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Genetic and metabolic associations with preterm birth

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GENETIC AND METABOLIC ASSOCIATIONS WITH PRETERM BIRTH

by

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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To my parents, for raising me to believe that I can accomplish anything I set my mind to.

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ABSTRACT

Preterm birth is defined as delivery prior to 37 weeks' completed gestation. It affects an average of 11% of pregnancies worldwide and is the leading cause of death in children under age 5. Many studies have identified associations between pregnancy lipid levels and increased risk for preterm birth. This thesis investigates the role of genetic variability associated with lipids and its relationship with preterm birth, and the relationship between pre-pregnancy dyslipidemia and risk for preterm birth.

Genetic variability in the form of single-nucleotide polymorphisms, previously identified by genome-wide association studies for association with lipid levels, was analyzed for association with risk for preterm birth. The study population included 992 women in California with banked 2nd trimester serum samples. Serum lipid levels and DNA were used. Genetic risk scores were constructed for each subject using published SNPs associated with lipid levels as an indicator of genetic burden. These genetic risk scores were then analyzed for association with risk for preterm birth. The GRS were not associated with the overall risk for preterm birth. However, a higher HDL-C GRS was associated with increased risk for spontaneous preterm birth. Higher triglyceride and total cholesterol GRS were associated with decreased risk for spontaneous preterm birth.

The relationship between pre-pregnancy dyslipidemia and risk for preterm birth was assessed in a cohort of 2,962,434 women giving birth in the state of California from 2007-2012. Dyslipidemia, as defined by medical diagnostic codes, was associated with a 1.5-fold increase in risk for preterm birth. This association was consistent across race/ethnicity, body mass index, type of dyslipidemia, and type of preterm birth.

This thesis identified counter-intuitive associations between lipid GRS and spontaneous preterm birth, while also identifying a strong relationship between pre-pregnancy dyslipidemia and all types of preterm birth including spontaneous. Together, these findings suggest that the previously reported associations between lipids and preterm birth may be reflecting unidentified dyslipidemias. One possible interpretation of the counter-intuitive genetic findings is that while

extreme dyslipidemia predisposes to preterm birth a genetic predisposition to low total cholesterol also confers increased risk for spontaneous preterm birth. An alternative explanation is that these results are simply an artefact of the data and additional genetic loci and lifestyle factors confer stronger effects on risk for spontaneous PTB than the effects of the genetic loci included in this thesis.

PUBLIC ABSTRACT

Preterm birth (PTB) affects an average of 11% of pregnancies worldwide and is the leading cause of death in children under age 5. Lipid levels (cholesterol) change during pregnancy to support the growing fetus. Many studies have identified associations between *extreme* pregnancy lipid levels and increased risk for PTB. In all people, genes contribute to lipid levels. Some people have higher genetic risk for extreme lipid levels. This thesis investigates the role of genetic variability associated with lipids and its relationship with PTB, and the relationship between pre-pregnancy dyslipidemia (high cholesterol) and risk for PTB.

Genetic variability that contributes to lipid levels was analyzed for association with risk for PTB. The study population included 992 women in California with banked 2nd trimester blood samples. Lipid levels and DNA were used. Genetic risk for higher than average lipid levels was calculated for each subject. These genetic risk scores were then analyzed for association PTB risk. Genetic predisposition to higher HDL-C and lower triglyceride or total cholesterol were associated with increased risk for spontaneous PTB. This is counter-intuitive to previous literature.

The relationship between pre-pregnancy dyslipidemia and risk for PTB was assessed in a cohort of 2,962,434 women giving birth in California. Dyslipidemia, as defined by medical codes, was associated with a 1.5-fold increase in PTB risk.

Together, these findings suggest that the previously reported associations between lipids and PTB may be reflecting unidentified dyslipidemias, and that alternative genes and lifestyle factors confer stronger effects on risk for spontaneous PTB.

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LIST OF ABBREVIATIONS

ABCA1 ATP binding cassette subfamily A member 1 transporter

ABCG1 ATP binding cassette subfamily G member 1 transporter

BMI body mass index

BV bacterial vaginosis

CHD coronary heart disease

CI confidence interval

ER endoplasmic reticulum

GWAS genome-wide association study

HDL high density lipoprotein

IDL intermediate density lipoprotein

LDL low density lipoprotein

OR odds ratio

PTB preterm birth

RR relative risk

SCAP SREBP-cleavage and activating protein

SNP single nucleotide polymorphism

SR-BI scavenger receptor class B member1

SRE sterol regulatory element

SREBP sterol regulatory element binding protein

TC total cholesterol

TG triglyceride

USA United States of America

VLDL very low density lipoprotein

CHAPTER I: The Origins and Fates of Human Lipids

Lipids are a class of compounds that are present throughout the body in various forms. Lipids include compounds of both endogenous and exogenous origin. In the context of human metabolism and clinical care, lipids can be considered in two distinct categories: cholesterol and triglyceride, which are bound to lipoproteins for transport in blood circulation. The origins and fates of these compounds will be discussed in this chapter.

Cholesterol

Cholesterol is an amphipathic sterol compound that is vital to cellular structure and function. It is ubiquitously expressed in all cell types throughout Kingdom Animalia. Analogous compounds are present in the Kingdom Fungi as ergosterols and in the Kingdom Plantae as phytosterols, where they are comparably critical to cellular structure and function.[1,2]

Cholesterol Synthesis

In addition to cholesterol's necessity at the cellular level, cholesterol intermediates are the biochemical precursors to bile salts, steroid hormones and vitamin D.[3] Bile salts emulsify dietary fats to prepare them for further metabolism. Steroid hormones are cell signaling molecules that control diverse bodily functions. Vitamin D is synthesized in skin cells when a cholesterol intermediate is exposed to ultraviolet radiation.[3]

Cholesterol is synthesized at the interface of the cytoplasm and endoplasmic reticulum.[4] It is synthesized by a series of enzyme-mediated reactions to produce the 27-carbon cholesterol molecule.[4] The rate-limiting step is at the level of HMG-CoA reductase, which reduces HMG-CoA to mevalonic acid.[5] This enzyme is the target of statin drugs.[6]

Cholesterol is synthesized by virtually all mammalian cell types.[6] Due to its necessity, cholesterol synthesis is a highly conserved cellular process by which cells regulate the synthesis and localization of cholesterol.

Sources of Cholesterol

The two sources of bodily cholesterol include endogenously synthesized cholesterol and dietary cholesterol. The average Western diet includes the consumption of ~300-500mg of cholesterol per day, while the human body synthesizes ~800-1200mg of cholesterol per day.[6] Another estimate is that ~80% of the bodily cholesterol pool derives from endogenous synthesis.[7] Thus, endogenous cholesterol constitutes most of the bodily cholesterol pool.

The two sources of cholesterol are associated with different chemical properties affecting their biological activity. Biliary cholesterol is unesterified and can thus be readily absorbed by cells.[6] By contrast, dietary cholesterol is partly esterified and must be hydrolyzed by pancreatic carboxyl ester lipase prior to absorption by enterocytes.[6]

Excess dietary cholesterol has long been assumed to contribute to atherosclerosis and increased risk for cardiovascular disease. However, epidemiologic studies have refuted this claim. Indeed, the 2015 Scientific Report of the Dietary Guidelines Advisory Committee recanted its previous recommendations regarding dietary cholesterol limits.[8] Citing lack of evidence between dietary cholesterol consumption and serum cholesterol levels, the Committee no longer considers cholesterol a nutrient of concern for overconsumption.[8]

Cholesterol Homeostasis

Cholesterol synthesis is tightly regulated at the cellular level. The enzyme responsible for the rate-limiting step of cholesterol synthesis, HMG CoA reductase, is also the level at which cellular cholesterol synthesis is regulated via a proteolytic pathway.[4,9,10] The sterol regulatory element (SRE), is a DNA sequence that regulates HMG CoA reductase synthesis.[9] The sterol regulatory element binding protein (SREBP) is a transcription factor localized to the endoplasmic reticulum (ER).[9] It is co-localized with SREBP cleavage and activating protein (SCAP), which is an ER transmembrane protein.[9] SCAP senses and responds to cellular cholesterol levels. When cellular cholesterol is low, SCAP and SREBP are cleaved from the ER and directed to the Golgi apparatus.[11] The Golgi apparatus further cleaves SREBP so it can enter the nucleus where it binds SRE and triggers increased HMG CoA reductase synthesis, ultimately increasing the amount of cholesterol that can be synthesized.[11] When cellular cholesterol is sufficient, the SCAP/SREBP complex remain intact. Translation of HMG CoA reductase mRNA is inhibited by sufficient dietary cholesterol intake and by post-mevalonate metabolites in a feedback loop.[12] The HMG CoA reductase enzyme is ultimately localized to the ER as a transmembrane protein whose cytosolic domain confers reductase activity and membrane domain contains a sterol-sensing domain.[11] Sufficient cellular cholesterol causes ubiquitination of the enzyme, ultimately resulting in its degradation.[11]

Cholesterol is primarily excreted via the biliary system, in which cholesterol and bile salts are excreted in feces.[13] Cholesterol delivered to the liver from peripheral tissues is transported to the small intestine via bile. Cholesterol that is not reabsorbed by the small intestine is ultimately excreted in feces.[6]

Cholesterol efflux involves active transport via the ATP binding cassette subfamily A member 1 transporter (ABCA1) and ATP binding cassette subfamily G member 1 transporter (ABCG1), and facilitated diffusion via scavenger receptor class B member1 (SR-BI).[14]

ABCA1 is a transmembrane protein localized to the late endosome/lysosome, the Golgi complex and plasma membrane, allowing it to facilitate the flow of intracellular cholesterol from these organelles to the plasma membrane.[14] ABCA1 contributes to nascent HDL formation by facilitating the transfer of plasma membrane lipids to lipid-poor apolipoproteins. Thus, the rate of ABCA1-mediated cellular lipid depletion influences cholesterol synthesis and LDL-receptor expression to maintain cellular cholesterol homeostasis. ABCA1 expression is triggered by cellular cholesterol accumulation.[15] ABCA1-mediated transport is the primary mechanism of cholesterol efflux in cultured human foam cells.[13]

ABCG1 is localized to endosomes where it facilitates the transport of cholesterol from the endoplasmic reticulum to the plasma membrane. Its localization in the plasma membrane is a matter of debate.[14]

SR-BI is a transmembrane protein localized to the plasma membrane, most abundantly in hepatocytes.[14] This receptor is responsible for the selective uptake of the core HDL cholesteryl ester, without requiring endocytic uptake of the entire HDL particle.[13] Thus, hepatocytes receive cholesterol via HDL. Hepatocytes then process the cholesterol and direct it toward recirculation via LDL or degradation via the biliary system.

Lipoproteins

The cholesterol and triglyceride-carrying lipoprotein complexes, including VLDL, IDL, LDL, HDL and chylomicrons, are characterized by the presence of different apolipoproteins. Apolipoproteins, including ApoA1, ApoB-100, ApoB-48, ApoC and ApoE, are key components of lipid-carrying particles.

Apolipoprotein A1, encoded by the *APOA* gene, is the characteristic apolipoprotein of high density lipoprotein (HDL). HDL binds cholesterol from peripheral tissues and transports it to the liver. The primary mechanism of nascent HDL particle formation occurs in hepatocytes and enterocytes.[16] Nascent HDL containing ApoA1 enter venous circulation where they interact with peripheral cells and arterial macrophages via the ABCA1 protein.[16] HDL ApoA1 binding to ABCA1 triggers cells to transport un-esterified cholesterol to the HDL particle, where the cholesterol is rapidly esterified to prevent its exit from the particle.[16] As HDL particles circulate,

they pick up more cholesterol from cells. Upon reaching the liver, cholesterol-rich HDL particles are taken up by hepatocytes where they are broken down to release their cholesterol content.[7] This cholesterol can then be packaged into new lipoprotein particles for circulation or directed to bile for degradation and excretion in feces.[13]

The apolipoprotein B gene, *APOB*, encodes two different apolipoproteins via alternative splicing.[17] The larger protein, Apo B-100, is the characteristic apolipoprotein of LDL, VLDL and IDL.[16] ApoB-100 on LDL particles interacts with the extracellular matrix of the arterial intima, allowing LDL particles to embed.[18] Trapped LDL particles oxidize, triggering recruitment of circulating monocytes to the intima.[19] These monocytes differentiate into macrophages and continue to take up oxidized LDL via scavenger receptors.[20] As arterial macrophages take up more ox-LDL, they are known as foam cells, which progress to atherosclerotic plaques.[19]

Very-low density lipoprotein (VLDL) also contains ApoB-100. These particles deliver stored triglyceride (TG) from the liver to peripheral tissues. In hepatocytes, ApoB-100 and TG are directed to the Golgi apparatus where they are packaged into nascent VLDL.[16] These particles enter the bloodstream directly from the liver and deliver TG to peripheral tissues in much the same manner as chylomicrons.[16]

ApoB-48 is unique to chylomicrons. In the fed state, fatty acids enter intestinal epithelial cells (enterocytes) where they are converted to TG.[7] ApoB-48 and TG are directed to the Golgi apparatus where they are packaged into nascent chylomicrons and ultimately deliver TG to peripheral tissues.[7]

Apolipoproteins C and E, encoded by *APOC* and *APOE*, respectively, are present in HDL, LDL and VLDL, where they facilitate the exchange of fatty acids and cholesterol.[16]

Triglyceride

Dietary fats are initially emulsified by lingual lipases in the mouth and gastric lipases in the stomach.[21] When dietary fats exit the stomach and enter the small intestine, bile emulsifies the large fatty droplets into smaller pieces.[21] Pancreatic lipases localized to the apical membrane of intestinal cells (enterocytes) hydrolyze triglyceride (TG), cleaving the ester linkages resulting in three carboxylic acids, known as fatty acids, and a glycerol backbone.[22] Fatty acids diffuse across the enterocyte membrane, entering the enterocytes where they are rapidly esterified back into TG.[7] TGs are then packaged into chylomicrons, containing ApoB-48, ApoE and cholesterol.[7] Unlike proteins and carbohydrates, which exit the small intestine and go directly to the liver, TG

packaged in chylomicrons enter the lymphatic system and venous system prior to entering the liver.[7] Chylomicrons are too large to diffuse into the capillaries that surround the basolateral side of the small intestine, so instead they enter the vessels of the lymphatic system, known as lacteals, which are also located on the basolateral side of the small intestine.[23] Chylomicrons are exocytosed from enterocytes, enter lacteals, and travel through the lymphatic system until they reach the thoracic lymphatic duct, which drains into the venous system.[23] Of note, the exact mechanism by which chylomicrons enter lacteals remains an active area of research, although evidence suggests it may occur via vesicular transport or via diffusion through open junctions.[23]

Once the chylomicrons have entered the venous system, they eventually enter arterial blood flow, which lead to capillary beds of peripheral tissues. Lipoprotein lipases, which are localized to the luminal side of capillaries, break the TG into free fatty acids and glycerol backbones.[24] These free fatty acids and glycerol molecules diffuse across the cell membrane of peripheral tissues, such as myocytes and adipocytes. In myocytes, fatty acids are oxidized to yield ATP.[7] In adipocytes, fatty acids and glycerol are re-esterified into TG.[7]

Once chylomicrons have travelled through capillaries and reach the liver, the fat-depleted chylomicrons are known as chylomicron remnants.[25] These are taken up by the liver via receptor-mediated endocytosis, in which ApoE located on chylomicrons is recognized by the LDL-receptor on hepatocytes.[25]

Genetic Determinants of Lipid Levels

Endogenous synthesis of cholesterol and lipoproteins are genetically determined by the biochemical activity of the enzymes involved in the synthesis processes. Although dietary intake of cholesterol and fats contribute to homeostasis, and thus affect the degree of endogenous synthesis required to maintain homeostasis, the success of the chemical reactions can be influenced by genetic variability in enzyme-encoding genes.

Common genetic variation in a population is identified by single-nucleotide polymorphisms (SNPs), which are loci within the genome whose nucleotides are highly variable.[26] This variability is the target of genome-wide association studies (GWAS), which genotype hundreds of thousands of SNPs and analyze them for association with a disease or trait.[26] Although GWAS identify many SNPs whose genes can inform the pathophysiology of a given condition, each individual SNP typically confers a small effect on the trait. In contrast, genetic mutations implicated in Mendelian disorders typically alter the protein structure and function, which manifest as an extreme phenotype.

The GWAS design has been applied to lipid levels in several populations, which have identified many SNPs and genes whose variability influence lipid levels.[27-29] A meta-analysis pooling data from 46 GWAS, representing over 100,000 individuals of European ancestry, identified 95 SNPs that were significantly associated with serum lipid levels.[28] Of the 95 SNPs reported, 59 had not been previously identified in association with lipids. These novel loci were located near, but not within, lipid-regulating genes and in genomic regions not previously associated with lipid metabolism.[28]

Dramatic lipid levels are characteristic of dyslipidemias, a group of diseases which are typically the result of Mendelian-inherited gene mutations. These mutations alter protein structure and function, which manifest as extreme lipid levels. Different dyslipidemias are characterized by different lipid component thresholds. Genes associated with dyslipidemias are presented in Table A.

The lipid-associated genes identified by GWAS, to be used in the design of Chapter 3, are presented in Table B. This table includes the genes that have been reported as mutated in association with dyslipidemias. Of the 67 genes identified by GWAS, to be used in Chapter 3, only two are implicated in dyslipidemias. A possible explanation for the lack of overlap is that the genes associated with dyslipidemias are more highly conserved, and thus less prone to common variation, than those identified by GWAS. Another possible explanation could be the homogeneous ancestry of the GWAS meta-analysis. Specifically, because all subjects included in this meta-analysis were of European ancestry, SNPs within gene dyslipidemias may be more common among other ancestral groups and were thus not identified by GWAS.

Laboratory Measurement of Lipids

A clinical lipid panel typically includes four lipid components: total cholesterol, HDL-C, LDL-C, and triglycerides. It is important to note that a standard lipid panel does not measure lipoproteins; rather, the assays target either cholesterol or triglycerides.

The total cholesterol measurement includes all cholesterol present in the blood serum, including cholesterol that is bound to HDL, LDL, IDL, VLDL, chylomicrons or unbound as un-esterified free cholesterol. The standard assay for measuring total cholesterol requires hydrolysis in the presence of cholesteryl ester hydrolase to produce free cholesterol, followed by oxidation in the presence of cholesterol oxidase to yield hydrogen peroxide. The hydrogen peroxide, which is derived from cholesterol, is measured by a reaction requiring peroxidase. This reaction produces

a color that can be measured by colorimetry, in which the color's intensity is proportional to the amount of cholesterol.[30]

The triglyceride measurement includes all triglycerides present in the blood serum, including triglycerides that are bound to HDL, LDL, IDL, VLDL, or chylomicrons. The standard assay for measuring triglyceride requires hydrolysis in the presence of lipase to produce glycerol. The glycerol is exposed to glycerokinase followed by glycerophosphate oxidase hydrogen peroxide. The hydrogen peroxide is measured in the same manner as for total cholesterol, in which the color's intensity is proportional to the amount of triglyceride.[30]

HDL is measured by binding ApoB-containing lipoproteins so that they are non-reactive with the cholesterol assay reagents. ApoB is a characteristic component of LDL, VLDL and IDL, and it is not present in HDL; thus, the only cholesterol that is assayed is that which is bound to HDL. HDL-C is de-esterified by an esterase that is specific to HDL-C and then oxidized by an oxidase to form hydrogen peroxide. The hydrogen peroxide is measured in the same manner as for total cholesterol, in which the color's intensity is proportional to the amount of HDL-C.[30]

LDL-C is typically calculated as [total cholesterol – HDL-C – (TG/5)], rather than measured directly. Triglycerides are divided by five and subtracted from total cholesterol to account for cholesterol present in VLDL. Alternatively, VLDL-C can be measured and substituted for (TG/5) in the formula.[30]

Although the lipid panel only measures forms of cholesterol and triglycerides as an indicator of cardiovascular disease risk, there is some interest in LDL and HDL *particle number* and their role in disease. Barter et al. suggest a paradigm wherein LDL particle number, as measured by Apo-B rather than cholesterol saturation of LDL-C, is the superior predictor of CVD risk.[31] This is based on the logic that a greater number of LDL particles increases the odds of a particle invading the arterial wall and triggering the atherosclerotic response. Conveniently, LDL, VLDL and IDL each contain one copy of the Apo B-100 lipoprotein per particle, allowing for the deduction of LDL particle number.[31] Multiple methods exist for assaying LDL and Apo B-100.[32] LDL can be measured by gel electrophoresis or nuclear magnetic resonance. Apo B-100 can be measured by immunoassay. Similarly, low HDL particle number is associated with increased CVD risk, due to the reduced opportunity for cholesterol clearance from arterial walls. HDL particle number can be assayed by nuclear magnetic resonance; a Clinical and Laboratory Standards Institute (CLSI) approved assay is now commercially available.[33] These assays are often referred to as advanced

lipid testing, which is currently performed in high risk populations per physician preference and is not formally recommended by ACC/AHA.[34,35]

Clinical Dyslipidemias and Cardiovascular Disease

Clinical dyslipidemias include multiple disorders of lipid metabolism, which are characterized by extremely aberrant lipid profiles. Many dyslipidemias are caused by mutations in a single gene related to lipid metabolism (Table A).[36] However, some forms of dyslipidemia are not the result of pathogenic mutations in a single gene, but are the combined result of common genetic variability and lifestyle factors.[37,38]

Familial hypercholesterolemia, characterized by elevated LDL-C, affects an estimated 1 in 250 people.[39] Familial hypertriglyceridemia is characterized by elevated triglycerides; its prevalence is difficult to estimate.[38] Familial combined hyperlipidemia, characterized by elevated triglycerides and elevated apolipoprotein B, affects an estimated 1 in 100 people.[40]

As stated previously, high LDL-C is associated with increased risk for cardiovascular disease (CVD).[34] LDL-C contributes to CVD via its role in atherosclerosis, in which cholesterol-enriched atherosclerotic plaques form in the arterial wall. As LDL-C particles invade the arterial wall, typically at curved or branched parts of the artery, they undergo modifications that trigger activation of the otherwise dormant epithelial tissue.[19] Endothelial activation triggers an inflammatory response, which recruits monocytes to the location of the injury. Once monocytes invade the arterial wall, they differentiate into macrophages. The macrophages endocytose oxidized LDL-C particles via their binding to macrophage scavenger receptors. As macrophages continue to take up and store LDL-C, they are called foam cells, which are the constituents of early atherosclerotic lesions known as fatty streaks.[41]

As atherosclerotic plaques increase in mass, they can obstruct the artery, reducing blood flow to downstream tissues. When atherosclerotic plaques rupture, their contents, which include clotting factors, form a blood clot which may result in myocardial infarction or ischemic stroke.[42]

Table A. List of known genes causing dyslipidemias.

ICD-9 Diagnosis	Specific Disease	Lipid Components Affected	Gene(s) with known mutations	Inheritance Pattern	Source
Pure hypercholesterolemia 272.0	Familial hypercholesterolemia	LDL	*APOA2, LDLR, ABCA1, ITIH4, GHR, GSBS, EPHX2*	AD	OMIM #143890
	Autosomal recessive hypercholesterolemia	LDL	*LDLRAP1*	AR	OMIM #603813
	Hyperalphalipoproteinemia 1	HDL	*CETP*	AD	OMIM #143470
	Hyperalphalipoproteinemia 2	HDL	*APOC3*	Not specified	Genetic Testing Registry. NCBI.
Pure hyperglyceridemia 272.1	Familial hypertriglyceridemia	Triglyceride	*APOA5, LIPI*	AR, AD	OMIM #145750
	Type I hyperlipoproteinemia	Triglyceride	*LPL, APOC2*	Varied	MeSH
Mixed hyperlipidemia 272.2	Type III hyperlipoproteinemia	VLDL, chylomicrons	*APOE*	AR	OMIM #617347 MeSH
	Familial combined hyperlipidemia	VLDL, LDL	*LPL*	AD	OMIM #144250
Hyperchylomicronemia 272.3	Type I hyperlipoproteinemia	Chylomicrons	*LPL*	AR	MeSH
	Burger-Grutz syndrome (Familial lipoprotein lipase deficiency)	Chylomicrons	*LPL*	Not specified	MeSH

Table B. Comparison of dyslipidemia genes to GWAS-identified genes used to construct genetic risk scores.

Dyslipidemia Genes	Genetic Risk Score Genes	Genetic Risk Score Genes – Continued
*ABCA1**	*ABCA1**	*LOC101929615*
APOA2	*ABCG8, LOC102725159*	*LOC102724766*
APOA5	*AFF1*	*LOC105372112*
APOC2	*APOB*	*LOC105372618*
APOC3	*ARL15*	*LOC105375508*
APOE	*BAZ1B*	*LOC105375745*
*CETP**	*C5orf67*	*LOC157273*
EPHX2	*CAPN3*	*MARC1*
GHR	*CELSR2*	*MIR1322,PINX1*
GSBS	*CETP**	*MYLIP*
ITIH4	*COBLL1*	*NAT2*
LDLR	*DNAH11*	*PABPC4*
LDLRAP1	*DNAH11, LOC105375183*	*PCSK9*
LIPI	*DOCK6*	*PLEC*
	DOCK7	*PSKH1*
	EVI5	*R3HDM1*
	F2	*R3HDM2*
	FADS1	*RAF1*
	FADS2	*SLC22A1*
	FER1L4	*SLC39A8*
	FRK, LOC101927818	*ST3GAL4*
	FRMD5	*STARD3*
	FUT2, LOC105447645	*SUGP1*
	GALNT2	*TBL2*
	GCKR	*TIMD4*
	GPAM	*TMEM57*
	HAVCR1	*TNF*
	HFE	*TNFAIP3*
	HMGCR	*TRPS1*
	HNF4A	*TTC39B*
	HPR	*ZHX3*
	LINC01132	*ZNF664,ZNF664-FAM101A*
	LIPC, LOC101928694	*ZPR1*
	LOC100287329, LTA, TNF	

*Indicates genes associated with dyslipidemia and included in genetic risk scores

CHAPTER II: Metabolic Changes in Human Pregnancy, Parturition and Preterm Birth

Pregnancy is a complex physiological state that involves metabolic, immune, hormonal, and inflammatory changes. This chapter will discuss the metabolic state of pregnancy, the physiologic process of parturition, and the phenomenon of preterm birth.

Metabolic Changes and Dyslipidemia in Pregnancy

Metabolic changes are characteristic of pregnancy. Such changes primarily include changes in glucose metabolism, insulin sensitivity and circulating lipids.

It has been widely observed that changes in glucose metabolism during pregnancy occur in two phases. In early pregnancy, fasting glucose levels fall, while in later pregnancy there is an increase in post-prandial glucose.[43] The increase in post-prandial glucose levels in late pregnancy are thought to serve the increased glucose needs of the fetus during the period of tremendous growth. Although the increased fetal metabolic demands do not increase the mother's energy needs until the third trimester of pregnancy, women typically experience a significant increase in fat mass in early pregnancy.[44,45] This is facilitated by upregulation of insulin receptors on adipocytes early in pregnancy, resulting in increased fat storage.[43]

Changes in lipid metabolism during pregnancy manifest as increased lipid levels and changes in lipoprotein composition. Total cholesterol, LDL, HDL and triglycerides (TG) all increase during pregnancy.[43] The increase in HDL is thought to confer a protective effect against the otherwise atherogenic effect of increased LDL.[45] Changes in TG levels are the most profound, increasing two- to four-fold compared to pre-pregnancy.[43-45] Increased circulating VLDL and reduced VLDL clearance via decreased hepatic lipase activity contribute to increased circulating TG.[43,46] The cholesterol content and TG content of lipoprotein particles both increase in pregnancy.[46]

Human Parturition

Human parturition, which is the process of the onset of labor and delivery, relies on the coordination of many signaling cascade events, with the ultimate goals of cervical ripening, myometrial contraction and rupture of fetal membranes.[47] Despite decades of research into the biological stimuli that trigger human parturition, the exact network and sequence of events is not fully understood. However, it is generally accepted to result from a combination of inflammatory and hormonal signals, from both maternal and fetal tissues.

Parturition is generally considered to consist of several processes, including progesterone withdrawal, cervical ripening, and HPA axis signaling.

Progesterone Withdrawal

In non-primate mammals, the onset of labor is preceded by a decrease in circulating progesterone levels. In humans, however, progesterone levels remain constant throughout the third trimester and during labor.[47] During pregnancy, progesterone suppresses myometrium sensitivity to inflammatory signals, thus allowing for the maintenance of gestation.

It has been proposed that human parturition is triggered by a state of 'progesterone withdrawal' which has the effect of reducing progesterone activity while maintaining constant progesterone levels. There are two mechanisms by which progesterone withdrawal may occur. One mechanism involves altered progesterone metabolism, in which progesterone is metabolized into a less active form, known as 20a-dihydroprogesterone, resulting in lower progesterone-to-estrogen ratio.[48] The more supported mechanism involves altered expression of progesterone receptor isoforms, PR-A and PR-B. These isoforms are encoded by the same gene; their transcription is controlled by independent promoters.[49] The PR-B isoform is the predominant receptor during non-pregnancy and during pregnancy up to parturition, and binding of progesterone to PR-B activates progesterone-responsive gene expression, conferring an anti-inflammatory effect.[50] Several studies have observed a shift toward PR-A expression at parturition in laboring tissues, including the myometrium and decidua.[50-52] Increased PR-A expression relative to PR-B induces pro-inflammatory gene expression, allowing the myometrium to be responsive to inflammatory signals that are characteristic of parturition.[53] The PR-A isoform inhibits PR-B activity, thus allowing for the propagation of parturition.[47,54]

Cervical Ripening

Cervical ripening refers to the remodeling of cervical tissue prior to parturition. Such changes include collagen breakdown, which is facilitated by leukocyte invasion.[47] Leukocytes secrete inflammatory cytokines, which trigger the release of degradative enzymes.[47,55]

Hyptothalamic-Pituitary-Adrenal (HPA) Axis Signaling

The hypothalamic-pituitary-adrenal (HPA) axis is a network of endocrine structures and signals that control myriad physiological functions. Specifically, the endocrine glands involved include the hypothalamus and pituitary, both located in the brain, and the adrenal glands, located anterior to the kidneys. The hypothalamus receives signals from peripheral tissues and sends the appropriate signals to the pituitary, which sends appropriate signals to the adrenal glands.[56] The hypothalamus secretes corticotropin-releasing hormone (CRH) which stimulates the pituitary to

secrete adrenocorticotropic hormone (ACTH), which stimulates the adrenal glands to secrete glucocorticoids, specifically cortisol.[56]

In the context of parturition, the glucocorticoid signaling cascade contributes to the initiation and maintenance of labor. Both maternal and fetal HPA axes contribute to parturition.[57] Corticotropin-releasing hormone (CRH) is secreted by the maternal and fetal membranes and its circulating levels increase toward the end of pregnancy.[55] This increase is due in part to the decrease in expression of CRH binding protein, which results in increased levels of free CRH.[55] It has been proposed that CRH expression functions as a placental clock in conjunction with fetal glucocorticoid production.[47] Increased CRH triggers increased cortisol production, which initiates prostaglandin release by the placenta and the myometrium.[55,58]

Prostaglandins are locally synthesized hormones at the site of tissue trauma and are considered the initiators of the acute inflammatory response.[59] Increased prostaglandins contribute to uterine contractility.[58] Uterine prostaglandins trigger increased expression of oxytocin receptors, making the myometrium more responsive to the contractile effects of oxytocin.[58]

Human Preterm Birth

The full gestational period of human females is 40 weeks, or 280 days, when defined by the first day of the woman's last menstrual period.[60] Pregnancy is often first identified by a missed menstrual period. Colloquially, this has resulted in a societal definition of pregnancy as lasting nine months. In developing countries, last menstrual period is frequently used for gestational age estimation, where it is subject to recall bias and maternal illiteracy.[61] It is also used in developed countries, including the United States, although it is now standard practice to corroborate this estimate by ultrasound.[62] Ultrasound-informed estimation of gestational age is known as the 'best obstetric estimate.'[62] Accurate and consistent estimation of gestational age is necessary for accurate estimation of preterm birth rates.[63]

Preterm birth is defined as delivery at <37 weeks' gestation and globally it is the leading cause of death among children less than five years of age.[64] The incidence of PTB ranges from 5-18% by country.[64] In the United States, PTB occurs in approximately 12% of pregnancies.[65] Increasing rates of PTB and differences in PTB rates between countries can be largely attributed to differences in clinical practice. Such differences include differences in gestational age estimation and PTB classification, increasing maternal age, increasing maternal complications such as diabetes and hypertension, and differences in obstetric intervention.[64,65]

Globally, women of lower socioeconomic status are more likely to experience PTB.[64] In the United States, rates of PTB are consistently higher among non-Hispanic Black women compared to non-Hispanic White women.[66] Disparities in PTB rates between non-Hispanic White and non-Hispanic Black or Aboriginal women have also been observed in Canada, the United Kingdom, Australia and New Zealand.[67-70] The causes of these disparities, particularly the sociodemographic versus biological contributions, and mechanisms for alleviating the disparities are an area of active research.

PTB is typically categorized as one of three subtypes: spontaneous PTB, preterm premature rupture of membranes (PPROM) or medically-indicated PTB.[71] Spontaneous PTB accounts for approximately half of PTB. It is characterized by spontaneous preterm labor without rupture of membranes. PPROM accounts for approximately one quarter of PTB. It is characterized by preterm rupture of membranes. Medically-indicated PTB accounts for approximately one quarter of PTB. Indications include maternal hypertension, preeclampsia, intrauterine growth restriction or fetal distress.[71]

The biological mechanisms responsible for spontaneous PTB and PPROM are unclear, and the majority of these preterm births are considered idiopathic.[47] However, substantial evidence supports the role of maternal infection in spontaneous PTB and PPROM.

Maternal Infection

Spontaneous PTB and PPROM can often be attributed to diagnosed or subclinical maternal infection.[72-74]

Several studies have investigated the role of the maternal microbiome in preterm birth. The non-pregnant, health vaginal microflora is dominated by *Lactobacillus* species.[75] These bacteria produce lactic acid, which results in an acidic environment that is protective against potentially pathogenic bacteria. The healthy vaginal pH is approximately 4.5. Lactic acid is specifically responsible for inhibiting growth of pathogenic bacteria, as compared to other acids of the same pH.[75,76] Specifically, the D-lactic acid isomer, which is produced by *Lactobacillus* but not by human cells, is what confers the protective effect.[77]

The most common mechanism by which intrauterine infections take hold is when bacteria from the vagina or cervix ascend into the uterine cavity. This is typically preceded by a shift in the vaginal microflora and a rise in vaginal pH above 4.7.[78] Bacterial vaginosis (BV) is characterized by a change in vaginal microflora from *Lactobacillus* species to anaerobic species, causing a

subsequent rise in vaginal pH.[79] BV affects ~20% of women of reproductive age, with a similar prevalence among pregnant women.[79]

Bacteria that have been isolated from the amnion of preterm placenta are typically of low pathogenicity. They are non-*Lactobacillus* species that are commonly present in the vaginal microbiome; however their excessive growth and subsequent rise in pH trigger leukocyte invasion and release of inflammatory cytokines and prostaglandins.[75,80] In this manner, intrauterine infections mimic the process of parturition, thus resulting in preterm birth.[80] A Cochrane review of two clinical trials of antibiotic treatment during pregnancy found that antibiotic treatment of BV significantly reduced the risk of preterm birth by half.[81]

Maternal Lipid Levels and Risk for Preterm Birth

In light of the metabolic changes that occur during pregnancy, numerous epidemiological studies have identified associations between aberrant maternal lipid profiles and increased risk for preterm birth. However, the lipid components and their effect sizes are inconsistent between studies. Heterogeneity among the study populations, gestational age at testing, fasting status at testing, and lipid components measured likely contribute to inconsistency among the literature.

Fourteen studies have investigated the association between individual lipid components and risk for preterm birth. The study design characteristics and results of these studies are summarized in Table C. Many studies did not measure all four standard lipid components – LDL, HDL, total cholesterol (TC) and triglycerides (TG). Differences in lipid components that were measured are a source of bias that could explain the inconsistencies between studies. By not measuring a lipid component, that study is unable to test that lipid for association with preterm birth – thus, studies require closer examination of the methods to determine which lipid components demonstrated no association with preterm birth, and which lipid components were not included in the study. Studies that measured all four lipid components provide the most insight into the potential associations between maternal lipids and PTB.

Of the seven studies that measured all four lipid components, four studies failed to identify an association between individual lipid components and risk for preterm birth. Jin *et al.* performed a prospective cohort study of 934 women in Hangzhou, China.[82] Lipids were measured during each trimester but only third trimester lipid levels were analyzed for association with PTB. None of the four lipid components were associated with PTB after adjusting for confounders; unadjusted analyses were not reported.[82] Chatzi *et al*. performed a prospective cohort study of 625 women in Crete, Greece, measured at ≤15 weeks' gestation.[83] None of the four lipid components were

associated with PTB; however, LDL/HDL ratio was significantly associated with PTB (RR: 1.19; 95%CI 1.02, 1.39).[83] Emet *et al*. performed a prospective cohort study of 801 women in Rize, Turkey measured at <14 weeks and >28 weeks' gestation.[84] TC, HDL and LDL were not correlated with PTB; TG was significantly correlated with PTB although the correlation was minimal (correlation coefficient: 0.032, $p<0.05$).[84] Alleman *et al*. performed a prospective cohort including 2,699 women from Iowa, USA, measured in both first and second trimester of pregnancy.[85] First trimester TC was marginally associated with PTB (OR: 1.14; 95%CI: 0.99, 1.31); LDL, HDL and TG in the first trimester were not associated with PTB. None of the four lipid components in the second trimester were associated with PTB.[85] However, first trimester TC and change in TC between first and second trimester were both significant predictors in the authors' final prediction model for PTB.[85]

Three studies that measured all four lipid components identified significant associations between maternal lipid levels and risk for PTB. Niromanesh *et al*. performed a prospective cohort of 395 women in Tehran, Iran measured between 16-20 weeks' gestation.[86] TG >195 mg/dl was associated with increased risk for PTB independently (OR: 5.1; 95%CI: 1.9, 13.8) and after adjusting for GA at testing (OR:10.9; 95%CI: 1.6, 74.4). TC, HDL and LDL were not associated with risk for PTB.[86] Mudd *et al*. performed a prospective cohort study of 1,309 women in Michigan, USA, measured between 15-27 weeks' gestation (mean GA: 22.4 weeks).[87] They identified associations between all four lipid components and preterm birth, although these associations were only significant after adjustment for confounders.[87] Specifically, high TC (OR: 1.51; 95%CI: 1.06, 2.15) and high TG (OR: 1.90; 95%CI: 1.21, 2.97) were associated with increased risk for spontaneous PTB.[87] In contrast, low TC (OR: 2.04; 95%CI: 1.12, 3.71), low HDL (OR: 1.89; 95%CI: 1.04, 3.42), and low LDL (OR: 1.96; 95%CI: 1.09, 3.54), were associated with increased risk for medically indicated PTB.[87] Jelliffe-Pawlowski *et al*. performed a nested case-control in two independent populations; Iowa, USA (57 cases, 677 controls) and California, US (72 cases, 32 controls). Lipid profiles were measured between 15-20 weeks' gestation.[88] Low HDL [(Iowa OR: 2.2; 95%CI: (1.3, 3.8); California OR: 2.4; 95%CI: (1.0, 5.9)] and high TG [(Iowa OR: 2.1; 95%CI: (1.2, 3.7); California OR: 3.4; 95%CI: (1.4, 8.4)] were associated with increased risk for PTB in both populations after adjusting for confounders.[88]

One study measured only LDL, HDL and TC. Kramer *et al*. performed a nested case-control of 207 preterm cases and 444 term controls from multiple hospitals in Quebec, Canada.[89] They measured LDL, TC and HDL between 24-26 weeks' gestation and found that HDL above the

median was protective against preterm birth (OR: 0.5; 95%CI: 0.3, 0.8). LDL and TC were not significantly associated with preterm birth risk.[89]

One study measured only TC and TG. Vrijkotte *et al*. performed a prospective cohort study of 4008 women in Amsterdam, Netherlands measured at 12 weeks' gestation.[90] Neither TG nor TC were associated with overall PTB; however, TG was associated with increased risk of induced PTB (OR: 1.69; 95%CI: 1.16, 2.45).[90]

One study measured only TG and HDL. Lei *et al*. performed a prospective cohort study of 5,535 women in Guangdong Province in China.[91] Elevated TG was significantly associated with increased risk for PTB (OR: 1.51; 95%CI: 1.23, 1.86).[91] Low HDL was associated with increased risk for PTB (OR: 1.38; 95%CI: 1.12, 1.69).[91]

Three studies measured only TC during the second trimester. Edison *et al*. performed a prospective cohort study in South Carolina, USA that included 118 women with low TC and 940 women with mid or high TC between 13-23 weeks' gestation (mean GA: 17.3 weeks).[92] Of these women, 677 were White and 381 were Black. TC below the 10th percentile was associated with increased risk for preterm birth among all women (OR: 2.93; 95%CI: 1.54, 5.56) and among White women (OR: 5.63; 95%CI: 2.58, 12.30) when stratified by race. TC above the 90th percentile was associated with increased risk for preterm birth among White women (OR: 2.74; 95%CI: 1.22, 6.18). All associations were adjusted for confounders; unadjusted analyses were not reported. TC was not associated with PTB risk among Black women. Summation of the numbers of women in each TC category by race suggest that Edison *et al*. applied the percentiles for the entire population to each racial group rather than deriving percentiles within each race category individually.[92] Failure to do so may explain why an association was identified in the larger racial group, White women, and not in the smaller racial group, Black women, if TC distributions differed between these groups. Maymunah *et al*. performed a prospective cohort of 287 women screened between 14-20 weeks' gestation in Lagos, Nigeria.[93] TC >239 mg/dl was independently associated with increased risk for preterm birth (RR: 6.89; 95%CI: 2.39, 11.34).[93] Oluwole *et al*. performed a prospective cohort of 261 women screened between 14-20 weeks' gestation in Lagos, Nigeria.[94] TC <200 mg/dl was independently associated with increased risk for preterm birth (RR: 4.83; 95%CI: 3.79, 5.87).[94] Of note, the study by Oluwole *et al*. included 287 women, of which 26 were excluded for having TC >239 mg/dl, and features Maymunah as an author. Thus, it would appear that these two studies were derived from the same study population.

A recent meta-analysis pooled the results from 11 studies investigating pregnancy lipids and preterm birth.[95] Jiang *et al*. identified significant pooled associations between elevated TC (OR: 1.71; 95%CI: 1.05, 2.79), elevated TG (OR: 1.55 95%CI: 1.13, 2.12) and low HDL (OR: 1.33; 95%CI: 1.14, 1.56) and risk for PTB.[95] The pooled association for elevated LDL was not significant (OR: 1.19; 95%CI: 0.95, 1.48).[95] The aforementioned studies by Emet *et al*., Jin *et al*., and Vrijkotte *et al*. were not included in the meta-analysis. It should also be noted that two studies included in the meta-analysis are likely duplicate studies of the same study population, in which the total cholesterol levels were selectively analyzed to produce two publications rather than one.[93,94]

Other studies have investigated the association between features of lipid metabolism and risk for preterm birth. Catov *et al*. performed a prospective cohort study at multiple sites in the USA and characterized pregnant women as having dyslipidemia based on elevated TC or elevated TG.[96] Dyslipidemia in the absence of inflammation was associated with increased risk for PTB between 34 and 37 weeks (adjusted OR: 2.0; 95%CI: 1.0, 4.2).[96] Dyslipidemia in the presence of inflammation was associated with increased risk for PTB between 34 and 37 weeks (adjusted OR: 4.0; 95%CI: 1.4, 11.8) and <34 weeks (adjusted OR: 6.4; 95%CI: 1.7, 24.1).[96] Laughon *et al*. performed a nested case-control study of women in New York, USA and assessed the association between change in TC and TG during pregnancy and PTB.[97] Rate of change of TC or TG were not significantly associated with PTB risk.[97] Toliekyte *et al*. studied the risk of preterm birth among women with familial hypercholesterolemia (FH) in a retrospective study of the Medical Birth Registry of Norway.[98] Maternal FH was not significantly associated with risk for preterm birth.[98] Chen *et al*. studied the association between third trimester free fatty acids and risk for PTB among women in New Jersey, USA.[99] Higher levels of free fatty acids were associated with increased risk for overall preterm birth and with spontaneous PTB.[99]

Overall, there is much inconsistency among studies investigating the associations between lipids and PTB both in their design and in their findings. Although heterogeneity among studies can result in greater generalizability of their findings when such findings are consistent, in the case of lipids and PTB, heterogeneity among studies has contributed to heterogeneity of findings. Previous studies have identified contradictory associations between specific lipid components and their direction of effect. More research is warranted to identify the relationship between lipid levels, dyslipidemia and PTB risk.

Table C. Summary of previous studies of the association between maternal lipids and risk for preterm birth.

Author	Population	Study Design	Sample Size	Exclusions	Fasted Samples	GA at sampling	Lipids Tested
Alleman et al.	Iowa Maternal Serum Screening Program	Prospective cohort	2699 total	Less than two screenings	Not specified	1st and 2nd trimester	LDL, HDL, TC and TG
Chatzi et al.	Crete, Greece	Prospective cohort	625 total - 74 preterm	Multiples	Yes	<=15 weeks	LDL, HDL, TC and TG
Emet et al.	Turkey	Prospective cohort	801 total	Not specified	Yes	<14 weeks; >28 weeks	LDL, HDL, TC and TG
Jelliffe-Pawlowski et al.	Iowa and California	Nested case-control	Iowa = 57 cases, 677 controls CA = 72 cases, 36 controls	IA and CA - singletons CA - chromosomal or structural defects, smoking, diabetes, or amniotic fluid abnormalities	No	15-20 weeks (both IA and CA)	LDL, HDL, TC and TG
Jin et al.	China	Prospective cohort	934 total	Multiples, diabetes, metabolic disease	Yes	1) 7-10 weeks 2) 21-24 weeks 3) 33-37 weeks	LDL, HDL, TC and TG
Mudd et al.	Michigan	Prospective cohort	1309 total	Multiples, fetal anomaly, diabetes	No	15-27 weeks (mean 22.4)	LDL, HDL, TC and TG
Niromanesh et al.	Iran	Prospective cohort	395 total	History of PTB, PE or GDM, primigravida, BMI>25 and age>35	Yes	16-20 weeks	LDL, HDL, TC and TG
Kramer et al.	Multicenter in Quebec, Canada	Nested case-control	444 controls, 207 cases	Multiples, fetal anomaly	No	24-26 weeks	LDL, HDL, TC
Vrijkotte et al.	Amsterdam Born Children and their Development	Prospective cohort	4008 total	Multiples, diabetes, lipid-altering medication	No	~12 weeks	TC and TG
Lei et al.	Guangdong Province, China	Prospective cohort	5535 total	Multiples, assisted reproductive technology, ischemic heart disease, stroke, peripheral vascular disease, dyslipidemia, pre-existing diabetes or hypertension	Yes	<20 weeks	TG and HDL
Edison et al.	South Carolina	Prospective cohort	118 with low TC, 940 mid or high TC	Multiples, infection, preeclampsia, genetic syndrome in neonate	Not specified	13-23 weeks (mean 17.6)	TC
Maymunah et al.	Lagos, Nigeria	Prospective cohort	287 total	Multiples, medically indicated PTB, gestational HTN, GDM, heart defect	Yes	14-20 weeks	TC
Oluwole et al.	Lagos, Nigeria	Prospective cohort	261 total	Multiples, diabetes, hypertension, HIV, current or previous smoking, other substance use, previous abnormal pregnancy	Yes	14-20 weeks	TC

Table C – Continued

Author	Factors Included in Adjustment	Unadjusted OR	Adjusted OR	Notes
Alleman et al.	Not applicable	None	None	
Chatzi et al.	Maternal age, maternal education, maternal smoking during pregnancy	LDL/HDL RR all preterm 1.19 (1.02, 1.39)		
Emet et al.	Not applicable	None	None	All lipids increased throughout pregnancy
Jelliffe-Pawlowski et al.	Gestational week at serum draw and maternal weight		CA HDL Q1 = 2.4 (1.0, 5.9) IA HDL Q1 = 2.2 (1.3, 3.8) CA TG Q4 = 3.4 (1.4, 8.4) IA TG Q4 = 2.1 (1.2, 3.7) Q4 TNF + dyslipidemia IA = 2.7 (1.1, 6.3) Q4 TNF + dyslipidemia CA = 4.0 (1.1, 16.3)	Restricted to PTB <30 weeks
Jin et al.	Maternal age, pre-pregnancy BMI, gestational weight gain, parity, maternal education, family income, cigarette exposure	None	None	
Mudd et al.	Maternal race, parity, gestational week at time of blood draw	None	Spon TC >70th: 1.51 (1.06, 2.15) Spon TG Q3: 1.90 (1.21, 2.97) vs Q1 Spon TG Q4: 1.72 (1.06, 2.78) vs Q1 Indicated TC <10th: 2.04 (1.12, 3.72) Indicated HDL <10th: 1.89 (1.04, 3.42) Indicated LDL <10th: 1.96 (1.09, 3.54)	Comparison group is 10-70th percentile
Niromanesh et al.	Gestational age	High TG: 5.1 (1.9, 13.8)	Adjusted for GA high TG: 10.9 (1.6, 74.4)	High TG > 195 mg/dl
Kramer et al.	Maternal age, primiparity, marital status, place of birth, language, maternal education, family income, smoking, pre-pregnancy BMI, height	HDL 0.5 (0.3, 0.8) (> vs <= median)	HDL 0.5 (0.3, 0.9) (> vs <= median)	
Vrijkotte et al.	Maternal age, ethnicity, parity, pre-pregnancy BMI, maternal education, physical activity, smoking during pregnancy, chronic hypertension	No association between TG or TC and PTB		
Lei et al.	Maternal age, parity	Elevated TG: 1.51 (1.23, 1.86) Low HDL: 1.38 (1.12, 1.69)	None	Elevated TG ≥ 3.49 mmol/L; Referent TG < 3.49 mmol/L Low HDL < 1.3 mmol/L Referent HDL ≥ 1.3 mmol/L

Table C – Continued

Edison et al.	Maternal race, maternal weight group, age group, infant gender, presence of IUGR	None	All mothers TC <10th: 2.93 (1.54, 5.56) White TC<10th: 5.63 (2.58, 12.3) White TC>90th : 2.74 (1.22, 6.18)	Comparison group is mid-TC
Maymunah et al.	Not applicable	High TC: 6.89 (2.39, 11.34)	None	
Oluwole et al.	Not applicable	Low vs mid-range TC RR: 4.83 (3.79, 5.87)	None	Low TC < 200 mg/dL compared to mid-range TC 200-239 mg/dL Women with high TC (>239 mg/dL) were excluded from analysis

CHAPTER III: Genetic Risk Scores for Lipid Levels and Their Association with Preterm Birth

Abstract

Background: Maternal lipid profiles are associated with risk for preterm birth, although the lipid component and effect size are inconsistent between studies. It is unclear whether these associations are the result of excessive changes in lipid metabolism during pregnancy or genetic variability in genes controlling basal lipid metabolism. This study investigates the association between genetic risk scores (GRS) for four lipid components (HDL-C, LDL-C, triglycerides (TG) and total cholesterol (TC)) and preterm birth (PTB).

Methods: Subjects included 992 pregnant women from California for whom second trimester serum samples were available, of which 495 delivered preterm and 497 delivered at term. We genotyped ninety-six single-nucleotide polymorphisms (SNPs) which were selected from two genome-wide association studies (GWAS) of lipid levels in adult populations. Lipid-specific GRS were constructed for HDL-C, LDL-C, TG and TC. The associations between GRS and PTB were analyzed using logistic regression.

Results: GRS were not associated with overall risk for preterm birth. However, a higher HDL-C GRS was associated with increased risk for spontaneous PTB. Higher TG and TC GRS were associated with decreased risk for spontaneous PTB.

Conclusions: This study identifies counter-intuitive associations between lipid GRS and spontaneous PTB. One possible interpretation is that genetic predisposition to low total cholesterol confers increased risk for spontaneous PTB. An alternative explanation is that these results are simply an artefact of the data and additional genetic loci and lifestyle factors confer stronger effects on risk for spontaneous PTB than the effects of the genetic loci included in this study.

Introduction

Pregnancy induces maternal metabolic changes that are necessary for successful fetal development. Metabolic changes include altered glucose tolerance and increased circulating lipids, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), total cholesterol (TC) and triglycerides (TG).[43,46]

Several studies have shown maternal lipid profiles, which include HDL, LDL, triglycerides and total cholesterol, to be associated with risk for preterm birth. Preterm birth is defined as delivery at less than 37 weeks of gestation, in contrast to full term which is an average of 40 weeks of gestation.[100,101] Globally, it affects ~11% of births, and is the leading cause of neonatal morbidity and mortality.[100,101]

The associations between lipid components and preterm birth have been inconsistent across multiple studies.[82-94] These studies are centered on the hypothesis that extreme metabolic changes in pregnancy, as reflected by lipid profiles, confer increased risk for preterm birth. These studies vary greatly in the lipid components that were measured, the gestational age at which they were measured, the inclusion and exclusion criteria, and the fasting status of the measurements, which likely contribute to their inconsistent results. Studies of maternal lipid profiles in late-first to early-second trimester found that low HDL-C[88,89,91], high TG[86-88,91], low TC[92,94], high TC[87,93], and high LDL-C:HDL-C[83] were associated with increased risk for preterm birth. A recent meta-analysis of these studies found that elevated TC, elevated TG and low HDL-C were significantly associated with increased risk for preterm birth, with significant odds ratios ranging from 1.33 to 1.71.[95] Elevated LDL-C was not significantly associated with risk for preterm birth.[95]

It is unclear whether changes in lipid levels due to pregnancy-induced metabolic changes contribute to preterm birth or whether lipid levels contribute directly to preterm birth. Causality of lipid exposures can begin to be addressed by examining preterm birth risk in relation to genetic predisposition toward certain lipid profiles. Multiple genome-wide association studies (GWAS) have investigated the genetic contribution to adult lipid levels.[27,28,102-104] These studies have identified many single nucleotide polymorphisms (SNPs), which represent common genetic variability that influences adult lipid levels. In contrast to monogenic mutations that cause profoundly abnormal lipid levels, many of the SNPs identified by GWAS each confer a small, but consistent influence on lipids.

The use of genetic risk scores (GRS) as an indicator of an individual's genetic risk for a trait was first proposed by Horne *et al*. in 2005.[105] This approach leverages GWAS findings for an

intermediate phenotype and applies them to a terminal phenotype. Given the inconsistent associations between pregnancy lipids and preterm birth, and the GWAS-identified SNPs that contribute to non-pregnancy lipid levels, this study aims to construct GRS for HDL, LDL, total cholesterol and triglycerides, and investigate their association with preterm birth. We hypothesize that the GRS will be significantly associated with preterm birth (PTB).

Methods

Study Population and Serum Samples

Study subjects included a nested case-control of 992 California women with banked 2nd trimester serum and lipid levels, as collected by the California Genetic Diseases Screening Program. Regions with banked serum samples include the California Central Valley, Los Angeles and Orange County, and San Diego County.[88] Serum samples were drawn between 15-20 weeks' gestation and were not fasted. Subjects were categorized as having a preterm birth based on a gestational age of <37 weeks. Subjects giving birth ≥37 weeks were categorized as having a term birth. Covariates include gestational age at serum draw, maternal age at delivery, pre-pregnancy body mass index (BMI), and race/ethnicity. Serum samples were stored at 4°Celsius. Serum lipid components including HDL-C, LDL-C, total cholesterol (TC) and triglyceride (TG) were measured at the Iowa State Hygienic Laboratory in Coralville, Iowa, USA. Specifically, lipid components were measured using separate enzymatic colorimetric tests on a Roche® Cobas c111 instrument. Lipid levels are reported in mg/dl.

SNP Selection and Genotyping

Single nucleotide polymorphisms (SNPs) were identified in the literature from a genome-wide association study (GWAS) of predominantly fasted lipid levels in adult populations.[28] This multinational study pooled data from 46 studies for a combined total population of >100,000 individuals of European ancestry.[28] Ninety-six SNPs were selected based on having the most statistically significant association with one or more of the following lipid components: LDL, HDL, triglycerides and total cholesterol. The list of genotyped SNPs is presented in the **Supplemental Table 3.1**.

Genotyping methods have been described previously.[106] Subjects were genotyped for 96 SNPs using TaqMan assays (Applied Biosystems, Foster City, CA) using the EP1 SNP Genotyping System and GT 192.24 Dynamic Array Integrated Fluidic Circuits (Fluidigm, San Francisco, CA, USA). All genotyping reactions were performed according to the standard protocol provided by

Fluidigm. Three CEPH-CEU individuals (1000 Genomes Project) served as positive controls and double-distilled water served as a negative control.

Statistical Analysis

Thirty-seven subjects were excluded due to genotyping efficiency <90%, which equates to successful genotype calling at less than or equal to 86/96 SNPs, resulting in a final analysis set of 955 subjects.

Genetic risk scores (GRS) were calculated using PLINK software (Broad Institute, Cambridge, MA).[107] GRS for LDL, HDL, triglycerides and total cholesterol were constructed for each subject based on the β-coefficients of GWAS-significant variants from a GWAS for lipid levels.[28] The GRS for LDL, HDL, triglycerides and total cholesterol included 20, 25, 21 and 31 SNPs, respectively. SNPs and beta coefficients used to construct each GRS are listed in the **Supplemental Table 3.1**. Specifically, GRS were constructed based on the reported β-coefficient for the effect allele at the given SNP. Alleles were coded such that the reported beta coefficient would be a positive value; for SNPs where the source allele had a negative beta coefficient, the other allele was used and the beta coefficient was made positive. The beta coefficient of each effect allele was summed for each subject based on their genotypes, such that individuals homozygous for the non-coded allele would receive a value of zero for that locus, heterozygous individuals would receive the value of the beta coefficient at that locus, and individuals homozygous for the coded allele would receive double the beta coefficient for that locus.

The associations between GRS, lipids, and preterm birth were analyzed using Statistical Analysis Software (SAS®) version 9.4 (Cary, NC, USA). Our primary analysis was to assess the association between 4 constructed GRS and preterm birth. The association between GRS and preterm birth was tested using logistic regression. Analyses were performed both unadjusted and adjusted for gestational age at serum collection, maternal age at delivery, and maternal pre-pregnancy BMI. BMI was categorized according to standard cut-points (underweight [<18.5 kg/m^2], normal [18.5-24.9 kg/m^2], overweight [25-29.9 kg/m^2], or obese [≥30 kg/m^2]).[108] Several secondary analyses were performed for evaluating the GRS with PTB risk. These include: 1) stratification by racial/ethnic category (Asian, Black, White, and Hispanic); 2) stratification by preterm birth subtypes (spontaneous, preterm premature rupture of membranes (PROM), and medically indicated); and 3) the association between GRS quartiles and preterm birth.

We also assessed the relationship between each GRS and its respective lipid level using linear regression. Lipid levels were transformed to their natural logarithm for analysis. Lastly, we

performed several secondary analyses further investigating the relationship between lipids, the GRS and preterm birth. These include: 1) the association between individual SNPs and preterm birth; 2) the association between non-HDL cholesterol and preterm birth; and 3) the association between dyslipidemia and preterm birth. The presence of dyslipidemia was determined according to the Adult Treatment Panel III guidelines for cholesterol and lipid levels.[109] Dyslipidemia was assigned if a women met one or more of the following criteria: LDL ≥160 mg/dl, HDL <40 mg/dl, total cholesterol ≥240, or triglyceride ≥200 mg/dl.

Results

Demographic characteristics of the study population are reported in **Table 3.1**. Mothers of term and preterm infants did not significantly differ by age group, race, or 2nd trimester lipid components, including HDL, LDL, and TC. TG was marginally higher among mothers of preterm infants compared to mothers of term infants (p=0.015). Mothers of preterm infants had marginally higher BMI compared to mothers of term infants (p=0.016). Mean and standard deviation of GRS values are presented for mothers of term and preterm infants.

The results of the association between GRS and preterm birth are presented in **Table 3.2**. None of the four GRS were associated with preterm birth, either independently or after adjusting for gestational age at serum collection, maternal age at delivery, or maternal pre-pregnancy BMI. Nor were any of the four GRS associated with preterm birth when stratifying by race. The associations between GRS quartiles and overall preterm birth risk are presented in **Supplemental Table 3.2**. The 4th quartile of the TG GRS was associated with increased risk for preterm birth. When quartiles were analyzed as a continuous variable, a higher TG GRS was associated with increased risk for preterm birth. However, these associations did not remain significant after correction for multiple comparisons. All other associations were non-significant. The associations between GRS and preterm birth subtypes are presented in **Supplemental Table 3.3**. Three GRS were associated with spontaneous preterm birth. When examining by preterm birth subtype, higher HDL-C GRS was associated with increased risk for spontaneous preterm birth, whereas higher TC and TG GRS were associated with decreased risk for spontaneous preterm birth. The associations between GRS quartiles and preterm birth subtypes are presented in **Supplemental Table 3.4**. The second quartile of the TG GRS was associated with decreased risk for PPROM. The third quartile of the HDL-C GRS was associated with increased risk for spontaneous preterm birth. The fourth quartiles of the TG and TC GRS were associated with decreased risk for spontaneous preterm birth, consistent with the results treating the GRS as a continuous measurement. None of the

individual SNPs used to construct the GRS were independently associated with preterm birth after correction for multiple testing (data not shown).

The results of the association between GRS and lipid levels are presented in **Table 3.3**. As expected, all GRS were associated with their respective lipid level in the total population, and among Asian and White subjects. The GRS for HDL and triglycerides were significantly associated with their respective lipid levels among Hispanic subjects, but the GRS for LDL and total cholesterol were not significant among Hispanic subjects ($p>0.05$). GRS were not associated with their respective lipid levels among Black subjects ($p>0.05$), which is an expected artefact of the small number of Black subjects in this analysis.

The results of the association between lipid levels and preterm birth are presented in **Table 3.4**. None of the four lipid components were significantly associated with preterm birth, either independently or after adjusting for gestational age at serum collection, maternal age at delivery, or maternal pre-pregnancy BMI. The associations between lipid quartiles and preterm birth, stratified by race/ethnicity, are presented in **Supplemental Table 3.5**. The highest quartile of TG was associated with increased risk for preterm birth in the total population and among White women, consistent with the results treating the lipid as a continuous measurement. The associations between lipids and preterm birth subtypes are presented in **Supplemental Table 3.6**. None of the four lipid components were associated with preterm birth subtypes. The associations between lipid quartiles and preterm birth subtypes are presented in **Supplemental Table 3.7**. None of the lipid quartiles were associated with any of the preterm birth subtypes. The associations between non-HDL cholesterol and preterm birth are presented in **Supplemental Table 3.8**. Non-HDL cholesterol was not significantly associated with risk for preterm birth. The associations between assigned dyslipidemia and preterm birth are presented in **Supplemental Table 3.9**. Half of the women who delivered preterm met the criteria for dyslipidemia and half of the women who delivered term met the criteria for dyslipidemia. Neither dyslipidemia nor its component criteria were significantly associated with preterm birth.

Discussion

This is the first study to investigate a genetic risk score for lipid levels and its association with preterm birth. All four GRS were significantly associated with their respective lipid components, indicating validity of the GRS. Genetic risk scores for lipid levels were not associated with risk for preterm birth overall or when stratified by race. When analyzing by preterm birth subtype the GRS were only associated with spontaneous preterm birth. A higher HDL-C GRS was associated

with increased risk for spontaneous preterm birth, whereas higher TG and TC GRS were associated with decreased risk for spontaneous preterm birth when adjusted for race/ethnicity. Overall we found no association between any lipid measurement and preterm birth. The only association was observed stratified by race where White women with higher TG levels were at an increased risk for preterm birth. This effect is consistent with previous studies, in which higher TG is associated with increased risk for preterm birth.[86-89,91]

However, some of the directions of the associations between the lipid GRS and risk for spontaneous preterm birth is inconsistent with the hypothesized directions of effect. In particular, a higher HDL-C GRS would be expected to confer a protective effect and higher TG and TC GRS would be expected to confer increased risk for spontaneous preterm birth. One interpretation of the findings is that genetic predisposition to high HDL-C, low triglyceride and low total cholesterol confer increased risk for spontaneous preterm birth. This is supported by studies which have found increased risk for spontaneous preterm birth among women with low total cholesterol during pregnancy or at delivery.[92,94,110] However, associations between high HDL-C or low triglyceride and increased risk for preterm birth have not been previously identified. Another potential explanation for these unexpected findings may lie in the degree to which the GRS explain variability in the lipid profile. When combined, the SNPs used to construct the various GRS explain ~11% of the total variance in non-pregnant lipid levels.[28] Thus, if the GRS do confer contradictory effects on risk for preterm birth, lipid levels during pregnancy are likely to be influenced by additional genetic and lifestyle factors, which, when combined, produce the routinely observed associations between pregnancy lipid levels and risk for preterm birth. Lastly, these associations could be an artifact of multiple testing as the effects are modest and may not hold up to replication in additional populations.

A strength of this study is the racial and ethnic diversity of the study population, which was predominantly comprised of Asian, Hispanic, and White women, although the small number of Black women in the study population is a limitation, as they are disproportionately affected by preterm birth.[111] The GWAS on which the GRS were based was initially performed on a large population of European ancestry.[28] However, Teslovich *et al*. also performed GWAS analyses on populations of East Asians, South Asians, and Blacks and found that nearly all significant SNPs identified in the European population were significant in the other ancestral groups.[28] A genetic fine-mapping study of Hispanic Americans and other minority populations found that SNP-lipid associations identified in European Americans generalized to these populations.[112] Thus, our

application of the GRS to different racial and ethnic groups is valid, and the diversity of our study population allowed us to investigate effect modification by race.

A common criticism of the GWAS approach is that the significant SNPs do not necessarily confer a biologically *causal* effect on the disease or trait being studied.[113] Rather, GWAS findings may be merely correlational, or the significant SNPs may be in high linkage disequilibrium with truly causal markers that were not assayed on the GWAS chip.[113] However, we have demonstrated that the GRS we constructed based on GWAS findings are strongly associated with their respective lipid components during pregnancy (**Table 3**). For the purpose of risk prediction, the SNPs within GRS do not need to be biologically causal, they simply need to be strongly associated with the intermediate phenotype of interest, which in the case of our study is lipid components during pregnancy.

A limitation of this study is the lack of pre-pregnancy lipid levels. Such information would allow us to examine the role of the change in lipid levels during pregnancy in preterm birth. Another limitation is the fact that the serum lipid measurements were not taken after fasting, whereas the GWAS of lipid levels from which SNPs were selected was performed on predominantly fasted lipids. However, this is unlikely to affect the results given that the GRS were strongly associated with their respective lipid measurements.

This study identifies counter-intuitive associations between genetic risk scores for lipid levels and risk for spontaneous preterm birth. Genetic risk for higher HDL-C was associated with increased risk for spontaneous preterm birth while genetic risk for higher triglyceride and total cholesterol were associated with decreased risk for spontaneous preterm birth. One possible interpretation is that genetic predisposition to low total cholesterol confers increased risk for spontaneous preterm birth. An alternative explanation is that these results are simply an artefact of the data and additional genetic loci and lifestyle factors confer stronger effects on risk for spontaneous preterm birth than the effects of the genetic loci included in this study.

Tables

Table 3.1. Demographic characteristics of study population.

	Preterm (N=477)	**Term (N=478)**	**P Value**
GA at sampling*	16.5 ± 1.12	16.5 ± 1.09	0.660
Age			0.572
<18 years	7 (1.5%)	4 (0.8%)	
18-34	331 (69.5%)	340 (71.3%)	
>34 years	138 (29.0%)	133 (27.9%)	
Race			0.601
Asian	74 (15.5%)	75 (15.7%)	
Black	13 (2.7%)	7 (1.5%)	
Hispanic	236 (49.5%)	238 (49.8%)	
White	154 (32.3%)	158 (33.0%)	
BMI*	26.0 ± 6.4	25.3 ± 5.7	**0.016**
Lipids*			
2nd trimester HDL	73.8 ± 17.4	75.5 ± 16.7	0.372
2nd trimester LDL	122.4 ± 34.6	122.4 ± 32.9	0.276
2nd trimester TG	207.2 ± 77.2	199.2 ± 69.0	**0.015**
2nd trimester TC	210.1 ± 36.9	209.5 ± 34.9	0.236
Genetic Risk Scores			
HDL Score	23.2 ± 2.4	23.3 ± 2.5	0.500
LDL Score	31.3 ± 5.0	31.5 ± 5.5	0.509
TG Score	96.8 ± 15.7	95.7 ± 15.6	0.299
TC Score	61.4 ± 6.8	61.4 ± 7.2	0.926

*Presented as Mean ± Standard Deviation. All other data are presented as N (%)

Table 3.2. Association between GRS and preterm birth.

GRS vs PTB	Overall (N=955)	Asian (N=149)	Black (N=20)	Hispanic (N=474)	White (N=312)
LDL	1.008 (0.984, 1.033)	1.016 (0.954, 1.083)	0.889 (0.737, 1.072)	1.006 (0.970, 1.043)	1.009 (0.969, 1.051)
Adjusted LDL*	0.994 (0.970, 1.019)	0.985 (0.923, 1.052)	1.186 (0.917, 1.534)	1.000 (0.963, 1.038)	0.992 (0.951, 1.034)
HDL	1.022 (0.970, 1.077)	0.972 (0.849, 1.112)	1.258 (0.908, 1.741)	1.037 (0.964, 1.116)	1.001 (0.911, 1.101)
Adjusted HDL*	0.980 (0.929, 1.033)	1.039 (0.900, 1.200)	0.829 (0.573, 1.200)	0.963 (0.894, 1.037)	0.998 (0.905, 1.100)
Total	0.999 (0.981, 1.017)	1.044 (0.993, 1.098)	0.872 (0.745, 1.021)	0.988 (0.962, 1.015)	0.993 (0.961, 1.026)
Adjusted Total*	1.003 (0.984, 1.022)	0.968 (0.919, 1.020)	1.333 (0.950, 1.870)	1.017 (0.989, 1.046)	1.005 (0.972, 1.039)
TRIG	0.996 (0.988, 1.004)	1.016 (0.995, 1.037)	0.946 (0.884, 1.013)	0.993 (0.982, 1.005)	0.991 (0.977, 1.006)
Adjusted Trig*	1.004 (0.995, 1.012)	0.987 (0.966, 1.008)	1.043 (0.970, 1.120)	1.006 (0.994, 1.018)	1.010 (0.994, 1.026)

Data are presented as OR (95% CI)

*Adjusted for gestational age at serum collection, maternal age at delivery and pre-pregnancy body mass index

Table 3.3. Association between GRS and respective lipid measurement.

GRS and Lipid	Overall (N=955)	Asian (N=149)	Black (N=20)	Hispanic (N=474)	White (N=312)
LDL	0.879 (<0.0001)	1.490 (0.0030)	0.928 (0.535)	0.531 (0.0966)	0.730 (0.0322)
HDL	1.122 (<0.0001)	1.382 (0.0096)	0.634 (0.722)	1.000 (0.0017)	1.157 (0.0032)
TC	0.506 (0.0022)	0.843 (0.0373)	-0.120 (0.871)	0.300 (0.223)	0.762 (0.0116)
TG	0.984 (<0.0001)	1.247 (0.0008)	0.114 (0.871)	0.956 (<0.0001)	0.520 (0.0129)

Data are presented as β coefficient (P Value). Lipids measured in mg/dl

Table 3.4. Association between lipids and PTB.

Lipid vs PTB	Overall (N=955)	Asian (N=149)	Black (N=20)	Hispanic (N=474)	White (N=312)
LDL	1.000 (0.996, 1.004)	1.005 (0.995, 1.015)	0.987 (0.959, 1.016)	0.999 (0.994, 1.005)	1.000 (0.993, 1.007)
Adjusted LDL*	1.000 (0.997, 1.004)	1.005 (0.994, 1.016)	0.982 (0.939, 1.028)	1.000 (0.994, 1.005)	1.000 (0.993, 1.007)
HDL	0.994 (0.987, 1.002)	0.987 (0.966, 1.008)	1.020 (0.975, 1.067)	0.994 (0.984, 1.005)	0.992 (0.979, 1.006)
Adjusted HDL*	0.997 (0.989, 1.005)	0.993 (0.970, 1.016)	1.026 (0.970, 1.084)	0.994 (0.983, 1.005)	0.998 (0.983, 1.012)
TC	1.000 (0.997, 1.004)	1.006 (0.996, 1.017)	1.002 (0.978, 1.027)	0.999 (0.994, 1.004)	1.001 (0.995, 1.007)
Adjusted TC*	1.001 (0.997, 1.004)	1.006 (0.995, 1.017)	1.008 (0.970, 1.046)	0.998 (0.993, 1.004)	1.001 (0.994, 1.007)
TG	1.002 (1.000, 1.003)	1.003 (0.998, 1.007)	1.025 (0.994, 1.056)	1.000 (0.998, 1.002)	1.005 (1.001, 1.009)
Adjusted TG*	1.001 (0.999, 1.003)	1.002 (0.997, 1.007)	1.018 (0.982, 1.056)	1.000 (0.998, 1.002)	1.004 (1.000, 1.008)

Lipids measured in mg/dl

*Adjusted for gestational age at serum collection, maternal age at delivery and pre-pregnancy body mass index

Supplemental Table 3.1A. List of genotyped SNPs.

SNP	Gene	Chromosome	Base Pair	Variant Type	Clinical Significance
rs10195252	*LOC101929615*	2	164656581	unknown	
rs1030431		8	58399138	unknown	
rs10401969	*SUGP1*	19	19296909	intron	
rs1042034	*APOB*	2	21002409	missense	other
rs10808546	*LOC105375745*	8	125483576	unknown	
rs10832963		11	18642694	unknown	
rs11136341	*PLEC*	8	143969375	intron	
rs11153594	*FRK,LOC101927818*	6	116033428	intron	
rs11220463	*ST3GAL4*	11	126378316	intron	
rs1129555	*GPAM*	10	112150963	3 prime utr	
rs11613352	*R3HDM2*	12	57398797	intron	
rs11649653		16	30907166	unknown	
rs11776767	*MIR1322,PINX1*	8	10826419	intron	
rs12027135	*TMEM57*	1	25449242	intron	
rs12310367	*ZNF664,ZNF664-FAM101A*	12	124002131	intron	
rs12328675	*COBLL1*	2	164684290	3 prime utr	
rs1260326	*GCKR*	2	27508073	missense, downstream	other
rs12670798	*DNAH11,LOC105375183*	7	21567734	intron	
rs12678919		8	19986711	unknown	
rs12916	*HMGCR*	5	75360714	3 prime utr	
rs13107325	*SLC39A8*	4	102267552	missense	
rs1321257	*GALNT2*	1	230169566	intron	
rs1367117	*APOB*	2	21041028	missense	other
rs1495743		8	18415790	unknown	
rs1532085	*LOC102724766*	15	58391167	intron	
rs1553318	*HAVCR1*	5	157052312	intron	
rs1564348	*SLC22A1*	6	160157828	intron	
rs16942887	*PSKH1*	16	67894139	intron	
rs17145738	*TBL2*	7	73568544	downstream	
rs174546	*FADS1*	11	61802358	3 prime utr	
rs174550	*FADS1*	11	61804006	intron	
rs174583	*FADS2*	11	61842278	intron	
rs174601	*FADS2*	11	61855668	intron	
rs1799964	*LOC100287329,LTA,TNF*	6	31574531	upstream	
rs1800562	*HFE*	6	26092913	missense	Pathogenic
rs1800629	*TNF*	6	31575254	upstream	drug-response
rs1800961	*HNF4A*	20	44413724	missense	other
rs1883025	*ABCA1*	9	104902020	intron	
rs1961456	*NAT2*	8	18398199	intron	
rs2000999	*HPR*	16	72074194	intron	
rs2068888		10	93079885	unknown	
rs2126259	*LOC157273*	8	9327636	intron	
rs2131925	*DOCK7*	1	62560271	intron	

Supplemental Table 3.1A – Continued

rs2255141	*GPAM*	10	112174128	intron	
rs2277862	*FER1L4*	20	35564866	nc transcript	
rs2285942	*DNAH11*	7	21543299	synonymous codon	Likely benign
rs2290159	*RAF1*	3	12587421	intron	
rs2412710	*CAPN3*	15	42391589	intron	
rs247616		16	56955678	unknown	
rs2479409	*PCSK9*	1	55038977	upstream	
rs261342	*LIPC,LOC101928694*	15	58438954	intron	
rs2737229	*TRPS1*	8	115636338	intron	
rs2807834	*MARC1*	1	220797251	intron	
rs2814944		6	34585020	unknown	
rs2902940	*LOC105372618*	20	40462847	intron	
rs2902941	*LOC105372618*	20	40462874	intron	
rs2929282	*FRMD5*	15	43953733	intron	
rs2943645		2	226234464	unknown	
rs2954022	*LOC105375745*	8	125470379	unknown	
rs2954029	*LOC105375745*	8	125478730	unknown	
rs3136441	*F2*	11	46721697	intron	
rs3757354	*MYLIP*	6	16127176	upstream	
rs3850634	*DOCK7*	1	62584927	intron	
rs386000		19	263646	unknown	
rs4297946	*ZHX3*	20	41182635	3 prime utr	
rs4299376	*ABCG8,LOC102725159*	2	43845437	intron	
rs442177	*AFF1*	4	87109109	intron	
rs4660293	*PABPC4*	1	39562508	intron	
rs4731702	*LOC105375508*	7	130748625	intron	
rs4810479		20	45916409	unknown	
rs4846914	*GALNT2*	1	230159944	intron	
rs492602	*FUT2,LOC105447645*	19	48703160	synonymous codon	
rs514230	*LINC01132*	1	234722850	upstream	
rs581080	*TTC39B*	9	15305380	intron	
rs6065906		20	45925376	unknown	
rs610604	*TNFAIP3*	6	137878280	intron	
rs629301	*CELSR2*	1	109275684	3 prime utr	
rs643531	*TTC39B*	9	15296036	intron	
rs6450176	*ARL15*	5	54002195	intron	
rs645040		3	136207780	unknown	
rs651007		9	133278431	unknown	
rs6759321	*R3HDM1*	2	135565106	intron	
rs6882076	*TIMD4*	5	156963286	upstream	
rs7205804	*CETP*	16	56970977	intron	
rs7239867	*LOC105372112*	18	49638347	intron	
rs7241918	*LOC105372112*	18	49634583	intron	
rs737337	*DOCK6*	19	11236817	synonymous codon	
rs7515577	*EVI5*	1	92543881	intron	

Supplemental Table 3.1A – Continued

rs7811265	*BAZ1B*	7	73520180	intron	
rs7941030		11	122651667	unknown	
rs838880		12	124777047	unknown	
rs881844	*STARD3*	17	39653965	intron	
rs909802	*ZHX3*	20	41308175	intron	
rs964184	*ZPR1*	11	116778201	3 prime utr	
rs9686661	*C5orf67*	5	56565959	intron	
rs9987289	*LOC157273*	8	9325848	intron	

Supplemental Table 3.1B. SNPs included in GRS for LDL-C.

SNP	Chromosome	Gene	Effect Allele	β Coeffieicent
rs12027135	1	*TMEM57*	T	1.1
rs2479409	1	*PCSK9*	G	2.01
rs2131925	1	*DOCK7*	T	1.59
rs629301	1	*CELSR2*	T	5.65
rs514230	1	*LINC01132*	T	1.13
rs1367117	2	*APOB*	A	4.05
rs4299376	2	*ABCG8,LOC102725159*	G	2.75
rs12916	5	*HMGCR*	C	2.45
rs6882076	5	*TIMD4*	C	1.67
rs3757354	6	*MYLIP*	C	1.43
rs1800562	6	*HFE*	G	2.22
rs1564348	6	*SLC22A1*	C	1.95
rs12670798	7	*DNAH11,LOC105375183*	C	1.26
rs11136341	8	*PLEC*	G	1.4
rs2255141	10	*GPAM*	A	1.08
rs174546	11	*FADS1*	C	1.71
rs964184	11	*ZPR1*	G	2.85
rs2000999	16	*HPR*	A	2
rs10401969	19	*SUGP1*	T	3.11
rs2902940	20	*LOC105372618*	A	0.98

Supplemental Table 3.1C. SNPs included in GRS for HDL-C.

SNP	Chromosome	Gene	Effect Allele	β Coeffieicent
rs4660293	1	*PABPC4*	A	0.48
rs4846914	1	*GALNT2*	A	0.61
rs1042034	2	*APOB*	C	0.9
rs12328675	2	*COBLL1*	C	0.68
rs13107325	4	*SLC39A8*	C	0.84
rs6450176	5	*ARL15*	G	0.49
rs2814944	6		G	0.49
rs17145738	7	*TBL2*	T	0.57
rs4731702	7	*LOC105375508*	T	0.59
rs9987289	8	*LOC157273*	G	1.21
rs12678919	8		G	2.25
rs2954029	8	*LOC105375745*	T	0.61
rs1883025	9	*ABCA1*	C	0.94
rs3136441	11	*F2*	C	0.78
rs174546	11	*FADS1*	C	0.73
rs964184	11	*ZPR1*	C	1.5
rs11613352	12	*R3HDM2*	T	0.46
rs838880	12		C	0.61
rs1532085	15	*LOC102724766*	A	1.45
rs16942887	16	*PSKH1*	A	1.27
rs7241918	18	*LOC105372112*	T	1.31
rs737337	19	*DOCK6*	T	0.64
rs386000	19		C	0.83
rs1800961	20	*HNF4A*	C	1.88
rs6065906	20		T	0.93

Supplemental Table 3.1D. SNPs included in GRS for triglyceride.

SNP	Chromosome	Gene	Effect Allele	β Coeffieicent
rs2131925	1	*DOCK7*	T	4.94
rs4846914	1	*GALNT2*	G	2.76
rs1042034	2	*APOB*	T	5.99
rs1260326	2	*GCKR*	T	8.76
rs10195252	2	*LOC101929615*	T	2.01
rs645040	3		T	2.22
rs442177	4	*AFF1*	T	2.25
rs9686661	5	*C5orf67*	T	2.57
rs11776767	8	*MIR1322,PINX1*	C	2.01
rs12678919	8		A	13.64
rs2954029	8	*LOC105375745*	A	5.64
rs2068888	10		G	2.28
rs174546	11	*FADS1*	T	3.82
rs964184	11	*ZPR1*	G	16.95
rs11613352	12	*R3HDM2*	C	2.7
rs2412710	15	*CAPN3*	A	7
rs2929282	15	*FRMD5*	T	5.13
rs1532085	15	*LOC102724766*	G	2.99
rs11649653	16		C	2.13
rs10401969	19	*SUGP1*	T	7.83
rs6065906	20		C	3.32

Supplemental Table 3.1E. SNPs included in GRS for total cholesterol.

SNP	Chromosome	Gene	Effect Allele	β Coeffieicent
rs12027135	1	*TMEM57*	T	1.22
rs2479409	1	*PCSK9*	G	1.96
rs2131925	1	*DOCK7*	T	2.6
rs7515577	1	*EVI5*	A	1.18
rs629301	1	*CELSR2*	T	5.41
rs514230	1	*LINC01132*	T	1.36
rs1367117	2	*APOB*	A	4.16
rs1260326	2	*GCKR*	T	1.91
rs4299376	2	*ABCG8,LOC102725159*	G	3.01
rs2290159	3	*RAF1*	G	1.42
rs12916	5	*HMGCR*	C	2.84
rs6882076	5	*TIMD4*	C	1.98
rs3757354	6	*MYLIP*	C	1.46
rs1800562	6	*HFE*	G	2.16
rs1564348	6	*SLC22A1*	C	2.18
rs12670798	7	*DNAH11,LOC105375183*	T	1.7
rs2737229	8	*TRPS1*	A	1.11
rs11136341	8	*PLEC*	G	1.34
rs581080	9	*TTC39B*	C	1.57
rs1883025	9	*ABCA1*	C	2.24
rs2255141	10	*GPAM*	A	1.14
rs174546	11	*FADS1*	T	1.78
rs964184	11	*ZPR1*	G	4.68
rs7941030	11		C	0.97
rs1532085	15	*LOC102724766*	A	1.54
rs2000999	16	*HPR*	A	2.34
rs10401969	19	*SUGP1*	T	4.74
rs492602	19	*FUT2,LOC105447645*	G	1.27
rs2277862	20	*FER1L4*	C	1.19
rs2902940	20	*LOC105372618*	A	1.38
rs1800961	20	*HNF4A*	C	4.73

Supplemental Table 3.2. Association between GRS quartiles and preterm birth.

GRS Quartile	LDL	HDL	TC	TG
Q4 vs. Q1.	0.974 (0.674, 1.408)	0.782 (0.546, 1.122)	1.070 (0.746, 1.533)	**1.491 (1.035, 2.149)**
Q3 vs Q1.	1.097 (0.767, 1.568)	0.821 (0.577, 1.169)	1.161 (0.810, 1.664)	1.329 (0.920, 1.919)
Q2 vs. Q1.	0.891 (0.621, 1.279)	0.787 (0.553, 1.121)	0.882 (0.614, 1.267)	1.234 (0.851, 1.790)
Quartiles as linear	1.015 (0.904, 1.140)	0.931 (0.831, 1.043)	1.049 (0.936, 1.176)	**1.134 (1.011, 1.272)**

Data are presented as OR (95% CI)

Supplemental Table 3.3. Associations between GRS and subtypes of preterm birth.

	LDL GRS		**HDL GRS**		**TC GRS**		**TG GRS**	
	Cases Mean ± SD	**Controls Mean ± SD**	**Cases Mean ± SD**	**Controls Mean ± SD**	**Cases Mean ± SD**	**Controls Mean ± SD**	**Cases Mean ± SD**	**Controls Mean ± SD**
Indicated vs Term	31.2 ± 5.0	31.6 ± 5.4	23.2 ± 2.4	23.1 ± 2.5	61.1 ± 6.7	62.0 ± 7.3	96.4 ± 15.7	97.5 ± 15.6
OR (95% CI)	0.984 (0.940, 1.029)		1.018 (0.924, 1.121)		0.981 (0.949, 1.015)		0.995 (0.980, 1.010)	
PPROM vs Term	31.1 ± 5.0	31.6 ± 5.4	23.3 ± 2.3	23.1 ± 2.5	61.0 ± 6.7	62.0 ± 7.3	95.7 ± 17.0	97.5 ± 15.6
OR (95% CI)	0.982 (0.948, 1.017)		1.044 (0.968, 1.126)		0.979 (0.954, 1.006)		0.993 (0.981, 1.004)	
Spontaneous vs Term	31.4 ± 5.2	31.6 ± 5.4	23.6 ± 2.4	23.1 ± 2.5	60.6 ± 6.7	62.0 ± 7.3	93.9 ± 14.7	97.5 ± 15.6
OR (95% CI)	0.991 (0.962, 1.021)		**1.092 (1.023, 1.165)**		**0.972 (0.950, 0.994)**		**0.985 (0.975, 0.995)**	

Supplemental Table 3.4. Associations between GRS quartiles and preterm birth subtypes.

GRS	PTB Subtype	OR	95% CI	
HDL-C Q2 vs Q1	**Indicated**	0.682	0.358	1.298
HDL-C Q2 vs Q1	**PPROM**	1.234	0.737	2.066
HDL-C Q2 vs Q1	**Spontaneous**	1.023	0.647	1.617
HDL-C Q3 vs Q1	**Indicated**	0.807	0.422	1.543
HDL-C Q3 vs Q1	**PPROM**	1.425	0.845	2.403
HDL-C Q3 vs Q1	**Spontaneous**	**1.639**	**1.053**	**2.549**
HDL-C Q4 vs Q1	**Indicated**	0.838	0.438	1.604
HDL-C Q4 vs Q1	**PPROM**	1.331	0.780	2.271
HDL-C Q4 vs Q1	**Spontaneous**	1.454	0.924	2.290
LDL-C Q2 vs Q1	**Indicated**	0.596	0.305	1.163
LDL-C Q2 vs Q1	**PPROM**	0.922	0.551	1.543
LDL-C Q2 vs Q1	**Spontaneous**	1.071	0.687	1.670
LDL-C Q3 vs Q1	**Indicated**	0.559	0.287	1.089
LDL-C Q3 vs Q1	**PPROM**	0.843	0.504	1.412
LDL-C Q3 vs Q1	**Spontaneous**	0.898	0.573	1.408
LDL-C Q4 vs Q1	**Indicated**	0.784	0.418	1.472
LDL-C Q4 vs Q1	**PPROM**	0.797	0.468	1.356
LDL-C Q4 vs Q1	**Spontaneous**	0.841	0.529	1.338
TG Q2 vs Q1	**Indicated**	0.498	0.238	1.045
TG Q2 vs Q1	**PPROM**	**0.537**	**0.315**	**0.914**
TG Q2 vs Q1	**Spontaneous**	0.818	0.527	1.269
TG Q3 vs Q1	**Indicated**	0.832	0.430	1.611
TG Q3 vs Q1	**PPROM**	0.610	0.362	1.029
TG Q3 vs Q1	**Spontaneous**	0.725	0.461	1.138
TG Q4 vs Q1	**Indicated**	0.915	0.487	1.719
TG Q4 vs Q1	**PPROM**	0.706	0.432	1.155
TG Q4 vs Q1	**Spontaneous**	**0.608**	**0.387**	**0.955**
TC Q2 vs Q1	**Indicated**	0.977	0.494	1.934
TC Q2 vs Q1	**PPROM**	1.371	0.825	2.281
TC Q2 vs Q1	**Spontaneous**	0.918	0.589	1.431
TC Q3 vs Q1	**Indicated**	1.005	0.527	1.917
TC Q3 vs Q1	**PPROM**	0.767	0.449	1.313
TC Q3 vs Q1	**Spontaneous**	0.747	0.483	1.156

Supplemental Table 3.4 – Continued

TC Q4 vs Q1	**Indicated**	0.731	0.368	1.451
TC Q4 vs Q1	**PPROM**	0.767	0.450	1.307
TC Q4 vs Q1	**Spontaneous**	**0.612**	**0.391**	**0.959**

Supplemental Table 3.5. Associations between lipid quartiles and preterm birth.

Lipid vs PTB	Overall (N=955)	Asian (N=149)	Black (N=20)	Hispanic (N=474)	White (N=312)
LDL-C					
Q4 vs Q1	0.974 (0.674, 1.408)	1.464 (0.538, 3.979)	0.400 (0.031, 5.151)	0.966 (0.576, 1.619)	0.953 (0.491, 1.852)
Q3 vs Q1	1.097 (0.767, 1.568)	1.278 (0.544, 3.002)	1.200 (0.073, 19.621)	0.997 (0.600, 1.654)	1.238 (0.643, 2.384)
Q2 vs Q1	0.981 (0.621, 1.279)	0.697 (0.295, 1.649)	0.600 (0.053, 6.795)	0.872 (0.521, 1.460)	1.103 (0.568, 2.142)
HDL-C					
Q4 vs Q1	0.782 (0.546, 1.122)	0.509 (0.200, 1.291)	<0.001 (<0.001, >999.999)	0.835 (0.503, 1.387)	0.748 (0.384, 1.455)
Q3 vs Q1	0.821 (0.577, 1.169)	0.765 (0.295, 1.981)	<0.001 (<0.001, >999.999)	0.926 (0.567, 1.513)	0.729 (0.385, 1.379)
Q2 vs Q1	0.787 (0.553, 1.121)	0.473 (0.183, 1.222)	<0.001 (<0.001, >999.999)	0.878 (0.541, 1.425)	0.833 (0.435, 1.596)
TC					
Q4 vs Q1	1.070 (0.746, 1.533)	2.288 (0.809, 6.470)	2.000 (0.224, 17.894)	0.861 (0.518, 1.430)	1.055 (0.555, 2.008)
Q3 vs Q1	1.161 (0.810, 1.664)	1.618 (0.682, 3.838)	2.000 (0.125, 31.975)	1.033 (0.625, 1.709)	1.148 (0.585, 2.253)
Q2 vs Q1	0.882 (0.614, 1.267)	1.471 (0.620, 3.487)	>999.999 (<0.001, >999.999)	0.719 (0.430, 1.202)	0.845 (0.434, 1.649)
TG					
Q4 vs Q1	**1.491 (1.035, 2.149)**	1.541 (0.633, 3.748)	>999.999 (<0.001, >999.999)	1.214 (0.698, 2.113)	**2.557 (1.263, 5.178)**
Q3 vs Q1	1.329 (0.920, 1.919)	0.480 (0.188, 1.228)	>999.999 (<0.001, >999.999)	1.749 (0.979, 3.128)	1.407 (0.776, 2.550)
Q2 vs Q1	1.234 (0.851, 1.790)	0.537 (0.204, 1.416)	3.000 (0.239, 37.672)	1.367 (0.764, 2.445)	1.508 (0.825, 2.758)

Supplemental Table 3.6. Associations between lipid levels and subtypes of preterm birth.

	LDL	HDL	TC	TG
Indicated vs Term	1.000 (0.993, 1.007)	1.007 (0.994, 1.021)	1.002 (0.995, 1.008)	1.001 (0.998, 1.004)
PPROM vs Term	1.000 (0.994, 1.005)	1.010 (0.999, 1.020)	1.000 (0.995, 1.006)	0.999 (0.997, 1.002)
Spontaneous vs Term	1.000 (0.995, 1.005)	1.007 (0.998, 1.017)	1.001 (0.996, 1.005)	1.001 (0.999, 1.003)

Data are presented as OR (95% CI)

Supplemental Table 3.7. Associations between lipid quartiles and preterm birth subtypes.

Lipid Quartile	PTB Subtype	OR	95% CI	
HDL-C Q2 vs Q1	**Spontaneous**	1.152	0.742	1.790
HDL-C Q2 vs Q1	**PPROM**	1.334	0.791	2.250
HDL-C Q2 vs Q1	**Indicated**	1.458	0.747	2.847
HDL-C Q3 vs Q1	**Spontaneous**	1.299	0.840	2.007
HDL-C Q3 vs Q1	**PPROM**	1.489	0.888	2.497
HDL-C Q3 vs Q1	**Indicated**	1.303	0.654	2.598
HDL-C Q4 vs Q1	**Spontaneous**	1.360	0.868	2.132
HDL-C Q4 vs Q1	**PPROM**	1.603	0.944	2.721
HDL-C Q4 vs Q1	**Indicated**	1.753	0.894	3.439
LDL-C Q2 vs Q1	**Spontaneous**	1.323	0.852	2.054
LDL-C Q2 vs Q1	**PPROM**	1.450	0.858	2.451
LDL-C Q2 vs Q1	**Indicated**	1.113	0.555	2.231
LDL-C Q3 vs Q1	**Spontaneous**	0.738	0.467	1.166
LDL-C Q3 vs Q1	**PPROM**	1.010	0.595	1.713
LDL-C Q3 vs Q1	**Indicated**	1.059	0.545	2.057
LDL-C Q4 vs Q1	**Spontaneous**	1.103	0.701	1.736
LDL-C Q4 vs Q1	**PPROM**	1.197	0.695	2.061
LDL-C Q4 vs Q1	**Indicated**	1.242	0.628	2.455
TG Q2 vs Q1	**Spontaneous**	0.749	0.469	1.199
TG Q2 vs Q1	**PPROM**	0.773	0.453	1.318
TG Q2 vs Q1	**Indicated**	0.837	0.424	1.653
TG Q3 vs Q1	**Spontaneous**	0.971	0.618	1.527
TG Q3 vs Q1	**PPROM**	0.882	0.523	1.490
TG Q3 vs Q1	**Indicated**	0.815	0.409	1.621
TG Q4 vs Q1	**Spontaneous**	1.058	0.676	1.656
TG Q4 vs Q1	**PPROM**	0.935	0.556	1.572
TG Q4 vs Q1	**Indicated**	0.994	0.513	1.927
TC Q2 vs Q1	**Spontaneous**	1.447	0.935	2.240
TC Q2 vs Q1	**PPROM**	1.272	0.752	2.151
TC Q2 vs Q1	**Indicated**	1.021	0.505	2.066
TC Q3 vs Q1	**Spontaneous**	0.737	0.463	1.171
TC Q3 vs Q1	**PPROM**	0.969	0.574	1.636
TC Q3 vs Q1	**Indicated**	1.110	0.576	2.138

Supplemental Table 3.7 – Continued

TC Q4 vs Q1	**Spontaneous**	1.080	0.691	1.689
TC Q4 vs Q1	**PPROM**	1.192	0.709	2.006
TC Q4 vs Q1	**Indicated**	1.208	0.621	2.348

Supplemental Table 3.8. Associations between non-HDL cholesterol and preterm birth.

	Unadjusted OR (95%CI)	**Adjusted OR (95% CI)***
Dyslipidemia	1.056 (0.819, 1.362)	0.948 (0.727, 1.236)
High LDL	0.945 (0.642, 1.392)	0.876 (0.587, 1.308)
High Cholesterol	1.031 (0.741, 1.435)	1.026 (0.732, 1.439)
High Triglyceride	1.122 (0.868, 1.450)	1.001 (0.766, 1.309)
Low HDL (low vs. normal)	1.476 (0.464, 4.690)	0.936 (0.665, 1.318)

Data are presented as OR (95% CI)
†Referent group is <5th percentile
*Quartiles run as continuous

Supplemental Table 3.9. Associations between dyslipidemia and preterm birth.

	Unadjusted	**Adjusted for BMI**	**Adjusted for BMI Category**	**Model with BMI alone**
Non-HDL	1.002 (0.998, 1.006)	1.018 (0.996, 1.040)	1.308 (0.925, 1.851)	1.019 (0.997, 1.041)
Non-HDL 5th vs 95th†	1.059 (0.477, 2.351)	0.981 (0.925, 1.041)	0.884 (0.286, 2.731)	1.019 (0.997, 1.041)
Non-HDL quartiles	1.071 (0.954, 1.203)*	1.061 (0.941, 1.196)*	1.069 (0.950, 1.202)*	1.019 (0.997, 1.041)
2 vs. 1	0.845 (0.581, 1.227)	0.835 (0.569, 1.225)	0.839 (0.577, 1.221)	
3 vs. 1	1.376 (0.969, 1.955)	1.386 (0.967, 1.985)	1.365 (0.958, 1.946)	
4 vs. 1	1.055 (0.730, 1.524)	1.013 (0.995, 1.040)	1.047 (0.722, 1.519)	

*Adjusted for GA at screening, body mass index and maternal age

CHAPTER IV: Pre-pregnancy Maternal Dyslipidemia and Risk for Preterm Birth

Abstract

Background: Maternal lipid profiles during pregnancy are associated with risk for preterm birth. Few studies have investigated the association between chronic pre-pregnancy dyslipidemia and risk for preterm birth. This study investigates the association between maternal dyslipidemia and subsequent preterm birth among pregnant women in the state of California.

Methods: Births were identified from California birth certificate and hospital discharge records from 2007-2012 (N=2,865,987). Preterm birth was defined as <37 weeks completed gestation and dyslipidemia was defined by diagnostic codes. Subtypes of preterm birth were classified as preterm premature rupture of membranes (PPROM), spontaneous labor, and medically indicated, according to birth certificate data and diagnostic codes. The association between dyslipidemia and preterm birth was tested using logistic regression. Stratified models were fit comparing preterm birth overall and each subtype to term birth. Models were adjusted for maternal age at delivery, race/ethnicity, hypertension, pre-pregnancy body mass index, insurance type, and education.

Results: Pre-pregnancy dyslipidemia was significantly associated with increased odds of preterm birth (adjusted OR: 1.49, 95%CI: 1.39, 1.59). This finding was consistent across all subtypes of preterm birth, including PPROM (adjusted OR: 1.54, 95%CI: 1.34, 1.76), spontaneous (adjusted OR: 1.51, 95%CI: 1.39, 1.65), and medically indicated (adjusted OR: 1.454, 95%CI: 1.282, 1.649).

Conclusions: This study suggests that pre-pregnancy dyslipidemia is associated with increased risk for all types of preterm birth. Dyslipidemia diagnoses may be a useful predictor of increased risk of preterm birth.

Introduction

Preterm birth is defined as delivery prior to 37 weeks of completed gestation. The World Health Organization estimates that preterm birth affects 11% of pregnancies worldwide, representing nearly 15 million births in 2010.[100] It is the second leading cause of death in children under age 5.[100] Despite decades of research into the causes of preterm birth, the biological causes of preterm birth remain largely unknown.[114]

Normal pregnancy is accompanied by metabolic changes, particularly in carbohydrate and lipid metabolism. The benefit of these changes is presumably to increase circulating glucose and triglycerides to nourish the growing fetus. Changes in carbohydrate metabolism are bimodal, in which fasting plasma glucose is decreased in early pregnancy, and impaired glucose tolerance occurs in late pregnancy.[43] Circulating lipids, including high density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol, and triglycerides, increase throughout pregnancy, with the greatest increase observed for triglycerides.[43] Although much previous research has been devoted to glucose metabolism during pregnancy due to the hazards of gestational diabetes mellitus,[115] increasing interest in lipid levels during pregnancy has revealed associations between maternal lipid levels and adverse pregnancy outcomes, including preterm birth.

Many studies have investigated associations between maternal lipid levels during pregnancy and risk for preterm birth, although the lipid components and effect sizes have been inconsistent across studies.[82-94] One previous study investigated the association between dyslipidemia, as defined by prenatal screening lipid levels, and found increased risks for preterm birth with mid-trimester hyperlipidemia in combination with elevated levels of tumor necrosis alpha.[88] The present study investigates the association between a clinical diagnosis of maternal pre-pregnancy dyslipidemia and subsequent preterm birth among pregnant women in the state of California. To our knowledge, the present study is the first to investigate the association between pre-pregnancy dyslipidemia irrespective of cause, identified by medical codes, and the risk for preterm birth.

Methods

Study Population

Births were identified from California birth certificate and hospital discharge records from 2007-2012 (N=2,962,434) as collected by the California Office of Statewide Health Planning and Development. Inclusion criteria included singleton pregnancy, availability of linked records, gestational age between 20-44 weeks and absence of hypertensive disease (ICD-9 402-405).

Dyslipidemia was defined by the International Classification of Diseases and Related Health Problems (ICD-9)[116] codes 272.0-272.4, which were recorded on hospital admissions one year prior to delivery and one year post-delivery. Specifically, these codes include pure hypercholesterolemia (ICD-9 272.0), pure hyperglyceridemia (ICD-9 272.1), mixed hyperlipidemia (ICD-9 272.2), hyperchylomicronemia (ICD-9 272.3), and other unspecified hyperlipidemia (ICD-9 272.4). A separate variable indicating 'maternal lipid disorder prior to delivery' was coded when a woman had an ICD-9 code 272.0-272.4 on a hospital admission prior to the delivery date.

Preterm birth was defined as gestational age at delivery <37 weeks and term birth was defined as gestational age at delivery ≥37 weeks, according to best obstetric estimate. Births were further categorized into early preterm birth (<32 weeks), late preterm birth (32-36 $^{6}/_{7}$weeks) and term birth (≥37 weeks). Subtypes of preterm birth were classified as preterm premature rupture of membranes (PPROM), spontaneous, and medically indicated, according to birth certificate data or hospital discharge records as previously described.[117] Specifically, preterm births with indication of premature rupture of membranes were classified as PPROM and births with indication of preterm labor or tocolytic medication AND absence of PPROM were classified as spontaneous. Births with absence of premature rupture of membranes, premature labor and tocolytic medication AND a code for 'medical induction' or 'artificial rupture of membranes' or cesarean delivery without such codes were classified as medically indicated.

Statistical Analysis

All analyses were performed using Statistical Analysis Software (SAS®) version 9.4 (SAS Institute, Cary, NC, USA). The associations between dyslipidemia and preterm birth were tested using logistic regression (PROC LOGISTIC). Dyslipidemia was modeled as a composite variable and as individual diagnostic codes. The association between dyslipidemia and preterm birth was also stratified by race/ethnicity. The associations were tested without adjustment and with adjustment for maternal age at delivery, hypertension, race/ethnicity, BMI, insurance type, and education. Maternal age at delivery was analyzed as a linear variable. Hypertension was coded as a binary variable and the absence of hypertension was used as the referent group. Race/ethnicity was categorized as Black, Asian, White or Hispanic, and White was used as the referent group. BMI was categorized according to standard cut-points (underweight [<18.5], normal [18.5-24.9] , overweight [25-29.9], or obese [≥30]), and 'normal' was used as the referent group.[108] Insurance type was categorized as Medi-Cal, private, self-pay, or other, and 'private' which was used as the referent group. Medi-Cal is California's Medicaid program, which provides health insurance and

health care services for low-income individuals. Education was categorized as <12 years, exactly 12 years (completion of high school diploma), or >12 years, which was used as the referent group.

We considered maternal age at delivery as a potential confounder, wherein we hypothesized that advanced maternal age would be associated with increased likelihood of diagnosis of dyslipidemia and an increased likelihood of delivering preterm. We also considered BMI as a potential confounder, in which overweight and obesity would be associated with increased likelihood of diagnosis of dyslipidemia and increased likelihood of delivering preterm. Other potential confounders including race/ethnicity, maternal age at delivery, hypertension (includes both pre-pregnancy and pregnancy diagnoses), pre-pregnancy body mass index (BMI), insurance type, and education were available from birth certificate and hospital discharge records.

Several supplemental analyses were performed. These included: 1) stratification of analyses by BMI category; 2) consolidation of ICD-9 codes into cholesterol dyslipidemia and triglyceride dyslipidemia for analytic purposes; and 3) examination of the association between obesity and medically indicated preterm birth. These stratified analyses were performed with statistical power >0.999 to detect an odds ratio of 1.5.

Results

Demographic characteristics of the study population are presented in **Table 1**. The analysis included 9,162 women with dyslipidemia and 2,953,272 women without dyslipidemia. Women with dyslipidemia differed from women without dyslipidemia at term by race/ethnicity, BMI, insurance status, education, and maternal age at delivery (all at $p<0.0001$). Specifically, the group of women who gave birth preterm included more Black women, were less likely to have a normal BMI, were more likely to receive Medi-Cal insurance and less likely to receive private insurance, were less likely to have completed more than 12 years of education, and were slightly older than the group of women who gave birth at term.

Results of the traditional logistic regression analyses are presented in **Table 2**. Three different outcomes are presented: preterm versus term, early and late preterm versus term, and PPROM, spontaneous, and medically indicated versus term. These analyses were performed in the total population (N=2,962,434) and in a sensitivity subset in which women aged <18 and >44 years old were excluded (N=2,870,449) to alleviate potential confounding by age. In the total population, dyslipidemia was significantly associated with preterm birth, both before and after adjusting for race/ethnicity, maternal age at delivery, hypertension, BMI, insurance type, and education. In the

age-restricted population, dyslipidemia was significantly associated with preterm birth, both before and after adjusting for race/ethnicity, maternal age at delivery, hypertension, BMI, insurance type, and education. The associations in the age-restricted population did not differ from their respective association among the total population.

Results of the traditional logistic regression analyses, stratified by type of dyslipidemia, are presented in **Table 3**. Hyperchylomicronemia (ICD-9 272.3) was not analyzed due to low sample size (N=3). Each type of dyslipidemia was significantly associated with preterm birth, both before and after adjusting for race/ethnicity, maternal age at delivery, hypertension, BMI, insurance type, and education.

Results of the traditional logistic regression analyses, stratified by race/ethnicity, are presented in **Table 4**. Within each racial/ethnic group, dyslipidemia was significantly associated with preterm birth. After adjusting for maternal age at delivery, hypertension, BMI, insurance type, and education, dyslipidemia was significantly associated with preterm birth among Asians, Whites, and Hispanics, but not among Black women.

Results of the traditional logistic regression analyses, stratified by BMI category, are presented in **Supplemental Table 1**. Within each BMI category, dyslipidemia was significantly associated with preterm birth. After adjusting for maternal age at delivery, hypertension, race/ethnicity, insurance type, and education, dyslipidemia was significantly associated with preterm birth among normal weight, overweight, and obese women, but not among underweight women. Obesity itself was associated with a 1.6-fold increase in risk for medically indicated preterm birth compared to normal BMI (OR: 1.61; 95%CI: 1.57, 1.65).

Results of the consolidation of ICD-9 codes into cholesterol dyslipidemia and triglyceride dyslipidemia are presented in **Supplemental Table 4.2**. Cholesterol dyslipidemia, which included pure hypercholesterolemia and mixed dyslipidemia, was significantly associated with preterm birth before and after adjustment. Triglyceride dyslipidemia, which included pure hyperglyceridemia and hyperchylomicronemia, was significantly associated with preterm birth before and after adjustment.

To investigate the individual impact of confounders, including race/ethnicity, hypertension, BMI, insurance type, maternal education and maternal age, on the association between dyslipidemia and preterm birth, each confounder was individually added to the logistic regression models (**Supplemental Table 4.3**). Adjusting for hypertension alone showed the greatest attenuation of the association between dyslipidemia and preterm birth of all the individual confounders (OR:

1.53; 95%CI: 1.45, 1.63). Adjusting for other confounders did not affect the odds ratios compared to the unadjusted models.

Discussion

In this prospective cohort of 2.9 million pregnant women in California, a pre-pregnancy diagnosis of dyslipidemia was significantly associated with increased risk for preterm birth. To the best of our knowledge, this study represents the largest investigation of the association between clinical dyslipidemia and risk for preterm birth done to date and it is the only study that we know of to utilize hospital diagnostic codes to define dyslipidemia, which include both familial and non-familial forms of dyslipidemia. The size and diversity of the study population allowed for the investigation of the association between dyslipidemia and preterm birth, stratified by subtype of dyslipidemia, by race/ethnicity, and by BMI category. These analyses revealed differential associations between subtypes of dyslipidemia, race/ethnicity and BMI category.

Several previous studies have investigated associations between maternal lipid levels during pregnancy and risk for preterm birth.[82-94] These studies varied in the lipid components they measured, the gestational age at which they were measured, and fasting status, which may explain their discordant findings. For example, of the seven studies that measured all four lipid components,[82-88] four studies failed to identify an association between individual lipid components and risk for preterm birth. Of the three studies that measured only total cholesterol (TC),[92-94] one identified a positive association between elevated TC and preterm birth and two identified associations between both low and high TC and preterm birth. A recent meta-analysis identified significant pooled associations between elevated TC, elevated TG and low HDL and preterm birth.[95] All previous studies have used lipid levels as a continuous exposure, although some categorized lipid levels by percentiles. However, this does not mean that these studies sampled women who would have met criteria for dyslipidemia. Thus, our study is unique in its use of a clinically significant exposure.

Of particular interest in the present study is the consistency of effect sizes across all subtypes of preterm birth, after adjusting for potential confounders. These adjusted odds ratios ranged from 1.4-1.6 (**Table 2**), providing strong and consistent evidence that women with pre-pregnancy dyslipidemia are approximately one-and-a-half times more likely to deliver preterm than comparable women without dyslipidemia regardless of preterm birth subtype.

With respect to medically indicated preterm birth, our data suggest that dyslipidemia may mediate the previously identified association between obesity and increased risk for medically indicated

preterm birth.[118] Dyslipidemia is often comorbid with obesity,[119] and obesity has long been known to increase the risk for pregnancy complications such as gestational diabetes mellitus and preeclampsia.[120] It may be that dyslipidemia severe enough to warrant a clinical diagnosis is a marker for more severely disturbed cardiometabolic milieu. Gestational diabetes mellitus and preeclampsia can become toxic to the mother and child and can require preterm induction of labor and/or caesarean section to save them, which is often reported as an association between obesity and medically indicated preterm birth.[121] Obesity is also independently associated with increased risk for caesarean section.[122]

Obesity is an independent risk factor for all types of preterm birth, although the mechanism by which obesity causes spontaneous preterm birth and PPROM is unclear. One proposed mechanism is the secretion of inflammatory cytokines by adipose tissue.[123] Human parturition progresses as reproductive tissues respond to inflammatory cytokines and hormonal signals. In particular, inflammatory cytokines induce thinning of fetal membranes, cervical ripening and increased myometrial contractility.[47] Thus, increased production of inflammatory cytokines in obese women may result in PPROM or spontaneous onset of labor and subsequent preterm birth. A similar mechanism may explain how dyslipidemia induces preterm birth. Chronic dyslipidemia is accompanied by inflammation, and acute inflammation triggers altered lipid metabolism.[124] Stratification by BMI category did not reveal stronger associations between dyslipidemia and preterm birth among overweight or obese women (**Supplemental Table 4.1**). This further suggests that dyslipidemia is independently associated with increased risk for preterm birth.

A limitation of this study is the lack of information regarding dyslipidemia diagnostic practices. Heterogeneity exists among practitioners in terms of the degree of follow-up testing of lipid levels. Thus, some women may have received a diagnosis after a single abnormal lipid panel, with no repeat testing, while other women may have received a diagnosis following multiple abnormal panels. Some women with dyslipidemia may not have a diagnosis because they have never had their lipid levels tested, which would result in non-differential misclassification of exposed and unexposed women. However, non-differential misclassification would bias the results toward the null. It is unlikely that women were treated with cholesterol-lowering drugs such as statins or niacin, since these drugs are contraindicated during pregnancy.[34]

It should also be noted that an important limitation of the study is the lack of lipid level information. Such data would have allowed for discrimination between familial, monogenic dyslipidemias, which are characterized by markedly abnormal lipid levels, and non-familial, polygenic dyslipidemias, which typically manifest as less drastic changes in lipid levels.

However, a Norwegian study of 895 women with familial hypercholesterolemia (FH) found no association between FH and risk for adverse pregnancy outcomes, including preterm birth.[98] Thus, we can infer that the association between pure hypercholesterolemia and preterm birth is driven by the non-familial form, which may be exacerbated by obesity. Additionally, assuming a prevalence of 1 in 250 for heterozygous FH,[39] only ten women with pure hypercholesterolemia would be expected to have FH in our study, which would likely not influence the results. Further, the detection of small differences in lipids between women who deliver term and preterm is unlikely to be clinically meaningful. In contrast, dyslipidemia is a clinically-validated medical condition that could be readily identified as a risk factor for preterm birth.

Dyslipidemia, as both an aggregate exposure and individual subtypes, was significantly associated with a 1.5-fold increased risk for preterm birth after adjusting for potential confounders. These findings suggest dyslipidemia may be a potential factor in the etiology of preterm birth, and may serve as a marker of increased risk for preterm birth. The identification of dyslipidemia as a risk factor for preterm birth is impactful for several reasons: 1) There are few known causal risk factors for preterm birth, as the causes of parturition and preterm birth remain largely unknown, 2) dyslipidemia may be modified by lifestyle changes and medication,[34] which could result in the prevention of preterm birth and 3) severe dyslipidemia receiving a clinical diagnosis may be easy to incorporate into clinical-decision making in the era of electronic medical records. Findings from this study support lipid screening among women of reproductive age to diagnose and treat dyslipidemia.

Tables

Table 4.1. Demographic characteristics of study population.

	Dyslipidemia (N=9162)	**No Dyslipidemia (N=2,953,272)**	**P Value**
Maternal Age at Delivery*	32.4 ± 5.97	28.3 ± 6.29	<0.0001
Race			<0.0001
Black	646 (7.8%)	157,917 (5.8%)	
Asian	1305 (15.7%)	365,274 (13.4%)	
White	2293 (27.5%)	770,805 (28.2%)	
Hispanic	4086 (49.0%)	1,963,803 (52.7%)	
Missing (N=218,664)			
BMI			<0.0001
Underweight	190 (2.2%)	144,146 (5.2%)	
Normal	2278 (26.7%)	1,349,503 (49.0%)	
Overweight	2308 (27.1%)	701,674 (25.5%)	
Obese	3754 (44.0%)	558,825 (20.3%)	
Missing (N=199,124)			
Insurance			<0.0001
MediCal	2473 (27.0%)	1,427,199 (48.4%)	
Private	6413 (70.1%)	1,366,516 (46.4%)	
Self-Pay	58 (0.6%)	59,778 (2.0%)	
Other	210 (2.3%)	95,014 (3.2%)	
Missing (N=4765)			
Education			<0.0001
<12 years	1350 (15.3%)	707,119 (24.9%)	
High school	2208 (25.0%)	755,196 (26.6%)	
>12 years	5292 (59.8%)	1,381,901 (48.6%)	
Missing (N=109,056)			
Preterm			<0.0001
Yes	1369 (14.9%)	209,717 (7.1%)	
No	7793 (85.1%)	2,743,555 (92.9%)	

*Data are presented as mean ± standard deviation. All other data are presented as N (%)

Table 4.2. Association between dyslipidemia and preterm birth.

	Excluding age <18 and >44 (N=2,870,449)		**Total population (N=2,962,434)**	
	Unadjusted OR (95% CI)	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**
Outcome 1[a]	**2.31 (2.18, 2.45)**	**1.48 (1.38, 1.58)**	**2.30 (2.17, 2.44)**	**1.49 (1.39, 1.59)**
Outcome 2[b]				
<32 weeks vs. Term	**2.97 (2.61, 3.38)**	**1.59 (1.37, 1.84)**	**2.97 (2.61, 3.37)**	**1.63 (1.41, 1.89)**
32-36 weeks vs. Term	**2.20 (2.06, 2.34)**	**1.45 (1.35, 1.56)**	**2.19 (2.06, 2.33)**	**1.46 (1.36, 1.57)**
Outcome 3				
PPROM vs. term	**1.94 (1.71, 2.20)**	**1.55 (1.35, 1.78)**	**1.92 (1.69, 2.17)**	**1.54 (1.34, 1.76)**
Spon. vs. term	**2.44 (2.26, 2.63)**	**1.49 (1.37, 1.63)**	**2.43 (2.26, 2.62)**	**1.51 (1.39, 1.65)**
Indicated vs. term	**2.83 (2.53, 3.15)**	**1.45 (1.28, 1.64)**	**2.85 (2.55, 3.17)**	**1.45 (1.28, 1.65)**

*Adjusted for race, maternal age at delivery, hypertension, body mass index, insurance type, and education

[a]Preterm birth (<37 weeks) vs. term birth (≥37 weeks)

[b]Early and late preterm birth

Table 4.3. Association between types of dyslipidemia and preterm birth.

	Pure hypercholesterolemia ICD-9 272.0 (N=2599)		**Pure hyperglyceridemia ICD-9 272.1 (N=681)**		**Mixed hyperlipidemia ICD-9 272.2 (N=379)**		**Other unspecified hyperlipidemia ICD-9 272.4 (N=6088)**		**Maternal lipid disorder before delivery (N=6816)**	
	Unadjusted OR (95% CI)	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**
Outcome 1[a]	**2.16 (1.93, 2.41)**	**1.30 (1.14, 1.47)**	**2.54 (2.07, 3.12)**	**1.64 (1.29, 2.09)**	**2.41 (1.82, 3.18)**	**1.77 (1.29, 2.43)**	**2.39 (2.23, 2.57)**	**1.53 (1.41, 1.66)**	**2.32 (2.17, 2.48)**	**1.63 (1.50, 1.76)**
Outcome 2[b]										
<32 weeks vs. Term	**2.92 (2.30, 3.70)**	**1.40 (1.07, 1.83)**	**3.43 (2.22, 5.31)**	**2.07 (1.26, 3.39)**	**2.94 (1.57, 5.50)**	**2.03 (1.03, 3.99)**	**3.07 (2.63, 3.58)**	**1.67 (1.40, 2.00)**	**2.89 (2.49, 3.36)**	**1.79 (1.51, 2.13)**
32-36 weeks vs. Term	**2.04 (1.81, 2.30)**	**1.28 (1.11, 1.46)**	**2.39 (1.92, 2.99)**	**1.56 (1.20, 2.03)**	**2.32 (1.72, 3.14)**	**1.72 (1.22, 2.42)**	**2.28 (2.12, 2.46)**	**1.50 (1.38, 1.64)**	**2.23 (2.07, 2.39)**	**1.59 (1.47, 1.73)**
Outcome 3										
PPROM vs. term	**1.64 (1.28, 2.11)**	**1.41 (1.08, 1.83)**	**2.05 (1.31, 3.21)**	**1.86 (1.16, 2.99)**	1.81 (0.97, 3.40)	1.43 (0.71, 2.88)	**2.06 (1.77, 2.39)**	**1.57 (1.33, 1.86)**	**1.99 (1.73, 2.29)**	**1.61 (1.38, 1.89)**
Spon. vs. term	**2.43 (2.11, 2.79)**	**1.38 (1.17, 1.61)**	**3.23 (2.53, 4.13)**	**1.76 (1.30, 2.40)**	**2.59 (1.81, 3.71)**	**1.99 (1.33, 2.97)**	**2.42 (2.21, 2.65)**	**1.50 (1.34, 1.67)**	**2.45 (2.25, 2.68)**	**1.70 (1.54, 1.88)**
Indicated vs. term	**2.46 (1.98, 3.05)**	1.13 (0.89, 1.44)	**2.50 (1.63, 3.83)**	1.56 (0.99, 2.46)	**3.05 (1.82, 5.10)**	1.62 (0.88, 2.98)	**3.23 (2.85, 3.66)**	**1.65 (1.43, 1.91)**	**2.84 (2.50, 3.22)**	**1.53 (1.32, 1.77)**

*Adjusted for race, maternal age at delivery, hypertension, body mass index, insurance type, and education

[a]Preterm birth (<37 weeks) vs. term birth (≥37 weeks)

[b]Early and late preterm birth

Table 4.4. Association between dyslipidemia and preterm birth, stratified by race.

	Black (N=158,563)		**Asian (N=366,579)**		**White (N=773,098)**		**Hispanic (N=1,444,698)**	
	Unadjusted OR (95% CI)	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**
Outcome 1[a]	**1.88 (1.53, 2.30)**	1.20 (0.96, 1.50)	**2.25 (1.92, 2.62)**	**1.32 (1.11, 1.57)**	**2.38 (2.11, 2.68)**	**1.64 (1.44, 1.86)**	**2.26 (2.08, 2.47)**	**1.44 (1.31, 1.58)**
Outcome 2[b]								
<32 weeks vs. Term	**1.58 (1.01, 2.48)**	0.86 (0.52, 1.41)	**3.11 (2.18, 4.43)**	**1.395 (0.95, 2.04)**	**3.30 (2.50, 4.35)**	**1.91 (1.41, 2.57)**	**3.18 (2.65, 3.82)**	**1.66 (1.36, 2.04)**
32-36 weeks vs. Term	**1.95 (1.57, 2.44)**	1.31 (1.03, 1.66)	**2.13 (1.80, 2.51)**	**1.30 (1.08, 1.57)**	**2.25 (1.98, 2.56)**	**1.59 (1.39, 1.83)**	**2.11 (1.92, 2.32)**	**1.39 (1.25, 1.55)**
Outcome 3								
PPROM vs. term	1.27 (0.77, 2.09)	1.128 (0.68, 1.868)	**2.10 (1.56, 2.83)**	**1.61 (1.17, 2.22)**	**1.91 (1.49, 2.44)**	**1.68 (1.30, 2.17)**	**1.91 (1.57, 2.32)**	**1.44 (1.17, 1.77)**
Spon. vs. term	**1.95 (1.506, 2.53)**	1.22 (0.92, 1.62)	**2.48 (2.03, 3.02)**	**1.38 (1.11, 1.72)**	**2.39 (2.03, 2.82)**	**1.61 (1.35, 1.92)**	**2.40 (2.14, 2.68)**	**1.47 (1.30, 1.67)**
Indicated vs. term	**2.68 (1.87, 3.86)**	1.281 (0.86, 1.899)	**2.08 (1.49, 2.92)**	0.97 (0.67, 1.40)	**3.32 (2.68, 4.11)**	**1.69 (1.33, 2.15)**	**2.80 (2.39, 3.23)**	**1.45 (1.21, 1.73)**

*****Adjusted for maternal age at delivery, body mass index, hypertension, insurance type, and education

[a]Preterm birth defined by gestational age

[b]Early and late preterm birth

Supplemental Table 4.1. Association between dyslipidemia and preterm birth, stratified by BMI category.

	Underweight (N=144,336)		**Normal (N=1,351,781)**		**Overweight (N=703,982)**		**Obese (N=562,579)**	
	Unadjusted OR (95% CI)	**Adjusted*OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted*OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted*OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted* OR (95% CI)**
Outcome 1[a]	**2.30 (1.55, 3.42)**	**1.59 (1.02, 2.48)**	**2.17 (1.91, 2.46)**	**1.50 (1.30, 1.73)**	**2.43 (2.16, 2.73)**	**1.55 (1.36, 1.76)**	**2.27 (2.08, 2.48)**	**1.40 (1.27, 1.55)**
Outcome 2[b]								
<32 weeks vs. Term	2.249 (0.833, 6.073)	1.412 (0.505, 3.952)	**3.380 (2.602, 4.391)**	**2.045 (1.527, 2.737)**	**2.925 (2.260, 3.786)**	**1.560 (1.166, 2.087)**	**2.455 (2.020, 2.983)**	**1.415 (1.144, 1.751)**
32-36 weeks vs. Term	**2.075 (1.361, 3.164)**	1.474 (0.918, 2.366)	**1.911 (1.669, 2.190)**	**1.411 (1.214, 1.639)**	**2.204 (1.943, 2.501)**	**1.481 (1.287, 1.703)**	**2.181 (1.983, 2.398)**	**1.382 (1.244, 1.537)**
Outcome 3								
PPROM vs. normal	1.42 (0.53, 3.84)	1.01 (0.32, 3.19)	**1.73 (1.32, 2.25)**	**1.41 (1.06, 1.89)**	**2.32 (1.83, 2.90)**	**1.89 (1.48, 2.41)**	**1.76 (1.45, 2.14)**	**1.36 (1.01, 1.68)**
Spon. vs. normal	**2.84 (1.81, 4.48)**	**2.02 (1.21, 3.36)**	**2.37 (2.03, 2.77)**	**1.67 (1.40, 1.98)**	**2.38 (2.04, 2.78)**	**1.42 (1.19, 1.69)**	**2.33 (2.08, 2.61)**	**1.38 (1.21, 1.57)**
Indicated vs. normal	1.69 (0.62, 4.55)	1.04 (0.38, 2.88)	**2.17 (1.66, 2.83)**	1.17 (0.86, 1.59)	**2.67 (2.14, 3.33)**	**1.53 (1.20, 1.95)**	**2.63 (2.25, 3.08)**	**1.48 (1.25, 1.76)**

*Adjusted for maternal age at delivery, race, hypertension, insurance type, and education

[a]Preterm birth defined by ICD-9 codes

[b]Preterm birth defined by gestational age

Supplemental Table 4.2. Analysis of consolidation of ICD-9 dyslipidemia codes.

Dyslipidemia Type	Unadjusted OR (95% CI)	Adjusted OR (95% CI)
Cholesterol (272.0, 272.2) N=2,965	2.178 (1.97, 2.41)	1.343 (1.20, 1.51)
Triglyceride (272.1, 272.3) N=683	2.560 (2.09, 3.14)	1.639 (1.29, 2.09)

Supplemental Table 4.3. Analysis of the individual impact of confounders.

Outcome	BMI	Hypertension	Race	Insurance	Education	Maternal Age
Outcome 1[a]	2.21 (2.08, 2.35)	1.54 (1.45, 1.63)	2.25 (2.12, 2.39)	2.37 (2.24, 2.51)	2.34 (2.20, 2.48)	2.22 (2.10, 2.35)
Outcome 2[b]						
<32 weeks vs. Term	2.76 (2.42, 3.15)	1.61 (1.41, 1.84)	2.94 (2.57, 3.37)	3.15 (2.78, 3.58)	3.03 (2.66, 3.46)	2.90 (2.55, 3.29)
32-36 weeks vs. Term	2.12 (1.98, 2.26)	1.52 (1.42, 1.62)	2.14 (2.00, 2.28)	2.25 (2.11, 2.39)	2.23 (2.09, 2.37)	2.11 (1.98, 2.25)
Outcome 3						
PPROM vs. normal	1.88 (1.66, 2.14)	1.67 (1.47, 1.89)	1.88 (1.65, 2.14)	1.87 (1.65, 2.12)	1.89 (1.67, 2.15)	1.77 (1.56, 1.56)
Spon. vs. normal	2.36 (2.18, 2.55)	1.53 (1.41, 1.65)	2.36 (2.18, 2.56)	2.55 (2.36, 2.75)	2.49 (2.30, 2.69)	2.40 (2.23, 2.59)
Indicated vs. normal	2.52 (2.25, 2.83)	1.65 (1.48, 1.85)	2.80 (2.50, 3.19)	2.92 (2.62, 3.26)	2.85 (2.55, 3.19)	2.54 (2.28, 2.84)

[a]Preterm birth defined by gestational age
[b]Early and late preterm birth

CHAPTER V: Conclusions

The results of Chapter III demonstrate that genetic variability associated with lipid levels, in the form of single nucleotide polymorphisms, is not associated with risk for preterm birth. The results of Chapter IV demonstrate the clinically-defined dyslipidemia is associated with approximately 1.5-fold increased risk for preterm birth. This association was largely consistent across demographic and clinical factors, including race/ethnicity, body mass index, type of dyslipidemia, and type of preterm birth. Together, these findings suggest that the previously reported associations between lipids and preterm birth may be reflecting unidentified dyslipidemias, rather than variability in single nucleotide polymorphisms (SNP).

The seemingly discordant findings between Chapters III and IV may be explained by the genetic underpinnings of the two traits. The lipid-associated SNPs utilized in Chapter III contained very little genetic overlap with known dyslipidemia mutations, as presumed in Chapter IV. Table B illustrates the genes known to be mutated in dyslipidemias contrasted with the genes represented by the genetic risk scores utilized in Chapter III. Specifically, of the 67 genes identified used in Chapter III, only two are implicated in dyslipidemias. A possible explanation for the lack of overlap is that the genes associated with dyslipidemias are more highly conserved, and thus less prone to common variation, than those identified by GWAS. Another possible explanation could be the homogeneous ancestry of the GWAS meta-analysis. Specifically, because all subjects included in this meta-analysis were of European ancestry, SNPs within gene dyslipidemias may be more common among other ancestral groups and were thus not identified by GWAS. An alternative explanation is that additional genetic loci and lifestyle factors confer stronger effects on risk for spontaneous preterm birth which override the effects of the genetic loci included in this GRS.

Another key difference between the lipid measurements utilized in Chapter III and dyslipidemia in Chapter IV are the origin of the lipid components. The supplemental analysis in Chapter III assigning dyslipidemia based on clinical criteria identified approximately half of the study population as having dyslipidemia. This greatly exceeds prevalence estimates and thus is likely a reflection of metabolic changes in pregnancy. In contrast, dyslipidemias in Chapter IV were diagnosed prior to pregnancy, representing chronic exposure to extreme lipid phenotypes. This cumulative exposure may contribute to the risk for preterm birth, whereas acute pregnancy-induced lipid changes are less impactful on preterm birth risk.

Known genetic variability associated with lipid levels was not associated with risk for preterm birth. One possible explanation is that additional genetic variability contributing to lipid

levels exists but has yet to be identified. Another possibility is that lipid-associated SNPs interact with other genetic loci or environmental factors which were not investigated in this work.

Under-diagnosis of dyslipidemia among women of reproductive age is plausible given the recommended age of lipid screening. The US Preventive Services Task Force (USPSTF) recommends lipid screening in women at increased risk for coronary heart disease (CHD) between ages 20-45.[125] Although this age range encompasses the typical reproductive time period for most women, this recommendation only applies to women of *known* increased risk for CHD. However, many dyslipidemias may occur in women without known risk. For example, dyslipidemias may arise from recessive mutations, novel mutations not carried by a woman's family, or she may have incomplete or unknown family history of CHD. Furthermore, the USPSTF makes no recommendations for lipid screening for women aged 20-35 *not* at increased risk for CHD.[125] Given that most people in this age range are not at increased risk for CHD, this recommendation would fail to identify many young women who have dyslipidemia.

The author concludes that dyslipidemia should be considered a novel risk factor for preterm birth. Prior to initiating clinical uptake of this new risk factor, similar studies should be repeated in additional populations within the USA and in other countries to further validate the association. If dyslipidemia continues to be associated with increased risk for preterm birth, lipid screening among women of reproductive age should be considered as a public health measure to reduce the burden of preterm birth.

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