-1β	IL-1RAP	rs759783	CC vs CT/TT	0.013	0.030	0.030	0.048
IL-1β	IL-1RAP	rs7628250	AA vs AG/GG	0.008	0.302	0.265	0.007
IL-1β	IL-1RAP	rs9290939	AA/AG vs GG	0.008	0.010	0.013	0.459
IL-6	IL-6	rs2069840	CC/CG vs GG	0.142	0.319	0.106	0.027
IL-6	IL-6R	rs4075015	AA/AT vs TT	0.029	0.048	0.083	0.004

[A] Full model p-value. Full model is transformed cytokine concentration = $\mu + \alpha(\text{SNP}) + \beta(\text{BV status}) + \gamma(\text{BV status*SNP}) + \varepsilon$

[B] BV status p-value adjusted for SNP and interaction

[C] SNP p-value adjusted for BV status and interaction

[D] Interaction p-value adjusted for BV status and SNP

[E] Indicates that Kruskal-Wallis non-parametric tests were used and therefore only the interaction p-value was calculated

Appendix Table 8. SNP positional information and ethnic comparisons for toll-like receptors

Marker	Gene	Position	Allele	*Allele Frequency* EA	AA	*AA vs EA* Allelic	Genotypic	Genic Region
rs1898830	TLR2	chr4:154966058	G	0.37	0.12	<0.001	<0.001	Promoter
rs4696483	TLR2	chr4:154976860	T	0.11	0.43	<0.001	<0.001	Intron
rs7656411	TLR2	chr4:154985260	G	0.24	0.57	<0.001	<0.001	3' UTR
rs1337	TLR2	chr4:154989192	C	0.23	0.07	0.001	0.002	3' UTR
rs10759930	TLR4	chr9:117541175	T	0.40*	0.08	<0.001	<0.001	Promoter
rs16906053	TLR4	chr9:117541736	C	0.01	0.25	<0.001	<0.001	Promoter
rs2770150	TLR4	chr9:117542693	C	0.30	0.17	0.014	0.070	Promoter
rs10759932	TLR4	chr9:117544698	C	0.13	0.25	0.009	0.037	Promoter
rs1927911	TLR4	chr9:117549608	T	0.25	0.62	<0.001	<0.001	Intron
rs1927907	TLR4	chr9:117552318	C	0.13	0.25	0.013	0.060	Intron
rs2149356	TLR4	chr9:117553753	A	0.29	0.74	<0.001	<0.001	Intron
rs7869402	TLR4	chr9:117557586	T	0.02	0.24	<0.001	<0.001	Exon
rs11536889	TLR4	chr9:117557685	C	0.13	0.04	0.012	<0.001	3' UTR
rs1927906	TLR4	chr9:117559669	G	0.05	0.41	<0.001	<0.001	3' UTR
rs11536898	TLR4	chr9:117559764	A	0.12	0.18	0.147	0.020	3' UTR
rs1554973	TLR4	chr9:117560366	C	0.20	0.66	<0.001	<0.001	3' UTR
rs7856729	TLR4	chr9:117561410	T	0.16*	0.27	0.034	0.004	3' UTR

* indicates deviation from HWE <0.05

Appendix Table 9: Significant SNP or interaction ANOVA models for toll-like receptors a) AA, b) EA

a)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-10	TLR4	rs1927906	AA vs AG/GG	0.024	0.033	0.036	0.735
IL-10	TLR4	rs10759932	CC/CT vs TT	0.027	0.038	0.026	0.603
IL-12(p40)	TLR4	rs7869402	CC vs CT/TT	0.046	0.025	0.148	0.347
IL-12(p70)	TLR2	rs7656411	GG vs GT/TT	0.004	0.257	0.004	0.008
IL-12(p70)	TLR2	rs7656411	GG/GT vs TT	0.019	0.466	0.056	0.027
IL-13	TLR2	rs4696483	CC vs CT/TT	0.046	0.126	0.917	0.020
IL-13	TLR4	rs10759932	CC/CT vs TT	0.055	0.018	0.206	0.727
IL-13	TLR4	rs1927907	AA/AG vs GG	0.077	0.024	0.152	0.967
IL-13	TLR4	rs1927911	CC/CT vs TT	0.077	0.033	0.106	0.497
IP-10	TLR4	rs11536898	AA/AC vs CC	0.026	0.006	0.990	0.499
IP-10	TLR4	rs7856729	GG vs GT/TT	0.057	0.009	0.744	0.952
IP-10	TLR4	rs7869402	CC vs CT/TT	0.176	0.738	0.525	0.044
TNF-α[E]	TLR2	rs7656411	GG vs GT/TT	-	-	-	0.004
TNF-α[E]	TLR2	rs7656411	Additive	-	-	-	0.005
TNF-α[E]	TLR4	rs2149356	AA vs AC/CC	-	-	-	0.045

b)

Cytokine	*Gene*	*RS#*	*Model*	*Model*[A]	*SNP*[B]	*BV*[C]	*Intxn*[D]
IL-10	TLR4	rs2149356	AA/AC vs CC	0.113	0.033	0.269	0.133
IL-12(p40)	TLR4	rs2770150	CC/CT vs TT	0.043	0.009	0.988	0.816
IL-12(p40)	TLR4	rs2149356	AA/AC vs CC	0.193	0.040	0.762	0.690
IL-12(p40)	TLR4	rs1927911	CC vs CT/TT	0.205	0.045	0.770	0.752
IL-12(p70)	TLR2	rs1337	CC/CG vs GG	0.027	0.023	0.749	0.006
IL-12(p70)	TLR2	rs1337	CC/CG vs GG	0.027	0.023	0.749	0.006
IL-12(p70)	TLR4	rs2149356	AA/AC vs CC	0.071	0.009	0.848	0.316
IL-12(p70)	TLR4	rs1927911	CC vs CT/TT	0.117	0.016	0.781	0.297
IL-12(p70)	TLR4	rs2770150	CC/CT vs TT	0.126	0.020	0.687	0.581
IL-13	TLR2	rs1337	CC/CG vs GG	0.168	0.090	0.716	0.045
IL-13	TLR4	rs10759930	CC vs CT/TT	0.234	0.907	0.511	0.045
IL-1β	TLR4	rs1554973	CC/CT vs TT	0.000	0.000	0.090	0.940
IL-1β	TLR4	rs2149356	AA/AC vs CC	0.001	0.004	0.027	0.352
IL-1β	TLR4	rs1927911	CC vs CT/TT	0.006	0.013	0.028	0.664
IL-1β	TLR4	rs2770150	CC/CT vs TT	0.010	0.042	0.053	0.400
IL-6	TLR4	rs2149356	AA/AC vs CC	0.005	0.009	0.559	0.280
IL-6	TLR4	rs1554973	CC/CT vs TT	0.030	0.011	0.413	0.993
IL-6	TLR4	rs1927911	CC vs CT/TT	0.023	0.018	0.648	0.470
TNF-α[E]	TLR4	rs2149356	AA/AC vs CC	-	-	-	0.0375
TNF-α[E]	TLR4	rs1927911	CC vs CT/TT	-	-	-	0.048

[A] Full model p-value. Full model is transformed cytokine concentration = $\mu + \alpha(SNP) + \beta(BV\ status) + \gamma(BV\ status*SNP) + \varepsilon$

[B] BV status p-value adjusted for SNP and interaction

[C] SNP p-value adjusted for BV status and interaction

[D] Interaction p-value adjusted for BV status and SNP

[E] Indicates that Kruskal-Wallis non-parametric tests were used and therefore only the interaction p-value was calculated

Appendix Table 10. TaqSNPs genotyped for study.

RS#	Gene	RS#	Gene	RS#	Gene
rs4295	ACE	rs543810	IL18	rs4975220	PGRMC2
rs4305	ACE	rs5744280	IL18	rs4975180	PGRMC2
rs4311	ACE	rs360722	IL18	rs3733260	PGRMC2
rs4362	ACE	rs4937113	IL18	rs3820185	PLA2G4A
rs4461142	ACE	rs2043055	IL18	rs4651330	PLA2G4A
rs4267385	ACE	rs17561	IL1A	rs2076075	PLA2G4A
rs4459610	ACE	rs2856838	IL1A	rs6696406	PLA2G4A
rs12507573	ADH1B	rs1878321	IL1A	rs12404877	PLA2G4A
rs1042026	ADH1B	rs2853550	IL1B	rs12720497	PLA2G4A
rs17033	ADH1B	rs1143643	IL1B	rs6685652	PLA2G4A
rs13133908	ADH1B	rs1143634	IL1B	rs2223307	PLA2G4A
rs1789882	ADH1B	rs1143630	IL1B	rs17591814	PLA2G4A
rs1693457	ADH1B	rs1143627	IL1B	rs2223309	PLA2G4A
rs4147536	ADH1B	rs1143623	IL1B	rs1980444	PLA2G4A
rs1353621	ADH1B	rs4848306	IL1B	rs2049963	PLA2G4A
rs1159918	ADH1B	rs3917225	IL1R1	rs10911946	PLA2G4A
rs1229982	ADH1B	rs949963	IL1R1	rs12749354	PLA2G4A
rs1229980	ADH1C	rs3771202	IL1R1	rs7540602	PLA2G4A
rs1614972	ADH1C	rs2287047	IL1R1	rs6695515	PLA2G4A
rs904096	ADH1C	rs3917254	IL1R1	rs726706	PLA2G4A
rs3762896	ADH1C	rs3917273	IL1R1	rs6656909	PLA2G4A
rs17586163	ADH1C	rs3917292	IL1R1	rs1569479	PLA2G4A
rs1662037	ADH1C	rs2160227	IL1R1	rs6683515	PLA2G4A
rs1432622	ADRB2	rs3917296	IL1R1	rs12726519	PLA2G4A
rs12654778	ADRB2	rs951193	IL1R1	rs6683416	PLA2G4A
rs1042713	ADRB2	rs3917304	IL1R1	rs11587539	PLA2G4A
rs4705271	ADRB2	rs3917306	IL1R1	rs7555140	PLA2G4A
rs745686	AKAP5	rs3171845	IL1R1	rs932476	PLA2G4A
rs4581040	AP3M2	rs2110726	IL1R1	rs10157410	PLA2G4A
rs4471024	AP3M2	rs3732131	IL1R1	rs7545121	PLA2G4A
rs2373929	ATG9B	rs3917332	IL1R1	rs4402086	PLA2G4A
rs2227551	C10orf55	rs11884283	IL1R2	rs7526089	PLA2G4A
rs2471980	C6orf48	rs6543105	IL1R2	rs761517	PLA2G4A
rs4785224	CARD15	rs12467316	IL1R2	rs2020922	PLAT
rs2067085	CARD15	rs4141134	IL1R2	rs879293	PLAT
rs17312836	CARD15	rs4851520	IL1R2	rs2299609	PLAT
rs2066843	CARD15	rs4851521	IL1R2	rs7837156	PLAT
rs751271	CARD15	rs4851522	IL1R2	rs2020919	PLAT
rs5743289	CARD15	rs4321386	IL1R2	rs2227562	PLAU
rs5743291	CARD15	rs1108338	IL1R2	rs2227564	PLAU
rs7203344	CARD15	rs7561191	IL1R2	rs34930250	PLAU
rs8056611	CARD15	rs4851526	IL1R2	rs3805118	PLAU
rs706208	CBS	rs4851527	IL1R2	rs2461863	PLAU
rs1051319	CBS	rs2302589	IL1R2	rs4251938	PLAUR
rs3788050	CBS	rs3218861	IL1R2	rs2302524	PLAUR
rs2124459	CBS	rs2160140	IL1R2	rs4251864	PLAUR
rs8132811	CBS	rs3218883	IL1R2	rs2239372	PLAUR

rs1005584	CBS	rs3218927	IL1R2	rs2283628	PLAUR
rs6586282	CBS	rs2072474	IL1R2	rs397374	PLAUR
rs6586283	CBS	rs3218979	IL1R2	rs4251854	PLAUR
rs11203172	CBS	rs733498	IL1R2	rs4251831	PLAUR
rs12329764	CBS	rs7589525	IL1R2	rs344787	PLAUR
rs234705	CBS	rs4851531	IL1R2	rs2286960	PLAUR
rs234709	CBS	rs9817203	IL1RAP	rs344781	PLAUR
rs2851391	CBS	rs6800625	IL1RAP	rs344779	PLAUR
rs234715	CBS	rs7628333	IL1RAP	rs740841	PLEKHG6
rs11701048	CBS	rs3821744	IL1RAP	rs2302350	PLEKHG6
rs1788484	CBS	rs7615368	IL1RAP	rs783144	PLG
rs1024611	CCL2	rs7615533	IL1RAP	rs2144723	PLG
rs1024610	CCL2	rs3773994	IL1RAP	rs2314852	PLG
rs3760396	CCL2	rs9290936	IL1RAP	rs1950562	PLG
rs4586	CCL2	rs9849030	IL1RAP	rs9458011	PLG
rs991804	CCL2	rs2059020	IL1RAP	rs1652508	PLG
rs1634491	CCL3	rs2361832	IL1RAP	rs4252092	PLG
rs1851503	CCL3	rs7626071	IL1RAP	rs9295131	PLG
rs9972960	CCL3	rs3773990	IL1RAP	rs783147	PLG
rs1634502	CCL3	rs2193880	IL1RAP	rs4252125	PLG
rs885691	CCL8	rs3773989	IL1RAP	rs813641	PLG
rs1233650	CCL8	rs1988743	IL1RAP	rs4252151	PLG
rs3138034	CCL8	rs4686554	IL1RAP	rs4252159	PLG
rs3138035	CCL8	rs16865597	IL1RAP	rs4252166	PLG
rs11575060	CCL8	rs2885370	IL1RAP	rs783176	PLG
rs3138039	CCL8	rs3773983	IL1RAP	rs11060	PLG
rs4794999	CCL8	rs3773982	IL1RAP	rs9458023	PLG
rs4914	CD14	rs3773981	IL1RAP	rs783166	PLG
rs2569190	CD14	rs9883249	IL1RAP	rs6713532	POMC
rs2569193	CD14	rs9845825	IL1RAP	rs854547	PON1
rs1061237	COL1A1	rs2241343	IL1RAP	rs8491	PON1
rs2277632	COL1A1	rs9877268	IL1RAP	rs854548	PON1
rs2586488	COL1A1	rs3773977	IL1RAP	rs3735590	PON1
rs2075559	COL1A1	rs3773976	IL1RAP	rs854551	PON1
rs2857396	COL1A1	rs4687151	IL1RAP	rs854552	PON1
rs2696247	COL1A1	rs11929157	IL1RAP	rs854555	PON1
rs2269336	COL1A1	rs10937439	IL1RAP	rs3917550	PON1
rs1107946	COL1A1	rs10937442	IL1RAP	rs2269829	PON1
rs11765563	COL1A2	rs1035347	IL1RAP	rs3917542	PON1
rs388625	COL1A2	rs1559018	IL1RAP	rs662	PON1
rs3814967	COL1A2	rs12053868	IL1RAP	rs3917538	PON1
rs1800222	COL1A2	rs6444435	IL1RAP	rs854560	PON1
rs411717	COL1A2	rs4687154	IL1RAP	rs854561	PON1
rs420257	COL1A2	rs10513854	IL1RAP	rs2272365	PON1
rs760043	COL1A2	rs7628250	IL1RAP	rs3917490	PON1
rs406226	COL1A2	rs3773958	IL1RAP	rs2049649	PON1
rs3763466	COL1A2	rs3773953	IL1RAP	rs2299260	PON1
rs17166249	COL1A2	rs1469007	IL1RAP	rs2299261	PON1
rs389328	COL1A2	rs9875362	IL1RAP	rs854568	PON1
rs42521	COL1A2	rs6781037	IL1RAP	rs2299262	PON1

rs42523	COL1A2	rs6765375	IL1RAP	rs854569	PON1
rs42524	COL1A2	rs9821002	IL1RAP	rs2237583	PON1
rs2621213	COL1A2	rs759783	IL1RAP	rs757158	PON1
rs2521205	COL1A2	rs4140711	IL1RAP	rs17166818	PON1
rs7781954	COL1A2	rs9290939	IL1RAP	rs11977702	PON2
rs3736638	COL1A2	rs1015704	IL1RAP	rs9641164	PON2
rs42527	COL1A2	rs1015705	IL1RAP	rs987539	PON2
rs369982	COL1A2	rs4687163	IL1RAP	rs2286232	PON2
rs42528	COL1A2	rs929729	IL1RAP	rs2299266	PON2
rs4266	COL1A2	rs1024941	IL1RAP	rs2237585	PON2
rs2472	COL1A2	rs1024946	IL1RAP	rs2286233	PON2
rs42531	COL1A2	rs1024949	IL1RAP	rs11981433	PON2
rs441051	COL1A2	rs7626795	IL1RAP	rs7802018	PON2
rs400218	COL1A2	rs4624606	IL1RAP	rs2299267	PON2
rs7804898	COL1A2	rs9847868	IL1RAP	rs730365	PON2
rs413826	COL1A2	rs9821122	IL1RAP	rs43037	PON2
rs10046552	COL1A2	rs4320092	IL1RAP	rs6978425	PON2
rs6465412	COL1A2	rs9831803	IL1RAP	rs16896153	PTCRA
rs12668754	COL1A2	rs7650510	IL1RAP	rs9471960	PTCRA
rs11764718	COL1A2	rs11915384	IL1RAP	rs9471966	PTCRA
rs1062394	COL1A2	rs17042917	IL1RN	rs6901007	PTCRA
rs11982782	COL1A2	rs315920	IL1RN	rs1390376	PTGER2
rs13234022	COL1A2	rs4251961	IL1RN	rs1254600	PTGER2
rs2138533	COL3A1	rs2637988	IL1RN	rs1254593	PTGER2
rs1878201	COL3A1	rs928940	IL1RN	rs708498	PTGER2
rs1516454	COL3A1	rs3213448	IL1RN	rs12147805	PTGER2
rs1914037	COL3A1	rs1794066	IL1RN	rs708505	PTGER2
rs17358825	COL3A1	rs380092	IL1RN	rs708506	PTGER2
rs16830973	COL3A1	rs579543	IL1RN	rs959	PTGER3
rs7576108	COL3A1	rs315951	IL1RN	rs1327460	PTGER3
rs3736487	COL3A1	rs315949	IL1RN	rs6656853	PTGER3
rs3134656	COL3A1	rs315946	IL1RN	rs7530345	PTGER3
rs12693525	COL3A1	rs315943	IL1RN	rs6685546	PTGER3
rs13306267	COL3A1	rs315942	IL1RN	rs17131465	PTGER3
rs7579903	COL3A1	rs10027390	IL2	rs12119442	PTGER3
rs2271682	COL3A1	rs2069772	IL2	rs5702	PTGER3
rs10204508	COL3A1	rs2069771	IL2	rs1409985	PTGER3
rs2203602	COL3A1	rs2069779	IL2	rs1327449	PTGER3
rs3134646	COL3A1	rs2069778	IL2	rs4649932	PTGER3
rs4667256	COL3A1	rs2069762	IL2	rs1409981	PTGER3
rs4667258	COL3A1	rs4833248	IL2	rs4147115	PTGER3
rs12002679	COL5A1	rs10795737	IL2RA	rs4998697	PTGER3
rs4341231	COL5A1	rs12359875	IL2RA	rs7541092	PTGER3
rs3124291	COL5A1	rs12722605	IL2RA	rs1409165	PTGER3
rs3128597	COL5A1	rs12244380	IL2RA	rs17131487	PTGER3
rs3124311	COL5A1	rs9663421	IL2RA	rs4650094	PTGER3
rs4842151	COL5A1	rs12722596	IL2RA	rs875727	PTGER3
rs11103509	COL5A1	rs2386841	IL2RA	rs17541722	PTGER3
rs4842157	COL5A1	rs12722588	IL2RA	rs1327466	PTGER3
rs4842158	COL5A1	rs2076846	IL2RA	rs1887404	PTGER3

rs4842161	COL5A1	rs7093069	IL2RA	rs17542063	PTGER3
rs3124932	COL5A1	rs942200	IL2RA	rs6424410	PTGER3
rs12005720	COL5A1	rs2031229	IL2RA	rs602383	PTGER3
rs3128621	COL5A1	rs2228150	IL2RA	rs578096	PTGER3
rs4842167	COL5A1	rs12722563	IL2RA	rs6670616	PTGER3
rs3811161	COL5A1	rs7910961	IL2RA	rs2421735	PTGER3
rs11999194	COL5A1	rs6602392	IL2RA	rs977214	PTGER3
rs3811153	COL5A1	rs7072398	IL2RA	rs6665776	PTGER3
rs3811152	COL5A1	rs11256457	IL2RA	rs594454	PTGER3
rs3811151	COL5A1	rs6602398	IL2RA	rs2300161	PTGER3
rs10776908	COL5A1	rs11598648	IL2RA	rs5697	PTGER3
rs10745387	COL5A1	rs4749926	IL2RA	rs2072947	PTGER3
rs3827848	COL5A1	rs1107345	IL2RA	rs481940	PTGER3
rs4842172	COL5A1	rs11256497	IL2RA	rs3819783	PTGER3
rs4841937	COL5A1	rs791589	IL2RA	rs3819790	PTGER3
rs4842174	COL5A1	rs791590	IL2RA	rs726764	PTGER3
rs10114036	COL5A1	rs2476491	IL2RA	rs1409164	PTGER3
rs11103543	COL5A1	rs706779	IL2RA	rs2256385	PTGER3
rs13946	COL5A1	rs706778	IL2RA	rs2300164	PTGER3
rs6760780	COL5A2	rs3134883	IL2RA	rs2050066	PTGER3
rs3923384	COL5A2	rs12722486	IL2RA	rs6424414	PTGER3
rs6434317	COL5A2	rs7072793	IL2RA	rs2300167	PTGER3
rs6434322	COL5A2	rs7073236	IL2RA	rs6678886	PTGER3
rs10165260	COL5A2	rs2743827	IL2RB	rs10789314	PTGER3
rs7420331	COL5A2	rs228937	IL2RB	rs5693	PTGER3
rs13024858	COL5A2	rs3218339	IL2RB	rs5680	PTGER3
rs6752781	COL5A2	rs3218329	IL2RB	rs5673	PTGER3
rs1515864	COL5A2	rs84458	IL2RB	rs11209736	PTGER3
rs9288163	COL5A2	rs84460	IL2RB	rs8179390	PTGER3
rs10191420	COL5A2	rs228945	IL2RB	rs2817864	PTGER3
rs1399991	COL5A2	rs228947	IL2RB	rs3000466	PTGER3
rs10497699	COL5A2	rs2072861	IL2RB	rs11999368	PTGES
rs12611950	COL5A2	rs3218315	IL2RB	rs4636306	PTGES
rs11691604	COL5A2	rs3218312	IL2RB	rs2302821	PTGES
rs10105164	CRH	rs228953	IL2RB	rs4837405	PTGES
rs6996265	CRH	rs3218297	IL2RB	rs10739757	PTGES
rs3176921	CRH	rs228954	IL2RB	rs2241270	PTGES
rs6472257	CRH	rs3218295	IL2RB	rs10988496	PTGES
rs7839698	CRH	rs3218292	IL2RB	rs3766354	PTGFR
rs10098823	CRH	rs228957	IL2RB	rs1555541	PTGFR
rs32897	CRHBP	rs2281094	IL2RB	rs1322934	PTGFR
rs6453267	CRHBP	rs228968	IL2RB	rs1322931	PTGFR
rs10055255	CRHBP	rs1362904	IL2RB	rs6424776	PTGFR
rs1875999	CRHBP	rs1003694	IL2RB	rs6701594	PTGFR
rs10514082	CRHBP	rs2235330	IL2RB	rs12725125	PTGFR
rs17689966	CRHR1	rs3218264	IL2RB	rs3766345	PTGFR
rs4722999	CRHR2	rs228975	IL2RB	rs668005	PTGFR
rs12701020	CRHR2	rs3218258	IL2RB	rs622346	PTGFR
rs973002	CRHR2	rs2243248	IL4	rs3766333	PTGFR
rs929377	CRHR2	rs2070874	IL4	rs12074883	PTGFR

rs2240404	CRHR2	rs2227284	IL4	rs1330344	PTGS1
rs2190242	CRHR2	rs2243263	IL4	rs1213266	PTGS1
rs2284217	CRHR2	rs2243268	IL4	rs10306135	PTGS1
rs6462219	CRHR2	rs2243274	IL4	rs7866582	PTGS1
rs2284219	CRHR2	rs2243290	IL4	rs3842788	PTGS1
rs2267716	CRHR2	rs2057768	IL4R	rs10306150	PTGS1
rs2267717	CRHR2	rs6498012	IL4R	rs4273915	PTGS1
rs3093066	CRP	rs4787948	IL4R	rs4240474	PTGS1
rs1800947	CRP	rs3024530	IL4R	rs3842798	PTGS1
rs1417938	CRP	rs3024537	IL4R	rs10306153	PTGS1
rs231770	CTLA4	rs3024547	IL4R	rs4836885	PTGS1
rs16840252	CTLA4	rs3024548	IL4R	rs9299282	PTGS1
rs11571317	CTLA4	rs3024560	IL4R	rs12238505	PTGS1
rs5742909	CTLA4	rs2239349	IL4R	rs3842801	PTGS1
rs231777	CTLA4	rs2239347	IL4R	rs10306188	PTGS1
rs231779	CTLA4	rs3024585	IL4R	rs10306202	PTGS1
rs3087243	CTLA4	rs3024623	IL4R	rs2066826	PTGS2
rs231726	CTLA4	rs4787423	IL4R	rs2745557	PTGS2
rs231727	CTLA4	rs3024648	IL4R	rs689466	PTGS2
rs4775932	CYP19A1	rs3024658	IL4R	rs689462	PTGS2
rs4275794	CYP19A1	rs3024668	IL4R	rs12042763	PTGS2
rs2899470	CYP19A1	rs3024675	IL4R	rs10911905	PTGS2
rs16964201	CYP19A1	rs3024676	IL4R	rs2179555	PTGS2
rs2899472	CYP19A1	rs2234898	IL4R	rs352143	PTK9L
rs12439137	CYP19A1	rs1805015	IL4R	rs2476601	PTPN22
rs4775934	CYP19A1	rs1801275	IL4R	rs10897270	SCGB1A1
rs2899473	CYP19A1	rs1805016	IL4R	rs3741240	SCGB1A1
rs4775935	CYP19A1	rs8832	IL4R	rs17145874	SCGB1A1
rs3784308	CYP19A1	rs1029489	IL4R	rs3764874	SCNN1A
rs700518	CYP19A1	rs4787956	IL4R	rs3917854	SELP
rs17703883	CYP19A1	rs743562	IL5	rs6950982	SERPINE1
rs726547	CYP19A1	rs739719	IL5	rs2227631	SERPINE1
rs10519295	CYP19A1	rs739718	IL5	rs2227667	SERPINE1
rs727479	CYP19A1	rs2069812	IL5	rs2227672	SERPINE1
rs10459592	CYP19A1	rs1880243	IL6	rs2070682	SERPINE1
rs12591359	CYP19A1	rs12700386	IL6	rs1050813	SERPINE1
rs767199	CYP19A1	rs1800797	IL6	rs11560324	SERPINE1
rs12911554	CYP19A1	rs1800795	IL6	rs646474	SERPINH1
rs17703982	CYP19A1	rs2069840	IL6	rs649257	SERPINH1
rs7172156	CYP19A1	rs1554606	IL6	rs7526293	SHE
rs11856927	CYP19A1	rs11766273	IL6	rs11868708	SHMT1
rs4545755	CYP19A1	rs952146	IL6R	rs2273027	SHMT1
rs1054984	CYP19A1	rs6427641	IL6R	rs2273026	SHMT1
rs4614671	CYP19A1	rs11265610	IL6R	rs6596471	SLC23A1
rs12050767	CYP19A1	rs1386821	IL6R	rs6596473	SLC23A1
rs749292	CYP19A1	rs4075015	IL6R	rs11950646	SLC23A1
rs17647719	CYP19A1	rs4601580	IL6R	rs1875324	SLC35B2
rs2305707	CYP19A1	rs4845618	IL6R	rs520639	SLC35B2
rs1902586	CYP19A1	rs6687726	IL6R	rs2282151	SLC35B2
rs16953058	CYP19A1	rs7549338	IL6R	rs7224199	SLC6A4

rs936306	CYP19A1	rs4553185	IL6R	rs1042173	SLC6A4
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rs2470176	CYP19A1	rs4845623	IL6R	rs140701	SLC6A4
rs3764221	CYP19A1	rs4537545	IL6R	rs140700	SLC6A4
rs17523880	CYP19A1	rs4845625	IL6R	rs2020942	SLC6A4
rs2470152	CYP19A1	rs4845374	IL6R	rs6354	SLC6A4
rs17523922	CYP19A1	rs11265618	IL6R	rs25528	SLC6A4
rs16964258	CYP19A1	rs10752641	IL6R	rs2020936	SLC6A4
rs3751592	CYP19A1	rs4329505	IL6R	rs12150214	SLC6A4
rs3751591	CYP19A1	rs2229238	IL6R	rs4251417	SLC6A4
rs2470150	CYP19A1	rs4072391	IL6R	rs9903602	SLC6A4
rs1902584	CYP19A1	rs4694178	IL8	rs16965628	SLC6A4
rs1004983	CYP19A1	rs4694637	IL8	rs2020933	SLC6A4
rs1004982	CYP19A1	rs1008563	IL8RA	rs1979277	SMCR8
rs1902585	CYP19A1	rs1008562	IL8RA	rs2267163	TCN2
rs10163138	CYP19A1	rs2854386	IL8RA	rs1946519	TEX12
rs7174997	CYP19A1	rs878887	IMP5	rs5744222	TEX12
rs8025191	CYP19A1	rs12451328	KCNH6	rs1293344	TEX12
rs1961177	CYP19A1	rs4790355	KIAA0664	rs12693471	TFPI
rs4646421	CYP1A1	rs4790356	KIAA0664	rs5940	TFPI
rs2470893	CYP1A1	rs398655	KL	rs8176541	TFPI
rs5758589	CYP2D6	rs495392	KL	rs7586970	TFPI
rs2977814	DEFA1	rs2283368	KL	rs3213739	TFPI
rs5028372	DEFA1	rs526906	KL	rs8176508	TFPI
rs11137085	DEFA3	rs9526983	KL	rs8176605	TFPI
rs4841815	DEFA3	rs9536239	KL	rs34047509	TFPI
rs4284061	DEFA3	rs577912	KL	rs2041778	TFPI
rs17466573	DEFA3	rs1207362	KL	rs2192824	TFPI
rs7825750	DEFA3	rs685417	KL	rs3755248	TFPI
rs4512398	DEFA3	rs1334928	KL	rs12613071	TFPI
rs12517451	DHFR	rs1888057	KL	rs2192825	TFPI
rs1650723	DHFR	rs657049	KL	rs16829086	TFPI
rs1677693	DHFR	rs9527025	KL	rs7573488	TFPI
rs10072026	DHFR	rs2149860	KL	rs7594359	TFPI
rs1650697	DHFR	rs522796	KL	rs10179730	TFPI
rs380691	DHFR	rs648202	KL	rs6434222	TFPI
rs12069358	EDN2	rs643780	KL	rs10187622	TFPI
rs1407550	EDN2	rs659117	KL	rs8179181	TGFB1
rs6690839	EDN2	rs582524	KL	rs5754289	TIMP3
rs734359	EDN2	rs2844482	LTA	rs9606994	TIMP3
rs11572340	EDN2	rs1800683	LTA	rs9619311	TIMP3
rs3754287	EDN2	rs2229094	LTA	rs130274	TIMP3
rs1077218	EDN2	rs1799964	LTA	rs11704261	TIMP3
rs883304	EDN2	rs769178	LTB	rs738992	TIMP3
rs12718439	EDN2	rs16933062	MBL2	rs242089	TIMP3
rs4660541	EDN2	rs12771266	MBL2	rs130287	TIMP3
rs2854450	EPHX1	rs2506	MBL2	rs242088	TIMP3
rs3753658	EPHX1	rs10082466	MBL2	rs130290	TIMP3
rs1877724	EPHX1	rs930507	MBL2	rs130293	TIMP3
rs2671272	EPHX1	rs1838065	MBL2	rs5749524	TIMP3

rs1051740	EPHX1	rs1838066	MBL2	rs242082	TIMP3
rs2260863	EPHX1	rs10824793	MBL2	rs80272	TIMP3
rs2740168	EPHX1	rs11003123	MBL2	rs242078	TIMP3
rs2740170	EPHX1	rs10824796	MBL2	rs242076	TIMP3
rs2234922	EPHX1	rs11003129	MBL2	rs130300	TIMP3
rs1051741	EPHX1	rs10417924	MGC20255	rs130301	TIMP3
rs3753663	EPHX1	rs1982072	MGC4093	rs242072	TIMP3
rs360063	EPHX1	rs16879258	MGC5297	rs4504	TIMP3
rs4149239	EPHX2	rs17184211	MGC5297	rs135029	TIMP3
rs2741334	EPHX2	rs1801394	MGC5297	rs5754312	TIMP3
rs17057288	EPHX2	rs1939008	MMP1	rs2267183	TIMP3
rs7816586	EPHX2	rs17293823	MMP1	rs1427378	TIMP3
rs891401	EPHX2	rs7945189	MMP1	rs1427376	TIMP3
rs721619	EPHX2	rs2071230	MMP1	rs9862	TIMP3
rs10503812	EPHX2	rs470747	MMP1	rs137485	TIMP3
rs17057312	EPHX2	rs7125062	MMP1	rs5749527	TIMP3
rs4149252	EPHX2	rs5031036	MMP1	rs137487	TIMP3
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rs13269963	EPHX2	rs470358	MMP1	rs137489	TIMP3
rs1042064	EPHX2	rs514921	MMP1	rs2040435	TIMP3
rs4149259	EPHX2	rs1155764	MMP1	rs3773364	TIMP4
rs4149260	EPHX2	rs484915	MMP1	rs4684841	TIMP4
rs7341557	EPHX2	rs243866	MMP2	rs99365	TIMP4
rs2640726	EPHX2	rs1477017	MMP2	rs3755724	TIMP4
rs3212998	F10	rs865094	MMP2	rs1898830	TLR2
rs474810	F10	rs11646643	MMP2	rs4696483	TLR2
rs3211744	F10	rs1053605	MMP2	rs7656411	TLR2
rs547138	F10	rs866770	MMP2	rs1337	TLR2
rs3211764	F10	rs2241145	MMP2	rs4862632	TLR3
rs2026160	F10	rs243845	MMP2	rs5743303	TLR3
rs3211770	F10	rs243842	MMP2	rs5743305	TLR3
rs776897	F10	rs183112	MMP2	rs11721827	TLR3
rs3213004	F10	rs1992116	MMP2	rs5743312	TLR3
rs9549675	F10	rs11639960	MMP2	rs7668666	TLR3
rs559054	F10	rs243836	MMP2	rs3775292	TLR3
rs5960	F10	rs243834	MMP2	rs3775291	TLR3
rs2070852	F2	rs11541998	MMP2	rs10025405	TLR3
rs3136485	F2	rs243832	MMP2	rs4862633	TLR3
rs2227744	F2R	rs243831	MMP2	rs10759930	TLR4
rs27593	F2R	rs9922534	MMP2	rs16906053	TLR4
rs37249	F2R	rs2241148	MMP2	rs2770150	TLR4
rs2227827	F2R	rs8054459	MMP2	rs10759932	TLR4
rs153311	F2R	rs2192853	MMP2	rs1927911	TLR4
rs250738	F2R	rs569444	MMP3	rs1927907	TLR4
rs11954573	F2R	rs650108	MMP3	rs2149356	TLR4
rs250731	F2R	rs520540	MMP3	rs7869402	TLR4
rs6453251	F2RL1	rs522616	MMP3	rs11536889	TLR4
rs639342	F2RL1	rs645419	MMP3	rs1927906	TLR4
rs2242991	F2RL1	rs1276284	MMP8	rs11536898	TLR4
rs2243073	F2RL1	rs2508383	MMP8	rs1554973	TLR4

rs2243004	F2RL1	rs1939020	MMP8	rs7856729	TLR4
rs2243010	F2RL1	rs17099443	MMP8	rs5741880	TLR7
rs34308580	F2RL1	rs1940475	MMP8	rs1731478	TLR7
rs631465	F2RL1	rs11225394	MMP8	rs179021	TLR7
rs2243083	F2RL1	rs6590985	MMP8	rs1731479	TLR7
rs2243066	F2RL1	rs10895354	MMP8	rs5743740	TLR7
rs6453253	F2RL1	rs4810482	MMP9	rs179016	TLR7
rs773902	F2RL3	rs8113877	MMP9	rs179012	TLR7
rs2227356	F2RL3	rs6104420	MMP9	rs179011	TLR7
rs773901	F2RL3	rs1956545	MTHFD1	rs179009	TLR7
rs1054533	F2RL3	rs3783731	MTHFD1	rs179008	TLR7
rs2608732	F2RL3	rs1950902	MTHFD1	rs864058	TLR7
rs762485	F3	rs17751556	MTHFD1	rs179007	TLR7
rs762484	F3	rs2295640	MTHFD1	rs179006	TLR7
rs696619	F3	rs17824591	MTHFD1	rs5935438	TLR7
rs28672143	F3	rs1885031	MTHFD1	rs178996	TLR8
rs2187952	F5	rs8016556	MTHFD1	rs3761621	TLR8
rs2420369	F5	rs2236225	MTHFD1	rs3761623	TLR8
rs2213865	F5	rs3818239	MTHFD1	rs5741883	TLR8
rs3766103	F5	rs11849530	MTHFD1	rs3764880	TLR8
rs12131397	F5	rs1256146	MTHFD1	rs17256081	TLR8
rs9332624	F5	rs4846048	MTHFR	rs1548731	TLR8
rs9332618	F5	rs4846049	MTHFR	rs4830807	TLR8
rs6427197	F5	rs1476413	MTHFR	rs5741886	TLR8
rs4656687	F5	rs1801131	MTHFR	rs5744068	TLR8
rs1557572	F5	rs12121543	MTHFR	rs2159377	TLR8
rs6020	F5	rs1994798	MTHFR	rs2407992	TLR8
rs6035	F5	rs1801133	MTHFR	rs3747414	TLR8
rs12120605	F5	rs17421462	MTHFR	rs5741890	TLR8
rs6427198	F5	rs17421511	MTHFR	rs16987224	TLR8
rs1894697	F5	rs4846052	MTHFR	rs1800629	TNF
rs9332575	F5	rs11121832	MTHFR	rs1800693	TNFRSF1A
rs6022	F5	rs9651118	MTHFR	rs1860545	TNFRSF1A
rs7534848	F5	rs17367504	MTHFR	rs4149578	TNFRSF1A
rs9287095	F5	rs3753582	MTHFR	rs4149577	TNFRSF1A
rs2298908	F5	rs3737964	MTHFR	rs4149622	TNFRSF1A
rs6019	F5	rs12759827	MTR	rs4149570	TNFRSF1A
rs12755775	F5	rs4659723	MTR	rs11064145	TNFRSF1A
rs10489185	F5	rs4077829	MTR	rs590368	TNFRSF1B
rs555212	F7	rs1805087	MTR	rs522807	TNFRSF1B
rs1475931	F7	rs4659743	MTR	rs652625	TNFRSF1B
rs488703	F7	rs7730643	MTRR	rs496888	TNFRSF1B
rs6045	F7	rs326121	MTRR	rs976881	TNFRSF1B
rs6046	F7	rs326123	MTRR	rs3766730	TNFRSF1B
rs3093261	F7	rs326124	MTRR	rs616645	TNFRSF1B
rs3211719	F7	rs1532268	MTRR	rs816050	TNFRSF1B
rs983751	FAS	rs7703033	MTRR	rs474247	TNFRSF1B
rs4934434	FAS	rs162031	MTRR	rs1201157	TNFRSF1B
rs3758483	FAS	rs162033	MTRR	rs653667	TNFRSF1B
rs6586165	FAS	rs162036	MTRR	rs1061622	TNFRSF1B

rs1571011	FAS	rs3815743	MTRR	rs5746051	TNFRSF1B
rs9658727	FAS	rs10380	MTRR	rs5746053	TNFRSF1B
rs9658742	FAS	rs8659	MTRR	rs235219	TNFRSF1B
rs7901656	FAS	rs10888150	NAT1	rs1061624	TNFRSF1B
rs2031611	FAS	rs7017402	NAT1	rs1061628	TNFRSF1B
rs9658761	FAS	rs4298522	NAT1	rs1061631	TNFRSF1B
rs982764	FAS	rs9325827	NAT1	rs235214	TNFRSF1B
rs2234978	FAS	rs17126350	NAT1	rs2234185	TNRC5
rs1051070	FAS	rs4921880	NAT1	rs6519132	TOMM22
rs7915235	FAS	rs7003890	NAT1	rs5757231	TOMM22
rs2859242	FASLG	rs8190837	NAT1	rs6001193	TOMM22
rs2639614	FASLG	rs8190870	NAT1	rs10781520	TRAF2
rs6700734	FASLG	rs4646246	NAT2	rs2784075	TRAF2
rs17370527	FASLG	rs7832071	NAT2	rs908831	TRAF2
rs5030772	FASLG	rs1801280	NAT2	rs16894387	TREM1
rs12041613	FASLG	rs1799929	NAT2	rs2234243	TREM1
rs17389016	G0S2	rs1208	NAT2	rs1817537	TREM1
rs947895	GSTP1	rs721398	NAT2	rs3804277	TREM1
rs140199	GSTT2	rs721399	NAT2	rs4711668	TREM1
rs2586482	HILS1	rs980455	NFKB1	rs6910730	TREM1
rs2586485	HILS1	rs3774933	NFKB1	rs6940092	TREM1
rs2235543	HSD11B1	rs1599961	NFKB1	rs6939973	TREM1
rs4844880	HSD11B1	rs1585213	NFKB1	rs3827632	TREM1
rs846910	HSD11B1	rs230528	NFKB1	rs3789204	TREM1
rs3753519	HSD11B1	rs4648011	NFKB1	rs1385105	TREM1
rs846911	HSD11B1	rs13117745	NFKB1	rs8009058	TSHR
rs11799643	HSD11B1	rs1801	NFKB1	rs2268451	TSHR
rs12040780	HSD11B1	rs4648058	NFKB1	rs12892567	TSHR
rs846906	HSD11B1	rs3755867	NFKB1	rs179247	TSHR
rs6672256	HSD11B1	rs4648090	NFKB1	rs179259	TSHR
rs9430012	HSD11B1	rs3774968	NFKB1	rs179260	TSHR
rs932335	HSD11B1	rs3817685	NFKB1	rs179261	TSHR
rs1780019	HSD17B7	rs4648135	NFKB1	rs2110696	TSHR
rs11589262	HSD17B7	rs4648141	NFKB1	rs1035145	TSHR
rs4656381	HSD17B7	rs1609798	NFKB1	rs3783950	TSHR
rs1039874	HSD17B7	rs7674640	NFKB1	rs3783948	TSHR
rs2805053	HSD17B7	rs997476	NFKB1	rs7143071	TSHR
rs2803865	HSD17B7	rs10489113	NFKB1	rs17111361	TSHR
rs10906772	HSPA14	rs11574845	NFKB2	rs6574616	TSHR
rs11593057	HSPA14	rs7897947	NFKB2	rs724169	TSHR
rs17155992	HSPA14	rs1056890	NFKB2	rs2300520	TSHR
rs7894284	HSPA14	rs3138056	NFKBIA	rs4903964	TSHR
rs9787671	HSPA14	rs696	NFKBIA	rs917986	TSHR
rs7905174	HSPA14	rs3138045	NFKBIA	rs17545310	TSHR
rs10906774	HSPA14	rs11575002	NFKBIB	rs3783943	TSHR
rs1043618	HSPA1A	rs2053071	NFKBIB	rs8012937	TSHR
rs2763979	HSPA1A	rs2241704	NFKBIB	rs10483973	TSHR
rs2075800	HSPA1L	rs2241705	NFKBIB	rs2300521	TSHR
rs2227956	HSPA1L	rs3136641	NFKBIB	rs12881268	TSHR
rs4574536	HSPA4	rs9636109	NFKBIB	rs2268466	TSHR

rs4705990	HSPA4	rs11083487	NFKBIB	rs4903967	TSHR
rs4616886	HSPA4	rs3136646	NFKBIB	rs7161100	TSHR
rs7730747	HSPA4	rs730775	NFKBIE	rs2300525	TSHR
rs10075878	HSPA4	rs483536	NFKBIE	rs2110697	TSHR
rs9427401	HSPA6	rs10277237	NOS3	rs1005292	TSHR
rs12129787	HSPA6	rs12703107	NOS3	rs722540	TSHR
rs4657053	HSPA6	rs1800783	NOS3	rs17111394	TSHR
rs4657054	HSPA6	rs1799983	NOS3	rs2268474	TSHR
rs404508	HSPA6	rs3918227	NOS3	rs2300528	TSHR
rs2099684	HSPA6	rs743507	NOS3	rs2284735	TSHR
rs10878763	IFNG	rs17287758	NR3C1	rs2075173	TSHR
rs2069727	IFNG	rs10482682	NR3C1	rs17111431	TSHR
rs2069718	IFNG	rs4986593	NR3C1	rs10129380	TSHR
rs2069716	IFNG	rs33388	NR3C1	rs17111481	TSHR
rs2069705	IFNG	rs17100236	NR3C1	rs7150670	TSHR
rs1520220	IGF1	rs2918417	NR3C1	rs2268475	TSHR
rs2471551	IGFBP3	rs2963155	NR3C1	rs11159491	TSHR
rs4803648	IGSF4C	rs9324918	NR3C1	rs2024426	TSHR
rs4802189	IGSF4C	rs9324921	NR3C1	rs11845715	TSHR
rs3024498	IL10	rs4634384	NR3C1	rs12885526	TSHR
rs3024496	IL10	rs9324924	NR3C1	rs8017455	TSHR
rs1800872	IL10	rs7701443	NR3C1	rs917984	TSHR
rs1800896	IL10	rs4244032	NR3C1	rs7158881	TSHR
rs1800890	IL10	rs4607376	NR3C1	rs6574629	TSHR
rs4936414	IL10RA	rs13182800	NR3C1	rs2268476	TSHR
rs2512143	IL10RA	rs4912911	NR3C1	rs3783938	TSHR
rs4252254	IL10RA	rs12656106	NR3C1	rs17111530	TSHR
rs4252270	IL10RA	rs12655166	NR3C1	rs930099	TSHR
rs2229113	IL10RA	rs6502385	PAFAH1B1	rs7157900	TSHR
rs9610	IL10RA	rs7209407	PAFAH1B1	rs1957547	TSHR
rs2508445	IL10RA	rs7213463	PAFAH1B1	rs2300540	TSHR
rs947889	IL10RA	rs3785958	PAFAH1B1	rs1991517	TSHR
rs4938467	IL10RA	rs1029744	PAFAH1B1	rs2268477	TSHR
rs11216666	IL10RA	rs11078302	PAFAH1B1	rs7144481	TSHR
rs17121510	IL10RA	rs12938775	PAFAH1B1	rs17630128	TSHR
rs2284552	IL10RB	rs2317297	PAFAH1B1	rs2288493	TSHR
rs962859	IL10RB	rs7223411	PAFAH1B1	rs12883801	TSHR
rs2834168	IL10RB	rs4790353	PAFAH1B1	rs6742078	UGT1A1
rs2834170	IL10RB	rs4938347	PAFAH1B2	rs4148324	UGT1A1
rs2243498	IL10RB	rs3736120	PAFAH1B2	rs12479045	UGT1A1
rs2834172	IL10RB	rs6001188	PGEA1	rs4663971	UGT1A1
rs765429	IL10RB	rs11224561	PGR	rs929596	UGT1A1
rs2276223	IL10RB	rs471767	PGR	rs2302538	UGT1A1
rs735299	IL10RB	rs563656	PGR	rs4148328	UGT1A1
rs999261	IL10RB	rs504372	PGR	rs11888492	UGT1A1
rs999259	IL10RB	rs578029	PGR	rs10929303	UGT1A1
rs1058867	IL10RB	rs635984	PGR	rs8330	UGT1A1
rs6517158	IL10RB	rs11224575	PGR	rs4148329	UGT1A1
rs2834175	IL10RB	rs492457	PGR	rs6717546	UGT1A1
rs3091307	IL13	rs518382	PGR	rs1500482	UGT1A1

rs1295686	IL13	rs553272	PGR	rs6719561	UGT1A1
rs848	IL13	rs660149	PGR	rs4663972	UGT1A1
rs1295683	IL13	rs653752	PGR	rs7586006	UGT1A1
rs2243204	IL13	rs538915	PGR	rs1500477	UGT1A1
rs12508866	IL15	rs503362	PGR	rs3755319	UGT1A3
rs1519551	IL15	rs542384	PGR	rs699947	VEGF
rs17461269	IL15	rs555572	PGR	rs833068	VEGF
rs1519552	IL15	rs11224589	PGR	rs833069	VEGF
rs7698675	IL15	rs619487	PGR	rs3025010	VEGF
rs13117878	IL15	rs537681	PGR	rs3025033	VEGF
rs12498901	IL15	rs518162	PGR	rs3025035	VEGF
rs6850492	IL15	rs507141	PGR	rs998584	VEGF
rs1907949	IL15	rs2499043	PGRMC1	rs6900017	VEGF
rs17007610	IL15	rs2428757	PGRMC1		
rs6537061	IL15	rs11726595	PGRMC2		
rs10833	IL15	rs2036687	PGRMC2		

Appendix Table 11. Significant maternal and fetal single locus results.

MATERNAL SAMPLES				*FETAL SAMPLES*			
Gene	*RS #*	*Allele p*	*Genotype p*	*Gene*	*RS #*	*Allele p*	*Genotype p*
ADH1B	rs17033	0.09	0.02	ADH1B	rs17033	0.04	0.05
ADRB2	rs1432622	0.05	0.13	C6orf48	rs2471980	0.02	3.8E-03
ADRB2	rs12654778	0.03	0.09	COL1A2	rs388625	0.01	0.01
AP3M2	rs4581040	0.04	0.11	COL1A2	rs411717	0.01	0.01
CARD15	rs8056611	0.11	0.04	COL1A2	rs420257	1.4E-03	9.6E-04
CBS	rs6586282	0.22	4.7E-03	COL1A2	rs389328	0.01	0.01
CBS	rs11203172	0.32	0.05	COL1A2	rs42524	0.01	0.02
COL1A1	rs1061237	0.33	0.04	COL1A2	rs2521205	0.04	0.13
COL1A2	rs2521205	0.02	0.05	COL1A2	rs42528	0.01	0.02
COL1A2	rs2472	1.6E-03	1.2E-03	COL1A2	rs2472	0.01	0.01
COL1A2	rs7804898	0.21	0.02	COL1A2	rs441051	2.8E-03	0.01
COL3A1	rs2271682	0.04	0.05	COL3A1	rs2138533	0.32	0.01
COL5A1	rs12005720	0.28	0.04	COL5A1	rs3124932	0.03	0.06
COL5A1	rs4842167	0.01	0.04	COL5A2	rs7420331	0.07	0.01
COL5A1	rs3811161	0.01	0.04	CRH	rs6996265	0.03	0.01
COL5A1	rs10745387	0.03	0.10	CRH	rs3176921	0.03	0.01
COL5A1	rs13946	0.04	0.12	CRH	rs6472257	0.02	4.2E-03
CRHR2	rs4722999	5.0E-03	0.02	CRHBP	rs10514082	0.34	0.01
CRHR2	rs12701020	0.09	0.02	CRHR2	rs2190242	0.82	0.05
CRHR2	rs929377	0.01	0.03	CYP19A1	rs8025191	0.04	0.05
CRHR2	rs2190242	0.03	0.09	EPHX2	rs4149239	0.58	0.03
CRHR2	rs2284219	0.01	0.04	EPHX2	rs891401	0.57	0.03
CYP19A1	rs727479	0.69	0.05	EPHX2	rs10503812	0.57	0.03
CYP19A1	rs17647719	0.04	0.08	EPHX2	rs4149252	0.58	0.03
CYP19A1	rs3751592	0.43	0.03	EPHX2	rs4149259	0.42	0.02
EDN2	rs4660541	0.86	0.05	F2R	rs27593	0.33	0.04
EPHX1	rs2740168	0.85	0.02	F5	rs1557572	0.54	0.05
F10	rs3211744	0.07	0.05	G0S2	rs17389016	0.01	0.02
F3	rs762484	0.01	0.04	HSD11B1	rs3753519	0.01	1.5E-03
F3	rs696619	0.05	0.09	HSD17B7	rs4656381	0.03	2.9E-03
F7	rs1475931	0.46	2.3E-03	HSPA1A	rs2763979	0.03	0.01
FAS	rs9658742	0.62	0.03	HSPA6	rs9427401	0.01	0.02
GSTP1	rs947895	0.07	0.04	IGSF4C	rs4803648	0.95	0.01
IL18	rs543810	0.31	0.04	IGSF4C	rs4802189	0.72	0.01
IL1B	rs1143630	0.03	0.04	IL10RB	rs999261	0.01	0.03
IL1R1	rs3917273	0.04	0.02	IL10RB	rs6517158	0.04	0.09
IL1R1	rs2110726	0.43	0.03	IL13	rs1295683	0.02	0.04
IL1R2	rs11884283	0.17	0.02	IL15	rs17461269	0.03	0.01
IL1R2	rs12467316	0.06	0.01	IL15	rs1519552	0.02	0.06
IL1R2	rs1108338	0.02	0.01	IL15	rs7698675	0.03	0.07
IL1RAP	rs7628333	1.8E-03	4.7E-03	IL15	rs13117878	0.02	0.05
IL1RAP	rs3821744	4.7E-03	0.01	IL15	rs6850492	0.04	0.09
IL1RAP	rs9883249	0.03	0.07	IL15	rs17007610	0.03	0.07
IL1RN	rs315920	4.9E-03	0.01	IL15	rs6537061	0.01	0.03

IL1RN	rs4251961	0.03	0.08	IL1A	rs17561	0.02	0.06
IL1RN	rs315946	0.01	0.03	IL1A	rs2856838	0.03	0.02
IL2RA	rs11598648	0.25	0.02	IL1A	rs1878321	0.02	0.06
IL2RA	rs1107345	0.04	0.06	IL1R1	rs3917225	0.03	0.08
IL2RA	rs11256497	0.03	0.10	IL1R1	rs2287047	0.01	0.04
IL2RA	rs706778	0.05	0.11	IL1R1	rs3917273	0.01	0.03
IL2RA	rs3134883	0.04	0.10	IL1R1	rs2160227	0.02	0.08
IL2RB	rs228954	0.01	0.03	IL1R1	rs3917304	0.02	0.07
IL2RB	rs228957	0.01	0.03	IL1RAP	rs7628333	0.18	0.02
IL2RB	rs2281094	0.03	0.08	IL1RAP	rs2193880	0.03	0.08
IL4R	rs3024548	0.13	0.04	IL1RAP	rs9877268	0.24	0.05
IL4R	rs3024623	0.05	0.02	IL1RAP	rs759783	0.03	0.09
IL6R	rs4845374	0.04	0.03	IL1RAP	rs4140711	0.03	0.09
IL6R	rs4329505	0.04	0.03	IL1RAP	rs1015704	0.02	0.04
IL8RA	rs1008562	0.05	0.04	IL1RAP	rs1015705	0.03	0.09
KL	rs495392	0.85	0.02	IL1RAP	rs4687163	0.03	0.09
KL	rs522796	0.16	0.04	IL1RAP	rs929729	0.04	0.10
MMP2	rs1053605	0.04	0.07	IL2RA	rs12722596	0.03	0.08
MTHFD1	rs17824591	0.03	0.11	IL2RA	rs11256497	0.01	0.03
MTHFD1	rs2236225	0.97	0.04	IL2RA	rs2476491	0.03	0.02
MTHFR	rs11121832	0.03	0.05	IL2RA	rs706778	0.03	0.09
MTHFR	rs3737964	0.02	0.03	IL2RA	rs3134883	0.04	0.09
MTRR	rs162031	0.02	0.05	IL4	rs2070874	0.02	0.03
MTRR	rs3815743	0.03	0.01	IL4	rs2227284	0.01	0.03
NAT1	rs7017402	0.04	0.05	IL4	rs2243268	0.02	0.04
NAT1	rs9325827	0.05	0.08	IL4	rs2243274	0.02	0.03
NAT1	rs4921880	0.52	0.02	IL4	rs2243290	0.02	0.04
NFKB1	rs10489113	0.39	0.04	IL4R	rs3024530	0.03	0.05
NR3C1	rs33388	0.77	0.01	IL4R	rs3024537	0.06	0.02
NR3C1	rs2918417	0.97	0.04	IL4R	rs3024547	0.06	0.02
NR3C1	rs4634384	0.72	0.01	IL4R	rs3024548	0.02	0.05
PAFAH1	rs7213463	0.02	0.06	IL4R	rs3024560	0.02	0.06
PGR	rs555572	0.01	0.03	IL4R	rs2239347	1.4E-03	3.8E-03
PGR	rs11224589	0.01	0.02	IL4R	rs3024676	0.03	0.08
PGR	rs619487	0.04	0.02	IL4R	rs1805015	0.04	0.10
PGRMC2	rs11726595	0.11	4.7E-03	MMP8	rs1939020	0.03	0.06
PLA2G4A	rs2076075	0.02	0.06	MTHFR	rs4846048	0.05	0.10
PLAT	rs2020922	0.03	0.08	MTHFR	rs1476413	0.05	0.02
PLG	rs4252092	0.01	0.03	MTHFR	rs1994798	3.9E-03	0.01
PLG	rs783147	0.03	0.06	MTHFR	rs17421462	0.01	0.01
PLG	rs4252166	0.04	0.01	MTHFR	rs17421511	0.25	0.01
PON1	rs2299260	0.34	0.03	MTHFR	rs4846052	0.01	0.01
PON1	rs854569	0.04	4.4E-03	MTHFR	rs11121832	0.01	0.02
PON2	rs43037	0.17	0.03	MTHFR	rs9651118	2.5E-03	0.01
PTGER3	rs959	0.58	0.03	MTHFR	rs3737964	0.01	0.02
PTGER3	rs6685546	0.03	0.08	MTRR	rs1532268	0.88	0.05
PTGER3	rs17131465	0.03	0.06	NFKB1	rs13117745	0.01	0.02
PTGER3	rs12119442	0.03	0.06	NFKB1	rs4648090	0.02	0.03
PTGER3	rs5702	0.57	0.02	NFKB1	rs4648141	0.05	0.10

PTGER3	rs7541092	0.03	0.03
PTGER3	rs1409165	0.02	0.03
PTGER3	rs17131487	0.03	0.09
PTGER3	rs875727	0.18	0.05
PTGER3	rs977214	0.06	0.04
PTGER3	rs6665776	0.06	0.04
PTGER3	rs2072947	0.20	0.03
PTGES	rs2302821	0.05	0.01
PTGES	rs2241270	0.05	0.11
PTGFR	rs3766345	0.03	0.08
PTGFR	rs668005	0.04	0.13
SLC35B2	rs2282151	0.02	0.06
SLC6A4	rs4251417	0.01	0.01
TFPI	rs12693471	0.01	0.01
TFPI	rs8176541	0.01	0.01
TFPI	rs7586970	0.01	0.01
TFPI	rs3213739	0.03	2.4E-03
TFPI	rs8176508	0.16	0.05
TFPI	rs2041778	0.04	0.06
TFPI	rs3755248	0.01	0.02
TFPI	rs7573488	0.01	0.01
TIMP3	rs2040435	0.83	0.05
TREM1	rs16894387	0.04	0.12
TREM1	rs4711668	2.9E-03	0.01
TREM1	rs6940092	0.02	0.05
TSHR	rs1035145	0.05	0.17
TSHR	rs2300520	0.04	0.08
TSHR	rs2110697	0.40	0.04
TSHR	rs11845715	0.78	0.02
TSHR	rs3783938	0.05	0.17
UGT1A1	rs4148329	0.43	0.03
UGT1A1	rs6719561	0.07	0.01
NFKBIB	rs3136646	0.07	0.03
NR3C1	rs9324918	0.38	0.04
PGR	rs518162	0.01	0.02
PGRMC1	rs2428757	0.07	0.03
PLAUR	rs4251854	0.21	0.03
PLG	rs9458011	0.03	0.07
PON1	rs854547	0.05	0.10
PON1	rs854548	2.5E-03	0.01
PON1	rs854551	0.01	0.02
PON1	rs854552	1.8E-03	0.01
PON1	rs2272365	0.04	0.12
PON1	rs757158	0.01	0.01
PON2	rs2286233	0.04	0.11
PTGER2	rs1254600	0.11	0.04
PTGER3	rs17131465	0.05	0.04
PTGER3	rs2256385	0.03	0.10
PTGER3	rs6424414	0.03	0.08
PTGER3	rs2300167	0.06	0.01
PTGFR	rs1322934	0.11	0.02
PTGFR	rs668005	0.02	0.01
SERPINE	rs2227667	0.03	0.05
SLC6A4	rs7224199	0.01	0.03
SLC6A4	rs1042173	0.01	0.03
SLC6A4	rs3794808	0.02	0.06
SLC6A4	rs140701	0.02	0.07
SLC6A4	rs2020942	0.02	0.06
SLC6A4	rs4251417	8.0E-04	2.7E-03
SMCR8	rs1979277	0.02	0.01
TCN2	rs2267163	0.03	0.07
TFPI	rs12693471	4.0E-03	0.01
TFPI	rs8176541	4.3E-03	0.01
TFPI	rs7586970	3.7E-03	0.01
TFPI	rs3213739	4.4E-03	0.01
TFPI	rs8176508	3.7E-03	0.01
TFPI	rs2041778	0.03	0.08
TFPI	rs3755248	0.03	0.09
TFPI	rs7573488	0.02	0.06
TFPI	rs6434222	5.8E-05	1.0E-04
TIMP3	rs5754289	0.87	0.03
TIMP4	rs3773364	0.29	0.04
TLR4	rs7869402	0.04	0.05
TOMM22	rs6519132	0.05	0.05
TREM1	rs6939973	0.02	0.04
TSHR	rs4903964	0.44	0.03
TSHR	rs3783943	0.05	0.14
TSHR	rs930099	0.03	0.07
UGT1A1	rs929596	0.22	0.03
UGT1A1	rs2302538	0.86	0.04
UGT1A1	rs11888492	0.45	0.03

Appendix Table 12. Significant haplotype results. a) Maternal, b) Fetal

a)

Gene	Haplotype	p
COL1A2	rs369982 rs42528 rs4266 rs2472	0.04
COL1A2	rs42528 rs4266 rs2472	4.9E-03
COL1A2	rs42528 rs4266 rs2472 rs42531	0.02
COL1A2	rs4266 rs2472	0.01
COL1A2	rs4266 rs2472 rs42531 rs441051	0.01
COL1A2	rs4266 rs2472 rs42531	0.01
COL1A2	rs2472 rs42531 rs441051	3.1E-03
COL1A2	rs2472 rs42531	4.9E-03
COL1A2	rs2472 rs42531 rs441051 rs400218	0.01
COL1A2	rs12668754 rs11764718 rs1062394	0.01
COL1A2	rs12668754 rs11764718 rs1062394 rs11982782	0.03
CRHR2	rs4722999 rs12701020	0.02
CRHR2	rs4722999 rs12701020 rs973002	0.04
CRHR2	rs973002 rs929377	0.03
CRHR2	rs973002 rs929377 rs2240404	0.05
CRHR2	rs929377 rs2240404	0.04
CRHR2	rs6462219 rs2284219	0.03
CRHR2	rs6462219 rs2284219 rs2267716	0.04
CRHR2	rs2284219 rs2267716	0.04
IL1RAP	rs7628333 rs3821744	1.8E-03
IL1RAP	rs7628333 rs3821744 rs9290936	0.01
IL1RAP	rs7628333 rs3821744 rs9290936 rs9849030	0.03
IL1RAP	rs3821744 rs9290936	0.02
IL1RN	rs17042917 rs315920 rs4251961	3.2E-03
IL1RN	rs17042917 rs315920	0.01
IL1RN	rs17042917 rs315920 rs4251961 rs2637988	0.01
IL1RN	rs315920 rs4251961	0.01
IL1RN	rs315920 rs4251961 rs2637988	0.01
IL1RN	rs315920 rs4251961 rs2637988 rs928940	0.02
IL1RN	rs4251961 rs2637988	0.01
IL1RN	rs4251961 rs2637988 rs928940 rs3213448	0.01
IL1RN	rs4251961 rs2637988 rs928940	0.01
IL1RN	rs928940 rs3213448 rs1794066 rs380092	0.01
IL1RN	rs3213448 rs1794066 rs380092	0.01
IL1RN	rs3213448 rs1794066 rs380092 rs579543	0.01
IL1RN	rs1794066 rs380092 rs579543	0.01
IL1RN	rs1794066 rs380092 rs579543 rs315951	0.01
IL1RN	rs1794066 rs380092	0.02
IL1RN	rs579543 rs315951 rs315949 rs315946	0.01
IL1RN	rs315951 rs315949 rs315946	0.01
IL1RN	rs315951 rs315949 rs315946 rs315943	0.01
IL1RN	rs315949 rs315946	0.02
IL1RN	rs315949 rs315946 rs315943	0.02
IL1RN	rs315949 rs315946 rs315943 rs315942	0.04
IL1RN	rs315946 rs315943 rs315942 rs12693471	3.2E-03

IL1RN	rs315946 rs315943	0.02
IL1RN	rs315946 rs315943 rs315942	0.04
IL1RN	rs315943 rs315942 rs12693471	0.02
IL1RN	rs315943 rs315942 rs12693471 rs8176541	0.02
IL1RN	rs315942 rs12693471	0.05
IL1RN	rs315942 rs12693471 rs8176541	0.05
IL1RN	rs315942 rs12693471 rs8176541 rs7586970	0.05
TFPI	rs12693471 rs8176541	0.01
TFPI	rs12693471 rs8176541 rs7586970	0.01
TFPI	rs12693471 rs8176541 rs7586970 rs3213739	0.03
TFPI	rs8176541 rs7586970	0.01
TFPI	rs8176541 rs7586970 rs3213739	0.03
TFPI	rs7586970 rs3213739	0.03
TFPI	rs2041778 rs2192824 rs3755248	0.04
TFPI	rs2192824 rs3755248	0.02
TFPI	rs2192824 rs3755248 rs12613071	0.04
TFPI	rs12613071 rs16829086 rs7573488	0.02
TFPI	rs16829086 rs7573488	0.03
TFPI	rs7573488 rs7594359	0.03
TFPI	rs7573488 rs7594359 rs10179730	0.04
TREM1	rs16894387 rs2234243 rs1817537	0.04
TREM1	rs16894387 rs2234243 rs1817537 rs3804277	0.04
TREM1	rs2234243 rs1817537 rs3804277 rs4711668	0.02
TREM1	rs2234243 rs1817537	0.03
TREM1	rs2234243 rs1817537 rs3804277	0.03
TREM1	rs1817537 rs3804277 rs4711668	0.01
TREM1	rs1817537 rs3804277 rs4711668 rs6910730	0.02
TREM1	rs3804277 rs4711668	0.01
TREM1	rs3804277 rs4711668 rs6910730	0.02
TREM1	rs3804277 rs4711668 rs6910730 rs6940092	0.02
TREM1	rs4711668 rs6910730 rs6940092 rs6939973	0.01
TREM1	rs4711668 rs6910730	0.01
TREM1	rs4711668 rs6910730 rs6940092	0.01
TREM1	rs6910730 rs6940092 rs6939973 rs3827632	0.01
TREM1	rs6910730 rs6940092 rs6939973	0.01
TREM1	rs6910730 rs6940092	0.01
TREM1	rs6940092 rs6939973 rs3827632	0.01
TREM1	rs6940092 rs6939973 rs3827632 rs1385105	0.01
TREM1	rs6940092 rs6939973	0.02

b)

Gene	Haplotype	p
C6orf48	rs2471980 rs16894387	0.02
C6orf48	rs2471980 rs16894387 rs2234243	0.02
C6orf48	rs2471980 rs16894387 rs2234243 rs1817537	0.04
COL1A2	rs11765563 rs388625 rs1800222	0.01
COL1A2	rs11765563 rs388625 rs1800222 rs411717	0.01
COL1A2	rs11765563 rs388625	0.02
COL1A2	rs388625 rs1800222	4.4E-03

COL1A2	rs388625 rs1800222 rs411717	4.4E-03
COL1A2	rs388625 rs1800222 rs411717 rs420257	0.01
COL1A2	rs1800222 rs411717	4.4E-03
COL1A2	rs1800222 rs411717 rs420257	0.01
COL1A2	rs1800222 rs411717 rs420257 rs760043	0.01
COL1A2	rs411717 rs420257	0.01
COL1A2	rs411717 rs420257 rs760043	0.01
COL1A2	rs411717 rs420257 rs760043 rs406226	0.03
COL1A2	rs420257 rs760043	0.01
COL1A2	rs420257 rs760043 rs406226	0.01
COL1A2	rs420257 rs760043 rs406226 rs3763466	0.04
COL1A2	rs760043 rs406226 rs3763466 rs17166249	0.05
COL1A2	rs406226 rs3763466	0.04
COL1A2	rs17166249 rs389328	0.04
COL1A2	rs389328 rs42521	0.04
COL1A2	rs42523 rs42524	0.03
COL1A2	rs42524 rs2621213	0.03
COL1A2	rs369982 rs42528 rs4266 rs2472	1.0E-03
COL1A2	rs369982 rs42528	0.02
COL1A2	rs369982 rs42528 rs4266	0.03
COL1A2	rs42528 rs4266 rs2472	4.2E-04
COL1A2	rs42528 rs4266 rs2472 rs42531	5.2E-04
COL1A2	rs42528 rs4266	0.01
COL1A2	rs4266 rs2472 rs42531 rs441051	3.9E-04
COL1A2	rs4266 rs2472 rs42531	1.7E-03
COL1A2	rs4266 rs2472	0.01
COL1A2	rs2472 rs42531 rs441051	1.9E-04
COL1A2	rs2472 rs42531 rs441051 rs400218	5.5E-04
COL1A2	rs2472 rs42531	2.4E-03
COL1A2	rs42531 rs441051	0.01
COL1A2	rs42531 rs441051 rs400218	0.03
COL1A2	rs441051 rs400218	0.01
COL1A2	rs441051 rs400218 rs7804898 rs10046552	0.03
COL1A2	rs441051 rs400218 rs7804898	0.03
COL1A2	rs400218 rs7804898 rs10046552	0.03
COL1A2	rs13234022 rs854547 rs8491 rs854548	0.02
CRH	rs6996265 rs3176921 rs6472257	0.02
CRH	rs6996265 rs3176921	0.03
CRH	rs3176921 rs6472257	0.02
HSD11B1	rs2235543 rs4844880 rs846910 rs3753519	0.01
HSD11B1	rs4844880 rs846910 rs3753519	0.04
HSD11B1	rs4844880 rs846910 rs3753519 rs6672256	0.04
HSD11B1	rs846910 rs3753519 rs6672256	0.01
HSD11B1	rs846910 rs3753519 rs6672256 rs9430012	0.01
HSD11B1	rs846910 rs3753519	0.03
HSD11B1	rs3753519 rs6672256	0.01
HSD11B1	rs3753519 rs6672256 rs9430012	0.01
HSD11B1	rs3753519 rs6672256 rs9430012 rs932335	0.01
IL4R	rs4787948 rs3024530 rs3024537	0.04

IL4R	rs4787948 rs3024530 rs3024537 rs3024547	0.04
IL4R	rs4787948 rs3024530	0.05
IL4R	rs3024537 rs3024547 rs3024548	0.04
IL4R	rs3024547 rs3024548 rs3024560 rs2239347	0.04
IL4R	rs3024547 rs3024548	0.04
IL4R	rs3024548 rs3024560 rs2239347	0.01
IL4R	rs3024548 rs3024560 rs2239347 rs3024623	0.01
IL4R	rs3024548 rs3024560	0.05
IL4R	rs3024560 rs2239347	1.8E-03
IL4R	rs3024560 rs2239347 rs3024623 rs4787423	3.7E-03
IL4R	rs3024560 rs2239347 rs3024623	0.01
IL4R	rs2239347 rs3024623 rs4787423 rs3024676	2.1E-03
IL4R	rs2239347 rs3024623 rs4787423	2.7E-03
IL4R	rs2239347 rs3024623	0.01
MTHFR	rs4846048 rs4846049 rs1476413	1.4E-03
MTHFR	rs4846048 rs4846049 rs1476413 rs1801131	2.0E-03
MTHFR	rs4846048 rs4846049	2.8E-03
MTHFR	rs1476413 rs1801131 rs12121543 rs1994798	1.7E-03
MTHFR	rs1801131 rs12121543 rs1994798	3.1E-03
MTHFR	rs1801131 rs12121543 rs1994798 rs1801133	0.02
MTHFR	rs12121543 rs1994798 rs1801133	0.01
MTHFR	rs12121543 rs1994798	0.01
MTHFR	rs12121543 rs1994798 rs1801133	0.03
MTHFR	rs1994798 rs1801133 rs17421462	0.01
MTHFR	rs1994798 rs1801133	0.02
MTHFR	rs1994798 rs1801133 rs17421462	0.02
MTHFR	rs1801133 rs17421462 rs17421511	0.02
MTHFR	rs1801133 rs17421462 rs17421511	0.02
MTHFR	rs1801133 rs17421462	0.03
MTHFR	rs17421462 rs17421511	0.01
MTHFR	rs17421462 rs17421511 rs4846052	0.01
MTHFR	rs17421462 rs17421511 rs4846052	0.01
MTHFR	rs17421511 rs4846052 rs11121832	3.9E-03
MTHFR	rs17421511 rs4846052 rs11121832	0.01
MTHFR	rs17421511 rs4846052	0.03
MTHFR	rs4846052 rs11121832 rs9651118	0.01
MTHFR	rs4846052 rs11121832 rs9651118	0.01
MTHFR	rs4846052 rs11121832	0.02
MTHFR	rs11121832 rs9651118	2.3E-03
MTHFR	rs11121832 rs9651118 rs17367504	2.8E-03
MTHFR	rs11121832 rs9651118 rs17367504	0.01
MTHFR	rs9651118 rs17367504 rs3753582 rs3737964	2.5E-03
MTHFR	rs9651118 rs17367504 rs3753582	4.6E-03
MTHFR	rs9651118 rs17367504	0.01
MTHFR	rs17367504 rs3753582 rs3737964	4.7E-03
MTHFR	rs17367504 rs3753582 rs3737964 rs590368	0.01
MTHFR	rs3753582 rs3737964	2.8E-03
MTHFR	rs3753582 rs3737964 rs590368	0.01
MTHFR	rs3753582 rs3737964 rs590368 rs652625	0.04

MTHFR	rs3737964 rs590368	0.05
PON1	rs854547 rs8491 rs854548	0.02
PON1	rs854547 rs8491 rs854548 rs854551	0.03
PON1	rs8491 rs854548	0.01
PON1	rs8491 rs854548 rs854551 rs854552	0.01
PON1	rs8491 rs854548 rs854551	0.01
PON1	rs854548 rs854551 rs854552	3.2E-03
PON1	rs854548 rs854551	3.6E-03
PON1	rs854548 rs854551 rs854552 rs854555	0.01
PON1	rs854551 rs854552	0.01
PON1	rs854551 rs854552 rs854555	0.01
PON1	rs854551 rs854552 rs854555 rs3917550	0.02
PON1	rs854552 rs854555	3.1E-03
PON1	rs854552 rs854555 rs3917550	0.01
PON1	rs854552 rs854555 rs3917550 rs2269829	0.01
PON1	rs854555 rs3917550 rs2269829 rs3917542	0.01
PON1	rs854555 rs3917550 rs2269829	0.02
PON1	rs3917550 rs2269829 rs3917542 rs662	0.05
PON1	rs2299262 rs854569 rs2237583 rs757158	0.03
PON1	rs854569 rs2237583 rs757158 rs11977702	0.03
PON1	rs854569 rs2237583 rs757158	0.04
PON1	rs2237583 rs757158	0.02
PON1	rs757158 rs11977702	0.01
SLC6A4	rs7224199 rs1042173	0.01
SLC6A4	rs7224199 rs1042173 rs3794808	0.01
SLC6A4	rs7224199 rs1042173 rs3794808 rs140701	0.01
SLC6A4	rs1042173 rs3794808	0.01
SLC6A4	rs1042173 rs3794808 rs140701	0.01
SLC6A4	rs1042173 rs3794808 rs140701 rs140700	0.02
SLC6A4	rs3794808 rs140701	0.02
SLC6A4	rs3794808 rs140701 rs140700	0.04
SLC6A4	rs140701 rs140700	0.04
SLC6A4	rs2020942 rs6354 rs2020936	0.05
SLC6A4	rs6354 rs2020936 rs12150214 rs4251417	3.2E-03
SLC6A4	rs2020936 rs12150214 rs4251417	2.7E-03
SLC6A4	rs2020936 rs12150214 rs4251417	0.01
SLC6A4	rs12150214 rs4251417	2.0E-03
SLC6A4	rs12150214 rs4251417 rs16965628	0.01
SLC6A4	rs12150214 rs4251417 rs16965628	0.02
SLC6A4	rs4251417 rs16965628 rs2020933	2.8E-03
SLC6A4	rs4251417 rs16965628	3.3E-03
SLC6A4	rs4251417 rs16965628 rs2020933 rs1024610	0.01
SLC6A4	rs2020933 rs1024610	0.05
TFPI	rs12693471 rs8176541	4.5E-03
TFPI	rs12693471 rs8176541 rs7586970	4.5E-03
TFPI	rs12693471 rs8176541 rs7586970 rs3213739	0.01
TFPI	rs8176541 rs7586970	4.2E-03
TFPI	rs8176541 rs7586970 rs3213739	0.01
TFPI	rs8176541 rs7586970 rs3213739 rs8176508	0.01

TFPI	rs7586970 rs3213739	0.01
TFPI	rs7586970 rs3213739 rs8176508	0.01
TFPI	rs7586970 rs3213739 rs8176508 rs2041778	0.01
TFPI	rs3213739 rs8176508 rs2041778 rs2192824	0.01
TFPI	rs3213739 rs8176508	0.01
TFPI	rs3213739 rs8176508 rs2041778	0.02
TFPI	rs8176508 rs2041778 rs2192824 rs3755248	3.5E-03
TFPI	rs8176508 rs2041778 rs2192824	3.5E-03
TFPI	rs8176508 rs2041778	0.01
TFPI	rs2041778 rs2192824 rs3755248 rs12613071	0.04
TFPI	rs2192824 rs3755248 rs12613071	0.03
TFPI	rs2192824 rs3755248 rs12613071	0.05
TFPI	rs3755248 rs12613071 rs16829086	0.02
TFPI	rs12613071 rs16829086 rs7573488	4.9E-04
TFPI	rs12613071 rs16829086 rs7573488	0.01
TFPI	rs16829086 rs7573488 rs7594359	2.9E-04
TFPI	rs16829086 rs7573488 rs7594359	4.1E-04
TFPI	rs16829086 rs7573488	0.01
TFPI	rs7573488 rs7594359 rs10179730 rs6434222	2.9E-04
TFPI	rs7573488 rs7594359 rs10179730	5.1E-04
TFPI	rs7573488 rs7594359	0.01
TFPI	rs7594359 rs10179730 rs6434222	2.9E-04
TFPI	rs7594359 rs10179730 rs6434222	4.7E-04
TFPI	rs10179730 rs6434222 rs10187622	2.3E-04
TFPI	rs10179730 rs6434222	2.7E-04
TFPI	rs6434222 rs10187622	2.7E-04

Appendix Table 13. **Replication of maternal and fetal results.** a) maternal samples, b) fetal samples

a)

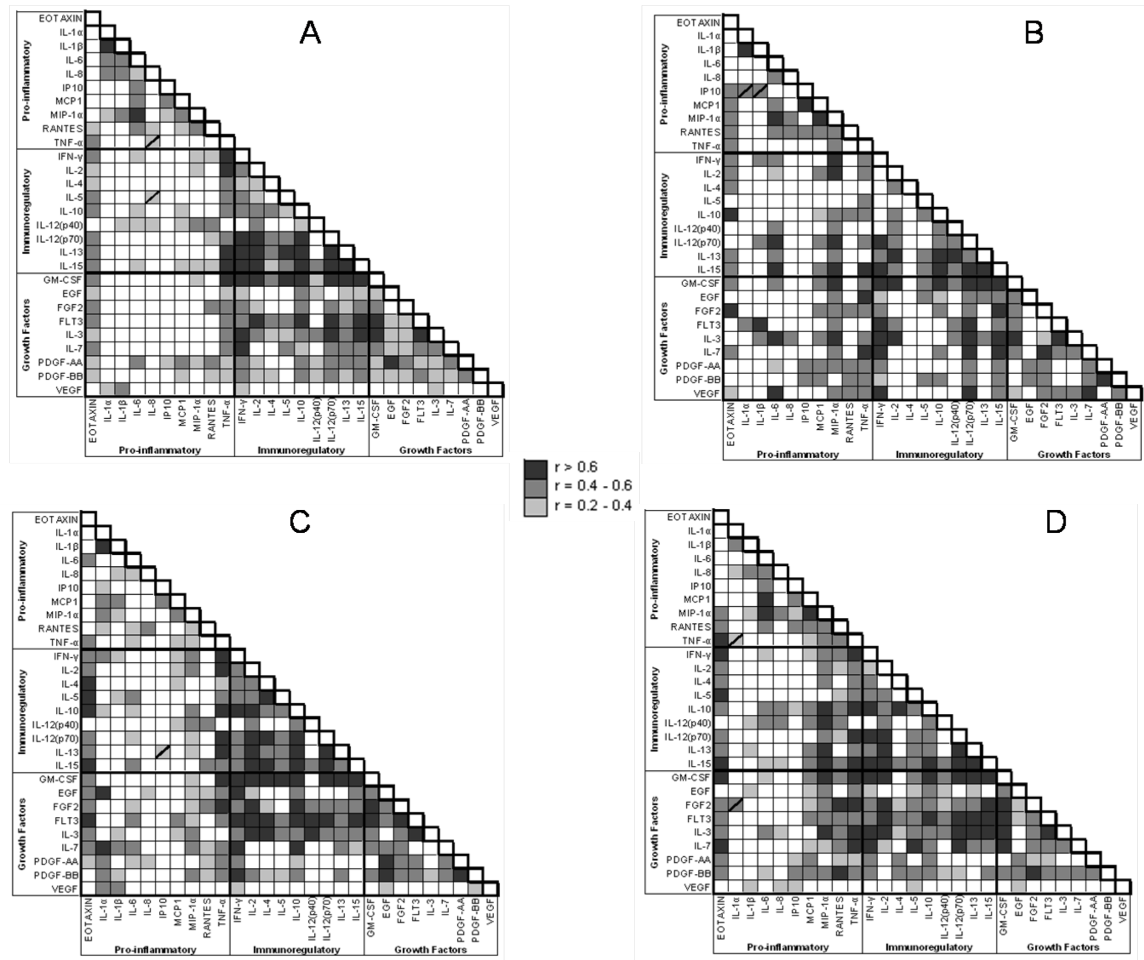
			Centennial Study				Norway MoBa Study				Pooled Data			
			Allele Freq.		Association		Allele Freq.		Association		Allele Freq.		Association	
Gene	SNP	Allele	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype
ADRB2	rs1432622	T	0.47	0.42	0.195	0.264	0.45	0.38	0.048	0.128	0.46	0.40	0.023	0.048
ADRB2	rs12654778	A	0.33	0.40	0.083	0.129	0.37	0.45	0.028	0.088	0.36	0.42	0.008	0.019
AP3M2	rs4581040	G	0.32	0.24	0.042	0.131	0.30	0.24	0.041	0.112	0.31	0.24	0.004	0.017
CBS	rs6586282	T	0.19	0.19	0.837	0.165	0.18	0.15	0.219	0.005	0.19	0.17	0.330	0.585
CBS	rs11203172	T	0.22	0.16	0.092	0.176	0.17	0.20	0.318	0.049	0.19	0.18	0.714	0.591
CCL2	rs1024610	A	0.21	0.20	0.657	0.007	0.18	0.15	0.184	0.180	0.19	0.17	0.258	0.185
COL1A1	rs1061237	C	0.23	0.28	0.090	0.055	0.26	0.29	0.332	0.044	0.24	0.29	0.069	0.003
COL1A2	rs420257	C	0.36	0.29	0.042	0.128	0.23	0.29	0.064	0.111	0.29	0.29	0.924	0.637
COL1A2	rs389328	T	0.20	0.13	0.031	0.083	0.12	0.16	0.131	0.334	0.15	0.15	0.760	0.851
COL1A2	rs2521205	G	0.57	0.47	0.015	0.066	0.43	0.51	0.021	0.045	0.49	0.49	0.832	0.758
COL1A2	rs7804898	G	0.08	0.12	0.050	0.105	0.17	0.14	0.214	0.015	0.13	0.13	0.984	0.059
COL3A1	rs2271682	A	0.75	0.71	0.195	0.181	0.25	0.31	0.040	0.053	0.46	0.50	0.099	0.302
COL5A1	rs12005720	C	0.11	0.17	0.032	0.091	0.19	0.16	0.284	0.041	0.16	0.17	0.681	0.122
COL5A1	rs4842167	C	0.48	0.38	0.009	0.021	0.36	0.44	0.011	0.037	0.41	0.41	0.845	0.526
COL5A1	rs3811161	C	0.43	0.51	0.028	0.087	0.53	0.44	0.012	0.044	0.49	0.48	0.646	0.897
COL5A1	rs10745387	A	0.54	0.61	0.110	0.150	0.37	0.44	0.031	0.101	0.44	0.52	0.003	0.009
CRHBP	rs10055255	A	0.67	0.55	0.001	0.006	0.41	0.36	0.125	0.286	0.52	0.45	0.006	0.026
CRHBP	rs1875999	G	0.29	0.40	0.005	0.023	0.38	0.33	0.113	0.250	0.34	0.36	0.485	0.784
CRHR2	rs12701020	T	0.16	0.15	0.551	0.191	0.18	0.14	0.086	0.015	0.17	0.14	0.090	0.006
CYP19A1	rs17703982	T	0.09	0.06	0.141	0.037	0.07	0.04	0.060	0.086	0.07	0.05	0.023	0.017
F5	rs2187952	A	0.24	0.31	0.045	0.098	0.24	0.29	0.126	0.242	0.24	0.30	0.012	0.026
IL1B	rs1143630	A	0.09	0.07	0.436	0.168	0.04	0.08	0.025	0.044	0.06	0.08	0.263	0.131
IL1R1	rs3917273	T	0.39	0.47	0.034	0.092	0.42	0.35	0.036	0.015	0.41	0.41	1.000	0.396
IL1R1	rs2110726	T	0.44	0.34	0.007	0.014	0.40	0.43	0.425	0.026	0.42	0.38	0.216	0.211
IL1R2	rs4851520	C	0.15	0.10	0.046	0.103	0.15	0.11	0.059	0.162	0.15	0.11	0.006	0.016
IL1R2	rs4851522	T	0.16	0.11	0.042	0.072	0.15	0.11	0.090	0.233	0.16	0.11	0.009	0.022
IL1R2	rs1108338	C	0.28	0.21	0.038	0.082	0.29	0.23	0.022	0.012	0.29	0.22	0.002	0.001
IL1RAP	rs7628333	T	0.21	0.24	0.358	0.080	0.22	0.32	0.002	0.005	0.22	0.28	0.004	0.012

IL2RA	rs2031229	A	0.19	0.26	0.036	0.142	0.28	0.23	0.088	0.074	0.25	0.25	0.999	0.408
IL2RA	rs1107345	A	0.21	0.25	0.198	0.471	0.28	0.22	0.042	0.063	0.25	0.23	0.464	0.443
IL2RA	rs11256497	A	0.32	0.37	0.167	0.314	0.31	0.38	0.035	0.104	0.31	0.37	0.012	0.045
IL4R	rs3024530	G	0.52	0.44	0.025	0.062	0.47	0.42	0.157	0.061	0.49	0.43	0.014	0.010
IL4R	rs3024548	C	0.49	0.57	0.039	0.075	0.46	0.41	0.132	0.038	0.47	0.49	0.637	0.048
IL6R	rs4845374	T	0.87	0.82	0.139	0.110	0.12	0.17	0.042	0.027	0.43	0.48	0.051	0.002
IL6R	rs4329505	C	0.13	0.17	0.129	0.067	0.12	0.17	0.042	0.027	0.13	0.17	0.011	0.003
MMP2	rs1053605	T	0.04	0.06	0.184	0.304	0.09	0.05	0.037	0.068	0.07	0.06	0.383	0.482
NAT1	rs7017402	A	0.15	0.10	0.038	0.088	0.13	0.09	0.043	0.048	0.14	0.09	0.004	0.013
NAT1	rs9325827	C	0.18	0.11	0.012	0.040	0.16	0.11	0.048	0.083	0.17	0.11	0.002	0.008
NAT1	rs4921880	T	0.28	0.23	0.147	0.055	0.27	0.25	0.523	0.021	0.27	0.24	0.149	0.290
PGR	rs555572	C	0.30	0.34	0.243	0.130	0.35	0.27	0.007	0.026	0.33	0.30	0.235	0.135
PGR	rs11224589	C	0.30	0.35	0.210	0.138	0.37	0.28	0.007	0.021	0.34	0.31	0.253	0.125
PGR	rs619487	G	0.37	0.31	0.102	0.132	0.33	0.39	0.040	0.018	0.34	0.35	0.682	0.065
PLA2G4A	rs2076075	A	0.14	0.10	0.110	0.236	0.17	0.12	0.020	0.056	0.16	0.11	0.004	0.016
PLAT	rs2020922	A	0.32	0.24	0.029	0.095	0.30	0.24	0.034	0.081	0.31	0.24	0.003	0.009
PTGER3	rs959	G	0.25	0.25	0.858	0.055	0.25	0.24	0.581	0.035	0.25	0.24	0.780	0.955
PTGER3	rs5702	T	0.24	0.25	0.681	0.154	0.27	0.25	0.575	0.020	0.25	0.25	0.852	0.862
PTGER3	rs1409165	C	0.14	0.11	0.169	0.432	0.09	0.14	0.024	0.031	0.11	0.12	0.443	0.763
PTGER3	rs977214	G	0.09	0.13	0.166	0.005	0.06	0.09	0.064	0.039	0.07	0.11	0.016	0.000
PTGER3	rs6665776	A	0.09	0.12	0.235	0.010	0.06	0.09	0.064	0.039	0.07	0.11	0.024	0.000
PTGER3	rs2072947	C	0.53	0.44	0.017	0.034	0.41	0.46	0.205	0.027	0.46	0.45	0.582	0.039
TIMP3	rs130290	T	0.06	0.11	0.013	0.022	0.08	0.05	0.151	0.234	0.07	0.08	0.373	0.644
TIMP3	rs130293	C	0.05	0.11	0.011	0.025	0.08	0.05	0.150	0.228	0.07	0.08	0.378	0.636
TLR2	rs1898830	G	0.29	0.38	0.015	0.052	0.33	0.38	0.152	0.345	0.31	0.38	0.008	0.025
TNFRSF1A	rs4149578	A	0.12	0.07	0.033	0.044	0.14	0.10	0.156	0.314	0.13	0.09	0.012	0.028
TSHR	rs11845715	T	0.22	0.15	0.025	0.099	0.17	0.17	0.779	0.023	0.19	0.16	0.093	0.025
TSHR	rs17630128	C	0.33	0.25	0.031	0.023	0.36	0.30	0.072	0.192	0.35	0.28	0.004	0.010
TSHR	rs12883801	G	0.41	0.52	0.005	0.011	0.37	0.43	0.107	0.137	0.39	0.47	0.001	0.001

b)

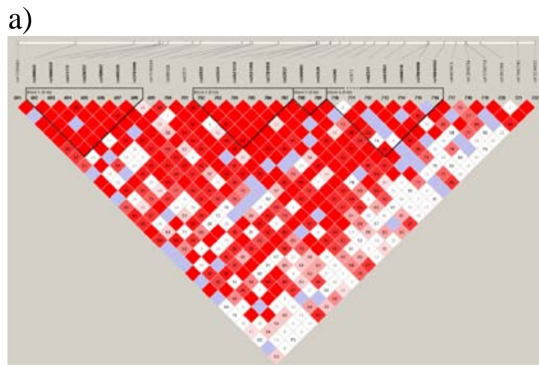
Gene	SNP	Allele	Centennial Study				Norway MoBa Study				Pooled Data			
			Allele Freq.		Association		Allele Freq.		Association		Allele Freq.		Association	
			Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype	Case	Cont	Allele	Genotype
C6orf48	rs2471980	G	0.36	0.31	0.239	0.170	0.28	0.36	0.019	0.004	0.31	0.34	0.316	0.458
COL1A2	rs42524	G	0.77	0.79	0.440	0.055	0.19	0.26	0.010	0.022	0.43	0.50	0.003	0.001
COL1A2	rs42528	T	0.24	0.23	0.699	0.135	0.20	0.28	0.008	0.018	0.21	0.25	0.079	0.025
COL3A1	rs2271682	A	0.76	0.71	0.120	0.045	0.26	0.32	0.062	0.108	0.47	0.49	0.251	0.008
COL3A1	rs3134656	A	0.50	0.39	0.005	0.026	0.47	0.41	0.067	0.098	0.48	0.40	0.001	0.004
COL5A2	rs9288163	A	0.11	0.06	0.025	0.034	0.08	0.09	0.467	0.192	0.09	0.08	0.361	0.057
EDN2	rs4660541	T	0.24	0.28	0.254	0.012	0.27	0.28	0.648	0.159	0.26	0.28	0.287	0.538
EPHX2	rs10503812	A	0.21	0.15	0.040	0.118	0.15	0.13	0.574	0.029	0.17	0.14	0.082	0.025
EPHX2	rs4149239	G	0.21	0.15	0.047	0.110	0.15	0.13	0.575	0.028	0.17	0.14	0.092	0.017
EPHX2	rs4149252	T	0.20	0.15	0.052	0.153	0.15	0.13	0.575	0.028	0.17	0.14	0.097	0.029
EPHX2	rs4149259	T	0.21	0.14	0.024	0.053	0.15	0.13	0.420	0.015	0.17	0.14	0.040	0.005
EPHX2	rs891401	G	0.21	0.15	0.030	0.081	0.15	0.13	0.574	0.029	0.17	0.14	0.070	0.025
HSPA6	rs9427401	C	0.86	0.89	0.160	0.381	0.15	0.09	0.007	0.022	0.44	0.45	0.508	0.027
IL1A	rs17561	T	0.24	0.33	0.012	0.043	0.35	0.28	0.025	0.058	0.30	0.30	0.887	0.891
IL1A	rs1878321	C	0.24	0.33	0.014	0.056	0.35	0.28	0.025	0.058	0.31	0.30	0.875	0.814
IL1A	rs2856838	T	0.42	0.35	0.073	0.158	0.32	0.40	0.027	0.023	0.36	0.38	0.607	0.547
IL1B	rs1143630	A	0.10	0.05	0.036	0.025	0.05	0.08	0.151	0.147	0.07	0.07	0.740	0.747
IL1R1	rs3917225	G	0.47	0.41	0.169	0.351	0.45	0.53	0.031	0.083	0.46	0.47	0.515	0.799
IL1RAP	rs9845825	A	0.40	0.30	0.015	0.011	0.35	0.30	0.132	0.311	0.37	0.30	0.007	0.018
IL1RN	rs315920	T	0.24	0.21	0.276	0.040	0.18	0.22	0.198	0.108	0.21	0.21	0.783	0.013
IL2RA	rs12722596	G	0.08	0.13	0.026	0.084	0.13	0.08	0.035	0.081	0.11	0.11	0.924	0.847
IL4R	rs1805015	C	0.23	0.18	0.096	0.228	0.16	0.11	0.039	0.103	0.19	0.14	0.013	0.044
IL4R	rs3024676	A	0.24	0.19	0.099	0.149	0.17	0.11	0.031	0.076	0.20	0.15	0.012	0.038
MMP1	rs1155764	G	0.20	0.12	0.011	0.019	0.11	0.15	0.109	0.091	0.15	0.14	0.618	0.558
MTHFR	rs1476413	A	0.26	0.31	0.197	0.427	0.25	0.31	0.049	0.021	0.26	0.31	0.019	0.026
MTHFR	rs1994798	C	0.39	0.45	0.131	0.281	0.37	0.47	0.004	0.013	0.38	0.46	0.001	0.006
MTHFR	rs4846048	G	0.28	0.33	0.189	0.352	0.25	0.31	0.047	0.101	0.26	0.32	0.016	0.054
MTHFR	rs4846052	T	0.39	0.46	0.085	0.205	0.36	0.45	0.009	0.010	0.37	0.46	0.002	0.003
NFKB1	rs13117745	T	0.18	0.14	0.149	0.375	0.17	0.11	0.011	0.019	0.18	0.12	0.005	0.016
NFKB1	rs4648141	A	0.20	0.15	0.067	0.140	0.19	0.14	0.047	0.102	0.19	0.14	0.007	0.026
NR3C1	rs9324918	G	0.21	0.14	0.031	0.076	0.21	0.18	0.380	0.036	0.21	0.16	0.036	0.005

PGR	rs11224589	C	0.26	0.34	0.024	0.078	0.34	0.28	0.085	0.231	0.31	0.31	0.875	0.752
PGR	rs555572	C	0.26	0.34	0.023	0.055	0.33	0.28	0.117	0.294	0.30	0.31	0.755	0.690
PON1	rs2272365	G	0.19	0.13	0.046	0.052	0.17	0.12	0.041	0.121	0.18	0.13	0.005	0.014
PON1	rs854552	C	0.22	0.28	0.120	0.248	0.21	0.30	0.002	0.006	0.21	0.29	0.001	0.002
PON2	rs2286233	A	0.91	0.84	0.009	0.041	0.10	0.15	0.037	0.106	0.43	0.46	0.236	0.018
PTGER2	rs1254600	T	0.15	0.20	0.131	0.157	0.20	0.16	0.112	0.042	0.18	0.18	0.825	0.089
SLC6A4	rs1042173	G	0.40	0.47	0.093	0.211	0.40	0.49	0.008	0.032	0.40	0.48	0.002	0.008
SLC6A4	rs140701	A	0.38	0.43	0.159	0.322	0.39	0.47	0.022	0.074	0.38	0.45	0.009	0.028
SLC6A4	rs3794808	A	0.37	0.45	0.044	0.109	0.39	0.47	0.018	0.062	0.38	0.46	0.002	0.007
SLC6A4	rs7224199	T	0.42	0.47	0.175	0.359	0.40	0.49	0.008	0.032	0.41	0.48	0.004	0.013
TREM1	rs4711668	T	0.27	0.36	0.017	0.030	0.34	0.28	0.072	0.134	0.31	0.32	0.818	0.382
TSHR	rs179247	G	0.51	0.42	0.024	0.024	0.42	0.46	0.174	0.141	0.46	0.45	0.680	0.121
UGT1A1	rs11888492	C	0.85	0.90	0.044	0.117	0.13	0.11	0.449	0.033	0.42	0.47	0.070	0.041



Appendix Figure 1: Significant cytokine correlation patterns a) BV^- EA, b) BV^+ EA, c) BV^- AA and d) BV^+ AA. Shaded cell denotes a significant correlation for given pair of cytokines with the darker shade representing stronger correlation coefficients as indicated in the key. White boxes denote that the correlation between the two cytokines was not significant. Sideways slash denotes a negative correlation.

a)



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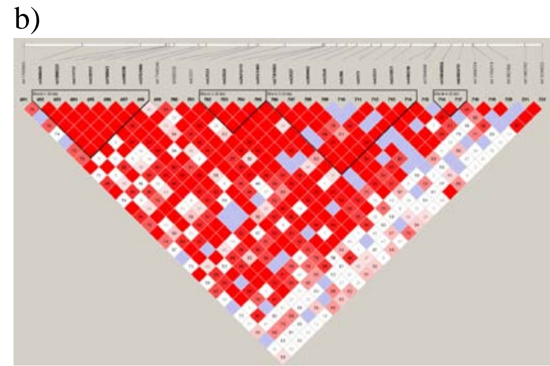

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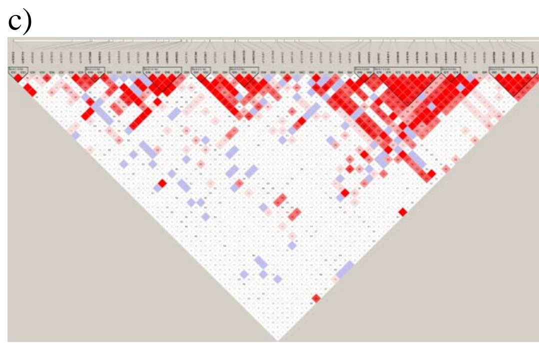

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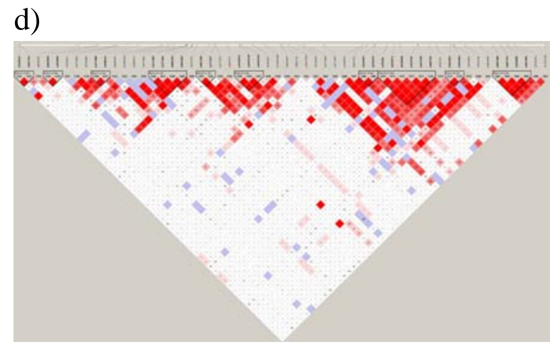
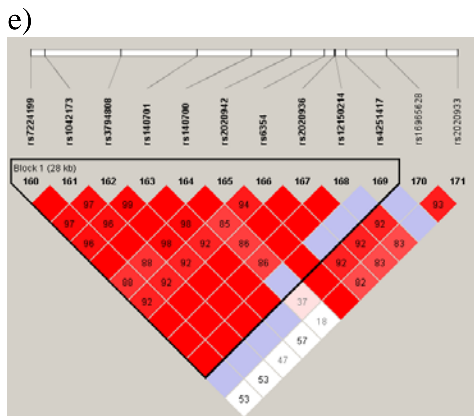


e)



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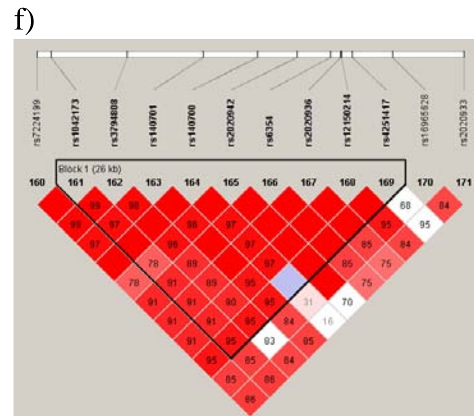

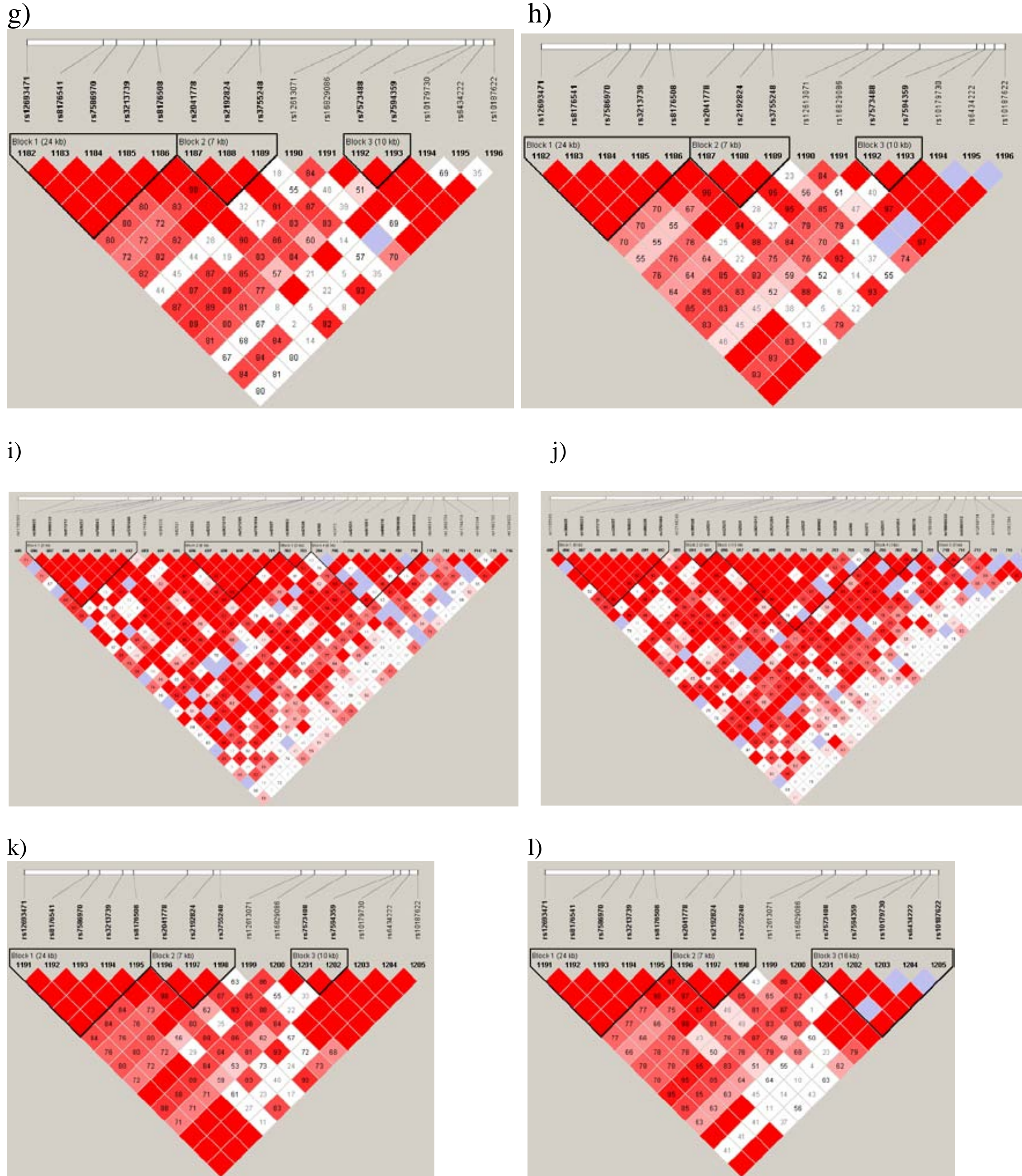

Appendix Figure 2: LD patterns. a) maternal COL1A2 cases b) maternal COL1A2 controls c) maternal IL1RAP cases d) maternal IL1RAP controls e) fetal SLC6A4 cases f) fetal SLC6A4 controls g) fetal TFPI cases h) fetal TFPI controls, i) fetal COL1A2 cases, j) fetal COL1A2 controls k) maternal TFPI cases, l) maternal TFPI controls. Red shading denotes strength of LD. D′ values are shown.

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