USING PHARMACOEPIDEMIOLOGIC METHODS TO STUDY PHARMACOTHERAPY IN PREGNANCY: APPLICATION TO OPIOID MAINTENANCE THERAPY AND 17-OHPC FOR PREVENTION OF PRETERM BIRTH

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Lara Siminerio Lemon

PharmD, University of Pittsburgh School of Pharmacy, 2011

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by

Lara Siminerio Lemon

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and approved by

Dissertation Advisor:
Lisa M. Bodnar, PhD, MPH, RD
Associate Professor
Departments of Epidemiology and Obstetrics, Gynecology of Reproductive Sciences
Graduate School of Public Health and School of Medicine
University of Pittsburgh

Committee Members:

Steve N. Caritis, MD
Professor
Department of Obstetrics, Gynecology, and Reproductive Sciences
School of Medicine
University of Pittsburgh

Catherine L. Carlson Haggerty, PhD, MPH
Associate Professor
Department of Epidemiology
Graduate School of Public Health
University of Pittsburgh

Raman Venkataramanan, PhD
Professor
Departments of Pharmaceutical Sciences and Pathology
School of Pharmacy and School of Medicine
University of Pittsburgh

Ada Youk, PhD
Associate Professor
Departments of Biostatistics, Epidemiology and Clinical & Translational Science
Graduate School of Public Health and School of Medicine.
University of Pittsburgh

Lisa M. Bodnar, PhD, MPH, RD

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ABSTRACT

We used pharmacoepidemiologic methods to overcome barriers innate to studying medication use in pregnancy. We applied these methods to two topics of high public health relevance: 1) optimal opioid maintenance therapy aimed at decreased neonatal abstinence syndrome (NAS), and 2) directing treatment with 17-OHPC for recurrent preterm delivery to women most likely to benefit.

Using a clinical dataset of women exposed to methadone or buprenorphine at Magee-Womens Hospital, Pittsburgh, PA (2013-2015, n=716), we performed a probabilistic bias analysis informed from an internal validation cohort to account for unmeasured confounding by maternal addiction severity. The historical increased risk of NAS associated with methadone compared with buprenorphine was not entirely attributable to maternal addiction severity [conventional adjRR: 1.3 (1.1, 1.5); bias adjRR: 1.2 (1.0, 1.4)]. Next, using an inverse probability weighted marginal structural model, we found that the association between treatment and NAS was mediated to a considerable degree through preterm birth (~25%). Because infants born preterm have lower rates of NAS, and methadone is associated with increased rates of preterm delivery, the increased risk of NAS associated with methadone was stronger among term births. For every 100 infants born to treated mothers, methadone was associated with 14 excess

cases of NAS overall, which increased to 17 excess cases among term births [adjRD: 16.7 (9.3, 24.0)].

To study 17-OHPC, we built models inclusive of significant interactions between obstetric history factors to predict the risk of recurrent spontaneous preterm delivery (sptd) in a cohort derived from the NICHD MFM Omega-3 trial. This randomized controlled trial found no significant effect of omega-3 supplementation on recurrent sptd in addition to administration of 17-OHPC. Using the treated women in this trial (n=754) and an externally validated predictive model, we found that risk of recurrence increased with earliest gestational ages of prior delivery only in women with ≥2 previous spontaneous preterm deliveries. These findings support the argument that more information, beyond having one previous spontaneous preterm delivery, is needed to target therapy to those most likely to benefit. This is of utmost public health importance as preterm birth remains the primary contributor to neonatal morbidity and mortality.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

I would like to first thank my incredibly dedicated and inspirational advisor, Dr. Lisa Bodnar. She has advised me both in my research and life, for which I am forever grateful. I am also thankful for my advisors Dr. Caritis and Dr. Venkat for their support and confidence in me. Together, all three have afforded me opportunities that put me where I am today. To my committee as a whole, the quality of my work is a direct result of your dedication to my learning and guided challenges at each step.

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1.0 INTRODUCTION

1.1 BACKGROUND

Medication use in pregnancy is on the rise, despite limited data on safety and efficacy in this population [1]. Research in obstetric pharmacology is innately complex, as many barriers exist to the study of medication use in pregnant women including the additional risk to the fetus associated with treatment, and the changing physiology of pregnancy which affects many aspects of the medication's pharmacology. Pharmacoepidemiology provides a safe and feasible approach to the study of medication use and its associated risks and benefits in pregnancy. The goal of this project is to apply pharmacoepidemiologic methods to two relevant clinical areas during pregnancy where pharmacotherapy is central to treatment: 1) opioid use disorder treatment, 2) prevention of recurrent preterm birth.

A rising issue over the past decade in the U.S., not limited to pregnancy, is the opioid epidemic [2, 3]. There are two recommended opioid maintenance therapies in pregnancy, methadone and buprenorphine [4]. Current comparative literature of these treatments is plagued with biases related to unmeasured confounding from prescribing preferences and inadequate accounting for the impact of gestational age.

Preterm birth, a relatively common obstetric complication, persists as one of the leading causes of infant mortality in the U.S. [5, 6]. Women with previous spontaneous preterm births

are considered to be at high risk for recurrent preterm birth. The American Congress of Obstetricians and Gynecologists (ACOG) recommends prophylactic treatment with progestin supplementation in those who are candidates [7]. This treatment practice reduces the risk of recurrence by approximately 33% [8]. Previous work identifying women most likely to respond to prophylactic treatment has failed to evaluate the interplay between various pregnancy history patterns.

Using pharmacoepidemiologic approaches to study these issues is a feasible approach to address issues of high public health priority.

1.2 SPECIFIC AIMS

The purpose of this dissertation is to apply pharmacoepidemiologic methods to relevant clinical issues encountered by obstetric care providers when prescribing medication in pregnancy. Specifically we will address, 1) preferred treatment for opioid use dependence in pregnancy, and 2) optimizing prophylactic treatment with 17-alpha-hydroxyprogesterone caproate (17-OHPC) for recurrent preterm birth with risk assessments based on pregnancy history. We will accomplish the following aims using two data sets. To address specific aims one and two, described below, we will use pharmacy-billing claims and chart data from all women with diagnosed drug-dependent deliveries at Magee-Womens Hospital, Pittsburgh, Pennsylvania, from 2013-2015 (n=716). To study our third aim, we will analyze data from 'The Omega-3 Trial', a multi-center, randomized controlled trial conducted at 13 centers by the National Institute of Child Health and Human Development (NICHD) Maternal Fetal Medicine Units (MFMU) Network from January 2005 to October 2006 (n=754).

Specific Aim 1. To estimate the association between in utero exposure to methadone versus buprenorphine and neonatal abstinence syndrome after accounting for unmeasured confounding by severity of maternal addiction.

Hypothesis: The greater historical risk of neonatal abstinence syndrome with methadone compared with buprenorphine-exposed infants will be reduced after accounting for unmeasured confounding by severity of maternal addiction.

Specific Aim 2. To describe and quantify the role of preterm birth in the association between opioid maintenance therapy and neonatal abstinence syndrome.

Hypothesis: The increased risk of neonatal abstinence syndrome associated with methadone exposure compared with buprenorphine will be stronger in infants delivered after 36 completed weeks of gestation because of preterm birth's role as a mediator.

Specific Aim 3. To build a model predictive of recurrent preterm birth that elucidates the interrelationship between pregnancy histories on the risk of recurrent spontaneous preterm birth among women treated prophylactically with weekly 17-alpha-hydroxyprogesterone caproate injections.

Hypothesis: Predicted risk of recurrent preterm delivery will be most influenced by the gestational age of the earliest previous spontaneous preterm deliveries, but this effect will be modified by the number of previous spontaneous preterm births in women treated with 17-alpha-hydroxyprogesterone caproate intramuscular injections.

Overall Impact: These results may alter treatment preferences for medications used for women with an opioid use disorder in pregnancy. Additionally, for use of 17-OHPC, these results will stratify risk and facilitate a patient specific risk profile which will enable treatment to

be better targeted to those most likely to benefit. Taken together, this work will demonstrate the utility of applying epidemiologic methods to the study of medication use in pregnancy.

2.0 LITERATURE REVIEW

2.1 INTRODUCTION

2.1.1 Pharmacotherapy in pregnancy

Medication use in pregnancy is on the rise. More than 90% of pregnant women in the U.S. used at least one over the counter or prescription medication during pregnancy in 2006-2008; with an average of 4 medications [1]. The number of women entering pregnancy already receiving treatment for chronic conditions, such as hypertension or opioid use disorder, and women receiving medication therapy for gestation-related conditions such as preeclampsia, gestational diabetes or preterm labor, have both recently increased.

Despite these increases in utilization, **very little is known about medication safety and efficacy in pregnancy**. There are several factors contributing to the paucity of data in pregnancy. A major barrier is the enormous liability concerns that discourage pharmaceutical companies and academics from studying pregnant women. The additional risk posed to both mother and fetus is often enough to prohibit randomized clinical trials and to discourage participant involvement [9]. An additional disincentive to the pharmaceutical industry is that a pregnant woman's care provider often will continue to provide medications if the clinical circumstances demand that the medication be used, despite a lack of precise pharmacological information.

Further complicating research in this population is the need for long-term follow-up for childhood outcomes. To fully quantify the risk of outcomes such as attention deficit hyperactive disorder, follow-up must be adequate. In addition to long lengths of follow-up, large sample sizes are required to document associations with rare birth defects. For example, the most common birth defect diagnosed in the U.S., congenital heart defects, occur at a rate of about 1% with an estimated 40,000 affected births each year in the U.S. [10, 11]. In order to demonstrate an increased risk resulting from medication exposure, investigators may need thousands of pregnant women with documented exposure to the medication of interest.

As a way to overcome the aforementioned challenges, large exposure registries in pregnancy that facilitate pharmacoepidemiologic research should be supported. This is a practice now being implemented through post marketing surveillance and the new Food and Drug Administration (FDA) Pregnancy and Lactation Labeling Rule. Utilizing large, extant databases minimizes a number of the previously described limitations. Benefitting from the fact that pregnant women often continue medication use in pregnancy, and analyzing this data retrospectively is a safe approach to address the risks and benefits of treatment in large populations. Epidemiologic and statistical methods are then needed to overcome the new biases inherent in observational data. Only when these studies, along with pharmacokinetic and pharmacodynamic studies, are performed more regularly, will we have enough data to define the associated efficacy and risks of medication use in pregnancy. Such large data repositories were used in this project to study medication use and optimization in pregnancy.

2.2 OPIOID USE DISORDER IN PREGNANCY

The use of prescription opioids and heroin has dramatically increased over the past decade in the U.S. [2, 12]— a trend that is consistent not only in women of childbearing age [13] but also extends to pregnancy [3, 14]. From 1998 to 2011 the reported prevalence of opioid abuse or dependence in pregnancy increased by 127% [14]. Opioid use disorder includes abuse of or dependence on prescription opioids and/or heroin. The DSM-IV and DSM-V criteria for opioid use disorder were used to create the International Classification of Disease (ICD) codes indicating opioid use disorder.

2.2.1 Medication treatment options

Current guidelines suggest replacement therapy, termed opioid maintenance therapy, over detoxification in pregnancy due risk of fetal demise and maternal relapse [4, 15]. Maintaining opioid exposure throughout pregnancy with such treatments decreases risk of withdrawal and relapse while also reducing risky behaviors associated with obtaining opioids illegally. The American Congress of Obstetricians and Gynecologists (ACOG) guidelines support the use of methadone or buprenorphine.

Methadone is a Schedule II, full opioid agonist and has been the mainstay of care for opioid replacement since the 1970s. Conversion to methadone treatment can be done at a licensed outpatient program, or through inpatient services contingent on transfer to a methadone clinic post-discharge [4]. Initial doses are tailored to withdrawal symptoms, with subsequent dose changes throughout pregnancy accommodating altered physiologic and pharmacokinetic

states that occur in pregnancy. In the U.S., methadone treatment must be obtained through a daily visit to a licensed methadone provider [16].

In 2002, buprenorphine, a partial opioid agonist, was approved for treatment of opioid use dependence. Benefits gained with this drug's arrival on the market are less drug-drug interactions, less abuse and overdose potential, and availability at outpatient clinics [17]. Buprenorphine is available as its pure form (Subutex®), or in combination with an opioid antagonist, naloxone, intended to decrease diversion and abuse (Suboxone®). Buprenorphine alone (Subutex®) is generally preferred in pregnancy to avoid exposure to naloxone which has unknown fetal safety data. Buprenorphine is classified as a schedule III narcotic, and can therefore be prescribed with a prescription in an office setting by a specifically licensed prescriber for up to 30 days [18]. **Use of buprenorphine in pregnancy has been on the rise since its FDA approval** [17] **likely due to its potentially superior perinatal outcomes** [17, 19].

2.2.2 Neonatal abstinence syndrome

Neonatal abstinence syndrome (NAS) is the term used for postnatal opioid withdrawal that can occur among infants exposed in utero to opioids, including maintenance therapies [20]. Not surprisingly, increasing trends in NAS have paralleled the opioid epidemic in the U.S. with a nearly three-fold increase in prevalence from 2000 to 2009 [21]. NAS is expected to occur in up to 94% of infants exposed to opioids in utero [22].

The exact pathophysiology of NAS remains unknown [23]. NAS may present as a variety of symptoms originating from the autonomic and central nervous systems. Such symptoms include, but are not limited to, irritability, tremors, seizures, diarrhea, sleep disturbances,

respiratory distress, feeding difficulties, failure to thrive, and rarely neonatal demise [20, 22, 23]. These symptoms are monitored using the Finnegan Scale for NAS diagnosis.

The Finnegan Scale is the most commonly used tool to evaluate neonates for NAS [20, 24]. Though it has been argued to be too subjective, no objective scoring instrument exists at this time. The American Academy of Pediatrics recommends that all infants with known in utero exposure to opioids be monitored in the hospital for 5 to 7 days postpartum. This extended period allows health professionals to administer the Finnegan Scale over many days, as timing of NAS presentation varies by opioid type [20].

The most commonly used pharmacologic treatment in infants requiring medication in the U.S. is morphine or methadone administration, with dosing tailored to symptoms of withdrawal [20]. Non-pharmacologic care for NAS is largely undefined [20], and pharmacologic protocols vary by institution [25].

In addition to the discomfort and short-term risks resulting from NAS, it is also associated with long-term adverse effects. There have been documented associations with decreased visual acuity [26], ear infections and behavioral and cognitive impairments [27]. Finally, NAS is a large burden on our healthcare system. In 2009 alone, hospital charges associated with NAS totaled an estimated $720 million [21].

2.2.3 State of the current evidence

The landmark randomized controlled trial (RCT) comparing opioid maintenance therapies in pregnancy is the MOTHER (Maternal Opioid Treatment: Human Experimental Research) Trial. The findings of this RCT demonstrated superior perinatal outcomes with use of buprenorphine compared with methadone including less severe NAS. The MOTHER

Trial was a double-blind, flexible-dosing, RCT, that randomized opioid dependent pregnant women to treatment with buprenorphine or methadone at 8 international cites from 2005 to 2008. In the final analysis of 73 pregnancies exposed to methadone and 58 to buprenorphine, authors found significantly lower NAS treatment doses (mean dose, 1.1 mg vs. 10.4 mg) with shorter duration (4.1 days vs. 9.9 days), and shorter hospital stays (10.0 days vs. 17.5 days) in the buprenorphine-treated group. Despite these differences, there was no statistically significant difference in the incidence of NAS nor the peak NAS score (47 vs. 57%, buprenorphine vs. methadone, respectively).

Comparative studies of methadone versus buprenorphine in pregnancy have been recently summarized in two large meta-analyses, both inclusive of the seminal MOTHER Trial [28]. **The findings of these meta-analyses summarizing the past fifteen years of publications are in general agreement that buprenorphine is associated with superior perinatal outcomes compared with methadone.**

In a meta-analysis conducted in 2014 by Brogly et al., authors assessed the risk of a variety of outcomes associated with each treatment. Authors combined and analyzed 12 studies comprised of 855 in utero exposures to methadone and 515 in utero exposures to buprenorphine from 1996 to 2012. They found that infants exposed to buprenorphine compared with methadone had longer gestations, higher birth weights, lengths and head circumferences at birth. These authors also found that the risk of NAS was lower in buprenorphine treated pregnancies [Risk Ratio (RR) = 0.90, 95% CI: 0.81, 0.98], with lower treatment doses (−3.60 mg, 95% CI: −7.26, 0.07), shorter durations of treatment (−8.46 days, 95% CI: −14.48, −2.44) and shorter hospital stays (−7.23 days, 95% CI: −10.64, −3.83). Results also demonstrated that mothers receiving buprenorphine treatment were less likely to use illicit opioids near time of delivery [19].

A meta-analysis conducted by Zedler et al. two years later, comprised of three RCTs (n=233) and 15 observational studies (n=1923), supported these findings showing buprenorphine to be associated with a lower risk of preterm birth [RCT: RR = 0.40, 95% CI: 0.18, 0.91; observational (OBS): RR = 0.67, 95% CI: 0.50, 0.90], increased birth weight [RCT: weighted mean difference (WMD) = 277 g, 95% CI: 104, 450; OBS WMD = 265 g, 95% CI: 196, 335] and larger head circumference (RCT WMD = 0.90 cm, 95% CI: 0.14, 1.66; OBS WMD = 0.68 cm, 95% CI: 0.41, 0.94) compared with methadone. This review did not address risk of NAS [17]. Zedler found no significant difference between fetal death nor congenital malformations, and Brogly et al. did not include this in their outcome assessments.

2.2.4 Major limitations in the literature

2.2.4.1 Unmeasured confounding

A significant bias in the comparison of methadone and buprenorphine is inherent in this research due primarily to the differing delivery systems in the U.S. Because the delivery system for buprenorphine allows for more diversion, often women who are considered more 'trustworthy' by the provider and less likely to relapse and divert the drug are prescribed buprenorphine. Conversely, women who are considered less reliable, likely with more severe addiction, are frequently thought to benefit from the daily observed therapy that methadone treatment mandates. Moreover, women with severe addiction will often choose to be treated with methadone as they feel it is a more powerful opioid agonist. This creates the potential for confounding by severity of opioid addiction — a factor that contributes both to opioid maintenance therapy selection and potentially to risk of NAS. In observational studies, it is difficult to collect data on severity of addiction and prescribing preferences partially due to the

strong stigma associated with opioid use disorder in pregnancy. Therefore, retrospective datasets used to study this topic are typically limited by an absence of detailed information on both addiction and treatment trajectories. Even in prospective studies, this information is generally lacking as this population is notoriously difficult to follow. **The resulting, often unmeasurable, differences in treated patient populations makes it difficult to compare the direct effects of these medications using standard statistical methods.**

Even the landmark MOTHER RCT [28] was plagued with this intrinsic bias. In this RCT that found buprenorphine to be associated with less severe NAS compared with methadone, 33% of women randomized to buprenorphine discontinued treatment, with 71% of them reporting "dissatisfaction" with treatment. This is in stark contrast to only 18% of methadone patients discontinuing treatment, of which only 13% reported "dissatisfaction" with treatment. These women were considered "lost to follow-up" and their treatment trajectories were not followed; however, in clinical practice patients can be converted from buprenorphine to methadone but not vice versa due to buprenorphine's antagonist effect which precipitates withdrawal. The differences in attrition by treatment group introduce the possibility that women with more severe addiction remained only in the methadone-treated group. If this occurred, benefits seen with buprenorphine treatment may in fact be attributable to the severity of opioid dependence in the methadone-treated group. Investigators had very few markers of severity of addiction and were therefore unable to assess if severity differed by treatment in the final analytic population, which was limited to women compliant with randomized medication. Furthermore, despite randomization, women allocated to methadone treatment had longer cumulative lifetime drug use. These factors result in similar biases seen in observational studies. Authors of the MOTHER Trial attempted to address the bias by repeating analyses in a sub-cohort limited to women

receiving an arbitrarily determined 'high-dose' of methadone (≥100mg), as a marker of severe addiction, compared with buprenorphine. They found no difference in results, but were underpowered to detect a difference.

This bias in prescribing preferences, whether referred to as confounding by severity or indication, is acknowledged widely in the literature today, but is rarely addressed [29-31]. The majority of existing cohort studies conducted unadjusted analyses [32-35] or adjusted for the limited variables available in their data sets that may be related to severity of addiction such as education, race, indication for opioid maintenance, maternal age, and/or smoking status but few to no direct markers of severity (e.g. documented relapse) [36, 37]. Unfortunately a few extended to control for variables that may be on the causal pathway of the association between opioid maintenance therapy and NAS, such as adequacy of prenatal care, gestational age at initiation of treatment, and heroin use in pregnancy [38, 39].

To our knowledge, the only study beyond the MOTHER Trial that attempted to address unmeasured confounding, despite it being commonly mentioned as a limitation of publications, was in the meta-analysis conducted by Brogly et al. The authors conducted a sensitivity analysis to address the role of unmeasured confounding, applying this specifically to the association between opioid maintenance therapy and NAS. The results of this analysis were limited as authors chose subjective parameters derived from extant literature alone that were admittedly based on strong assumptions. The association of treatment with NAS became null in the results of the sensitivity analysis. Similar bias analyses have been replicated with slight changes in the bias inputs that resulted in minimal changes from the original results, contrary to Brogly's findings [31]. **Up to this point, the only study using epidemiologic methods to address this well-documented bias, was informed entirely from extant literature.**

Our study is innovative as it is the first to utilize empirical data to inform the parameters of the bias analysis. This approach eliminates the subjectivity of applying data from the literature, and allows us to use tighter ranges of potential values based upon surrogate markers of severity of addiction in our internal validation cohort.

2.2.4.2 Role of gestational age

The current literature comparing buprenorphine with methadone use in pregnancy generally ignores the role of gestational age, or inappropriately attempts to account for it by adjusting for gestational age [39, 40]. Gestational age is inherently a complex issue in perinatal research, one that is frequently ignored despite the fact that it can have significant impact on findings and conclusions [41, 42]. Gestational age plays an important role in the association between opioid maintenance therapy and NAS.

Methadone is associated with preterm birth, both in comparison with no treatment or with buprenorphine [17, 37, 39, 43, 44]. **Preterm birth is associated with a lower frequency and less severe NAS after exposure to methadone** [45-47]. Therefore, gestational age may in fact lie on the causal pathway of this association and therefore should be assessed as a mediator. When gestational age is adjusted for, this assumes that gestational age is associated with both treatment and NAS. However, due to the temporality of these relationships, gestational age cannot contribute to treatment choice and therefore does not meet the criteria of a confounder. When gestational age is included in the model as a confounder, the association is generally biased away from the null, conferring an even higher benefit associated with buprenorphine use compared with methadone. Alternatively, not addressing gestational age through adjustment nor methods like a survival analysis, demonstrates the association that is a result of both treatment and gestational age together.

No study to date has attempted to isolate the role of gestational age in the association between methadone and NAS compared with buprenorphine. Because preterm birth may mediate the association, the true association between methadone and NAS compared with buprenorphine may in fact be stronger than is documented when the association is studied among term births alone. By evaluating the role of gestational age as mediator, **our study will be the first to quantify the risk of NAS associated with opioid maintenance therapy independent of prematurity.**

2.3 SPONTANEOUS PRETERM BIRTH IN THE U.S.

Preterm birth remains a leading cause of neonatal and infant mortality, contributing to an estimated 75% of all perinatal deaths [5, 6, 48, 49]. Preterm birth is defined as delivery prior to 37 weeks' gestation. Nearly 1 out of every 10 live born infants is delivered preterm in the United States [50], costing an estimated $26 billion in 2006 [51]. Preterm birth carries with it severe short and long term sequelae including respiratory difficulties, intracranial hemorrhage, infection, necrotizing enterocolitis, and developmental disabilities to name a few [52]. Preterm birth can be defined generally as medically necessary (30-35%) for maternal or fetal indications, or as spontaneous (65-75%) [49]. This work will focus solely on spontaneous preterm births. Generally, spontaneous preterm birth is caused by preterm labor or preterm premature rupture of membranes (pPROM). Preterm labor, the more common cause of spontaneous preterm birth, is defined as "regular contractions of the uterus resulting in changes in the cervix that start before 37 weeks of pregnancy" [48]. The exact mechanism triggering preterm labor is unknown. Similarly, the cause of the majority of pPROM cases are also unknown. PPROM is defined as

spontaneous rupture of the membranes prior to 37 weeks' gestation. Preterm labor and pPROM share the majority of their known risk factors, with pPROM occurring more commonly in women with undiagnosed intrauterine infections [49].

2.3.1 Obstetric history as a risk factor for preterm birth

Predicting preterm birth is difficult as the pathophysiology is not well-defined and is likely multi-factorial. Despite this, it is accepted that previous spontaneous preterm birth is one of the strongest predictors of recurrent preterm birth [7, 53-56]. Along with obstetric history, risk factors range from genetic and biologic differences to behaviors and factors unique to the current pregnancy. Each of these broad categories has been studied with conflicting results. This project will focus on obstetric history and its role in predicting recurrent spontaneous preterm birth.

The characteristics of obstetric history that are most commonly studied and thought to contribute to risk of recurrent spontaneous preterm birth are: the gestational age at which previous preterm birth(s) occur(s), the number of previous preterm and term births, the outcome of the most recent pregnancy, and the cause(s) of previous preterm birth(s).

Studying which of these obstetric history factors is the most predictive of recurrent preterm birth and their influence on each other is critical as this could optimize treatment in those women most likely to respond. This approach may also elucidate potential mechanisms of preterm birth that are consistent across pregnancies.

2.3.2 17-alpha-hydroxyprogesterone caproate

17-alpha-hydroxyprogesterone caproate (17-OHPC) is currently the only FDA-approved formulation of injectable progestin with the indication of prevention of recurrent spontaneous preterm birth in singleton pregnancies with a history of a singleton spontaneous preterm birth. For decades, there was no effective treatment for the prevention of preterm birth. Today, only one medication therapy-beyond physical interventions such as cerclage and cervical pessaries-has been proven to be efficacious in preventing recurrent preterm birth in high-risk women: progestin.

Progesterone, a hormone occurring naturally and sustaining early pregnancy, is known to suppress uterine contractions and promote uterine quiescence, though the exact mechanism is unknown [57]. A commonly accepted biologic mechanism of preterm birth stems from evidence that anti-progestins increase myometrial contractions [58]. By extension, progesterone withdrawal may contribute to labor. These hypotheses fueled research into 17-OHPC as a prophylactic treatment for preterm birth. Progestins have been tested for preterm birth prevention as both an intramuscular weekly injection, supplied as 17-OHPC, and vaginally in micronized form. In 2003, a seminal trial conducted by Meis et al., described in detail below (section 2.3.3.1), demonstrated a decreased risk of recurrent preterm birth by approximately 34% in women with a prior spontaneous preterm birth who were treated prophylactically with 17-OHPC intramuscular injections [8]. Largely as a result of these findings and expert opinions, adoption of 17-OHPC use in this high risk population has been widely accepted and implemented.

In fact, current **ACOG guidelines recommend that women with singleton gestations and a history of at least one prior spontaneous preterm singleton birth be treated with 17-OHPC injections.** This treatment is administered as a weekly 250 mg intramuscular injection

initiated at 16 to 24 weeks gestation and continued until 36 weeks of completed gestation or delivery, whichever occurs first. In keeping with clinical practice, this project will focus on the use of 17-OHPC injections as prophylactic treatment for spontaneous preterm birth in women with a history of spontaneous preterm birth of a singleton.

2.3.3 Review of the current evidence of 17-alpha-hydroxyprogesterone caproate in the prevention of recurrent preterm birth

It is a public health priority to better identify women at high risk for recurrent preterm birth, and furthermore those most likely to benefit from prophylactic treatment with 17-OHPC as it is the only currently available therapeutic option. Consistent with the aims of this project, this review will focus on studies identifying women most likely to respond to 17-OHPC injection therapy based on obstetric history. Current research aimed at decreasing preterm birth has 3 general concentrations: 1) identification of women at the highest risk for preterm birth, 2) identifying women most likely to benefit from treatment, and 3) developing further therapeutic options to prevent preterm birth. Our project primarily addresses the first two arms of this research. Each study included in the review defined preterm birth as delivery prior to 36 weeks of completed gestation unless otherwise noted.

2.3.3.1 Studies assessing the effect of gestational age of earliest spontaneous preterm birth, number of previous spontaneous preterm births, and gestational age of penultimate pregnancy

The landmark trial assessing the utility of 17-OHPC, mentioned previously, was conducted by Meis et al. in 2003 [8]. This was a double-blind, placebo-controlled RCT conducted at 19 clinical

centers from 1998-1999. Recruitment was limited to women with at least one previous spontaneous preterm delivery between 20 and 36^6 weeks of gestation. Participants were randomized in a 2:1 manner to treatment with weekly 250mg injections of 17-OHPC (n=310) or inert oil placebo (n=153) beginning at 16 to 20^6 weeks gestation and continuing through 36^6 weeks gestation or until delivery. These authors found that **17-OHPC significantly decreased the risk of recurrent preterm birth prior to 37 weeks (RR 0.66, 95% CI: 0.54, 0.81) and at less than 32 weeks (RR 0.58, 95% CI: 0.37, 0.91).**

Two secondary analyses of this cohort were conducted by Meis et al. and Spong et al. in 2005 [53, 59]. The objective of the first, conducted by Meis et al., was to assess how maternal age, race, parity, prepregnancy body mass index (BMI), education, smoking status, alcohol use, number of previous preterm deliveries, number of previous abortions or miscarriages, number of total previous deliveries, interval since preterm delivery, history of a previous term delivery, and gestational age of penultimate pregnancy (term vs. preterm) influenced the risk of recurrent preterm birth and further how these factors influenced the effectiveness of treatment with 17-OHPC [53]. Authors conducted all univariate logistic regressions separately by exposure to 17-OHPC, assessing which variables were associated with recurrent preterm birth in each group. Their final models showed that women treated with placebo had increased risk of recurrent preterm birth if they experienced more than 1 previous preterm delivery, the penultimate delivery was preterm, and if the patient was obese prior to pregnancy. In the women treated with 17-OHPC, the increased risk attributed to a history of more than one previous preterm birth was eliminated and only the increased risk associated with a preterm penultimate pregnancy remained. These authors concluded that treatment with 17-OHPC principally reduces the risk

associated with more than one previous preterm birth, in addition to an overall benefit of decreased preterm birth risk in women with a history of previous spontaneous preterm birth.

Again utilizing the data from the seminal trial, Spong et al. evaluated if the effectiveness of 17-OHPC varied by gestational age of the earliest previous spontaneous preterm delivery, classified as 20-27.9, 28-33.9, and 34-36.9 weeks [59]. Analysis consisted of statistical comparisons between groups of earliest gestational ages, logistic regressions within each subgroup adjusted for African American race, Hispanic ethnicity, gestational age at randomization, and more than one previous spontaneous birth, and a Cox Proportional Hazards survival analysis with censoring at 37 weeks. Findings from this secondary analysis supported an overall decrease in risk of recurrent spontaneous birth with 17-OHPC use, and further found the greatest benefit of treatment in women with earliest gestational ages of <34 weeks.

Taken together, these secondary analyses demonstrated that in addition to an overall benefit of decreased preterm birth risk in women with a history of previous spontaneous preterm birth, that treatment with **17-OHPC principally reduced the risk associated with more than one previous preterm birth and was most effective in women with a prior spontaneous preterm birth before 34 weeks' gestation**.

In an additional retrospective cohort, investigators analyzed records from women with a singleton gestation and at least one prior spontaneous preterm birth with 17-OHPC delivered according to provider discretion as routine care [60]. These authors shared the same primary aim as Spong et al, to determine how the earliest gestational age of a previous spontaneous preterm birth (classified as 20-27.9, 28-33.9, and 34-36.9 weeks) impacted the effectiveness of 17-OHPC. A total of 2978 women received treatment with 17-OHPC and 1260 did not, despite history of spontaneous preterm birth. Contrary to Spong et al. [59], findings demonstrated that

17-OHPC showed similar benefit and decreased rates of spontaneous preterm birth regardless of gestational age at earliest preterm birth. Incidence of recurrent preterm birth in the 17-OHPC treated compared with the untreated were: 32.2% vs 40.7% in 20-27.9 weeks, 34.1% vs 45.5% in 28-33.9 weeks, and 29.3% vs 38.8% in 34-36.9 weeks. These results are likely biased as, without randomization, women who received treatment with 17-OHPC were different than those who opted for no treatment. Specifically, the group with the earliest documented gestational ages at 20-27.9 weeks in previous pregnancies were more likely to receive treatment with 17-OHPC compared with those with an earliest age of 34.9-36 weeks. This confounding relationship is unaccounted for; however, other potential confounders such as Black race, more than 1 previous preterm delivery, and smoking status were adjusted for.

Incorporating appropriate weights for each study's biases and limitations, the results of these studies support the overall benefit of 17-OHPC implementation with an additional benefit gained in women with earlier previous gestational ages.

2.3.3.2 Studies comparing the risk of recurrent preterm birth by indication of previous preterm birth

The difference in risk of recurrent spontaneous preterm birth by indication of previous spontaneous preterm birth, preterm labor versus preterm premature rupture of membranes (pPROM), is another obstetric history characteristic of interest. This factor was not addressed in the seminal RCT, which included women with either type of spontaneous preterm birth history. Gonzalez-Quintero approached this question using a dataset of 2,123 women with singleton gestations and a history of one prior spontaneous preterm birth being administered weekly 17-OHPC [61]. Findings from unadjusted logistic regressions demonstrated that women with preterm labor had higher rates of recurrent spontaneous preterm birth compared with those

delivered for pPROM at both 37 (29.7 vs. 2,2.9%) and 32 weeks (5.9 vs. 3.3%). However, in this study women with a history of preterm labor also had a higher incidence of previous preterm births at 20 to 27.9 weeks compared with 28 to 33.9 weeks in the pPROM group. Therefore, the conclusions of these investigators that the previous clinical preterm birth presentation influences the risk of recurrent preterm delivery may in fact be reflective of the impact of gestational age. The authors went further and concluded that beyond the type of previous spontaneous preterm birth, the gestational age of the prior preterm birth was also associated with recurrence- though the effect of previous gestational age varied by previous clinical presentation. In women with a history of preterm labor there was no association between previous gestational age and recurrent preterm birth. Conversely, in women with a history of pPROM, the rate of recurrence was highest in those with a previous delivery at a gestational age of 28-33.9 compared with both deliveries <28 weeks and ≥34 weeks. This association is likely an artifact of their unadjusted approach and lack of placebo-controlled group for comparison. Though rates of recurrence are typically higher in the earlier previous age groups, women in these earlier groups may benefit more from treatment [59].

Similar to Gonzalez-Quintero et al., Coleman et al. used a database inclusive of 1,183 singleton pregnancies with a history of a preterm birth in the previous pregnancy and current treatment with 17-OHPC. They assessed the risk of recurrent preterm birth by the clinical presentation of the penultimate preterm birth—i.e. preterm labor versus pPROM [56]. Using unadjusted logistic regression models they found three obstetric history variables that significantly increased the risk of recurrent preterm birth despite treatment: more than one previous preterm birth (OR 1.80, 95% CI: 1.33, 2.44), a penultimate birth at 28 to 33.9 weeks' gestation compared with 34–36.9 weeks' gestation (OR 1.61, 95% CI: 1.22, 2.13), and

penultimate delivery presenting as preterm labor compared with pPROM (OR 1.66, 95% CI: 1.16, 2.37). These findings are generally consistent with Gonzalez-Quintero's work above, demonstrating **an increased risk of recurrent preterm birth despite treatment with 17-OHPC in women with a history of preterm labor compared with pPROM and in those with earlier gestational age of most recent preterm birth. It is important to note that the results from each of these studies are derived from unadjusted analyses.**

2.3.3.3 Studies evaluating "response" versus "no response" to 17-alpha-hydroxyprogesterone caproate

Recently a group of authors redefined treatment 'response' to 17-OHPC by classifying the difference in gestational age from the 17-OHPC treated pregnancy and the woman's earliest spontaneous preterm birth. Prolongation of gestational age by three or more weeks from a subject's earliest spontaneous preterm birth was considered 'response'. Authors were attempting to better classify utility of 17-OHPC, but in doing so may have lost clinical relevance. Under this definition infants born at 24 weeks would be classified as 'responders' if the earliest previous preterm birth occurred at 20 weeks. In practice these infants remain at risk for the negative effects associated with preterm birth and therefore may not have benefitted significantly from treatment.

This new outcome was applied using data from *Eunice Kennedy Shriver* NICHD Genomic and Proteomic Network for Preterm Birth Research. Manuck et al. performed a retrospective study evaluating the effect of pregnancy history, maternal demographics and antenatal factors on the gestational age in pregnancy treated with 17-OHPC [62]. 'Responders' (n=118) were compared with 'non-responders' (n=37) for statistical differences in characteristics. In this study authors used a stepwise backward elimination process to define the

logistic regression model, including all variables with a p-value <0.20 in the final model. Only two obstetric history variables were tested, 1) gestational age of the earliest previous spontaneous preterm birth, and 2) a history of abruption in a previous pregnancy. Results showed that the only pregnancy history factor that predicted treatment response was the gestational age of the earliest previous spontaneous preterm birth (OR 0.68, 95% CI: 0.56, 0.82). Results were consistent when the cohort was limited to women with a penultimate preterm birth, as this was associated with nonresponse to treatment. Additional factors, beyond obstetric histories, that remained predictive in the final model included vaginal bleeding or abruption in the current pregnancy and first degree family history of spontaneous preterm birth- consistent with the authors' previous findings [63].

An additional secondary analysis, conducted by the same group, again used the "response vs. nonresponse" outcome. This study utilized a cohort of women all treated with 17-OHPC from the NICHD MFMU RCT titled "Omega-3 Trial", the same cohort used in this project [63]. This RCT compared the effect of omega-3 supplementation with placebo in a cohort of 852 women with a history of spontaneous preterm birth and treated with weekly 17-OHPC injections. Omega-3 supplementation had no significant effect on outcomes or effect of 17-OHPC. The objective of this secondary analysis was to create a clinical risk scoring system based on the novel definition of 'response' to treatment in place of the standard preterm birth definition. The final analytic sample consisted of 595 'responders' (of which 27% still had a preterm birth) and 159 'nonresponders', all treated with 17-OHPC. Using multivariate logistic regression they identified risk factors for 'nonresponse' which included two obstetric history variables: each additional week of gestation of earliest previous preterm birth (OR 1.23, 95% CI: 1.17, 1.30) and a penultimate preterm birth (OR 2.10, 95% CI: 1.03, 4.25). No other obstetric history variables

were tested in the multivariable analysis. Variables independently significant in predicting nonresponse beyond obstetric history included placental abruption or significant vaginal bleeding in the current pregnancy, diagnosis of gonorrhea and/or chlamydia, and having a male fetus. When this prediction model was validated in a separate sample it had a sensitivity and specificity of only 65% and 67%, respectively, but findings were consistent with their previous study.

Applying the new outcome definition of 'response' findings continued to support the association between gestational age of earliest preterm birth and outcome of penultimate pregnancy with recurrent preterm birth. Similar to the two secondary analyses of the Meis trial [8], both studies concluded that **women with earlier previous gestational ages, and penultimate term births are more likely to 'respond' to treatment with 17-OHPC, and therefore are less likely to have a preterm birth.**

Notwithstanding significant biases, the extant literature taken together is consistent and supports an effect of obstetric history on the effectiveness of 17-OHPC.

2.3.3.4 Major gaps in the literature

Notwithstanding the vast amount of literature assessing the role of obstetric history in recurrent preterm birth despite treatment with 17-OHPC, no study to date has assessed the interplay between these characteristics. It is reasonable to expect that the risk associated with one factor, for example number of previous spontaneous preterm births, would be modified by another such as the age at which the earliest occurred. Assessing each of these individually rather than in concert is a gap in the literature that we will fill by completing this aim. **We will address this critical gap by evaluating interactions between each available obstetric history variable.**

Furthermore, **the majority of the extant literature did not adjust for crucial confounders when assessing various risk factors.** In simple epidemiologic terms, all factors known to be associated with both the exposure (17-OHPC or obstetric history variable) and the outcome (recurrent preterm birth) that are not on the causal pathway should be considered potential confounders and subsequently adjusted for. It has been well-documented that despite ACOG guidelines and proven evidence of effect, many women do not receive treatment with 17-OHPC, regardless of a history of spontaneous preterm birth and accessibility [64, 65]. Research has indicated that treatment with 17-OHPC is lower in women with later gestational ages of previous preterm deliveries and in those whose penultimate pregnancy ended in a term delivery [64, 65]. These results suggest that these factors, potentially in addition to others, may then be associated with both exposure and outcome and are therefore critical confounders of the 17-OHPC and recurrent birth relationship. This bias is of critical importance in retrospective studies that include both treated and untreated women who are not randomized [60].

In regards to obstetric history as the exposure of interest, there are likely numerous factors that contribute to both the previous and recurrent spontaneous preterm delivery that remain unidentified. The undefined pathophysiology of preterm birth makes it challenging to identify shared causes of each delivery. This is a limitation that exists in all retrospective studies on this topic, but can be addressed by using a probabilistic bias analysis for unmeasured confounding. It is important to note that it may not be prudent to eliminate the effects of these other unknown characteristics, but rather identify which of those factors are shared to determine the mechanisms through which preterm birth may be occurring. **We will address the biases introduced by these commonly ignored characteristics through our model building**

approaches including all available potential confounders identified a priori through direct acyclic graphs in our original model [66].

Finally, **observational studies focused on efficacy of 17-OHPC are limited by documentation of compliance with the intensive weekly regimen.** 17-OHPC is administered as a weekly intramuscular injection initiated between 16^0 and 20^6 weeks' gestation and continued until 37 weeks or delivery, whichever occurs first. The injections cause local pain at the site of injection in more than 30% of women receiving treatment (Makena Package Insert). This burdensome treatment regimen along with frequent side effects often decreases compliance. In many observational studies the adherence to treatment is not recorded, particularly in women who administer injections at home. **We will overcome this limitation in our work by utilizing data from a RCT with detailed information on adherence to the treatment regimen, allowing us to limit the cohort to those with >50% compliance.**

3.0 MANUSCRIPT 1: METHADONE VERSUS BUPRENORPHINE FOR OPIOID USE DEPENDENCE AND RISK OF NEONATAL ABSTINENCE SYNDROME

Lara S. Lemon[1,2,*]
Steve N. Caritis[3]
Raman Venkataramanan[2,4]
Robert W. Platt[5]
Lisa M. Bodnar[1,3]

1 Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh
2 Department of Pharmaceutical Science, School of Pharmacy, University of Pittsburgh
3 Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine, University of Pittsburgh
4 Department of Pathology, School of Medicine, University of Pittsburgh
5 Department of Epidemiology, Biostatistics and Occupational Health, McGill University

3.1 ABSTRACT

Background Our objective was to estimate the association between methadone and neonatal abstinence syndrome (NAS) compared with buprenorphine using a probabilistic bias analysis to account for unmeasured confounding by severity of addiction.

Methods We used a cohort of live-born infants exposed in utero to methadone or buprenorphine for maternal opioid maintenance therapy at Magee-Womens Hospital in Pittsburgh, PA from 2013–2015 (n=716). Exposure and outcome status were determined using pharmacy billing claims. Log-binomial regression models were used to assess the association of treatment with NAS after adjusting for parity, maternal race, age, delivery year, employment, hepatitis C,

smoking, marital, and insurance status. We implemented a probabilistic bias analysis, informed by an internal validation study, to assess the impact of unmeasured confounding by severity of addiction.

Results Infants exposed to methadone in utero were more likely to experience NAS compared with those exposed to buprenorphine [RR: 1.3, 95% CI: 1.2, 1.5]. After adjustment, infants exposed to methadone were 30% (adjusted RR 1.3, 95% CI: 1.1, 1.5) more likely than infants exposed to buprenorphine to have NAS. In the validation cohort (n=200), severe addiction was more common in the methadone- compared with buprenorphine-exposed deliveries (77% vs. 32%). However, adjustment for severe addiction in the bias analysis only slightly attenuated the association (RR 1.2, 95% CI: 1.0, 1.4), supporting the conventional analysis.

Conclusions Methadone is associated with increased risk of NAS compared with buprenorphine in infants exposed in utero. This association is subject to minimal bias due to unmeasured confounding by severity of addiction.

3.2 INTRODUCTION

Pregnant women are not immune from the opioid epidemic in the U.S.[2, 12, 67, 68] The trend of rising opioid use in pregnancy parallels simultaneous increases in the number of cases of neonatal abstinence syndrome (NAS). NAS is a clinical condition in which the infants exposed to opioids in utero manifest symptoms of withdrawal from the drug postnatally.[22, 69, 70] NAS is costly to treat[71] and it has long term sequelae for the child, including neurocognitive and behavioral issues[27] along with decreases in visual acuity.[72] To lessen the risk of NAS and a host of other poor maternal and child health outcomes, pregnant women with opioid use

dependence are treated with either methadone or buprenorphine as opioid maintenance therapy.[4] Literature has consistently shown that buprenorphine use is associated with less NAS and shorter duration of neonatal treatment compared with the use of methadone.[28, 37-40] However, these findings may be biased because large databases often used for this research typically do not contain data on the severity of the mother's addiction—a potential confounding variable.[19, 29]

In the U.S., women who suffer from more severe opioid addiction are often allocated to methadone treatment, while women with lower risk of relapse and drug diversion tend to be treated with buprenorphine. This prescribing preference exists in part because methadone and buprenorphine are delivered with different systems of care in the U.S. Women prescribed methadone must attend a clinic daily to obtain medication under direct observation, eliminating the chance of diversion. Alternatively, women treated with buprenorphine are legally permitted a supply of medication for administration at home through outpatient providers.[18] Therefore, it is critical to account for factors that determine this prescribing preference in comparative treatment studies.

Our objective was to estimate the association between methadone versus buprenorphine exposure as opioid maintenance therapy and NAS after accounting for unmeasured confounding by severity of addiction.

3.3 METHODS

We used data on all singleton pregnancies delivered at 20 to 42 weeks of gestation with live-born infants exposed to in utero methadone or buprenorphine opioid maintenance therapy at Magee-

Womens Hospital (MWH) in Pittsburgh, PA from 2013-2015. MWH delivers over 10,000 infants annually and cares for opioid addicted mothers with treatment protocols similar to those at other U.S. institutions.[32, 33, 39, 40] Buprenorphine is administered through prescription by a certified buprenorphine provider while methadone treatment requires daily visits to an opioid treatment clinic.[73] The protocol is described in detail in the Appendix A.

International Classification of Diseases, Ninth (ICD-9) and Tenth Revision (ICD-10) codes in pharmacy billing claims were used to identify drug-dependent (ICD-9 64831) or drug-complicated deliveries (ICD-10 O99324). Billing claims that specifically documented exposure to methadone or buprenorphine as opioid maintenance therapy were then confirmed with dosing information extracted from the medical chart. Buprenorphine-exposed infants were those whose mothers were treated with Subutex® (buprenorphine, n=299) (Reckitt Benckiser Pharmaceuticals Inc., VA) or Suboxone® (buprenorphine + naloxone, n=10) (Reckitt Benckiser Pharmaceuticals Inc., VA). The exposure window of interest was the day of delivery because medication effect on NAS is most influential closest to delivery [74] and we lacked access to the entire treatment trajectories including treatment initiation dates.

We identified cases of NAS from pharmacy billing codes indicating treatment with morphine after delivery. At MWH, all infants with known or suspected opioid exposure in utero are kept for NAS observation for 5 to 7 days. Infants are scored using the Finnegan Scale every 3 to 4 hours.[24] When the average of 3 consecutive scores is ≥8 on the Finnegan Scale, infants are treated with morphine. In our cohort, morphine treatment was highly correlated with ICD code indicative of “Drug Withdrawal Syndrome in Newborn” (kappa>0.99).

Maternal characteristics and birth outcomes were obtained first from the MWH electronic pharmacy records comprised primarily of billing and ICD codes, and were informed with data

provided by the birth record when data were missing. These data are a combination of self-report, clinical billing codes, and chart documentation by a health professional. Information on maternal race (Black, White, other), education level (less than high school, high school or equivalent, some college, college graduate), employment (yes, no), marital status (married, unmarried), insurance type (private, public), prepregnancy weight and height, parity, smoking during pregnancy (yes, no), and hepatitis C status (positive, negative) were available. Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared and was categorized as underweight (<18.5), normal weight (BMI 18.5 to <25), overweight (BMI 25 to <30), or obese (BMI ≥30).[75] Birth outcome data included gestational age at delivery, infant length of stay (days), birthweight, congenital anomalies (yes, no), admission into the NICU (yes, no), and number of prenatal visits. Gestational age was determined using the best obstetric estimate in the chart from ultrasound or last menstrual period when ultrasound was not available. This study was approved by our Institutional Review Board.

3.3.1 Validation Cohort

Severity of addiction is a potential confounder that was unmeasured in our dataset. We therefore performed a validation study to collect indicators of addiction severity from medical chart abstraction on a random sample of 100 buprenorphine- and 100 methadone- treated women in our cohort. The study team identified four indicators of severity of addiction that were based on literature [19, 29, 34, 76] and clinical expertise (details in Appendix A). One reviewer (LSL), who was blinded to outcome but not exposure status, performed the medical chart abstractions and entered data into an electronic database. The majority of information was abstracted from physicians' notes and the social workers' discharge plans.

We defined severe addiction as having any one of the 4 following indicators documented in the chart: 1) conversion to opioid maintenance therapy during pregnancy, 2) documented relapse during pregnancy, 3) use of illicit substances at delivery, and 4) use of benzodiazepines in pregnancy. When there was no documentation of conversion to opioid maintenance therapy in the chart, women were assumed to have conceived on the same treatment noted at delivery. All other lack of documentation was recorded as missing unless explicitly noted that the patient did not have the indicator (e.g. “patient did not relapse in this pregnancy”). Reconversion to therapy within one pregnancy was recorded as a relapse. Illicit substance use at the time of delivery included any of the following: marijuana, benzodiazepines, illicit buprenorphine, cocaine, nondescript intravenous drugs, heroin, or illicit opiate pills.

3.3.2 Statistical Analysis

Multivariable log-binomial regression models were used to estimate the independent association between NAS and methadone compared with buprenorphine while accounting for clustering within each woman (25 contributed multiple pregnancies).[77] We calculated risk ratios (RR), risk differences (RD), and their 95% confidence intervals (CI). Risk differences were calculated using marginal standardization.[78] We identified potential confounders *a priori* using theory-based conceptual models: maternal indication for opioid maintenance therapy, gestational age at opioid maintenance therapy initiation, duration of opioid dependence, maternal age, race, employment status, smoking status, marital status, insurance type, hepatitis C status, parity and year of delivery. The final model was limited to maternal age, race, employment status, smoking status, marital status, insurance type, hepatitis C status, parity and year of delivery, based on availability of data. We did not adjust for adequacy of prenatal care, total visits to the emergency

room during the pregnancy, and gestational age because they are likely on the causal pathway.[29]

Probabilistic Bias Analysis

To quantify the extent to which unmeasured confounding by severity of addiction biased the association between opioid maintenance therapy and NAS, we performed a probabilistic bias analysis. This approach is based on a set of methods developed and described in detail previously by Lash et al.[79, 80] The parameters for this analysis were informed using data indicative of addiction severity from our internal validation study. We defined the limits of the relative risk due to confounding using the Flanders and Khoury method.[81] This method involved fitting two logistic regressions in the subcohort: the first modeling the odds of treatment type by severity of addiction, the second modeling the odds of NAS by severity of addiction. The Flanders and Khoury method also incorporates the prevalence of severity in each treatment group (Appendix A). This information was used to determine the limits of the trapezoidal distribution used to parameterize the risk. We sampled the risk due to confounding from 100,000 simulated data sets using a Monte Carlo approach. Results were presented as bootstrapped point estimates with an interval defined as the 2.5^{th} and 97.5^{th} percentiles. This interval corresponds to the 95% confidence interval obtained in a conventional analysis but incorporates both systematic and random error. The results from the probabilistic bias analysis were then compared with the risk ratios and 95% confidence intervals from the conventional model.

3.4 RESULTS

There were a total of 872 drug-dependent pregnancies in the study period. Of these, 745 (85%) received either methadone or buprenorphine as opioid maintenance therapy on the day of delivery and were eligible for this study (Figure 1). We excluded 9 women with multi-fetal gestations (18 infants), 6 with a fetal death, and five who had stopped all medication due to relapse or weaning prior to delivery. Our final sample consisted of 716 pregnancies.

Slightly more than half of pregnancies on opioid maintenance therapy were treated with methadone (57%) and the remaining with buprenorphine (43%). Women treated with methadone were more likely than their buprenorphine-treated counterparts to be unmarried, unemployed, hepatitis C positive, multiparous, and to have less than a high school education (Table 1). Methadone-treated pregnancies on average had shorter gestations and infants with lower birthweights. Race, age, prepregnancy BMI, and smoking status were not meaningfully different between treatment groups.

NAS occurred in 58% of the infants (n=415). Infants with treatment for NAS were more likely to be born to unmarried, unemployed, hepatitis C positive mothers with less than a high school education and a normal prepregnancy BMI (Appendix A: Table 12). Infants diagnosed with NAS were also more likely to be born at a later gestational age without a congenital anomaly compared with their counterparts not requiring treatment.

The incidence of NAS was 65% in infants exposed in utero to methadone compared with 49% in infants exposed to buprenorphine. Infants exposed to methadone in utero were 30% more likely than infants exposed to buprenorphine to be treated for NAS (unadjusted RR 1.3, 95% CI: 1.2, 1.5). After adjustment for parity, maternal race, employment status, hepatitis C status, age, year of delivery, smoking status, marital status, and insurance, the association did not change

(adjusted RR 1.3, 95% CI: 1.1, 1.5). On the absolute scale, the adjusted RD was 0.14 (95% CI: 0.059, 0.22), indicating that methadone was associated with 14 excess cases of NAS for every 100 live-born infants born to mothers treated with methadone compared with buprenorphine (Table 3).

Though there were expected significant differences comparing the study cohort of opioid dependent mothers to all births at MWH from 2013-2014 (Appendix A: Table 10), the validation subsample was similar to the full study cohort (Appendix A: Table 11). In the validation subsample, methadone-treated women were more likely than buprenorphine-treated women to have converted to opioid maintenance treatment during pregnancy (58% vs 12%; median gestational age at conversion: 12 weeks vs before conception), relapsed in pregnancy (23% vs 4%), used any illicit substance at delivery (24% vs 15%), or used benzodiazepines during pregnancy (28% vs 8%) (Table 4). Prevalence of having any one of the indicators of severe addiction was higher in the methadone group compared with buprenorphine (77% vs. 32%). This composite of addiction severity was associated with a slightly higher risk of NAS (odds ratio 1.2, 95% CI: 0.7, 2.1). These results were robust to removal of each individual factor included in the severity index (data not shown).

There was a large amount of missing data in the validation cohort that varied by treatment (Appendix A: Table 13). Women treated with buprenorphine were more likely than methadone-treated women to have missing data for more than one indicator of severity. Despite the difference in rate of missing data, women treated with buprenorphine also had documentation indicating less severity (e.g. “patient did not relapse in pregnancy”) more often than methadone-treated women. This is true for each severity indicator excluding benzodiazepine use (Table 4).

After accounting for unmeasured confounding by severity of addiction in the probabilistic bias analysis, the association between methadone and NAS was slightly attenuated from the conventional results [point estimate 1.2 (95% simulation interval: 1.0, 1.4; Table 5)]. The bootstrapped 5^{th} and 95^{th} percentiles in the bias analysis were slightly wider than the conventional confidence intervals as they accounted for both systematic and random error.

3.5 DISCUSSION

There is agreement in the literature that buprenorphine confers benefits over methadone for opioid maintenance therapy in pregnancy, including decreased risk of NAS in the infants exposed in utero.[19, 37, 39, 40] Nonetheless, there is a potential for these findings to be biased due to unmeasured confounding.[19, 29] Our conventional analysis results suggested that the risk of NAS in infants exposed to in utero methadone was 30% higher compared with buprenorphine-exposed infants. The results from the probabilistic bias analysis suggest that unmeasured confounding by severity of addiction only slightly biased the conventional results away from the null. Although we found that women receiving methadone had more indicators of severe addiction than women receiving buprenorphine, the relatively weak relationship between addiction severity and NAS reduced the potential for prescribing differences to confound the primary association.

The ideal approach to eliminate unmeasured confounding is to conduct a randomized controlled trial. However, the largest double-blinded, flexible-dosing, randomized controlled trial comparing methadone and buprenorphine use in pregnancy (Maternal Opioid Treatment: Human Experimental Research trial) was plagued with the same biases faced in observational

research.[28] Analyzing only women who remained on randomized treatment, Jones et al.[28] found no significant difference in percent of infants requiring treatment for NAS between treatment groups, though more morphine (mean dose 10.4 vs. 1.1 mg) and longer hospital stays (17.5 vs. 10.0 days) were needed for infants exposed to methadone in utero. Importantly, investigators found that 33% of women randomized to buprenorphine discontinued treatment, with 71% of them reporting "dissatisfaction" with treatment. This is in stark contrast to only 18% of methadone patients discontinuing treatment, of whom only 13% reported "dissatisfaction" with treatment. Only those women who continued allocated treatment were included in the final analyses. Furthermore, despite randomization, women who remained on methadone treatment had longer cumulative lifetime drug use. Together, these findings demonstrate a similar bias to unmeasured confounding as addiction severity may have influenced treatment choice and continuation regardless of randomization.

Our results are consistent with a large meta-analysis of 11 studies including 855 methadone-treated women and 515 buprenorphine-treated women for opioid dependence and risk of NAS.[19] These authors described a summary estimate of 1.11 (95% CI: 1.02, 1.23) reported as an increased risk of NAS by 10% in infants exposed to methadone compared with buprenorphine in utero. The authors conducted a sensitivity analysis for unmeasured confounding by indication applying the VanderWeele and Arah [82] approach for unmeasured confounding. Unlike our analysis, which was informed by an internal validation study, these authors used bias parameters informed by the extant literature. They found that after accounting for unmeasured confounding by indication, the risk of NAS associated with methadone treatment in the conventional analysis was biased away from the null (50^{th} percentile adjusted RR 1.01, 95% CI: 0.92, 1.11). Consistent with our conceptual model, bias parameters reflected values for

unmeasured confounding that conferred increased risk for poor neonatal outcomes in the methadone treated women [RR of confounder-NAS association (RR_{CD}) 1.05-1.25] that was reversed in the buprenorphine patients (RR_{CD} 0.80-0.95). Prevalence of unmeasured confounding by indication was assumed to be 40% in both treatment groups. Inputs for this bias analysis have been previously questioned as the assumptions informing these are subjective and results vary by slight changes in their inputs.[31] Our findings extend this work by using an internal validation study to inform the bias parameters and draw conclusions from one study center limiting heterogeneity in treatment practices. Using more conservative bias parameters informed from the validation cohort slightly weakened the impact of unmeasured confounding on our results by comparison.

In our probabilistic bias analysis, informed from the validation cohort, the RR for NAS associated with methadone compared with buprenorphine marginally decreased from 1.3 (95% CI: 1.1, 1.5) to 1.2 (95% CI: 1.0, 1.4) when limits were defined by the Flanders and Khoury method.[81] We therefore maintain that the risk of NAS associated with methadone treatment even after accounting for severity, may not be fully explained by unmeasured confounding.

It was surprising that accounting for severity of addiction did not further attenuate the association between methadone treatment and NAS compared with buprenorphine. However, the impact of addiction severity on the association between opioid maintenance therapy and NAS is likely limited by the weak relationship between addiction severity and NAS. Of note, women actively abusing heroin during pregnancy have a lower risk of NAS compared with women receiving methadone as replacement therapy.[37, 83] Therefore, behaviors associated with more severe addiction such as relapse and later conversion to opioid maintenance therapy may not increase the risk of NAS. It is important to note that the lower risk of NAS with active abuse

does not negate other potential risks such as reduced prenatal care. Opioid maintenance therapy should undoubtedly remain the standard care.[4, 84]

Our findings must be interpreted within the bounds of their limitations. We used a large administrative database that lacked detailed information on treatment and addiction histories. Without information on the initiation, timing, and duration of exposure to medication, we were unable to appropriately assess how these factors influence the development of NAS. We relied on the dose and medication treatment on the day of delivery as a relatively crude measure of exposure, as it is thought that treatment closest to the time of delivery has the strongest impact on NAS.[74] Though using this approach allows for misclassification of exposure, this unlikely impacts our findings as only 6 of 200 women in our validation cohort had documentation of ever changing treatment (inclusive of prior to pregnancy). The lack of information on addiction history contributes considerably to the unmeasured confounding remaining in the analysis. Furthermore, by using treatment for NAS as our outcome measurement, we restricted our analysis to only the more severe cases of NAS. Though having a gradient of Finnegan scores or morphine dose may be informative, those receiving treatment incur the largest costs and this approach is subject to less misclassification due to the subjectivity of the Finnegan Scale.

The lack of adjustment for prescribing preferences by severity of addiction, which is typically unmeasured, is one of the greatest shortcomings in the current literature. Our probabilistic bias analysis aimed to minimize this limitation using information from our internal validation cohort. As was expected due to the nature and sensitivity of this topic, upon chart review there was a substantial amount of missing data in the validation cohort with a missingness that differed by opioid maintenance type. Differential missingness was likely driven by more buprenorphine treated patients entering into pregnancy on treatment and potentially having less

interaction with the healthcare system due to an overall superior health profile. Both may contribute to less documentation in their charts. Though we are the first to use an internal validation cohort to derive information on severity to adjust for unmeasured confounding, our findings are subject to the limitations of the data available to us and to the parameterization the severity index. Future research with the aim of developing a robust severity index or using a clinically validated scale is warranted. Nevertheless, this approach is preferable to deriving effect estimates exclusively from the literature.

Prescribing preferences for opioid maintenance therapy are often warranted as many women benefit from the different methods of delivery of care in the U.S. However, in many places in the U.S. patients do not have access to both treatment options due to both a lack of clinics and licensed providers in addition to limitations imposed by insurance. Lack of treatment options can result in structural confounding in other studies. In our study population, it is unlikely that non-positivity impacted our results as women had access to both treatment options and both were covered under Pennsylvania Medicaid, the primary insurer of this population.

As both observational studies and randomized trials are subject to the biases inherent in opioid maintenance treatment choices, it is imperative to account for this unmeasured confounding when comparing methadone with buprenorphine exposures in pregnancy to advocate for availability of both options if one is superior. Our results suggest that the previous findings that buprenorphine is associated with less NAS compared with methadone in infants exposed in utero are subject to minimal bias from unmeasured confounding. Applying similar bias analyses to the association of these treatments with other neonatal outcomes is necessary to fully inform treatment decisions.

3.6 TABLES AND FIGURES

Table 1. Maternal Characteristics by Opioid Maintenance Treatment Type, Magee-Womens Hospital, 2013- 2015 (n=716).

Characteristic	Methadone N (%) n=407	Buprenorphine N (%) n=309
Race		
White	381 (93.6)	294 (95.1)
Black	19 (4.7)	8 (2.6)
Missing	7 (1.5)	7 (2.3)
Mother's age [Mean (SD)]	29.1 (4.7)	28.5 (4.9)
Mother's Education		
Less than high school	83 (20.4)	45 (14.6)
High school graduate or GED completed	165 (40.5)	139 (45.0)
Some college credit	78 (19.2)	68 (22.0)
College graduate	66 (16.2)	54 (17.4)
Missing	15 (3.7)	3 (1.0)
Prepregnancy BMI [Mean (SD)][a]	24.6 (5.3)	24.2 (6.1)
BMI category[b]		
Underweight (<18.5kg/m^2)	11 (2.7)	17 (5.5)
Normal weight	116 (28.5)	94 (30.4)
Overweight	35 (8.6)	30 (9.7)
Obese	29 (7.1)	24 (7.7)
Missing	216 (53.1)	144 (46.6)
Married	35 (8.6)	58 (18.8)
Employed	139 (34.2)	135 (43.7)
Smoked during pregnancy	336 (82.6)	250 (80.9)
Parity		
Nulliparous	118 (29.0)	106 (34.3)
1-2 previous pregnancies	208 (51.1)	151 (48.9)
Greater than 2 pregnancies	81 (19.9)	52 (16.8)
Hepatitis c positive	61 (15.0)	31 (10.0)
Gestational age at delivery [Mean (SD)]	37.4 (2.9)	38.5 (2.5)
Birthweight [Mean (SD)]	2734 (619.3)	2999 (591.2)
Infant with congenital anomaly	50 (12.3)	27 (8.7)

[a]Prepregnancy BMI based on n=356.

[b]Prepregnancy BMI defined as underweight (<18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), obese (≥30 kg/m^2).

GED=general education development, SD=standard deviation, BMI=body mass index

Table 2. Maternal Characteristics by Opioid Maintenance Treatment Type in a Validation Subcohort, Magee-Womens Hospital, 2013-2015 (n=200).

Characteristic	Methadone N (%) n=100	Buprenorphine N (%) n=100
Race		
White	97	97
Black	3	2
Missing	0	1
Mother's age [Mean (SD)]	28.6 (5.1)	28.2 (5.2)
Mother's Education		
Less than high school	19	9
High school graduate or GED completed	40	52
Some college credit	23	18
College graduate	14	19
Missing	4	2
Prepregnancy BMI [Mean (SD)][a]	24.6 (5.9)	23.7 (5.3)
Married	8	21
Employed	31	43
Smoked during pregnancy	84	80
Parity		
Nulliparous	31	39
1-2 previous pregnancies	47	46
Greater than 2 pregnancies	22	15
Hepatitis c positive	12	10
Gestational age at delivery [Mean (SD)]	37.3 (3.2)	39.1 (1.8)
Birthweight [Mean (SD)]	2695 (631.6)	3147 (472.4)
Infant with congenital anomaly	15	10
Severe maternal addiction	77	32

[a]Prepregnancy BMI based on n=43 in methadone treated women and n=54 in buprenorphine treated women.

GED=general education development, SD=standard deviation, BMI=body mass index

Table 3. Results from conventional analyses of the risk of neonatal abstinence syndrome associated with methadone compared with buprenorphine as opioid maintenance therapy, at Magee-Womens Hospital, Pittsburgh, Pennsylvania (2013-2015).

Opioid maintenance therapy	Events (n)	Population at risk	Unadjusted risk per 100 livebirths	Unadjusted risk difference per 100 live-born infants (95% confidence interval)	Adjusted[a] risk difference per 100 live-born infants (95% confidence interval)
Buprenorphine	152	309	49	Reference	Reference
Methadone	263	407	65	15 (8.1, 23)	14 (5.9, 22)
				Unadjusted relative risk (95% confidence interval)	**Adjusted[a] relative risk (95% confidence interval)**
Buprenorphine				Reference	Reference
Methadone				1.3 (1.2, 1.5)	1.3 (1.1, 1.5)

[a]Adjusted for parity, maternal race, employment status, hepatitis c status, age, year of delivery, smoking status, marital status, and insurance.

Table 4. Characteristics of a subsample of opioid use dependent singleton pregnancies with severity of addiction indicators abstracted from medical charts at Magee-Womens Hospital in Pittsburgh, 2013-2015 (n=200).

Characteristic	Methadone n=100	Buprenorphine n=100
Converted to opioid maintenance therapy in pregnancy		
Yes	58	12
No	18	30
Missing	24	58
Gestational age at conversion (Median, IQR), weeks	12 (5, 22)	Prior to conception (prior, 4)
Relapse in pregnancy		
Yes	23	4
No	9	24
Missing	68	72
Using illicit substance at time of delivery		
Yes	24	15
No	34	71
Missing	42	14
Used benzodiazepines in pregnancy		
Yes	28	8
No	33	22
Missing	39	70
Neonatal abstinence syndrome		
Yes	61	54
No	39	46

IQR=interquartile range

Table 5. Comparison of results from adjusted conventional and probabilistic bias analyses accounting for unmeasured confounding by severity of addiction on the risk of neonatal abstinence syndrome associated with methadone compared with buprenorphine as opioid maintenance therapy, at Magee-Womens Hospital, Pittsburgh, Pennsylvania (2013-2015).

Opioid maintenance therapy	Conventional analysis: Adjusted[a] relative risk (95% confidence interval)	Bias Analysis 1: Adjusted[a] point estimate (95% bootstrapped simulation interval)[b]
Buprenorphine	Reference	Reference
Methadone	1.3 (1.1, 1.5)	1.2 (1.0, 1.4)

[a]Adjusted for parity, maternal race, employment status, hepatitis c status, age, year of delivery, smoking status, marital status, and private vs. public insurance.

[b]minimum RR_C=1.0, mode 1=1.02, mode 2=1.11, maximum RR_C=1.13

RRc=relative risk due to confounding

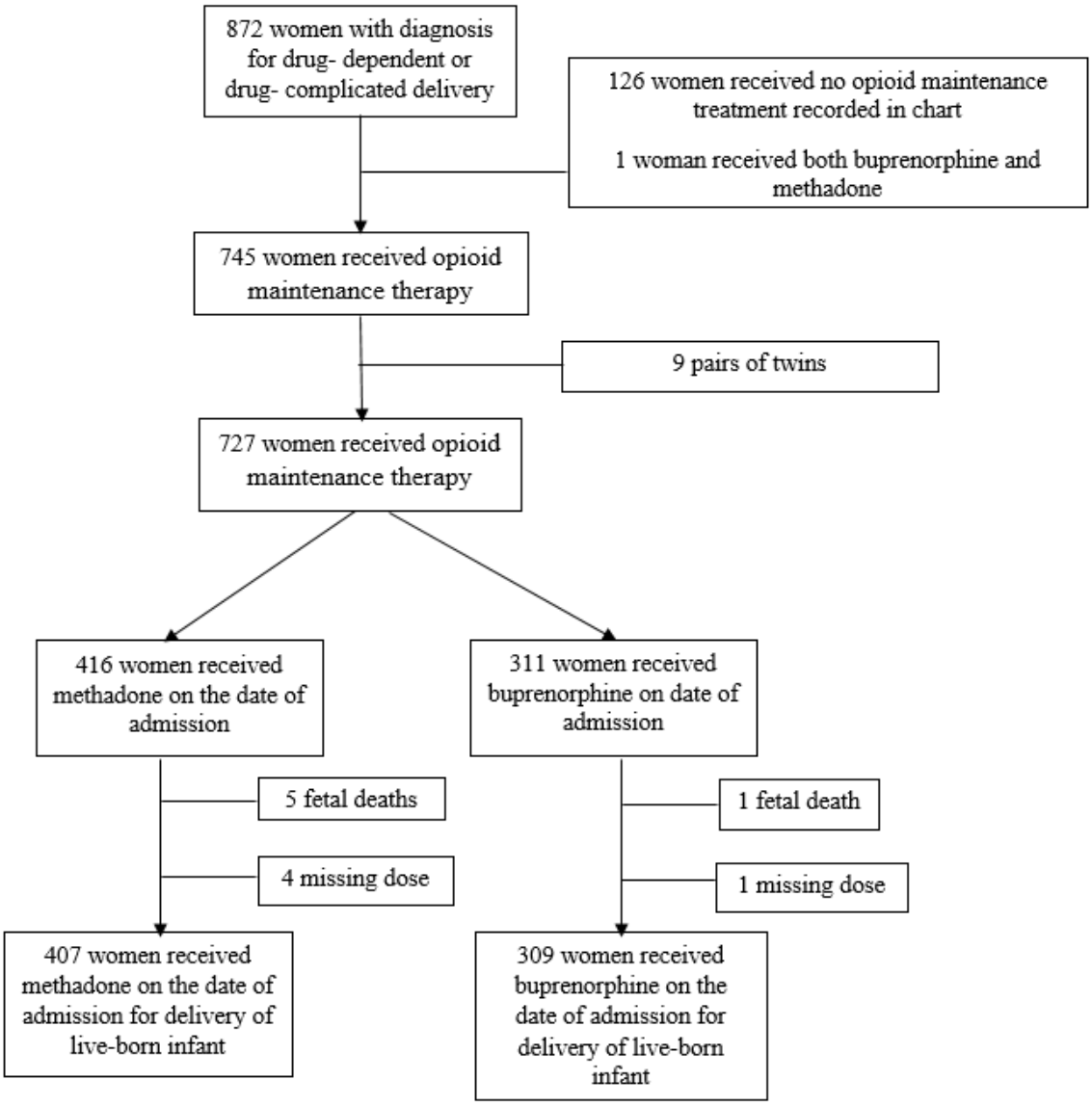

Figure 1. Flow diagram describing sample population (n=716, 2013-2015*Note: 25 women with 2 pregnancies).

4.0 MANUSCRIPT 2: THE ROLE OF PREMATURITY IN ASSOCIATION BETWEEN OPIOID MAINTENANCE THERAPY AND NEONATAL ABSTINENCE SYNDROME

Lara S. Lemon[a,b]
Ashley Naimi[a]
Steve N. Caritis[c]
Robert W. Platt[d]
Raman Venkataraman[b,e]
Lisa M. Bodnar[a,c]

[a] Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh
[b] Department of Pharmaceutical Science, School of Pharmacy, University of Pittsburgh
[c] Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine, University of Pittsburgh
[d] Departments of Pediatrics and Epidemiology, Biostatistics and Occupation Health, McGill University
[e] Department of Pathology, University of Pittsburgh

4.1 ABSTRACT

Background Pregnant women treated with methadone as opioid maintenance therapy are more likely than women treated with buprenorphine to deliver preterm. Preterm birth is associated with less risk of neonatal abstinence syndrome (NAS). We sought to assess the role of preterm birth as a mediator of the relationship between in utero exposure to methadone and NAS compared with buprenorphine.

Methods We studied 716 women receiving methadone or buprenorphine as opioid maintenance therapy and delivering live-born infants at Magee-Womens Hospital, Pittsburgh, Pennsylvania (2013-2015). We implemented inverse probability weighted marginal structural models to isolate the role of preterm birth (≤37 weeks' gestation). Weights accounted for confounding by maternal age, race, insurance, parity, delivery year, marital, employment, hepatitis C, and smoking status.

Results Approximately 57% of the cohort was treated with methadone. Preterm birth was more common in methadone exposed pregnancies (25% vs. 14%). The incidence of NAS was higher in methadone-compared with buprenorphine-exposed infants (65% vs 49%), and term compared with preterm births (64% vs 36%). For every 100 infants live-born to mothers treated for opioid dependence, there were 13 excess cases of NAS among infants exposed to methadone compared with buprenorphine [adjusted RD=13.3, 95% CI: (5.7, 20.9)]. Among term births, this increased to 17 excess cases of NAS in methadone- compared with buprenorphine-exposed [16.7 (9.3, 24.0)]. Findings were similar on the relative scale.

Conclusion The further increased risk of NAS associated with methadone use versus buprenorphine in term deliveries emphasizes the utility of buprenorphine in clinical settings aimed at decreasing NAS.

4.2 INTRODUCTION

Neonatal abstinence syndrome (NAS), or postnatal opioid withdrawal, affected nearly 6 of every 1,000 live-born U.S. infants in 2012 [85]— a five-fold increase since 2000 [21]. NAS is associated with long-term physical and behavioral complications [27], and cost the U.S. health system an estimated $1.5 billion in 2012 alone [85]. The marked increase in NAS parallels

increases in opioid use dependence in pregnancy [14] and use of the two recommended opioid maintenance therapies, buprenorphine and methadone [4]. Determining which treatment regimen will optimize maternal and infant outcomes, including reducing risk of NAS, is a public health priority.

Literature suggests that buprenorphine is associated with superior perinatal outcomes compared with methadone, most notably reporting less NAS and shorter duration of neonatal treatment [17, 19, 28, 86]. Though the majority of extant studies agree, methadone remains the mainstay of care in the U.S. [87] and barriers to access buprenorphine treatment persist [88]. Prescribing of buprenorphine as opioid maintenance therapy in an outpatient setting requires that the physician obtain a waiver from the Controlled Substances Act [89]. In 2012, only 2.2% of all physicians in the U.S. received the waiver and were able to treat patients with buprenorphine [90].

Inherent biases in prescribing preferences, access to treatment options, and necessity of tailoring treatment to the individual beyond risk of NAS, have continued to fuel the debate on optimal treatment in pregnancy. The role of gestational age in the relationship between opioid maintenance therapy and NAS, however, has not been investigated. Ignoring or mishandling gestational age is a problem frequently encountered in perinatal epidemiologic studies [41]. Researchers frequently adjust for gestational age in regression models or ignore its impact altogether. Such approaches ignore the complexities of this relationship. Methadone treatment has been associated with an increased risk of preterm birth compared with buprenorphine treatment or no treatment [17, 43] [37, 39, 44], and preterm infants exhibit a lower incidence and reduced severity of NAS compared with term infants [45-47]. Prematurity is likely a mediator of

this relation, so the estimated increased risk of NAS associated with methadone compared with buprenorphine may be an underestimate among term infants- the ideal gestational length.

In this study, we aimed to estimate the association between opioid maintenance therapy and NAS, independent of the effect of opioid maintenance therapy on prematurity. We hypothesized that the increased risk of NAS associated with methadone exposure compared with buprenorphine would be stronger among term than preterm births. If true, these results will support the expansion of buprenorphine use and access in pregnancy, making more treatment options available to individualize treatment.

4.3 METHODS

Magee-Womens Hospital is one of the largest maternity hospitals in Pennsylvania with approximately 10 000 deliveries annually. Pregnant women initiating new opioid maintenance therapy at Magee-Womens Hospital can self-select treatment with methadone or buprenorphine in accordance with the American Congress of Obstetricians and Gynecologists recommendations provided they meet prescribing requirements for both [4]. Women who conceive while receiving opioid maintenance therapy are normally maintained on their medication regimen.

Study cohort

The study cohort consisted of all live-born, singleton deliveries to women exposed to methadone or buprenorphine as opioid maintenance therapy on the day of delivery at Magee-Womens Hospital from 2013-2015. Using *International Classification of Diseases,* Ninth and Tenth Revision codes for drug- dependent (ICD-9 64831) or drug- complicated delivery (ICD-10 O99324) we identified 872 drug-dependent pregnancies (Figure 1). Of these, 745 had

documentation of opioid maintenance therapy with either buprenorphine or methadone on the day of delivery. We restricted the cohort further to live-born, singleton pregnancies and therefore excluded 6 fetal deaths and 9 pairs of twins. We retained 716 pregnancies (691 women) in our final analytic sample. The Institutional Review Board at the University of Pittsburgh approved this study.

Opioid maintenance medications

We determined maternal exposure to opioid maintenance therapy with pharmacy billing claims, then extracted dosing information directly from the medical chart. Women treated with Subutex® (buprenorphine, n=299) (Reckitt Benckiser Pharmaceuticals Inc., VA) or Suboxone® (buprenorphine + naloxone, n=10) (Reckitt Benckiser Pharmaceuticals Inc., VA) were considered buprenorphine-treated. In utero exposure to buprenorphine was the referent in all analyses. We selected treatment on the day of delivery as the exposure of interest and used this as a surrogate of pregnancy exposure as opioid exposure closest to the time of delivery is thought to have the highest impact on NAS risk [74] and we lacked data on entire treatment trajectories.

Neonatal abstinence syndrome

We identified cases of NAS using pharmacy-billing codes indicating infant pharmacologic treatment with morphine. At Magee-Womens Hospital all infants with known exposure to opioids, both illicit and maintenance, remain in the hospital for 5 to 7 days post-delivery for continuous monitoring for NAS. Infants are scored using the Finnegan Neonatal Abstinence Scoring Tool [24] every 3 to 4 hours; those with an average score of eight or greater for 3 consecutive assessments receive treatment with morphine. In our cohort, receipt of morphine was

highly correlated with ICD code indicating "Drug Withdrawal Syndrome in Newborn" (kappa>0.99).

Preterm birth

Preterm birth was the mediator in each analysis. For consistency with the literature, we defined preterm birth as live-born delivery prior to 37 weeks gestation documented in the pharmacy billing records [91]. We were unable to discern between spontaneous and induced labor and therefore considered both in our definition of preterm birth. Gestational age was determined using the best obstetric estimate from ultrasound or last menstrual period when ultrasound was unavailable. All pregnancies had documented gestational age from 20 to 42 weeks at delivery.

Preterm birth meets the criteria as a potential mediator of the association between opioid maintenance therapy and NAS as: 1) methadone has been shown to be associated with preterm birth both in comparison to buprenorphine [17, 39, 44] and to no opioid maintenance therapy [37, 43], and 2) preterm infants develop less, or less severe, NAS compared with term infants after exposure to methadone [45-47, 92].

Covariates

We obtained data on medication use, maternal characteristics and pregnancy outcomes from electronic pharmacy records at Magee-Womens Hospital. Information missing from electronic pharmacy records was informed directly from patient charts and birth certificates. Data were therefore a combination of clinical billing codes, documentation by a health professional, and self-report.

Maternal characteristics in our cohort included maternal age, race (Black, White, other), education (less than high school, high school graduate or equivalent, some college, college

graduate), marital status (yes/no), employment status (employed vs unemployed), type of insurance (private vs public), and prepregnancy body mass index (BMI). BMI was calculated as prepregnancy weight in kilograms divided by height in meters squared and was categorized as underweight (BMI <18.5), normal weight (BMI 18.5 to <25), overweight (BMI 25 to <30), or obese (BMI ≥30) [75]. Data pertinent to the pregnancy included parity, hepatitis c status (positive vs negative), smoking status (smoked at any point in pregnancy), birthweight, congenital anomalies, and year of delivery.

Statistical analysis

Our analytic strategy was to assess the total adjusted association between opioid maintenance therapy and NAS, then to define the controlled direct effect of treatment on NAS removing the effect of preterm birth [93]. The difference between these associations represented the effect of preterm birth.

Conceptual models were used to identify potential confounders of the overall relationship between opioid maintenance therapy and NAS, and of the preterm birth-NAS association [66]. Variables identified as potential confounders of the opioid maintenance therapy-NAS total association in our cohort included maternal age, race, marital status, employment status, type of insurance, parity, hepatitis c status, smoking status, and year of delivery. Final models of the preterm birth- NAS association were adjusted for parity, maternal race, age, smoking status, and marital status.

We first assessed interactions between treatment and preterm birth. Log-binomial models regressing NAS against treatment (methadone vs buprenorphine) were performed with and without main effect and interaction terms for preterm birth. Because the risk ratio changed by

less than 10% with the inclusion of the interaction term, interaction between exposure and mediator was considered insignificant and was not included in the final models.

Our primary analysis evaluating mediation by preterm birth, adjusted for confounders marginally using inverse probability weighting [93]. This approach can estimate direct effects in the presence or absence of exposure-induced mediator-outcome confounding. To execute this analytic approach, we used two log-binomial regression models weighted by stabilized inverse probability weights. The weights were generated from modeling methadone exposure then preterm birth as a mediator. Weights were calculated as:

$$sw = \begin{cases} \frac{P(X=1)}{P(X=1|Cxy)} \times \frac{P(M=1)}{P(M=1|Cmy)}, & if\ X=M=1 \\ \frac{P(X=0)}{P(X=0|Cxy)} \times \frac{P(M=1)}{P(M=1|Cmy)}, & if\ X=0 \text{ and } M=1 \\ \frac{P(X=1)}{P(X=1|Cxy)} \times \frac{P(M=0)}{P(M=0|Cmy)}, & if\ X=1 \text{ and } M=0 \\ \frac{P(X=0)}{P(X=0|Cxy)} \times \frac{P(M=0)}{P(M=0|Cmy)}, & if\ X=M=0 \end{cases}$$

where X denotes treatment (X=1 if methadone, X=0 if buprenorphine), M represents gestational age (M=1 if term birth, M=0 if preterm birth), y indicates NAS (y=1 if infant treated for NAS, y=0 no treatment for NAS), and C represents potential confounders included in the model (described above). The numerators represent the predicted probabilities from logistic regression models of treatment and preterm birth and the denominators replicate this model but adjusted for the confounding variables. All weights had a mean of one with no extreme values.

Weights were then incorporated into two binomial regression models: 1) modeling the total effect of methadone treatment on NAS compared with buprenorphine, 2) the controlled direct effect of methadone on NAS among term births. Results were reported on both the risk difference (RD) and risk ratio (RR) scale. Standard errors were obtained using robust variance

estimators [77]. Finally, the proportion increase in the association in term births was calculated as the absolute value of: [(Total Effect-Controlled Direct Effect)/Total Effect] x 100 [94].

To triangulate our results, we then implemented a mediation analysis conditionally adjusting for the same variables using the generalized product method [95]. While this approach can accommodate exposure-mediator interactions, it cannot account for mediator-outcome confounders affected by the exposure. However, our conceptual models suggested that no such mediator-outcome confounders were present, and our analyses suggested no exposure-mediator interactions. Therefore, the generalized product method should yield results identical to the inverse probability weighted approach. All analysis was conducted in Stata Version 14 (StataCorp, College Station, TX).

4.4 RESULTS

In the cohort, 57% (n=407) women were treated with methadone and the remaining 43% (n=309) with buprenorphine on the day of delivery. Nearly 20% of the final sample was born preterm, and 58% developed NAS. Women with a preterm delivery were more likely than women with a term delivery to have less than a high school education, smoke during pregnancy, have a higher parity (Table 6). Race, maternal age, prepregnancy BMI, marital, employment, and hepatitis C status were not different between groups. The preterm infants were lighter at birth and more often had a birth defect than infants delivered at term. The incidence of NAS was higher in methadone compared with buprenorphine-exposed infants (65% vs. 49%), and term infants compared with preterm infants (64% vs. 36%) (Table 7). Rates preterm birth were also higher in methadone versus buprenorphine treated women (25% vs. 14%) (Table 7).

Crude and adjusted associations between type of opioid maintenance treatment and NAS are displayed in Table 8. On the RD scale, for every 100 live-born infants exposed to opioid maintenance therapy in utero there were 13 more cases of NAS among infants exposed to methadone compared with buprenorphine [RD=13.3, 95% CI: 5.7, 20.9). When the mediating role of preterm birth was accounted for, the RD increased to 16.7 (95% CI: 9.3, 24.0). These findings suggested an estimated 25% increase in the association among term deliveries.

Assessing the associations on a relative scale resulted in a total increased relative risk of NAS of 1.26 (95% CI: 1.10, 1.45) for women treated with methadone compared with buprenorphine. When the mediating role of preterm birth was accounted for, the relative risk of NAS increased to 1.34 (95% CI: 1.17, 1.53). The results on the relative scale support the findings of an increased risk of NAS with methadone compared with buprenorphine that was stronger among term births. Results were not meaningfully different when the generalized product method was used to assess mediation (Appendix B: Table 15).

4.5 DISCUSSION

Our results support previous findings that risk of NAS is decreased in buprenorphine- compared with methadone-exposed infants. We advanced this research by further decomposing the association between methadone treatment and NAS compared with buprenorphine and found that the association was stronger among term births compared with preterm births. As prolongation of pregnancy to term delivery is preferable when possible, this conclusion supports expanded use of, and access to, buprenorphine in women eligible for this therapy.

Our study expands upon previous work arguing the need to properly address gestational age in studying the association between opioid maintenance therapy and NAS [29], by being the first to describe the mediating role of preterm birth and to quantify to what extent it may influence the association. Regression adjustment for gestational age, an approach often implemented in the extant literature [28, 40], is inappropriate. Due to temporality, gestational age at delivery is a potential *result* of opioid maintenance therapy- and cannot be a *predictor* of treatment type.

As with all studies using observational data, interpreting our associations causally requires assumptions of positivity, no interference, exchangeability, and counterfactual consistency [96]. In this work, positivity and no interference pose little to no threats to the validity of our inferences. Positivity requires the presence of both exposed and unexposed term and preterm infants in all confounder strata. This assumption is verifiable, and held in our setting, as evidenced by the distribution of our stabilized inverse probability weights. No interference requires that the outcome of any given infant is not affected by the opioid maintenance therapy or preterm birth status of any other infant, and is a reasonable assumption to make.

Exchangeability requires no uncontrolled information, selection, or confounding bias. As with other studies, we were unable to control for the prescribing preference for methadone versus buprenorphine. However, our previous work found that unmeasured confounding by severity of addiction had little impact on the association between methadone and NAS compared with buprenorphine (L. Lemon, University of Pittsburgh, unpublished data). We also lacked information on treatment trajectories and gestational age at initiation and therefore assumed that treatment remained constant throughout pregnancy. This could introduce immortal time bias if

women receiving opioid maintenance therapy were not converted to treatment until after 37 weeks [42]. However, a detailed chart review we undertook in a subset of this cohort (n=200) found that no women were initiated on treatment after 36 weeks (L. Lemon, University of Pittsburgh, unpublished data). The absence of trajectories also prohibited the presentation of true 'directed' acyclic graphs. Because data were cross-sectional, we were unable to establish temporality of certain associations. For example, it is reasonable to assume that lack of insurance could increase a woman's addiction severity if she cannot afford treatment. Conversely, it is also possible that a woman with severe addiction will be less likely to be employed and therefore have no insurance. Finally, a lack of treatment history also prevented the evaluation of cumulative exposure. If NAS is influenced by a cumulative effect or sensitive exposure window we were unable to asses this. We chose to utilize the day of delivery as our exposure of interest as complete pregnancy treatment data were unavailable and because this is thought to be the most strongly associated with NAS [74].

A noteworthy limitation of this work is our assumption that the relationships between opioid maintenance therapy-preterm birth and preterm birth-NAS are causal. Though a large body of work supports the notion that methadone affects preterm birth [17, 37, 39, 43, 44], research devoted to better understanding the mechanisms by which gestational age influences NAS is needed. Information on this relationship is limited because the pathophysiologic response associated with NAS is not fully understood. Preterm infants may experience less NAS due to alterations in their opioid receptor network immaturity, differential development of neurotransmitters, increased placental transfer of the opioid as pregnancy progresses, less fatty tissues available for methadone distribution in preterm infants, and/or less cumulative exposure to opioids [23]. However, it should be noted that NAS is defined using Finnegan Scores

developed in term infants only, and thus, this assessment tool may not be appropriate in preterm infants. This complication jeopardizes the validity of the counterfactual consistency assumption, an assumption that is commonly violated in studies of preterm birth [97].

Despite these limitations, our approach is characterized by several strengths. First, we found the same results using inverse probability weighted regression and the generalized product method, which suggests that our findings are robust to model misspecification. Second, we relied on pharmacy records only for identification of women receiving opioid maintenance therapy; each treatment type and dose was confirmed by extraction directly from medical records for all subjects. Finally, this is the largest study to date that compares these opioid maintenance therapies in actively treated pregnant women at one institution in the U.S.

It is crucial to accurately assess the risk of NAS associated with methadone and buprenorphine, while appropriately accounting for gestational age, in order to inform clinical practice and guide treatment decisions for pregnant women initiating care. Though previous research has established less risk of NAS associated with buprenorphine, we found that the increased risk of NAS after methadone exposure in utero compared with buprenorphine was stronger among a population of term than preterm births. These results support expanded use of buprenorphine as opioid maintenance therapy with the aim of decreasing NAS, adding additional incentive to providers and insurance companies to expand access through prescribing availability and medication coverage.

4.6 TABLES AND FIGURES

Table 6. Demographics of women diagnosed with drug-dependent deliveries of singletons by preterm vs. term status at Magee-Women Hospital in Pittsburgh, Pennsylvania (2013-2015, n=716).

Characteristic	Preterm N (%) n=146	Term N (%) n=570
Opioid maintenance therapy		
Buprenorphine	43 (29.5)	266 (46.7)
Methadone	103 (70.5)	304 (53.3)
Race		
White	136 (93.2)	539 (94.6)
Black	7 (4.8)	20 (3.5)
Other/Unknown	3 (2.0)	11 (1.9)
Mother's age [Mean (SD)]	29.2 (4.7)	28.7 (4.8)
Mother's education		
Less than high school	35 (24.0)	93 (16.3)
High school graduate or GED completed	52 (35.6)	252 (44.2)
Some college credit	34 (23.3)	112 (19.7)
College graduate	20 (13.7)	100 (17.5)
Unknown	5 (3.4)	13 (2.3)
Prepregnancy BMI [Mean (SD)][a]	24.6 (6.5)	24.4 (5.5)
BMI category[a,b]		
Underweight	5 (7.8)	23 (7.9)
Normal weight	38 (59.4)	172 (58.9)
Overweight	21 (32.8)	97 (33.2)
Obese	8 (5.5)	45 (7.9)
Married	19 (13.0)	74 (13.0)
Employed	52 (35.6)	222 (39.0)
Smoked during pregnancy	129 (88.4)	456 (80.0)
Parity		
Nulliparous	43 (29.5)	181 (31.8)
1-2 previous pregnancies	67 (45.9)	292 (51.2)

(Table 6 continued)

Greater than 2 pregnancies	36 (24.6)	97 (17.0)
Hepatitis C positive	15 (10.3)	77 (13.5)
Birthweight [Mean (SD)]	2091.0 (588.1)	3043.1 (459.3)
Gestational age at delivery [Mean (SD)]	33.6 (3.0)	39.0 (1.2)
Diagnosed with congenital anomaly	21 (14.4)	56 (9.8)

[a]Prepregnancy BMI based on n=356.

[b]Prepregnancy BMI defined as underweight (<18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), obese (≥30 kg/m^2).

SD=standard deviation, GED=general educational development, BMI=body mass index

Table 7. Rate of neonatal abstinence syndrome by opioid maintenance treatment and preterm birth status.

Treatment	Term with NAS	Term without NAS	Preterm with NAS	Preterm without NAS	Total
Methadone	223	81	40	63	407
Buprenorphine	140	126	12	31	309
Total	363	207	52	94	716

NAS=neonatal abstinence syndrome

Table 8. Opioid maintenance therapy-NAS association and opioid maintenance therapy-NAS association not attributable to preterm birth in women exposed to opioid maintenance therapy at Magee-Womens Hospital, 2013 to 2015 using inverse probability weighted marginal structural models (n=716).

				Risk Differences[a]: RD per 100 live born infants (95% CI)		Risk Ratios[b]: RR (95% CI)		Proportion Explained on RD Scale
	Events (n)	Population at risk (n)	Unadjusted risk per 100 live born infants	Total association: OMT-NAS	Association not attributed to preterm birth:	Total association: OMT-NAS	Association not attributed to preterm birth:	Proportion of total avoided by preterm birth
Methadone	263	407	64.6	13.3 (5.7, 20.9)	16.7 (9.3, 24.0)	1.26 (1.10, 1.45)	1.34 (1.17, 1.53)	24.9%
Buprenorphine	152	309	49.2	Reference	Reference	Reference	Reference	

[a] Linear risk models adjusted for parity, maternal race, age, employment status, smoking status, marital status, hepatitis C status, private vs. public insurance, and year of delivery.
[b] Poisson regression models adjusted for parity, maternal race, age, employment status, smoking status, marital status, hepatitis C status, private vs. public insurance, and year of delivery.

OMT=opioid maintenance therapy, NAS=neonatal abstinence syndrome, RD=risk difference, RR=risk ratio, CI=confidence interval

5.0 MANUSCRIPT 3: PREDICTING THE RISK OF RECURRENT SPONTANEOUS PRETERM BIRTH BASED ON OBSTETRIC HISTORY IN WOMEN TREATED WITH 17-OHPC

Lara S. Lemon[1,2*]
Alisse Hauspurg-Janicki[3]
Lisa M. Bodnar[1,3]
Raman Venkataraman[2,4]
Ada Youk[1]
Maisa Feghali[3]
Steve N. Caritis[3]

1 Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh
2 Department of Pharmaceutical Science, School of Pharmacy, University of Pittsburgh
3 Department of Obstetrics, Gynecology, and Reproductive Sciences, School of Medicine, University of Pittsburgh
4 Department of Pathology, School of Medicine, University of Pittsburgh

5.1 ABSTRACT

Objective To predict risk of recurrent spontaneous preterm birth based on obstetric history, accounting for interplay between variables, in women treated with 17-OHPC.

Design Secondary analysis of a randomized controlled trial (Omega-3 Trial; 2005-2006).

Setting 13 Maternal-Fetal Medicine Units across the U.S.

Population Women with prior spontaneous preterm delivery (sptd) at 20^0 to 36^6 receiving weekly injections of 17-OHPC (n=754).

Methods Backwards, chunk-wise, model building methods were used to create log binomial regression models predictive of recurrent preterm birth. Models included interaction terms between obstetric history variables and were externally validated for discrimination and calibration.

Main Outcome Measures Spontaneous delivery prior to 37 weeks' gestation.

Results Approximately 35% (n=267) of the pregnancies ended in a recurrent sptd. The predictive model had moderate discrimination with good calibration. Predicted risk of recurrence was higher in women with more prior preterm deliveries, fewer term deliveries, and earlier gestational ages at delivery of the most recent pregnancy. Risk also increased with earliest gestational ages of prior delivery, however this effect was only seen in women with two or more previous spontaneous preterm deliveries. An average women with 1 previous sptd and no prior term deliveries had a risk ranging from 27.0 (95% CI: 18.4, 35.6) to 31.5 (95% CI: 24.7, 38.3) regardless of the age of her earliest delivery. However, predicted risk increased significantly with earlier gestational ages of prior delivery in women with two or more previous sptds [(risk for average woman with 2 prior sptds and earliest at 20 weeks: 47.4 (29.1, 65.7); risk for average woman with 2 prior sptds and earliest at 36 weeks: 19.4 (7.7, 31.2)].

Conclusions Factors in obstetric history have an important impact on one another. Risk of recurrent preterm delivery is similar for women with only one prior sptd, regardless of gestational at which it occurred. More work is needed to target treatment with 17-OHPC beyond identification of women with a single previous sptd.

5.2 INTRODUCTION

Nearly 1 of every 10 live born infants is delivered preterm (<37 weeks) in the United States [50]. Preterm birth continues to be one of the leading the causes of infant morbidity and mortality contributing to an estimated 75% of all perinatal deaths, despite progress in preventive treatment [5, 6, 48].

It has been well established that the greater number of prior spontaneous preterm births a woman has, the more likely she is to have a recurrent preterm birth [53-56]. The American College of Obstetricians and Gynecologists (ACOG) recommends administration of 17- alpha hydroxyprogesterone caproate (17-OHPC) injections for women with a prior spontaneous preterm delivery to reduce the risk of recurrence [7, 8, 98]. However, response rates are highly variable with about 30-40% 'failing' treatment and delivering preterm [8].

Targeting this pharmacologic intervention to women most likely to respond has been a recent priority in the field of obstetrics [99, 100], but has been complicated by the fact that both the pathophysiology of preterm birth and mechanism of action 17-OHPC remain unknown. A few known obstetric history factors that influence the risk of recurrence include gestational age at earliest preterm delivery [59, 62], number and pattern of previous preterm and term deliveries [54], gestational age of the most recent previous pregnancy [53, 56, 63], and clinical presentation of previous spontaneous preterm delivery [56, 61]. However, extant literature does not consistently agree on which factors are most important in predicting risk despite treatment nor do they adequately address the interplay between various pregnancy history characteristics.

It would be valuable to both provider and patient to estimate a woman's risk of recurrent preterm delivery while receiving 17-OHPC. Differentiating between inherent risks of various obstetric history patterns will 1) identify which patients are at a highest risk of recurrence, and 2)

lead to the question: “Is it appropriate to administer identical pharmacotherapy with 17-OHPC to all women with one previous spontaneous preterm delivery?”

We sought to address these questions by creating an externally validated predictive model, inclusive of interactions between pregnancy histories, using the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units (NICHD MFMU) Omega-3 Trial [101]. This model will help to granulate risk profiles and tailor the predicted risk of recurrent spontaneous preterm birth to the woman’s obstetric history pattern, ultimately targeting treatment to those most likely to respond.

5.3 METHODS

Population

We performed a secondary analysis of the NICHD MFM ‘Omega-3 Trial’. This was a double-blind, randomized, placebo-controlled trial of omega-3 fatty acid supplementation to prevent preterm birth in addition to 17-OHPC administration in high risk pregnancies. The original trial has been described in detail previously [101]. Briefly, a total of 852 pregnant women with at least one previous preterm delivery (between 20^0 and 36^6 weeks gestation) were administered weekly injections of 250mg 17-OHPC from randomization until 36^6 weeks or delivery, whichever occurred first. In addition to this treatment, women were randomized to receive a daily supplement of omega-3 long-chain polyunsaturated fatty acids or placebo. Authors found no significant effect of omega-3 supplementation on recurrent preterm birth [101].

In the randomized controlled trial (RCT), data pertinent to inclusion and exclusion were reviewed directly in the patient’s chart. All demographic and history variables (medical,

obstetric, social) were obtained in patient interview at randomization followed by a review of the patient's chart. This retrospective, secondary analysis was exempt from our Institutional Review Board's approval

Predictors

We defined primary exposure as gestational age of the earliest spontaneous preterm birth (20-27, 28-33, 34-36 weeks per RCT data). The following pregnancy history characteristics were identified a priori as potential modifiers of the effect of the primary exposure based on the literature: number of previous spontaneous preterm deliveries (1, 2, or ≥3), gestational age of most recent pregnancy (<20, 20-36, ≥37 weeks), and the percent of previous births delivered preterm. After evaluation for effect modification, those obstetric history variables not classified as interactions were included in the final model as exposures of interest.

Potential baseline factors confounding the association between gestational age of earliest previous spontaneous preterm delivery and recurrent spontaneous preterm delivery were grouped into three categories: 1) maternal demographics (maternal race, age, marital status, years of schooling), 2) clinical conditions (prepregnancy BMI, diabetes, chlamydia or gonorrhea), and 3) behavioral history (smoking status, consumption of alcoholic drinks, street drug use, marijuana use).

Obstetric history variables of interest included total number of pregnancies, total number of term deliveries (none, 1, ≥2), history of previous spontaneous loss prior to 20 weeks, history of previous elective termination, classification of most recent previous pregnancy [delivery ≥37 weeks, spontaneous preterm labor with delivery, preterm premature rupture of membranes (pPROM) leading to spontaneous preterm delivery, preterm delivery for fetal or maternal

indications, spontaneous loss <20 weeks, preterm intrapartum stillbirth], and the gestational age of most recent pregnancy.

Outcomes

We defined our primary outcome as spontaneous preterm delivery prior to 37 weeks' gestation as this dictates administration of 17-OHPC in subsequent pregnancies in the US [7]. Gestational age was estimated using a combination of last menstrual period and the earliest dating ultrasound. Secondary outcomes included more clinically relevant preterm birth defined as delivery prior to 35 weeks and prior to 32 weeks. These were referred to as 'late-' and 'early-' preterm birth, respectively, in this paper.

5.3.1 Analysis

Associated risks were assessed using log binomial regression models, or Poisson distribution when the model would not converge, to estimate risk ratios with associated 95% confidence intervals. We assessed interaction between the gestational age of earliest spontaneous preterm birth (primary exposure) and the potential effect modifiers on both additive and multiplicative scales. The synergy index was used to assess additive interaction, and a change in the risk ratio of >10% after addition of the interaction term indicated significant interaction on the multiplicative scale. Wald tests were evaluated to confirm significance of each interaction term.

Model building

Model building was completed by hand using a chunk-wise, backwards variable selection process. Covariates were added by grouping described above: 1) maternal demographics, 2) clinical conditions, then 3) behavioral history. All variables with a p-value greater than 0.2 were

removed from the model. Variables remaining significant in the model from each group were then combined with the significant interaction terms to create the optimal, parsimonious predictive model. All obstetric history variables were included in the final model to facilitate risk predictions for various history patterns.

Model validation and performance assessment

The predictive model was externally validated using data from treated women in the landmark randomized controlled trial conducted by Meis et al. through the NICHD MFMU Network (details in Appendix C).[8]

We assessed the calibration of the model graphically by comparing the predicted preterm delivery rates to the observed in both the original study and in the Meis trial. Discrimination of the model was assessed using receiver operating characteristic (ROC) curves and corresponding c-statistics. We used the final model to predict the risk of recurrent spontaneous preterm delivery in hypothetical women with various obstetric history patterns using the margins command.

Model building and prediction of risk was then repeated with late- and early- preterm birth as the outcome (delivery prior to 35 and 32 weeks' gestation). All analyses were conducted in Stata 14.0 (StataCorp, Texas).

5.4 RESULTS

Further limiting the original Omega-3 cohort (n=854), we excluded women with a compliance of less than 50% with the 17-OHPC injection (n=41), a cervical length of less than 2.5 cm (n=13), those missing a gestational age at delivery (n=1), all indicated preterm deliveries (n=42), and 1

woman with no documented previous spontaneous preterm deliveries. Our final analytic sample included 754 women (Appendix C: Figure 5).

Slightly over one-third (35%) of the pregnancies treated with 17-OHPC ended in a spontaneous preterm delivery (n=267 at <37 weeks; n=128 at <35 weeks; n=67 at <32 weeks). Women with a recurrent preterm delivery were more likely to be non-Hispanic White, have less education, and to smoke at baseline (Table 9). Women with term deliveries had less previous spontaneous preterm deliveries, occurring at later gestational ages, more previous term deliveries, with their last pregnancy more likely to have ended in a term delivery (Table 9). Maternal age, pre-pregnancy body mass index (BMI), marital status, parity, number of elected terminations and spontaneous losses, and route of delivery of current pregnancy were similar between groups.

The only significant interaction on an additive and multiplicative scale was between gestational age of earliest spontaneous preterm delivery and number of previous spontaneous preterm deliveries. This interaction term demonstrated a significant Wald test ($p<0.0001$), a synergy index of 5.8 (-19.0, 30.6), and a contour graph displaying curvature indicative of interaction with 2 or more previous spontaneous preterm deliveries (Appendix C: Figure 6).

Recurrent Spontaneous Preterm Delivery

Backwards, step-wise model building resulted in a final regression modeling the risk of recurrent spontaneous preterm delivery by the variables described in Figure 7 (Appendix C). The final model of recurrent spontaneous preterm delivery prior to 37 weeks was well calibrated, demonstrating similar predicted and observed outcomes even in the lowest and highest deciles of predicted risk (Figure 2). The final model exhibited moderate discrimination in the Omega-3 data with a c-statistic of 0.71 (95% CI: 0.67, 0.74). In the external validation the calibration was

slightly decreased, but discrimination was not significantly different from the original data with a c-statistic of 0.70 (95% CI: 0.64, 0.77) (Figure 2).

Recurrent Late- and Early- Spontaneous Preterm Delivery

Backwards, step-wise model building modeling for late- and early- recurrent spontaneous preterm delivery resulted in similar final models (Appendix C: Figures 8 and 9). When externally validated in the Meis trial, which had a total of 57 late- and 33 early- spontaneous preterm deliveries, the model demonstrated reasonable calibration and increased ROCs compared to the primary analysis (Appendix C: Figures 8 and 9). The c-statistic was increased in both the original and external data.

Predicting Risk

Using the final model, predicted risk of recurrent spontaneous preterm delivery before 37 weeks is shown, 1) for significant predictors of recurrence independently (Figure 3), and 2) with the interplay of these variables in women with no previous term deliveries (Figure 4).

Women with earlier previous spontaneous preterm deliveries (≤33 weeks) incurred significant additional risk of recurrence compared with 34-36 weeks only in women with two or more previous spontaneous preterm deliveries (Figure 3, Panel A). Risk of recurrence was inversely related to the number of previous term deliveries (Figure 3, Panel B) and gestational age of most recent delivery (Figure 3, Panel C). Women with no previous term deliveries (n=491) and those delivering before 20 weeks in their most recent pregnancy (n=99) had the highest predicted risk. The associations with number of term births and earliest gestational age were consistent irrespective of number of previous spontaneous preterm deliveries.

Figure 4 demonstrates again that predicted risk of recurrence decreased when the earliest previous spontaneous preterm delivery occurred later (34-36 weeks), but only in women with two or more previous spontaneous preterm deliveries. The risk of recurrent preterm delivery was similar for women with only one previous spontaneous preterm and no previous term deliveries ranging from 27.0 (95% CI: 18.4, 35.6) to 31.5 (95% CI: 24.7, 38.4), regardless of earliest gestational age (Figure 4). The protective effect of previous term deliveries slightly decreased all risks similarly (Appendix C: Figure 10).

Three women in the study met the criteria for highest hypothetical risk based on obstetric history: ≥3 prior spontaneous preterm deliveries, earliest occurring at 20-27 weeks, the most recent pregnancy delivered before 20 weeks with no history of a term birth. The predicted risk of recurrent spontaneous preterm delivery in these women was 100% which correlated exactly with the observed rate (n=3) in this cohort, despite treatment. A woman with the same high risk history but the earliest previous spontaneous preterm delivery occurring between 34 to 36 weeks had a predicted risk of recurrence of 57%. No women in this cohort had this pattern of obstetric history.

Conversely, women were expected to be at the lowest risk of recurrence if they had only 1 previous spontaneous preterm delivery, the most recent pregnancy was a term delivery, and 2 or more previous term deliveries (n=12). Regardless of the gestational of the earliest spontaneous preterm delivery, women with this pattern had a predicted risk of recurrence 15 to 18% and an observed rate from 0 to 25%.

Predictions of recurrence prior to 35- and 32- weeks followed similar patterns with consistently lower risk predictions. Confidence intervals of risk estimates were wider for these less frequent outcomes.

5.5 DISCUSSION

Main findings

We have developed models to predict the risk of recurrent preterm term birth (prior to 37-, 35-, and 32- weeks) while receiving 17-OHPC, based primarily on a woman's obstetric history highlighting the interplay between these factors. With good discrimination and calibration, these models were used to estimate risk of recurrence when receiving standard treatment and dosing of 17-OHPC. Our results demonstrate that women with only one previous spontaneous preterm delivery have similar risk of recurrence regardless of the age of their earliest pregnancy. The increased risk associated with earliest gestational age has the most impact after two or more previous spontaneous preterm deliveries. Conversely, the protective effect seen with increased number of prior term deliveries and the increased risk with earlier gestational age of most recent pregnancy are consistent irrespective of number of previous spontaneous preterm deliveries.

Strengths and limitations

As with all observational studies, our findings must be viewed within the bounds of its limitations. The primary restrictions in this paper result directly from our study cohort. First, without a placebo group we were unable to evaluate absolute risk reductions and therefore could not assess efficacy of 17-OHPC. This is important as women with a predicted risk of 30% and 70% may have the same risk reduction and benefit from 17-OHPC, depending on their inherent initial risk. Second, and perhaps most importantly, there is sparse data in certain history patterns resulting in wide confidence intervals of risk predictions. For instance, only 45 women in the cohort have had 3 or more previous spontaneous preterm births, and only 5 of those had an earliest gestational age in the range of 34 to 36 weeks. Though we categorized variables to

maintain sufficient samples in each pattern, this limited our ability to create a robust clinical tool for risk prediction. Additionally, beta coefficients which have been used in the past to compare risk impact, were not interpretable in our model due to the interaction term.

In addition, the original RCT did not collect documentation of preventative treatment in previous pregnancies. This is unlikely to impact our findings as the majority of women in this trial only had one previous spontaneous preterm delivery, making the study pregnancy the first eligible for treatment. Additionally, the landmark Meis trial was not published until 2003 leaving little time for 17-OHPC to be adopted as standard practice before this trial.

Despite these limitations, our study also had a few notable strengths. Notwithstanding the vast amount of literature assessing the role of obstetric history in recurrent preterm birth, no study to date has assessed the interplay between these characteristics. It is reasonable to expect that the risk associated with one factor, for example number of previous spontaneous preterm births, would be modified by another such as the age at which the earliest occurred. Assessing each of these individually rather than in concert is a gap in the literature that we filled by including significant interactions in our predictive model along with generating predictions for individual patterns rather than interpreting individual beta coefficients.

Our modeling approaches utilizing binomial regression instead of a logistic model, which is commonly used, were less likely to overestimate the risk. Furthermore, our modeling allowed focus on obstetric history while including significant demographic, clinical, and behavioral characteristics that are readily available in the woman's chart. All obstetric variables were included in the model to facilitate predictions for a large array of history patterns-regardless of statistical significance. We were also not limited by documentation of compliance with the

burdensome weekly regimen as this was collected in the RCT and we limited the sample to women with >50% compliance.

Interpretation

Our overall findings of associations between recurrence and number of previous spontaneous preterm deliveries, gestational age of earliest and most recent pregnancy, and number of term deliveries is generally consistent with extant literature. Nonetheless, contrary to our results, in a secondary analysis of the landmark Meis trial [8], authors found that the risk associated with number of previous spontaneous preterm births was only present in the placebo group and not those treated with 17-OHPC [53]. Their results suggest that treatment may be most beneficial in women with phenotypes shared for recurrent preterm births. Opposite these findings, in our study cohort of treated women the association between number of prior spontaneous preterm deliveries and recurrent preterm delivery remained significant. Moreover, we found that women with high recurrence numbers and early ages were in fact most likely to have a subsequent preterm delivery despite treatment.

Another study of interest, utilizing the Omega-3 trial data but with major differences in objective, was conducted by Manuck et al [63]. These authors aimed to develop a clinical scoring method to predict non-response to treatment with 17-OHPC. In an attempt to better classify utility of 17-OHPC, they defined response as prolongation of gestation by 3 or more weeks past a patient's earliest preterm birth. The final analytic sample in this study consisted of 595 'responders' (of which 27% still had a preterm birth) and 159 'nonresponders'. Using multivariate logistic regression they identified two obstetric history variables associated with 'nonresponse': each additional week of gestation of earliest previous preterm birth (OR 1.23, 95% CI: 1.17, 1.30) and a penultimate preterm birth (OR 2.10, 95% CI: 1.03, 4.25). No further

obstetric history variables were tested in the multivariable analysis. When this prediction model was externally validated it had a sensitivity and specificity of 65% and 67%, respectively. Distinct from our results, authors concluded that additional weeks of gestation in the earliest pregnancy increased the woman's likely to *not* respond. This opposite conclusion is likely due to the disregard of the interplay between characteristics and simply interpreting odds ratios of independent risk factors, or it may be a result of their unique outcome definition. Despite these limitations, an added value of this work is the exploration of genetic risks significant in predicting nonresponse [62].

The similar risk for women with one previous spontaneous preterm delivery regardless of the gestational age at which it occurs is a novel finding. We are confident in these findings as sample size is largest for women with only one previous spontaneous preterm delivery and the predicted and observed risks both support this conclusion. As a clinical example, consider Patient A as an average woman with only one prior pregnancy ending in a spontaneous preterm delivery at 20 weeks. Her predicted risk of recurrence is 29.2 (95% CI: 20.6, 37.7). In that same woman, if the previous delivery instead occurred at 36 weeks her risk would slightly decrease to 25.8 (19.2, 32.4). However, if instead she had 2 prior spontaneous preterm deliveries, then when the earliest delivery occurred at 20 weeks the predicted risk increases to 47.4 (29.1, 65.7) compared with a risk of 19.4 (7.7, 31.2) when the earliest delivery occurred at 36 weeks. These findings suggest that women with only one prior spontaneous preterm delivery are too diverse a group, comprised of multiple phenotypes of preterm birth, which results in a similar average risk profile. Conversely, women with a history of recurrence may share phenotypes that are potentially resistant to treatment, particularly for phenotypes associated with early preterm delivery.

We are less confident in results indicative of "100% predicted risk" as few women comprise these high risk profiles, resulting in unstable estimates with wide confidence intervals. Nevertheless, in women who remain at a 100% predicted and observed risk of recurrence there are two possible explanations for treatment failure. The first explanation, as preterm delivery is an outcome with numerous causes, it is possible that 17-OHPC does not target certain phenotypes through its mechanism of action. 17-OHPC may act on a pathway that is different and more amenable to treatment than to that which is shared with recurrent, early preterm deliveries. The second possibility is an insufficient dose based on demographic or phenotypic characteristics.

Conclusions

Our findings contribute to the argument that utilization of a blanket treatment protocol of 17-OHPC for all women with a history of only one previous spontaneous preterm birth is inefficient. With more research in larger populations to confirm and expand these findings, we hope to better target treatment by individual risk and phenotype beyond a single previous spontaneous preterm birth. This paper adds clarity and begins to stratify risk by obstetric history, facilitating a patient-specific profile which will direct treatment to those most likely to benefit.

5.6 TABLES AND FIGURES

Table 9. Baseline demographics of women in study cohort by recurrent spontaneous delivery prior to 37 weeks' gestation (n=754).

Characteristic	Spontaneous Preterm Delivery N (%) n=267	Term Delivery N (%) n=487
Race		
NH White	151 (56.5)	230 (47.2)
NH Black	92 (34.5)	152 (31.2)
Hispanic	19 (7.1)	89 (18.3)
Other	5 (1.9)	16 (3.3)
Maternal age [Mean (SD)]	27.1 (5.4)	28.0 (5.5)
Years of maternal schooling		
≤6 years	2 (0.8)	6 (1.2)
7-12 years	131(49.0)	201 (41.3)
≥13 years	134 (50.2)	280 (57.5)
Pre-pregnancy BMI [Mean (SD)]	26.3 (6.9)	26.6 (6.6)
Married	192 (71.9)	337 (69.2)
Smoker	50 (18.3)	65 (13.4)
Parity		
1 previous pregnancy	126 (47.2)	228 (46.8)
2 previous pregnancies	75 (28.1)	153 (31.4)
3 or more pregnancies	66 (24.7)	106 (21.8)
Diabetes	3 (1.1)	8 (1.6)
Street drug use	5 (1.9)	9 (1.9)
Number of previous SPTDs		
1	176 (65.9)	386 (79.1)
2	59 (22.1)	88 (18.0)
≥3	32 (12.0)	13 (2.7)
Gestational age of earliest sptd		
20-27 weeks	83 (31.1)	130 (26.6)
28-33 weeks	109 (40.8)	167 (34.2)
34-26 weeks	75 (28.1)	190 (38.9)
Gestational age of most recent pregnancy		
<20 weeks	38 (14.2)	61 (12.5)
20- 27 weeks	48 (18.0)	88 (18.1)
28-33 weeks	86 (32.2)	111 (22.8)
34-36 weeks	76 (28.5)	162 (33.3)

(Table 9 continued)

≥37 weeks	19 (7.1)	65 (13.3)
Number of previous term deliveries		
0	192 (71.9)	299 (61.4)
1	50 (18.7)	119 (24.4)
≥2	25 (9.4)	69 (14.2)
History of elected termination(s)	36 (13.5)	74 (15.2)
History of spontaneous loss(es) at <20 weeks	86 (32.2)	153 (31.4)
Delivery classification of most recent pregnancy		
Delivery ≥37 weeks	19 (7.1)	65 (13.4)
Spontaneous PTL with delivery	137 (51.3)	211 (43.3)
PROM leading to spontaneous PTD	66 (24.7)	131 (26.9)
PTD for fetal indications	0 (0)	5 (1.0)
PTD for maternal indications	2 (0.8)	4 (0.8)
Spontaneous loss <20 weeks	38 (14.2)	61 (12.5)
Preterm intrapartum stillbirth	5 (1.9)	10 (2.1)

[a]NH=non-Hispanic, SD=standard deviation, SPTD=spontaneous preterm delivery; SD=standard deviation; ptd=preterm delivery; PTL=preterm labor; PROM=premature rupture of membranes

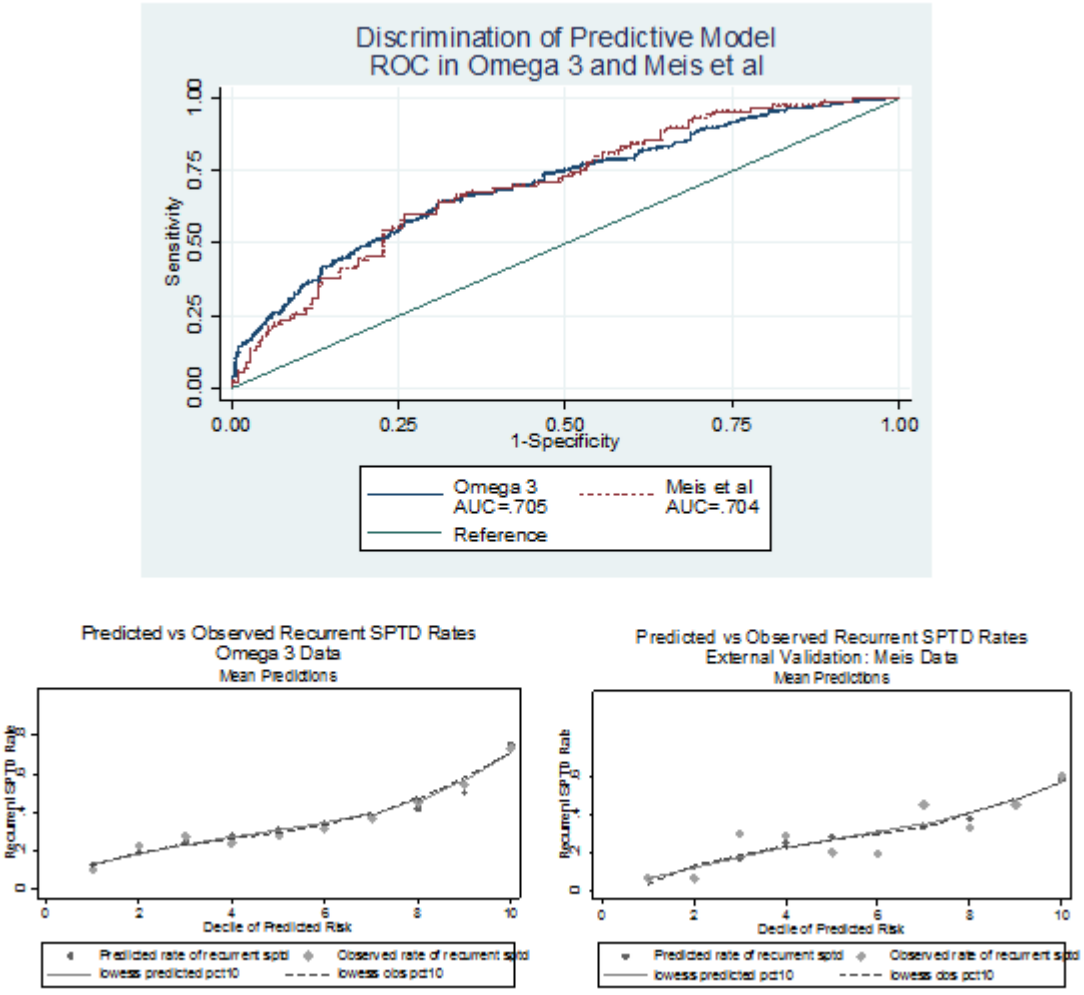

Figure 2. Discrimination and calibration of the predictive model for recurrent spontaneous preterm delivery prior to 37 weeks.

*ROC= receiver operating characteristic curve; AUC= area under the curve; SPTD= spontaneous preterm delivery

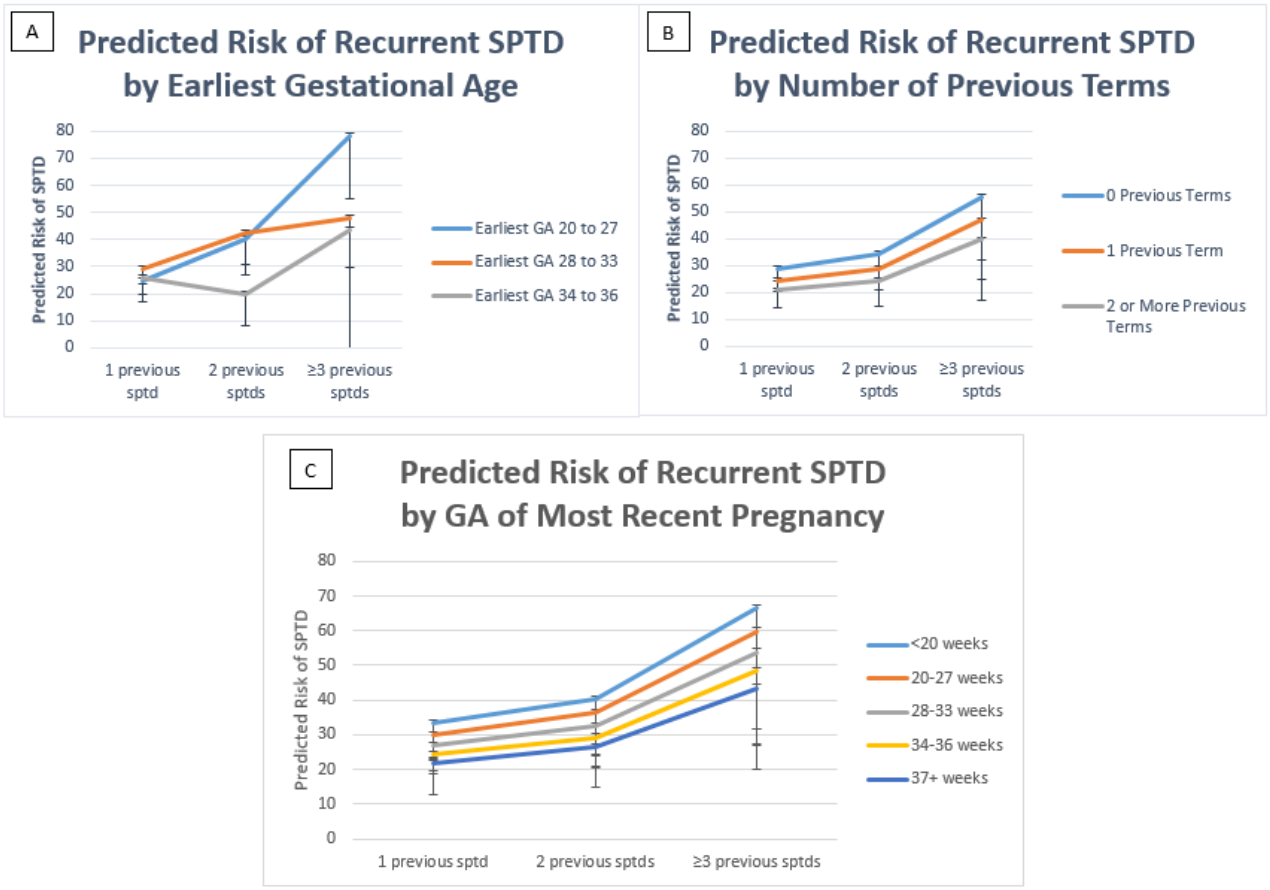

Figure 3. Risk of recurrent sptd before 37 weeks gestation by number of prior sptds, earliest gestational age (Panel A), number of previous term deliveries (Panel B), and gestational age of the most recent pregnancy (Panel C) with all other variables at means.

SPTD=spontaneous preterm delivery, GA=gestational age

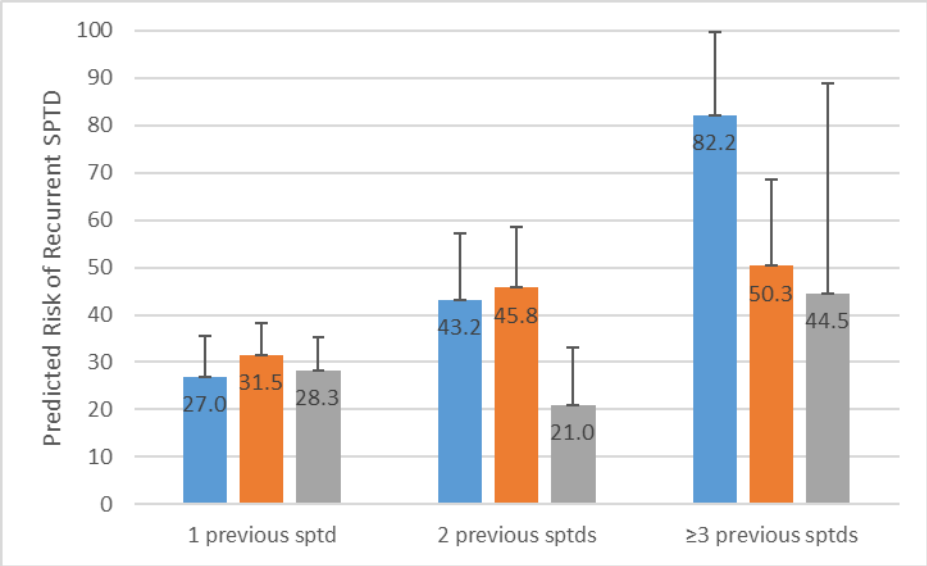

Figure 4. Risk of recurrent sptd before 37 weeks by number of prior sptds and earliest gestational age in women with no previous term deliveries with all other variables at their mean.

SPTD=spontaneous preterm delivery, GA=gestational age

6.0 SYNTHESIS

6.1 OVERVIEW OF FINDINGS

The global purpose of this dissertation was to apply pharmacoepidemiologic methods to relevant clinical issues encountered by obstetric care providers when prescribing medication in pregnancy. We did so by addressing optimization of two medication therapies used in pregnancy, 1) opioid maintenance therapy, and 2) 17-OHPC for secondary prevention of preterm birth. Using a retrospective cohort of 716 women treated with opioid maintenance therapy at Magee-Womens Hospital, Pittsburgh we addressed aims 1 and 2. To assess aim 3 we utilized data from 754 women enrolled in the National Institute of Child Health and Human Development (NICHD) Maternal Fetal Medicine Units (MFMU) Network Omega-3 trial. The findings of this work are summarized below.

Specific Aim 1. To estimate the association between in utero exposure to methadone compared with buprenorphine and neonatal abstinence syndrome after accounting for unmeasured confounding by severity of maternal addiction.

Using an internal validation cohort with intensive chart review, we informed a probabilistic bias analysis accounting for maternal addiction severity. Methadone and buprenorphine exposure were determined using pharmacy billing codes with confirmation in chart review. Neonatal abstinence syndrome (NAS) was defined as neonatal treatment with

morphine according to pharmacy billing. We found, contrary to our hypothesis, that the historical decreased risk of NAS associated with buprenorphine was not fully explained by unmeasured confounding attributable to maternal addiction severity.

Specific Aim 2. To describe and quantify the role of preterm birth in the association between opioid maintenance therapy and neonatal abstinence syndrome.

Implementing a marginal structural model with inverse probability weighting to account for confounding, we assessed the impact of preterm birth as a mediator of the relationship between opioid maintenance therapy and NAS. Our hypothesis was based on the following associations: 1) methadone is associated with preterm birth, and 2) preterm birth is associated with decreased risk of NAS. Results confirmed our hypothesis that the increased risk of NAS after methadone exposure in utero compared with buprenorphine was stronger among a population of term compared with preterm births.

Specific Aim 3. To build a predictive model that elucidates the interrelationship between pregnancy histories on the risk of recurrent spontaneous preterm birth among women treated prophylactically with weekly 17-alpha-hydroxyprogesterone caproate injections.

Using backwards, chunk-wise, model building approaches after assessing interactions between obstetric history variables, we built a predictive model for recurrent spontaneous preterm delivery (at <37 weeks, <35 weeks, and <32 weeks) despite treatment with 17-OHPC. Using this model we predicted risks for various obstetric historical patterns. Our analysis demonstrated that women with only 1 previous spontaneous preterm delivery had similar predicted risk regardless of the gestational age at which it occurred and the amount of previous term deliveries. Put differently, risk attributable to earliest gestational age in a previous pregnancy was only seen in women with 2 or more previous spontaneous preterm deliveries.

Other confirmed associations did not vary by the amount of previous spontaneous preterm deliveries.

6.2 STRENGTHS AND LIMITATIONS

As with all research, our conclusions must be considered in light of their limitations, but also has notable strengths.

6.2.1 Opioid maintenance therapies

Misclassification

Opioid maintenance therapy documented on the day of delivery or up to one week prior to delivery was used for classification of exposure. This method was used as the majority of women do not receive their opioid maintenance medication directly from the hospital and we lacked information on retail claims. It is therefore possible that exposure for the entire pregnancy period was misclassified, particularly if therapy was changed from buprenorphine to methadone as the reverse is clinically contraindicated. Misclassification would result in an underestimate of the observed association for Chapters 3 and 4 (Manuscripts 1 and 2). However, in our validation subcohort, only 6 of 200 women had documentation of switching therapies, including changing therapies from a previous pregnancy or prior to knowing they were pregnant. It is therefore unlikely that many women were exposed to both treatments in one pregnancy and consequently is not expected to significantly impact the results.

Defining NAS as neonatal treatment with morphine limits assessment to the most severe cases of NAS. We chose to focus on the treated infants for three reasons. First, the Finnegan Scale has been criticized for being too subjective, thus potentially resulting in additional misclassification of outcome. Second, treated infants typically have the longer stays and are therefore associated with the highest costs. Third, we did not have access to Finnegan scores nor the morphine dosing required to treat withdrawal. Therefore, though having a gradient of Finnegan scores or morphine dose may be preferable, those receiving treatment incur the largest costs and this approach is subject to less misclassification due to the subjectivity of the Finnegan Scale.

Unmeasured confounding

Though the aim of Chapter 3 was to estimate the effect without unmeasured confounding, some likely remains in both Chapters 3 and 4. Accounting for all potential confounders is particularly difficult because the pathophysiology of NAS is unknown. Therefore, identifying factors that are associated with NAS and treatment, and further differentiating confounders from mediators is challenging. Under the rules of causal inference in the mediation analysis, residual unmeasured confounding violates the assumption of exchangeability. This is a limitation we share with most, if not all, observational studies.

Parameterizing maternal addiction

We are the first to use an internal validation cohort to derive information on severity to adjust for unmeasured confounding; however, our findings are subject to the bounds of the data available to us and to the parameterization the severity index. Our severity index was restricted by the variables recorded in the chart and was further limited by differential missingness by treatment.

Because of sample size, we relied on any marker of severity as indicative as “severe addiction”. This approach was shown to be robust regardless of which variable was used to create the index. Ideally, a future prospective study will evaluate severity of each woman’s addiction using a validated scale such as the Addiction Severity Index [102]. Despite these limitations, our approach is preferable to deriving effect estimates exclusively from the literature.

Missing data: Lacking detailed treatment and addiction trajectories

The social judgement incurred by this population was a primary contributor to the amount of missing data in the validation cohort (Chapter 3). The stigma associated with opioid use dependence is a finding consistent across studies of similar populations, one that is exacerbated in pregnancy.

The amount of missing data and the differences by treatment in the validation cohort was a primary limitation of this analysis. To assess the impact of missing data in Chapter 3 we performed the analysis with ‘bound’ estimates in the validation cohort. When we performed our analysis as a “worst case” scenario where all missing in the methadone group was assumed to be “yes” and all missing in the buprenorphine group was coded as “no”, 97% of the methadone group was classified as having severe addiction compared with 32% in the buprenorphine group. Using the Flanders and Khoury method with this new severity index under the “worst case” scenario assumptions, the lowest association was increased to 1.38 (compared with 1.13 using the original index). Performing the probabilistic bias analysis informed using this bias parameter provided results similar to those using the literature values. Conclusions were the same demonstrating that confounding by maternal addiction did not fully explain the association between methadone and NAS. For Chapter 4 there was very minimal missing data in the total cohort and therefore had little impact on the results.

Because we lacked detailed information on treatment and addiction trajectories in our cross sectional data, we were unable to define the temporality of certain relationships. Therefore, the adjustment represented only one hypothetical relationship among the variables. For example, it is reasonable to assume that lack of insurance could increase a woman's addiction severity if she cannot afford treatment. Conversely, it is also reasonable to assume that a woman with severe addiction may be less likely to be employed and therefore have no insurance. Not knowing the temporality of these associations prohibited the development of a true direct acyclic graph to dictate adjustments. Moreover, without information on the initiation, timing, and duration of exposure to medication, we were unable to appropriately assess how these factors influence the development of NAS.

In Chapter 4, the lack of temporality and information on treatment trajectories and gestational age at initiation could have introduced immortal time bias if women receiving opioid maintenance therapy were not converted to treatment until after 37 weeks [42]. However, the validation cohort from Chapter 3 found that no women were initiated on treatment after 36 weeks eliminating this concern.

Causal assumption violations

Interpreting the mediation analysis (Chapter 4), must be done within the confines of four primary assumptions: positivity, no interference, exchangeability, and counterfactual consistency [96]. Of these, only exchangeability (described above) and counterfactual consistency are potentially violated in our study.

Counterfactual consistency requires that both the relationship between treatment-preterm birth and preterm birth-NAS are causal. A large amount of extant data support the relationship between methadone and preterm birth [17, 37, 39, 43, 44], providing us with confidence in this

association. However, the relationship between preterm birth-NAS presented a larger challenge as the pathophysiology of both are unknown. We were therefore forced to make the assumption that this relationship is causal based on the commonly accepted biologic plausibility that the association results from immature opioid-receptor development in the neonate [23].

6.2.2 17-OHPC

Study cohort

Using data collected prospectively from a randomized controlled trial is a strength of this study as there is very little missing data. Moreover, demographics were obtained from patient interview then confirmed in the medical record, decreasing the likelihood of misclassification. Nevertheless, the primary limitation in Chapter 5 results directly from the sample size and design of this cohort.

The majority of the women in the cohort (75%) had only 1 previous spontaneous preterm delivery. Therefore very few women had obstetric history patterns with at least 3 previous preterm births and this varied by gestational age of the earliest preterm delivery. For example, of the 45 women with 3 or more previous spontaneous preterm deliveries, only 5 had an earliest gestational age of 34 to 36 weeks. This was expected as women with more previous preterm deliveries typically have poorer history profiles and are associated with earlier gestational ages (i.e. <34 weeks). However, the sparseness of data in specific historical patterns resulted in unstable estimates with wide confidence intervals. This prohibited the development of a clinical tool for recurrence prediction.

No placebo group

Our study was limited to women with a previous spontaneous preterm delivery who were treated with 17-OHPC. This approach is clinically relevant as this is standard care recommended by ACOG [7]. However, without a placebo group we were unable to assess the efficacy of 17-OHPC. In order to evaluate the true effect of the drug we would need to assess absolute, rather than relative, risk reductions. For instance, a women with a poor history profile (e.g. earliest gestational age of 20 weeks, 3 previous preterm deliveries, and no previous terms) may have a much higher inherent risk than a women with only 1 previous preterm delivery at 36 weeks. Both could have a risk reduction of 20% due to use of 17-OHPC and the risk of the first patient still remain at 70% compared to only 10% for the woman with a more favorable history. Unfortunately we could not address this factor with the data available to us.

6.3 PUBLIC HEALTH AND CLINICAL IMPLICATIONS

Contributing to a field that suffers from a dearth of data to guide clinical decisions is indisputably important. We applied pharmacoepidemiologic methods to contribute to two frequently encountered and high public health priorities in pregnancy: opioid use dependence and recurrent preterm birth.

Opioid use, both prescription and heroin, has reached epidemic proportions in the U.S. [12]. This marked increase in abuse and dependence has impacted women of reproductive age including those who are pregnant [103]. Because opioid maintenance therapy is recommended over drug detoxification in pregnancy [4], determining which treatment provides optimal outcomes for both mother and fetus is of the utmost importance. Withdrawal in the infant after in

utero exposure, termed neonatal abstinence syndrome (NAS), is one of the most costly and detrimental outcomes associated with treatment [85]. This dissertation adds to the argument for treatment availability of both approved opioid maintenance therapies as buprenorphine is associated with less NAS compared with methadone. Decreasing the incidence of NAS suffered by these infants improves long term outcomes for these children, reduces maternal fear due to neonatal exposure, and reduces healthcare costs by avoiding longer NICU stays and treatment. This work advocates for expansion of buprenorphine use in pregnancy through extended access and increased number of licensed buprenorphine providers.

Another obstetric challenge that has yet to be overcome despite treatment is preterm birth. In the U.S. preterm birth occurs at a rate of about 10% and contributes to 75% of all perinatal deaths [5]. The public health significance of this occurrence cannot be overstated. Though treatment with 17-OHPC has been widely adopted for prevention of recurrence in women with a previous spontaneous preterm delivery, targeting this treatment to women most likely to respond has not been successful. The predictive models created in this dissertation began to stratify the risk of treatment failure by obstetric history. In women with 100% predicted recurrence, new therapies targeting different mechanisms of action need to be explored.

This work may alter treatment preferences for women with an opioid use disorder in pregnancy and begins to facilitate a patient-specific risk profile targeting treatment with 17-OHPC in various formulations to those most likely to benefit. Overall this work demonstrated the utility of applying epidemiologic methods to the study of medication use in pregnancy.

6.4 FUTURE RESEARCH

Future studies should apply our approach to minimize unmeasured confounding by severity of maternal addiction to other outcomes associated with opioid maintenance therapy that are of public health significance such as fetal growth, length of stay in the NICU, NAS severity and others. Impact of maternal addiction severity may vary for each outcome, all of which contribute to clinical decision making and determining optimal treatment. Performing such studies in a population with documented addiction and medication histories would be valuable to evaluate cumulative effects of exposure. These results can be strengthened in a cohort utilizing a validated tool assessing maternal addiction severity. Subsequent health policy and economic studies evaluating the effect of expanded access to treatment options are also warranted.

Basic science studies evaluating how each opioid maintenance therapy effects the placenta are also needed. It is biologically plausible that the increased risk of preterm birth associated with methadone results from morphologic changes in the placenta [104]. Furthermore, neonatal exposure to the opioid agonists may vary by structure of each medication. Defining these mechanisms and elucidating the pathophysiology of NAS will guide methods to decrease neonatal risk.

Furthermore, dosing for both mother and neonate should be studied at a basic science level. Current maternal dosing, both initiation and maintenance, are based on subjective measures and tools such as the Clinical Opioid Withdrawal (COW) score. A critical contribution to the field would be development of a new tool to objectively evaluate withdrawal and dose to appropriate blood concentration levels to avoid this. This same approach would be extremely beneficial in administration of morphine to infants. The ability to determine doses and length of

treatment on objective measures, such as blood concentration, rather than Finnegan Scores could help to avoid excess exposure of the infant and periods of withdrawal.

The predictive models for recurrent preterm birth need to be replicated in a population with more women in the highest risk strata, while still assessing for interaction between the variables. Ideally, models will be built in both a treated and non-treated group with similar histories, though this is not likely feasible as women with later previous preterm deliveries more frequently refuse prophylactic treatment. A novel approach to address this inherent bias with an observational cohort of treated and untreated women (both with documented previous spontaneous preterm delivery), would be to implement a propensity score to assess the true efficacy of the medication in a matched sample.

To provide the most clinically applicable results, similar studies should be repeated assessing prolongation of gestation rather than binary preterm-term birth. Predicting gestational age rather than risk of recurrence, provides the healthcare provider valuable information to evaluate benefit. For instance, in a women with an earliest previous gestational age of 21 weeks, prolongation of gestation in her next pregnancy to 34 weeks is clinically beneficial, though still classified as a recurrent preterm delivery.

Finally, a critical area of importance for future work is to define both the mechanism of action of 17-OHPC and the pathophysiology of preterm birth. Pharmacologic and physiologic studies elucidating the various phenotypes will allow for more tailored preventative therapies to be developed and applied. These studies also have the potential to identify necessary dose alterations after establishing a dose-concentration-response relationship. For example, women with higher body mass indices may require a larger dose of 17-OHPC to reach effect due to a larger distribution of the drug and subsequent lower concentration.

Though the opportunity and need for future research on these topics is vast— this dissertation work provides a significant contribution. Our research on both opioid maintenance therapies and 17-OHPC can be utilized by clinical organizations such as ACOG to help inform future treatment practice recommendations.

APPENDIX A: MANUSCRIPT 1 – SUPPLEMENTAL CONTENT

Opioid maintenance therapy selection protocol at Magee-Womens Hospital

At Magee-Womens Hospital, initiation of opioid maintenance therapy with methadone or buprenorphine is determined by the patient, provided the patient is not already on a stable opioid maintenance regimen and does not concurrently use benzodiazepines (self-reported or on urine drug screen). Women who are using benzodiazepines, a sedative drug, are allocated to methadone treatment as they will require inpatient tapering from both opioids and benzodiazepines. Methadone-treated patients are converted to methadone during an inpatient admission where the dose is titrated to the mother's Clinical Opioid Withdrawal (COW) Score. After discharge they are provided the medication daily at one of several outpatient opioid treatment clinics.

After the establishment of the Pregnancy Recovery Center at Magee-Womens Hospital in July of 2014, eligible women treated with buprenorphine were able to receive their medication at a hospital-based outpatient clinic. The treatment protocol for buprenorphine is also based on the COW score. Once a stable dose is achieved, women are provided up to a 2 week supply of medication allowing them to dose themselves daily at home without a need for hospitalization or a daily clinic visit. Prior to the Pregnancy Recovery Center, women were referred to outside buprenorphine clinics with DEA approved providers for treatment. Outside clinics remain a utilized option at this institution. Women who are discontinued from the Pregnancy Recovery

Center due to signs of ongoing illicit substance abuse will be converted to methadone at Magee-Womens Hospital or transferred to an outside buprenorphine provider. Methadone treated women will not be converted to buprenorphine in pregnancy.

Selecting bias analysis parameters

The first severity indicator determined by the research team, time of conversion, was determined based on clinical experience and consistent with previous literature that women with more controlled addiction were more likely to conceive while receiving opioid maintenance therapy. Women with uncontrolled addiction were determined to be more likely to avoid seeking treatment until later in pregnancy. These women with more uncontrolled addiction were also more likely to relapse and to continue to use illicit substances despite treatment. These factors were considered indicative of a more severe addiction with higher degrees of maladaptive compulsive drug-seeking behavior.

Benzodiazepines are sedative prescription drugs that can accentuate the “high” associated with opioid abuse while also increasing risk of overdose when used concomitantly with opioids. It has been found that persons abusing both substances have more social dysfunction which can potentially be used clinically as a marker of a more severe addiction.

Table 10. Demographics of women in study cohort vs. all deliveries Magee-Women Hospital in Pittsburgh from 2013 through 2014.

Characteristic	Study cohort N (%) n=716	All deliveries at MWH N (%) n= 20,565
Race		
White	675 (94.3)	14,835 (72.1)
Black	27 (3.8)	4, 085 (19.9)
Other	1 (0.1)	771 (3.8)
Missing	13 (1.8)	874 (4.2)
Mother's age [Mean (SD)]	28.8 (4.8)	29.1 (5.6)
Mother's Education		
Less than high school	128 (17.8)	1,129 (5.5)
High school graduate or GED completed	304 (42.5)	3,329 (16.2)
Some college credit	146 (20.4)	2,087 (10.2)
College graduate	120 (16.8)	9,591 (46.6)
Missing	18 (2.5)	4,429 (21.5)
Prepregnancy BMI [Mean (SD)][a,b]	24.4 (5.7)	26.4 (6.6)
Married[c]	93 (13.0)	11,938 (58.1)
Smoked during pregnancy	586 (81.8)	1,891 (9.2)
Parity[d]		
Nulliparous	224 (31.3)	9,473 (46.1)
1-2 previous pregnancies	359 (50.1)	9,493 (46.2)
Greater than 2 pregnancies	133 (18.6)	1,586 (7.7)
Gestational age at delivery [Mean (SD)][e]	37.9 (2.8)	38.5 (2.6)
Birthweight [Mean (SD)][f]	2849 (621)	3269 (620)
Infant with congenital anomaly	77 (10.8)	2 (0.01)

[a]Prepregnancy BMI in full cohort based on n=356.
[b]Prepregnancy BMI in all deliveries based on n=14,753.
[c]Married in all deliveries based on n=20,432.
[d]Parity in all deliveries based on n=20,552.
[e]Gestational age in all deliveries based on n=20,302
[f]Birthweight in all deliveries based on n=20,073
MWH=Magee-Womens Hospital, GED=general education development, SD=standard deviation, BMI=body mass index

Table 11. Demographics of women in validation cohort vs. total study cohort at Magee-Womens Hospital in Pittsburgh from 2013 through 2015.

Characteristic	Validation cohort N (%) n=200	Full cohort N (%) n=716
Race		
White	194 (97.0)	675 (94.3)
Black	5 (2.5)	27 (3.8)
Filipino	0 (0)	1 (0.1)
Missing	1 (0.5)	13 (1.8)
Mother's age [Mean (SD)]	28.4 (5.1)	28.8 (4.8)
Mother's Education		
Less than high school	28 (14.1)	128 (17.8)
High school graduate or GED completed	92 (46.2)	304 (42.5)
Some college credit	41 (20.6)	146 (20.4)
College graduate	33 (16.6)	120 (16.8)
Missing	6 (2.5)	18 (2.5)
Prepregnancy BMI [Mean (SD)][a,b]	24.1 (5.5)	24.4 (5.7)
Married	29 (14.6)	93 (13.0)
Employed	74 (37.2)	274 (38.3)
Smoked during pregnancy	163 (82.7)	586 (81.8)
Parity		
Nulliparous	70 (35.2)	224 (31.3)
1-2 previous pregnancies	94 (46.7)	359 (50.1)
Greater than 2 pregnancies	36 (18.1)	133 (18.6)
Hepatitis c positive	22 (11.1)	92 (12.9)
Gestational age at delivery [Mean (SD)]	38.2 (2.7)	37.9 (2.8)
Birthweight [Mean (SD)]	2920 (601)	2849 (621)
Infant with congenital anomaly	25 (12.6)	77 (10.8)

[a]Prepregnancy BMI in validation cohort based on n=97.

[b]Prepregnancy BMI in full cohort based on n=356.

GED=general education development, SD=standard deviation, BMI=body mass index

Table 12. Demographics of women diagnosed with drug-dependent deliveries of singletons by treatment for neonatal abstinence treatment from 2013 through 2015 (n=716).

Characteristic	Treated for NAS N (%) n=415	Not Treatment for NAS N (%) n=301
Race		
White	389 (93.7)	286 (95.0)
Black	17 (4.1)	10 (3.3)
Missing	9 (2.2)	5 (1.7)
Mother's age [Mean (SD)]	28.8 (4.8)	28.8 (4.8)
Mother's Education		
Less than high school	80 (19.3)	48 (15.9)
High school graduate or GED completed	179 (43.1)	125 (41.5)
Some college credit	79 (19.0)	67 (22.3)
College graduate	67 (16.2)	53 (17.6)
Missing	10 (2.4)	8 (2.7)
Prepregnancy BMI [Mean (SD)] [a]	24.6 (5.3)	24.2 (6.1)
BMI category[b]		
Underweight (<18.5kg/m^2)	17 (4.1)	11 (3.6)
Normal weight	130 (31.3)	80 (26.7)
Overweight	35 (8.5)	30 (9.9)
Obese	25 (6.0)	28 (9.3)
Missing	208 (50.1)	152 (50.5)
Married	49 (11.8)	44 (14.6)
Employed	150 (36.1)	124 (41.2)
Smoked during pregnancy	336 (81.0)	250 (83.1)
Parity		
Nulliparous	129 (31.1)	95 (31.6)
1-2 previous pregnancies	212 (51.1)	147 (48.8)
Greater than 2 pregnancies	74 (17.8)	59 (19.6)
Hepatitis c positive	62 (14.9)	30 (10.0)
Gestational age at delivery [Mean (SD)]	38.5 (1.8)	37.1 (3.6)
Birthweight [Mean (SD)]	2953 (506.6)	2704 (727.6)
Infant with congenital anomaly	35 (8.4)	42 (14.0)

[a]Prepregnancy BMI based on n=356.

[b]Prepregnancy BMI defined as underweight (<18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), obese (≥30 kg/m^2).

NAS=neonatal abstinence syndrome, GED=general education development, SD=standard deviation, BMI=body mass index

Table 13. Amount of missing data on severity indices abstracted from medical charts per woman by opioid maintenance type in a subsample of opioid use dependent women at Magee-Women Hospital in Pittsburgh, 2013-2015 (n=200).

Number of Variables Missing	Methadone N=100	Buprenorphine N=100
None	16	11
1 variable	32	17
2 variables	25	28
3 variables	17	35
All 4 variables	10	9

Description of Flanders and Khoury method. Data informing these associations was obtained directly from logistic regressions in the validation subcohort.

- OR_{EC} =7.11
- OR_{DC} =1.21
- $1/P_{C+E-}$ =3.13
- $OR_{DC}/ (Q_{C+E-} + OR_{DC} * P_{C+E-})$ =1.13
- $OR_{EC} / (Q_{C+E-} + OR_{EC} * P_{C+E-})$ =2.41

Per the Flanders and Khoury method, the minimum of these values is the upper limit of RR due to confounding. Therefore, 1.13 becomes the maximum value of RR due to confounding thereby becoming the upper limit of the trapezoidal distribution.

*OR=odds ratio, EC=exposure-confounder, DC=disease-confounder, P=prevalence, Q=1-prevalence, RR=relative risk

Worst case scenario approach of addressing missing data is defined as all missing data coded as “yes” in the methadone group and “no” in the buprenorphine group.

Sensitivity of bias analysis (worst case scenario of missing data severity index):

- OR_{EC} = 68.71 (20.22, 233.51)
- OR_{DC} = 1.68 (0.94, 3.02)
- $1/P_{C+E-}$ = 3.13
- $OR_{DC}/ (Q_{C+E-} + OR_{DC} * P_{C+E-})$ =1.38
- $OR_{EC} / (Q_{C+E-} + OR_{EC} * P_{C+E-})$ =3.03
- The upper limit of the RR due to confounding is increased to 1.38

*OR=odds ratio, EC=exposure-confounder, DC=disease-confounder, P=prevalence, Q=1-prevalence, RR=relative risk

Table 14. Comparison of results from adjusted conventional and probabilistic bias analyses accounting for unmeasured confounding by severity of addiction on the risk of NAS associated with methadone compared with buprenorphine, at MWH, Pittsburgh, Pennsylvania.

Opioid maintenance therapy	Conventional analysis: Adjusted[a] relative risk (95% confidence interval)	Bias Analysis 1: Adjusted[a] point estimate (95% bootstrapped simulation interval)[b]	Bias Analysis 2: Adjusted[a] point estimate (95% bootstrapped simulation interval)[c]
Buprenorphine	Reference	Reference	Reference
Methadone	1.3 (1.1, 1.5)	1.1 (0.9, 1.3)	1.1 (0.9, 1.3)

[a]Adjusted for parity, maternal race, employment status, hepatitis c status, age, year of delivery, smoking status, marital status, and private vs. public insurance.

[b]minimum RR_C=1.0, mode 1=1.08, mode 2=1.3, maximum RR_C=1.38

[c]minimum RR_C=1.0, mode 1=1.1, mode 2=1.28, maximum RR_C=1.38

RRc=relative risk due to confounding

These results demonstrate findings using a "worst case" scenario approach in which all missing data is coded as "yes" in the methadone group and "no" in the buprenorphine group.

APPENDIX B: MANUSCRIPT 2 – MEDIATION SUPPLEMENTARY TABLE

Table 15. Opioid maintenance therapy-NAS association and opioid maintenance therapy-NAS association not attributable to preterm birth in women exposed to opioid maintenance therapy at Magee-Womens Hospital, 2013 to 2015 using the generalized product method (n=716).[a]

				Risk Differences[b]: RD per 100 live born infants (95% CI)		Risk Ratios[c]: RR (95% CI)		Proportion Explained on RD Scale
	Events (n)	Population at risk (n)	Unadjusted risk per 100 live born infants	Total association: OMT-NAS	Association not attributed to preterm birth:	Total association: OMT-NAS	Association not attributed to preterm birth:	Proportion of total avoided by preterm birth
Methadone	263	407	64.6	13.3 (5.4, 20.6)	16.6 (9.2, 23.5)	1.23 (0.17, 8.73)	1.25 (0.17, 8.85)	24.8%
Buprenorphine	152	309	49.2	Reference	Reference	Reference	Reference	

[a] All results using the generalized product method are bootstrapped.
[b] Linear risk models adjusted for parity, maternal race, age, employment status, smoking status, marital status, hepatitis c status, private vs. public insurance, and year of delivery.
[c] Poisson regression models adjusted for parity, maternal race, age, employment status, smoking status, marital status, hepatitis c status, private vs. public insurance, and year of delivery.

OMT=opioid maintenance therapy, NAS=neonatal abstinence syndrome, RD= risk difference, RR= risk ratio, CI= confidence interval

APPENDIX C: MANUSCRIPT 3 – 17 OHPC SUPPLEMENTAL CONTENT

Description of Meis Trial

The landmark trial assessing the utility of 17-OHPC was conducted by Meis et al. in 2003. This was a double-blind, placebo-controlled RCT conducted at 19 clinical centers from 1998-1999. Recruitment was limited to women with at least one previous spontaneous preterm delivery between 20 and 36^6 weeks of gestation. Participants were randomized in a 2:1 manner to treatment with weekly 250mg injections of 17-OHPC (n=310) or inert oil placebo (n=153) beginning at 16 to 20^6 weeks gestation and continuing through 36^6 weeks gestation or until delivery. These authors found that 17-OHPC significantly decreased the risk of recurrent preterm birth prior to 37 weeks (RR 0.66, 95% CI: 0.54, 0.81) and at less than 32 weeks (RR 0.58, 95% CI: 0.37, 0.91).

Table 16. Demographics of women in Omega-3 cohort compared with Meis trial at baseline.

Characteristic	Omega-3 N (%) n=754	Meis et al. N (%) n=310
Race		
NH White	381 (50.5)	79 (25.5)
NH Black	244 (32.4)	183 (59.0)
Hispanic	108 (14.3)	43 (13.9)
Other	21 (2.8)	5 (1.6)
Mother's age [Mean (SD)]	27.7 (5.5)	26 (5.6)
Years of maternal schooling		
≤6 years	8 (1.2)	11 (3.6)
7-12 years	332 (44.0)	216 (69.7)
≥13 years	414 (54.9)	83 (26.8)
Prepregnancy BMI [Mean (SD)]	26.5 (6.7)	26.7 (7.4)
BMI category[a]		
Underweight	39 (5.2)	26 (8.4)
Normal weight	326 (43.2)	115 (36.8)
Overweight	177 (23.5)	65 (21.0)
Grade 1 obesity	113 (15.0)	46 (14.8)
Grade 2 obesity	51 (6.8)	22 (7.1)
Grade 3 obesity	40 (5.3)	25 (8.1)
Missing	8 (1.1)	12 (3.9)
Married	529 (70.2)	159 (51.3)
Smoked	115 (25.3)	70 (22.6)
Chlamydia or gonorrhea	24 (3.2)	14 (4.5)
Marijuana use	12 (1.6)	9 (2.9)
Alcohol use	59 (7.8)	27 (8.7)
Parity		
1 previous pregnancy	354 (47.0)	105 (33.9)
2 previous pregnancies	228 (30.2)	105 (33.9)
3 or more pregnancies	172 (22.8)	100 (32.2)
Diabetes	11 (1.5)	13 (4.2)
Street drug use prior to randomization	14 (1.9)	11 (3.6)

[a]Prepregnancy BMI defined as underweight (<18.5 kg/m^2), normal weight (18.5 to <25 kg/m^2), overweight (25 to <30 kg/m^2), grade 1 obesity (30 to <35 kg/m^2), grade 2 obesity (35to <40 kg/m^2), grade 3 obesity (≥40 kg/m^2).

NH=non-Hispanic, SD=standard deviation, BMI=body mass index

Table 17. Pregnancy histories in Omega-3 trial compared with Meis et al. trial.

Characteristic	Omega-3 N (%) n=754	Meis et al. N (%) n=310
Number of previous sptds		
1 previous sptds	562 (74.5)	235 (75.8)
2 previous sptds	147 (19.5)	51 (16.5)
3 or more previous sptds	45 (6.0)	24 (7.7)
Gestational age of earliest sptd [Mean(SD)]	30.3 (4.6)	29.8 (4.9)
Class of gestational age of earliest sptd		
20 to 27 weeks	213 (28.3)	98 (31.6)
28 to 33 weeks	276 (36.6)	106 (34.2)
34 to 36 weeks	265 (35.1)	106 (34.2)
Gestational age of penultimate pregnancy	29.5 (8.0)	32.1 (5.5)
Class of gestational age of penultimate pregnancy		
<20 weeks	99 (13.1)	----
20 to 27 weeks	136 (18.0)	70 (22.6)
28 to 33 weeks	197 (26.1)	84 (27.1)
34 to 36 weeks	238 (31.6)	87 (28.1)
37+ weeks	84 (11.1)	69 (22.3)
Number of previous term deliveries		
0 previous term	491 (65.1)	157 (50.7)
1 previous term	169 (22.4)	93 (30.0)
2 or more previous terms	94 (12.5)	60 (19.3)
Number of previous PTDs		
1 previous ptds	540 (71.6)	224 (72.2)
2 previous ptds	166 (22.0)	56 (18.1)
3 or more previous ptds	48 (6.4)	30 (9.7)
Number of previous elected terminations		
0 previous terminations	644 (85.4)	259 (84.4)
1 previous termination	66 (8.8)	38 (12.4)
2 or more previous terminations	44 (5.8)	10 (3.2)
Number of previous spontaneous loss at <20 weeks		
0 losses	515 (68.3)	217 (73.8)
1 loss	164 (21.8)	59 (20.1)
2 or more losses	75 (9.9)	18 (6.1)
Gestational age of qualifying pregnancy [Mean(SD)]	31.0 (4.5)	30.6 (4.6)
Route of delivery		
Vaginal	577 (76.5)	229 (74.8)
Cesarean section	177 (23.5)	77 (25.2)

SPTD=spontaneous preterm delivery, SD=standard deviation, PTD=preterm delivery

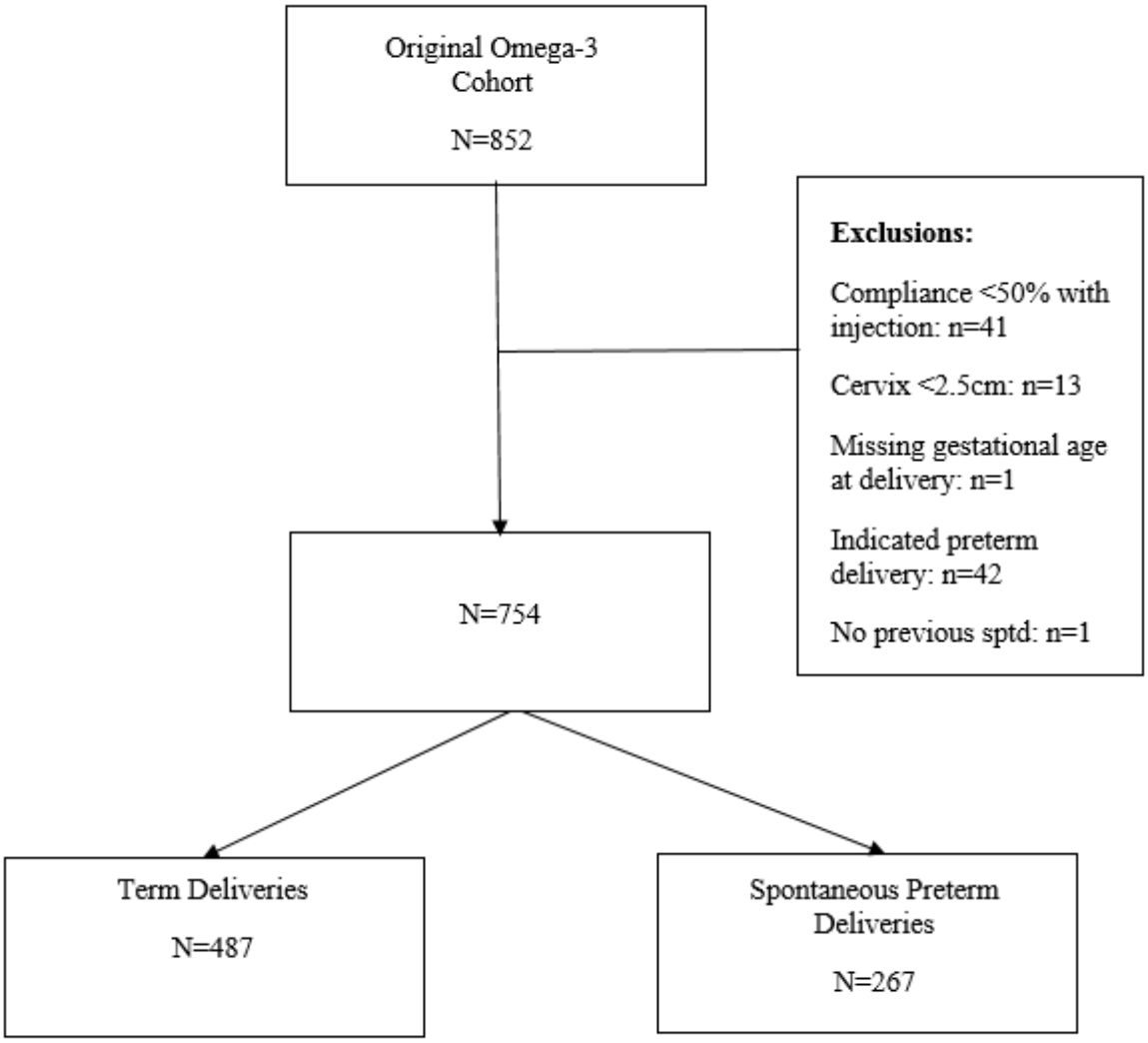

Figure 5. Flow diagram describing sample population included in secondary analysis (n=754, 2005-2006).

sptd= spontaneous preterm delivery

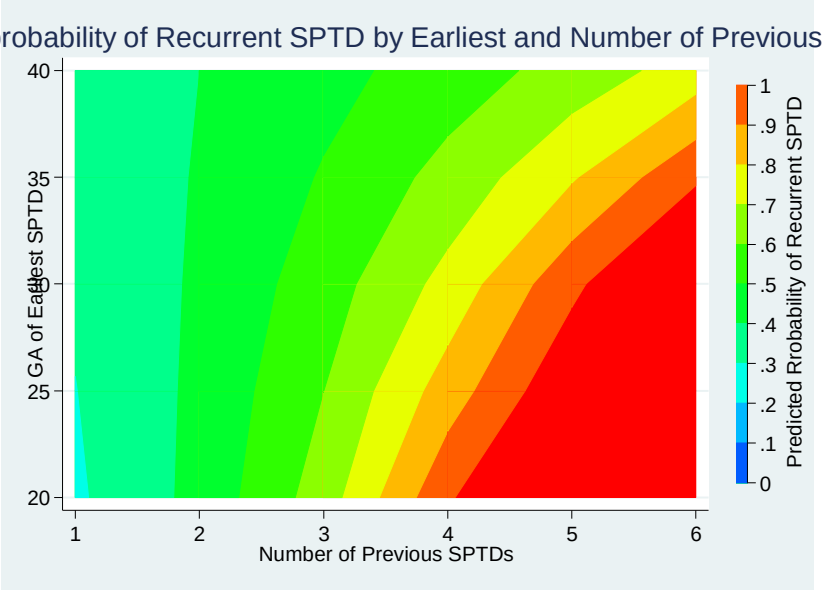

Figure 6. Interaction between gestational age of earliest spontaneous preterm delivery and number of previous spontaneous preterm deliveries.

SPTD= spontaneous preterm delivery

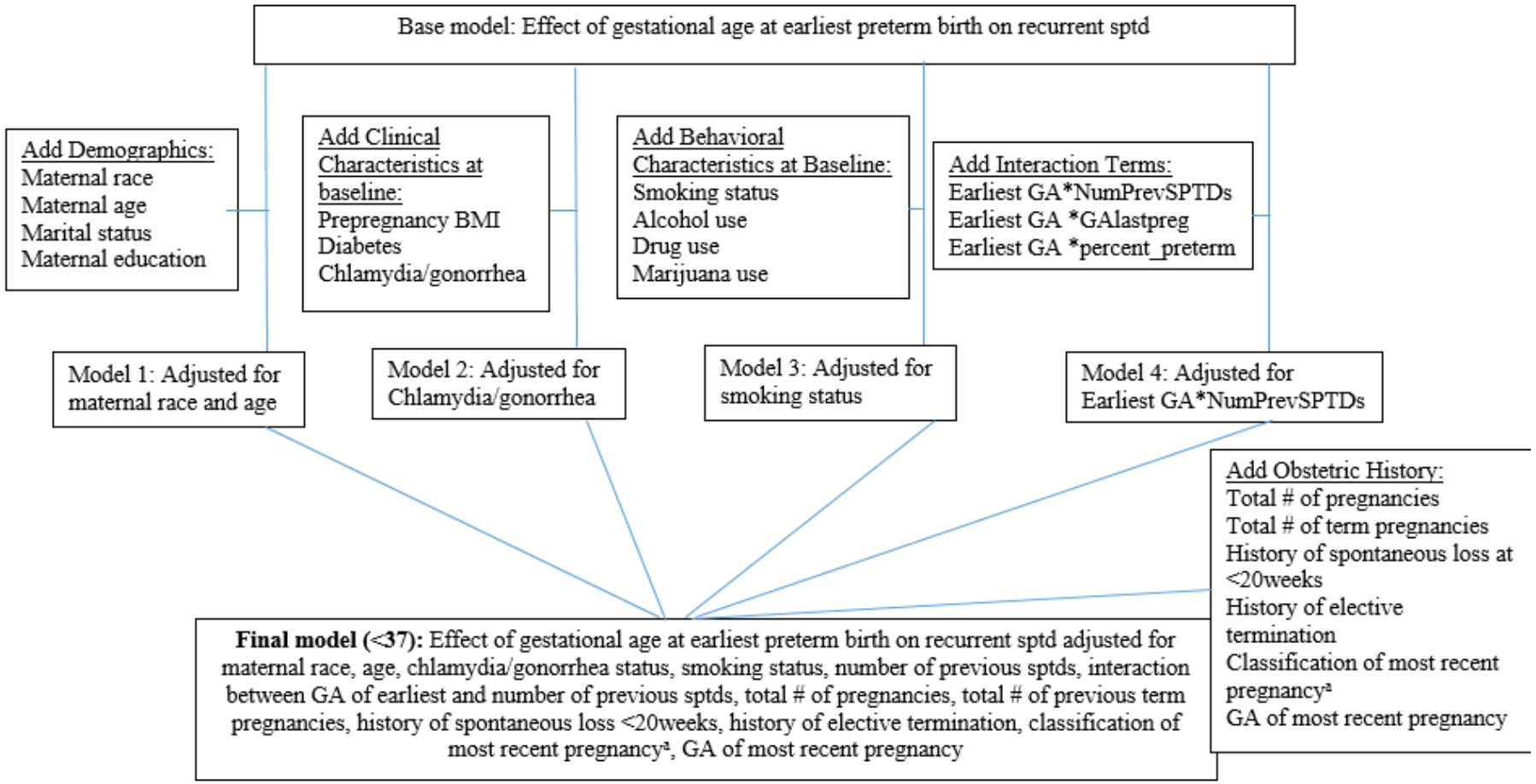

Figure 7. Model building: outcome= spontaneous preterm delivery <37 weeks; primary exposure: gestational age of earliest previous spontaneous preterm birth. Using generalized linear models with Poisson distribution.

[a]Classification of most recent pregnancy: Delivery ≥37 weeks, spontaneous PTL with delivery, PROM leading to spontaneous PTD, PTD for fetal indications, PTD for maternal indications, spontaneous loss <20 weeks, preterm intrapartum stillbirth
[b]sptd=spontaneous preterm delivery, BMI=body mass index, GA=gestational age, PTL=preterm labor, PROM=premature rupture of membranes

Final model (<35): Effect of gestational age at earliest preterm birth on recurrent sptd adjusted for maternal race, age, marital status, chlamydia/gonorrhea status, smoking status, number of previous sptds, interaction between GA of earliest and number of previous sptds, total # of pregnancies, total # of previous term pregnancies, history of spontaneous loss(es) at <20weeks, history of elective termination(s), Classification of most recent pregnancy[a], GA of most recent pregnancy

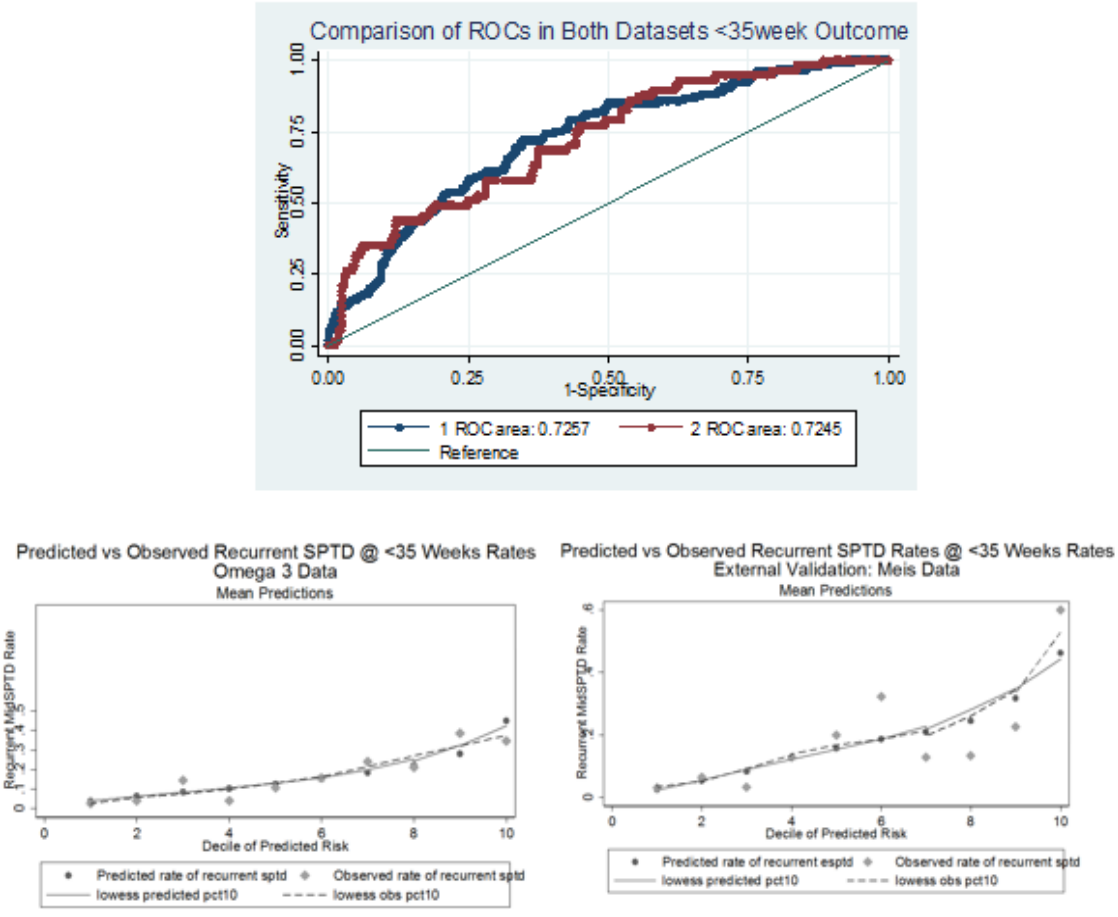

Figure 8. Discrimination and calibration of the predictive model for recurrent mid-sptd prior to 35 weeks.

*ROC= receiver operating characteristic curve; AUC= area under the curve; SPTD= spontaneous preterm delivery

Final model (<32): Effect of gestational age at earliest preterm birth on recurrent sptd adjusted for maternal age, prepregnancy BMI, chlamydia/gonorrhea status, smoking status, number of previous sptds, interaction between GA of earliest and number of previous sptds, total # of pregnancies, total # of previous term pregnancies, history of spontaneous loss(es) at <20weeks, history of elective termination(s), Classification of most recent pregnancy[a], GA of most recent pregnancy

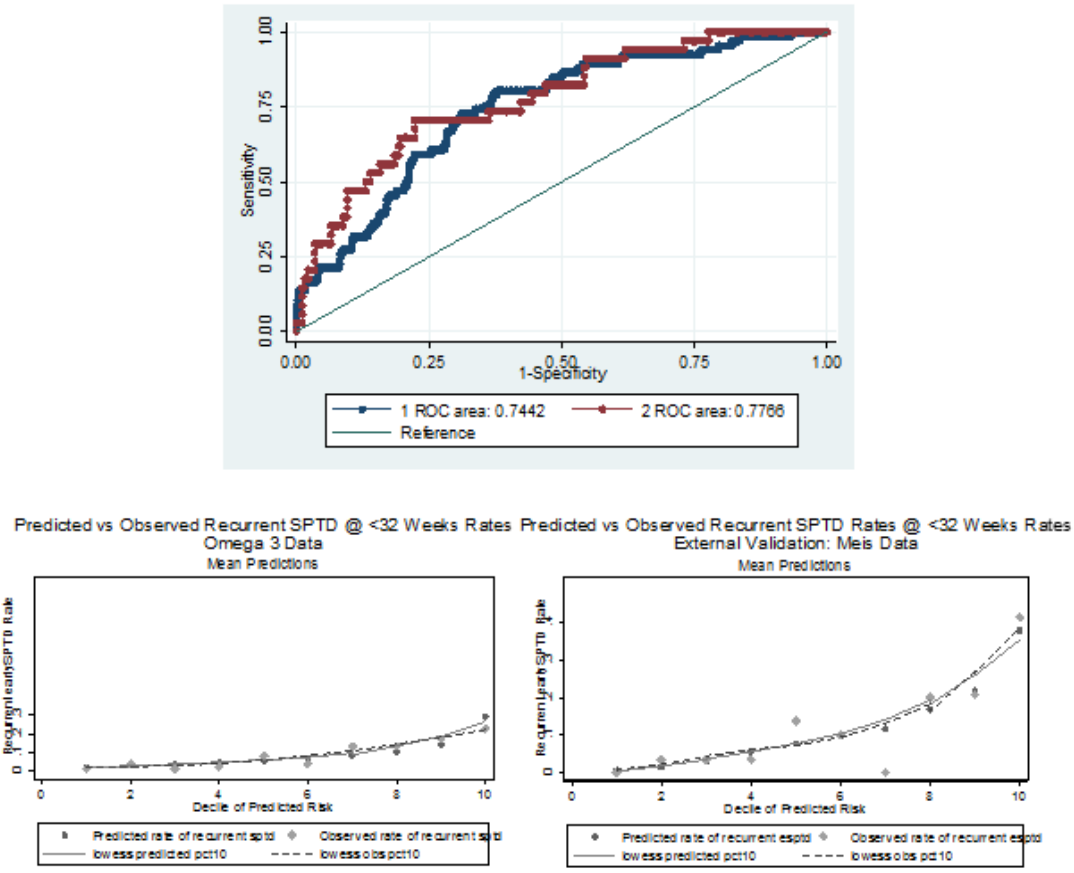

Figure 9. Discrimination and calibration of the predictive model for recurrent early- sptd prior to 32 weeks.

*ROC= receiver operating characteristic curve; AUC= area under the curve; SPTD= spontaneous preterm delivery

Figure 10. Risk of recurrent sptd before 37 weeks gestation by number of prior sptds, earliest gestational age and number of previous term deliveries taken together with all other variables at their mean.

SPTD=spontaneous preterm delivery, GA=gestational age

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