University of Alberta

Establishing a Pregnancy Cohort for the Study of Spontaneous Preterm Birth

by

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Abstract

Preterm birth is a global challenge. Research on etiologies and risk factors for spontaneous preterm birth appear to be stagnant and circular. New discoveries are needed as are new study approaches that examine gene-environment interactions as etiologies of preterm birth. Guidelines for studies of discovery using '-omics' technologies for preterm birth research are suggested. A prospective community pregnancy cohort is established to integrate '-omics' technologies and environmental data for the study of spontaneous preterm birth and its multifactorial etiological associations. Representative assessments of the Cohort for the provincial pregnant and parenting populations inform scope of generalizability of study findings, assessments current pregnancy and birth cohorts lack. The study design and representativeness assessment presented, as an example of adherence to the guidelines suggested, will serve as a model for future studies on the etiologies of spontaneous preterm birth.

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List of Abbreviations

Preterm Birth	PTB
Preterm Premature Rupture of the Membranes	PPROM
Iatrogenic Preterm Birth	IPTB
Spontaneous Preterm Birth	SPTB
Socioeconomic Status	SES
Tumor Necrosis Factor Alpha	TNF-α
Single Nucleotide Polymorphism	SNP
Threatened Preterm Labour	tPTL
Fetal Fibronectin	FFN
Avon Longitudinal Study of Parents and Children	ALSPAC
Ribonucleic Acid	RNA
Preterm Birth Genome Project	PGP
Preterm Birth International Collaborative	PREBIC
Genome-Wide Association Studies	GWAS
Preterm Birth Biomarker Project	PBP
Interleukin	IL
Corticotropin Releasing Hormone	CRH
Alberta Perinatal Health	APH
Maternity Experiences Survey	MES
Confidence Interval	CI

Chapter 1: Introduction

1.1.0 Overview

Preterm birth is a global challenge. A review of the literature indicates that both environmental and genetic factors are associated with risk of preterm birth yet they fail to reliably predict timing of delivery in clinical settings. Similarly, biomarkers can be used to screen for preterm birth but consistently low positive predictive values hinder their utility in medical decisions regarding interventions. Investigations focused on discovery may reveal novel markers predictive of preterm birth. The large sample sizes required to support discovery research can be obtained through cohort studies, a study design that also enables the longitudinal examination of genetic and environmental factors as they relate to preterm birth and fertility in the offspring. Cohort studies have been employed in maternal-fetal-newborn health research in the past. However, there remains a need to assess cohorts for their representativeness of the population from which they were created to inform generalizability of results.

1.2.0 Clinical Problem of Preterm Birth

The World Health Organization defines preterm birth as birth at less than 37 weeks (less than 259 days) of pregnancy.[1] Clinically, preterm birth (PTB) is often further distinguished by gestational age at delivery into extremely preterm (<28 weeks), very or severe preterm (28-<32 weeks), moderate preterm (32-<34 weeks) and late preterm births (34-<37 weeks).[2] Premature births have effects at individual, family, community, and societal levels since PTBs are associated with 75% of perinatal mortality.[3] Infants born preterm are at increased risk of acute health complications and long term morbidities including apnea, patent ductus arteriosus, gastroesophageal reflux, necrotizing enterocolitis, respiratory distress syndrome, chronic long disease or bronchopulmonary displasia, hearing and

vision impairments, cerebral palsy and neurological delays.[4] These complications are the result of immature organ systems being forced to function in the extrauterine environment. Risk of complications is inversely related to gestational age at delivery. Even late preterm infants are still at increased risk compared to their term counterparts.[5]

As medical technology continues to advance, so does the ability to support infants born at decreasing gestational ages and to offset some of the severity of PTB associated morbidities. Antenatal administration of corticosteroids to help rapidly develop immature lungs reduces respiratory distress syndrome by 50%.[6] Surfactant treatment immediately postpartum is also associated with decreased neonatal mortality and chronic lung disease at 36 weeks gestation[7] and has doubled the survival rate of infants born at 24 to 26 weeks gestation.[8] Indomethacin is administered in three doses following a PTB event and is a non-surgical approach used to close a patent ductus arteriosus, reducing or avoiding many of the negative consequences which result from surgery including vocal cord paralysis, feeding difficulties and increased risk of retinopathy of prematurity.[9] Despite these improvements to neonatal care and the increased survival rates of preterm infants, risks and incidence of morbidities in preterm infants remain high with focus turning to the cognitive impairments apparent in school-aged children.[10, 11] While it is undeniable that infants born preterm experience long-term morbidities, the relative contribution of low birthweight (or small for gestational age), altered growth trajectories or environmental influences on the development of morbidities, specifically cognitive developmental delays, have yet to be separated.

1.2.1 Global Issue of Preterm Birth

The lower limit of PTB continues to be undefined, reflecting the global variability in threshold of viability and access to medical technology. This

lack of consensus around the PTB definition makes a true assessment of continental and global PTB rates difficult and contributes to the wide ranges reported. Deliveries at early gestational ages (22-30 weeks) may be classified as miscarriage, abortion, stillbirth or preterm birth.[12] This is especially true in the developing world where as many as one third of all births occur in the home where minimal if any record of gestational age, birth weight, survival or even occurrence of the birth event is made.[12] However, PTB is of such paramount importance that national and global rates are continually measured and estimated as best as possible. Current estimates indicate a PTB outcome in 5-12% of all births in the developed world including Canada, the United States of America and many European Nations.[2] Rates have been rising for the past three decades[2] with infants born between 32^{0} and 36^{6} weeks comprising most of the observed increase from 1990 to 2005.[13] Current perinatal mortality rates are unavailable. However, the 2010 infant mortality rates[14] indicate that Canada has not shown the same decreases in infant mortality as other developed nations despite having a similar socioeconomic profile and is currently ranked 39th compared to 17th in 2005.[15] Preterm birth, as the largest contributor to perinatal mortality (and therefore a contributor to infant mortality), is likely to be contributing to Canada's dismal performance as PTB rates continue to climb.

1.2.2 Economic Burden of Preterm Birth

Assessing the annual or lifetime burden of PTB and its sequelae is challenging and detailed economic or financial assessments of costs associated with prematurity are relatively rare in the literature. The annual estimated cost of PTB in 2005 in the United States was in excess of $26.2 billion[13] and has likely increased in the past six years. This estimate was far from comprehensive, including only costs associated with increased medical care required for delivery and treatment until four years of age, early intervention services, special education services, and lost household

productivity in the labour market. In 2003 the Centre for Disease Control in the United States estimated that for individuals with cerebral palsy (just one of the disabilities associated with PTB), the average lifetime costs are $921,000[16] when including medical, educational and support services needed to care for these infants throughout their lives. Both cost estimates do not include estimates of the familial and psychosocial burdens that are incurred by individuals, parents, couples, siblings or entire families who raise infants born prematurely. Studies have shown some negative impacts to families of preterm infants over the early childhood period[17, 18] with recent studies suggesting that these stresses have been mostly overcome by late adolescence.[19] The strain on or deterioration of marriages and parental relationships, struggles of caregivers to maintain employment while meeting the needs of a prematurely born child, the need for additional and perhaps specialized caregivers, and the negative emotional impacts on families cannot easily be assign monetary value. Additionally, infants born preterm may not attain their full potential as a consequence of lifelong morbidities. Loss of individual potential is impractical to measure yet is likely a cost of PTB. These overlooked consequences that develop over the lifetime are potential costs experience by each family of a preterm infant and may suggest that the current figures for the annual burden of PTB are grossly underestimated.

1.3.0 Etiology of Preterm Birth

Multifetal pregnancies are at increased risk for preterm delivery with nearly 60% of twins being born preterm.[2, 20, 21] Uterine overdistension caused by multiple growing fetuses is a suspected cause with probable physiologic mechanisms reviewed elsewhere.[22] However, recent study findings are inconsistent.[23] After excluding multifetal pregnancies, PTB of singleton pregnancies can be phenotypically classified into three broad categories, namely (1) iatrogenic when delivery is for maternal or fetal indications (30-

35%); (2) preterm premature rupture of the membranes (PPROM) (25-30%) or (3) spontaneous (sometimes referred to idiopathic) with intact membranes (40-45%).[2] These three phenotypic classifications are differentiated by maternal, fetal and uterine characteristics at the time of presentation to a health care provider and are associated with different etiologies. Detailed phenotyping of PTB cases is essential as the heterogeneity contained within and between the broad categories can dilute and potentially prevent the discovery of significant subtype-specific associations.

1.3.1 Iatrogenic Preterm Birth

It is not always in the best interest of mother nor fetus to continue a pregnancy to term. When the benefits of delivery outweigh the benefits of continuing a pregnancy, the PTB is iatrogenic (IPTB). Common pregnancy complications warranting an IPTB include gestational diabetes mellitus, preeclampsia or ecclampsia, intrauterine growth restriction and fetal distress.[13] Pre-existing maternal medical conditions can increase the risk of an IPTB outcome and include maternal renal disorders, maternal cardiac disease, diabetes mellitus, and immune/autoimmune disorders such as lupus.[13, 24, 25] With medical advancements, the ability to detect and monitor these conditions has improved and, not surprising, so has the rate of IPTBs increased.[2] In Canada specifically, much of the increase in the overall PTB rate is suggested to be the result of the more aggressive use of caesarean sections as an intervention for suboptimal fetal growth[26] and therefore an increase in IPTBs. Iatrogenic preterm births are a category of PTBs for which prevention of early delivery is an unlikely goal as intervention in the form of delivery gives both the mother and the infant the best prognoses.

1.3.2 Preterm Premature Rupture of the Membranes (PPROM)

PPROM is defined as the rupture of the uterine membranes before 37 completed weeks of gestation and before the onset of labour.[27] It complicates 2-5% of all pregnancies[28] or approximately one quarter of all PTB cases.[2] The

fetus is at increased risk for infection and sepsis when the uterine membranes are no longer intact and cannot preserve the sterile intrauterine environment. The cause of PPROM remains unclear. However, ascending vaginal infections or inflammatory processes triggered by insults elsewhere in the maternal body may contribute to the rupture.[29, 30] Women (and their unborn fetuses) who experience a pregnancy complicated by PPROM may benefit from prolonging pregnancy temporarily but intervening with the goal of a term delivery may be of questionable benefit as the risk of fetal infection increases the longer membranes are ruptured. A large prospective study of 4826 women with PPROM who were randomly assigned to receive antibiotics or a placebo showed that erythromycin was associated with prolongation of pregnancy and a reduction in adverse neonatal outcomes.[31] A mere six months following the release of these results, 50% of the maternity units in the United Kingdom changed their clinical practice to include erythromycin antibiotics for women presenting with PPROM.[32] Disappointingly however, were the findings from the seven-year follow-up studies as the short-term benefits observed in the neonatal period did not extend into childhood. No improvements to or decreases in cognitive function in children were associated with maternal receipt of erythromycin during pregnancy.[33, 34] These childhood findings create concern for coupling administration of antibiotics with expectant management as a clinical practice because long-term outcomes may be negatively effected. Additionally, three small randomized control trials comparing expectant management to immediate delivery in PPROM cases have shown increases in infection rates in groups being treated with expectant management.[35-37] While no differences in neonatal outcomes were seen between groups, all three trials were of insufficient sample size to adequately assess neonatal outcomes, and none examined gestational ages less than 30 weeks. A recent Cochrane review included an additional four randomized control trials and found insufficient evidence for expectant management nor immediate delivery as the ideal clinical practice.[38] While more studies are needed,

unless the rupture itself can be prevented, intervening for a term delivery is unreasonable. Temporary treatment with subsequent preterm delivery may be the clinical management strategy most plausible for this group, striking a balance between increased risk of infection with prolonged rupture and increased severity of morbidities at decreasing gestational ages.

1.3.3 Spontaneous Preterm Birth (SPTB)

Spontaneous preterm labour is the occurrence of regular uterine contractions in the presence of cervical changes prior to 37 completed weeks of pregnancy. When these contractions result in delivery, SPTB occurs. Spontaneous preterm birth (also referred to as idiopathic preterm birth) is often combined with PPROM to achieve appropriate sample sizes or power in research studies. However, rupture of uterine membranes prior to onset of labour may arguably be the result of different physiological pathways, processes or causes; an argument supported by the recurrence of PPROM leading to PTB in black women and preterm labour with intact membranes leading to SPTB in white women.[39] Often, there is the absence of risk factors or known etiologies that can explain or predict the spontaneous labour and subsequent birth event. Given that SPTB occurs in as many as 45% of all preterm births,[2] the need to gain a more detailed understanding of the etiologies and pathways leading to this outcome is in immediate need of research focus. Importantly, while PTBs occurring for iatrogenic indications or in the presence of PPROM may not warrant interventions that lead to a term delivery, SPTBs may benefit from prevention. However, it is unlikely that all SPTBs result from the same etiology and as a result, it is naïve to anticipate that all SPTBs should be prevented. Similar to PPROM, SPTBs occurring in the presence of infection might be best managed by temporary treatment followed by PTB while cases not complicated by infections might benefit from interventions focused on preventing the preterm delivery. Discovery research study designs that incorporate a sample size sufficient to

study SPTB and that collect detailed phenotypic information that facilitates further sub-classification of this broad category may be the key to unveil the mechanisms of SPTB and identify new therapeutic targets for interventions for a clinical class (or subtypes within a class) of PTBs that could benefit from prevention.

1.4.0 Previous Research on Prediction of Preterm Birth

Preterm birth is multifactorial and known risk factors have been recently reviewed in detail[40] with both environmental and genetic factors being implicated. Spontaneous preterm birth is of particular interest as these deliveries often occur in the absence of pre-existing medical conditions or clinical cues, making the clinical reliance on risk factors to determine at-risk pregnancies central to obstetrical care.

1.4.1 Environmental Risk Factors

Many environmental factors are associated with SPTB including maternal age, socioeconomic status, marital status, short interpregnancy intervals, and infections. Developed countries have seen a marked increase in delayed childbearing as more women are waiting to have children until their late thirties.[41] Women over the age of 35 are at increased risk for PTB and this risk persists when controlling for educational attainment and parity.[42] Little has been offered as an explanation for this observed increase in risk. Advanced maternal age however is associated with obstetrical complications and adverse pregnancy outcomes; PTB may be another obstetrical outcome resulting from the decreased fertility associated with aging. Advanced maternal age has been offered as a partial explanation for the racial disparities of PTB. While advanced maternal age increases risk across women, the risk is greatest in African Americans and begins to rise at younger ages than in Caucasian women.[13] A particular theory has been offered to account for the increased risk of advanced maternal age linked to

ethnicity. Weathering is the concept that social inequalities accumulate over time and have physical consequences manifested as early health deterioration.[43] Physiological evidence supporting this theory remains inconclusive. However, the maternal stress associated with a disadvantaged lifestyle may impact fertility. Maternal stress and low social support are known risk factors for PTB and have been reviewed in detail elsewhere.[13, 44]

Socioeconomic status (SES) is a combined measure of income, education and occupation used to determine relative social position and may be a proxy for a disadvantaged lifestyle. A low socioeconomic status has been identified as a risk factor for PTB for several decades. A case-control study of 175 mothers of preterm infants and 313 mothers of term infants showed a low SES as assessed by self-report questionnaires with medical record confirmation was associated with PTB throughout pregnancy.[45] Similarly, a Canadian study examined mother's education level and household income as independent measures and showed that both remained significantly associated with PTB in multivariate modeling.[46] Employment status and household income may fluctuate throughout adult life while maternal educational attainment tends to remain more constant or possibly increase. A comparative study of maternal educational attainment and PTB risk in Denmark, Finland, Norway and Sweden showed that despite differences in trends in socio-economic inequalities during a period of large economic changes (economic recession and recovery), low maternal educational attainment remained a risk factor for early and moderate PTBs[47] though annual household income and current employment status were not examined. Multiple SES measures combined together likely generate the most appropriate measure for SES[48] and the most reliable proxy for a disadvantaged lifestyle as stable and changing factors are considered. Further, while biological mechanisms of low SES and PTB remain unclear, maternal stress and weathering hypothesized to mediate PTB in advanced maternal age are likely also at play in women of low SES.

Marital status trends, similar to maternal age at childbearing, have been changing in developed countries. Between 1980 and 2007, the number of women delivering infants outside of wedlock in the United States had increased between 1.5 and 5 fold.[49] Similarly, in Quebec Canada, 44% of all births were to common-law mothers in 1997, more than double that of 1990.[50] A recent meta-analysis examined marital status in relation to PTB, small for gestational age and low birthweight outcomes.[51] While some studies define marital status as married, common law, divorced, widowed, separated or single, others use more broad classifications of married, cohabiting or single. The meta-analyses examined all studies in terms of the broader classifications and concluded that unmarried women were at increased risk for adverse birth outcomes including PTB.[51] Even in nations or regions where common-law/cohabitating arrangements are common, the risk for PTB and adverse pregnancy outcomes are still elevated in non-married women.[50, 52] While it is likely not the lack of the marriage license specifically that increases risk of PTB, unmarried mothers may experience less financial and emotional security[52] and may lack the social and emotional support received by their married counterparts. As stress and low social support are known risk factors for PTB, these factors may be the underlying environmental effects contributing to PTB in unmarried women.

Both a short interpregnancy interval, defined as the time between a birth event and subsequent conception, and a short interbirth interval, defined as the time between the birth events of two subsequent pregnancies, have been associated with increased risk of PTB in the later pregnancy.[53] A large meta-analysis of the optimal time between pregnancies revealed a 1.9% increase in the risk of PTB for each month in intervals less than 18 months between pregnancies[54] yet should be interpreted cautiously as interpregnancy and interbirth intervals were not kept distinct. Despite study limitations in terms of defining the intervals between gestations, the evidence does support a short interval as a risk factor for PTB. Most studies fail to offer insights into

why this may be the case. It can be speculated that the uterine and overall maternal physiology have not had time to properly return to a prepregnancy state or prepare for another pregnancy if the interval is too short. Depleted nutrients or energy stores may limit the ability to carry a pregnancy to term, a speculation supported by research showing that women with low prepregnancy body mass indexes[55] and therefore presumably low nutrient and energy stores are also at risk for delivering their pregnancies preterm. Alternatively, the psychological and/or physical stress and demands of caring for a young toddler while pregnant may also negatively impact gestation length albeit the physiology remains unclear.

Infections, be it vaginal or intrauterine, are highly associated with PTB especially at earlier gestational ages. The rate of infection increases with decreasing gestational age with bacterial colonization rates as high as 80% in births before 24 weeks compared to 10% in moderate preterm deliverys.[56, 57] Bacterial vaginosis is a shift in the bacterial composition of the vagina[40] and was first shown to associate with preterm labour in 1986.[58] Since this initial finding, much research has been done on bacterial vaginosis and other infections in their relation to true preterm labour and PTB. The Preterm Prediction Study was conducted by the National Institute for Child Health and Human Development Maternal Medicine Units Network in the 1990s to assess predictors of preterm birth.[59] Participants were enrolled from ten centres across the United States between October 1992 and July 1994.[60] Participants with a positive test for bacterial vaginosis at 28 weeks gestation had an elevated risk for PTB (odds ratio 1.84) while the other vaginal infections examined, *Trichomonas vaginalis* and *Candida* were not significantly associated with PTB.[61] It has been hypothesized that bacterial vaginosis may ascend to infect the upper genital tract and eventually the uterine membranes and intrauterine environment[58] and that infections here would trigger preterm labour. However, a Cochrane review of fifteen randomized control trials in which antibiotics were used to treat bacterial

vaginosis showed that collectively antibiotics did not reduce the risk of PTB despite effectively clearing bacterial vaginosis infections.[62] Additionally, infection is often not confirmed in clinical cultures of amniotic fluid from PTBs but histological evidence of inflammation is apparent in the uterine membranes or umbilical cord.[40] Inflammation, with or without the presence of detectable infections, may signify the early activation of normal parturition cascades in which inflammatory factors increase with the progression towards active labour.[63] Currently there is a lack of evidence indicating that infections alone are causal for PTB; both infections and inflammation remain strongly associated with PTB.

1.4.2 Genetic Risk Factors

Important risk factors for PTB include a previous preterm birth or relative delivering preterm and a black ethnicity, suggesting a role for genetic factors. Among multiparious women, risk of PTB in each pregnancy increases with each recurrence of a preterm delivery with a risk of 15% of recurrence with one previous PTB and up to 30% recurrence risk with two previous PTBs.[64] This risk of recurrence is observed whether the first PTB is spontaneous or iatrogenic and while the risk of the same clinical subtype of PTB recurring is highest, risk of an alternate clinical subtype is also increased.[65] Recurrence risk has also been observed for women with a history of postterm deliveries.[66] Together, these findings suggest that gestational length may be predetermined within each individual woman, perhaps at the genetic level. However, for nulliparous women, pregnancy history has no utility as a there has not been a previous birth event. For these women, familial history can also suggest risk of PTB. Women who were born preterm, whose mothers were born preterm or whose sister has delivered an infant preterm, have an increased risk of delivering their pregnancies preterm.[67] These familial tendencies are attributed to genetic predispositions for PTB. In addition, twin studies have suggested that the heritability, or genetic risk for PTB

ranges between 17 and 40%.[68, 69] These studies often overlook or discount that families tend to live in similar if not the same environments and therefore these familial trends in PTB may be genetic, environmental, or more likely, a combination of both.

Interestingly, the familial risk of preterm birth only appears in females. If a man is born preterm, his risk of fathering a preterm infant does not seem to be affected.[70] Changing female partners for each child decreases a man's risk of fathering additional preterm infants, yet a woman's risk remains the same despite changes in male partners.[71] This risk might be simply explained by the uterus being an organ of females. However, it has been suggested that these patterns might be the result of the women's nuclear DNA; that each women has a genetic determinant of gestational length.[40] Muglia and Katz also suggest that the mitochondrial DNA might influence this female familial risk pattern as mitochondrial DNA is passed solely through females and therefore is present in both the mother and growing fetus.[40] These might in some way work to determine the timing of birth and influence the function of the uterus. Each woman also has her own compilation of microbes inhabiting her person and in particular her genital track. This microbiome might also on its own or with the maternal nuclear or mitochondrial DNA work to mediate a PTB outcome. It remains to be understood how and why PTB recurs in related females. However, a genetic role is likely involved.

Another risk factor for PTB suggesting a genetic role is maternal ethnicity. Women of black ethnicity, specifically African American ancestry, are more likely to deliver preterm compared to white women. In the United States in 2007, nearly 19% of infants born to black mothers were born preterm compared to nearly 12% of those born to Non-Hispanic white mothers.[72] This difference in apparent risk of PTB has been the focus of much research, especially in the genetic context. A recurring theme in the literature is the need for genome-wide association studies in the study of preterm birth. Most

studies published to date have used the candidate gene approach in which specific genes believed or known to be important in process of term parturition are examined. Anum *et al.* provide a detailed summary of the candidate genes studied that have revealed variants associated with PTB in African Americans.[73] For example, Tumor Necrosis Factor Alpha (TNF-α) is a proinflammatory cytokine associated with PTB.[74] Soluble receptors for TNF-α differ between African Americans and Caucasians[75] and may in part explain how TNF-α can behave differently at the cellular level in these ethnic groups. In a detailed candidate gene examination of TNF-α, TNF-receptor 1 and TNF-receptor 2, several small DNA differences, single nucleotide polymorphisms (SNPs), were shown to exist between African Americans and Caucasians.[76] However, when associated with PTB, neither SNPs nor genes held significant associations with PTB risk. This does not automatically suggest that these genetic disparities are not relevant to PTB. Rather, it can be hypothesized that research has yet to focus on the correct candidate genes or that some of these differences may be predispositions to PTB risk but that through gene-environment interactions, a PTB outcome results or is protected against.

1.4.3 Rationale for Research on Prediction of Preterm Birth

Many of the risk factors discussed above were included in a risk of preterm delivery scoring system developed in the 1970s.[77] Points were assigned to risk factors related to SES, previous pregnancy history, daily habits and current pregnancy complications to create a score indicating low, medium or high risk of PTB. Although the test was quick to administer, its predictive value was quite low. Resources would be wasted with intensive observation and clinical follow-up of the medium and/or high risk pregnancies for which less than one-quarter would deliver preterm, despite accounting for 80% of the PTBs.[78] The false positives hampered clinical utility and contributed to the lack of implementation of the tool.[79] While E. Papiernik was instrumental in developing the risk scoring system, he recognized that many components

of the scoring system were non-modifiable. However, a work environment with heavy lifting or physical exertion can be modified: pregnant women could take a leave from these work environments during pregnancy. He supported a work leave program in France that began in 1971.[79] Haguenau was selected as a follow-up site after implementation of the program. Over a 12 year period, there was a one-third reduction in the PTBs between 33 and 34 weeks gestation and a greater than 50% decrease in births between 28-32 weeks gestation.[79] This was not seen in women with a previous history of PTB or with a high risk pregnancy,[79] suggesting that not all PTBs can be prevented. Despite the apparent success of the program in France, a true controlled study was not published. The program was implemented nationwide and therefore causal relationships could not be assessed.[80] Education provided around current pregnancy symptoms and not the leave from work might be the major contributor to the decline in PTBs. This suggestion is partially supported by the results observed during the 12 year follow-up: less births to teen mothers and to mothers of advanced maternal age as well as a general increase in females' education level.[80] However researchers attempted to control for these changes statistically with no apparent effect. The work leave program has not been embraced in North America and its feasibility may be limited by financial constraints, market productivity, and the personal motivation to work throughout pregnancy.

Despite the failure of the risk of preterm delivery scoring system and the methodological weaknesses in the work leave program in France, research on the risk factors identified more than forty years ago continues. As an example, the Institute of Health Economics Alberta Canada recently released a report summarizing the evidence of determinants and prevention of low birth weight from other reviews,[81] re-enforcing existing knowledge. The main summary points related to maternal demographic factors include an interpregnancy interval of less than 18 months and being unmarried as risk factors for PTB, knowledge well established. The outcomes examined in the

report were limited to low birth weight, small for gestational age and PTB. While these outcomes are important, the resulting childhood and lifelong morbidities were unfortunately missed in the meta-analyses. For instance, the report suggests that low alcohol consumption during pregnancy is protective of low birth weight but fetal alcohol spectrum disorder was not considered. While these meta-analyses provide clear summaries of current knowledge, they also serve as reminders that research on risk factors for PTB appears stagnant and repetitive to the point of being circular.

Examining gene-environment interactions as etiologies of PTB from the perspectives of discovery research and PTB prediction might build upon the known risk factors and propel the field forward. Admittedly, if new discoveries lead to screening methods predictive of PTB, the clinical utility is controversial. Interventions to prevent a PTB if it were predicted early in pregnancy are not currently available. However, the ability to predict early delivery would provide information to allow clinicians, expecting women and families to better plan. Clinicians might transfer women to the care of an obstetrician to manage these high-risk pregnancies, women may alter scheduled maternity leaves, and rural families might arrange to be closer to an urban center with tertiary care services for the preterm infant. Health care professionals and families could work together to alter birth plans and identify the resources and knowledge necessary to care for a preterm infant. These potential benefits from the ability to successfully predict PTB provide individual, familial and clinical utility in the absence of preventative interventions. The new knowledge gained about PTB etiologies from the study of gene-environment interactions through investigations focused on prediction and discovery might also unveil new therapeutic targets for which preventative interventions can be designed. Individual to societal benefits might result from predictive studies of PTB.

1.5.0 Biomarkers for Preterm Birth

A diagnostic test is one that is positive in a high proportion of patients with the disease and negative in a large proportion of patients without the disease.[82] Diagnostic tests are typically used to confirm the presence of disease or to rule it out. Comparatively, a screening test is one that detects the disease in patients without signs or symptoms of the condition.[82] Screening tests are used to identify those who will be afflicted with the disease and to eliminate those who will not. The ability to conclusively diagnose threatened preterm labour and screen for preterm birth remain central challenges in obstetrics. Threatened preterm labour (tPTL) occurs when women experience signs and symptoms of labour before 37 completed weeks of pregnancy. These contractions are often more intense than the Braxton-Hicks contractions experienced in pregnancies. The diagnosis of true preterm labour is of great clinical importance as tPTL accounts for up to a third of hospital admissions during pregnancy yet less than 25% of women will deliver before 35 weeks gestation regardless of medical treatment received.[83, 84] These hospital admissions are costly to the healthcare system with many being arguably unnecessary. For those women who are in true preterm labour, the need for quick access to medical care is beneficial to both mother and fetus as the PTB outcome is the main concern. Diagnostic studies of true preterm labour are loosely termed 'symptomatic' as women already have signs and symptoms of preterm labour at study enrollment. Conducting studies at this stage enables shorter study timelines as participants need not be followed throughout gestation and reduces costs as fewer participants need to be enrolled to ensure adequate numbers of PTBs. However, these studies also suggest that once the labour cascade has begun very few current clinical interventions slow, reverse or stop preterm labour. This is highlighted in reviews of the inconsistent performance of tocolytics,[85-87] drugs designed to quiet preterm contractions in symptomatic women.

In comparison, screening studies enroll women prior to the onset of signs and symptoms of tPTL and seek to predict PTB early in pregnancy. The underlying research questions are therefore inherently different from those of symptomatic studies: diagnosing true preterm labour that leads to PTB (symptomatic studies) compared to screening for the development of PTB early in pregnancy (asymptomatic studies). The ultimate goal is the same: identify women who will deliver preterm. Asymptomatic studies have distinct disadvantages compared to symptomatic studies. Recruiting women early in pregnancy and following them throughout gestation increases both research time and costs. These studies are still aimed at determining factors predictive of PTB, necessitating large sample sizes to ensure PTB numbers as the majority of study participants will deliver at term. Asymptomatic studies focused on discovery may reveal the processes involved in initiation of the preterm labour (and eventual birth) cascades and lead to the development of screening tests for SPTB that are so desperately sought. The information discovered may open up new avenues to develop therapeutic targets for interventions to prevent SPTB.

1.5.1 The Gold Standards of Biomarker Tests for Preterm Birth

Fetal fibronectin (FFN) and a short cervical length are biomarkers that have the greatest clinical utility to date for diagnosing true preterm labour and screening for PTB, albeit both have limitations.

FFN is an isoform belonging to the family of glycoproteins called fibronectins and is produced by the fetal tissues during pregnancy where it can be found in the placenta, chorio-decidual tissues and in the amniotic fluid.[88] Prior to 20 weeks gestation, FFN is commonly found in cervical and vaginal secretions and again in the cervical secretions near term. However, after 20 weeks of pregnancy and prior to term, only about 4% of women have detectable levels of FFN in their cervical and vaginal secretions.[89]

In 1991, Lockwood *et al.* measured FFN in cervicovaginal fluid from 117 women with tPTL and intact membranes.[90] A positive FFN test correctly diagnosed true preterm labour with approximately 82% sensitivity and 83% specificity and was in stark contrast to the less than 5% of term delivering women who had positive FFN tests. However, the other clinical presentations of these women, namely average gestational age of nearly 30 weeks, a cervical dilation of >2cm and approximately 10 contractions per hour, strongly suggest true preterm labour. It may be questioned whether the FFN tests added anything diagnostically. Subsequent studies of FFN in symptomatic women[91-95] report similar specificities of 85% or higher, varying sensitivities of 44-100%, high negative predictive values of 76-100% and disappointingly low positive predictive values of well less than 50%. In a review of these studies, Khan *et al.*[96] determined that nearly two-thirds of the studies over estimated the diagnostic value of FFN, further limiting the clinical utility. The FFN test itself is sensitive to contaminations that can yield false positive results. Vaginal bleeding or rupture of the membranes can both yield positive results as FFN is present in detectable levels in amniotic fluid[90, 97] and plasma. These FFN sources can obscure results.[98]

To summarize these studies in a clinical context, a clinician can be fairly confident that a woman with preterm signs and symptoms of labour who has a negative FFN test is not experiencing true preterm labour, is at low risk for imminent delivery and does not need to be admitted to hospital immediately (ie diagnostically rule out 'disease'). A woman with the same presentation and a positive FFN test is non-diagnostic with the likelihood of the woman being in true preterm labour and at risk of imminent preterm delivery comparable to flipping a coin. It is the low positive predictive value of FFN and the ease with which a test can be compromised that undercuts FFN as the ideal diagnostic test for true preterm labour. The strong negative predictive value is of diagnostic utility for clinically ruling out true preterm labour in many cases.

FFN has also been examined in asymptomatic women as a screen for PTB. The first study, conducted by Lockwood *et al.*, measured cervical and vaginal FFN between 24 and 37 weeks gestation in 429 women.[99] A threshold of >60ng/ml of FFN was predictive of PTB before 37 weeks of pregnancy with a sensitivity of 73%, a specificity of 72%, a negative predictive value of 95% and a extremely low positive predictive value of 25%. The Preterm Prediction Study yielded similar, albeit more disappointing, results.[60] FFN was measured every two weeks between 24 and 30 weeks of pregnancy in nearly 3000 asymptomatic women. For both cervical and vaginal FFN positive tests, the specificities were greater than 96% for predicting preterm birth at <34 weeks. However the sensitivity was 19-29% and the positive predictive value 13-25%, limitations comparable to those of symptomatic studies. Interestingly, FFN was a stronger predictor for PTB if tests were positive at earlier gestational ages (closer to 24 weeks) than positive tests at later gestational ages (closer to 30 weeks) for early and moderate PTBs respectively. This difference may be reflective of different etiologies at play in early compared to moderate or late PTBs. Spontaneous early preterm births are often associated with genital tract infections[100] and these infections may breakdown or weaken the uterine membranes such that FFN is present and detectable in the cervicovaginal fluids. It is anticipated that future asymptomatic studies will not reveal the same risk factors or predictive screens for the various gestational age categories of preterm birth similar to that seen for FFN as a screen for PTB.

Cervical length has also been examined in the diagnosis of true preterm labour. True preterm labour is often associated with cervical ripening, that is, the cervix shortens and begins to efface prior to dilation.[101] These cervical measures are therefore part of the test for true preterm labour. Upon manual examinations, cervical length and effacement can be difficult to assess with variabilities of >50% between observers.[102] A study by Gomez *et al.* in 1994 showed that the use of transvaginal ultrasound to examine the cervix was a

better predictor of PTB risk in symptomatic woman than the digital examinations formerly used.[103] Many studies have since examined transvaginal ultrasound assessment of the uterine cervix for its utility in diagnosing true preterm labour. A study of 216 women with tPTL at 24-36 weeks showed that when taking into account demographic characteristics, the only statistically significant predictor of birth within 7 days of clinical presentation was a short cervical length of 0 – 14mm as assessed by transvaginal ultrasound.[104] Another study of 200 symptomatic women also found transvaginal ultrasound of the cervix to be predictive of PTB when cervical length was <30mm but their inclusion/exclusion criteria were less straightforward.[105] These researchers included women who were admitted to hospital with a diagnosis of preterm labour but excluded women who had PPROM, cervical dilations of >3cm and whom delivered within 24 hours of hospital admission. The inclusion criteria of diagnosed preterm labour is therefore confusing as their exclusion criteria reflects the clinical presentations often attributed to true preterm labour. Tekesin *et al.* examined 68 patients in tPTL and determined transvaginal ultrasound of the cervix to have a sensitivity of 82.1%, a specificity of 72.5%, a positive predictive value of 67.6% and a negative predictive value of 85.3% for preterm delivery when the cervical length was <25 mm.[106] While each study defines cervical length thresholds differently, there is a recurrent association of a short cervical length on transvaginal ultrasound assessment with preterm delivery.

Findings related to cervical length are inconsistent in studies of asymptomatic women and should be interpreted with caution as a screen for PTB. Ozdemir *et al.* used transvaginal ultrasound to examine cervical length at 10-14 weeks and 20-24 weeks of pregnancy in 152 asymptomatic women.[107] A short cervical length at 10-14 weeks was not predictive of PTB (at <35 weeks) though a cervical length of <27 mm at 20-24 weeks predicted preterm delivery with a sensitivity of 81.2%, a specificity of 99.3% and a

positive predictive value of 92.9%. These findings are similar to those of other investigators[108-110] although each study used a different cutoff for 'short' cervical length, a different cutoff for gestational age of PTB, measured the cervical length at different time points in pregnancy and used different inclusion criteria resulting in low to high risk subjects being included. These differences make the studies difficult to compare and the clinical utility of the findings unclear. Furthermore, while the odds ratios for PTB risk with a short cervix in the second trimester may have seemed convincing (OR 24.3, 95% CI 12.9-45.9), they translate into a positive predictive value of <50% and a sensitivity of <10% despite a negative predictive value and a specificity of >95%.[108]

1.5.2 Rationale for Research on Biomarkers of Preterm Birth

FFN and cervical length are not causes of PTB. Their utility early in pregnancy might therefore be limited because the changes from a non-labouring to labouring uterus have yet to start with these markers not detectable. While a true preterm labour diagnosis warrants hospital admission and expectant patient management for the impending PTB, little can be done to prevent preterm delivery for women in true preterm labour. The earlier in pregnancy women can be screened for PTB, the greater the potential for development of therapeutic interventions that may prevent a SPTB outcome. There is a strong need for robust screens for risk of SPTB early in pregnancy which represent or are very closely associated with the etiologies of SPTB. Screening for an etiology may more reliably and consistently predict SPTB. Discovery research concentrating on unveiling SPTB etiologies and assessing the predictive values of newly discovered factors more directly related to these causes should be a focal point of future SPTB research. Asymptomatic studies focusing on genetics and environmental risk factors of SPTB may hold the answer. While a short cervical length and a positive FFN screen are the clinical gold standards for

diagnosing true preterm labour, both factors fail to take into account the best predictor of risk for SPTB – a personal or family history of preterm birth. Because DNA, and therefore its expression (RNA), is inherited in each generation and cohabiting families have similar environments, studies focusing on these factors from the perspective of prediction and etiology and risk discovery might present the necessary next step for SPTB research.

"-Omics" technology provides methodologies for the study of genes and their functions (genomics), the study of the complete set of RNA transcripts produced by the genome at one time (transcriptomics), the study of the complete set of proteins produced by a species (proteomics) and the study of small-molecule metabolite profiles generated by cellular processes (metabolomics). These methodologies have been applied to the study of preterm birth in isolation for the testing of specific hypotheses.[111] The result has been the absence of findings that translate into clinical utility.[111] High throughput systems biology refers to large sample sets, processed rapidly, that are analyzed by integrating the various components of cellular or organism function to understand and model interaction networks.[112] The development of systems biology has enabled the integration of "-omics" technologies such that endeavours focused on discovery rather than hypothesis testing are possible. PTB research may experience progress for prediction and etiology and risk discovery if integrated "-omics" technology is applied to its study. To maximize the success of "-omics" investigations into PTB, researchers will have to work together, across international borders, to create merged sample sets appropriate for these methodologies. Guidelines designed to create consistency in the collection of samples and data will support international consortia as a means of facilitating discovery-focused investigations of PTB. These guidelines will be essential to the effective and appropriate implementation of such studies. "-Omics" methodologies are informed by environmental context and data. Risk factors predictive of SPTB and its' etiologies might be discovered by the proper

application of "-omics" technologies, beginning to fill the gap that currently exists between basic science research and clinical utility.

1.6.0 Cohorts for Gene-Environment Studies of Spontaneous Preterm Birth

Assessing gene-environment interactions or conducting detailed assessments of genetic or environmental etiologies of PTB require large sample sizes in order to evaluate clinically relevant relationships. While genetic composition is relatively stable over time, the environment is more prone to fluctuations and individuals may recall environmental factors differently over time. A recent study on recall bias of maternal depression and medication use during pregnancy demonstrated only a moderate agreement between the prospective assessment and retrospective reporting of prenatal depression.[113] Golding emphasizes that mothers may not be able to accurately remember many environmental factors as time passes and that recall may be biased once birth outcome and the health of the child is known.[114] While the time lapsed between pregnancy and the post-partum period is relatively short compared to lifelong exposures and outcomes, the changes from a pregnant to a non-pregnant state and the physical, emotional and mental demands of caring for a newborn may influence recall. Collecting environmental information prospectively during pregnancy may be more accurate and reliable in studies of environmental factors as etiologies and/or predictors for SPTB. Prospective cohorts are a study design that can accommodate large sample sizes needed to study genetic and environmental etiologies of SPTB.

1.6.1 Advantages of Cohort Studies

A cohort study is defined as a group of people moving through time from an exposure to an outcome or set of outcomes.[115] Cohort studies are uniquely advantageous. The appeal of cohort studies includes but is not limited to: (1)

the assessment of a wide range of exposures (environmental and/or genetic) and the assessment of a variety of outcomes are possible;[116] (2) temporal order between a potential exposure and an outcome is clear;[115] (3) calculations of incidence rates, relative risks and confidence intervals can be done;[115] and (4) cohorts are appropriate when randomization is not possible, desirable or appropriate.[117] Preterm birth is an outcome that is likely the result of a variety of exposures that occur by chance (e.g. genetics) or by choice (e.g. smoking) and by those which are continuous (e.g. stress or body mass index). A cohort design enables the examination of all of these factors as they relate to PTB, its clinical subtypes and other birth outcomes such as low birth weight, small for gestational age and postpartum depression. A prospective cohort study is uniquely advantageous as exposures can be measured at a baseline[118] (e.g. early in pregnancy or pre-conception) and changes can be followed over time leading to the outcome(s) (e.g. preterm birth).

1.6.2 Limitations of Cohort Studies

Cohort studies have important limitations to consider. They cannot be used to definitively determine causality[118] of PTB. Because exposures are not randomly assigned, associations rather than causes can be revealed as confounders may be present. However, in such complex situations as SPTB where the causes are largely unknown, cohorts may provide an opportunity to identify strong associations that can be investigated for causation in subsequent studies. Cohort studies may also suffer from selection bias.[115, 118] This bias can occur if enrollment or response rate is low or if eligibility is determined in such a way that participants systematically differ from those not in the cohort. Loss to follow-up is a recurrent issue in cohort studies[118] and can bias results. Assessing bias associated with those who enroll and continue to participate compared to those who do not is important to generalizability of results. The limitations of cohort studies should be

considered in the design of pregnancy cohorts past, present and future in order to maximize utility of the information collected and the results generated.

1.6.3 Previous Pregnancy Cohorts for the Study Maternal-Fetal-Newborn Health

Numerous small and large cohorts have been established for the study of maternal-fetal-newborn health. Through follow-ups over time, these cohorts also provide insights into child and adult health and how life long health can be linked to the prenatal period. A selection of pregnancy cohorts is described below.

The RAINE Study began as a research study on ultrasound imaging during pregnancy.[119] Between 1989 and 1992, 2900 pregnant women who were between 16 and 20 weeks gestation were enrolled from King Edward Memorial Hospital in Perth, Western Australia, Australia.[119, 120] Women and their partners completed questionnaires during pregnancy, ultrasounds were conducted at intervals throughout mid and late pregnancy and information on delivery and birth outcomes collected from hospital records. The children born into the RAINE Study have been followed up at 1, 2, 3, 5, 8, 10, 14, 17, and 20 years of age. This study seeks to understand how pregnancy events impact health outcomes in childhood and adult life. Health outcomes studied include dental health, asthma, allergies, epigenetics, language development, mental health, growth and nutrition.[121]

The Avon Longitudinal Study of Parents and Children (ALSPAC) was established to investigate how genotypes and the environment combine to influence health and development during pregnancy and into childhood.[122] Study methodology has been published.[122] Enrollment began September 1990 and included women living in Avon, United Kingdom during their

pregnancy and who had estimated due dates between April 1st 1991 and December 31st 1992. Women were recruited via community poster and media campaigns, by study staff at ultrasound exams, by midwives at intake interviews and on maternity wards for women missed during pregnancy. More than 14,000 women were enrolled. Detailed longitudinal information currently exists on over 10,000 children and their parents. ALSPAC is a cohort study in which women completed four questionnaires during pregnancy; biological samples were collected from women, their partners and the children born into the cohort; environmental samples were collected from participants' homes (e.g. air pollutant samples); and information was extracted from medical and educational records. Families have been followed up with parental and child questionnaires. Biological samples and environmental data collection are ongoing. ALSPAC investigates a range of health outcomes including childhood weight gain, dental health, nutrition, school performance, and maternal fertility that have generated over 300 scientific publications.[123]

Generation R is a pregnancy cohort study of 9778 women based in Rotterdam, Netherlands and has been described in detail.[124] Eligible mothers were residents of Rotterdam with estimated due dates between April 2002 and January 2006. Recruitment occurred early in pregnancy (<18 weeks gestation) when women were given information about the study at their first prenatal visit and then followed-up for enrollment into the study and consent to be contacted for all five study phases: (1) pregnancy; (2) birth-4 years; (3) 4-12 years; (4) 12-16 years; and (5) 16 years onwards. The women and their partners completed questionnaires, provided blood samples and had prenatal visits throughout pregnancy, establishing a large set of genetic and environmental data. Follow-up questionnaires have been administered at 12, 18, 24, 36 and 48 months of age focusing on the areas of healthcare, childhood diseases, growth, physical, behavioural and cognitive development.[125]

1.6.4 Rationale for Assessing Representativeness of Pregnancy Cohorts

The pregnancy cohorts described are samples of the pregnant population in a geographic region of a nation during a specified time period. It is therefore important for the interpretation of findings to assess and understand who is represented in the cohort and to whom the findings may be generalized. The RAINE Study has not released manuscripts that clearly document their protocol from recruitment strategies to detailed specifics of sample and data collection. As a result it is difficult to assess if biases were present in the recruitment of participants. The brief summaries of RAINE Study recruitment provide some detail on rates of exclusion in potential participants as a result of failing to meet the eligibility criteria, with 50% of presenting women eligible for the study with 90% of them enrolled.[119] Similarities between the randomized groups of participants have been discussed in the literature, but not the representativeness of participants to the pregnant and parenting population in Perth Australia or in Australia as a nation. With such a high enrollment rate it is unlikely that strong differences exist between the cohort participants and the eligible pregnant population in Perth at the time of study recruitment but without the comparison such a conclusion cannot be reached.

Both ALSPAC and Generation R have published detailed study protocols.[122, 124] ALSPAC published pilot phase details in addition to the criteria established by the World Health Organization for the European Longitudinal Study of Pregnancy and Childhood as it related to the development and planning of the main ALSPAC study.[122] The variety of recruitment strategies minimized bias as women could learn of the study independent of access to prenatal care or healthcare services. The published details enable the independent assessment of study design by the scientific community.

Unfortunately, the study methodology did not establish the cohort representativeness of the Avon pregnant and parenting population, albeit it is estimated that 85-90% of eligible women enrolled. Similar to the RAINE Study, with such a high enrollment rate it is unlikely that cohort participants differed significantly from the eligible pregnant population in Avon but cannot be conclusively stated.

The Generation R Study primarily recruited women at their first prenatal visit. This may have introduced a bias. However the investigators also recruited missed women from hospitals at the time of birth and may have captured women not accessing routine prenatal care. The recruitment process was informed by a pilot phase, and the published details suggest a high degree of thought and logistical planning. Further, Generation R was compared to the Rotterdam population using variables established by Statistics Netherlands.[124] The investigators found that overall the Generation R participants (estimated to include 61% of eligible women) tended to have a higher SES than the general population but that this may partially be accounted for by information not being reported (questions left blank) by participants. As there is no pregnancy registry in Rotterdam, a direct comparison to the exact pregnant population is difficult if not impossible. The attempt to compare the cohort to the population of women delivering during the same time as study recruitment and with the same eligibility criteria was a commendable undertaking and adds insights to the generalizability of findings from the cohort. Future pregnancy cohorts can learn from representativeness assessments conducted by Generation R. Generalizability of new discoveries and the strategic design of reproducibility attempts will be informed by representativeness assessments. The result will be the ability to maximize the utility of findings from cohort studies of PTB.

1.7.0 Objectives

Despite a general lack of assessing representativeness of pregnancy cohorts, great progress has been made in the area of maternal-fetal-newborn health through the use of cohorts as a study design. In the field of preterm birth, cohorts may be a useful study design in the quest to understand genetic and environmental etiologies leading to and risk factors predictive of SPTB. The objectives of this thesis are (1) to introduce guidelines for studies of discovery using '-omics' technologies for preterm birth research; (2) to establish prospectively a community pregnancy cohort for the study of spontaneous preterm birth and its prediction; and (3) to characterize the All Our Babies Study Cohort and assess the representativeness of the sample for the provincial pregnant and parenting population.

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Chapter 2: Discovery-Focused Investigations of Preterm Birth

2.1.0 Overview

High throughput systems biology, referred to as "-omics" technology, is a discovery-focused set of research methodologies. Genomics or the study of genes and their functions, transcriptomics or the study of the complete set of ribonucleic acid (RNA) transcripts produced by the genome at one time, proteomics or the study of the complete set of proteins produced by a species and metabolomics or the study of small-molecule metabolite profiles generated by cellular processes, comprise current "-omics" technology. As evidence increases for a genetic contribution to PTB, so does the need to explore genomics, transcriptomics, proteomics and metabolomics in its study. These molecular studies may generate new understandings of the mechanisms of SPTB and reveal new factors predictive of SPTB. "-Omics" technologies require enormous sample sizes for initial investigations and subsequent replication of results in order to elucidate biologically important information rather than significant findings based on the mere mathematical probability of large data sets. Conducting "-omics" research is costly and together with the large sample sizes, it is nearly impossible for individual investigators or small research groups to carry out "-omics" investigations of PTB. However, in order to pool samples, data and methodologies from individual studies, guidelines are needed to ensure consistency that is adequate for sharing and merging smaller studies. This review manuscript suggests research guidelines for the conduct of "-omics" investigations into PTB with the expectation that this will facilitate the appropriate sharing of samples and data internationally through consortia, generating the power needed to study PTB using integrated "-omics" technologies. The issues addressed include: (1) integrated "-omics" approaches, (2) phenotyping, (3) sample collection, (4) data management-integrative databases, (5) international consortia and (6) translational feasibility. This manuscript is

the product of discussions initiated by the "-Omics" Working Group at the Preterm Birth International Collaborative Meeting held at the World Health Organization, Geneva, Switzerland in April 2009.*

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2.2.0 An integrated systems biology approach to the study of preterm birth using "-omic" technology - a guideline for research

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2.3.0 Background

Preterm birth, (PTB – birth before 37 weeks gestation), is the leading cause of neonatal mortality and is associated with up to 75% of long-term morbidity including developmental delay, cerebral palsy, retinopathy of prematurity and hearing and vision problems.[1, 2] Despite medical advances and better understanding of uterine activation and parturition, the rates of PTB have been increasing over the past three decades in developed countries[3] with current rates ranging from 5-7%[4] and 9.6% of all births worldwide.[5] Late PTBs, defined as delivery at 34^{+0} weeks to 36^{+6} weeks of pregnancy[6], have risen 25% since 1990,[7] now accounting for three quarters of preterm deliveries. This stark increase may be attributed to fetal indications, preterm premature rupture of membranes (PPROM) and its associated risks, and the increase in multiple pregnancies associated with assisted reproductive technology.[8]

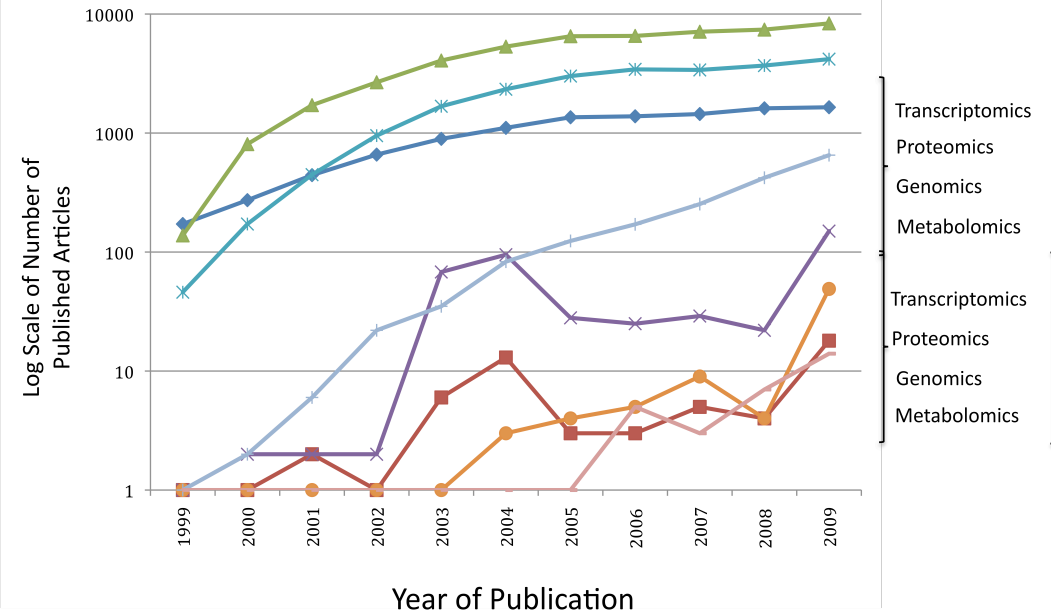
Complicating our understanding of PTB is that it is multifactorial in etiology, probably varying by gestational age. Among factors associated with increased risk of PTB are maternal smoking during pregnancy,[9, 10] advanced maternal age,[11, 12] sub-optimal weight gain during pregnancy,[13] maternal stress,[14-16] decidual thrombosis,[17] cervical insufficiency[18, 19] and the presence of infection.[20-22] In addition to the variety of environmental factors that may mediate PTB, a role of genetics is virtually certain; however the genetic effect size is not clear. In the United States, PTB occurs disproportionately in women of African ancestry[23, 24] even when controlling for social confounders.

Twin studies suggest that the heritability of PTB may be 17-36%.[25, 26] Clinically, the best predictor of PTB is a prior history,[27, 28] where recurrence risk increases by approximately 15% with each PTB.[29] Further, data suggest that the risk of PTB is inherited across generations.[30] As evidence increases for a genetic contribution to PTB, so does the need to explore genomics, transcriptomics, proteomics and metabolomics in its study.

High throughput systems biology, referred to as "-omics" technology has revolutionized research methodologies. Through these high throughput technologies and the generation of massive data sets, it is now possible to do in an afternoon what previously took several years and yet our understanding of the complex phenotypes of PTB remain incomplete, inconsistent and without clinical clarity. The "-omics" era has seen many publications (>100,000) however only a limited number (~5,000) have been in reproductive medicine (Figure 2-1). Many of the "-omics" publications relating to PTB have assessed single classes of "-omics" data, utilizing genomics, transcriptomics or proteomics in isolation. The results of many of these "-omics" publications have failed to replicate and their practical value has been limited, failing to translate into clinical practice. The limited successes of singular approaches emphasize the need for integrated approaches to investigate complex phenotypes across "-omics" categories.

Figure 2-1: "-Omics" Publications in Relation to Pregnancy

Published articles utilizing selected systems biology approaches from 1999-2009. Those related to pregnancy generally less than 1% (note log scale) of the total published articles, and have only begun to increase in 2009. Data abstracted from PubMed with search terms: transcriptomics, transcriptomics + pregnancy, proteomics, proteomics + pregnancy, genomics, genomics + pregnancy, metabolomics, and metabolomics + pregnancy.


10000
1000
100
10
1
Log Scale of Number of Published Articles
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
Year of Publication
Transcriptomics
Proteomics
Genomics
Metabolomics
Transcriptomics
Proteomics
Genomics
Metabolomics

To support both singular and integrated systems biology approaches, the "-omics" movement has seen the development of multiple consortiums utilizing high throughput platforms to investigate complex phenotypes. Central to the study of complex phenotypes are accurate phenotype definitions. In the study of PTB, this necessitates collaboration among multiple research groups working synergistically to define phenotypes and to provide adequate sample size. An example of the success made possible from the mobilization of international collaborations is the Preterm birth Genome Project (PGP) consortium, part of the Preterm Birth International Collaborative (PREBIC).[31]

Consortia, by design, employ multiple sites for the collection of phenotype data and biological samples with the goal of creating sample sizes large enough to power studies at levels impossible for any single research group, institute or funding opportunity. Moreover, "-omics" technologies require high quality biologic samples with specific, consistent and precise collection and handling. Key to effective consortia is consistency in information gathered, specimen collection, storage and management without which merging of data is problematic.

There is a need for guidelines for the conduct of integrated "–omics" studies into PTB. The genomic, transcriptomic and proteomic working group from the Preterm Birth International Collaborative (PREBIC) meeting in 2009 propose these suggested guidelines. The aim of this article is to establish guidelines for "-omics" studies of PTB such that data and samples collected can be merged, compared and replicated through consortia capable of integrated systems biology methodologies. The issues to be addressed in this guideline include: (1) integrated "-omics" approaches, (2) phenotyping, (3) sample collection, (4) data management-integrative databases, (5) international consortia and (6) translational feasibility.

2.4.0 Integrated "-omics" Approaches

Until recently the "-omics" era consisted of studies in genomics (the study of genes and their functions), transcriptomics (the study of the complete set of RNA transcripts produced by the genome at one time), and proteomics (the study of the complete set of proteins produced by a species). Recently, through the development of new technologies, metabolomics (the study of small-molecule metabolite profiles generated by cellular processes) has further expanded the "–omics" field. The considerations for using each "–omics" platform in studies of PTB and its sequelae have been reviewed elsewhere.[32] Additionally, the limitations of investigations using "–omics" in isolation have been discussed[33] and emphasize the need for integrated "–omics" approaches as the future path of research.

The circle of discovery (Figure 2-2) is central to integrated "-omics" approaches yet without strategic implementation, integrating "-omics" fields may be plagued by limitations comparable to the utilization of singular approaches. Each step in the circle yields distinctly different information (Figure 2-3) yet has a place in discovery research. It is suggested that the circle of discovery be implemented beginning with the transcriptome but that all steps be integrated when utilizing "-omics" approaches for PTB research. Transcriptomics, not in isolation but rather as an entry point to the circle of discovery, presents unique advantages for the study of PTB and perhaps other complex phenotypes alike. Unlike genomics, transcriptomics provides a snapshot of what appears to be happening at a given point in time in a biological sample. Therefore, if patterns are observed which are specific to PTB phenotypes, the functional consequences (protein products) or genetic predisposition (single nucleotide polymorphisms - SNPs) may be ascertained and feedback interactions and processes explored. Proteomics and metabolomics are key to the circle of discovery, holding their promise as secondary and tertiary analytic steps essential to integrated discovery

research studies. These steps are able to build upon the patterns revealed by transcriptomics, as transcriptomes are putative precursors to the actual physiology.

Figure 2-2: The Circle of Discovery

DNA, RNA, proteins and metabolites can be analyzed in research. RNA is the suggested ideal starting place for discovery research. DNA, proteins and metabolites are best utilized in secondary and tertiary analyses integrated with transcriptomes.

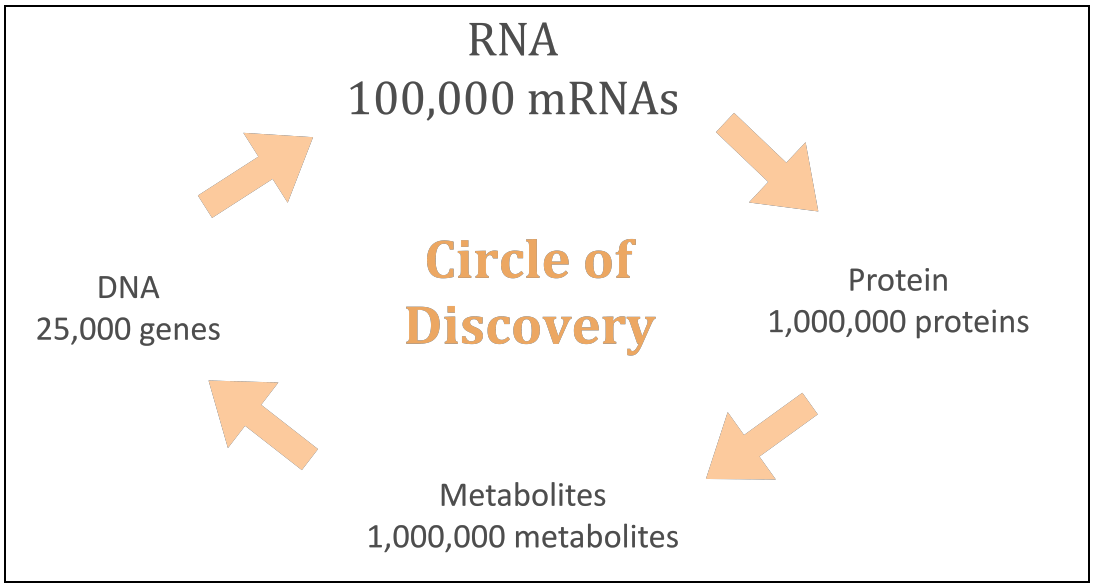
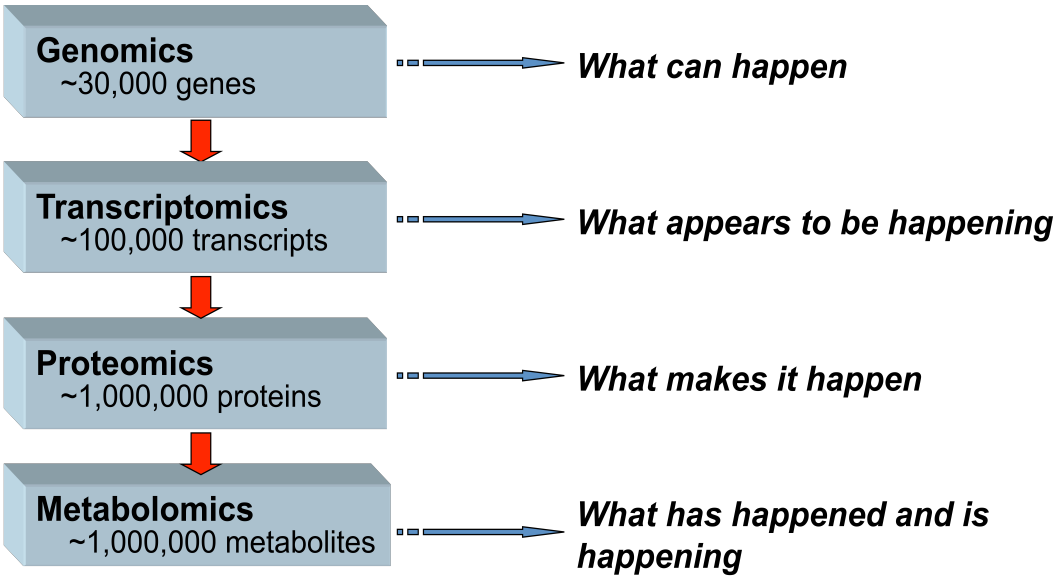

RNA
100,000 mRNAs
Protein
1,000,000 proteins
Metabolites
1,000,000 metabolites
DNA
25,000 genes
Circle of Discovery

Figure 2-3: Systems Biology Tools for Reproductive Medicine

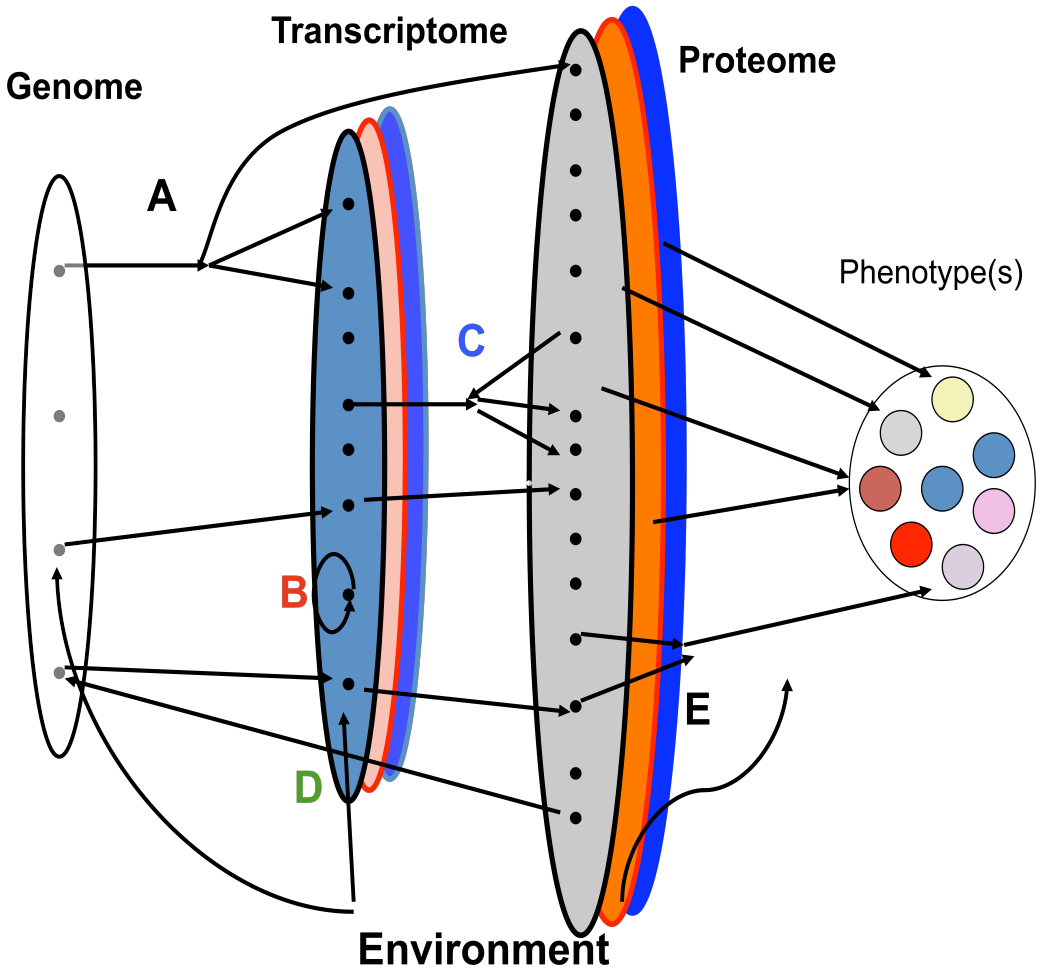
The four main systems biology categories vary in size and physiological information generated from their study. Together, a more complete understanding of PTB pathophysiology can be ascertained. Adapted from Dettmer K, Aronov PA, Hammock BD. Mass spectrometry-based metabolomics. Mass Spectrom Rev. 2007;26:51-78.[34]


Genomics
~30,000 genes
What can happen
Transcriptomics
~100,000 transcripts
What appears to be happening
Proteomics
~1,000,000 proteins
What makes it happen
Metabolomics
~1,000,000 metabolites
What has happened and is happening

However, the large sample sizes and still rapidly evolving technologies hinder the use of proteomics and metabolomics in revealing clinically relevant and significant information for discovery, as opposed to candidate driven research; hence it can be problematic to begin "-omics" investigations at these steps in the circle. In comparison, genomics is limited by the lack of linear associations between genetic variants and complex phenotypes (Figure 2-4). It holds its intrinsic value in secondary analyses and should also be included in integrated investigations. Studying all steps in the circle strategically in integrated studies may reveal the pathophysiological insights and clinical clarity PTB research seeks to discover.

Figure 2-4: A General Model of "–Omics" in Complex Disease.

Variation in the genome is represented in the transcriptome which is presented in the proteome. Each level is represented by an oval. For the genome each dot in the oval is a different gene or sequence variant. These variants are expressed as part of the transcriptome. However, unlike the genome which is essentially invariant among cells and tissues, the transcriptome can differ substantially. Different tissues are represented by overlapping ovals. Similarly, the transcriptome is translated into the proteome differently in different tissues (again represented as overlapping ovals). These ultimately influence the phenotype. This simple model is modified by multiple factors within and among levels noted on the figure as: A) Differential splicing that can be affected by the proteome; B) siRNA and/or micro RNA; C) post-translation modification of proteins; D) transcription factor binding; E) receptor ligand binding; F) environmentally induced factors such as epigenetic modifications, mutagenesis or modifier of gene expression.


Genome
Transcriptome
Proteome
Phenotype(s)
A
B
C
D
E
Environment

2.5.0 Phenotyping

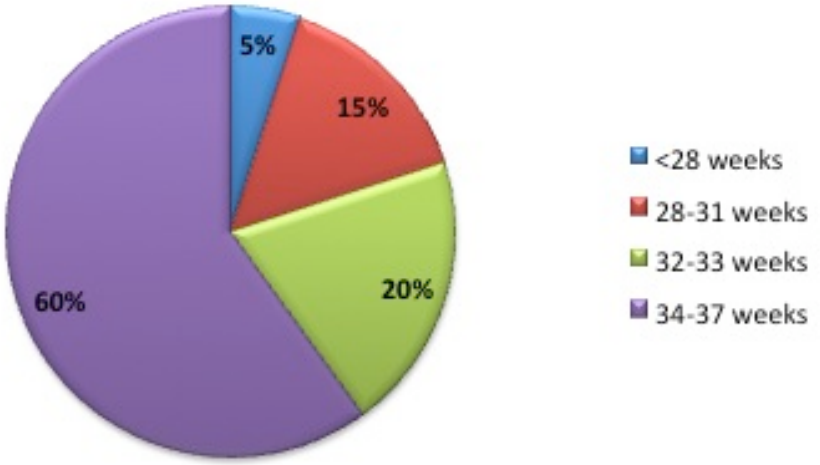
The World Health Organization defines PTB as "birth before 37 weeks (or 259 days) gestation";[35] however, as an obstetric syndrome, PTB represents a common end point to a wide variety of clinical conditions. PTB can be classified in a number of ways including: 1) gestational age at which delivery occurs; 2) clinical presentation resulting in PTB; and 3) pathophysiology (proposed to be) responsible for PTB. These classification systems are not mutually exclusive with each of them offering different benefits depending on the scientific or clinical question of interest.

2.5.1 Gestational Age PTB Phenotype

The most common classification system for PTB is based on gestational age at delivery where cases are classified into strata of extreme prematurity (<28 weeks gestation), severe prematurity (28-31 weeks gestation), moderate prematurity (32-33 weeks gestation) and near term prematurity (34-37 weeks gestation).[3] The majority of PTB occurs between 34 and 37 weeks gestation with smaller numbers occurring at lower gestational ages (Figure 2-5).[36]

Figure 2-5: Gestational Age Phenotyping of Preterm Birth

This figure represents the prevalence of preterm birth by gestational age in Australia 2006. Extreme PTB <28 weeks, Severe PTB 28-31 weeks, Moderate PTB 32-33 weeks, Late PTB 34-37 weeks. Adapted from Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet. 2008;371:75-84.[3]


5%
15%
20%
60%
<28 weeks
28-31 weeks
32-33 weeks
34-37 weeks

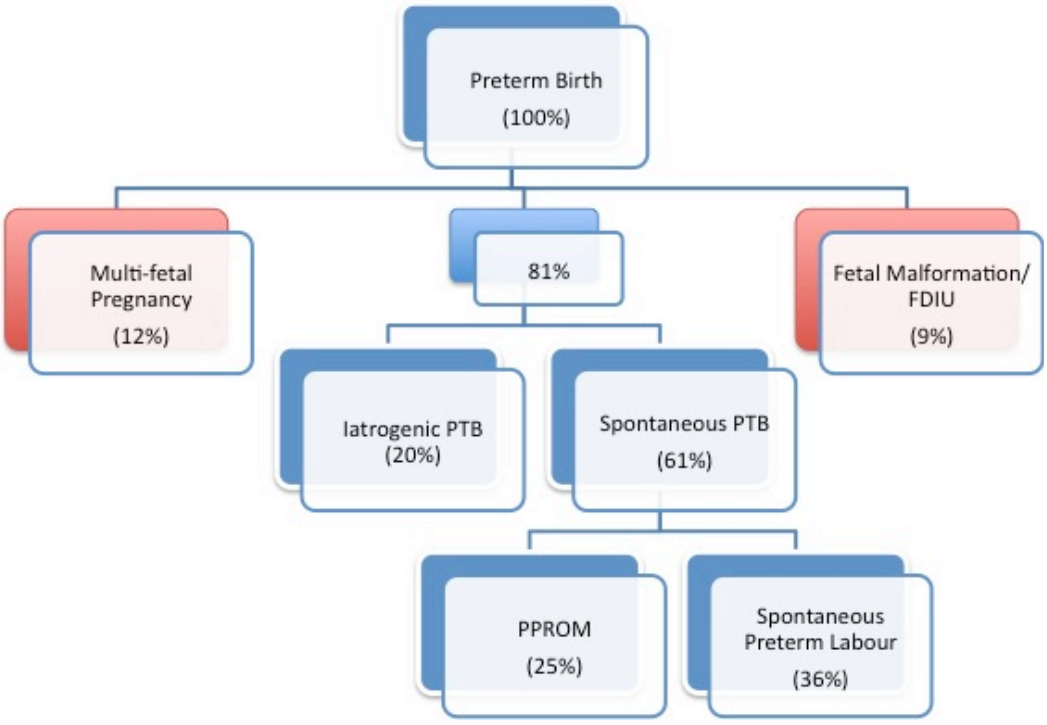
The severity of sequelae from PTB is directly related to gestational age (and therefore developmental state) at birth; hence, this classification system is ideal to evaluate health outcomes. Similarly, treatment strategies vary widely across the gestational age range, supporting the use of a gestational age classification system when evaluating interventions to improve health outcomes from preterm infants. When utilizing research techniques such as the "-omics" technologies to study the pathways and mechanisms leading to PTB, alternative classification systems are needed. Classifications based on clinical presentation or proposed pathophysiology are more likely to be of value in understanding the genetic and physiological processes that lead to PTB than using a classification system based on gestational age.

2.5.2 Clinical presentation PTB Phenotype

After excluding multi-fetal pregnancy, severe fetal malformations and fetal death in-utero, PTB can be classified into two broad clinical pathways – iatrogenic PTB and spontaneous PTB (Figure 2-6).

Figure 2-6: The Phenotypic Distribution of Preterm Birth

After excluding multifetal pregnancy and severe fetal malformations / fetal death in-utero, PTB can be classified into two broad clinical pathways – iatrogenic PTB and spontaneous PTB Adapted from Morken NH, Kallen K, Hagberg H, Jacobsson B. Preterm birth in Sweden 1973-2001: rate, subgroups, and effect of changing patterns in multiple births, maternal age and smoking. Acta Obstet Gynecol Scand. 2005;84:558-65[37] and PENNELL CE, JACOBSSON B, WILLIAMS SM, et al. Genetic epidemiologic studies of preterm birth: guidelines for research. Am J Obstet Gynecol 2007;196:107-18.[38]
FDIU: Fetal demise in utero.


Preterm Birth
(100%)
Multi-fetal Pregnancy
(12%)
81%
Fetal Malformation/ FDIU
(9%)
Iatrogenic PTB
(20%)
Spontaneous PTB
(61%)
PPROM
(25%)
Spontaneous Preterm Labour
(36%)

Iatrogenic preterm birth (IPTB) is indicated when the benefits to either the mother or fetus of delivery outweigh the benefits of continuing pregnancy. IPTB occurs in about 25% of all PTB with variations from 8.7% to 35.2% according to studied populations.[39] This clinical phenotype includes preeclampsia, diabetes, other maternal medical conditions and fetal growth restriction; a range of conditions with differing etiologies, risk factors and clinical outcomes. As a result of an increased ability to monitor fetal health during pregnancy and recognize the onset of maternal disease earlier, IPTB is becoming more common and the increasing rate of late PTB is thought to be largely attributable to iatrogenic causes.[3]

Spontaneous preterm birth (SPTB) can result from either spontaneous preterm labor (defined as regular contractions with cervical changes at less than 37 weeks gestation) or preterm pre-labor rupture of membranes (PPROM) defined as spontaneous rupture of the membranes at least 1 hour before the onset of labor and at less than 37 weeks gestation.[40] SPTB accounts for approximately 50% of all PTB (range 23.2% to 64.1%).[39, 41] It is more frequent in populations without any established risk factors in which it represents 50% to 70% of all preterm deliveries.[42, 43]

It is important to recognize that classifications of PTB based on clinical presentation and gestational age at delivery result in different study groups as the proportion of PTB that are iatrogenic and spontaneous varies between study populations,[38, 44-48] racial backgrounds[38, 44-48] and gestational age.[37, 38]

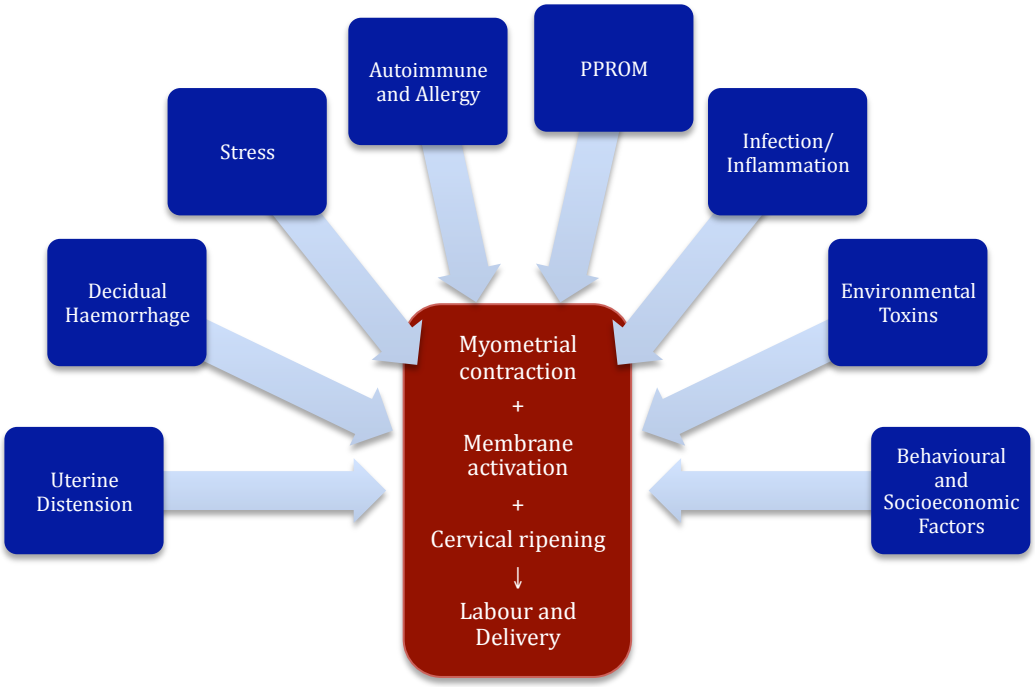
2.5.3 Pathophysiological PTB Phenotype

It has been hypothesized that PTB and term birth share the same final physiological pathway, but that this pathway is triggered early in PTB.[49] This common pathway of parturition involves the activation of various physiological processes including myometrial contraction, decidual

activation, membrane extracellular matrix degradation, weakening and rupture and cervical ripening resulting in labor and delivery.[40, 49] While the physiological triggers of this final pathway in term birth are still not well understood, the proposed pathological triggers involved with SPTB are outlined in Figure 2-7.

Figure 2-7: The Proposed Pathophysiological Pathways Leading to Preterm Birth

The pathophysiology of preterm birth is innately complex and incompletely understood. Several genetic, physiological and environmental factors are associated with preterm birth and contribute to uterine activation, labour and ultimately birth.


Autoimmune and Allergy
PPROM
Stress
Infection/ Inflammation
Decidual Haemorrhage
Environmental Toxins
Myometrial contraction
+
Membrane activation
+
Cervical ripening
↓
Labour and Delivery
Uterine Distension
Behavioural and Socioeconomic Factors

It is thought that activation of one (or more) of these triggers and their interaction with environmental factors and genetic susceptibility in the host can lead to activation of the common parturition pathway at an earlier gestational age and result in PTB.[40, 49]

With ongoing elucidation of the pathways leading to PTB, it has become clear that PTB is not a homogenous disease. This complexity is often disregarded in "-omics" research into PTB where spontaneous preterm labour and PPROM, or even IPTB and SPTB are commonly grouped together for analyses. The heterogeneity resulting from grouping known clinical presentations together decreases the sensitivity and power of many research studies. It is vital that PTB is distinguished by its different phenotypes prior to all analyses, including those utilizing "-omics" technologies. Although this may decrease numbers in any given study, it may increase biological homogeneity, thereby potentially replacing the lost statistical power.

2.6.0 Sample Collection

Sample collection for "–omics" research is not without it's own considerations, complications and detailed protocols. For PTB research, the biological sample collected is determined by the research question of interest, making cervical mucus, blood, urine, saliva, vaginal discharge, myometrium, and uterine tissues appropriate depending on the investigation. Collection of these specimens should be carefully planned a priori and the consistency of handling closely monitored to assure specimens are representative of the physiology rather than a reflection of ex vivo handling. To ensure samples are of maximum utility to individual and consortia investigations, extracted DNA, RNA, proteins or metabolites should be handled consistently. Table 2-1 provides an outline of approaches that can be utilized. The availability of biological specimens by itself is not sufficient for integrated "–omics" approaches to PTB research. Detailed documentation

of the phenotypes is, as noted above, essential. Regardless of the classification system for PTB employed (Section 2), each sample should have the minimal dataset available (Table 2-2[38]) and if possible, the optimal dataset (Table 2-3[38]) described originally for genetic epidemiology studies of PTB but which translate directly into integrated "–omics" approaches in general.

Table 2-1: Sample Handling for "-Omics" Studies on Preterm Birth

"-omics" Technology	Sample	Special Considerations
Genomics (DNA)	Blood Saliva	Stable at room temperature but best if refrigerated Salivette superior to buccal swab for high throughput genotyping
Transcriptomics (RNA)	Blood Amniotic fluid Cervico-vaginal fluid Myometrium Amnion Chorion Decidua	All samples require appropriate collection equipment (eg. PAXgene Blood RNA tube©), meticulous processing and sample storage to obtain high quality RNA for evaluation
Proteomics (protein)	Plasma Cervico-vaginal fluid Amniotic fluid Amnion Chorion Decidua	All samples require rapid preparation and preservation (+/- protease inhibitors) to optimize downstream evaluation
Metabolomics	Blood Amniotic fluid	??

Suggested biological samples and sample handling considerations for each stream of "-omics" technology for the study of preterm birth. The optimal sample to collect should be determined by the research question driving each investigation.

Table 2-2: The Minimal Dataset for "–Omics" Studies on Preterm Birth

Minimal Dataset
• Spontaneous initiation of PTB • Medically indicated PTB • Living fetus vs. intrauterine death when labor commences • Singleton of multi-fetal pregnancy • Gestational age at delivery • Smoking status during pregnancy • Use of drugs and/or alcohol during pregnancy • Maternal age • Parity • Past history of PTB • Ethnicity

The minimal dataset required for international merging of biological samples for "–omics" studies on preterm birth. Adapted from PENNELL CE, JACOBSSON B, WILLIAMS SM, et al. Genetic epidemiologic studies of preterm birth: guidelines for research. Am J Obstet Gynecol 2007;196:107-18.[38]

Table 2-3: The Optimal Dataset for "-Omics" Studies on Preterm Birth

Optimal Dataset
•Demographic Variables SES, maternal education •Clinical Variables spontaneous labour vs labour induction •Maternal Variables BMI, nutritional status, weight gain, uterine anomaly, psychologic stress, medication use, cerclage, mode of conception, evidence of infection, preexisting medical conditions, pregnancy complications •Fetal Variables birthweight, congenital anomaly, evidence of infection •Placental Histopathology infection, uteroplacental ischemia •Family History maternal gestational age at delivery, familial history of preterm birth

The optimal dataset suggested for international merging of biological samples for "–omics" studies on preterm birth. Adapted from PENNELL CE, JACOBSSON B, WILLIAMS SM, et al. Genetic epidemiologic studies of preterm birth: guidelines for research. Am J Obstet Gynecol 2007;196:107-18.[38]

2.7.0 Data Management – Integrative Databases

A major limitation to current progress in understanding the genetic predispositions to PTB is that only a limited number of genetic epidemiologic studies are available representing various ethnic/racial groups globally.[50] Therefore, there is a critical need for large and comprehensive clinical resources linked to biospecimen banks. At the level of individual investigators or small teams of researchers, clinical, environmental and biological data are continually being collected for studies with relatively small sample sizes. While it is possible to obtain high quality, mergeable data on large numbers of high-risk pregnancies, the use of this approach is limited (in part) by the absence of field standards and guidelines. Without these navigational beacons, the current use of inadequately sized cohorts or samples has resulted in inconsistent and possibly spurious initial findings for "-omics" results in PTB studies. This is likely due to the multi-genic/ multifactorial origin of PTB where any given factor/gene may contribute at most a few percent to the phenotypic variation.

If consistency is present in sample and data collection, an integrated international dataset becomes possible and transparent. The creation of integrated databases that contain both clinical (phenotype) data and biospecimen data has two additional major benefits: access and dissemination. This will allow researchers across the globe to work synergistically to attempt to answer many of the unanswered questions about PTB utilizing adequate sample sizes and the latest developments in technology.

2.8.0 International Consortia

PTB is a global problem with increasing rates in developed countries yet the vast majority of cases occur in the developing world (Table 2-4).[5]

International consortia are therefore needed to bring together resources, experts and data from low, middle and high-income countries to facilitate "-omics" research of PTB and to disseminate results to all who may benefit. An integrated "-omics" approach for PTB holds the potential for enormous scientific and, ultimately, clinical benefit. The ultimate goal of such research is the improvement of biological understanding leading to prevention, early diagnosis tools, and treatment for PTB and its associated outcomes.

Table 2-4: Regional Variation in Preterm Birth Rates.

Region	*Preterm Births (x1,000)*	*Preterm Birth Rate (%)*
World Total	**12,870**	**9.6**
By Economic Development		
Developed Regions	1,014	7.5
Less Developed Regions	7,685	8.8
Least Developed Regions	4,171	12.5
By Region		
Africa	4,047	11.9
Asia	6,907	9.1
Europe	466	6.2
Latin America	933	8.1
North America	480	10.6
Oceania (Australia/New Zealand)	20	6.4

The global burden of preterm birth varies by region with the highest rates occurring in developing regions. Adapted from BECK S, WOJDYLA D, SAY L, et al. The worldwide incidence of preterm birth: a systematic review of maternal mortality and morbidity. Bull World Health Organ;88:31-8.[5]

There are currently at least five international consortia established to investigate genome wide associations with PTB. All of these consortia are limited in their sample sizes due to the costs of genotyping and it is likely that meta-analyses of these data will be required to make substantial advances in our understanding of the genetic contributions of PTB. Most international consortia have an organized structure including an executive that contains both consortia leadership and members from each of the individual studies or data collection sites contributing data to the consortia. Detailed memorandums of agreement are required between both participating universities and researchers to facilitate smooth working of these consortia.

The Preterm Birth Genome Project (PGP) was initiated within PREBIC members in September 2007. This consortium includes investigators from four continents and has established a memorandum of agreement to collaborate on genome wide association studies (GWAS) by pooling resources (DNA) and establishing a database of phenotype definitions. The goals of the PGP consortium have been to 1) create a community of researchers to identify PTB susceptibility genes; 2) pool resources from multiple investigators to conduct GWAS across multiple geographic populations including detailed phenotypic and environmental data; 3) to establish a large pool of replication samples; and 4) to enable deep re-sequencing of genes with significant and/or interesting findings in GWAS. This consortium has been highly successful in both collecting resources (>5000 cases, >5000 controls) and also funding research into this rapidly evolving field.

A recent consortium established by the PREBIC biomarker working group is based on a systematic review of SPTB biomarker literature published between 1965-2008. Due to heterogeneities in study designs including the

issues detailed above of study designs, phenotype definitions and assay variability between different laboratories, no biomarker emerged as a risk predictor. Preterm Birth Biomarker Project (PBP) is setup to address these issues. This study will identify homogeneous studies/samples from around the globe to be tested on a panel of potential PTB biomarkers.

Similarly, further consortia will be required to utilize "-omics" technology and a systems biology approach to study PTB; however, those in existence rarely have adequate samples amenable to multiple "-omics" analyses. We hope that this paper will motivate others to increase the variety of biological samples collected to better address the major hurdles to the study of PTB.

2.9.0 Translational Feasibility – Barriers and Constraints

Despite the increasing number of publications documenting the utilization of "-omics" technologies for PTB prediction or preterm labor diagnosis, translation into clinical utility is absent despite its continued promise. This apparent gap in knowledge translation reveals both barriers and constraints to applying insights gained from "-omics" investigations of complex diseases. The inconsistencies in defining PTB phenotypes, sample handling methods and environmental variables have, not surprisingly, made reproducibility of study findings nearly impossible. These mixed messages plague the PTB literature and limit the interpretation of "-omics" generated knowledge, hindering translational feasibility. This is of course not unique to PTB. Genomic analyses of complex traits such as PTB implicitly and explicitly make assumptions regarding the nature of the risks conferred by genetic variants. The most important of these assumptions is that variants in the nucleotide level are linear (or nearly so) in terms of their effects on disease risk. Therefore, one can test for associations between single nucleotide polymorphisms (SNPs) and PTB with the expectation that the role of any given change is transparent to intermediate processes that are included in

the central dogma of molecular biology and its correlates (DNA to RNA to protein; Figure 2-2). Specifically, a gene is transcribed and the mRNA translated in such a way that changes in base pair composition in a gene encoding a critical protein are easily detectable at the phenotypic level. Although this is a very powerful model and in general approximately true (especially for Mendelian disorders), recent research has indicated that this unidirectional process is not universal and many non-linear processes are part of the progression (Figure 2-4). The failure of the linear model has many implications for the genetic/genomic analyses of PTB. Foremost is the fact that any changes in the primary DNA sequences are not necessarily directly or easily translated into phenotypic changes. Instead, a large number of intermediate processes modulate the effects of DNA variation. Therefore, changes in the DNA may be difficult to detect using a simple association methodology even though they play a key role in disease etiologies. The goal therefore is to more completely model the overall process of gene to phenotype. As a field, we need to recognize that this approach in time will lead to clinical advances to better predict disease and to the design of more effective preventative strategies and treatment. While it is universally accepted that translation is the goal, this is not a realistic deliverable nor tangible aim of any single investigation or approach, although this is often implicitly promised.

The goal of integrated international consortia using "-omics" generated data will further complicate translational feasibility if ethical considerations are not addressed at the individual study level. Ethics boards, participants and even researchers themselves are faced with new challenges when conducting integrated "-omics" investigations beyond that of the complexity of the huge data sets produced. The now clear need (as opposed to wish) to link data sets from multiple studies is pivotal to the progress of PTB research; however, obtaining informed consent from

participants at the time of enrolment to share samples with international consortia is sometimes difficult as the details of future joint ventures are often unknown or impossible to anticipate.

As ethical reviewers are tasked with protecting participants' and researchers' best interests, approval of international sharing of data and samples is often not addressed in current policies and therefore complicates the review process. It is problematic for ethics committees to assess ethical integrity as they cannot evaluate each unanticipated use of data. Participants themselves may be hesitant to partake in studies when the destiny of their samples and information is unknown. This leads to the reoccurring question "how can robust informed consent be sought from participants?"[51-53] The de-identification of biological samples and clinical information is designed to protect the confidentiality of individual participants. However, exactly what information collected by the original study team would be required by a secondary investigator to merge datasets? This may result in only the partial de-identification of participants. Therefore each study design must consider and clarify during the process of informed consent whether consent to share information internationally is optional or required for participation, a choice which may introduce participant bias into study populations. Furthermore, when samples and data are shared internationally, it becomes unclear upon whom the onus to maintain the security of the integrated databases should fall. From the secure storage of specimens to the protection of databases, security of internationally shared information is of huge ethical and legal consideration, a necessary yet daunting element of integrated international datasets.

Strategies for the sharing of information generated by integrated international datasets has been reviewed elsewhere in detail[54] and need to be considered at the various levels of target audiences – scientific

communities, clinicians, contributing investigators and the participants from whom the samples and data were originally collected. In regards to participants, when de-identification is in place to protect privacy, how can an ethical plan be designed to return results to biobank participants and/or a study community[51-53] and on whom should this responsibility lie? Consortia comprised of representatives from each of the contributing data pools may facilitate the dissemination of study results to their respective participants while still maintaining subject privacy at the consortia level. This would also enable the channeling of information to the original investigators and local communities whom supported the primary data and sample collection. The obligation to return consortia generated results to participants will depend upon the scope and duration of the relationship between the consortia, investigators and participants and therefore may not be possible in all cases. It may not even be recommended depending on the ethics committees involved as the return of findings to participants is not a universal requirement of institutional review boards. As integrated datasets come to the forefront of PTB research, the investments and interests of the participants, local investigators, local communities and international research communities cannot be forgotten. International consortia may be positioned to best preserve these interests. Sharing aggregate consortia generated results may be facilitated by password-protected web-based research updates and newsletters, to keep project level investigators and participants aware of ongoing aggregate findings. These can include contact information for participants and researchers with questions about the studies and aggregate findings. Such sites can also serve as a place for posting educational information about healthy pregnancies, child development or parenting strategies. Because of the psychological burden that attends PTB for women and parents globally, international consortia may be in a position to facilitate social networking among participants or communities by allowing voluntary anonymous participant-participant communication

through these websites. This is a way to engage participants in long-term studies and to provide benefit, when significant clinical findings (and therefore direct benefit) for individual participants are not expected.

Linking and integrating large data sets and their associated biospecimen banks is not inherently straightforward, nor is the dissemination of knowledge generated back to the original communities. These ethical challenges are not unique to PTB research but rather impact biobanking and international consortia efforts in all fields; as such models and lessons developed in relation to cancer research, for example, may be tailored to PTB research. PTB research also needs to consider the specific expectations and experience of participants. Women or couples who have suffered through one or more preterm births, pregnant women who have experienced a prior preterm or stillbirth, or have a family history of PTB, will likely experience heightened anxiety about their pregnancy that should be taken into consideration during the recruitment process.[54] Similarly, such women may have an expectation that by participating in research, they will "find a cure" to prevent preterm delivery in this pregnancy or a subsequent pregnancy.[55] Attention to these sensitive issues should shape the informed consent process and be considered by consortia utilizing data and specimens collected from these women. Addressing these ethical considerations proactively may facilitate knowledge translation rather than continue to be a barrier at the study design and implementation phases, as sharing with international consortia is addressed.

2.10.0 Conclusion

The "-omic"s era presents an exciting time for PTB research. Opportunities now exist to address complex biology utilizing technology that can achieve in a matter of hours what once took many years if possible at all. Although the "-omics" revolution has promise, there are important limitations and

constraints to these approaches that cannot go unnoticed. Critical needs at the current time include: 1) improved phenotyping for PTB; 2) large and well-characterized case and control samples with DNA; 3) one or more genome-wide association studies for PTB with broad replication across different populations; and 4) an international consortium for PTB "-omics". Only through the use of multicentre collaborations, careful, detailed phenotyping, specific and consistent sample collections, integrated systems biology approaches and the shedding of simplistic assumptions of the gene-to-phenotype cascade will "-omics" technologies be able to provide new insights into the complex pathophysiology of PTB. The possibilities are within reach and consortia may offer the answer to data management. These guidelines for research provide the direction necessary to harness the promises of "-omics" technologies for advances in the understanding, treatment and prevention of PTB.

Competing Interests
The authors declare they have no competing interests.

Author Contributions
All authors were part of the PREBIC "-Omics" Working Group and together contributed to the conception of the manuscript. SG, CP, SL, JM, SW, LP, MK and MG wrote subsections of the manuscript. SG, CP, SW and MG created or adapted all figures and tables in the manuscript. RM and GEO made substantial contributions to the editing of the manuscript. All authors have read and approved the final manuscript.

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Chapter 3: A Pregnancy Cohort Study Protocol for the Investigation of Spontaneous Preterm Birth

3.1.0 Overview

The pathophysiology of preterm birth is multifactorial, necessitating the study of genetics and environmental factors together to understand its etiologies and physiologic mechanisms. Current risk factors for preterm birth and available biomarkers have limited clinical utility to screen for preterm delivery. Prospectively established cohorts facilitate the examination of genotypic and phenotypic (environmental and medical) factors throughout gestation and may reveal factors and interactions, known or novel, that associate with preterm birth.

The All Our Babies Cohort is a community based longitudinal pregnancy cohort study. This study established a cohort of women that can be observed to investigate how womens' genetics and environment contribute to the pathophysiology of spontaneous preterm birth. Specifically, it was designed to examine the predictive potential of maternal white blood cells (leukocytes) for predicting preterm birth in non-labouring women through the examination of gene expression profiles (transcriptomics) and gene-environment interactions. The manuscript describes the design and protocols. The processes described include recruitment, sample and data collection, sample calculations as they relate to the planned transcriptomic analyses, efforts to protect against biases and a discussion of the significance of the study. The methodologies described* compliment the guidelines described in Chapter 2. Data and sample collections were conducted with the consistency required for sharing in international consortia. This pregnancy cohort illustrates the potential cohorts provide as a study design for discovery-focused investigations of preterm birth.

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3.2.0 Study Protocol: All Our Babies Cohort Study: Recruitment of a cohort to predict women at risk of preterm birth through the examination of gene expression profiles and the environment

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3.3.0 Background

Preterm birth remains poorly understood in modern society despite accounting for 75% of perinatal mortality.[1, 2] Moreover, the rate of preterm birth, that is birth before 37 completed weeks of gestation, has been on the rise for the past 3 decades in many developed countries including Canada, the USA and European nations where current rates range between 5-12%.[3] Infants born preterm are at increased risks for neonatal complications and associated long-term morbidity[4] such as developmental delays, hearing and vision impairments, respiratory distress syndrome, cerebral palsy and retinopathy of prematurity; prolonging the financial, emotional and stress-related costs of prematurity well beyond the care received within neonatal intensive care units. The risk for adverse neonatal outcomes is inversely related to gestational age at birth with the highest morbidity among those infants delivered prior to 28 completed weeks of pregnancy[5] (early preterm) despite technological advances and improved medical treatments having greatly increased the survival of these infants.[6, 7] The rate of late preterm births (34-36 weeks gestation) has also continued to climb and accounts for 75% of all preterm infants.[8] Recent studies have shown that these late preterm infants are at higher risk for adverse acute and long-term outcomes when compared to term infants.[9]

The pathophysiology of preterm birth is complex due to multi-factorial causes and its heterogeneous nature. Preterm birth is often categorized as

(1) iatrogenic when delivery is a consequence of medical intervention (30-35%); (2) spontaneous when it occurs after spontaneous labour with intact membranes (40-45%); or (3) the result of preterm premature rupture of the membranes (PPROM) (25-30%).[3] While gestational diabetes, preeclampsia and intrauterine growth restriction have all been associated with iatrogenic preterm birth, the underlying causes of spontaneous preterm birth are less clear. Reported risk factors are widespread and diverse, supporting gene-gene and gene-environment interactions in the pathophysiology of spontaneous preterm birth. Highly associated risk factors include a previous preterm birth or a relative with preterm birth[10] or a black racial background,[11] suggesting genetic causes. Other demographic factors such as low socioeconomic status[12] and maternal stress[13] have shown an association indicating that the environment contributes to the etiology of spontaneous preterm birth.

Evidence for a causal role of infection and inflammation in preterm birth has been reviewed in detail.[14] Both interleukin (IL)-1β and tumor necrosis factor (TNF)α are cytokines produced by the intrauterine tissues in response to bacterial infections[15, 16] which stimulate prostaglandin synthesis[17] and increase uterine contractility. Several groups have investigated the relationship between a single nucleotide polymorphism (SNP) located in the promoter of the TNFα gene (G-308>A) (referred to as the TNF-2 allele) and preterm birth, but inconsistent results have been reported.[18-21] A recent meta-analysis of seven studies[22] found no association between preterm parturition and the TNF-2 allele. Similarly, studies examining polymorphisms in IL-1β and IL-6 as well as other variants have also produced inconsistent results.[19, 23, 24]

These inconsistencies may be the result of gene-environment interactions impacting the effects of potential genetic predispositions to preterm birth.

For example, Macones *et al.* reported an increased risk of preterm delivery being associated with both the TNF-2 allele and the presence of bacterial vaginosis, but not with either factor alone[25] suggesting a synergistic interaction. These findings highlight the complexity of understanding preterm birth and the need for an interdisciplinary approach, a strategy embraced by the Preterm Birth International Collaborative (PREBIC) in recently published guidelines for preterm birth research.[26]

Prediction of risk for preterm birth is critical to successful clinical management. Understanding risk and protective factors is best accomplished through the development of a pregnancy cohort where asymptomatic women can be prospectively recruited and followed over time. Despite our growing awareness that infection, inflammation, stress and other risk factors contribute to the pathophysiology of preterm birth, predicting which women will deliver preterm remains an ongoing challenge. Many biomarkers for preterm birth have been suggested and explored such as fetal fibronectin,[27, 28] maternal corticotropin releasing hormone (CRH),[29] and cervical length.[30] All have had limitations such as low positive predictive values, low sensitivity or poor specificity[31] in addition to high inter-individual variability, which limit their translation into clinical settings. Bocking *et al.* have shown that white blood cell (leukocyte) counts[32] or the presence of infection[33] at 22-27 weeks gestation is an accurate predictor of preterm birth before 28 weeks gestation. These researchers have also shown CRH or combination of CRH and maternal age to be predictive for mid to late preterm deliveries respectively. Recent multiple-marker tests for risk of preterm birth show promise but continue to have limited predictive power for non-labouring women.[34]

A clinical screen for risk of preterm delivery before women have signs or symptoms of preterm labour is needed. The evidence also suggests that an

interdisciplinary approach is required to understand preterm birth pathophysiology. The purpose of this paper is to describe the methodology for community based recruitment of a pregnancy cohort, The All Our Babies Cohort Study, to examine genetic, environmental and pathophysiologic contributions to preterm birth.

3.4.0 Methods/Design

3.4.1 Objectives

The aims of the All Our Babies Cohort Study are

a) To investigate genetic components of preterm birth through comparisons of transcriptomic patterns at 18-22 weeks and 28-32 weeks of pregnancy between spontaneous preterm birth cases and term delivering controls.
b) To examine the association between environmental factors and preterm birth, including access to routine prenatal care and health services, maternal mental and psychosocial health during pregnancy, and;
c) To examine the relative impact of genes and environment on the risk of preterm birth by exploring the interactions between medical history, gene expression patterns and environmental variables.

3.4.2 Study Design

The All Our Babies Cohort Study is a community-based prospective pregnancy cohort study to determine the environmental and genetic risks for preterm birth.

3.4.3 Study Population

The study population is drawn from all women who receive prenatal viral serology testing in Calgary Alberta Canada. Participants reside within the

Calgary city limits and surrounding rural communities. Women are recruited through a partnership with the clinical laboratory service in Calgary.

3.4.4 Inclusion Criteria

Inclusion criteria for the All Our Babies Cohort Study are designed to ensure women most at risk of preterm birth are captured in the cohort.

1. Women <17 weeks 6 days gestation at time of recruitment
2. Women receiving prenatal care in Calgary
3. Women able to understand written and spoken English
4. Age 18 years or older at time of enrollment
5. One of the following pregnancy histories:
    a. Nulliparous or primiparous OR
    b. A personal or familial history of preterm birth
6. Singleton pregnancy

3.4.5 Exclusion Criteria

1. Planned to move outside of the greater Calgary area during their pregnancy
2. Known to be carrying multiples at the time of enrollment
3. Had any of the following pre-existing medical conditions
    a. Type I or Type II diabetes
    b. High blood pressure or hypertension
    c. Autoimmune/immune disorders: lupus, rheumatoid arthritis, Sjogren's syndrome
    d. Kidney disease, chronic renal disease, nephritis, nephropathy, dialysis
    e. A heart problem that was repaired by surgery
    f. Chronic infection: hepatitis, HIV

3.4.6 Recruitment Strategies

Prenatal clinical practice guidelines prompt evaluation of viral serology by public health laboratories. The sole provider of phlebotomy for this service in the Calgary region is Calgary Laboratory Service. Women with laboratory orders for prenatal viral serology tests are contacted by telephone by the clinical laboratory to ask for permission to release their contact information to the research staff. Women that consent to the release of their information are subsequently contacted by telephone to determine eligibility, inform them about the study and invite them to participate. Women who provide verbal consent to participate are enrolled in the study and are mailed a record of the consent form for their records. Written consent for blood collections and associated genetic analyses is obtained at the time of the first blood collection and prior to the blood draw.

3.4.7 Questionnaire Data Collection

Questionnaires were developed to address the objectives of the All Our Babies Cohort Study and included input from academics, stakeholders and decision makers. These questionnaires assess demographics, pregnancy history, service utilization, nutrition and exercise practices, mental health, social support, lifestyle and life history, and breastfeeding experiences through questions designed for this study as well as the following validated instruments: Edinburgh Postnatal Depression Scale,[35] Spielberger State Anxiety Scale,[36] MOS Social Support Scale,[37] Perceived Stress Scale,[38] T-ACE Screen for alcohol consumption risk,[39] Parenting Morale Index,[40] the Parental Expectations Scale for parenting self-efficacy,[41] and the MCH Feeding Scale (personal communication, M. Ramsay, Feeding Problem Questionnaire 2003).

The questionnaires were pilot tested prior to study commencement and revised for unclear wording. The questionnaires were designed using the

Cardiff Teleform software suite (Cardiff Teleform, Version 10.1, 2007) which enables the conversion to electronic data upon completion of paper-based copies.

The first questionnaire is mailed out at study enrollment for completion before 24 weeks of gestation. Women are given reminder calls beginning at 20 weeks gestation or 3 weeks after the questionnaire is mailed, whichever is later, to answer participants' questions and encourage completion and return of the questionnaire. The second questionnaire is mailed to all participants at 32 weeks gestation, to be completed between 34-36 weeks gestation. At 36 weeks gestation, women are contacted by telephone to provide a reminder about the questionnaire. A modified version of this questionnaire is used for women who delivered prior to receiving or completing the questionnaire. The third questionnaire is designed for completion at four months post-partum. Women are contacted two weeks after their expected due date to determine the birthdate of their infant(s). The questionnaire is mailed to participants at 3.5 months postpartum.

3.4.8 Obstetrical and Birth Record Data

The Alberta Perinatal Health (APH) database is an Alberta-based database that contains prenatal maternal and birth outcome information from all recorded births in the province. Study participants provide informed consent to access medical records. This enables the questionnaire data to be linked to their APH administrative data about birth outcomes, and to examine medical records to verify circumstances associated with preterm birth.

Due to the expected 18 month delay to populate and validate the APH database, study team members perform manual chart extractions for all preterm deliveries in the All Our Babies Cohort Study. This accelerates the collection of key medical record variables and is required to phenotype each

preterm birth and enable case selection for the genetic analyses.

3.4.9 Maternal Blood Specimens

Maternal blood samples are collected at 18-22 weeks gestation and at 28-32 weeks gestation either at a laboratory location or by a mobile phlebotomist. Participants are contacted at 17 and 27 weeks gestation by telephone and/or email to begin scheduling their first and second blood collections respectively. Collections can occur at any time of day and fasting is not required.

Four PAXgene™ blood RNA tubes (PreAnalytix / BD Canada, Mississauga, Ontario, Canada) are used at each blood collection. These tubes are specially designed to preserve RNA in whole blood at the time of collection. Blood is also collected into a heparin tube to isolate plasma (first collection), a serum collection tube (second collection) and into an EDTA tube for maternal DNA extraction.

3.4.10 Cord Blood Specimens

Umbilical cord blood (3-5ml) is routinely collected following hospital births to establish infant red blood cell antigens. Study participants provide informed consent to allow the researchers to access the unused portion of these blood specimens that would otherwise be discarded. These samples are stored at -80^{o}C as a source of fetal DNA.

3.4.11 Summary of Frequency and Duration of Follow-Up

All participants are asked to complete three questionnaires: at <24 weeks gestation, at 34-36 weeks gestation and at 4 months post-partum for the All Our Babies Cohort Study. Blood collections are completed between 18-22 weeks of pregnancy and between 28-32 weeks of pregnancy. Women are

contacted at approximately two weeks after their estimated due date to determine the delivery date. It is anticipated that participants of the All Our Babies Cohort Study will be followed-up every two to three years to provide the opportunity for life course research on maternal, child and family outcomes.

3.4.12 Methods of Protecting Against Sources of Bias

All women who receive prenatal viral serology phlebotomy by Calgary Laboratory Service and consent to be contacted by researchers are contacted via telephone. Should they meet the inclusion criteria, the women are invited to participate in the All Our Babies Cohort Study. This recruitment strategy minimizes selection bias due to self-referral, patient populations at specific clinical practices and enables a citywide and surrounding area sampling approach.

Questionnaire data is collected prospectively, eliminating the influence of birth outcome on responses and potential for differential recall bias. However, the repeat use of standardized scales may introduce a form of bias where women remember answering the questions before and past answers influence responses on subsequent questionnaires. We anticipate that misclassification errors in exposure data will not be an issue in the information collected and generated from this cohort due to well-spaced and timely use of questionnaires.

Blood collections are offered at both a permanent laboratory location and by mobile phlebotomists to ensure that women are able to provide the blood sample at a location and time that is comfortable and convenient. The mobile phlebotomist option enables evening and weekend blood collections such that work hours are not a constraint. The flexibility of the blood collection options minimizes participant loss due to inconvenience or personal

schedule demands.

3.4.13 Proposed Outcome Measures

a) Transcriptomic profiles from maternal blood samples collected at 18-22 weeks and 28-32 weeks of pregnancy will be compared between two groups (women who deliver at term and women who deliver preterm) for patterns predictive of preterm birth
b) Health service utilization, maternal social support, psychosocial well-being, mental health, breastfeeding and parenting support at four months postpartum will be compared between term and preterm deliveries
c) The relative contribution of the environmental risk and the genetic risk will be compared between term and preterm deliveries

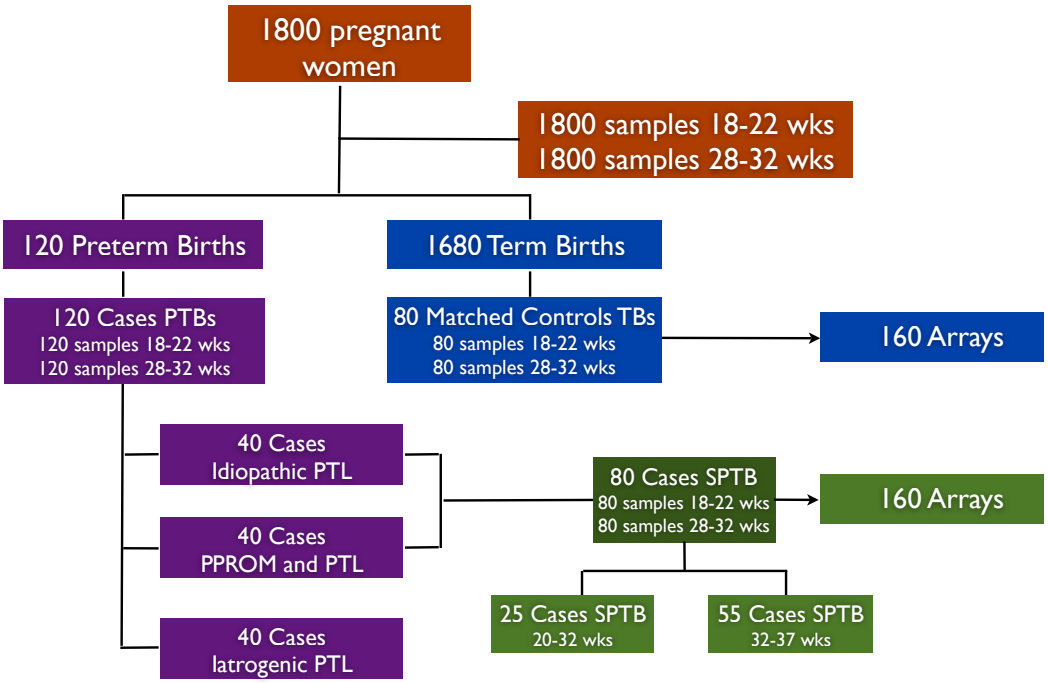
3.4.14 Sample Size

Sample size calculations for the All Our Babies Cohort Study required complex considerations as maternal blood samples are destined for microarray analyses. Several power calculations for microarray methodologies exist. Consequently sample size was determined and compared with two power calculation approaches. The first approach uses a two-class comparison model with a significance level of 0.001, a power of 0.95, one technical replicate per sample and estimated values of $\tau^2 + 2\gamma^2 = 0.25$ and $\tau^2 / \gamma^2 = 4$ (where τ^2 is the biological variance within class of log ratios and γ^2 is the technical variance of log ratios). The minimum sample size to identify a two-fold change in gene expression between classes is thus 25 patients in each study group.[42, 43] An alternate approach is to consider the sample size required to detect a two-fold change in the expression levels of the 90% and 75% least-variable genes for a given set of false positive rates and power.[42] Using previously published human tissue microarray data [43]

and setting the false positive rates at 0.001 and a power of 0.9, this approach gave a minimum of 18 and 13 patients in each group to detect a two-fold change in expression levels for the 90% and 75% least-variable genes, respectively. Microarrays will be performed on RNA samples collected from 80 cases of spontaneous preterm birth and 80 matched controls (160 biological replicates) from samples collected at two time points (18-22 weeks and 28-32 weeks): a total of 320 microarrays (Figure 3-1). This sample size will allow prediction of: 1) idiopathic PTB (n~40); 2) PPROM and PTB (n~40); 3) idiopathic PTB prior to 32 weeks gestation (n~13); 4) idiopathic PTB between 32 and 37 weeks gestation (n~27); 4) PPROM and PTB prior to 32 weeks gestation (n~13); 5) PPROM and PTB between 32 and 37 weeks gestation (n~27); 6) spontaneous PTB (n~80); 7) spontaneous PTB prior to 32 weeks (n~25); and 8) spontaneous PTB between 32 and 37 weeks gestation.

Figure 3-1: Planned Transcriptomic Analysis Design

A schematic of the planned transcriptomic analysis of preterm birth cases (excluding iatrogenic preterm births cases) and matched term controls resulting in 320 paired microarrays comparing RNA expression at two time points.


1800 pregnant women
1800 samples 18-22 wks
1800 samples 28-32 wks
120 Preterm Births
1680 Term Births
120 Cases PTBs
120 samples 18-22 wks
120 samples 28-32 wks
80 Matched Controls TBs
80 samples 18-22 wks
80 samples 28-32 wks
160 Arrays
40 Cases Idiopathic PTL
40 Cases PPROM and PTL
40 Cases Iatrogenic PTL
80 Cases SPTB
80 samples 18-22 wks
80 samples 28-32 wks
160 Arrays
25 Cases SPTB
20-32 wks
55 Cases SPTB
32-37 wks

Therefore, with a preterm birth rate of 9.1% in Alberta, an anticipated miscarriage rate of 5% and an anticipated loss to follow-up rate of 15% based on experience with other Alberta based cohorts,[44] 2200 women will be enrolled in the All Our Babies Cohort Study. This should ensure 1800 women complete the study of which approximately 180 will have preterm birth outcomes, approximately 120 will be spontaneous with or without PPROM.

The impact of access to routine prenatal care and health services on maternal variables at four months postpartum will be compared among the entire cohort. Although the sample size calculations for the All Our Babies Cohort Study were derived for the microarray analysis, the sample size of 1800 women is adequate to describe differences between groups with respect to health service utilization, maternal social support, psychosocial well-being, mental health, breastfeeding and parenting support using logistic regression.[45-47]

3.4.15 Planned Recruitment Rate

There are approximately 18,500 live births per year in Calgary.[48] Recruitment for the All Our Babies Cohort Study began September 2009 and is targeted for completion late 2010. The recruitment rate has averaged 36 participants/week through the community laboratory services and represents approximately 10% of births in Calgary during this period.

3.4.16 Study Compliance

Strategies used to maintain participant involvement include reminder phone calls for outstanding questionnaires and providing incentives including public library gift certificates and grocery gift certificates for completed questionnaires. Mailing the questionnaires and providing postage paid envelopes has minimized time and costs to participants.

The All Our Babies Cohort Study participants are provided with two options for blood collections. Women may schedule an appointment through the research team at a central community laboratory clinic within Calgary. For participants unable or unwilling to travel to the laboratory clinic, the research team will send a certified mobile phlebotomist to the women's home or other neutral location of her choice for the blood collections. In appreciation for their time and commitment to the study, women are provided with department store gift certificates at the time of their blood collection.

Umbilical cord blood samples collected at the time of birth at local hospital births are sent to a single transfusion medicine laboratory location. The research team is notified of delivery and obtains these specimens after all clinical testing is completed. Women and their health care providers therefore do not need to do anything at the time of delivery for the study. Study team members need not be present at delivery units.

3.4.17 Anticipated Rate of Loss To Follow-Up

The All Our Babies Cohort Study anticipates retaining 85% of women after completion of the first questionnaire and excluding miscarriages. Based on women's consent to participate in follow-up studies of 97% and with dedicated retention strategies, it is anticipated that no more than 10% of study participants will be lost to follow-up into the preschool years.

3.5.0 Proposed Type and Frequency of Analysis

3.5.1 Transcriptomic Analysis

RNA will be extracted from samples collected from identified cases (spontaneous preterm births with or without PPROM) and controls (term deliveries). Cases and controls will be 'loosely matched' such that each group

has a similar distribution of maternal ages, prepregnancy body mass indexes and smoking status. Extracted RNA will be aliquoted into three tubes – one for Agilent RNA quality assessment, one for the transcriptomic microarray, and one for validation of microarray findings by real time polymerase chain reaction. Samples will be run on Affymetrix microarrays in two batches of 40 cases and 40 controls. All statistical analyses will be conducted on the full dataset generated from the microarrays.

3.5.2 Statistical Analysis

Transcriptomic data, medical record data and self-report questionnaire data will first be analyzed univariately. Participant characteristics will be assessed and compared to the pregnant and parenting population in Alberta Canada and nationwide. Should transcriptomic signatures predictive of preterm delivery phenotypes be elucidated from the microarray analyses and validation, gene-environment interactions will be explored by integrating the transcriptomic data to the questionnaire and medical record datasets using appropriate statistical analytical techniques.

3.6.0 Potential Risks to the Safety of Participants Involved in the Study

3.6.1 Medical Risks

Participation in the All Our Babies Cohort Study does not alter women's medical care during pregnancy. Participants are being informed about the routine risks during phlebotomy (eg. risk of bruising or infection). There are also no direct medical benefits to the participants nor their infants for participating in the All Our Babies Cohort Study.

3.6.2 Confidentiality

A coded study ID is assigned to each participant at the time of enrollment.

This ID is used to identify all questionnaire data, biological specimens and extracted information from participants' medical records. The study ID key is contained in a password protected file on password protected computers in a secure office area. All questionnaires and biological specimens are stored in locked cabinets/freezers in secure areas. Only study team members have access to the identifying data collected in this study.

Information from participants' medical records is obtained through linkage with the APH database. At the study's end, Alberta Health and Wellness will extract the requested information from their database for our study participants. Published results will report only aggregate information and will not identify individual participants. Participants will be notified of aggregated study findings through the distribution of a study summary.

3.7.0 Significance of the Study

The rate of preterm birth is on the rise in the developed world. Alberta has the highest provincial preterm birth rate in Canada, the causes of which remain unexplained. The All Our Babies Cohort Study is strategically situated to examine the environmental, medical and genetic factors impacting preterm birth in Alberta and seeks to exploit their predictive properties for future clinical screening of all pregnant women. Should gene expression patterns predictive of spontaneous preterm birth be elucidated or gene-environment interactions protective against or synergistic for a spontaneous preterm birth outcome be discovered, the detailed phenotypic information will inform validation endeavors potentially expediting the journey from basic science discovery to clinical utility, a translation process which to date has had limited success in the applications of 'omics technologies in maternal-fetal newborn health. In addition, this study will improve our understanding of how social support and social services impact spontaneous preterm birth in Calgary.

The delivery of health services in the province of Alberta is currently undergoing reorganization making the assessment of women's needs during pregnancy very timely to inform service and program development and change of healthcare policies. The comprehensive questionnaire data obtained from participants in the All Our Babies Cohort Study correlated to birth outcomes through medical record linkage provides a concise record of local health practices and the impact of practice on maternal-fetal and newborn health. The results of the study will provide the opportunity to describe the mental health and psychosocial characteristics of mothers living in Calgary as well as estimates of the impact of these characteristics on birth and the parenting experience.

The All Our Babies Cohort Study will also record women's prenatal care experience in Calgary and identify barriers and facilitators to accessing prenatal care. In addition, the information will enable evaluation of the relationships between access to routine prenatal care on postpartum depression, parenting morale, anxiety, stress, social support, lifestyle choices such as smoking, drug use and alcohol consumption, breastfeeding initiation, duration and challenges and postpartum use of health care services. Furthermore the research team is uniquely positioned to report directly to Alberta Health Services stakeholders, ensuring knowledge gained from this research is used to inform programs and practice.

3.8.0 Ethics

This study was approved by the following ethics review boards: Health Research Ethics Board (University of Alberta, Edmonton, Canada; Ethics ID 7515), Conjoint Health Research Ethics Board (University of Calgary, Calgary; Ethics ID 22128), Health Records Services (Calgary; Ethics ID 2265), Child Health Research Office (Calgary; Ethics ID #E-22128), and the Calgary Laboratory Services Ethics and Privacy Office (Calgary).

Participants are asked to provide verbal consent to participate in the study over the phone at the time of recruitment, and completion and return of the questionnaires signifies implied consent. At the time of the first blood collection, participants are required to give signed consent to the blood collections, access to medical records and corresponding analyses. Participants are given copies of the consent forms to which they provided informed consent over the phone and in writing.

3.9.0 Study Timeline

The All Our Babies Cohort Study began in September 2009. Participants are involved in the study for approximately one year's time. It is anticipated that sample collections will be completed by spring 2011, all participants will have delivered their infant(s) by summer 2011 and data collection will be completed by fall 2011. Participants have been asked about their interest in participating in follow-up studies, potentially allowing for further research on early determinants of child and adult health outcomes.

3.10.0 Discussion

Complex preterm phenotypes are determined by genetic, physiological and environmental factors interacting at the cellular, organ, systemic, and lifestyle levels. The All Our Babies Cohort Study is gathering extensive detailed data on the gene expression profiles, medical history, lifestyle, demographics, physical and mental well-being, and neighbourhoods of the participants to predict preterm birth in asymptomatic women. The integration of these diverse data types will create an improved understanding of both maternal well-being in the perinatal period and of the cascade of the pathophysiology of preterm birth, the ultimate goal being improved maternal-fetal-newborn health outcomes.

The All Our Babies Cohort Study is the optimal study design to facilitate the integration of biological, medical and social data. The research team, community laboratory service and provincial health service provider are intimately partnered to facilitate multiple aspects of the study process. Recruitment, biological sample collection, and accessing medical record data are made possible by the academic-clinical collaborations that are the foundation of the All Our Babies Cohort Study. The implementation of this study protocol represents a successful example of how academic-clinical partnerships can increase efficiency in the research process, expediting the generation of study findings by streamlining the recruitment and collection phases of the study. Monthly meetings, routine newsletters, local and provincial committee membership and established relationships with key stakeholders including program designers and decision makers increases the likelihood of rapid translation of research findings into policy and programs.

Conflicts of Interest

Financial competing interests: In accordance with the conditions of the funding agency (Alberta Innovates – Health Solutions), the research will be evaluated by University Technologies Inc, Calgary AB and Technology Edmonton, AB for possible protection as intellectual property.

Author's Contributions

STough is responsible for the overall integrity, progress and timely completion of the study. STough, CPennell, ABocking, SLye, SDolan, ALyon, and DOlson are involved in the study design, the acquisition of funding, providing advice on methodological issues, and contributing to the interpretation of study results. STough, KHegadoren, HKehler, DMcNeil and JSiever were involved in the design, content and piloting of the questionnaires. ALyon is responsible for all lab-based queries. SMcDonald will be responsible for the management, coding, linkage and analysis of data.

SGracie was involved in the study design, is responsible for the daily management of the study, ensuring protocol compliance, high-quality and complete data collection, and will conduct analyses. SGracie wrote the manuscript. Study investigators are required to participate in meetings related to study issues and progress as needed. All authors have read and approved the final manuscript.

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Chapter 4: Assessing the Representativeness of the All Our Babies Cohort

4.1.0 Overview

The All Our Babies Cohort Study is a community based longitudinal pregnancy cohort study established to study spontaneous preterm birth by integrating genotypic and phenotypic (environmental and medical) data. Interpretation of findings generated from this cohort will be influenced by the scope of generalizability of the data. This chapter contains unpublished data that compares the All Our Babies Cohort participants to the pregnant and parenting populations in Alberta Canada. The analyses described assess the representativeness of the All Our Babies Cohort, an examination that most current pregnancy and birth cohorts lack.

4.2.0 Background

Past pregnancy and birth cohorts are largely lacking representativeness assessments for the populations from which they came. This oversight undercuts the ability to maximize utility of findings because generalizability of results is assumed. Consequently, findings may be inappropriately applied to populations for which they have not been tested or confirmed.

Interestingly, researchers consistently report descriptive characteristics of women participating in specific pregnancy or birth cohorts including measures of maternal age, parity, marital status, work status, income, ethnicity, and educational attainment.[1-4] It might be a natural approach for representativeness assessments to compare these routinely reported demographic variables to national or regional statistical sources, building upon an established reporting trend.

Identifying the best source for comparison for representativeness assessments is not a trivial undertaking. Generation R is the only known pregnancy cohort to attempt a representativeness assessment and their

efforts highlight this challenge. National and regional pregnancy registries containing demographic characteristics do not exist in the Netherlands, making comparisons of the cohort to all eligible women difficult.[2] Investigators resourcefully compared Generation R to the Netherlands population using variables established by their national statistics department, Statistics Netherlands. These variables included maternal age, parity, marital status, work status, income, ethnicity, and educational attainment. Results from the analyses showed the cohort to be more affluent than the national population.[2] This suggests that findings from Generation R should not be interpreted to apply to the Netherlands in its entirety. Caution must be used to extrapolate findings to less affluent groups nationally or internationally until findings can be properly examined for these groups.

Knowing the scope of generalizability strengthens the investigative outputs of Generation R because utility of results can be maximized appropriately. Other cohorts should strive to similarly maximize that utility of their research endeavours by adding representativeness assessments to their analyses. Admittedly, challenges are expected to arise when trying to complete these assessments. It is anticipated that cohorts based in other nations would similarly lack pregnancy registries as comparisons sources for representativeness assessments of local cohorts. Only Scandinavian countries (including Finland, Norway, Denmark and Sweden) are known to have national centralized registries.[5] These resources would be the ideal sources of comparison for representativeness assessments of cohorts from these countries. Detailed information from all pregnancies and births are available in such registries, minimizing biases that can be introduced from volunteer-based surveys or proxy measures of the pregnant population.

However, for the majority of developed and developing countries around the globe lacking this infrastructure, the approach used by Generation R might

be the best available strategy for conducting representativeness assessments. By using the national statistic department, researchers can obtain reliable data at regional and national levels. This data is typically updated every few years as part of national censuses and is internally validated by the statistics department. These validations may include weighing of the data to produced a weighted sample, a process in which numerical adjustment factors are applied to each case to adjust for probability of selection due to study (census) design and non-response.[6] Weighted samples more accurately reflect the profile of the population. Designing cohort study data collections of demographic variables such that measures coincide with those used nationally facilitates comparisons to these resources. This would enable researchers to continue their routine practice of reporting the descriptive characteristics of their cohorts but increase the richness of the data by assessing the representativeness of the cohort for the population from which it came.

For the study of PTB, the routinely reported demographic characteristics (maternal age, parity, marital status, work status, income, ethnicity, and educational attainment) are known to associate with risk of PTB (see Chapter 1). Importantly, representativeness assessments use demographic characteristics to compare the general characteristics of mothers in the sample to the general characteristics of mothers in the population. Analyses of the associations of demographic characteristics with birth outcomes such as preterm birth are separate considerations. Outcome associations should not be confused with the goal of assessing representativeness: determining scope of generalizability of results.

In summary, representativeness assessments should seek to compare routinely reported demographic characteristics (maternal age, parity, marital status, work status, income, ethnicity, and educational attainment) to (1)

national pregnancy and birth registries where available or (2) data from the national statistics department as the next best comparative data source. Canada, like the Netherlands, does not have a national pregnancy and birth registry. Some regional databases are populated, such as the Alberta Perinatal Health (APH) program database (see Chapter 3). However, these databases do not contain the detailed demographic information needed for assessments of representativeness of study cohorts. Data from Statistics Canada including associated surveys focused on maternal-fetal-newborn health are the best available data sources for comparison in representativeness assessments of Canadian pregnancy and birth cohorts. The All Our Babies Cohort will be compared for the routinely reported demographic characteristics (maternal age, parity, marital status, income, ethnicity, and educational attainment) to determine representativeness of the Cohort.

It is expected that the All Our Babies Cohort will systematically differ from the comparison populations because of the inclusion criteria used to establish the Cohort (see Chapter 3).[7] Specifically, all participants had to be able to communicate in English. Household language and maternal place of birth, in addition to ethnicity, will be analyzed to understand if and how language might have biased the ethnic diversity of the sample. Additionally, nulliparous/primiparous woman were primarily included. It is expected that multiparous women will be under represented in the Cohort. The analyses will determine the representativeness of the Cohort, avoiding unnecessary and potentially misplaced assumptions. The general applicability of any future study findings will be known.

4.3.0 Methods

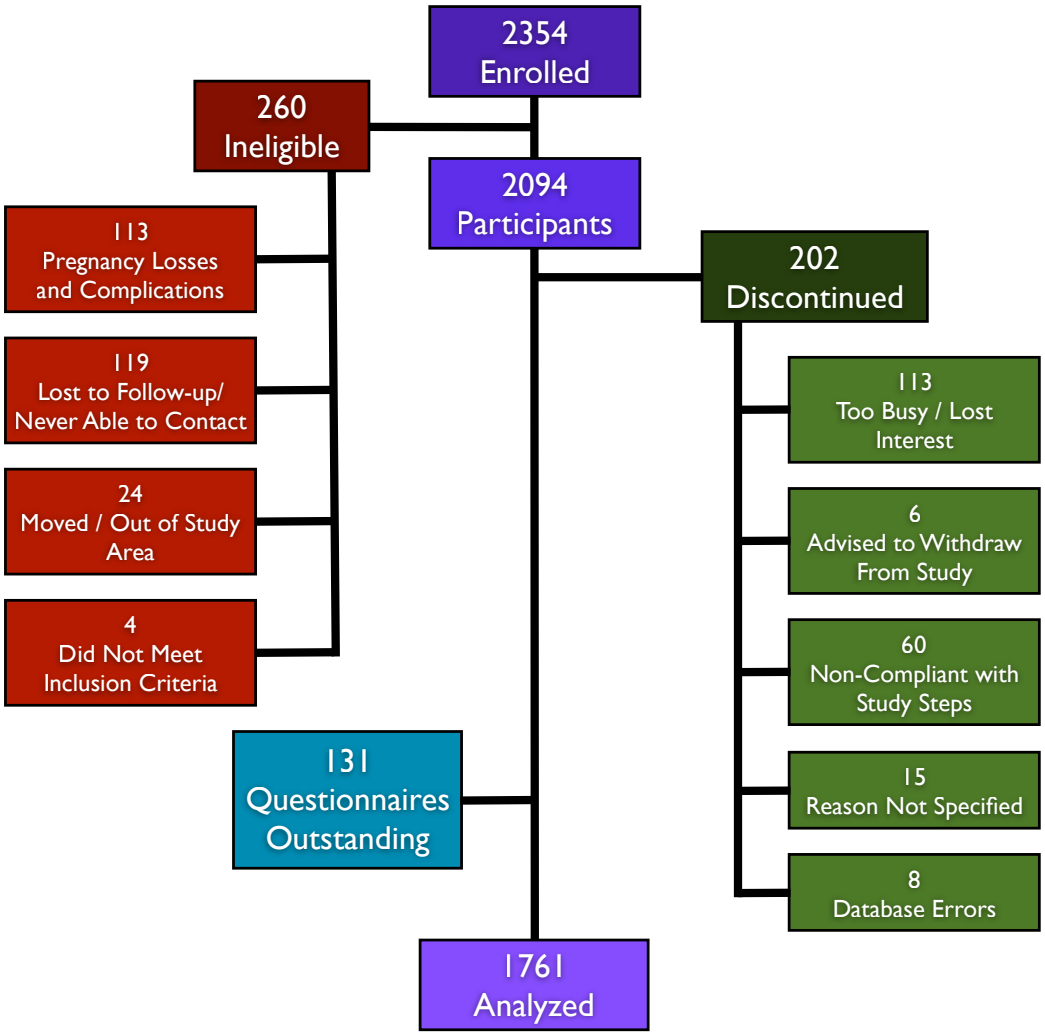
4.3.1 Data Collection

Data for this analysis were obtained from the first questionnaire from the All Our Babies Cohort Study described in Chapter 3. Recruitment was completed

November 29, 2010 with 2354 women enrolled. Not all women enrolled were eligible for the cohort study due to pregnancy loss, moving out of the study area, inability to contact after enrollment and not fulfilling the required inclusion criteria (see Figure 4-1). Retention rate is the proportion of eligible women who did not discontinue participation. The All Our Babies Cohort is a sample of 2094 pregnant women from Calgary Alberta Canada and the surrounding communities and has a retention rate of 90% (1892/2094). Response rate is the proportion of eligible participants who did not discontinue participation and for whom demographic data were available at the time of analysis. The sample for the current analysis (n=1761) included all eligible participants whom had not discontinued study participation and whom had a completed first questionnaire as of January 31 2011, a response rate of 93% (1761/1892) (see Figure 4-1). Demographic characteristics were assessed through the first questionnaire and included maternal age, marital status, maternal ethnicity and country of birth, maternal educational attainment, parity, household income, household language and home ownership (see Appendix A for questionnaire).

Figure 4-1 Selection of Participants for Representativeness Assessment

The assessment of representativeness for the All Our Babies Cohort included eligible, non-discontinued participants who had completed first questionnaires as of January 31 2011.


2354
Enrolled
260
Ineligible
2094
Participants
113
Pregnancy Losses
and Complications
119
Lost to Follow-up/
Never Able to Contact
24
Moved / Out of Study
Area
4
Did Not Meet
Inclusion Criteria
202
Discontinued
113
Too Busy / Lost
Interest
6
Advised to Withdraw
From Study
60
Non-Compliant with
Study Steps
15
Reason Not Specified
8
Database Errors
131
Questionnaires
Outstanding
1761
Analyzed

4.3.2 Maternity Experiences Survey

Published in 2009, the Maternity Experiences Survey (MES) is a national survey of Canadian mothers 15 years of age and older who delivered a singleton live birth in the three months prior to the 2006 Canadian Census.[8] Using the 2006 Canadian Census a stratified random sample of 8,244 eligible women were identified for the MES with 6,421 participating in the survey (response rate 78%).[8] The MES, conducted by a study group from the Canadian Perinatal Surveillance System, sought to address the gaps in understanding women's maternity experiences, perceptions, knowledge and practices from the preconception to early postpartum periods. The survey reports national and provincial demographic data for survey respondents. Only the data reported for the province of Alberta was used in the analysis. The MES data is a proxy for the pregnant Albertan population as it sampled women who had recently delivered infants, not pregnant women. This survey provides provincial data for comparison of maternal age and parity to the All Our Babies Cohort. The data reported in the MES are calculated as a national weighted sample of 76,508 women (or a weighted Albertan sample of approximately 7,415 women).

4.3.3 2006 Canadian Census

The most recent Canadian Census was conducted in 2006.[9] The census does not assess current pregnancy status. For this analysis all married, cohabiting and single-parent women in Alberta with an infant 0-1 year of age in their household were examined. The provincial data reported in the 2006 Census are calculated as a weighted sample of 75,950 women. These women can be used as a comparison for the population of women in Alberta parenting infants by focusing on those women who were presumably most recently pregnant prior to the census year. The 2006 Canadian Census was used for statistical analyses of the variables that could not be compared to the MES. Provincial data are available for household income, household language,

maternal ethnicity, martial status, maternal educational attainment, maternal country of birth, and home ownership (see Appendix B for variables used).

4.3.4 Statistical Analysis

Descriptive statistics were used to describe the characteristics of the All Our Babies Cohort. Categorical variables were expressed as frequencies and percentages with 95% confidence intervals (CI). Maternal age was calculated as a continuous variable to obtain maternal age at the estimated due date but recoded to be categorical for comparison to the MES data. The frequencies of missing data are reported for all data sources and missing data were omitted for statistical tests. The MES sample contains women who had recently delivered; therefore, all primiparous women in the MES would have been nulliparous during the pregnancy from which the delivery warranting their inclusion in the MES occurred. Nulliparous All Our Babies Cohort participants were compared to primiparous MES data. Primiparous and multiparous participants were compared to multiparous MES data as these MES respondents would have primiparous or multiparous during their pregnancies. Chi square tests were used to assess the representativeness of the All Our Babies Cohort participants for the provincial parenting population. A p-value of 0.05 was used to assess statistical significance. Descriptive statistics were generated using Stata SE version 10.[10] Statistical analyses were conducted using SPSS version 16.[11]

4.4.0 Results

Demographic data were analyzed from 1761 participants in the All Our Babies Cohort Study (response rate of 93%; 1761/1892) and is summarized in Table 4-1. The majority of women were married (83.9%), had household incomes greater than $60,000/year (83.0%), own their homes (77.5%), had completed post-secondary education (74.5%), were born in Canada (80.3%), primarily speak English and/or French in their homes (90.5%) and were not

visible minorities (84.4%). Nearly half of the participants were nulliparous (47.0%) and in their early thirties (42.0%, median age 31).

Table 4-1 – All Our Babies Cohort Study Participant Characteristics (n=1761)

Variable	N	%	95% CI
Maternal Age			
≤19 years	11	0.6%	0.3 – 0.1
20-24 years	111	6.3%	5.2 – 7.5
25-29 years	466	26.6%	24.5 – 28.7
30-34 years	735	42.0%	39.6 – 44.2
35-39 years	367	20.9%	19.0 – 22.8
≥ 40 years	63	3.6%	2.7 – 4.5
Missing	8		
Maternal Parity			
Nulliparous	827	47.0%	44.7 – 49.4
Primiparous/Multiparous	932	53.0%	50.6 – 55.3
Missing	2		
Marital Status			
Married	1475	83.9%	82.2 – 85.6
Never Legally Married/ Divorced/ Separated/ Widowed	283	16.1%	14.4 – 17.8
Missing	3		
Annual Household Income			
<$60,000	290	17.0%	15.2 – 18.8
≥$60,000	1417	83.0%	81.2 – 84.8
Missing	54		
Housing Ownership			
Own	1365	77.5%	75.6 – 79.5
Rent / Other	396	22.5%	20.5 – 24.4
Missing	0		
Maternal Educational Attainment			
Completed ≥ Post-Secondary	1309	74.5%	72.3 – 76.5
Completed < Post-Secondary	448	25.5%	23.5 – 27.5
Missing	4		
Maternal Birth Place			
Canadian Born	1411	80.3%	78.4 – 82.2
Foreign Born	346	19.7%	17.8 – 21.6
Missing	4		
Maternal Ethnicity			
Not a Visible Minority	1465	84.4%	82.7 – 86.1
Visible Minority	270	15.6%	13.9 – 17.3
Missing / Unknown	26		
Household Language			
Official Language(s)	1592	90.5%	89.1 – 91.8
Non-Official Language(s)	168	9.5%	8.2 – 10.9
Missing / Unknown	1		

Published results from the MES[8] (Table 4-2) were compared for maternal age and parity to the All Our Babies Cohort (Table 4-3). Statistical comparisons could not be conducted within the scope of this analysis as the raw data from the MES was unavailable. All Our Babies Cohort participants were more likely to be 30 years of age or older whereas the majority of MES respondents were less than 30 years old. The distribution of parity was comparable between study participants and the MES respondents.

Table 4-2 – Pregnant Albertan Women Characteristics (from the Maternity Experiences Survey[8])

Variable	N	%	95% CI
Maternal Age			
≤19 years	*	3.8	3.3 – 4.3
20-24 years	*	15.2	12.8 – 17.6
25-29 years	*	34.1	31.6 – 36.6
30-34 years	*	30.6	28.2 – 32.9
35-39 years	*	13.0	10.8 – 15.3
≥ 40 years	*	2.6†	1.4 – 3.7
Missing	*	0.8‡	0.1 – 1.4
Maternal Parity			
Nulliparous	*	46.0	42.4 – 49.7
Primiparous/Multiparous	*	53.8	50.2 – 57.4
Missing	*	*	*

* Not reported in published MES data tables

† MES reported a coefficient of variation of 16.6% to 33.3%

‡ MES reported a coefficient of variation > 33.3%

Table 4-3 Representativeness of the All Our Babies Cohort for the Population of Pregnant Albertan Women

Variable	AOB N (%)	MES N (%)
Maternal Age		
≤19 years	11 (0.6)	* (3.8)
20-24 years	111 (6.3)	* (15.2)
25-29 years	466 (26.6)	* (34.1)
30-34 years	735 (42.0)	* (30.6)
35-39 years	367 (20.9)	* (13.0)
≥ 40 years	63 (3.6)	* (2.6)
Maternal Parity		
Nulliparous	827 (47.0)	* (46.0)
Primiparous/Multiparous	932 (53.0)	* (53.8)

* Not reported in published MES data table

Demographic characteristics for Albertan women parenting an infant 0-1year of age from the 2006 Canadian Census are shown in Table 4-4. The All Our Babies Cohort was compared to the 2006 Canadian Census data to assess representativeness of the Cohort for the population of women parenting infants in Alberta (Table 4-5). Statistically significant differences were found with more study participants being married (χ^2=75.8, p<0.001), having household incomes of $60,000/year or more ($\chi^2$=339.2, p<0.001), owning their homes (χ^2=20.6, p<0.001), having a minimum of a post-secondary education (χ^2=76.0, p<0.001), and less study participants primarily speaking one or both of Canada's official languages in their household (χ^2=63.2, p<0.001). No statistical differences were found between study participants and the 2006 Census data for maternal ethnicity and maternal place of birth.

Table 4-4 – Albertan Women Parenting Infants Characteristics (from 2006 Canadian Census[9])

Variable	N	%	95% CI
Marital Status			
Married	56898	74.9%	73.0 – 76.8
Never Legally Married/ Divorced/ Separated/ Widowed	19052	25.1%	23.2 – 27.0
Missing	0		
Annual Household Income			
<$60,000	29374	38.7%	36.6 – 40.8
≥$60,000	46465	61.3%	59.2 – 63.4
Missing	111		
Housing Ownership			
Own	54900	72.7%	70.7 – 74.6
Rent / Other	20643	27.3%	25.4 – 29.3
Missing	407		
Maternal Educational Attainment			
Completed ≥ Post-Secondary	45503	60.1%	58.0 – 62.3
Completed < Post-Secondary	30151	39.9%	37.7 – 42.0
Missing	296		
Maternal Birth Place			
Canadian Born	59339	78.1%	76.3 – 79.9
Foreign Born	16611	21.9%	20.1 – 23.7
Missing	0		
Maternal Ethnicity			
Not a Visible Minority	62521	83.0%	81.4 – 84.6
Visible Minority	12800	17.0%	15.4 – 18.6
Missing / Unknown	629		
Household Language			
Official Language(s)	71917	94.7%	93.7 – 95.7
Non-Official Language(s)	4033	5.3%	4.3 – 6.3
Missing / Unknown	0		

Table 4-5 – Representativeness of the All Our Babies Cohort for the Albertan Population of Women Parenting Infants

Variable	Cohort N (%)	Census N (%)	Chi-Square	p-value
Marital Status				
Married	1475 (83.9)	56898 (74.9)	χ^2=75.8	p<0.001
Never Legally Married/ Divorced/ Separated/ Widowed	283 (16.1)	19052 (25.1)		
Annual Household Income				
<$60,000	290 (17.0)	29374 (38.7)	χ^2=339.2	p<0.001
≥$60,000	1417 (83.0)	46465 (61.3)		
Housing Ownership				
Own	1365 (77.5)	54900 (72.7)	χ^2=20.6	p<0.001
Rent / Other	396 (22.5)	20643 (27.3)		
Maternal Educational Attainment				
Completed ≥ Post-Secondary	1309 (74.5)	45503 (60.1)	χ^2=76.0	p<0.001
Completed < Post-Secondary	448 (25.5)	30151 (39.9)		
Maternal Birth Place				
Canadian Born	1411 (80.3)	59339 (78.1)	χ^2=5.0	p=0.025
Foreign Born	346 (19.7)	16611 (21.9)		
Maternal Ethnicity				
Not a Visible Minority	1465 (84.4)	62521 (83.0)	χ^2=2.5	p=0.111
Visible Minority	270 (15.6)	12800 (17.0)		
Household Language				
Official Language(s)	1592 (90.5)	71917 (94.7)	χ^2=63.2	p<0.001
Non-Official Language(s)	168 (9.5)	4033 (5.3)		

4.5.0 Discussion

The All Our Babies Cohort was compared to the most recent Canadian Census (2006) and the MES. In the absence of national pregnancy and birth registries, data from Statistics Canada are the best available comparative data sources for assessments of representativeness of the Cohort. The questionnaires used in the All Our Babies Cohort Study were designed to measure demographic variables similarly to Statistics Canada (see Appendix A and B), facilitating comparative analyses between the datasets.

Chi-square tests were utilized for statistical analyses in the representativeness assessment. As a non-parametric test, Chi-square analyses do not require assumptions about the distribution of the data. This statistic can be used for the analysis of large and small sample sizes for random categorical variables, requiring that the frequency of any observation is at least five.[6] It is possible that the statistical comparisons may have generated significant findings due to the fact that the All Our Babies Cohort is a small sample ($n<2000$) compared to that of the 2006 Census data ($n>70,000$). However, any demographic variables that displayed statistically significant disparities between the Cohort sample and the Census sample did not have overlap between their 95% CIs. This suggests that these statistical significances did have mathematical validity and were not spurious findings. The clinical significance of these statistical results is a separate issue that can be examined by considering possible explanations for the results found in the representativeness assessment.

Parity for participants in the All Our Babies Cohort and participants in the MES was very similar. Inclusion criteria for the All Our Babies Cohort Study included being nulliparous, or primiparous.[7] Multiparous women could participate in the study only if they had a personal or familial history of preterm birth.[7] The inclusion criteria for the All Our Babies Cohort Study did

not result in a disproportionate amount of nulliparous women participating in the study. The average Albertan family has 1.1 children.[12] That fact that most families have none, one or two children means that parity would not exclude most expectant mothers from the Cohort study. The Cohort is representative of the provincial population for the distribution of parity.

While parity was comparable, age of participants was not comparable to the age of respondents in the MES. This might in part be explained by the exclusion criteria for the All Our Babies Cohort Study. Women less than 18 years of age at enrollment were not eligible to participate[7] whereas the MES included women 15 years of age and older.[8] However, it was not only the youngest age group that differed. The majority (53%) of MES respondents tended to be less than 30 years of age whereas the majority (66.5%) of All Our Babies Cohort participants were 30 or older.

The differences in overall age distribution might partly be explained by a consideration of educational attainment. Significantly more Cohort participants had completed at least post-secondary studies when compared to the 2006 Census data. Completing advanced levels of education takes time and has been inconsistently linked to motivations for delayed childbearing (having children over 35 years of age).[13, 14] It may however, influence the decision to parent at more moderate ages (25-34 years of age). A study comparing France, Norway, and England and Wales found that educational attainment was the strongest differentiator of age at first childbearing across all three countries where one-third to one-half of women with secondary and higher levels of education were childless upon entering their thirties.[15] While education might explain the older age of Cohort participants, educational attainment itself must also be considered given the significant differences found. According to the Canadian Census, in 2006 33% of Canadian women between 25 and 34 years of age had a university degree and 56% of the

Albertan population held a trades certificate, college diploma or university degree.[16] Of note was that from 2001 to 2006 Alberta experienced a net inflow of adults from elsewhere in Canada who had post-secondary education.[16] If this trend continued beyond 2006, then it might be expected that the educational profile of the province is higher in 2010 than it was in 2006. Unfortunately more recent comparative data are not available for the educational attainment of women in Alberta. If education levels are assessed on the next census, an examination of provincial trends between 2006 and 2011 might inform the representativeness of the Cohort participants for parenting Albertan women during the time of study enrollment rather than women in 2005-2006.

Cohort participants were also significantly more likely to be married compared to the population of women parenting young infants in Alberta. The 2006 Census found, for the first time in Canadian history, that unmarried people outnumbered legally married people. Despite common-law arrangements increasing, it did not offset the decline in the number of couples.[17] However, 54.5% of women were part of a couple by their late twenties and nearly three-quarters by their late thirties.[17] Differences in provincial trends are important considerations for assessments of representativeness on a province to province basis. Alberta had the highest provincial growth rate for married-couples (9.6%), common-law couples (23.4%) and for households of couples with children (6.4%), resulting in nearly one-third of Alberta households consisting of a couple with at least one child.[17] Despite the trends in Alberta, Cohort participants were still significantly more likely to be married. This might have been influenced by the recruitment method used to establish the cohort. All women who received a prenatal rubella screen were contacted by delegates of the local laboratory services to obtain consent to release contact information to the researchers who could then explain the details of the study and invite eligible

women to participate.[7] Therefore women had to access baseline medical care to be identified as potentially eligible for the study. Studies have shown that women who do not access or who under-attend prenatal care tend to be unmarried, have low education levels and are younger in age than those who attend antenatal care.[18-20] It is possible that these same characteristics are associated with receiving baseline medical care when women suspect that they may be pregnant. The highly educated, older age and married status of the Cohort participants might be reflective of the use of baseline medical care to identify potential participants and might partially explain the differences observed when compared to the women of Alberta parenting an infant.

Compared to the sample from the 2006 Census, significantly more Cohort participants owned their homes and had household incomes of $60,000/year or greater. The decision to use a cutoff of $60,00/year for the analyses conducted was informed by the consideration of household incomes of couples with and without children. In 2005, the median earnings for couples without children at home was $59,834 compared to $82,943 for couples with children rising 21.6% and 14.6% since 1980 respectively.[21] As household earnings increase, the ability to own a home becomes more feasible. Alberta had the highest proportion of households with a mortgage in 2006 which was largely attributed to renters transitioning to homeownership.[22] Similar to the trends for increasing education, if couples' earnings and thus household incomes continued to rise together with the inflow of highly educated persons to Alberta, the distribution of household incomes for couples with children might be higher during the time of study enrollment than that seen in 2006.

Admittedly, it is unlikely that household incomes would have changed enough to eliminate the significant differences found in this analysis. Canada experienced a recession from 2007-2009.[23] Reports have yet to surface on

how the recession impacted family planning during this time. However, a study of birth rates in Japan during the period of economic recession in 1973 revealed a decline in birth rates during the time of economic hardship.[24] While the causes of the decline in birthrate are inconclusive, low income was negatively associated with age at first marriage and therefore might have contribute to delaying childbearing during the economic hardship. Until the impacts of the recent recession are known, the impact on family planning can only be speculated. The high-SES women participating in the Cohort might be reflective of the decision to delay childbearing in those most compromised during the recession, leaving the most financially stable individuals choosing to expand their families in difficult economic times. More simply put, couples with low incomes might decide that they cannot (or do not want to) afford a child. Additionally, income might not be a truly independent variable but rather is related to age and education. It might be expected that older, more highly educated women would earn a greater income as a result of their additional training and this additional income would make them more able to support the costs of raising a family. Considerations of economic trends coupled with identifying potential participants from those who access medical care, the high SES profile of the All Our Babies Cohort might be expected.

The high-SES profile of Cohort participants might also be explained by a consideration of urban setting from which the sample was established. Inclusion criteria for the All Our Babies Cohort Study included the requirement that women to be receiving prenatal care in Calgary Alberta.[7] This inclusion criteria might be expected to result in a sample of women who predominantly reside within the Calgary Metropolitan Area. Access to rural medical centres, access to transportation and time constraints might deter women in rural Southern Alberta from committing to prenatal care services in Calgary. The high-SES profile of the Cohort might therefore reflect the

higher ages, income, and education levels that might be found in this urban centre compared to rural communities. While a small proportion of Cohort participants were from rural Alberta (data not shown) this proportion might have been too small to offset the demographic characteristics of the women coming from the Calgary Metropolitan Area.

The inclusion criteria of being able to understand written and spoken English[7] was expected to bias the Cohort such that English speaking (including English as a second language) women were disproportionately represented. This anticipated finding was not confirmed in the analyses; Cohort participants were significantly less likely to primarily speak one or both of Canada's official languages (English and French) in their household (with most participants speaking English, data not shown) than the sample from the 2006 Census. Interestingly, while language was significantly different, maternal place of birth and maternal ethnicity were not statistically different between Cohort participants and the 2006 Census sample of Albertan women parenting an infant. This suggests that while language was statistically different, it is not of consequence (clinical significance) for the representativeness of the Cohort. This might be of consideration for future representativeness assessments of other cohorts also using language as an inclusion criteria. Maternal ethnicity and birthplace, not household language, are more informative of cohort representativeness. For interpretation of results, maternal ethnicity and birthplace might also provide additional information for considerations of frequencies of genetic factors represented in the sample. Results from the representativeness assessment of the All Our Babies Cohort find language to be a non-informative factor for determining representativeness.

4.5.1 Limitations

The MES is the best comparative sample to assess representativeness of the

All Our Babies Cohort because it specifically included mothers of singleton live births in the three months prior to the 2006 Census. Unfortunately, raw data were not available and as such statistical analyses could not be conducted for comparisons within the scope of this analysis. While the MES has data on marital status, maternal educational attainment and household income, these variables were not reported such that comparisons to the Cohort data could be done. Access to the MES raw data would have enabled recoding of variables and statistical comparisons made for several demographic characteristics of interest but were not possible for this analysis.

The 2006 Canadian Census was used for statistical analyses of the variables that could not be compared to the MES. Only data from Alberta women with an infant 0-1 year of age in their household were included. It is therefore possible that some women in the sample are not the biological mother of the infant but rather a female relative, a foster parent, an adoptive parent or a live-in caregiver. In 2006 Alberta had the lowest number of young adults (aged 20-29) living with their parents (31.7%).[25] However, the presence of an infant(s) in these households might undermine the statistical analyses conducted as characteristics of multiple women in the household would be present in the Census sample. This Census sample is a proxy for the population of Albertan women parenting an infant but cannot be assumed to represent mothers of live births in the year preceding the census. This unclear heterogeneity of the maternal relationships present in the Census sample is a limitation to the strength of the comparative results generated.

Both the MES and the 2006 Census were samples in which the data had been weighted. As a result these datasets are products of mathematical manipulations. The weighted sample sizes (MES: n=76,508; Census: n=75,950) are therefore larger than the number of true respondents in the

original sample. Sample weighting was done by Statistics Canada to adjust for biases in study design and non-response to make these more representative samples and therefore reflective of the provincial populations they are describing. However, even after sample weighting, these datasets may not be completely representative for the provincial pregnant and parenting populations. This is an importance limitation of using the best available, rather than the ideal, comparative datasets. National or provincial pregnancy and birth registries would have enabled comparisons to the Alberta population directly. Instead, the All Our Babies Cohort, a small sample, was compared to larger weighted samples. Knowing the results of the representativeness assessment and considering the inclusion criteria (study design) used to establish the cohort[7], statistical consultation might be sought in the future to determine if the All Our Babies Cohort can also be weighted and whether this would be an appropriate undertaking.

The All Our Babies Cohort Study recruited women who lived in Calgary or the surrounding communities.[7] MES data was not available for the Calgary region alone and therefore all comparisons were done at the provincial level. It might be unrealistic to assume that women coming from the southern metropolitan area and its surrounding communities can adequately represent the province of Alberta in its entirety. However, if Cohort participants are only representative of the Calgary region, the generalizability of findings produced from the Cohort is quite limited. While representativeness assessments at the provincial level may have been overly optimistic, it is the narrowest comparative level that still provides limited generalizable potential.

The 202 women who discontinued participation were not included in the representativeness assessment. The majority of these women did not complete the first questionnaire and therefore demographic information was

not available for these women. Because these women have discontinued participation and are not contributing to the biological samples or environmental datasets collected from this Cohort, any results or findings from the Cohort will not include these women. Therefore, their characteristics would not inform the scope of generalizability of study findings. Their characteristics are of importance for consideration of any biases that may be present in those women choosing to complete the study compared to those who did not. Without the demographic information from their first questionnaires other means would need to be used to examine the demographic characteristics of these discontinued women. These women had enrolled in the All Our Babies Cohort Study and as a result, the postal codes for their last known addresses are known. It may be possible to conduct postal code level comparisons to estimate demographic characteristics of these women. In addition, if these women carried their pregnancies to delivery and delivered in Alberta, it may be possible to examine their birth outcomes, their age and their parity through the APH database. These investigations into the characteristics of women who discontinued participation might reveal whether demographic variables differed between those women who discontinued study participation and those women who completed the All Our Babies Cohort Study.

4.5.2 Conclusion

The analyses conducted assessed the representativeness of the Cohort for the pregnant and parenting population in Alberta Canada in 2006. Overall, the Cohort sample has a higher SES profile than the populations in the comparative datasets, despite being representative for the provincial distributions of maternal ethnicity and parity. The representativeness of the Cohort cannot be neatly simplified to state that the Cohort represents the provincial population. Rather, the representativeness assessment results suggest that it may be inappropriate to generalize findings from the Cohort

to the province of Alberta in its entirety. The Cohort is a sample of women with a high-SES profile. Awareness of these demographic characteristics might inform study findings and should be considered when outcome data are interpreted. Findings from the Cohort should be generalized to high-SES populations. Generalizations to populations with low or middle SES profiles will require extreme caution as these populations were not well represented in the Cohort.

4.6.0 References

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Chapter 5: Discussion

5.1.0 Summary of Changes Needed to Progress Spontaneous Preterm Birth Research

Spontaneous preterm birth is a pathophysiology that is believed to be multifactorial in etiology. Research in this area has become stagnant as environmental and genetic factors continue to be analyzed in isolation. Integration across disciplines might inform the etiologies of SPTB, enabling prediction, intervention and ultimately prevention of this birth outcome. The prospective recruitment of a cohort and collection of biological and environmental data, analyzed retrospectively with discovery-focused approaches, is a stepping-stone that can begin to move SPTB research forward. To determine the applicable scope of any findings from a cohort, the sample needs to be characterized to determine what population is represented and if any systematic biases are present in the sample. This assessment will be essential to drawing insights from all findings produced from a cohort.

5.2.0 Study Significance

Large sample sizes are required to achieve the necessary power for interdisciplinary research approaches to the study of SPTB. This, in addition to the complexity of discovery-focused research, translates into costly research endeavours not supported by individual operating grants. Pooling data and biological samples gathered at the individual study level might generate the power necessary to study SPTB etiologies with a multifactorial approach. Merging data sets is appropriate if standardized environmental information is collected and standardized methods to collect biologicals are used. Chapter 2 suggests guidelines by which individual research groups can investigate their targeted research questions while incorporating in their study design and implementation the ability to contribute to international consortia in the future. The importance of Chapter 2 is to emphasize the

need to design current studies with the ability to merge data with future studies, eliminating the need to recollect, reinvest in and redo work that has already been done. These guidelines highlight the discovery-focused research approaches that are needed to progress SPTB research and calls upon researchers in the field to come together by following guidelines that enable merging of datasets.

A prospective pregnancy cohort is a study design in which the collection of environmental data, biological samples and medical outcome data is possible (ie the collection of genotypic and phenotypic data). Chapter 3 provides a detailed study design for a community-based pregnancy cohort, the All Our Babies Cohort Study, established to study SPTB etiological associations. This study collected environmental data that covers the optimal dataset designed by PREBIC[1] and emphasized in the guidelines in Chapter 2. The biological samples were also collected using methodology presented in the discovery-focused research guidelines (Chapter 2). Moreover, the consent form used in the All Our Babies Cohort Study obtained participants consent to share samples and related health records for research on PTB by other researchers. This is a noteworthy example of implementation of the main message from the guidelines: conduct local research with the foresight for merging through international consortia. This Cohort might be exemplary of the study design changes necessary at the individual study level to move SPTB research from stagnant circular repetition to discovery-focused progression through its incorporation of the following steps: collection of the optimal dataset designed,[1] collection of biological samples in accordance with newly proposed guidelines, obtaining consent for future sharing of data and studying SPTB by integrating genotypical and phenotypical information.

Success of discovery-focused research is founded upon appropriately interpreting results and the scope to which they can be generalized. Knowing

both the descriptive characteristics of study participants and whom they represent provides an extra level of information not articulated in the PREBIC guidelines. Chapter 1 highlighted that assessments of cohort representativeness are lacking in several already established cohorts designed to study maternal-fetal-newborn health and its impacts on health throughout the lifetime. Assessing representativeness of the All Our Babies Cohort for the pregnant and parenting populations in Alberta Canada informs interpretation of any results generated from this Cohort. Chapter 4 revealed that the All Our Babies Cohort participants have a higher SES-profile than the provincial datasets used for comparisons. Generation R, the only other known pregnancy cohort to attempt representative assessments, also found their cohort to be of a higher SES than the population from which it came.[2] The inability to generalize results to the entire population from which the sample was established does limit the impact of study findings. However, strength is added because generalizations are informed rather than assumed.

The observation that research attracts participants with high SES is not new. The Paris prospective birth cohort study began in 2003 for the study of asthma and allergies in childhood in France.[3] Researchers found that participation over the course of the study was higher for parents with high SES, for mothers over thirty years of age and for parents of French or European backgrounds.[3] The same trends are found in studies not using cohorts in their design. A recent report from the Chemical, Health and Pregnancy Study, indicated that despite using a variety of recruitment strategies, their participant sample was less ethnically diverse, had higher educational attainment and was more affluent than the background population in Vancouver.[4] Even a study specifically looking to examine women at high risk for unintended pregnancies found that they obtained a more affluent sample than desired until recruitment strategies were modified to target at-risk women.[5] The findings from representative

assessments in Chapter 4 are not surprising but rather align with samples of other pregnancy studies.

The tendency of research to attract high SES samples might further support the merging of datasets proposed in Chapter 2. As PTB research moves into an era where datasets are merged by international consortia, representativeness assessments of each contributing study will be essential to the interpretation of results and the successful replication of discovery-focused research findings. If available datasets are similarly biased for high SES samples then the generalizability of findings is limited to populations of individuals with high SES. Despite the international focus of consortia and the goal for globalized discoveries, findings could not be appropriately generalized to disadvantaged populations such as those in low and middle-income countries. This is of paramount importance for consideration. PTB is a pathophysiology that disproportionately affects disadvantaged populations. The under-representation of those most affected by PTB in studies designed to investigate its etiologies might miss important information. Special consideration must be used to avoid inappropriate application of discoveries from high SES samples to low or middle SES populations. Representativeness assessments inform these considerations. If representativeness assessments reveal differences in biases between the datasets and similar or consistent results are found, the findings become stronger and their global utility greater. It is recognized that future investigations of SPTB focused on both prediction and discovery-research might be most informative if less affluent populations can be attracted to participate in research studies, increasing the representativeness of study samples.

Motivations for study participation, like attendance to prenatal classes, might be differentially influenced by sociodemographic characteristics and might

highlight an innate challenge for research to produce representative samples. Studies have found that willingness to participate in studies is dependent on what is being asked of potential participants and, further, that demographic characteristics are associated with participation in different study designs. For example, a study of attitudes of pregnant women about perinatal epidemiological research revealed 83% of pregnant women were willing to participate in phone interviews and 60% for in-person interviews but that willingness, especially by highly educated women, was much lower if infant examinations (57%) and reviews of infant medical records (54%) were involved.[6] The All Our Babies Cohort Study used questionnaire mailouts for environmental data collection and the choice of home visits or appointments at lab clinics for the genetic sample collection (blood sample collection), resulting in a combination of complete anonymity and brief in-person contact with members of the research team. No examinations are conducted on the infants born to the Cohort participants and review of infant medical records occurs only if birth outcome information is missing from the mothers' obstetrical records. The low in-person requirements and lack of infant-focused study stages might have made the study attractive to women across demographic characteristics and was reinforced by offering incentives that could be utilized by women with varying socioeconomic standings. It has also been shown that sociodemographic factors were unrelated to willingness to consent to participate in genetic research among participants in a longitudinal community based survey.[7] In conjunction with these findings, it is speculated that the presence of the biological sample collection did not systematically deter women based on sociodemographic characteristics. However, motivations for study participation were not collected from the All Our Babies Cohort Study participants and therefore, the potential effects of study requirements on creating a biased sociodemographic profile in the participants cannot be ascertained. As discussed in Chapter 4, the major contributor to the high SES sample

comprising the All Our Babies Cohort Study might have been the use of the lab test and thus accessing baseline medical care to identify potential participants or the inclusion criteria of accessing prenatal care in Calgary. This might be the resulting trade-off for the ability to efficiently implement the complex prospective sample and data collections this Cohort required that were made possible by engaging clinical and community partners in all aspects of the study (see Chapter 3). Had other methods of indentifying potentially eligible women been used, retrospective data collection may have been biased by recall post-delivery and the collection of biological samples during pregnancy missed entirely. The high SES sample might be an unavoidable trade-off for the ability to efficiently conduct prospective cohort studies.

The representativeness assessment conducted compared the All Our Babies Cohort to the pregnant and parenting populations in the province of Alberta. However the original goal of the All Our Babies Cohort was not to establish a provincial pregnancy cohort. The purpose of the All Our Babies Cohort Study was to establish a pregnancy cohort that could be used to study spontaneous preterm birth. The inclusion criteria were designed to try and hone in on women who may be at slightly higher risk for a SPTB (as opposed to an IPTB) based on current knowledge of environmental risk factors for PTB (reviewed in Chapter 1). Interestingly, this systematic inclusion criteria was expected to influence the Cohort sample such that it would be non-representative of the provincial population for parity and maternal ethnicity. Surprisingly, the representativeness assessment showed the Cohort to be representative for the provincial population for both of these variables. This emphasizes the importance of examining demographic variables for representativeness assessments separate from analyses of demographic variables as they associate with outcomes such as PTB. Examinations of birth outcomes will be of intrinsic value for this cohort. The inclusion criteria sought to hone in on

women at elevated risk for spontaneous preterm birth and thereby produce a Cohort in which the preterm birth rate is higher than that of the province. However, the representativeness assessment suggests that, at least for some demographic variables, the Cohort did represent the provincial population. It will be of interest to see if the Cohort met the study goal of obtaining a sample of women with elevated rates of SPTB.

5.3.0 Study Limitations

The guidelines established in Chapter 2 are suggestions rather than rules and should be viewed as a living document as research progresses. The development of new technologies and the knowledge obtained from new discoveries might require these guidelines to grow and change as research advances. Should cohorts become the study design of choice for discovery-focused research into SPTB, the guidelines might need to be expanded to include representativeness assessments for cohorts in the minimum and/or optimal dataset described. Further, these guidelines do not hold all the answers to challenges that might arise when merging is attempted in the future. Ethical challenges involved in consenting participants to international sharing where details are unknown and the difficulties of protecting the interests of local participants and investigators are raised but few concrete solutions are provided. Ethical and legal experts are needed to inform these guidelines and provide practical tangible solutions to these anticipated challenges. These guidelines remain limited in their scope and can be viewed as incomplete until the ethical and legal components are added. Establishing ethical and legal precedents takes time, and SPTB research must move forward in parallel with these developments. Research progression can be served through the guidelines in their current version.

Obtaining consent upfront from participants in the All Our Babies Cohort Study does not circumvent the ethical and legal challenges anticipated should

sharing occur with international consortia in the future. It is recognized that once legal requirements for international sharing are detailed, this initial consent might be invalid and processes to re-consent Cohort participants might be required. The legal validity of the consent obtained is thus uncertain at this time. However, with consent also being obtained to contact for future studies, it may be possible to re-engage participants and obtain further consent if needed in the future. It is difficult if not impossible to obtain valid consent for data sharing in international consortia when the standards have yet to be established. The attempt in the All Our Babies Cohort Study is striving to move SPTB research forward and anticipate the shift toward discovery-focused research that might be coming.

Identifying appropriate comparative datasets to determine representativeness of the cohort and therefore generalizabilty of results is difficult. Representativeness assessments for the All Our Babies Cohort were conducted using the best comparative datasets available: the 2006 Canadian Census[8] and the MES.[9] Both datasets limited the strength of the assessments that could be conducted (see Chapter 4). Unfortunately a birth registry containing detailed maternal demographics is not available for Alberta. The APH database (described in Chapter 3) contains information on parity and ages of women delivering in Alberta but does not collect information on many sociodemographic variables[10] analyzed in Chapter 4. This database is therefore not a feasible comparative dataset for representativeness assessments focused on demographic characteristics of participants. The lack of optimal comparative datasets may in part explain why these representativeness assessments have been lacking in previous pregnancy and birth cohorts but should not justify their complete disregard. Despite the comparative samples introducing some limitations on the strengths of the representativeness assessments, the information gained about scope of generalizability is of value. Understanding the limitations of

representativeness analyses conducted and using the findings to inform interpretation of results might be of more benefit than omitting these comparisons altogether. Analyses in Chapter 4, like those of Generation R,[2] sought to address current gaps in pregnancy cohort research by recognizing that the use of the best available datasets for assessing sample representativeness, despite the limitations they present, can inform study findings. However, as this representativeness assessment used a similar strategy as that of Generation R, insights and lessons learned from other Cohorts in the future might provide better approaches to the conduct of representativeness assessments if national pregnancy and birth registries are unavailable.

5.4.0 Next Steps

The representativeness of the All Our Babies Cohort Study should be revisited when the data from outstanding questionnaires are available. It is not anticipated that these remaining questionnaires will change the results found in Chapter 4 but the analysis would be more complete. The representativeness assessment also currently lacks measures of women's work status. This information is collected on the second questionnaire (administered between 34-36 weeks of pregnancy) and was not available at the time of this analysis. To align the All Our Babies Cohort more closely with descriptive characteristics reported for other pregnancy cohort studies, this information should be considered for inclusion in the representativeness analysis prior to publication of data. Comparison data for maternal work status is available through the 2006 Canadian Census.[8] The findings from the representativeness assessment should be considered when interpreting all future results generated from the Cohort and should be made available if the aggregate dataset is shared with international consortia in the future.

Since the primary purpose of the All Our Babies Cohort Study is to examine

etiological associations of SPTB, special attention should be placed on the birth outcome data of this Cohort. The inclusion criteria were specifically designed to focus on women at slightly higher risk for SPTB than IPTB.[11] When available, analysis of birth outcome data will inform the effectiveness of the inclusion criteria to result in the SPTB outcome desired. Because the majority (68%) of Cohort participants were between 25-34 years of age, the maternal ages of preterm birth cases will be of interest as preterm birth rates are typically elevated in adolescents and in women of advanced maternal age.[12] Comparisons of the birth outcomes observed in the All Our Babies Cohort can be made to the APH database,[10] the Canadian Perinatal Health Report,[13] and the Alberta Reproductive Health Report.[12] The representative analyses conducted in this study should be considered when examining the prevalence of birth outcomes in the Cohort.

5.5.0 Conclusion

SPTB is a category of PTBs that might benefit from prediction and interventions designed for prevention. Understanding etiologies of SPTB is essential to working towards these clinical goals. The guidelines established seek to create standardization in individual research endeavours that will enable international consortia to merge datasets and achieve the power required for interdisciplinary discovery-focused research into SPTB etiologies. The All Our Babies Cohort Study is an example of a thoughtfully designed pregnancy cohort that has been established for the study of SPTB etiological associations. The known representativeness of the Cohort will inform interpretation and generalizability of all findings generated from the Cohort. It is hoped that the study design and representativeness assessment presented, as an example of adherence to the guidelines suggested, will serve as a model for future studies on the etiologies of SPTB.

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Appendix

Appendix A All Our Babies Cohort Study First Questionnaire

The first questionnaire for the All Our Babies Cohort Study is mailed to participants at the time of study enrollment for completion prior to 24 weeks of pregnancy. The data dictionary for the first questionnaire is presented below. Questions used for the analysis in Chapter 4 are italicized.

SECTION 1: MATERNAL PRENATAL HEALTH DATA

The first series of questions will ask about prenatal care and your thoughts about this pregnancy.

1. Has it been difficult for you to obtain prenatal care? [diffpnc]
1 - O Yes
2 - O No

If yes, what is the main reason it has been difficult for you to obtain prenatal care? Please select one. [whydiff]
1 - O Could not find a doctor or midwife accepting prenatal patients
2 - O Lack of available transport to get to clinic or office
3 - O Lack of finances
4 - O Lack of child care
5 - O Excessive stress
6 - O Cultural values and beliefs
7 - O Family not supportive of seeking prenatal care and services
8 - O Not aware of the health services available
9 - O Fear about your pregnancy
10- O Delay in suspecting pregnancy
11- O Did not see the need to go
12- O Other: [otherdiff]_____
_____

2. Have you been to a physician or midwife since first suspecting you were pregnant to confirm your pregnancy? [confirm]
1 - O Yes
2 - O No

If yes, how many weeks pregnant were you when you first visited a physician or midwife to confirm your pregnancy? Your best guess is ok. ___ weeks [confirmga]

3. Have you been to a doctor or midwife for at least one prenatal care visit? At this visit, the doctor may have given you a pelvic exam, gone over your pregnancy and health history and sent you for lab testing. If you are having your first visit today, please respond "Yes". [anypnc]

1 - O Yes
2 - O No

If no, please skip to Question 8.

4. Which of the following health care providers did you see for your first prenatal visit? [pnchcp]
1 - O A walk-in clinic doctor
2 - O A family doctor in an appointment based office
3 - O A doctor in a Low Risk Maternity Clinic
4 - O An obstetrician
5 - O A midwife
6 - O Other: [otherpnchcp]___

5. Approximately how many weeks pregnant were you for your first prenatal care visit? Your best guess is ok. Each month has approximately 4 weeks. For example, if you are 2 and a half months pregnant, you are approximately 10 weeks pregnant. **[pnc1ga]**

____ weeks

6. How many prenatal visits have you had so far? If you have a prenatal appointment today, please include this visit. **[numvisit]**

1 - O 1 2 - O 2 3 - O 3 4 - O 4 5 - O 5 6 - O 6+

7. During your prenatal visits, have you received advice on... Select all that apply. If you are having your first prenatal appointment today, please skip to the next question.

1 - O Nutrition? **[advnutri]**
1 - O Alcohol consumption during pregnancy? **[advalc]**
1 - O Appropriate amount of weight gain? **[advwt]**
1 - O Taking prescription non-prescription drugs **[advdrug]**
1 - O Taking vitamins or mineral supplements? **[advvita]**
1 - O Exercise or active living during pregnancy? **[advexer]**
1 - O Working during pregnancy? **[advwork]**
1 - O Cigarette smoking and second hand smoke? **[advsmoke]**

8. Have you been to a dentist in the past year? **[dentist]**

1 - O Yes
2 - O No

9. What is your height? feet ____ inches **OR** cm
[htft] **[htin]** **[htcm]**

10. How many weeks pregnant are you right now? ____ *weeks* ***[ganow]***

Your best guess is ok. Each month has approximately 4 weeks. For example, if you are 2 and a half months pregnant, you are approximately 10 weeks pregnant.

11. Thinking back to just before you became pregnant, how did you feel about the timing of your pregnancy? **[timing]**

1 - O I wanted to become pregnant earlier
2 - O I wanted to become pregnant at a later point in time
3 - O I wanted to become pregnant at this point in time
4 - O I didn't want to become pregnant then or any other time in the future

12. How did you feel when you found out you were pregnant? **[feltpreg]**

1 - O Very happy 2 - O Happy 3 - O Not sure 4 - O Unhappy 5 - O Very unhappy

13. How much did you weigh before getting pregnant? pounds **OR** kg
[prewtlb] **[prewtkg]**

14. How much do you weigh now? pounds **OR** kg
[wtlb] **[wtkg]**

SECTION 2: PREGNANCY HISTORY

1. Have you ever been pregnant before? ***[prevpreg]***
1 - O Yes
2 - O No *If no, skip to question 11.*

2. How many times have you been pregnant (not including this pregnancy)? ⌊__⌋ ***[numprevpreg]***

We would now like to ask you some questions about your previous pregnancies, including those you may have lost. Please tell us if you have experienced any of the following and the number of times you have experienced them.

Have you ever experienced...

3. A miscarriage in the first trimester? (ie. when you were less than or equal to 12 weeks pregnant) **[miscar1]**
2 - O No
1 - O Yes Number of times: ⌊__⌋ **[nummiscar1]**

4. A miscarriage in the second trimester? (ie. when you were 13 to 20 weeks pregnant) **[miscar2]**
2 - O No
1 - O Yes Number of times: ⌊__⌋ **[nummiscar2]**

5. A stillbirth? *(e.g. born dead over 20 weeks gestation or with a weight above 500 grams)* ***[still]***
2 - O No
1 - O Yes *Number of times:* ⌊__⌋ ***[numstill]***

6. An abortion? **[abort]**
2 - O No
1 - O Yes Number of times: ⌊__⌋ **[numabort]**

7. Neonatal death? *(death in the first 28 days after birth)* ***[death]***
1 - O No
2 - O Yes *Number of times:* ⌊__⌋ ***[numdeath]***

8. Live births? ***[child]***
1 - O No
2 - O Yes *Number of times:* ⌊__⌋ ***[numchild]***

How many months between when your last child was born (ie -delivery date) and the start of this pregnancy?
⌊__⌋ months **[interpreg]**

9. Were any of your children less than 2500 grams (5 lbs 5 oz) when they were born? [childlbw]

3 - ○ Don't Know
2 - ○ No
1 - ○ Yes **How many children?** ___ [numlbw]

10. Were any of your children born preterm (before 37 weeks gestation)? [numptb]

3 - ○ Don't Know
2 - ○ No
1 - ○ Yes **How many children?** ___ [numptb]

11. Were you born preterm (before 37 weeks gestation)? [momptb]

1 - ○ Yes 2 - ○ No 3 - ○ Don't Know

12. Were any of your brothers or sisters born preterm (before 37 weeks gestation)? [sibptb]

1 - ○ Yes 2 - ○ No 3 - ○ Don't Know 4 - ○ No brothers or sisters

13. Was your mother born preterm (before 37 weeks gestation)? [mom2ptb]

1 - ○ Yes 2 - ○ No 3 - ○ Don't Know

SECTION 3: PRE-PREGNANCY

The next questions will ask you about this pregnancy.

1. Before you became pregnant, did you receive any information about becoming pregnant or pregnancy planning from a healthcare professional? [preconcept]

1 - ○ Yes
2 - ○ No If no, please skip to Question 2.

If yes, which professionals gave you this information? Select all that apply.

1 - ○ A family doctor [prefamdr] 1 - ○ A Public Health Nurse or other nurse [prenurse]

1 - ○ A walk in clinic doctor [prewalkin] 1 - ○ An obstetrician/gynecologist [preobgyn]

1 - ○ Other: [preother]_____blank = [preother2]

Did you receive information about... (Select all that apply)

1 - ○ Nutrition? [preconnutri] 1 - ○ Physical Activity? [preconpa] 1 - ○ Smoking and pregnancy? [preconsmok] 1 - ○ Emotional health? [preconemo]
1 - ○ Folic Acid? [preconfolic] 1 - ○ Alcohol and pregnancy? [preconalc] 1 - ○ Oral health? [preconoral] 1 - ○ Sexually transmitted infections? [preconsti]

2. Which method(s) of birth control were you and your partner using most recently? Select all that apply.

1 - O : Abstinence **[abstinence]**
1 - O : Withdrawal **[withdraw]**
1 - O : Natural family planning **[famplan]**
1 - O : Female condom **[fcondom]**
1 - O : Male condom **[mcondom]**
1 - O : Diaphragm/Cervical cap **[cervcap]**
1 - O : We were not using any form of birth control **[nocontra]**
1 - O : Sponge **[sponge]**
1 - O : Spermicide **[spermicide]**
1 - O : Birth control pill **[bcp]**
1 - O : The shot (Depo-provera) **[depo]**
1 - O : The patch (Ortho Evra) **[patch]**
1 - O : The ring (NuvaRing) **[ring]**
1 - O : IUD (Intrauterine Device) **[iud]**
1 - O : Tubes tied (tubal ligation) **[tubes]**
1 - O : Vasectomy **[vasect]**
1 - O : Emergency contraceptive pill (the "morning after pill") **[emergpill]**
1 - O : Lea's Contraceptive **[leas]**
1 - O : Lactational amenorrhea method (LAM) **[lam]**

3. When you became pregnant, were you trying to get pregnant? [trying]

1 - O : Yes 2 - O : No

4. Were you or your partner doing anything to keep from getting pregnant? (ie -using at least one method of birth control) **[contra]**
1 - O : All of the time 2 - O : Most of the time 3 - O : Some of the time 4 - O : A little of the time 5 - O : None of the time

5. How many months did it take to get pregnant? ___ months **[nummonth]**

6. While you were trying to become pregnant, did you use any of the following? Select all that apply

1 - O : Menstrual cycle and ovulation tracking **[menstrack]**
1 - O : Cervical mucus monitoring **[mucus]**
1 - O : Acupuncture **[fertacupunc]**
1 - O : Temperature monitoring **[tempmonitor]**
1 - O : Ovulation prediction tests purchased at a pharmacy or clinic **[ovtests]**
1 - O : Naturopathic medicine **[naturo]**
1 - O : Fertility-enhancing drugs prescribed by a doctor **[fertdrug]**
(e.g. Clomid, Serophine, Gonal-F, Menopur, Repronex, or other drugs that stimulate ovulation)

1 - O : Artificial insemination or intrauterine insemination (IUI) **[fertai]**
(treatments in which sperm, but not eggs were collected and medically placed into a woman's body)
1 - O : Partner's sperm **[partsperm]**
1 - O : Donor sperm **[donorsperm]**
1 - O : Assisted reproductive technology **[fertart]**
(treatments in which both a woman's eggs and a man's sperm were handled in the laboratory)
1 - O : In vitro fertilization (IVF) **[fertivf]**
1 - O : Intracytoplasmic sperm injection (ICSI) **[ferticsi]**
1 - O : Superovulation/IUI **[fertiui]**
1 - O : Fresh embryo transfer **[fertembtrans]**
1 - O : Donor embryo transfer **[fertdonoremb]**
1 - O : Any other fertility treatment: **[fertother2]**_____ **[fertother]**
_____

SECTION 4: SERVICE UTILIZATION

The next questions will ask about health care providers you may have visited during your pregnancy.

Between the time you found out you were pregnant and now, have you visited any of the following for any reason? How many times each? Indicate all that apply.

1 - O : Visited a family doctor (for reasons other than a regular prenatal visit) **[famdr]**	Number of family doctor visits: ___ **[numfamdr]**
1 - O : Visited a walk-in clinic doctor **[walkin]**	Number of walk-in clinic visits: ___ **[numwalkin]**
1 - O : Visited an obstetrician **[obs]**	Number of obstetrician visits: ___ **[numobs]**
1 - O : Visited a specialist physician **[specdr]**	Number of specialist visits: ___ **[numspecdr]**
1 - O : Visited the hospital Emergency Department **[er]**	Number of ER visits: ___ **[numer]**
1 - O : Stayed overnight in a hospital **[hosp]**	Number of nights in hospital: ___ **[numhosp]**
1 - O : Saw a physiotherapist **[physio]**	Number of physiotherapist visits: ___ **[numphysio]**
1 - O : Saw a chiropractor **[chiro]**	Number of chiropractor visits: ___ **[numchiro]**
1 - O : Saw a psychologist or psychiatrist **[psych]**	Number of psychologist visits: ___ **[numpsych]**
1 - O : Saw a nutritionist/dietician **[nutri]**	Number of nutritionist visits: ___ **[numnutri]**
1 - O : Saw a social worker **[socwork]**	Number of social worker visits: ___ **[numsocwork]**
1 - O : Called Healthlink, the Calgary Health Region 24-hour help line You can call Healthlink anytime for any health concern at 943-LINK (5465) or 1-866-408-LINK (5465). **[link]**	Number of Healthlink calls: ___ **[numlink]**

1 - O : Saw any other type of health care provider(s) **[otherhcp]**

Please list here: **[otherhcp1]** _____ Number of times: ___ **[numotherhcp1]**

[otherhcp2] _____ Number of times: ___ **[numotherhcp2]**

[otherhcp3]_____ Number of times: ___ **[numotherhcp3]**

1 - O : No visits to healthcare providers **[nohcp]**

SECTION 5: FOOD, EXERCISE AND HOUSING

The next questions will ask you about your exercise and eating habits during pregnancy, as well as your housing situation.

1. At this time in your pregnancy, how often do you exercise for 15 to 30 minutes per day? Exercise includes activities such as fast walking, dancing and swimming. **[exer]**

1 - O 0 -2 times each week
2 - O 3 -5 times each week
3 - O 6 or more times each week

2. Since you have been pregnant, are you exercising: [exer2]

1 - O Less often
2 - O About the same
3 - O More often

3. At this time in your pregnancy, on an average day how much liquid (e.g. water, milk, juice, soup, etc.) do you have? Do not include caffeinated beverages such as coffee, tea, pop, cola, etc. **[fluid]**

1 - O None
2 - O 1 litre (4 cups) or less each day
3 - O 2.2 litres (4 -8 cups) each day
4 - O 2.3 litres (9 cups) or more each day

4. At this time in your pregnancy, on an average day how many servings of meat and alternatives do you eat? [meat]

1 - O None
2 - O One each day
3 - O 2 -3 each day
4 - O 4 or more each day

Examples of one serving would include:
-½ cup (125 mL) cooked fish, shellfish, poultry or lean meat
-¾ cup (175 mL) cooked beans-2 eggs
-2 Tbsp (30 mL) peanut butter.

5. At this time in your pregnancy, on an average day how many servings of milk and alternative products do you eat? [milk]

1 - O None
2 - O One each day
3 - O 2 -4 each day
4 - O 5 or more each day

Examples of one serving would include:
-1 cup (250 mL) milk or fortified soy beverage
-¾ cup (175 g) yogurt-50 g (1 ½ oz.) cheese

6. At this time in your pregnancy, on an average day how many servings of fruits and vegetables do you eat? [fruit]

1 - O None
2 - O 1 -3 each day
3 - O 4 -6 each day
4 - O 7 or more each day

Examples of one serving would include:
-½ cup (125 mL) fresh, frozen or canned vegetable or fruit or 100% juice
-1 cup (250 mL) leafy raw vegetables or salad
-1 piece of fruit.

7. At this time in your pregnancy, on an average day how many servings of grain products do you eat? [grain]

1 - O None
2 - O 1 -5 each day
3 - O 6 -7 each day
4 - O 8 or more each day

Examples of one serving would include:
-1 slice (35 g) bread
-½ bagel (45 g), ½ pita (35 g)
-½ tortilla (35 g)
-½ cup (125 mL) cooked rice, pasta, or couscous
-30 g cold cereal
-¾ cup (75 mL) hot cereal

8. At any point in your pregnancy, have you engaged in a fast (i.e. refrained from eating for at least 8 hours while you were awake)? [fast]
1 - ○ Yes
2 - ○ No

If yes, please describe when you fasted and the main reasons why. **[whyfast]**

_____
_____
_____

9. In this pregnancy, on average, how often do you take a prenatal vitamin? [pnvit]

1 - ○ Never
2 - ○ 1 -3 times a week
3 - ○ 4 -7 times a week

10. How much weight do you think YOU should gain during your pregnancy to help you have a healthy baby? [wtgain]

1 - ○ 15 -25 lbs (7.0 -11.5 kg)
2 - ○ 25 -35 lbs (11.5 -16.0 kg)
3 - ○ 28 -40 lbs (12.5 -18.0 kg)

11. In the past year, have you experienced a time that the food you bought didn't last and you didn't have money to get more? [foodsec1]

1 - ○ Often
2 - ○ Sometimes
3 - ○ Never

12. In the past 6 months, has anyone in your household ever received food from a food bank, soup kitchen or other charitable agency? [foodsec2]

1 - ○ Yes
2 - ○ No

13. What kind of housing are you currently living in? [accom]
1 - ○ House
2 - ○ Apartment
3 - ○ Duplex / Four-plex
4 - ○ Group dwelling
(e.g. hotel, shelter, boarding house, colony)
5 - ○ Townhouse
6 - ○ Condominium
7 - ○ Other: **[otheraccom]**_____

14. Do you rent or own the housing you are currently living in? [own]

1 - ○ Rent *2 - ○ Own* *3 - ○ Living with family (no rent)* *4 - ○ Other: **[otherown]**_____*

15. Including yourself, how many people currently live in your household? ___ persons **[numhousehold]**
People who live in your household on a part-time basis also count, but please do not include pets.

16. How may times have you moved in the past two years (including moves within the city)? [nummove]

1 - ○ None 2 - ○ 1 -2 times 3 - ○ 3 -4 times 4 - ○ 5 or more times

17. Has it been difficult for you to find stable housing? [stable]
1 - ○ Yes
2 - ○ No

SECTION 6: SOCIAL SUPPORT

Next are some questions about the support that is available to you.

1. How many close friends and/or close relatives do you have that you feel at ease with and can talk to about what is on your mind? ___ person(s) **[numss]**

2. People sometimes look to others for companionship, assistance, or other types of support.

How often is each of the following kinds of support available to you if you need it?	*Fill in only one circle for each line* None of the Time 1	A little of the Time 2	Some of the Time 3	Most of the Time 4	All of the Time 5
Someone to help you if you were confined to bed **[ssbed]**	O	O	O	O	O
Someone you can count on to listen to you when you need to talk **[sslisten]**	O	O	O	O	O
Someone to give you good advice about a crisis **[sscrisis]**	O	O	O	O	O
Someone to take you to the doctor if you needed it **[ssdr]**	O	O	O	O	O
Someone who shows you love and affection **[sslove]**	O	O	O	O	O
Someone to have a good time with **[ssgood]**	O	O	O	O	O
Someone to give you information to help you understand a situation **[ssinfo]**	O	O	O	O	O
Someone to confide in or talk to about yourself or your problems **[ssconfide]**	O	O	O	O	O
Someone who hugs you **[sshug]**	O	O	O	O	O
Someone to get together with for relaxation **[ssrelax]**	O	O	O	O	O
Someone to prepare your meals if you were unable to do it yourself **[ssmeal]**	O	O	O	O	O
Someone whose advice you really want **[ssadvice]**	O	O	O	O	O
Someone to do things with to help you get your mind off things **[ssthings]**	O	O	O	O	O
Someone to help with daily chores if you were sick **[sschore]**	O	O	O	O	O
Someone to share your most private worries and fears with **[ssfear]**	O	O	O	O	O
Someone to turn to for suggestions about how to deal with a personal problem **[ssdeal]**	O	O	O	O	O
Someone to do something enjoyable with **[ssenjoy]**	O	O	O	O	O
Someone who understands your problems **[ssprob]**	O	O	O	O	O
Someone to love and make you feel wanted **[sswant]**	O	O	O	O	O
Someone available to confide in or talk about your pregnancy **[sspreg]**	O	O	O	O	O

3. How satisfied are you with the social and/or emotional support you receive from your family? [famsup]
1 - O Very satisfied 2 - O Satisfied 3 - O Unsatisfied 4 - O Very unsatisfied

4. Does your family support you in making healthy pregnancy choices? (ie. Getting enough rest, eating well, avoiding smoking and alcohol, attending prenatal appointments) **[famsuppreg]**
1 - O None of the time 2 - O A little of the time 3 - O Some of the time 4 - O Most of the time 5 - O All of the time

5. How satisfied are you with the social and/or emotional support you receive from your friends? [frsup]
1 - O Very satisfied 2 - O Satisfied 3 - O Unsatisfied 4 - O Very unsatisfied

6. Do your friends support you in making healthy pregnancy choices? (ie. Getting enough rest, eating well, avoiding smoking and alcohol, attending prenatal appointments) **[frsuppreg]**
1 - O None of the time 2 - O A little of the time 3 - O Some of the time 4 - O Most of the time 5 - O All of the time

7. How satisfied are you with the social and/or emotional support you receive from your health care providers? [hcpsup]
1 - O Very satisfied 2 - O Satisfied 3 - O Unsatisfied 4 - O Very unsatisfied

8. Do you currently have a partner? [partner]
1 - O Yes
2 - O No If no, please skip to Section 7: Your Emotional and Physical Health

9. How happy do you think your partner is that you are pregnant at this time? [partopinion]
1 - O Very happy
2 - O Happy
3 - O They have no opinion
4 - O A little unhappy
5 - O Not at all happy
6 - O They do not know I'm pregnant

10. In general, how would you describe your relationship with your partner? [parttens]
1 - O A lot of tension 2 - O Some tension 3 - O No tension

11. Do you and your partner work out arguments with: [partdiff]
1 - O Great difficulty 2 - O Some difficulty 3 - O No difficulty

12. At the present time, which best describes how often your partner smokes cigarettes? [partsmk]
1 - O Every day 2 - O Occasionally 3 - O Not at all

13. How satisfied are you with the social and/or emotional support you receive from your partner? [partsup]
1 - O Very satisfied 2 - O Satisfied 3 - O Unsatisfied 4 - O Very unsatisfied

14. Does your partner support you in making healthy pregnancy choices? (ie. Getting enough rest, eating well, avoiding smoking and alcohol, attending prenatal appointments) **[partsuppreg]**

1 - O None of the time 2 - O A little of the time 3 - O Some of the time 4 - O Most of the time 5 - O All of the time

15. Besides your family, friends and healthcare providers, do you have any other people in your life who are a source of support? If yes, please list: [othsup]

_____

_____

SECTION 7: YOUR EMOTIONAL & PHYSICAL HEALTH

We would like to ask you a series of questions about how you have been feeling.

Fill in only one circle for each line

1. In the past month, how often have you...	Never	Almost Never	Sometimes	Fairly Often	Often
	1	2	3	4	5
Felt upset by something that happened unexpectantly [psi1]	O	O	O	O	O
Felt unable to control important things in your life [psi2]	O	O	O	O	O
Felt nervous or stressed [psi3]	O	O	O	O	O
Felt confident in your ability to handle your personal problems [psi4]	O	O	O	O	O
Felt that things were going your way [psi5]	O	O	O	O	O
Felt unable to cope with all the things you had to do [psi6]	O	O	O	O	O
Felt able to control irritations in your life [psi7]	O	O	O	O	O
Felt on top of things [psi8]	O	O	O	O	O
Felt angry because of things that happened that you couldn't control [psi9]	O	O	O	O	O
Felt that difficulties were piling up so high that you couldn't overcome them [psi10]	O	O	O	O	O

For the next questions, please check the answer that comes closest to how you have felt in the past 7 days, not just how you felt today.

2. In the past 7 days, I have been able to laugh and see the funny side of things [edps1]

1 - O As much as I always could
2 - O Not quite so much now
3 - O Definitely not so much now
4 - O Not at all

3. In the past 7 days, I have looked forward with enjoyment to things [edps2]

1 - O As much as I ever did
2 - O Rather less than I used to
3 - O Definitely less than I used to
4 - O Hardly at all

4. In the past 7 days, I have blamed myself unnecessarily when things went wrong [edps3]

1 - O Yes, most of the time
2 - O Yes, some of the time
3 - O Not very often
4 - O No, never

5. In the past 7 days, I have been anxious or worried for no good reason [edps4]

1 - O No, not at all
2 - O Hardly ever
3 - O Yes, sometimes
4 - O Yes, very often

6. In the past 7 days, I have felt scared or panicky for no very good reason [edps5]

1 - ○ Yes, quite a lot

2 - ○ Yes, sometimes

3 - ○ No, not much

4 - ○ No, not at all

7. In the past 7 days, things have been getting on top of me [edps6]

1 - ○ Yes, most of the time I haven't been able to cope at all

2 - ○ Yes, sometimes I haven't been coping as well as usual

3 - ○ No, most of the time I have coped quite well

4 - ○ No, I have been coping as well as ever

8. In the past 7 days, I have been so unhappy that I have had difficulty sleeping [edps7]

1 - ○ Yes, most of the time

2 - ○ Yes, sometimes

3 - ○ Not very often

4 - ○ No, not at all

9. In the past 7 days, I have felt sad or miserable [edps8]

1 - ○ Yes, most of the time

2 - ○ Yes, sometimes

3 - ○ Not very often

4 - ○ No, not at all

10. In the past 7 days, I have been so unhappy that I have been crying [edps9]

1 - ○ Yes, most of the time

2 - ○ Yes, quite often

3 - ○ Only occasionally

4 - ○ No, never

11. In the past 7 days, the thought of harming myself has occurred to me [edps10]

1 - ○ Yes, quite often

2 - ○ Sometimes

3 - ○ Hardly ever

4 - ○ Never

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OR
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12. In general, how would you rate your emotional health? [emohlth]

1 - O Excellent
2 - O Very good
3 - O Good
4 - O Fair
5 - O Poor

13. In general, how would you rate your physical health? [sf1]

1 - O Excellent
2 - O Very good
3 - O Good
4 - O Fair
5 - O Poor

14. Here are 20 statements that people use to describe how they are feeling. Please select the that indicates how you feel **right now** (in this moment).

Fill in only one circle for each line response

	Not at all 1	Somewhat 2	Moderately So 3	Very much so 4
I feel calm. [anx1]	O	O	O	O
I feel secure. [anx2]	O	O	O	O
I am tense. [anx3]	O	O	O	O
I am regretful. [anx4]	O	O	O	O
I feel at ease. [anx5]	O	O	O	O
I feel upset. [anx6]	O	O	O	O
I am presently worrying over possible misfortunes. [anx7]	O	O	O	O
I feel rested. [anx8]	O	O	O	O
I feel anxious. [anx9]	O	O	O	O
I feel comfortable. [anx10]	O	O	O	O
I feel self-confident. [anx11]	O	O	O	O
I feel nervous. [anx12]	O	O	O	O
I am jittery. [anx13]	O	O	O	O
I feel "high strung". [anx14]	O	O	O	O
I am relaxed. [anx15]	O	O	O	O
I feel content. [anx16]	O	O	O	O
I am worried. [anx17]	O	O	O	O
I feel overexcited and rattled. [anx18]	O	O	O	O
I feel joyful. [anx19]	O	O	O	O
I feel pleasant. [anx20]	O	O	O	O

The next questions will ask you about your current health.

15. Does your health now limit you in moderate activities such as pushing a vacuum cleaner, bowling or playing golf? [sf2]

1 - ○ Yes, limited a lot 2 - ○ Yes, limited a little 3 - ○ No, not limited at all

16. Does your health now limit you in climbing several flights of stairs? [sf3]

1 - ○ Yes, limited a lot 2 - ○ Yes, limited a little 3 - ○ No, not limited at all

17. During the past 4 weeks, how much of the time have you accomplished less than you would like at your work or other regular daily activities as a result of your physical health? [sf4]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

18. During the past 4 weeks, how much of the time have you been limited in the kind of work or other daily activities that you can perform as a result of your physical health? [sf5]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

19. During the past 4 weeks, how much of the time have you accomplished less than you would like at your work or other regular daily activities as a result of any emotional problems? e.g. feeling depressed or anxious [sf6]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

20. During the past 4 weeks, how much of the time have you been limited in the kind of work or other daily activities that you can perform as a result of any emotional problems? e.g. feeling depressed or anxious [sf7]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

21. During the past 4 weeks, how much did pain interfere with your normal work? Include both work outside the home [sf8]

1 - ○ Not at all 2 - ○ A little bit 3 - ○ Moderately 4 - ○ Quite a bit 5 - ○ Extremely

22. During the <u>past 4 weeks</u>, how much of the time have you felt calm and peaceful? [sf9]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

23. During the past 4 weeks, how much of the time did you have a lot of energy? [sf10]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

24. During the past 4 weeks, how much of the time have you felt downhearted and depressed? [sf11]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

25. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with social activities? (e.g. visiting with friends, relatives, etc.) [sf12]

1 - ○ All of the time 2 - ○ Most of the time 3 - ○ Some of the time 4 - ○ A little of the time 5 - ○ None of the time

SECTION 8: HISTORY & LIFE EVENTS

The next questions will ask about events that may have happened to you in your life.

1. Have you ever had alcohol dependency problems? [alcprob]

1 - ○ Yes
2 - ○ No

If yes, have you ever sought treatment? [alctreat]
1 - ○ Yes
2 - ○ No

2. Have you ever had drug dependency problems (including prescription medications)? [drugprob]

1 - ○ Yes
2 - ○ No

If yes, have you ever sought treatment? [drugtreat]
1 - ○ Yes
2 - ○ No

3. Have you ever experienced not having a job for a long time when you wanted to be working? [nojob]

1 - ○ Yes
2 - ○ No

4. Have you ever experienced feeling underemployed at a job for your education or experience? [underjob]

1 - ○ Yes
2 - ○ No

5. Have you ever experienced feeling sad, blue, depressed or down for most of the time for at least 2 weeks? [depres]

1 - ○ Yes
2 - ○ No

If yes, have you ever sought treatment? [deprestreat]
1 - ○ Yes
2 - ○ No

6. Have you ever experienced other mental disorders such as generalized anxiety disorder, bipolar disorder, schizophrenia, or obsessive compulsive disorder? [mental]

1 - ○ Yes
2 - ○ No

If yes, have you ever sought treatment? [menttreat]
1 - ○ Yes
2 - ○ No

7. Have you ever had suicidal thoughts or attempts? [suicide]
1 - O Yes
2 - O No

If yes, have you ever sought treatment? [suictreat]
1 - O Yes
2 - O No

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OR
AADAC Help Line: 1-866-332-2322

SECTION 9: LIFESTYLE

The next few questions will ask you about your lifestyle.

1. In the 12 months before you became pregnant, did you drink alcohol? [prealc]

1 - O Yes
2 - O No If no, skip to Question 9

2. Do you feel the effects of alcohol after one drink? (e.g. tipsy or lightheaded) **[tace1a]**

1 - O Yes
2 - O No

If no, how many drinks does it take you to feel the effects of alcohol? ___ drinks **[tace1b]**

3. Have people ever annoyed you by criticizing your drinking? [tace2]

1 - O Yes
2 - O No

4. Have you ever felt you should cut down on your drinking? [tace3]

1 - O Yes
2 - O No

5. Have you ever had a drink first thing in the morning to steady your nerves or get rid of a hangover? [tace4]

1 - O Yes
2 - O No

6. In the 12 months before you became pregnant, on average, how many days per week did you drink alcohol? [tace5]

1 - O Less than 1 2 - O 1 3 - O 2 4 - O 3 5 - O 4 6 - O 5 7 - O 6 8 - O 7

7. On average, how many drinks would you typically have when you drank? [numdrink]

1 - ○ Less than 1 2 - ○ 1 3 - ○ 2 4 - ○ 3 5 - ○ 4 6 - ○ 5 or more

8. In the 12 months before you became pregnant, did you ever drink 5 or more drinks on any one occasion? [binge]

1 - ○ Yes

2 - ○ No

9. In the 12 months before you got pregnant, did you smoke cigarettes? [presmok]

1 - ○ Yes

2 - ○ No If no, skip to Question 12

10. In the 12 months before you became pregnant, on average, how many days per week did you smoke cigarettes? [dayssmok]

1 - ○ Less than 1 2 - ○ 1 3 - ○ 2 4 - ○ 3 5 - ○ 4 6 - ○ 5 7 - ○ 6 8 - ○ 7

11. In the 12 months before you became pregnant, on average, how many cigarettes did you smoke per day? [numsmok]

1 - ○ Less than 1

2 - ○ 1 -10 cigarettes

3 - ○ 11 -24 cigarettes

4 - ○ 25 cigarettes (1 pack) or more

12. Which best describes the way smoking is currently handled in your home? [homesmok]

1 - ○ No smoking inside or outside the house

2 - ○ No smoking inside the house

3 - ○ Not allowed when children are present

4 - ○ Confined to certain areas of the home

5 - ○ Permitted anywhere

13. In the 12 months before you got pregnant, did you use street drugs? (e.g. Marijuana, cocaine, crystal meth, etc.) [predrug]

1 - ○ Yes

2 - ○ No If no, skip to Section 10: Demographics

14. In the 12 months before you became pregnant, on average, how many days per week did you use street drugs? [daysdrug]

1 - ○ Less than 1 2 - ○ 1 3 - ○ 2 4 - ○ 3 5 - ○ 4 6 - ○ 5 7 - ○ 6 8 - ○ 7

SECTION 10: DEMOGRAPHICS

The next set of questions will help us to get a better picture of who is involved in the study.

1. How would you describe your current marital status? [mstat]

1 - O *Single*
2 - O *Single with partner*
3 - O *Married*
4 - O *Common law*
5 - O *Divorced*
6 - O *Separated*
7 - O *Widowed*

2. What is your birth date? ___/___/_____
MM DD YYYY [bday]

3. What is the highest level of education you have completed? [educ]

1 - O *Some Elementary or High School (Grades 1 -12)*
2 - O *Graduated High School*
3 - O *Some college, trade, university*
4 - O *Graduated college, trade, university*
5 - O *Some graduate school*
6 - O *Completed graduate school*

4. Were you born in Canada? [born]
1 - O *Yes*
2 - O *No* **If no, which country were you born in? [country]**_____

How long have you lived in Canada? ___ months **[mthcan]** OR ___ years **[yrcan]**

What was your status upon entering Canada? [statcan]
1 - O Immigrant
2 - O Dual Citizen
3 - O Refugee
4 - O Other: **[otherstatcan]**_____

5. How long have you lived in Calgary or the surrounding area? ___ months **[mthcal]** OR ___ years **[yrcal]**

6. How would you describe your ethnic background? [eth]
1 - O *White / Caucasian*
2 - O *Latin American*
3 - O *First Nations person registered (under the Indian Act of Canada)*
4 - O *First Nations person not registered*
5 - O *Inuit*
6 - O *Métis*
7 - O *Chinese*
8 - O *South Asian*
9 - O *Filipino*
10 - O *Black / African North American*
11 - O *Southeast Asian*
12 - O *Arab*
13 - O *West Asian*
14 - O *Korean*
15 - O *Japanese*
16 - O *Mixed / Other: **[othereth]*** _____

7. Where was your mother born?: [momborn]_____

8. How would you describe your mother's ethnic background? [mometh]

1 - O White / Caucasian
2 - O Latin American
3 - O First Nations person registered (under the Indian Act of Canada)
4 - O First Nations person not registered
5 - O Inuit
6 - O Métis
7 - O Chinese
8 - O South Asian
9 - O Filipino
10 - O Black / African North American
11 - O Southeast Asian
12 - O Arab
13 - O West Asian
14 - O Korean
15 - O Japanese
16 - O Mixed / Other: **[momethoth]** _____

9. Where was your father born?: [dadborn] _____

10. How would you describe your father's ethnic background? [dadeth]

1 - O White / Caucasian
2 - O Latin American
3 - O First Nations person registered (under the Indian Act of Canada)
4 - O First Nations person not registered
5 - O Inuit
6 - O Métis
7 - O Chinese
8 - O South Asian
9 - O Filipino
10 - O Black / African North American
11 - O Southeast Asian
12 - O Arab
13 - O West Asian
14 - O Korean
15 - O Japanese
16 - O Mixed / Other: **[dadethoth]** _____

11. Where was your baby's father born?: [patborn] _____

12. How would you describe your baby's father's ethnic background? [pateth]

1 - O White / Caucasian
2 - O Latin American
3 - O First Nations person registered (under the Indian Act of Canada)
4 - O First Nations person not registered
5 - O Inuit
6 - O Métis
7 - O Chinese
8 - O South Asian
9 - O Filipino
10 - O Black / African North American
11 - O Southeast Asian
12 - O Arab
13 - O West Asian
14 - O Korean
15 - O Japanese
16 - O Don't Know
17 - O Mixed / Other: **[patethoth]** _____

13. What is your baby's father's birth date? __/__/____

MM DD YYYY [patbday]

14. What language do you mainly speak at home? *Please select only one.* ***[lang]***

1 - O English	*10 - O Korean*
2 - O Cantonese	*11 - O Urdu*
3 - O Punjabi	*12 - O Nuer*
4 - O Vietnamese	*13 - O Dinka*
5 - O Mandarin	*14 - O Russian*
6 - O Arabic	*15 - O French*
7 - O Spanish	*16 - O Louw*
8 - O Farsi	*17 - O Other:* ***[langoth]*** _____
9 - O Hindi	

15. If necessary, are you able to communicate in English to people in your community? [english]

1 - O Yes

2 - O No

16. What is the total income, before taxes and deductions, of all household members from all sources in the past 12 months? *Your best guess is ok.* ***[income]***

1 - O Less than $10,000

2 - O $10,000 -$19,999

3 - O $20,000 -$29,999

4 - O $30,000 -$39,999

5 - O $40,000 -$49,999

6 - O $50,000 -$59,999

7 - O $60,000 -$69,999

8 - O $70,000 -$79,999

9 - O $80,000 -$89,999

10 - O $90,000 -$99,999

11 - O $100,000 or more

17. Do you receive income support from the government? [incsup]

1 - O Yes

2 - O No

18. Please enter today's date. ___/___/____

MM DD YYYY [today]

Thank you very much for taking the time to complete this survey! You will receive your $10 certificate for Superstore as soon as we receive your survey.

[studyid]

Appendix B: 2006 Canadian Census Variables

The variables used from the 2006 Canadian Census for the comparison analyses in Chapter 4 are presented below. The variable and the options available on the census are listed.

- Marital Status (marst)
  - Divorced
  - Legally married (and not separated)
  - Separated, but still legally married
  - Never legally married (single)
  - Widowed
- Household Income (hhinc)
  - Under $2,000
  - $2,000 to $4,999
  - $5,000 to $6,999
  - $7,000 to $9,999
  - $10,000 to $11,999
  - $12,000 to $14,999
  - $15,000 to $16,999
  - $17,000 to $19,999
  - $20,000 to $24,999
  - $25,000 to $29,999
  - $30,000 to $34,999
  - $35,000 to $39,999
  - $40,000 to $44,999
  - $45,000 to $49,999
  - $50,000 to $54,999
  - $55,000 to $59,999
  - $60,000 to $64,999
  - $65,000 to $69,999
  - $70,000 to $74,999
  - $75,000 to $79,999
  - $80,000 to $89,999
  - $90,000 to $99,999
  - $100,000 to $124,999
  - $125,000 to $149,999
  - $150,000 to $174,999
  - $175,000 to $199,999
  - $200,000 to $249,999
  - $250,000 and Over

- Housing Ownership (tenur)
    - Owned
    - Rented or Banded Housing
- Ethnicity (vismin)
    - Chinese
    - South Asian
    - Black
    - Filipino
    - Latin American
    - Southeast Asian
    - Arab
    - West Asian
    - Korean
    - Japanese
    - Visible Minority, n.i.e
    - Multiple Visible Minority
    - Not a Visible Minority
- Household Language (hlbno)
    - False, respondent did not report a non-official language as the language spoken at home on a regular basis
    - True, respondent reported a non-official language as the language spoken at home on a regular basis
- Country of Birth (pob)
    - Canada
    - United States of America
    - Central America
    - Jamaica
    - Other Caribbean and Bermuda
    - South America
    - United Kingdom
    - Germany
    - Other Northern and Western Europe
    - Poland
    - Other Eastern Europe
    - Italy
    - Portugal
    - Other Southern Europe
    - Eastern Africa
    - Northern Africa
    - Other Africa
    - West Central Asia and the Middle East
    - China, People's Republic of
    - Hong Kong, Special Administrative Region
    - Other Eastern Asia
    - Philippines
    - Other Southeast Asia

    - India
    - Pakistan
    - Other Southern Asia
    - Oceania and Others
- Education (hdgree)
    - None
    - High School Graduation Certificate or Equivalency Certificate
    - Other Trades Certificate or Diploma
    - Registered Apprenticeship Certificate
    - College, Cegep, or Other Non-University Certificate or Diploma from a Program of 3 months – less than 1 year
    - College, Cegep, or Other Non-University Certificate or Diploma from a Program of 1-2 years
    - College, Cegep, or Other Non-University Certificate or Diploma from a Program of more than 2 years
    - University Certificate or Diploma Below Bachelor's Level
    - Bachelor's Degree
    - University Certificate or Diploma above Bachelor's Level
    - Degree in Medicine, Dentistry, Veterinary Medicine or Optometry
    - Master's Degree
    - Earned Doctorate Degree