

TRANSLATIONAL RESEARCH IN OBSTETRIC
PHARMACOLOGY: HISTORICAL TRENDS, PRENATAL
PHARMACOGENOMICS, AND AN OPPORTUNISTIC STUDY OF
PLACENTAL ABCG2 AND FETAL GLYBURIDE EXPOSURE

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A DISSERTATION SUBMITTED IN PARTIAL
FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF:

DOCTOR OF PHILOSOPHY

UNIVERSITY OF WASHINGTON
2015

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PROGRAM AUTHORIZED TO OFFER DEGREE:
SCHOOL OF PUBLIC HEALTH
INSTITUTE FOR PUBLIC HEALTH GENETICS

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ABSTRACT

TRANSLATIONAL RESEARCH IN OBSTETRIC PHARMACOLOGY: HISTORICAL TRENDS, PRENATAL PHARMACOGENOMICS, AND AN OPPORTUNISTIC STUDY OF PLACENTAL ABCG2 AND FETAL GLYBURIDE EXPOSURE

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This dissertation is principally comprised of three distinct but related projects. The first is an assessment of the quantity and nature of obstetric pharmacology clinical trials conducted in the past decade, as well as a comparison of these trials to non-obstetric trials, in order to identify whether the challenges and complexities of conducting research in this field are reflected in the aggregate study data available through ClinicalTrials.gov. The analysis identified several significant differences between obstetric and non-obstetric trials, which have implications for program planning and funding needs. Second is an overview of the ways in which the fetal genome may be informative of fetal outcomes with regard to medication and other chemical exposures during pregnancy, and the attendant research prioritization of this area and clinical testing opportunities made possible by non-invasive prenatal genetic tests that utilize cell-free fetal DNA in a pregnant woman's blood. Finally, an opportunistic clinical study was conducted, assessing whether fetal genotype for the placental efflux transporter breast cancer resistance

protein (BCRP, ABCG2) is predictive of relative fetal exposure to the oral hypoglycemic agent glyburide, which is used, off-label as an alternative to insulin, to treat gestational diabetes. The results of the study showed no association between fetal *ABCG2* Q141K genotype and relative glyburide exposure at term, which was unexpected given the extensive, albeit indirect, supporting evidence that formed the basis of the initial hypothesis. This result has clinical implications related to the optimal use of glyburide to treat gestational diabetes, and also underscores the complexity of drug disposition during pregnancy and the need to study pregnant women directly.

ACKNOWLEDGEMENTS

I would like to thank all of the people who made this dissertation possible, starting with my professors at the University of Washington. Dr. Wylie Burke has supported me since I arrived here and has been a steadfast source of guidance and mentorship, and an invaluable sounding board as I developed the B-Side component of my dissertation. The clinical study that is the A-Side of my dissertation, and is my proudest accomplishment during graduate school, would not have been possible without the support of Dr. Mary Hebert. Her dedication and work-ethic are truly inspiring. Finally, I would like to thank my committee chair, Dr. Ken Thummel, who was also instrumental in the conception and execution of the clinical study. In addition to extensive tactical assistance, he has been as encouraging and responsive as I could have ever hoped for in a mentor.

I would also like to thank the other members of the clinical study research team at the University of Washington, the University of Texas Medical Branch Galveston and Columbia University. I am particularly grateful for the contributions of Dr. Holly West, Amina Lawrence, and members of the University of Washington Obstetric-Pharmacology Research Unit, including Dr. Tom Easterling, Anna Lemchen and Claudine Hernandez.

Over the past five years my classmates in the Institute for Public Health Genetics have provided comradeship and much-needed distractions that have encouraged me and helped me through the inevitable challenges of completing a doctorate. Lorelei Straub, Ursula Schick and Alie Fohner in particular have become close friends and have made this entire experience one that I will remember fondly.

Finally, I would like to thank my wonderful and family and dear friends, without whose love and support none of this would be possible.

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INTRODUCTION

This dissertation was inspired the parallel progress being made in the fields of pharmacogenomics and the noninvasive prenatal genetic testing technologies that utilize the presence of cell-free fetal DNA in the blood of a pregnant woman. Both of these areas represent ways that genomics is finally beginning to impact clinical practice and deliver upon the promises of personalized medicine that were first put forth over a decade ago with the completion of the Human Genome Project. The intersection between pharmacogenomics and prenatal genetic testing has thus far received limited attention. This is surprising given the extensive discussions taking place regarding the likelihood and implications of an expanded scope of prenatal genetic screening and testing resulting from the technical feasibility of sequencing a fetal genome using non-invasive methods. This dissertation explores the possible validity and utility of prenatal pharmacogenomic testing at a broad level, as well as a specific use case involving fetal *ABCG2* genotype and its involvement in fetal exposure to the medication glyburide. Important historical and contextual information about clinical research in obstetric pharmacology helps to ensure that the opportunities related to prenatal assessment of the fetal genome are not overemphasized at the expense of uncertainty regarding more fundamental aspects of medication disposition in pregnant women.

There is strong biological plausibility and mounting evidence that fetal genotype may make a significant contribution to inter-individual differences in fetal risk from exposure to medications and other chemicals, although the majority of the research to date has been performed in non-human animals or using preclinical designs. The primary mechanisms likely to be involved are placental drug transport, placental metabolism and fetal metabolism. Non-invasive prenatal

genetic tests can be performed in the first trimester of pregnancy. As a result, should further research confirm that statistically significant and clinically useful differences in fetal exposures and outcomes can be predicted on the basis of fetal genotype, prenatal pharmacogenetic testing is likely to be feasible at time points that are meaningful for predicting or interpreting fetal exposures. The evidentiary basis, research priorities and clinical testing opportunities for prenatal pharmacogenomics at a broad level form Chapter 2 of this dissertation.

The most robust data regarding fetal genomic contributions to fetal drug exposures are in the area of placental drug transport. Genes for transport proteins are expressed in tissues throughout the body, and the placenta has the highest levels of expression and activity for several efflux transporters, which move compounds out of cells and can act counter to passive diffusion to maintain a concentration gradient. The abundance of efflux transporters in the outermost-layer of the placenta, which faces maternal circulation, supports the hypothesis that they play an important role in protecting the developing fetus from exposure to potentially harmful endogenous and xenobiotic compounds in maternal blood.

A clinical study assessing the functional effect of a common variant in the gene *ABCG2*, which encodes the transporter Breast Cancer Resistance Protein (BCRP), on fetal exposure to the medication glyburide is presented in Chapter 3. Glyburide is commonly used, off-label, as an alternative to insulin in the treatment of gestational diabetes mellitus (GDM). While the medication has a comparable efficacy and safety profile to that of insulin, recent data has suggested that some women who fail therapy at current doses may be able to achieve and maintain glycemic control at higher doses than are currently used. This raises new questions

about fetal exposure and safety. Extensive animal and preclinical data supported the primary role of BCRP in the efflux of glyburide out of fetal circulation, and, in individuals with one or more copies of a common variant, the reduced transport phenotype for other BCRP substrate drugs as well as reduced placental BCRP content. In the largest study performed to date investigating the transplacental passage of glyburide, we assessed the role of fetal *ABCG2* genotype on relative fetal drug exposure and fetal outcomes. The results did not support our original hypothesis, but suggest important areas for future research that may help clinicians optimize the use of this medication.

Preceding the two chapters that focus on clinical pharmacogenomic applications of the fetal genome is a chapter which includes a summary of the numerous reasons it is necessary to study pregnant human women (as opposed to non-pregnant women or pregnant experimental animals) to truly understand medication safety and efficacy for this population, key historical events affecting obstetric pharmacology research, and a study assessing the volume and nature of obstetric pharmacology clinical trials conducted in the last 10 years and how these studies systematically differ from those focused on non-obstetric populations. This study helps to place the opportunities surrounding prenatal pharmacogenomics into the context of the broad gaps in understanding of the efficacy, safety and disposition of many medications when used in pregnant women. There is also growing interest in the intra-uterine environment and the possible fetal origins of adolescent- and adult-onset conditions. This is likely to result in increased research activity into fetal exposures, and given the numerous barriers to and challenges of conducting clinical research with pregnant women, it will behoove us to learn as much as possible from the experiences of the past.

CHAPTER 1: OBSTETRIC PHARMACOLOGY

1. WHY WE NEED TO STUDY MEDICATIONS IN PREGNANT WOMEN

All three of the principle elements of this dissertation are founded on a shared premise: that to understand the safety and efficacy of medications for use in pregnancy, and the fetal consequences of other chemical exposures, we must study pregnant women. This assumption requires justification, particularly given the ethical dilemmas of exposing developing embryos and fetuses to medications and chemicals that may be helpful, neutral, or have profound, unpredictable and irreversible effects. For numerous reasons, however, the pregnant human female is the only truly reliable model for evaluating the safety and efficacy of medications used during pregnancy. Animal data and data from non-pregnant women are useful, but insufficient to fully characterize and predict the pregnant human response. Furthermore, the absence of relevant empirical data can result in underuse of safe and effective medications, and continued use of drugs that are ineffective and harmful, both of which can harm women, fetuses and infants.

Limitations of Animal Data

Animal data are useful in assessing the human teratogenicity of medications, but for several reasons are often insufficient alone to predict maternal and fetal safety in humans. Teratogens often demonstrate species- and strain-specific sensitivity to chemical agents, and it is challenging to select the species that are most predictive of human response. The most famous example of this is the anti-nausea medication thalidomide, which was marketed outside of the US for the treatment of morning-sickness. Most strains of mice, rats and hamsters are resistant to the limb

malformations (phocomelia) that result from fetal exposure to the S-enantiomer early in gestation. New Zealand white rabbits are sensitive, but this was only discovered after the birth of more than 10,000 affected infants between 1957-1962. Even when an agent is teratogenic in humans and an experimental animal, there is commonly imperfect concordance between the specific morphological defects. Developmental effects are particularly challenging to evaluate in non-human animals and relevant endpoints are scarce. Chemical agents can also exhibit threshold effects, causing harm only at and above certain exposure levels. These thresholds can vary across species and testing often takes place at doses that far exceed therapeutic concentrations, giving the appearance of teratogenicity to medications that would be safe for use at therapeutic doses. These differences collectively derive from the multiple anatomical, physiological, biochemical and genetic differences between species. There are significant differences in placentation and gestation between humans and other species. Additionally, genes that are evolutionarily conserved across species do not remain identical and can have function-affecting differences in the DNA sequence, apart from differences in gene expression that result from epigenetics and post-translational regulation. The resulting differences in protein content, structure and function can alter the metabolism of drugs and other chemicals. Lastly, and critically, animal models rarely account for the inter-individual differences in susceptibility that result from human genetic heterogeneity affecting drug disposition.

Limitations of Data from Non-pregnant Populations

While fetal effects clearly cannot be assessed by studying non-pregnant adults, the question arises whether characterization of drug disposition, efficacy and safety for the pregnant women themselves requires direct study, or can be sufficiently understood by studying non-pregnant

men and women and generalizing the findings from those populations. The answer is that they cannot. Gender-based differences in drug response are common and gender-specific analyses are increasingly included, and sometimes required, when evaluating trial results. Studying non-pregnant women is insufficient due to the numerous pregnancy-induced changes in pharmacokinetics (PK) and pharmacodynamics, which are often dynamic across gestation. All four of the PK processes, absorption, distribution, metabolism, and excretion, have been reported to be altered in pregnancy.

Changes in gastric emptying and pH as well as small intestine motility affect the absorption of orally administered medications. Changes in pulmonary function can impact medications that are absorbed or eliminated via the lungs. Increased total body water and fat content, and changes in protein binding all alter drug distribution. An additional factor is the novel presence of the fetal compartment. Increased renal blood flow and glomerular filtration rate (GFR) affect the elimination of drugs cleared through the kidneys. Many drug-metabolizing enzymes are up-regulated or suppressed in pregnancy. The affects of these changes can dramatically alter a medication's safety and efficacy profile, as well as the dosing required to maintain therapeutic plasma drug concentrations across gestation. A relatively straightforward example of this is offered by considering lamotrigine, a drug used to treat seizure disorders. Epilepsy is routinely treated throughout pregnancy due to risk to the mother and fetus from seizures. Lamotrigine is predominantly metabolized by UGT1A4, which has increased activity in pregnancy. When used to treat either seizure disorders, the drug exhibits significantly increased clearance as early as the first trimester, and returns to pre-pregnancy baseline by 2-3 weeks postpartum. Maintaining the pre-pregnancy dose could result in subtherapeutic drug levels during pregnancy, reducing

efficacy while still exposing the developing fetus to the risks of medication exposure. These differences in drug response and appropriate changes to dose and medication selection can only be identified by studying pregnant women.

Evidence of Absence of Risk

Another consideration is the possibility that safe and effective medications may go unused as a result of incorrect assumptions regarding safety. Medications with potential obstetric applications can suffer a presumption of teratogenicity and go unused when other medications in the same drug class or other drugs that treat the same condition are known human teratogens. This is exemplified by the medication bendectin (pyridoxine/doxylamine), which is used to treat morning sickness. The drug was first marketed in US in 1956 (pre-thalidomide) and then voluntarily removed from market in 1983 following lawsuits alleging it caused birth defects. Extensive evidence from cohort and case control studies demonstrated no increase in risk of birth defects or other adverse pregnancy outcomes, and a reformulation of the drug finally returned to the market in the US in 2013, at which time it became the only FDA Pregnancy Category A medication for treatment of pregnancy-associated nausea and vomiting.

Obstetric Conditions

Incorrect assumptions can result in the continued use of ineffective and harmful medications as well. A pregnant woman may require treatment for a chronic or acute condition unrelated to her pregnancy, and the question is whether the available medications remain safe and effective now that she is pregnant. There are also obstetric conditions, such as preeclampsia or hyperemesis gravidarum, which are directly related to pregnancy. Treatment options for many obstetric

diseases are limited, and frequently “borrowed” from those of related, non-obstetric conditions. The disposition, efficacy and safety of medications when used to treat the obstetric condition can significantly differ as compared to the non-obstetric analog. An example of this is the treatment of hypertension during pregnancy. A separate issue is the repurposing of medications that were developed for treatment of an unrelated non-obstetric condition. Terbutaline is FDA approved to prevent and treat bronchospasm associated with asthma, bronchitis, and emphysema. It has also been used off-label to treat preterm labor and uterine hyperstimulation (acute) and to prevent recurrent preterm labor (prolonged treatment). In the 1990s, growing concerns regarding potentially serious maternal heart problems and death associated with prolonged use by women in labor resulted in an FDA ‘Dear Colleague’ letter and revised labeling discouraging the off-label use. This proved insufficient to halt the use of the drug that was ineffective and harmful for this indication and in 2011 the FDA issues a safety warning and added a Boxed Warning and Contraindication on the drug label.

2. HISTORICAL EVENTS AND CONTEXT

The current underrepresentation of pregnant women in biomedical research is largely the product of a small number of highly influential historical events, which are shown in timeline format in Figure 1. Three events that have had a profound influence are the thalidomide tragedy, the 1977 FDA guidance on ‘women of childbearing potential’ and Subpart B of the federal human subjects research protections.

Between 1957 and 1961 more than 10,000 children worldwide were born with phocomelia attributable to prenatal exposure to the anti-nausea drug. The event captured worldwide attention and raised awareness of the possible teratogenicity of maternally administered medications. In the United States, despite the medication never receiving FDA approval, it also generated concerns over the inadequacy of controls for ensuring medication efficacy and safety. The Kefauver-Harris Amendment to the Federal Food, Drug and Cosmetic Act in 1962, which for the first time required drug manufacturers to meet safety and efficacy requirements prior to FDA approval, was a direct response to the thalidomide experience. More than 50 years later, thalidomide continues to serve as a cautionary example of the harms that can result from medication exposure during pregnancy.

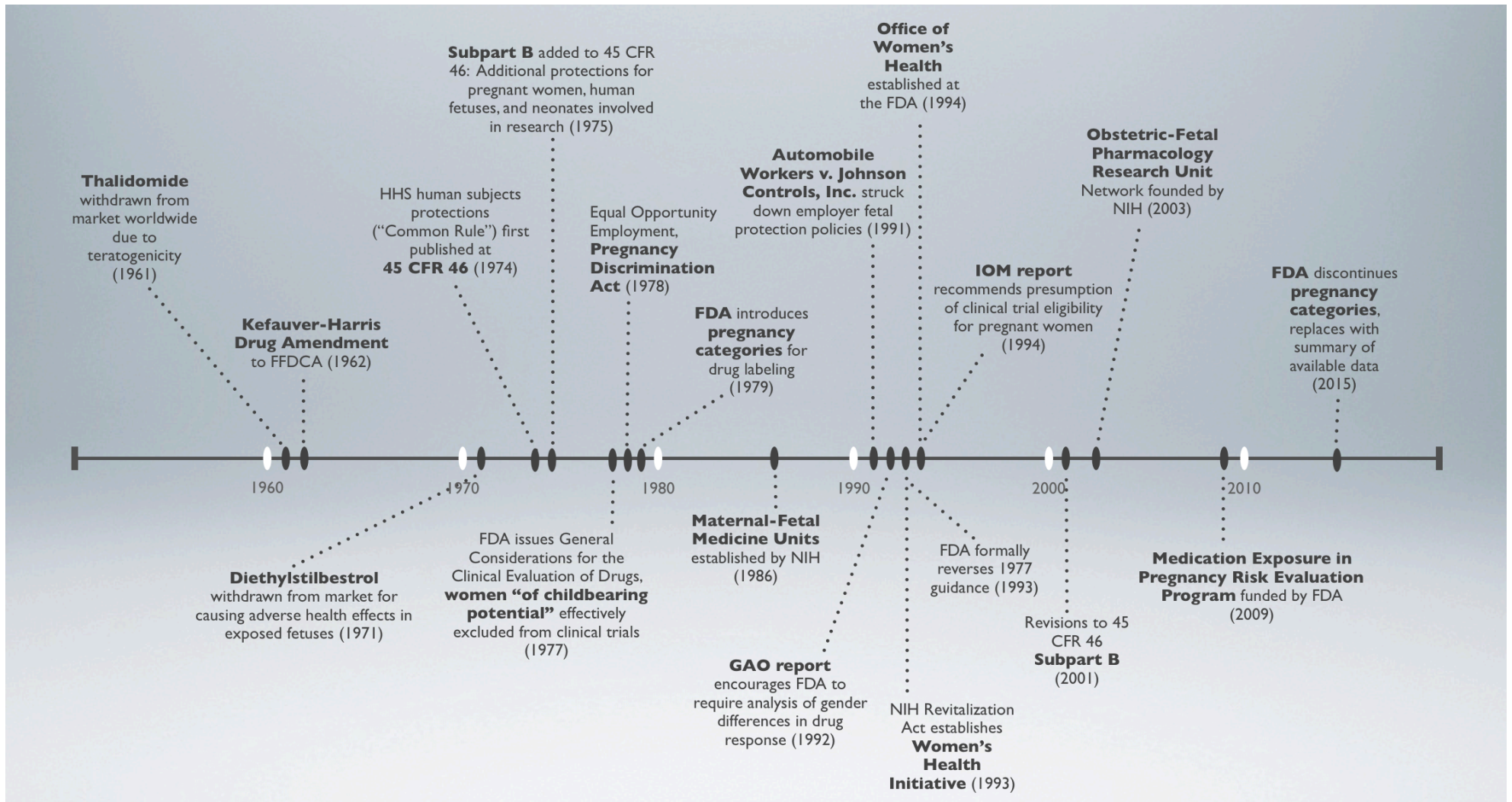
In 1977, concern that a research participant might become pregnant and inadvertently expose a developing embryo or fetus to an experimental therapy prompted the FDA to issue an industry guidance that included the recommendation that all ‘women of childbearing potential’ be excluded from drug trials. The result was an effective exclusion of women from clinical studies evaluating medications. It wasn’t until 1993, after a report from the General Accounting Office

the preceding year encouraging the FDA to require evaluation of gender-based differences in drug response, that this guidance was formally rescinded. The Women's Health Initiative at the NIH and the Office of Women's Health at the FDA were both established in that time period and significant progress has been made in achieving greater participation by women in clinical studies. The gains in women's representation in studies have not been matched by improvements in research participation by pregnant women. Although the Institutes of Medicine formally recommended that pregnant women be presumed eligible for clinical studies in 1994, this has had a minimal effect on the inclusion of pregnant women in studies.

Also highly influential on biomedical research practices are the additional protections for pregnant women, fetuses and neonates involved in research, known as "Subpart B" of the federal human subjects research protections. These additional research regulations were first introduced in 1975, and although they included 'pregnant women' in their scope, the additional regulations were largely motivated by the legalization of abortion in *Roe v. Wade* in 1973 and the resulting commencement of research involving aborted human fetuses. The initial wording of the Subpart B regulations appeared to permit only studies that met stringent and unprecedented requirements, such as the prospect of direct benefit to the fetus, and offered limited options for institutional review boards to approve other types of trials. It wasn't until 2001 that Subpart B was revised to promote a policy of presumed inclusion of pregnant women, permit the pregnant woman to be the sole decision-maker regarding her participation, and offer a process for consideration of studies that are not otherwise approvable.

Today there is growing interest in obstetric pharmacology. Earlier this year the FDA discontinued use of the FDA Pregnancy categories (A/B/C/D/X) in favor of a new system intended to encourage evaluation of each medication's available data on safety and efficacy. In addition, the NIH recently announced over \$40 million in funding for research on the placenta. As we move forward, it will be important to keep history in mind. Learning from the past and recognizing that some historical events and experiences continue to impact research and clinical practice will aid in our ability to identify and implement programs and policies likely to be effective in improving the representation of pregnant women in biomedical research.

FIGURE 1: HISTORICAL EVENTS OF IMPORTANCE TO OBSTETRIC PHARMACOLOGY



3. THE RIGHT TIME FOR OBSTETRIC PHARMACOLOGY

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ABSTRACT

Objective(s): Medication safety and efficacy during pregnancy is an understudied area and there are many barriers to research activity in this field. The unique challenges faced by investigators who conduct clinical trials in obstetric pharmacology have received limited formal study, but will be important for policy makers to understand if they hope to address the current knowledge deficit. Using data from ClinicalTrials.gov, we sought to comprehensively characterize the subset of obstetrics studies that specifically assessed the safety and efficacy of medications for use during pregnancy, and determine whether and in which ways these obstetric pharmacotherapy studies systematically differed from other types of trials.

Study Design: All Phase 3 and 4 clinical trials registered in ClinicalTrials.gov between 2007 and 2013 were evaluated for their obstetric status, pharmacotherapeutic status and substantive focus. Obstetric and obstetric pharmacotherapeutic trials were compared to non-obstetric trials on the basis of available parameters including study design, current activity status, time to study completion and time to results.

Results: Obstetric trials represented 3.3% of the 25,405 studies evaluated, and most commonly focused on labor and delivery, infertility and contraception. As compared to non-obstetric trials, obstetric studies were less likely to be completed (40.7% vs. 46.6%, $p=0.0008$), less likely to have results (9.4% vs. 17.7%, $p<0.0001$) and took 223.6 days longer, on average, to post results ($p=0.0001$). Obstetric pharmacotherapy trials comprised 1.3% of all studies evaluated and nearly half focused on labor and delivery. Obstetric pharmacotherapy studies were less likely to be completed (34.4% vs. 46.6%, $p<0.0001$) or to have reported results (6.9% vs. 17.7%, $p<0.0001$),

and more likely to be active but not yet recruiting (9.3% vs. 4.3%, $p < 0.0001$), as compared to non-obstetric trials.

Conclusion(s): These data support the presence of factors unique to or disproportionately affecting obstetrics research that prolong or otherwise complicate successful completion of these studies. Meaningful progress in addressing the evidence gaps in obstetric pharmacology will be most readily achieved through a multifaceted approach that addresses both the factors that hinder overall research activity in this field as well as the additional challenges that affect clinical studies once they are underway.

INTRODUCTION

Due to the long-standing and numerous challenges of conducting clinical research in obstetric pharmacology, pregnant women remain an under-studied population.^{1,2} Known barriers include fear stemming from historical events involving the teratogenic agents thalidomide and diethylstilbestrol, public relations risk and limited economic appeal for pharmaceutical companies, ethical dilemmas,³ additional research regulations on studies involving pregnant women⁴ and regulatory and clinical care environments permissive of off-label usage in this patient population. As a result, the safety for use in pregnancy is unknown for most medications, and is coupled with a lack of empirical data regarding drug efficacy.⁵

This knowledge deficit exists in a context of widespread and increasing medication use during pregnancy. Each year in the United States approximately 6.5 million women become pregnant, resulting in over four million live births.⁶ Ninety percent of these women take at least one medication while pregnant and 70% take at least one prescription medication. First trimester use of prescription medications has increased more than 60% over the last 30 years, and use of four or more medications during pregnancy tripled in the same timespan.⁷

The characteristics and publication patterns of obstetrics-focused studies registered in ClinicalTrials.gov were recently published by Stockmann et al, and provide summary data regarding study attributes such as phase, study design, primary funding source and recruitment status.⁸ These data provide a useful foundation for discussions of obstetric research as a whole. Still unexplored are the subset of obstetric studies that specifically seek to assess the disposition,

safety and efficacy of medications for use during pregnancy. Also unclear is whether these obstetric pharmacotherapy studies systematically differ from other types of trials.

Researchers conducting clinical trials in obstetric pharmacology have anecdotally shared the unique challenges they face. These include the difficulties designing optimal therapeutic strategies with minimal pharmacokinetic data and the frequent need to adjust drug regimens across gestation; the lengthy process of gaining institutional approvals; and the challenges of enrolling participants and having them complete study procedures with limited gestational windows for eligibility, common transfer of care in high-risk pregnancies and the unpredictability of the timing and location of delivery.⁹ In this study we sought to determine whether these factors were reflected in the study attributes available through ClinicalTrials.gov, with the hypothesis that obstetric pharmacotherapy studies, as compared to other trials, would take longer to begin enrolling participants, to complete study procedures and to report results. Improved characterization of the challenges that are unique to or that disproportionately affect obstetric pharmacotherapeutic trials will be of value to both researchers and policy-makers, as meaningful progress in addressing the evidence gaps in this field will be most readily achieved through a multi-faceted approach that addresses both the factors that hinder overall research activity as well as the additional challenges that affect clinical studies once they are underway.

MATERIALS & METHODS

SELECTION OF STUDIES

We identified all Phase 3 and Phase 4 clinical trials registered in ClinicalTrials.gov between January 1, 2007 and December 31, 2013. These phases were selected as new molecular entities are very seldom developed for use in pregnancy, and it is far more common for drugs already approved for other indications or populations to be re-evaluated for obstetric indications using a Phase 3 or Phase 4 trial design. The date range was selected for the reasons described by Stockmann et al, namely the 2007 enactment of a federal law that resulted in more comprehensive and consistent registration and reporting of results for interventional trials.¹⁰ The resulting 29,491 trials were then filtered to exclude those with start dates prior to 2007 (n=4,086), yielding 25,405 studies that were included in the analysis. All data were downloaded on November 12, 2014.

STUDY CATEGORIZATION

The 25,405 studies went through three rounds of obstetrics-related categorization of increasing specificity [Figure 1]. Studies were first evaluated for their overall obstetrics focus. This involved manual review of all women-only studies and of studies matching keyword searches that included the terms “abortion,” “cesarean,” “contraception,” “contraceptive,” “fertility,” “fertilization,” “fetal,” “fetus,” “gestation,” “infertility,” “in utero,” “IVF,” “labor,” “labour,” “lactation,” “maternity,” “obstetric,” “post-partum,” “postpartum,” “pregnancies,” “pregnancy,” “pregnant,” “prenatal.” Studies were categorized as obstetric when they (i) focused on obstetric conditions (e.g. gestational diabetes or chorioamnionitis), (ii) focused on obstetric periods (e.g. first trimester or post-partum), (iii) focused on obstetric procedures (e.g. cervical cerclage or epidural), or (iv) had a study inclusion criterion that participants be pregnant or actively trying to become pregnant. Manual review involved evaluation of study Title, Conditions, and Outcome

Measures from ClinicalTrials.gov data fields. The full study record was consulted when these three fields were insufficient for classification.

The resulting obstetric studies were then categorized into one or more of 10 substantive themes: contraception, fetal therapy, infertility, lactation, labor & delivery, non-obstetric conditions (including both infectious and non-communicable disease), obstetric conditions, recurrent spontaneous abortion and non-viable pregnancy (SAB, including both treatment and prophylaxis), termination, and other [Figure 2]. Trials categorized as ‘other’ included those focused on maternal weight gain, smoking cessation and substance abuse, oral health and maternal supplementation with vitamins and other micronutrients not tied to the treatment or prevention of a specific condition.

Each obstetric study was separately evaluated to determine its pharmacotherapeutic status. Obstetric pharmacotherapeutic status required a study to (i) involve a drug or biologic (as opposed to a surgical technique, device, behavioral intervention or dietary supplement) and (ii) have a maternal or fetal safety or efficacy endpoint unrelated to pregnancy termination, contraception, or conception.

STATISTICAL ANALYSES

All statistical analyses were conducted after the completion of study categorization. Comparisons between study types used the two-sample z-test for proportions and the two-sample t-test for comparison of two means, as appropriate. A p-value equal to or less than 0.05 was considered

statistically significant for this exploratory analysis. All statistical analyses were performed using Stata 11.2 (StataCorp LP, College Station, TX).

RESULTS

OBSTETRIC STUDIES

Out of 25,405 total studies evaluated, 830 (3.3%) were identified as obstetric. The proportion of all studies that were obstetric steadily increased over the date range evaluated, from a low of 1.8% in 2007 to a high of 3.9% for 2013 [Figure 3]. Studies related to labor and delivery (n=225, 27.1%) and infertility (n=225, 27.1%) were the most common, followed by contraception (109, 13.1%), non-obstetric conditions (n=108, 13.0%) and obstetric conditions (n=69, 8.3%). Of the obstetric studies, 40.7% were completed and 9.4% had reported results, as compared to 46.6% of non-obstetric studies completed and 17.7% with results ($p=0.0008$ and $p<0.0001$, respectively) [Table 1]. The proportion of obstetric trials using an observational study design (2.5%) was similar to that of non-obstetric studies (2.4%). Completed obstetric studies took an average of 25.1 days longer from start to completion as compared to non-obstetric studies (691.1 ± 481.1 days vs. 665.9 ± 461.9 days), although this difference was not statistically significant. Obstetric studies with results took 223.6 days longer on average from start to the posting of results as compared to non-obstetric studies with results ($1,366.9\pm 458.5$ days vs. $1,143.3\pm 507.0$ days, $p=0.0001$). Not-yet-completed obstetric studies were more likely to be active but not yet recruiting (7.5% vs. 4.3%, $p=0.043$) and more likely to be suspended, terminated or withdrawn (11.6% vs. 8.9%, $p=0.0075$), as compared to non-obstetric studies.

OBSTETRIC PHARMACOLOGY

Of the 830 obstetric studies, 334 met the inclusion criteria to be considered an obstetric pharmacotherapeutic study, representing 40.2% of all obstetric studies and 1.3% of all studies evaluated, a proportion that was fairly consistent over time. Among these, studies related to labor and delivery were by far the most common (n=163, 48.8%), followed by non-obstetric conditions (n=88, 26.4%) and obstetric conditions (n=59, 17.7%). The attributes of obstetric pharmacotherapeutic studies as compared to non-obstetric studies were similar to those of the obstetric studies, but generally more pronounced. Obstetric pharmacology studies were less likely to be completed (34.4% vs. 46.6%, $p<0.0001$) or to have reported results (6.9% vs. 17.7%, $p<0.0001$), and more likely to be active but not yet recruiting (9.3% vs. 4.3%, $p<0.0001$). Obstetric pharmacotherapeutic studies took an average of 212.5 additional days from the start of the study to the posting of results as compared to non-obstetric studies ($1,355.8\pm 503.2$ days vs. $1,143.3\pm 507.0$ days, $p=0.045$).

To isolate studies focusing on assessment of medication safety and efficacy in the treatment of pregnancies complicated by one or more obstetric or non-obstetric conditions from trials focused on routine aspects of labor and delivery, such as pain management and post-cesarean wound control, we performed an additional analysis of obstetric pharmacotherapeutic studies limited to the substantive themes of fetal therapy, lactation, non-obstetric conditions, obstetric conditions and SAB. The resulting subset of studies comprised just 0.7% of all studies evaluated, a rate that was consistent across sampled years. Just 27.1% of these 170 studies were completed and 4.7% had reported results ($p<0.0001$ for both as compared to non-obstetric studies). Completed studies took an average of 808.7 ± 496.4 days from start to finish, and $1,599.3\pm 480.2$ days from start to

the posting of results, which represents an excess of 142.8 days in time to completion and 456.0 days in time to results as compared to non-obstetric studies ($p=0.039$ and $p=0.011$, respectively).

COMMENT

Over three decades have passed since obstetrics ingloriously received the ‘wooden spoon’ award for the least evidence-based specialty in medicine.¹¹ While some progress has been made, what we know about the safety and efficacy of medication use during pregnancy remains dwarfed by unknowns. Many of the reasons for this are well recognized. Thalidomide and diethylstilbestrol continue to cast a long shadow, reminding pregnant women, providers and pharmaceutical companies of the unpredictable, profound and irreversible effects that medication exposure can have on a developing fetus. Despite a 1994 Institute of Medicine recommendation that pregnant women be presumed eligible for clinical trials,³ they remain effectively excluded from most studies,¹² a result of additional federal regulations on research involving pregnant women and fetuses, understandably cautious patients and providers, ethical dilemmas and the complexity of interpreting drug disposition data from pregnant women. The permissiveness of off-label prescribing and industry fears of financial losses due to legal liability and negative press further discourage companies from seeking Food and Drug Administration (FDA) approval for obstetric indications and conducting the post-marketing studies such approval would typically require.

These obstacles, while restrictive, are not insurmountable. In this study we found that 1.3% of the Phase 3 and Phase 4 trials registered in ClinicalTrials.gov, starting between 2007 and 2013, addressed obstetric pharmacology. Nearly half of these studies, however, were focused on the perinatal period immediately before and after delivery, a time point at which concerns about

teratogenicity are greatly reduced relative to earlier in gestation. Just 0.7% of trials studied the safety or efficacy of medication use in pregnancies complicated by an acute, chronic or obstetric condition.

One key concern when a pregnant woman takes a medication is fetal safety. However, the evidence available to characterize the presence, nature, and magnitude of any medication-associated fetal harm is highly limited. The expert advisory board members who review published human and relevant animal data to assign risk ratings for the Teratogen Information System (TERIS) had insufficient data to determine this risk for 168 of 172 (97.7%) drugs that received FDA approval between 2000 and 2010. Notably, there were no data at all for 73.3% of these drugs.⁵

Receiving even less formal study are the maternal efficacy and safety of medications. Pregnancy is accompanied by physiological and biochemical changes that can dramatically affect drug pharmacokinetics and pharmacodynamics, and these changes are frequently gestational age dependent.¹³ Pregnancy can also interact with non-obstetric conditions resulting in altered disease pathology relative to that of non-pregnant women. These interactions often have therapeutic implications.¹⁴

Women who would choose to discontinue or forego medication use during pregnancy due to these uncertainties regarding risk and benefit often don't get that opportunity. Over half of all pregnancies and about 37% of births in the United States are unintended at the time of conception, a rate that has been steady for over three decades.¹⁵ Safety data are needed not just to

prospectively guide pharmacotherapy decisions, but to assess fetal harm subsequent to an unintended exposure. Additionally, very few drugs are definitively teratogenic, and there is a growing number of instances where pharmacotherapy for a maternal condition is associated with superior fetal outcomes relative to non-treatment.¹⁶

Real progress in addressing the evidence gaps in obstetric pharmacology will require a multifaceted approach that considers both the barriers to conducting these studies at all, as well as factors unique to obstetrics research that prolong or otherwise complicate successful completion of these studies. These latter factors have received little formal study to date, but can affect the duration and likelihood of gaining the necessary institutional and ethics approvals, recruiting participants and having them complete study procedures, in addition to data analysis and interpretation.

Pregnancy-specific federal research regulations and additional caution by review boards can result in lengthy periods of review, significant requested changes to study protocols, and multiple rounds of deferral prior to approval even for observational and minimal risk studies. This is clearly reflected in our data. Obstetric studies are significantly more likely than non-obstetric studies to be open but not yet recruiting participants. For obstetric pharmacotherapy studies, the difference is even more pronounced, with a greater than two-fold increase in the likelihood of a study having this recruitment status.

Participant recruitment and retention is a laborious component of most if not all clinical studies. Obstetric pharmacology studies have all the same types of inclusion and exclusion criteria as

other trials, with additional requirements related to gestational age and status of the current pregnancy (e.g. it is common to exclude women with multiple gestation pregnancies or a known fetal abnormality), as well as a woman's obstetric history of gravidity, parity and complications. Many women with high-risk pregnancies initiate obstetric care with their local provider, and then have their care transferred to a facility more equipped to manage complex obstetric cases and deliveries. Delivery, which is often the first time to collect samples that are directly informative of fetal drug exposure and effects, is unpredictable in its timing and location, and delivery sample collection is frequently not possible. Neonatal diagnoses and care may take place at a separate facility from the one at which a woman received her obstetric care. Moreover, an infant's health records may be only partially-linked, or unlinked, administratively, to that of the mother, complicating researchers' ability to assess fetal outcomes.

The obstetric and obstetric pharmacology studies identified in this analysis were significantly less likely to be completed as compared to other trials, with the completion status defining whether data collection tied to the primary study outcome remained underway. This is likely attributable, at least in part, to the additional complexities of participant recruitment and sample and data collection just described. While completed obstetric and obstetric pharmacotherapy studies did not statistically differ from other trials in study duration from start to completion, when the analysis was restricted to only pharmacotherapeutic studies unrelated to labor and delivery (i.e. that evaluated the safety or efficacy of a medication used to treat one or more obstetric or non-obstetric conditions over the course of a woman's pregnancy), which would be most affected by these factors, there was a significant increase in study duration.

Both obstetric studies and obstetric pharmacotherapy studies were significantly less likely to have results available than were non-obstetric trials, to an even greater extent than would be explained by the difference in completion status. Many factors are likely contributing to this relative delay. Pregnancy-related data can be challenging to analyze and interpret. Trials with a drug safety component have to evaluate outcomes in the mother, developing fetus and infant. Adverse events in a pregnant woman may be caused by the medication, the condition being treated and the extent to which it is under therapeutic control. They may also result from a woman simply being pregnant, a combination of these factors interacting, or they may have a completely separate and unrelated cause. It is similarly difficult to assign cause for many birth defects and other adverse fetal outcomes. Pharmacokinetic, metabolic, genetic and other analyses often have to incorporate both the dynamic state of many physiological parameters across gestation, as well as the fetal compartment. These analyses and other data interpretation can be done using established methodologies,¹⁷ but this process may take longer, on average, for obstetric trials.

The main limitations of this study relate to the content and quality of data available in ClinicalTrials.gov. While there are legal requirements for registration and reporting, study data are self-reported by sponsors and investigators and their accuracy and completeness are difficult to ascertain. The data elements available through ClinicalTrials.gov do not allow for direct assessment of the challenges encountered by investigators conducting clinical trials in obstetric pharmacology. While their existence can only be inferred by the presence and magnitude of statistically significant differences in the available parameters, the observed differences were largely consistent with expectations based on anecdotal experiences shared by researchers.⁹

The obstacles to conducting clinical trials in obstetric pharmacology will remain, but the studies completed to date show that they can be managed and the researchers and funding agencies already working in this field deserve recognition. In particular, the Maternal-Fetal Medicine Units (MFMU) Network, first established and funded by the National Institute of Child Health and Development (NICHD) in 1986, continues to conduct well-designed clinical trials focused on both safety and efficacy. NICHD also funds the Obstetric-Fetal Pharmacology Research Units (OPRU) Network, which focuses on basic, translational and clinical pharmacology of drugs during pregnancy. The lessons learned in the process of conducting these trials will be highly valuable as we enter a period of increasing interest in the intrauterine environment and possible fetal origins of subsequent developmental delays and disease risk.¹⁸ This is an important opportunity to attract additional resources to address the knowledge deficits that exist in obstetric pharmacology. As this happens, it will be to the benefit of all to position researchers for success by acknowledging and, where possible, reducing both the obstacles that limit research activity in this field, as well as the challenges investigators will face once studies are underway.

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TABLE 1: STUDY CHARACTERISTICS BY TRIAL TYPE

| | TRIAL TYPE | | | |
|---------------------------------|---|-------------------------------------|---|---|
| | Non-Obstetric n = 24,577 | Obstetric n = 830 | Obstetric Pharmacotherapy n = 334 | Obstetric Pharmacotherapy (limited) ^a n = 170 |
| RECRUITMENT STATUS (%) | | | | |
| Completed | 46.6 | 40.7* | 34.4** | 27.1** |
| Active, not recruiting | 11.2 | 9.0 | 8.7 | 12.9 |
| Enrolling by invitation | 1.5 | 1.8 | 1.2 | 1.8 |
| Not yet recruiting | 4.3 | 7.1** | 9.3** | 10.6** |
| Recruiting | 29.6 | 27.4 | 34.1 | 35.9 |
| Suspended/terminated/withdrawn | 8.9 | 11.6* | 12.0 | 11.8 |
| RESULTS (% with results) | 17.7 | 9.4** | 6.9** | 4.7** |
| DESIGN (%) | | | | |
| Expanded Access | 0.1 | 0.1 | 0.3 | 0 |
| Interventional | 97.5 | 97.3 | 97.6 | 96.5 |
| Observational | 2.4 | 2.5 | 2.1 | 3.5 |
| PHASE (%) | | | | |
| Phase 2/3 | 8.2 | 10.7 | 10.5 | 13.0 |
| Phase 3 | 48.4 | 36.8 | 34.7 | 36.4 |
| Phase 4 | 43.4 | 52.5 | 54.8 | 50.6 |
| | n completed = 10,845 n results = 4,342 | n completed = 327 n results = 77 | n completed = 115 n results = 23 | n completed = 45 n results = 8 |
| TRIAL DURATION (days) | | | | |
| Time to completion | 665.9±461.9 | 691.1±481.1 | 632.2±500.9 | 808.7±496.4* |
| Time to results | 1,143.3±507.0 | 1,366.9±458.5** | 1,355±503.2* | 1,599.3±480.2* |

Duration values presented as mean±standard deviation.

^a Obstetric Pharmacology (limited) is the subset of obstetric pharmacotherapy studies with the substantive themes of fetal therapy, lactation, non-obstetric conditions, obstetric conditions and spontaneous abortion/non-viable pregnancy.

*Compared to non-obstetric studies, $p \leq 0.05$

**Compared to non-obstetric studies, $p \leq 0.000$.

FIGURE 1: STUDY INCLUSION CRITERIA & CATEGORIZATION PROCESS

Process and criteria for identifying trials, determining obstetric and obstetric pharmacotherapy status, and applying substantive themes. L&D: labor and delivery, SAB: spontaneous abortion.

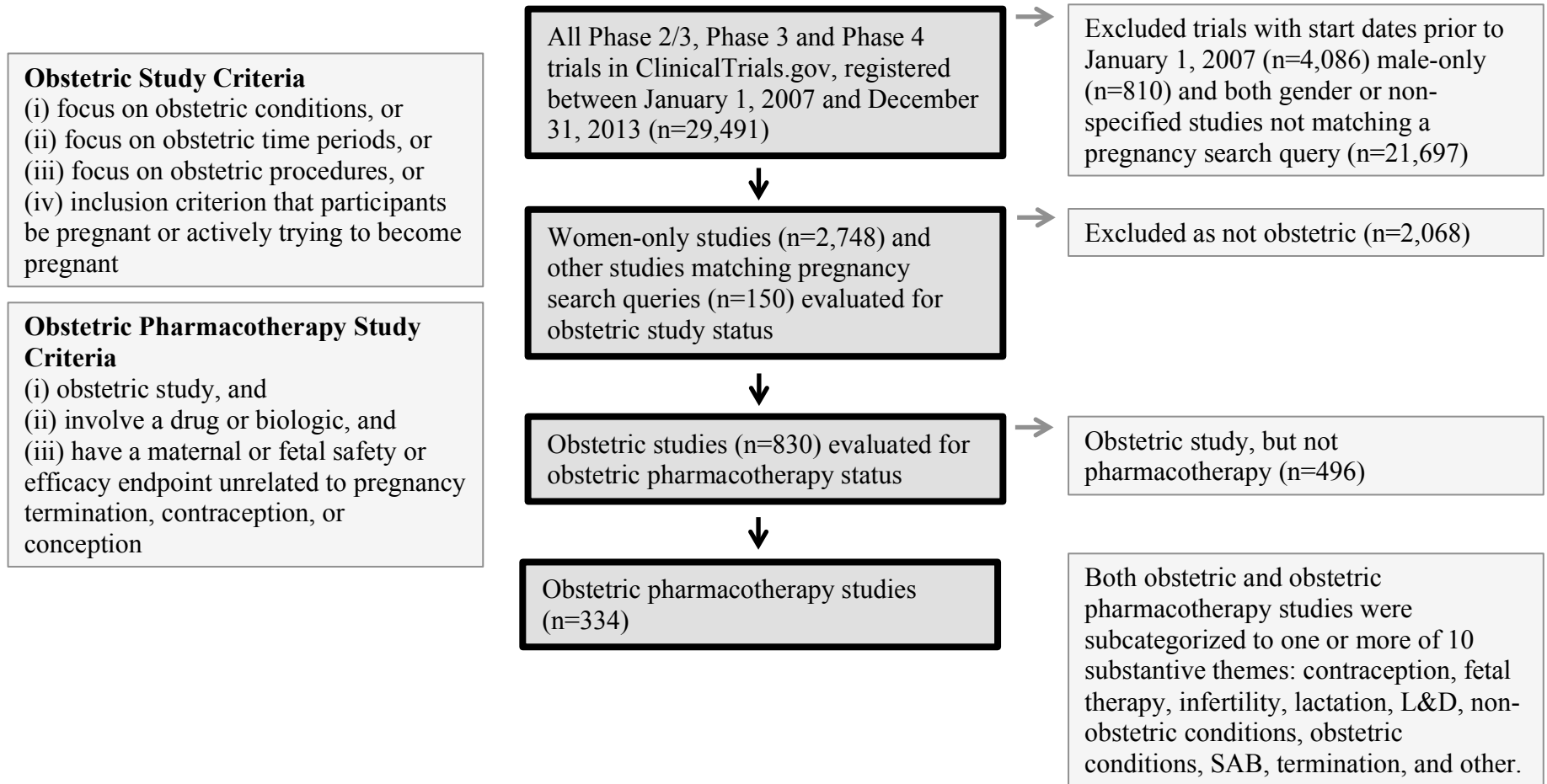
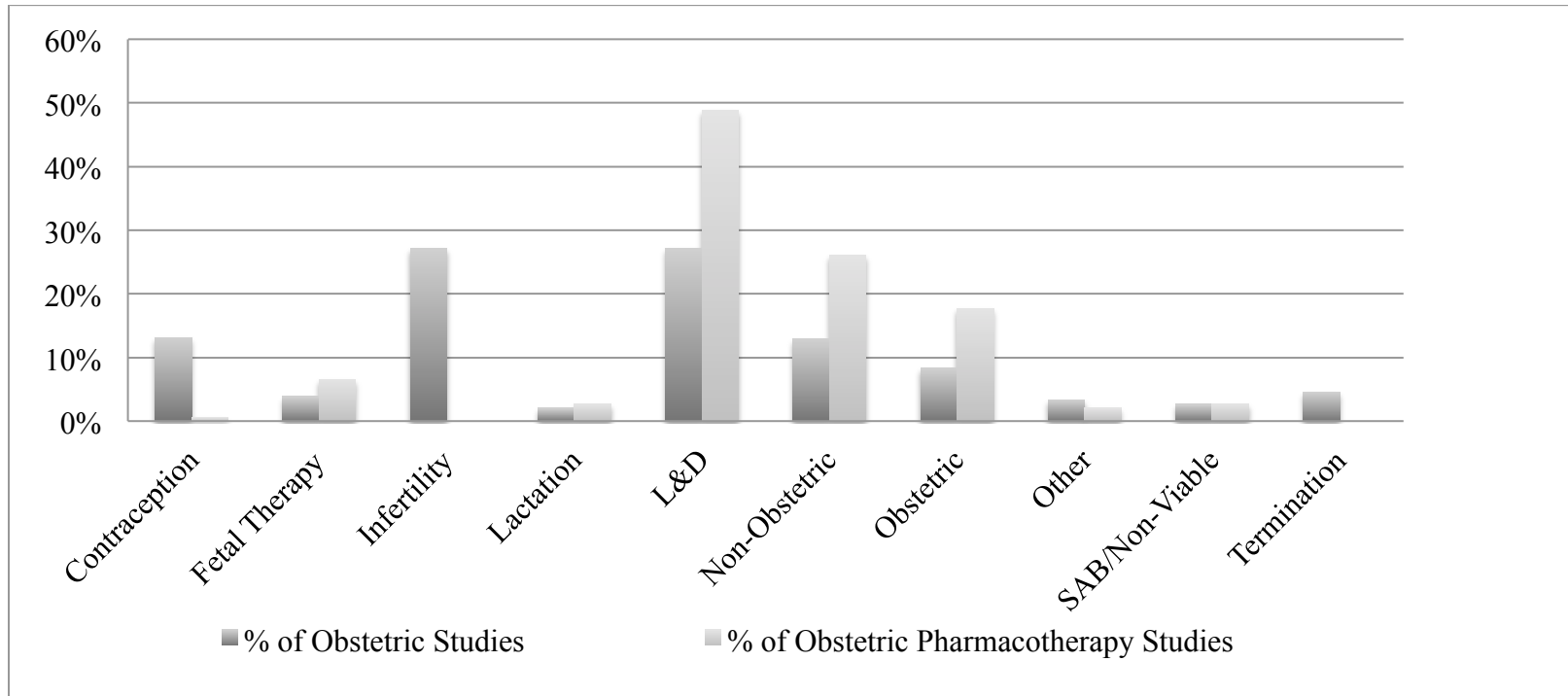
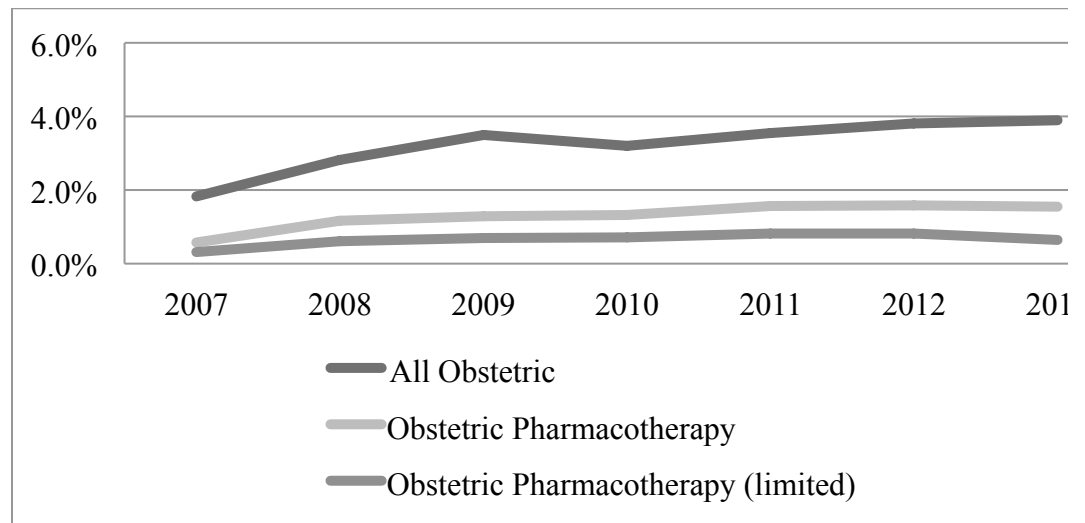


FIGURE 2: SUBSTANTIVE THEMES



Obstetric and obstetric pharmacotherapy studies organized by substantive theme. L&D: labor and delivery, SAB: spontaneous abortion.

FIGURE 3: TIME TRENDS



Obstetric and obstetric pharmacotherapy studies as a proportion of all studies, 2007-2013. Obstetric Pharmacotherapy (limited) is the subset of Obstetric Pharmacotherapy studies with the substantive themes of fetal therapy, lactation, non-obstetric conditions, obstetric conditions and spontaneous abortion/non-viable pregnancy.

CHAPTER 2: PRENATAL PHARMACOGENOMICS

1. PRENATAL PHARMACOGENOMICS: A PROMISING AREA FOR RESEARCH

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The demonstration of noninvasive prenatal sequencing of a fetal genome using cell-free fetal DNA¹ stimulated extensive discussion of the implications of this technological advancement, including the recent publication of a joint position statement from the European Society of Human Genetics and the American Society of Human Genetics with recommendations for responsible innovation in non-invasive prenatal testing (NIPT)². Noninvasive fetal genotyping and sequencing could enable expansion of prenatal genetic screening and testing far beyond common aneuploidies, to potentially include diagnosis of Mendelian disease and identification of carrier status and disease susceptibility markers. Much of this information has uncertain clinical utility, even for adults, and would include the identification of many gene variants of unknown clinical significance. It therefore raises ethical concerns. Left out of this discussion is the value fetal genomic data could offer to obstetric management of a current pregnancy, principally through pharmacogenomic and toxicogenomic effects, and the attendant research priorities and clinical testing opportunities.

There is a growing body of evidence to support the contribution of fetal genotype to inter-individual differences in placental drug transport as well as placental and fetal drug metabolism, all of which may influence the fetal safety profiles for maternally-administered medications and other chemical exposures. Nearly all drugs and xenobiotics pass through the placenta into fetal circulation to some extent, with neutral, lipophilic molecules of small molecular weight crossing most readily through passive diffusion. Fetal-origin efflux transport proteins in the apical (maternal-facing) brush border membrane of the placenta's syncytiotrophoblast are believed to play a major role in

limiting fetal exposures, and several transporter genes that are ubiquitously expressed throughout the body, including P-glycoprotein (*MDR1*, *ABCB1*) and breast cancer resistance protein (BCRP, encoded by the gene *ABCG2*), have their highest expression levels in this tissue³.

Few studies have directly assessed the relationship between fetal genotype and fetal exposure to medications and other xenobiotics in humans, but variants in genes for membrane transporters are of increasing clinical interest. The International Transporter Consortium recently identified two common polymorphisms, including one in *ABCG2*, with sufficient evidence of impact to medication disposition or response to warrant their incorporation into the drug development process⁴. Polymorphisms that reduce the efflux capacity of placental membrane transporters may result in higher fetal drug exposure levels and increased concerns for fetal safety for medications used to treat a condition affecting a pregnant woman. Clinical applications could include both prospective testing to identify pregnancies likely to have significantly altered placental transfer, allowing for changes in dosing and medication selection to optimize safety and efficacy, as well as retrospective testing to identify whether fetuses may have been previously exposed to significant risk. Less common but also relevant are situations where treatment of the fetus or the placenta is the goal, and characterization of variation in placental transfer may enable dosing adjustments to maintain desired therapeutic drug concentrations at the target site.

Inherited variability in drug metabolism has been the subject of the majority of pharmacogenetic research to date, as this pharmacokinetic parameter can significantly affect a drug's safety and efficacy profile, as well as the dosing required to achieve the same steady-state drug concentration across individuals. The human placenta and developing fetus each have some xenobiotic metabolizing capacity, although both are minor overall relative to that of the mother, and unlikely to significantly contribute to the total pharmacokinetics of a maternally administered medication. Placental, embryonic and fetal metabolism are, however, speculated to play a role in intrauterine toxicity, particularly through the local formation of teratogenic, mutagenic and carcinogenic metabolites by cytochrome P-450 (CYP)-mediated oxidative metabolism⁵.

In contrast to most species in animal studies, the human embryo and fetus express multiple CYP isoforms in both hepatic and extra-hepatic tissues, which are thought to be involved in normal fetal development. *CYP3A7* is the major CYP constituent in the fetal liver where it participates in synthesis of estrogens, and it may play a significant role in fetal metabolism of exogenous compounds. Over 100 missense and nonsense variants have been observed in this gene, as well as extensive variability in gene expression and protein catalytic activity in the fetal liver⁶, and multiple *CYP3A7* exogenous substrates have been identified, including several that are metabolically activated by the enzyme.

While xenobiotic metabolizing enzymes (XMEs) comprise only a small fraction of total CYP content in the placenta, several medications and other xenobiotic compounds including polycyclic aromatic hydrocarbons have been shown to undergo metabolism in

this tissue. Of particular interest is CYP1A1, or aryl hydrocarbon hydroxylase (AHH), which has low constitutive activity but is highly inducible by maternal cigarette smoking. Polymorphisms in *CYP1A1* have been shown to augment AHH inducibility and increase the risk of lung cancer in smokers⁷. Placental AHH inducibility has been positively associated with the risk of low birth weight among the offspring of women who smoked during pregnancy⁸, but the overall role of fetal *CYP1A1* in smoking-associated intrauterine toxicity remains uncertain and is likely complex, with the enzyme involved in both toxicity-inducing and protective detoxification processes.

The involvement of the fetal genome in exposure-related adverse pregnancy and neonatal outcomes has strong biological plausibility and a growing evidentiary basis, but additional research is needed to demonstrate specific instances of clinical validity and utility. The prioritization of this research is justified by the substantial knowledge deficits that exist in maternal-fetal medicine. Over 70% of pregnant women take at least one prescription medication⁹, and for over 90% of medications there is significant uncertainty regarding both safety and efficacy for use in pregnancy¹⁰. This is a long-standing and serious problem that has the potential to harm both women and infants. There is also growing interest in the intra-uterine environment and the possible fetal origins of adolescent- and adult-onset disease. Research focused on the effects of polymorphisms in placental transporters, as well as variants in XMEs expressed in the fetus and placenta, may contribute not only to a better understanding of exposure-associated adverse fetal outcomes, but also to long-term health. While caution is warranted as we consider an expanded scope of prenatal genetic screening and testing, cell-free fetal DNA-based

methods of genomic analysis may offer an opportunity to assess and avoid harmful fetal exposures. Responsible innovation involves both minimizing the harms and maximizing the benefits of new technologies, and NIPT for pharmacogenomic and toxicogenomic applications has the potential to offer significant benefit to women and children. This promising area for research merits more attention than it has thus received.

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CHAPTER 3: FETAL Q141K GENOTYPE AND FETAL GLYBURIDE EXPOSURE

1. FETAL ABCG2 Q141K GENOTYPE DOES NOT PREDICT FETAL GLYBURIDE EXPOSURE AT TERM

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ABSTRACT

This study evaluated the contribution of fetal *ABCG2* genotype to inter-individual differences in the transplacental passage of glyburide in human pregnancy at term. Maternal and venous umbilical cord blood samples were collected at delivery from 70 women receiving glyburide for treatment of gestational diabetes mellitus. Maternal and fetal DNA were genotyped for the *ABCG2* 421C>A allele, and glyburide and metabolite concentrations were measured in maternal and venous umbilical cord plasma. Umbilical cord-to-maternal glyburide and metabolite concentration ratios at the time of delivery, fetal metabolic biomarkers and fetal outcomes were compared on the basis of fetal *ABCG2* genotype. The fetal-to-maternal glyburide concentration ratios for homozygous reference and heterozygous or homozygous variant infants were 0.91 ± 0.53 ng/ml and 1.07 ± 0.71 ng/ml, respectively, and not significantly different ($p=0.34$). A difference in this metric at earlier stages of gestation cannot be ruled out.

INTRODUCTION

Gestational diabetes mellitus (GDM), defined as glucose intolerance that begins or is first recognized during pregnancy, currently affects between 4.6-9.2% of pregnancies in the United States(1), and the rate is increasing(2). GDM is associated with significant maternal, fetal and neonatal complications, which are reduced with treatment(3).

Treatments for GDM include dietary interventions, insulin injections and oral glucose lowering agents such as glyburide and metformin.

Glyburide was originally developed in the 1960s as a treatment for type 2 diabetes mellitus (T2DM), and became accepted as a treatment for GDM due to comparable efficacy and superior convenience and cost relative to insulin. In some practice settings, glyburide is now more commonly used than insulin for the treatment of GDM(4).

Glyburide is considered safe for use in pregnancy because of its perceived limited transplacental passage and similar maternal, fetal and neonatal outcomes as with insulin(5, 6). However, a recent study documented significant but variable fetal glyburide exposure during pregnancy, where the glyburide umbilical cord plasma concentration averaged 70% of the maternal plasma concentration at the time of delivery, contradicting previous findings of limited fetal transfer(7). The results of this study also suggested that for some women with GDM, current glyburide dosage strategies, which commonly follow those used for non-pregnant patients with T2DM, result in lower plasma drug concentrations than in dose-matched non-pregnant women with T2DM, and that higher doses and more frequent dosing may be needed(7). A randomized clinical trial investigating this approach is underway(8).

When considering fetal exposure to glyburide, active efflux of the drug from fetal to maternal circulation, countering concentration gradient-driven passive diffusion, is believed to occur primarily via the drug transporter breast cancer resistance protein (BCRP)(9, 10), which is found in abundance in the apical (maternal-facing) layer of the placental syncytiotrophoblast(11), a fetal-origin tissue. Over 100 mutations have been identified in *ABCG2*, the gene that encodes BCRP, including several common variants(12). The most studied variant, the 421C>A (rs#2231142) single nucleotide polymorphism (SNP), resulting in a glutamine to lysine (Q141K) amino acid change, has been shown to alter the level of BCRP expression in the placenta, affect the pharmacokinetics of several BCRP substrate drugs(13-15), and reduce the renal transport activity of BCRP for the endogenous substrate uric acid(16). Higher fetal glyburide concentrations have been observed in *Bcrp1* knockout mice as compared to wild-type controls(17), but no studies have investigated the effect of *ABCG2* variation on fetal glyburide exposure in humans.

The elucidation of the polymorphic nature of the placental drug transporter involved in the efflux of glyburide from fetal to maternal circulation, combined with recent data concerning glyburide pharmacokinetics suggesting higher doses could be used in pregnant women with GDM, raises new questions regarding fetal safety. In this observational study we sought to determine whether placental *ABCG2* Q141K genotype is predictive of fetal glyburide exposure at term. A greater understanding of the impact of

ABCG2 variation on placental BCRP function and fetal glyburide exposure will help to establish recommendations for optimal glyburide dosing in the treatment of GDM.

RESULTS

PARTICIPANT DEMOGRAPHICS

A total of 70 women and their infants completed study procedures between October 2, 2013 and December 30, 2014. Enrollment sites included the University of Washington (UW), University of Texas Medical Branch (UTMB), and Columbia University Medical Center (CUMC). Completed participants were on average 32.8 ± 5.5 years of age at the time of delivery, 81.3 ± 18.2 kg body weight pre-pregnancy and had a mean pregnancy weight gain of 8.9 ± 7.0 kg. Participating women were 67.1% Hispanic White, 22.9% non-Hispanic White, 4.3% non-Hispanic Black, 1.4% Asian, 1.4% multiple racial groups and 2.9% unknown.

DELIVERY AND SAMPLE COLLECTION

All pregnancies were delivered at term with an average of 39 weeks' gestation. Forty-one (58.6%) infants delivered by cesarean delivery, and 29 (41.4%) delivered vaginally.

Venous maternal blood and venous umbilical cord blood samples were collected immediately following delivery; typically within 30 minutes. Three maternal blood samples were collected more than one hour after delivery and were excluded from analyses for which non-simultaneous umbilical cord and maternal blood draws were likely to invalidate results.

GLYBURIDE AND METABOLITES

The glyburide doses for study participants were determined by each woman's provider based on clinical need without regard to the study. Prescribed daily dosages ranged from 1.25-15 mg (mean 4.1 ± 2.7 mg/day). Thirty-six women received glyburide once daily, 31 were dosed twice daily, and three were dosed three times per day. A mean of 22.2 ± 11.0 hours (range: 4-58h) had elapsed between the last administered dose of glyburide and blood sample collections. Total glyburide, and the five known metabolites, 4-trans-hydroxycyclohexyl glyburide (M1), 4-cis-hydroxycyclohexyl glyburide (M2a), 3-cis-hydroxycyclohexyl glyburide (M2b), 3-trans-hydroxycyclohexyl glyburide (M3), 2-trans-hydroxycyclohexyl glyburide (M4) and ethylene-hydroxylated glyburide (M5), were assayed in venous maternal and venous umbilical cord plasma. The limit of detection and quantification for both the drug and metabolite assays were 0.05 ng/ml and 0.10 ng/ml, respectively. Ten maternal samples and 10 venous umbilical cord samples were below the limit of detection for the parent drug.

The mean concentrations of glyburide in maternal blood and venous umbilical cord blood with detectable drug levels were 4.5 ± 7.3 ng/ml and 3.1 ± 3.5 ng/ml, respectively. The overall fetal-to-maternal glyburide concentration ratio, a commonly used metric for transplacental drug passage and relative fetal exposure, was 1.0 ± 0.6 , with an R^2 of 0.78. Measurable glyburide concentrations were available for both mother and umbilical cord in 55 dyads. In 22 (40%) of these pairs, the glyburide level in the umbilical cord sample was equal to or greater than that of the maternal sample. Both the maternal and umbilical

cord glyburide concentrations decreased as a function of time elapsed since the last dose ($p=0.002$ and $p<0.001$, respectively). The fetal-to-maternal glyburide concentration ratio showed a statistically significant increase at greater times between last dose and sample collection ($p=0.02$), but the effect was moderate and the overall fetal-to-maternal glyburide concentration ratio remained at 0.91 ± 0.4 ($n=39$) even when the analysis was restricted to samples collected within 24 hours of dosing. The ratio did not vary as a function of time between maternal and umbilical cord sample collection when both were within one hour of delivery. The ratio did not vary based on the study site, glyburide dose, glycemic control (assessed via the blood glucose test prior to delivery and the most recent hemoglobin A1c test result, if available), infant race or ethnicity or mode of delivery.

Two of the five known glyburide metabolites, M1 and M2b, are known to be pharmacologically active. Venous umbilical cord blood glyburide concentrations alone were positively associated with umbilical cord insulin and c-peptide levels ($p=0.001$ for both when insulin analysis was restricted to non-hemolyzed samples), and the strength of this association increased when the parent drug was combined with the active metabolites, adjusted for potency(18) ($p<0.001$ for both).

ABCG2 Q141K GENOTYPE

Both the maternal and fetal *ABCG2* Q141K genotypes were successfully determined for 67 of the 70 mother-fetal dyads. In all instances, the fetal genotype was consistent with that of the mother. Among the fetal genotypes, 46 homozygous reference, 21

heterozygous and one homozygous variant genotypes were observed. The variant allele frequency for all participants was 0.15, and the allele frequency among White-Hispanic participants (0.19) was comparable to 1,000 Genomes, Phase 3, allele frequencies from individuals in the Americas (0.14) and Mexico (0.20)(12). Also, the observed frequencies were in Hardy-Weinberg equilibrium.

The fetal-to-maternal glyburide concentration ratios for homozygous reference and heterozygous or homozygous variant infants were 0.91 ± 0.53 and 1.09 ± 0.73 , respectively (Figure 1). These differences were not statistically significant ($p=0.345$). Fetal genotype was not associated with maternal factors including glyburide dose, glycemic control, mode of delivery, or the time between dosing and sample collection. Adjusting for these factors, as well as infant race and ethnicity, in regression analyses did not affect the statistical significance of fetal genotype as a predictor of the umbilical cord-to-maternal plasma concentration ratio for glyburide.

Glyburide is more than 98% bound to albumin, and albumin levels change throughout gestation in both maternal and fetal circulation(19). To assess whether differences in maternal and fetal albumin levels were masking the effect of fetal Q141K genotype we divided each maternal and fetal glyburide concentration by the corresponding albumin level from the same specimen, and repeated the analysis with the resulting values. This adjustment did not alter the statistical significance of the results. However, it was interesting to note that the one dyad for which the maternal albumin level exceeded that of the umbilical cord sample included the one Q141K homozygous variant infant in

whom the greatest phenotypic effect on the umbilical cord-to-maternal concentration ratio would be expected, but which had a ratio of 0.7.

INFANT OUTCOMES

Infant outcomes evaluated in this study included neonatal hypoglycemia, shoulder dystocia and birth weight. These outcomes were selected as they are of interest for GDM and glyburide-exposed infants, and could be ascertained within a short period of time after birth. A total of 13 (18.6%) infants in the study received a clinical diagnosis of neonatal hypoglycemia (<60 mg/dl), of which 4 (5.7%) were severe (<40 mg/dl) (Table 1). These rates are comparable to those previously observed in studies of oral hypoglycemic agents, although there has been variability in definitions of and diagnostic criteria for neonatal hypoglycemia(20). Umbilical cord C-peptide concentrations were predictive of neonatal hypoglycemia risk (OR: 0.24 [0.09-0.39], $p=0.002$), as were umbilical cord insulin concentrations, although these associations were not independent. Umbilical cord glyburide concentrations, while associated with umbilical cord C-peptide concentrations, were not directly predictive of neonatal hypoglycemia risk ($p=0.174$ for parent drug only, $p=0.128$ for glyburide and active metabolites). There were no significant differences among infants with and without neonatal hypoglycemia in terms of the maternal dose of glyburide, time since last dose, infant birth weight or mode of delivery. Three infants in the study had shoulder dystocia. This is only a risk of vaginal delivery, and all of the affected infants were delivered via that mode. The strongest predictor of shoulder dystocia risk was infant birth weight, although this did not meet the threshold of statistical significance ($p=0.085$). Infant birth weights were normally

distributed and just four infants had macrosomia ($\geq 4,000\text{g}$). Neither birth weight nor macrosomia risk was predicted by any of the demographic, drug, or delivery related variables included in the study.

DISCUSSION

This is the largest study performed to date of the transplacental passage of glyburide at term, and the first study that has evaluated the effect of fetal *ABCG2* genotype on fetal glyburide exposure in humans. It was motivated by studies demonstrating the primary role of BCRP in glyburide efflux from fetal to maternal circulation(9), high levels of *ABCG2* expression in the apical layer of placental syncytiotrophoblast cells which form the interface between maternal and fetal circulation(11), decreased BCRP protein levels in 421A heterozygous and homozygous variant full-term human placental tissue(21), increased fetal glyburide exposure in *Bcrp1/Abcg2* knockout mice(17), and altered pharmacokinetics in Q141K heterozygous and homozygous variant individuals for BCRP substrate drugs including diflomotecan(13), sunitinib(14) and several statins(15), as well as the endogenous BCRP substrate uric acid(22). In our data, fetal Q141K genotype did not predict the fetal-to-maternal glyburide concentration ratio at term.

There are several possible explanations for this negative result that remain consistent with our original hypothesis and the supporting data. An important role of the placenta is its barrier function, protecting the developing fetus from potentially harmful xenobiotic and endogenous compounds in maternal blood. The near term and full term placenta has diminished barrier function relative to placenta in earlier gestation. The term placenta has

greater surface area for exchange, decreased diffusing distance between maternal and fetal circulation (50-100 μm early in pregnancy compared to 4-5 μm near term), larger and more capillaries in placental villi, and increased uterine blood flow (50 mL/min at 10 weeks gestation compared to 600 mL/min at term)(19). All of these factors facilitate the passive diffusion of glyburide, a relatively small and lipophilic molecule, between maternal and fetal circulations, and could reduce or eliminate the capacity of placental BCRP to maintain a concentration gradient at this point in gestation.

An additional consideration is *ABCG2* ontogeny in the placenta. Fetal susceptibility to teratogenic agents peaks between 15 and 60 days gestation, when significant organ and morphological development is taking place(23). The expression level of some placental membrane transporters, including P-glycoprotein (*MDR1*), also peaks during this window and decreases as the pregnancy nears full term and fetal vulnerability declines(24). The ontogeny of *ABCG2* in human placenta is less established, but several rodent studies and the largest human study to date indicate that both mRNA and protein levels peak prior to term(11). This attenuation of gene transcription activity at term relative to earlier stages of pregnancy could result in a lower and hence difficult to detect phenotypic distinction between the different *ABCG2* genotype groups at term when one did exist earlier in gestation.

ABCG2 is a polymorphic gene, with over 100 SNPs identified to date. A small number of variants apart from Q141K have been shown to affect BCRP function(11), and several others would be predicted to do so based on their location and nature. These variants are

very rare, but their presence in some of the Q141K homozygous reference allele infants could have resulted in misclassification and an apparent lack of phenotypic distinction across Q141K genotype groups.

Our results could also be explained by variable concordance between genotype and phenotype across tissue types for the 421A allele. BCRP is a half-transporter, having only one transmembrane domain and one ATP-binding region, and is believed to require homodimerization to transport substrates(25). Pharmacogenetic studies of other BCRP substrate drugs in other body tissues have consistently observed an altered pharmacokinetic phenotype in both heterozygous and homozygous variant individuals. The placenta has the highest *ABCG2* expression level of any tissue in the body and could, therefore, contain sufficient functional homodimer protein in heterozygous individuals that their glyburide transport phenotypes matched those of homozygous reference individuals. We had just one homozygous variant infant in our study. The fetal-to-maternal glyburide concentration ratio for this infant was lower than the mean values for both heterozygous and homozygous reference infants, which does not support this possible explanation.

Finally, while BCRP is believed to be the primary protein involved in the active transport of glyburide across cellular membranes, there has been some controversy about the role that P-glycoprotein may also play. If P-glycoprotein is also involved in glyburide efflux, it could become the dominant glyburide transporter at term, when placental BCRP content has declined. This scenario, however, would be better supported if the overall and

within Q141K genotype fetal-to-maternal glyburide concentration ratios observed in this study were all significantly less than one, which was not the case.

It is also possible, despite persuasive indirect evidence suggesting that *ABCG2* variation plays a strong role in fetal glyburide exposure for human pregnancy, that this is not the case, at least at term. The human placenta is qualitatively and quantitatively distinct from that of other laboratory animals, particularly the mouse. Significant differences include the extent of trophoblast invasion into uterine arteries, the labyrinthine (murine) or villous (human) placental exchange area, and the number of trophoblast layers at the interhemal barrier (three in mice compared to one in humans)(26). Additionally, the mouse studies that found higher fetal glyburide levels in heterozygous and homozygous variant mice were not conducted at term(17). Animal models remain a valuable source of information, particularly given the restrictions on performing pharmacology studies on pregnant human women, but inter-species differences in gestation and placentation often limit the ability to generalize findings from animal models of pregnancy to that of humans.

There are two main limitations to this study. A critical window of fetal vulnerability to *in utero* exposures occurs early in gestation, when morphogenesis and organogenesis are taking place. It is rarely possible, however, to collect samples that are directly informative of fetal exposure prior to delivery. The human placenta and the extent of placental drug transfer at term are distinct from earlier time points in gestation, and we cannot rule out the possibility that *ABCG2* genotype-based differences in fetal glyburide

exposure exist prior to term. Glyburide exhibits very high (>98%) binding to albumin. In this study we measured total glyburide, when it is the unbound drug that passes across membranes and exerts its pharmacological effect. Direct measurement of the unbound drug concentrations was not attempted as the results were expected to be well below the limit of detection for our assay. Instead we measured albumin concentrations in maternal and umbilical cord plasma and then divided total fetal and maternal glyburide by its respective protein concentration to provide a rough accounting for albumin variation in the analysis. This adjustment suggested that differences in binding protein were an unlikely explanation for our results.

In this study we sought to determine whether there is an association between fetal *ABCG2* genotype and the at-birth umbilical cord-to-maternal glyburide plasma concentration ratio in women with GDM. The most parsimonious interpretation of the data is that *ABCG2* genotype does not affect fetal glyburide exposure at term in women taking the drug to control GDM. This has important clinical implications. Although fetal exposure to glyburide earlier in pregnancy may elicit effects of unknown consequence, the primary concern for women with GDM is its role in neonatal hypoglycemia. For the most part, fetal glucose control before birth is determined by the degree of maternal glucose control. This control is lost at birth and the neonatal hyperinsulinemia caused by poor maternal glucose control as well as the presence of glyburide in neonatal blood could cause hypoglycemia. Neonatal hypoglycemia was observed in our study population, and was associated with elevated C-peptide levels, but was not directly predicted by umbilical cord glyburide concentrations. This suggests that glyburide-

induced increased fetal pancreatic insulin secretion may be transient in nature as compared to the islet cell hyperplasia that results from hyperglycemia, and that maternal glucose control during delivery may have a greater influence on the risk of this adverse fetal outcome. The association between umbilical cord insulin and C-peptide and fetal glyburide concentration does suggest that glyburide-induced neonatal hypoglycemia is still possible, and further study of the sources of inter-individual differences in fetal glyburide exposure may aid clinicians' ability to predict and avoid this adverse event. This is particularly important given data suggesting that dose escalation may enable more women with GDM to achieve glycemic control and remain on oral therapy, as opposed to treatment with insulin(7). While there is no strong evidence to suggest adverse fetal outcomes at current dosages, some compounds only display teratogenic or other adverse effects at or above exposure thresholds. Additionally, there is increasing awareness of possible non-morphological effects (e.g. behavioral, cognitive, developmental or metabolic) of prenatal exposures that may not present until adolescence or adulthood. Long-term studies following the developmental and metabolic outcomes of infants exposed to oral glucose lowering medications, as well as those exposed to poorly controlled and insulin-controlled GDM, are needed. Such studies, as well as those resulting in an improved understanding of the placental transfer of glyburide, particularly the sources of inter-individual variation in fetal drug exposure, will aid our ability to optimize the safe and effective use of this drug for the treatment of GDM.

METHODS

SUBJECTS. The study included 70 mother-infant dyads recruited in Seattle, WA; Galveston, TX, and New York City, NY. Women were eligible to be enrolled in the study if they (i) had a current clinical diagnosis of GDM and (ii) had an active prescription for glyburide. Women were excluded from the study if they (i) were receiving a medication known to interact with either glyburide or BCRP, (ii) had multiple gestations or a known major fetal abnormality, or (iii) were diagnosed with a condition known to alter the placenta (e.g. chorioamnionitis, preeclampsia).

All participant data were abstracted from medical records, with the exception of the samples collected and analyzed for the study, as well as the time of last glyburide dose prior to delivery and the infants' paternal race and ethnicity, which were obtained verbally from the participating women. Women enrolled in the study who (i) discontinued use of glyburide, (ii) met an exclusion criterion, or (iii) subsequently delivered preterm (<37 weeks) or postterm (>42 weeks) were withdrawn and any data collected prior to withdrawal was removed from the analysis.

The study was approved by the institutional review boards at the University of Washington, University of Texas Medical Branch in Galveston and Columbia University. Written informed consent was obtained from all participants.

SAMPLE ANALYSIS. All specimens were shipped to UW, which was the coordinating center for all sample and data analysis. Plasma concentrations of glyburide and its metabolites (M1, M2a, M2b, M3, M4 and M5) were determined using a validated high-performance liquid chromatography–mass spectrometry assay [Supplement 1]. DNA was

isolated from the buffy coat of each blood sample using a QIAamp DNA Mini Kit (Qiagen, Valencia, CA). The *ABCG2* Q141K (421C>A, rs2231142) genotype was determined for each sample using a validated Taqman® allelic discrimination assay (Applied Biosystems, Foster City, CA). The fluorescent 5'-nuclease assay results were analyzed on an ABI 7900HT Real-Time PCR System (Life Technologies, Carlsbad, CA). Fetal umbilical cord serum concentrations of insulin were determined using Access Ultrasensitive Insulin, a simultaneous one-step immunoenzymatic assay (Beckman Coulter, Brea, CA). Serum C-peptide concentrations were determined using the IMMULITE 1000 C-Peptide solid-phase, two-site chemiluminescent immunometric assay (Siemens Healthcare, Malvern, PA). Plasma albumin analysis was conducted using UniCel DxC 800 Synchron Clinical System (Beckman Coulter, Brea, CA).

STATISTICAL ANALYSIS. Sample size considerations for the study included the previously observed mean and standard deviation for the umbilical cord-to-maternal glyburide ratio(7), the pharmacokinetic effect size of the 421A variant seen in studies of other BCRP substrate drugs(13, 21), discussions with clinicians regarding a clinically meaningful difference in fetal exposure, and variant allele frequencies in the primary racial and ethnic groups represented in the recruitment areas(12). Based on these factors, 50 participants were expected to yield the minimum number of heterozygous and homozygous variant infants necessary to replicate, conservatively, an effect of similar magnitude to those previously observed. We increased this number to 70 to account for uncertainty and ensure a sufficient number of heterozygous and homozygous variant

infants. All statistical analyses were performed using Stata 11.2 (StataCorp LP, College Station, TX). A p-value of <0.05 was considered statistically significant.

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TABLE 1: INFANT CHARACTERISTICS & OUTCOMES, BY *ABCG2* GENOTYPE

| | ALL n=70 | REFERENCE^c n=48 | HETEROZYGOUS AND VARIANT^d n=22 |
|-------------------------------------|--------------------|--------------------------------------|---|
| RACE^a (%) | | | |
| White, non-Hispanic | 15.7 | 21.7 | 0 |
| White, Hispanic | 67.1 | 58.7 | 86.36 |
| African American | 4.3 | 4.4 | 0 |
| Asian | 1.4 | 2.2 | 0 |
| multiple | 10.0 | 10.9 | 9.1 |
| unknown | 1.4 | 2.7 | 0 |
| HYPOGLYCEMIA | | | |
| clinical (40-60mg/dl) | 9 | 6 | 3 |
| severe (>40mg/dl) | 4 | 4 | 0 |
| Shoulder Dystocia | 3 | 1 | 2 |
| Birth weight (g) | 3345.1±385.8 | 3455.2±388.4 | 3451.6±393.9 |
| Insulin^b (uIU/ml) | 10.1±8.9 | 10.7±10.2 | 8.8±6.3 |
| C-peptide (ng/ml) | 1.2±0.8 | 1.4±1.0 | 1.04±0.4 |
| Albumin (g/dl) | 2.9±0.4 | 2.6±0.4 | 3.0±0.2 |

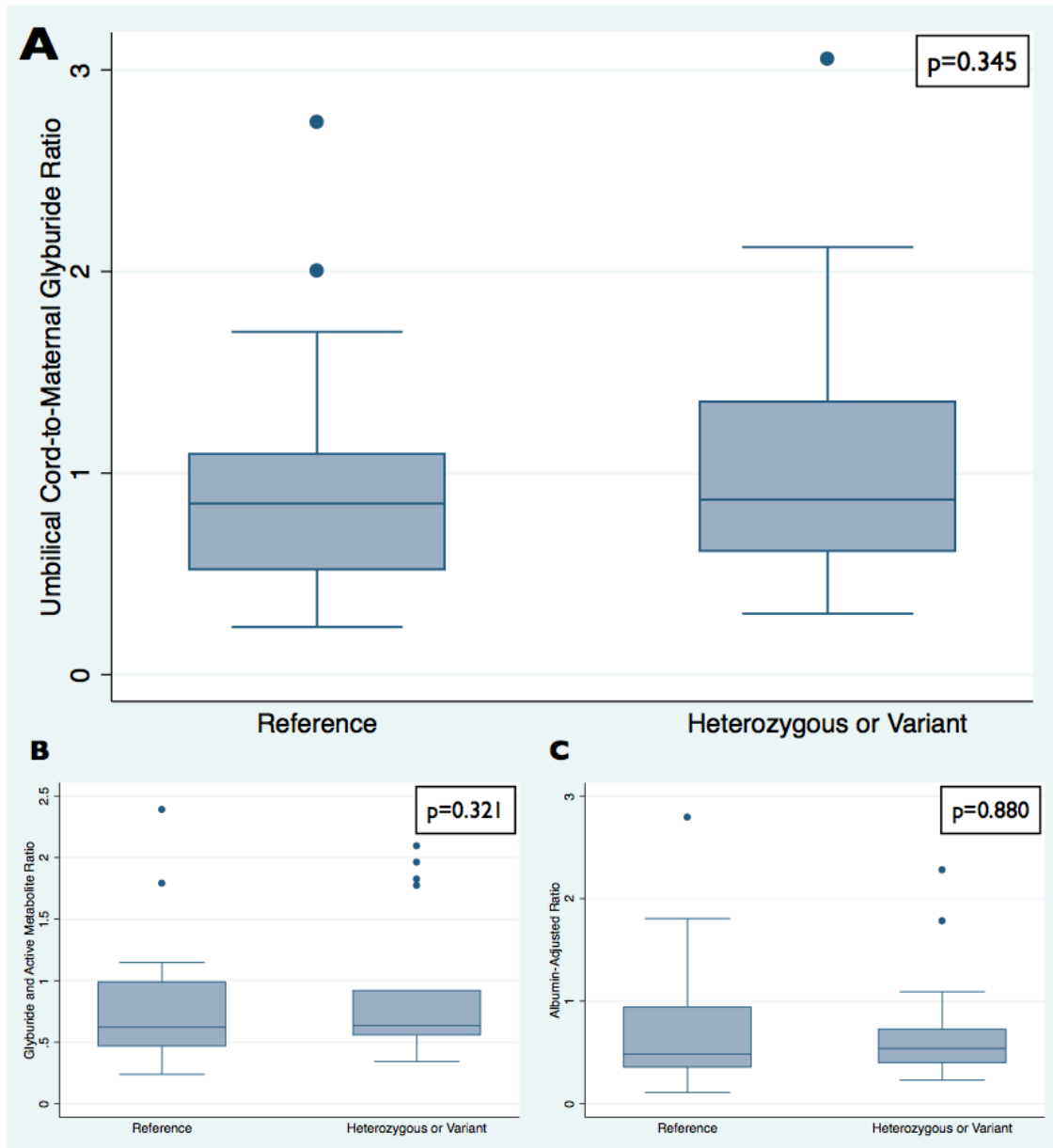
^a Infant race and Hispanic status were determined by combining the self-reported race and Hispanic status of the mother with that of the infant's father, as reported by the mother. When the paternal race and Hispanic status were unavailable, the infant's status was considered to match that of the mother.

^b Insulin analysis restricted to non-hemolyzed samples due to the degradational effects of hemolysis on insulin.

^c Infants that are homozygous reference for the *ABCG2* Q141K variant.

^d Infants that are heterozygous or homozygous variant for the Q141K variant.

FIGURE 1: UMBILICAL CORD-TO-MATERNAL GLYBURIDE PLASMA CONCENTRATION RATIOS



Umbilical cord-to-maternal concentration ratio for glyburide (A); glyburide and the two known active metabolites, adjusted for potency (B); and glyburide adjusted for the albumin concentration in each plasma sample, which provides an approximation of the concentration of unbound drug.

SUPPLEMENT 1: GLYBURIDE AND METABOLITE ASSAY

Plasma concentrations of glyburide, M1, M2a, M2b, M3, M4 and M5 were determined using a validated high-performance liquid chromatography–mass spectrometry assay. Briefly, 0.5 ml of plasma was spiked with internal standard solution (20 µl in methanol of 0.1 ng/µl each of glyburide-*d*₁₁ and 4-transhydroxy glyburide ¹³C-*d*₃) and mixed with 1 ml of water and 100 µl of 2 M HCl. Samples were applied to Agilent Technologies (Santa Clara, CA) Certify C18 25 mg SPE tubes which had been prepared with 2 ml of methanol followed by 2 ml of water. The SPE tubes were washed with 3 x 1 ml of water then dried with air for 30 seconds. SPE tubes were eluted with 1 mL of acetonitrile. The eluent was evaporated to dryness in air at 40° C. The residue was reconstituted in 75 µl of mobile phase. Five µl was injected onto the HPLC. Calibration curves were generated by spiking blank plasma from 0.1 to 10 ng/ml. No standards are commercially available for M4 and M5. Presence of these metabolites was ascertained based upon the expected mass transitions (Selvan Ravindran, *Biochemical Pharmacology* 72 (2006) 1730–1737).

An Agilent Technologies 1290 Series UPLC coupled to an Agilent G6410B triple-quadrupole mass spectrometer was used for the quantitation of glyburide and metabolites. The HPLC column was an ACE C8 150 mm x 2.1 mm x 3 µm (MAC-MOD, Chadds Ford, PA) maintained at 50° C. The mobile phase consisted of a binary mixture of 5 mM ammonium formate pH=6 and methanol at a flow rate of 0.4 ml/min according to the following gradient:

| Time (min) | % Methanol |
|------------|------------|
| 0 | 40 |
| 5 | 40 |
| 9 | 90 |
| 9.1 | 40 |

The total run time was 12 minutes.

The mass spectrometer was operated in the ESI+ mode. Nebulizer gas was nitrogen at 35 psi and drying gas was nitrogen at 350° C and 10 L/min. Capillary voltage was 1500 volts and the cell acceleration voltage was 7 volts. The first 3 minutes of the chromatographic run were diverted to waste.

The following mass transitions were monitored:

| Compound | Precursor ion (m/z) | Product ion (m/z) | Dwell (msec) | Fragmentor energy (volts) | Collision energy (volts) |
|---|---------------------|-------------------|--------------|---------------------------|--------------------------|
| OH-Glyburide- ¹³ Cd ₃ | 514.2 | 173 | 150 | 123 | 40 |
| OH-Glyburide | 510.2 | 169 | 150 | 110 | 40 |
| Glyburide | 494.2 | 169 | 150 | 110 | 40 |
| Glyburide-d ₁₁ | 505.3 | 169 | 150 | 110 | 40 |

2. BCRP AND FETAL GLYBURIDE EXPOSURE STUDY PROTOCOL

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GENERAL INFORMATION

Title: BCRP and Fetal Glyburide Exposure

Date: August 6, 2013

Sponsor/funder:

Drug Metabolism, Transport, and Pharmacogenetics Research Program
University of Washington School of Pharmacy, Department of Pharmaceutics

University of Washington Obstetric-fetal Pharmacology Research Unit (OPRU)
The Eunice Kennedy Shriver National Institute of Child Health and Human Development

Northwest-Alaska Pharmacogenomics Research Network (NWA-PGRN)
National Institute of General Medical Sciences

Center for Genomics and Healthcare Equality at the University of Washington
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Number of Subjects: Up to 200 women will be enrolled in order to complete study procedures for 100 participants

RATIONALE & BACKGROUND INFORMATION

Gestational diabetes mellitus (GDM) currently affects between 2-10% of pregnancies in the United States, and this rate is increasing¹. GDM is associated with significant maternal and perinatal complications, which are reduced with treatment². Treatments for GDM include dietary intervention, insulin, and oral glucose lowering agents such as glyburide (GLY) and metformin.

GLY was originally developed in the 1960s as a treatment for type 2 diabetes mellitus (T2DM), and became accepted as a treatment for GDM due to comparable efficacy and superior convenience and cost relative to insulin³. GLY has generally been considered safe for use in pregnancy due to limited fetal transfer and no apparent increase in the rate of fetal morbidity^{3,4}. However, elucidation of the placental drug transporters involved in efflux of GLY from fetal to maternal circulation, combined with recent data related to GLY pharmacokinetics in women with GDM, raises new questions regarding fetal safety.

Efflux of GLY from fetal to maternal circulation is dominated by the drug transporter breast cancer resistance protein (BCRP)^{5,6}. Over 80 single nucleotide polymorphisms (SNPs) have been identified in *ABCG2*, the gene that encodes BCRP⁷, including several common variants⁸. The most commonly studied SNP, 421C>A, results in a glutamine to lysine (Q141K) amino acid change, and has been shown to alter the amount of BCRP in the placenta, as well as reduce the transport activity of BCRP in human embryonic kidney cells^{9,10}. Recent data also suggest that for some women, current GLY dosage strategies, which most commonly follow those used for T2DM, may be inadequate to

achieve glycemic control during pregnancy¹¹. This same study found that the GLY umbilical cord plasma concentration averaged 70% of the maternal plasma concentration at the time of delivery, contradicting previous findings of limited fetal transfer.

There are no studies currently available that have evaluated the effect of *ABCG2* genotype on fetal GLY exposure, complicating efforts to predict the effects of GLY dose escalation on fetal GLY exposure and fetal outcomes. A greater understanding of the impact of *ABCG2* variation on placental BCRP function and fetal GLY exposure will help to inform investigations of optimal GLY usage in the treatment of GDM.

Additionally, placental BCRP/*ABCG2* is fetal origin, meaning that the *ABCG2* genotype of interest is that of the fetus and not the pregnant woman. Historically, prenatal genetic testing has been focused on detecting major chromosomal abnormalities and Mendelian diseases. Should specific *ABCG2* variants be found to significantly increase fetal GLY exposure, and if such exposure were associated with perinatal complications, it could present an opportunity for a new, pharmacogenetic application of prenatal genetic testing.

SPECIFIC AIMS

Specific Aim: To evaluate the relationship between fetal *ABCG2* genotype and the ratio of fetal to maternal concentration of GLY at the time of delivery, in women taking GLY for treatment of GDM.

Primary Endpoint: Determination of the strength of association between fetal *ABCG2* Q141K genotype and the at-delivery fetal to maternal concentration ratio of GLY in women taking GLY for treatment of GDM.

Hypothesis: Variant fetal *ABCG2* alleles will disrupt the function of placental BCRP, resulting in reduced GLY efflux and increased fetal GLY exposure.

Secondary Endpoints: Exploration of the impact of maternal and non-Q141K fetal *ABCG2* variation and variation in placental (fetal) drug uptake transporters, including OATs, OATPs and OCTs, on the at-delivery fetal to maternal concentration ratio of GLY in women taking GLY for treatment of GDM. Exploration of the impact of maternal and fetal genetic variation on select neonatal outcomes and biomarkers of fetal metabolism.

STUDY DESIGN

This observational study will enroll 200 women with gestational diabetes mellitus (GDM) who are receiving glyburide (GLY) in mono- or combination therapy at the time of delivery, with the expectation that 100 women will complete study procedures. The study is designed to evaluate whether variation in the gene *ABCG2*, which encodes the placental transport protein breast cancer resistance protein (BCRP), affects the level of fetal GLY exposure by comparing the fetal to maternal ratio of GLY concentration in participants with variant fetal *ABCG2* to that of participants with wild type fetal *ABCG2*.

Subject Selection Criteria:

Inclusion Criteria:

1. Pregnant women (singleton pregnancy)
2. Gestational diabetes mellitus, as diagnosed by obstetrician or perinatologist
3. Use of GLY in mono- or combination therapy for GDM at time of delivery
4. Term pregnancy at time of delivery, defined as ≥ 37 weeks and ≤ 42 weeks
5. Age 18 or older
6. Able to give written informed consent
7. Plan to deliver at the hospital/clinic where recruited/enrolled

Exclusion Criteria:

1. Women diagnosed with preeclampsia or chorioamnionitis
2. Pregnancies with a known fetal abnormality, e.g. Trisomy 21
3. Women taking or planning to take medications expected to induce or inhibit *ABCG2/BCRP*, or to otherwise interact with glyburide within 2 weeks of delivery [carbamazepine, rifampin, phenytoin, clopidogrel, disulfiram, fluconazole, fluoxetine, metronidazole, sulfamethoxazole, voriconazole, zafirlukast, cyclosporine, tipranavir, ritonavir, atazanavir, lopinavir, omeprazole, esomeprazole, lansoprazole, rabeprazole, pantoprazole, eltrombopag, gefitinib]

Duration of subject participation: >1 day, <5 months (enrollment can occur any time after diagnosis of GDM and initiation of GLY, but before delivery; GDM diagnosis typically occurs after 20 weeks gestation)

Duration of study: 2 years

Concurrent/Concomitant Medications: Women taking medications expected to induce or inhibit *ABCG2/BCRP*, or to otherwise interact with glyburide will be excluded from the study (see list above). All other usual disease or condition medications and treatments are allowed.

Prior to recruitment, participant medical records will be pre-screened to determine eligibility.

The following information will be collected on case report forms (Appendix C):

1. Age (years)
2. Race/Ethnicity of both the mother and the infant's father
3. Height (inches)
4. Weight (1. pre-pregnancy and 2. at delivery or last clinic visit prior to delivery)
5. Current glyburide dosage (mg/dose, dosing schedule)
6. Time of 1. Last GLY dose, 2. Delivery, 3. Maternal sample collection, and 4.

Umbilical cord sample collection. Every effort will be made to collect the maternal sample within 30 minutes of delivery.

7. Gestational age at delivery (weeks)
8. Concomitant medications in the week prior to delivery
9. Most recent pre-delivery HbA1c (maternal) and date
10. Most recent pre-delivery glucose level (maternal) and date

11. Mode of delivery
12. Infant weight at delivery (grams)
13. Infant APGAR score at delivery
14. Shoulder dystocia (y/n)
15. Neonatal hypoglycemia (glucose < 40 mg/dL) (y/n)

Samples of maternal venous and umbilical cord venous blood will be collected within 30 minutes after delivery for measurement of GLY (and metabolites) and for genotyping. Umbilical cord venous blood will additionally be analyzed for two markers of fetal metabolism: insulin, and C-peptide.

Participants for whom any circumstance of labor or delivery precludes the collection of either a maternal or umbilical cord blood sample will be excluded from any further sample collection.

All samples will be shipped to the University of Washington, which will be the coordinating center for all sample and data analysis. Samples will be shipped in accordance with the relevant guidelines for human biospecimen storage and tracking.

Sample shipping address:

Thummel Lab

C/O: Tina Calamia

4225 Roosevelt Way NE, Suite 100, Room 2310

Seattle, WA 98105

STUDY PROCEDURES

Consent and Screening Visit:

1. Obtain written informed consent and HIPAA authorization
2. Confirm subject eligibility

Delivery:

1. Within 30 minutes of delivery, collect:
 - 7 mLs of maternal venous blood in a LAVENDER TOP tube for genotyping and process per GLY and genotyping sample handling instructions (Appendix A).
 - 7 mLs of umbilical cord venous blood in LAVENDER TOP tube for genotyping, and process per GLY and genotyping sample handling instructions. (Appendix A)
 - 5 mLs of umbilical cord venous blood in RED TOP tube for insulin and C-peptide, and process per insulin and C-peptide sample handling instructions. (Appendix B)
2. Complete participant case report form (Appendix C).

GENOTYPING

Both maternal and umbilical cord venous blood samples will be genotyped for function-disrupting variants in genes that encode proteins directly or indirectly involved in the

disposition of and response to GLY. This includes the 421C>A SNP (rs2231142) in the *ABCG2* gene, that results in a glutamine to lysine (Q141K) amino acid change and which is strongly associated with reduced transporter function in other tissues.

Genotyping will be done at the Functional Genomics Core Laboratory at the University of Washington Center for Ecogenetics and Environmental Health.

RISK/BENEFIT ASSESSMENT

The overall maternal, fetal and neonatal risks of this study are similar to the risks expected with standard of care treatment for pregnant women with GDM. The risks of this study beyond standard of care are minimal.

Risks of blood drawing include discomfort or pain with the needle stick, bruising and rarely fainting or infection. Aseptic technique will be used with all blood drawing to limit the risk of infection. The total amount of blood to be drawn from the women with GDM is 7 mLs on a single draw.

The risks of samples collected from the umbilical cord at the time of delivery are minimal for both mother and neonate because the samples are collected after delivery of the placenta. Blood samples will not be collected if they will in any way hamper the clinical care of the mother or baby.

DNA will be used for experiments related to the genes directly or indirectly involved in GLY handling and response. This is not a study to find genes that cause GDM, but rather a study of women that have GDM and an evaluation of the genes that are involved in how the body handles and responds to GLY. The analysis will be limited to a small number of markers that are insufficient, on their own, to identify participants or make any statements regarding health or family relationships. The samples will be coded. The laboratory personnel that will conduct the genotype assays will not have access to the individual identifying information. The key to the code will be stored in a separate location and kept in locked files until clinical study completion and destruction of the link. The link between genetic information and study information will be maintained, but the link to identifying information will be destroyed after completion of the study and publication of results or no later than 1/1/20. After destruction of the identifying link, DNA will be stored anonymously for up to 20 years.

Participation in any research study has the potential for loss of confidentiality. While we cannot guarantee absolute confidentiality, research records will be handled as confidentially as possible within the law. Study data will be kept in locked files and will be safeguarded from illegitimate access by established procedures. Participants will be assigned code numbers and the key to the code will be destroyed following publication of study results, but no later than 1/1/20. Participants' identifiable information will be kept as paper records only, stored in a locked filing cabinet, and will not be revealed to family members, insurance companies, employers, other individuals or organizations.

Participants will receive no direct benefits from this study.

[Note: Glyburide is a FDA Pregnancy Category C drug (i.e. Animal reproduction studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans, but potential benefits may warrant use of the drug in pregnant women despite potential risks.) However, as this is an observational study, decisions related to the use of GLY will have been made prior to and independent of study enrollment and cannot be considered risks of this study.]

DISCONTINUATION

All subjects are free to withdraw from participation at any time, for any reason, specified or unspecified, and without prejudice. Reasonable attempts will be made by the investigator to obtain a reason for subject withdrawals. The reason for the subject's withdrawal from the study will be specified in the subject's source documents.

STATISTICAL METHODS AND CONSIDERATIONS

This study aims to determine whether there is an association between fetal *ABCG2* genotype and the at-birth fetal to maternal concentration ratio of GLY in women taking GLY for treatment of GDM.

Over 80 single nucleotide polymorphisms (SNPs) have been identified in *ABCG2*, including several common variants. For this study, both maternal and umbilical cord venous blood samples will be genotyped for function-disrupting variants in genes that encode proteins directly or indirectly involved in the disposition of and response to GLY.

This includes the 421C>A SNP (rs2231142) in the *ABCG2* gene, that results in a glutamine to lysine (Q141K) amino acid change and which is strongly associated with reduced transporter function in other tissues. The primary analysis will be restricted to the 421C>A SNP.

BCRP is a half-transporter, having only one transmembrane domain and one ATP-binding region, and is believed to require homodimerization to transport substrates¹². As a result, individuals who are heterozygous for a non-functional allele (i.e. have one copy) may exhibit reduced function, in addition to individuals who are homozygous for the variant allele. This is supported by several *in vivo* studies in humans. A study that evaluated the effect of the 421C>A SNP on BCRP levels in placental tissue found a 50% reduction in the mean protein level in individuals who were homozygous for the A421 allele as compared to C421, with heterozygous individuals displaying an intermediate value (n=99, p<.05)¹⁰. A study looking at the impact of the 421C>A SNP on plasma levels of diflomotecan, another drug that is transported by BCRP, found nearly three times the amount of drug in the plasma of individuals who were heterozygous for the A421 allele as compared to C421 homozygous individuals (n=20, p=.015)¹³.

The frequency of the 421C>A SNP varies among different ethnic groups. The highest frequencies are found in individuals of Chinese and Japanese ancestry, where approximately half of all individuals have at least one variant allele. Approximately one quarter of individuals of Caucasian, Pacific-Island, Hispanic, and Middle Eastern

ancestry have at least one variant allele. Lower frequencies are found in individuals of African American and Ashkenazi Jewish ancestry^{8,10}.

There are limited data available regarding the expected mean GLY concentration ratio and variation. A recent study that evaluated the at-birth fetal to maternal concentration ratio of GLY in 40 women taking GLY for treatment of GDM found a mean ratio of 0.7 ± 0.4 , after excluding one outlier.

This information regarding expected mean concentration ratio value and variation, allele frequency, and effect size forms the basis of the power analysis and sample size determination (see separate document for additional detail on this analysis). Assuming a minor allele frequency of .13, a sample of 100 participants completing the study is expected to yield twice the number of A421 carriers necessary reach 80% power to detect a difference in mean concentration ratio of .375 or larger, which allows for uncertainty in the expected effect size.

Up to 200 women will be enrolled in order to complete study procedures for 100 participants.

DATA ANALYSIS

We will compare the mean fetal to maternal GLY concentration ratio for participants with wild type fetal *ABCG2* to that of participants with at least one probable function-disrupting fetal *ABCG2* variant allele. The primary analysis will be limited to the *ABCG2*

Q141K variant. Secondary analysis will include other variation in *ABCG2* and regulatory regions, as well as alternative dependent variables (e.g. C-peptide, insulin, and fetal outcomes).

The primary analysis will be repeated using the maternal *ABCG2* genotypes, which will function as a control.

The analysis will be limited to data from participants with term pregnancies (≥ 37 weeks and ≤ 42 weeks).

Genotypes will be evaluated for concordance with Hardy-Weinberg Equilibrium, and participant race/ethnicity data will be used to assess and correct for any population stratification.

Demographic and maternal data such as duration of time between the last GLY dose and sample collection, age, height, weight, race/ethnicity and GLY dose will be used for exploratory multivariate analyses and data interpretation.

Glyburide and metabolite levels will be measured in the Clinical Pharmacokinetic Laboratory at the University of Washington (4225 Roosevelt Way NE, Suite 305, Seattle, Washington, 98105). Genotyping will be done at the Functional Genomics Core Laboratory at the University of Washington Center for Ecogenetics and Environmental

Health. Fetal insulin and C-peptide will be measured in the Department of Laboratory Medicine in the University of Washington School of Medicine.

DATA COLLECTION AND RETENTION

Data will be collected on standardized Case Report Forms. Study data will be reviewed for quality and accuracy on a regular basis by the principal investigator.

A file for each participant will be maintained at the University of Washington that includes the signed Informed Consent, HIPAA Authorization and copies of all source documentation related to that participant, and will be available on request to authorized representatives of the Sponsor (or designee), IRB/IEC, and Regulatory Agency (e.g., FDA) inspectors.

All study documents will be kept secured for 5 years after the completion of the study, at which time the link connecting study data with personally identifiable participant data will be destroyed, and study samples and reports will have their original study code removed and replaced with an unrelated reference number.

ADMINISTRATIVE, ETHICAL, AND REGULATORY CONSIDERATIONS

The study will be conducted according to the Declaration of Helsinki, Protection of Human Volunteers (21 CFR 50), Institutional Review Boards (21 CFR 56), and Obligations of Clinical Investigators (21 CFR 312).

To maintain confidentiality, all laboratory specimens, evaluation forms, reports and other records will be identified by a study site and subject coded number only. All study records will be kept in a locked file cabinet and code sheets linking a patient's name to a patient identification number will be stored separately in another locked file cabinet.

Clinical information will not be released, except as necessary for monitoring by the FDA and IRB. The Investigator will also comply with all applicable privacy regulations (e.g., Health Insurance Portability and Accountability Act of 1996, EU Data Protection Directive 95/46/EC).

The protocol and consent form will be reviewed and approved by the IRB/IEC of each participating center prior to study initiation. A protocol amendment intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRBs are notified within five working days. All other amendments must have IRB approval prior to implementation.

Any documents that the IRB/IEC may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB/IEC. The IRB/IECs written unconditional approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated. This approval must refer to the study by exact protocol title and should identify the documents reviewed and the date of review.

Protocol and/or informed consent modifications or changes will not be initiated without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the patients or when the change involves only logistical or administrative aspects of the study. Such modifications will be submitted to the IRB/IEC and written verification that the modification was submitted and subsequently approved will be obtained.

The IRB/IEC will be informed of revisions to other documents originally submitted for review; serious and/or unexpected adverse experiences occurring during the study in accordance with the standard operating procedures and policies of the IRB; new information that may affect adversely the safety of the patients in the study; an annual update and/or request for re-approval; and when the study has been completed.

Informed consent will be obtained in accordance with the Declaration of Helsinki, ICH GCP, US Code of Federal Regulations for Protection of Human Subjects (21 CFR 50.25[a,b], CFR 50.27, and CFR Part 56, Subpart A), the Health Insurance Portability and Accountability Act (HIPAA, if applicable), and local regulations.

The Investigator will prepare the informed consent form, assent and HIPAA authorization and provide the documents to the Sponsor or designee for approval prior to submission to the IRB/IEC. The consent form generated by the Investigator must be acceptable to the Sponsor and be approved by the IRB/IEC. The written consent document will embody the elements of informed consent as described in the International Conference on Harmonization and will also comply with local regulations.

A properly executed, written, informed consent will be obtained from each subject prior to entering the subject into the study. Information will be given in both oral and written form and subjects will be given ample opportunity to inquire about details of the study. A copy of the signed consent form will be given to the subject and the original will be maintained with the subject's research records.

RECRUITMENT AND SCREENING

The research sites will vary in terms of institutional requirements and challenges related to participant recruitment. The general approach will be to have participants sign the consent any time after receiving a diagnosis of GDM and initiating GLY therapy, but prior to delivery.

Recruitment will commence after receiving written IRB/IEC approval.

SUBJECT COMPENSATION

Subjects will be compensated for their time while participating in this study with a \$25 gift card to Target (or local equivalent for Columbia and UTMB). Only participants who successfully provide samples for analysis will receive the honorarium.

PUBLICATIONS

The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

All investigators will be offered co-authorship for publications resulting from the study. Study sponsors (the Northwest-Alaska Pharmacogenomics Research Network, and the Center for Genomics and Healthcare Equality at the University of Washington and appropriate local support for each site) will be identified for all publications resulting from the study.

STUDY TIME TABLE

Year 1: Subject recruitment and conduct

Year 2: Sample and data analysis

TRAINING AND CERTIFICATION

All clinical research staff will complete human subjects ethics training and HIPAA training. Where appropriate, clinical research staff will complete good clinical practice training.

APPENDIX A. GLY AND GENOTYPING SAMPLE HANDLING

INSTRUCTIONS

- Collect samples within 30 minutes after delivery.
- For each sample, collect 7 mLs blood into LAVENDER TOP tube (anticoagulant EDTA).
- Mix blood well to avoid clotting. Invert at least 5 times gently and be careful not to hemolyze the samples.
- Place samples on ice until processed.
- Centrifuge samples for 10 minutes at ~2000 g to fractionate.
- Use a transfer pipet to aspirate off the plasma being careful not to disturb the white blood cells.
- Split the plasma from each sample into 2 cryo vials.
- Store plasma samples in -80° C freezer until shipping (vials must be capable of storage at -80° C).
- After removing plasma, use a clean transfer pipette to remove the buffy coat and 1-2 mm. of the red cell layer from the remaining contents of the lavender top tube.
- Transfer the buffy coat to a 0.5 mL cryo vial.
- Store buffy coat samples in -80° C freezer until shipping (vials must be capable of storage at -80° C).

APPENDIX B. INSULIN AND C-PEPTIDE SAMPLE HANDLING INSTRUCTIONS

It is critical that insulin and C-peptide samples are processed per protocol.

- For each sample, collect 5 mLs blood into a red top tube.
- Samples must be processed within 2 hours of collection
- Let tube sit upright at room temperature for 30 minutes. Then, immediately centrifuge for 10 minutes at ~2000 g. As soon as centrifuge stops, immediately separate serum and aliquot into 2 cryovials, with at least 1 mL of serum in each vial.
- Freeze samples immediately at -80° C until shipping (vials must be capable of storage at -80° C).

BCRP and Fetal Glyburide Exposure

SUBJECT NUMBER:

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DATE:

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APPENDIX C: CASE REPORT FORM

CASE REPORT FORM (coordinator to complete) MATERNAL INFORMATION

| | | | | | | |
|----------------------|--|----------------|----------------------------------|-------------|--|---|
| Age | <input style="width: 95%;" type="text"/> | | <i>years</i> | | | |
| Race/Ethnicity | <input style="width: 95%;" type="text"/> | | | | | |
| Hispanic | YES | NO | | | | |
| Height | <input style="width: 95%;" type="text"/> | | <i>cm</i> | | | |
| Pre-pregnancy Weight | <input style="width: 95%;" type="text"/> | | <i>kg</i> | | | |
| Current Weight | <input style="width: 95%;" type="text"/> | | <i>kg, most recent available</i> | | | |
| HbA1c | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 20%;"><i>result</i></td><td style="width: 20%;"><i>units</i></td><td style="width: 20%;"><i>date</i></td></tr></table> | <i>result</i> | <i>units</i> | <i>date</i> | | <i>most recent pre-delivery measurement, if available</i> |
| <i>result</i> | <i>units</i> | <i>date</i> | | | | |
| Glucose | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 20%;"><i>result</i></td><td style="width: 20%;"><i>units</i></td><td style="width: 20%;"><i>date</i></td></tr></table> | <i>result</i> | <i>units</i> | <i>date</i> | | <i>most recent pre-delivery measurement, if available</i> |
| <i>result</i> | <i>units</i> | <i>date</i> | | | | |
| Current GLY Dose | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 50%;"><i>mg/dose</i></td><td style="width: 50%;"><i>frequency</i></td></tr></table> | <i>mg/dose</i> | <i>frequency</i> | | | |
| <i>mg/dose</i> | <i>frequency</i> | | | | | |

DELIVERY AND SAMPLE COLLECTION INFORMATION

| | | | | | |
|--|---|-------------|-------------|--|--|
| Mode of Delivery | <input style="width: 95%;" type="text"/> | | | | |
| Time of Last GLY Dose | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 50%;"><i>date</i></td><td style="width: 50%;"><i>time</i></td></tr></table> | <i>date</i> | <i>time</i> | | |
| <i>date</i> | <i>time</i> | | | | |
| Time of Maternal Blood Sample Collection | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 50%;"><i>date</i></td><td style="width: 50%;"><i>time</i></td></tr></table> | <i>date</i> | <i>time</i> | | <i>use military format for all times</i> |
| <i>date</i> | <i>time</i> | | | | |
| Time of Umbilical Cord Sample Collection | <table border="1" style="display: inline-table; border-collapse: collapse;"><tr><td style="width: 50%;"><i>date</i></td><td style="width: 50%;"><i>time</i></td></tr></table> | <i>date</i> | <i>time</i> | | |
| <i>date</i> | <i>time</i> | | | | |

Were samples collected within 30 minutes of delivery? YES NO: _____

BCRP and Fetal Glyburide Exposure

SUBJECT NUMBER:

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DATE:

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CASE REPORT FORM (L&D to complete)

PATERNAL INFORMATION

Race/Ethnicity

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*If paternal race/ethnicity or
Hispanic status is
uncertain/unknown, please
indicate this*

Hispanic

YES

NO

INFANT INFORMATION

Gestational Age at
Delivery

| |
|--|
| |
|--|

weeks

Infant Weight at
Delivery

| |
|--|
| |
|--|

grams

APGAR Score at
Delivery

| |
|--|
| |
|--|

Neonatal Hypoglycemia
glucose < 40mg/dL

YES

NO

Shoulder Dystocia

YES

NO

IMPORTANT:

Every effort must be made to collect the maternal and umbilical cord blood samples within 30 minutes of delivery. If not, indicate duration of time between delivery and sample collection for both maternal and umbilical cord blood samples.

BCRP and Fetal Glyburide Exposure

SUBJECT NUMBER:

DATE:

CONCOMITANT MEDICATION (participant to complete)

List all **prescription medications** you have taken in the last **week** (7 days).

| MEDICATION NAME | DOSE | ROUTE (How do you take it? E.g. oral, inhaled, subcutaneous) | FREQUENCY (How many times per day?) |
|-----------------|------|---|--|
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List all **over-the-counter medications, herbal supplements and vitamins** you have taken in the last **week** (7 days).

| PRODUCT NAME | DOSE | ROUTE (How do you take it? E.g. oral, inhaled, subcutaneous) | FREQUENCY (How many times per day?) |
|--------------|------|---|--|
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APPENDIX D: INITIAL PARTICIPANT CONTACT SCRIPT

Hello my name is _____. Your obstetrician, Dr. _____ let me know that you are willing to hear about a study that you may be eligible for. Would now be a good time to talk with you about the study?

(If it is a good time, continue with consent process; if not a good time, ascertain when would be a good time and collect contact information if appropriate (e.g. if preferred correspondence is by phone); if not interested in hearing about the study, thank her for her time and leave)

APPENDIX E: PARTICIPANT FOLLOW UP SCRIPT

Hello my name is _____ and I am a researcher from the study on glyburide and genetics that you are participating in. I am calling to follow up on how [infant name: _____] is doing. Would now be a good time to talk to you?

[if NOT a good time, ascertain when would be a good time and call back then]

Is he/she home with you? Has his/her [AE: _____] resolved?

[if infant at home and AE has resolved, thank her for her time]

Where is [infant name: _____] currently receiving treatment for [AE: _____]? May we have your permission for our research team to access his/her medical record at this hospital/clinic so that we can keep track of how his/her [AE: _____] resolves?

[if YES, contact treating physician at new location to initiate authorization process; if NO, thank her for her time]

APPENDIX F: L&D SAMPLE INSTRUCTIONS

ATTN L&D Nursing Staff: BCRP and Fetal Glyburide Exposure Study Subject

1. Please collect the following samples within 30 minutes of delivery:
 - 7 mL of **maternal venous blood** into pre-labeled 7 mL LAVENDER vacutainer.
 - 7 mL of **venous umbilical cord blood** into pre-labeled 7 mL LAVENDER vacutainer.
 - 5 mL of **venous umbilical cord blood** into pre-labeled 5 mL RED vacutainer.
2. Please complete the highlighted fields on the case report form
3. Place collected samples and completed forms in “soiled utility” room refrigerator and call PI for pickup at 650-387-8372 (cell)

ADDITIONAL INFORMATION:

- Original order included with sample collection tubes.
- Please notify delivery physician.
- A duplicate set of collection tubes (as a back-up) is stored in the dirty utility room on L&D. Patients are provided with a primary set in advance and asked to give tubes to L&D.

For study questions, please call:

PI: Elizabeth Dorfman 650-387-8372

If PI unavailable, please call Mary Hebert at 206-697-2138

APPENDIX G: L&D PROVIDER INSERVICING

Women taking Glyburide for Gestational Diabetes Needed for a Study

WHO

We are recruiting pregnant women who are taking glyburide to treat gestational diabetes mellitus (GDM) for a research study. Women taking glyburide in both mono- and combination therapy are eligible.

WHY

This research is being done to evaluate whether fetal or maternal genetic variation affects fetal glyburide exposure levels through altered transplacental glyburide passage. The results of this study will help to inform investigations of optimal glyburide usage in the treatment of GDM, particularly the possible effects of glyburide dose escalation on fetal glyburide exposure and fetal outcomes.

WHAT

Women who are eligible for this study will be asked to provide:

- Permission for researchers to collect information from her, her medical record and her infant's medical record
- 7mL venous blood drawn within 30 minutes of delivery
- 12mL umbilical cord blood drawn within 30 minutes of delivery

No visits other than those required for usual care are necessary to participate in this study. Study participants will receive a \$25 gift card to Target.

HOW

Potential participants will be identified through automated screening of medical records. A member of the research team will contact you if one of your patients may be eligible to participate in this study.

If you have any questions, please contact Elizabeth Dorfman for more information.

Elizabeth Dorfman
Principal Investigator
Doctoral Student, Public Health Genetics
University of Washington
edorfman@uw.edu
(650)387-8372

| | | |
|------------------------------|------------------------|------|
| STUDY IRB # 44794 | VISIT Labor & Delivery | DATE |
| PI Elizabeth Dorfman | CELL 650-387-8372 | |
| MD ON CALL Thomas Easterling | PAGER 206-991-4966 | |
| ALLERGIES | | |

APPENDIX H: EXAMPLE L&D ORDER FORM

**BCRP and Fetal Glyburide Exposure Study
Labor and Delivery Orders**

PI: Elizabeth Dorfman, 650-387-8372 (cell)

Please call PI immediately after sample collection:

PI: Elizabeth Dorfman, (650) 387-8372
Mary Hebert, (if PI unavailable), (206) 697-2138

UWMC L&D: Pre-labeled tubes are located in the dirty utility room on 6E
****PLEASE COLLECT SAMPLES AS INDICATED BELOW****

Subject Name: _____ U#: _____

- 1) IMMEDIATELY, within 30 min of delivery of placenta draw the following samples simultaneously:
 - 7 mL of **maternal venous blood** into pre-labeled 7 mL LAVENDER vacutainer.
 - 7 mL of **venous umbilical cord blood** into pre-labeled 7 mL LAVENDER vacutainer.
 - 5 mL of **venous umbilical cord blood** into pre-labeled 5 mL RED vacutainer.
- 2) Place all blood samples in **refrigerator** (UWMC L&D: fridge in dirty utility room on 6E) within **five** minutes.
- 3) **RECORD** all collection times and dates on the case report form.
- 4) **RECORD** the date, time, dose, and frequency of the subject's last dose of GLYBURIDE on the case report form.
- 5) **Please call the PI as soon as the samples are obtained.**

| PHYSICIAN/ARNP/PA SIGNATURE | PRINT NAME | PAGER | NPI | DATE | TIME |
|-----------------------------|------------|-------|-----|------|------|
|-----------------------------|------------|-------|-----|------|------|

PT.NO

NAME

DOB

UW Medicine Health System
Harborview Medical Center – UW Medical Center
Northwest Hospital & Medical Center – University of Washington Physicians
Seattle, Washington

GLY&BCRP L&D Order



U2722

MEDICAL RECORD
PHARMACY

UH2722 REV FEB 11

NURSING

105

REFERENCES

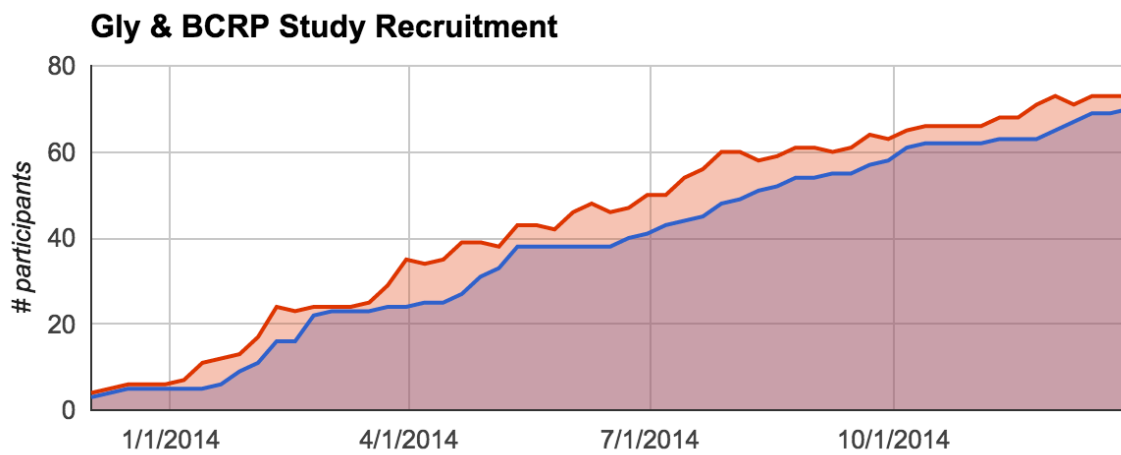
1. Getahun D, Nath C, Ananth CV, Chavez MR, Smulian JC. Gestational diabetes in the United States: temporal trends 1989 through 2004. *American journal of obstetrics and gynecology*. 2008;198(5):525 e521-525.
2. Crowther CA, Hiller JE, Moss JR, et al. Effect of treatment of gestational diabetes mellitus on pregnancy outcomes. *The New England journal of medicine*. 2005;352(24):2477-2486.
3. Langer O, Conway DL, Berkus MD, Xenakis EM, Gonzales O. A comparison of glyburide and insulin in women with gestational diabetes mellitus. *The New England journal of medicine*. 2000;343(16):1134-1138.
4. Kraemer J, Klein J, Lubetsky A, Koren G. Perfusion studies of glyburide transfer across the human placenta: implications for fetal safety. *American journal of obstetrics and gynecology*. 2006;195(1):270-274.
5. Gedeon C, Behravan J, Koren G, Piquette-Miller M. Transport of glyburide by placental ABC transporters: implications in fetal drug exposure. *Placenta*. 2006;27(11-12):1096-1102.
6. Pollex E, Lubetsky A, Koren G. The role of placental breast cancer resistance protein in the efflux of glyburide across the human placenta. *Placenta*. 2008;29(8):743-747.
7. Robey RW, To KK, Polgar O, et al. ABCG2: a perspective. *Advanced drug delivery reviews*. 2009;61(1):3-13.

8. Zamber CP, Lamba JK, Yasuda K, et al. Natural allelic variants of breast cancer resistance protein (BCRP) and their relationship to BCRP expression in human intestine. *Pharmacogenetics*. 2003;13(1):19-28.
9. Morisaki K, Robey RW, Ozvegy-Laczka C, et al. Single nucleotide polymorphisms modify the transporter activity of ABCG2. *Cancer chemotherapy and pharmacology*. 2005;56(2):161-172.
10. Kobayashi D, Ieiri I, Hirota T, et al. Functional assessment of ABCG2 (BCRP) gene polymorphisms to protein expression in human placenta. *Drug metabolism and disposition: the biological fate of chemicals*. 2005;33(1):94-101.
11. Hebert MF, Ma X, Narahariseti SB, et al. Are we optimizing gestational diabetes treatment with glyburide? The pharmacologic basis for better clinical practice. *Clinical pharmacology and therapeutics*. 2009;85(6):607-614.
12. Kage K, Tsukahara S, Sugiyama T, et al. Dominant-negative inhibition of breast cancer resistance protein as drug efflux pump through the inhibition of S-S dependent homodimerization. *International journal of cancer. Journal international du cancer*. 2002;97(5):626-630.
13. Sparreboom A, Gelderblom H, Marsh S, et al. Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. *Clinical pharmacology and therapeutics*. 2004;76(1):38-44.

3. ADDITIONAL DATA

FIGURE 1: PARTICIPANT RECRUITMENT

| | Enrolled | Completed | Withdrawn |
|-------|----------|-----------|-----------|
| UW | 14 | 10 | 4 |
| CU | 36 | 17 | 19 |
| UTMB | 50 | 44 | 6 |
| TOTAL | 100 | 71 | 29 |



Blue: completed participants

Red: completed + enrolled and still pregnant

Note: 2 incomplete (mother *or* infant samples only)

FIGURE 2: MATERNAL METRICS

| | all (n=70) | UW (n=9) | CU (n=17) | UTMB (n=44) |
|--|--------------------------|---------------------------|---------------------------|--------------------------|
| AGE (yrs) mean min - max | 32.8 [5.5] 19 - 43 | 34.6 [5.0] 28 - 43 | 32.9 [5.4] 23 - 42 | 32.4 [5.7] 19 - 43 |
| WEIGHT (kg) pre-pregnancy mean change mean | 81.3 [18.2] 8.9 [7.0] | 83.5 [21.5] 12.4 [8.3] | 74.8 [13.0] 10.3 [7.7] | 83.2 [19.0] 7.6 [6.2] |
| HEIGHT (cm) | 158.3 [7.0] | 165.4 [4.7] | 158.2 [8.0] | 157.0 [6.2] |
| HbA1c (%) | 5.5 [0.6] | 5.1 [0.3] | 5.3 [0.6] | 5.6 [0.6] |
| PoCT Glucose (mg/dl) | 89 [18] | 104 [26] | 87 [17] | 86 [14] |
| GLYBURIDE daily dose (mg) time since last dose (h) | 4.1 [2.7] 22.2 [11.0] | 4.2 [2.1] 24.9 [7.9] | 3.0 [1.8] 19.5 [12.3] | 4.5 [3.0] 22.7 [11.0] |

- HbA1c only provided if available (43 total, 7UW/7CU/29UTMB).
- Glucose value is the most recent pre-delivery value available in the medical record.

FIGURE 3: INFANT DEMOGRAPHICS

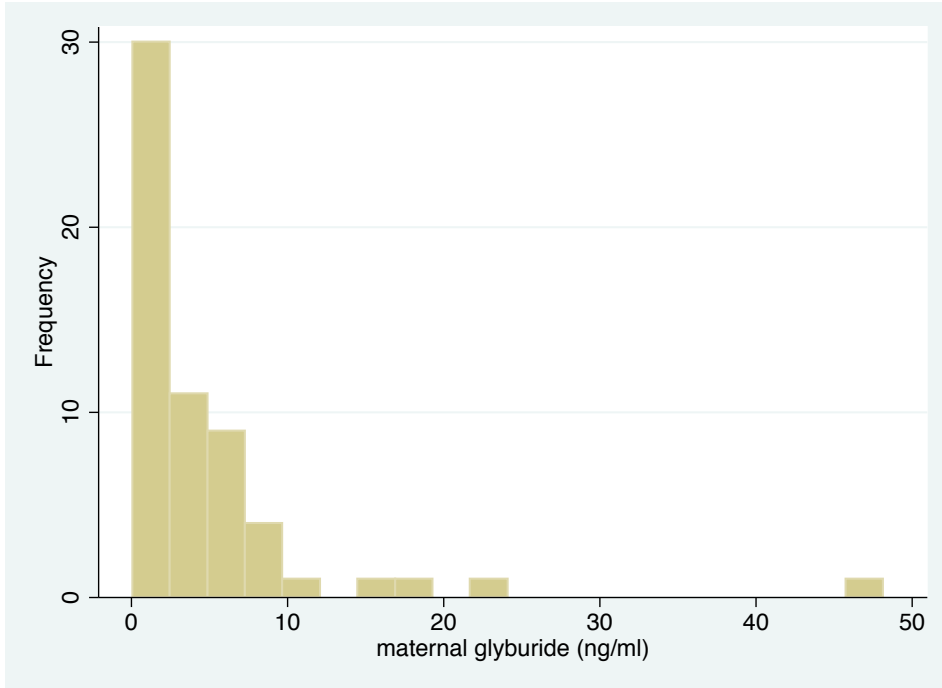
| Infant Race | Count | % | Count 2 | 2% |
|----------------------------|--------------|----------|----------------|-----------|
| White, non-Hispanic | 11 | 15.7% | 11 | 15.7% |
| White, Hispanic | 40 | 57.1% | 47 | 67.1% |
| African American | 3 | 4.3% | 3 | 4.3% |
| Asian | 1 | 1.4% | 1 | 1.4% |
| Multiple | 6 | 8.6% | 7 | 10.0% |
| Unknown | 9 | 12.9% | 1 | 1.4% |

- Maternal and paternal race were free text fields in the CRF, infant race determined by combining maternal and paternal entries.
- African American includes: African American, black, African
- Multiple: denotes non-matching maternal and paternal race field entries
- Unknown: denotes the race was not available for one (n=8) or both (n=1) parents
- Count 2/% 2 columns have categorized the 8 ‘unknown’ infants for whom race information was available for one parent to that of the known parent.

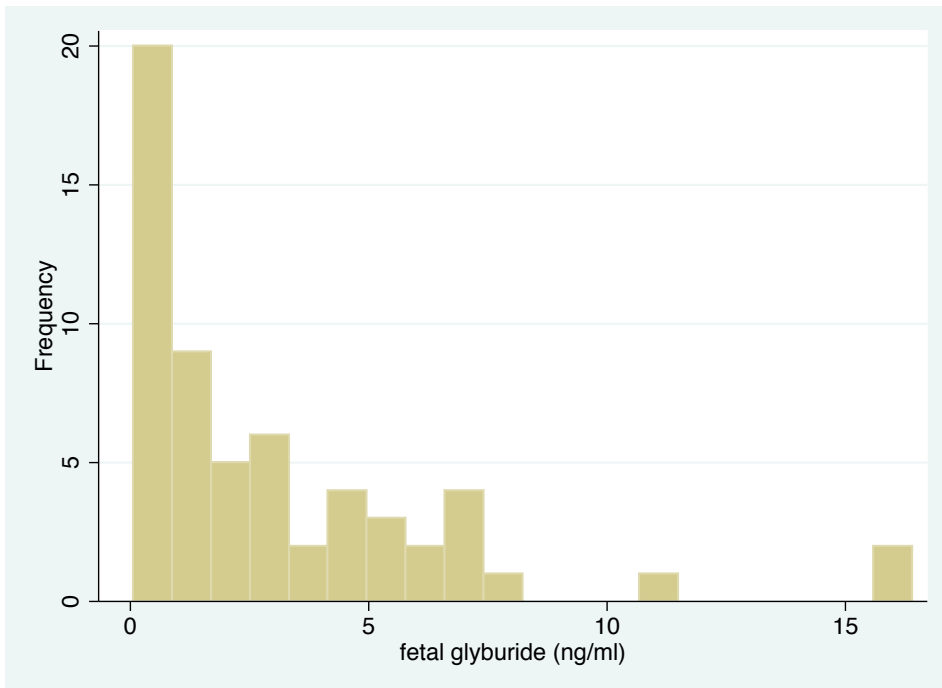
| Infant Hispanic Status | Count | % |
|-------------------------------|--------------|----------|
| Hispanic | 48 | 68.6% |
| Non-Hispanic | 18 | 25.7% |
| Partial Hispanic | 4 | 5.7% |
| Unknown | 0 | 0.0% |

- Hispanic status was a yes/no option on the CRF; infant hispanic status determined by combining maternal and paternal entries.
- Partial hispanic: denotes one parent yes one parent no.

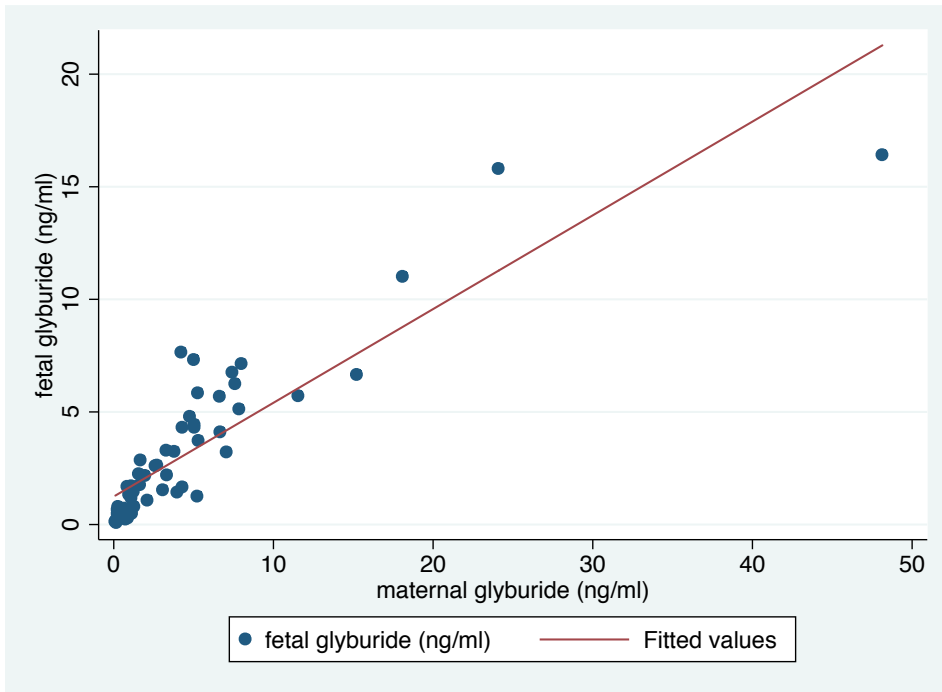
FIGURE 4: MATERNAL & FETAL GLYBURIDE



$\mu=4.5\pm 7.3\text{ng/ml}$
N=59



$\mu=3.1\pm 3.5\text{ng/ml}$
N= 59

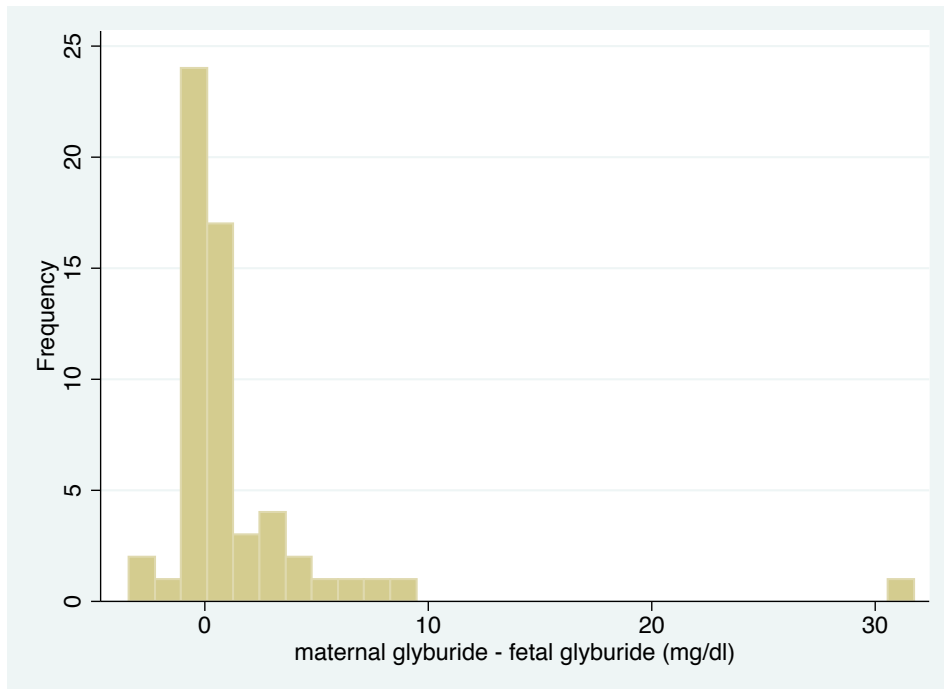


$R^2=0.778$

$N=58$

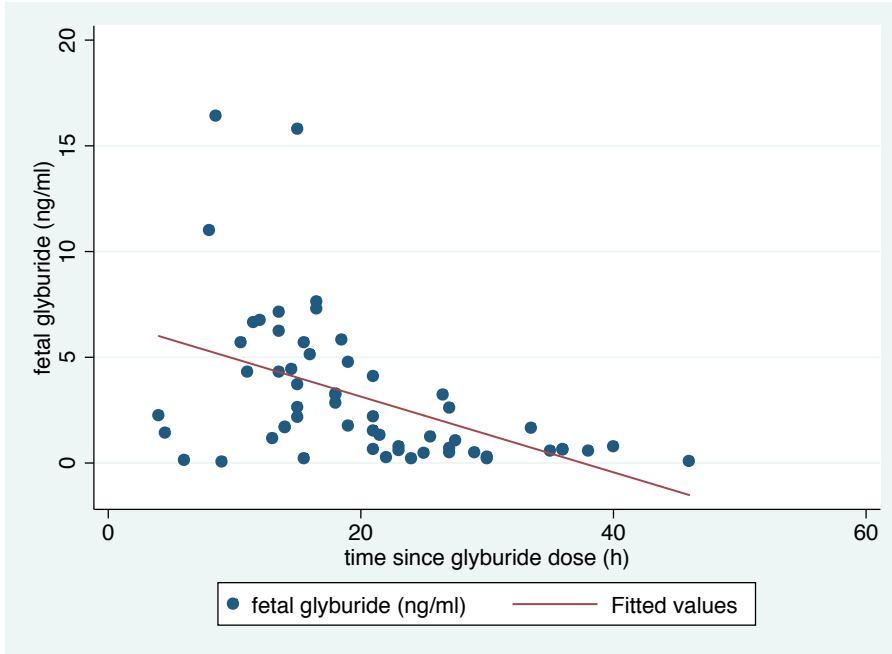
$p<0.001$

FIGURE 5: GLYBURIDE DIFFERENCE



$\mu=1.4\pm 4.6$ ($\mu=0.89\pm 2.2$ excluding outlier)
N=58 (57 excluding outlier)

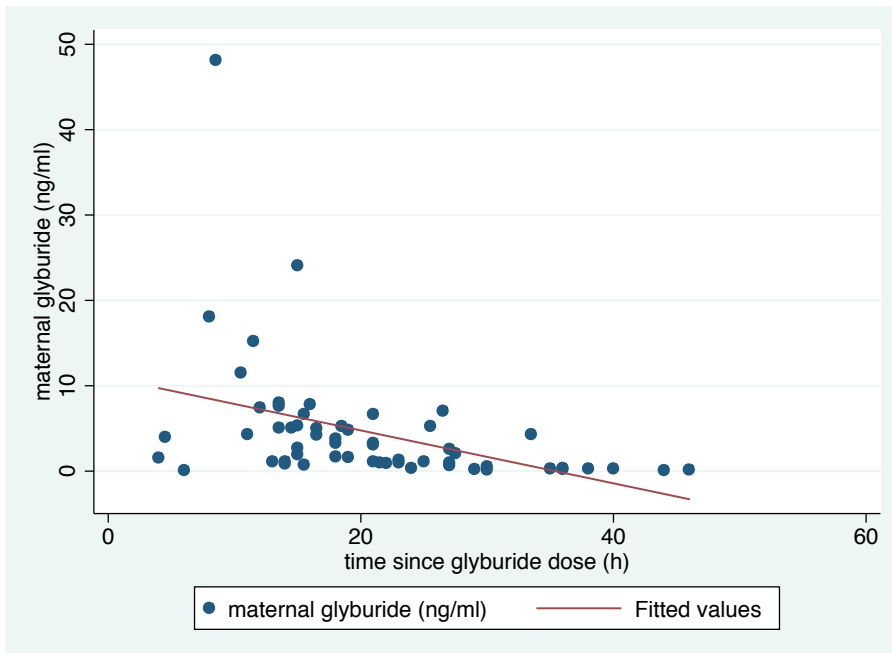
FIGURE 6: GLYBURIDE BY TIME



$R^2=0.221$

$N=58$

$p<0.001$

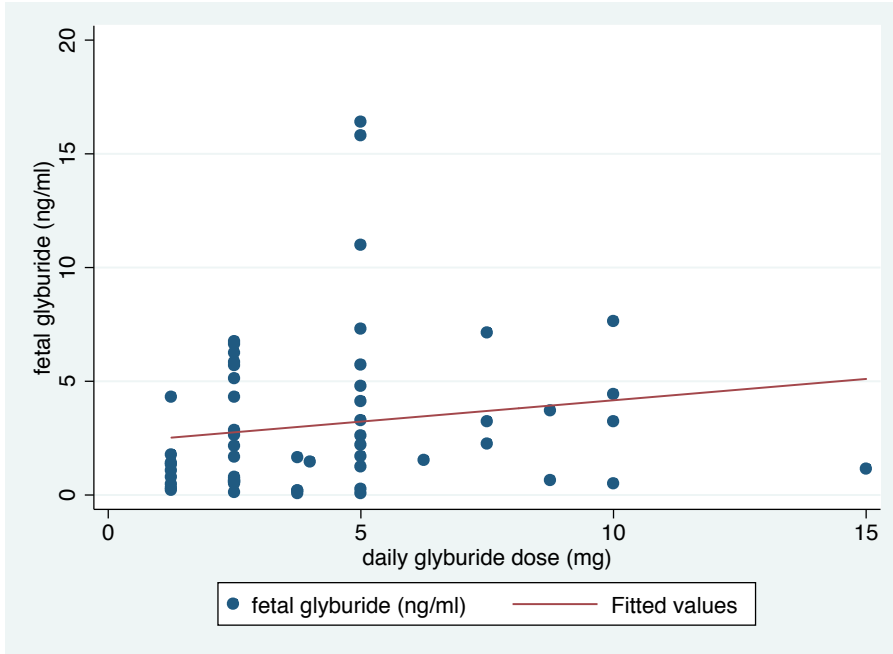


$R^2=0.162$

$N=58$

$p=0.002$

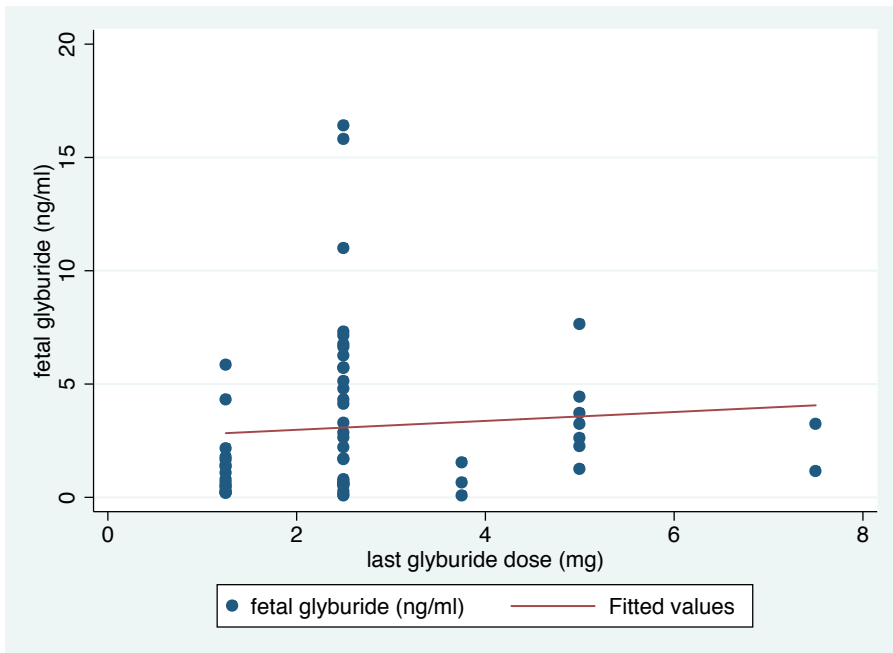
FIGURE 7: FETAL GLYBURIDE BY DOSE



$R^2=0.024$

$N=59$

$p=0.243$

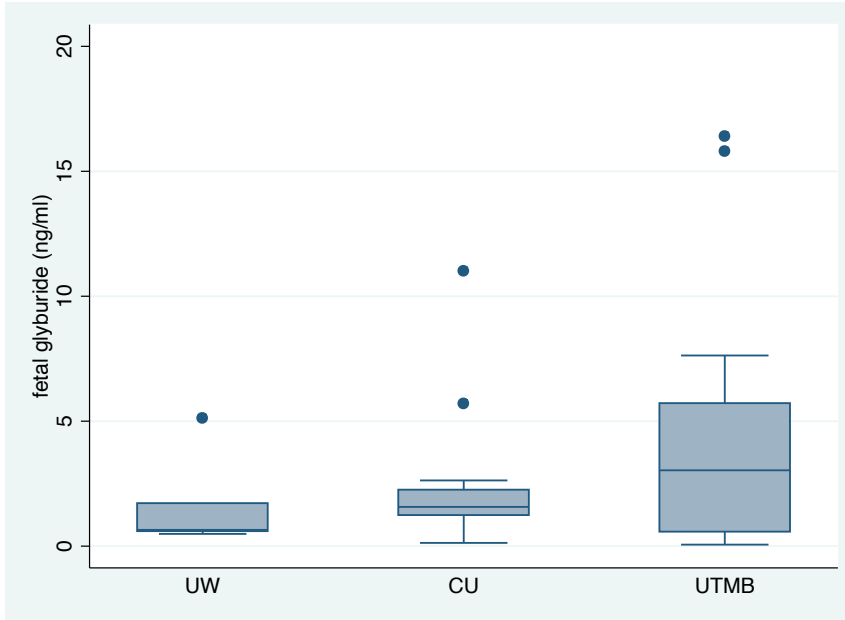


$R^2=0.007$

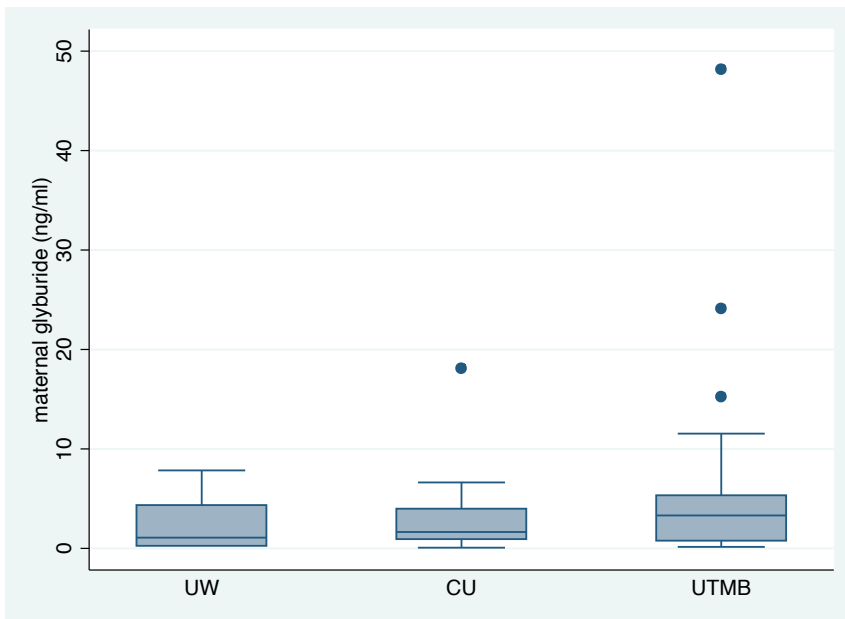
$N=58$

$p=0.541$

FIGURE 8: GLYBURIDE BY SITE

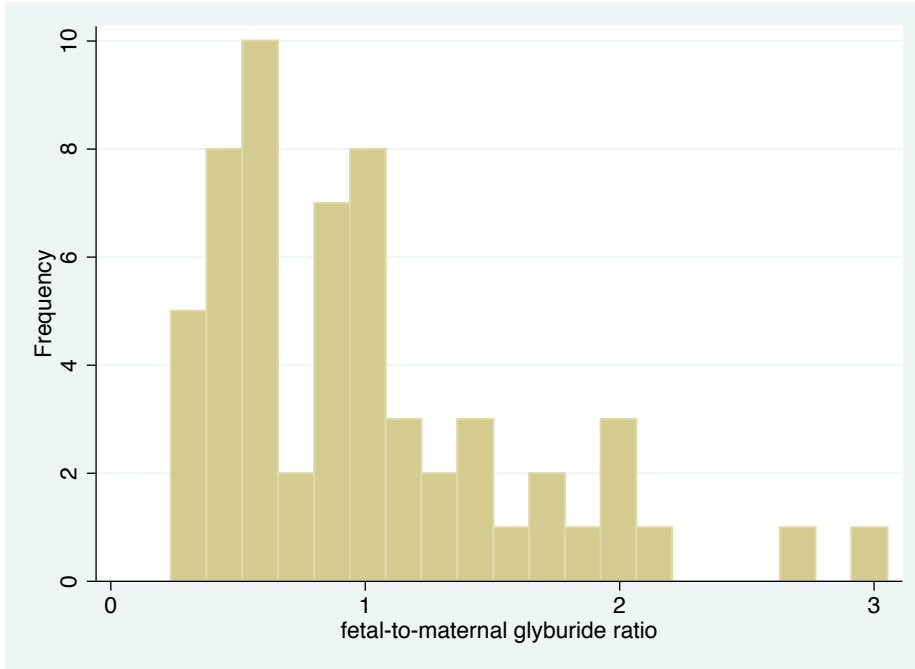


UW: $\mu=1.6\pm 1.7$ ng/ml, N=7
CU: $\mu=2.4\pm 2.8$ ng/ml, N=14
UTMB: $\mu=3.6\pm 3.9$ ng/ml, N=38
p=0.100

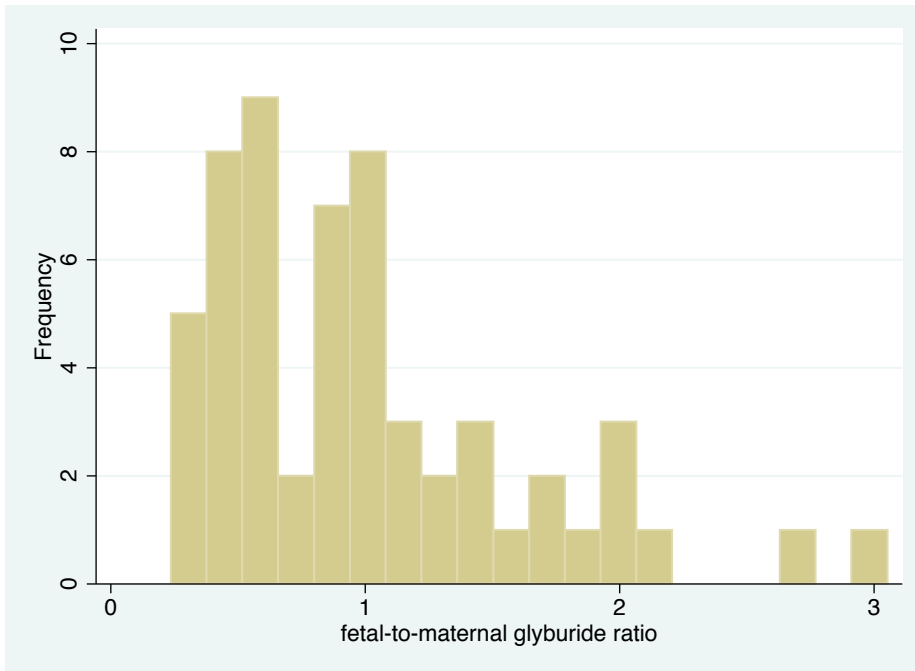


UW: $\mu=2.2\pm 2.8$ ng/ml, N=7
CU: $\mu=3.2\pm 4.5$ ng/ml, N=15
UTMB: $\mu=5.4\pm 8.7$ ng/ml, N=37
p=0.203

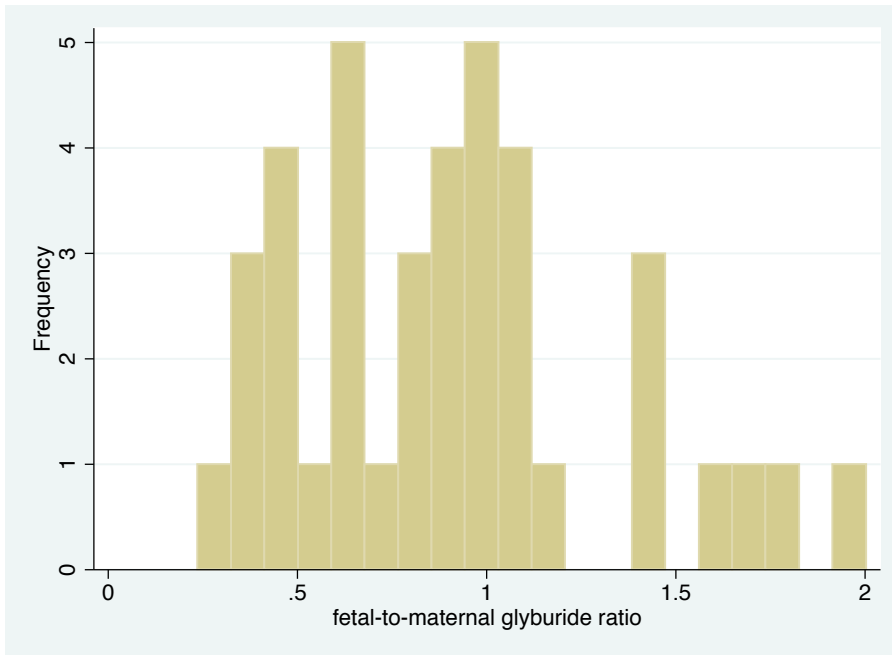
FIGURE 9: GLYBURIDE RATIO



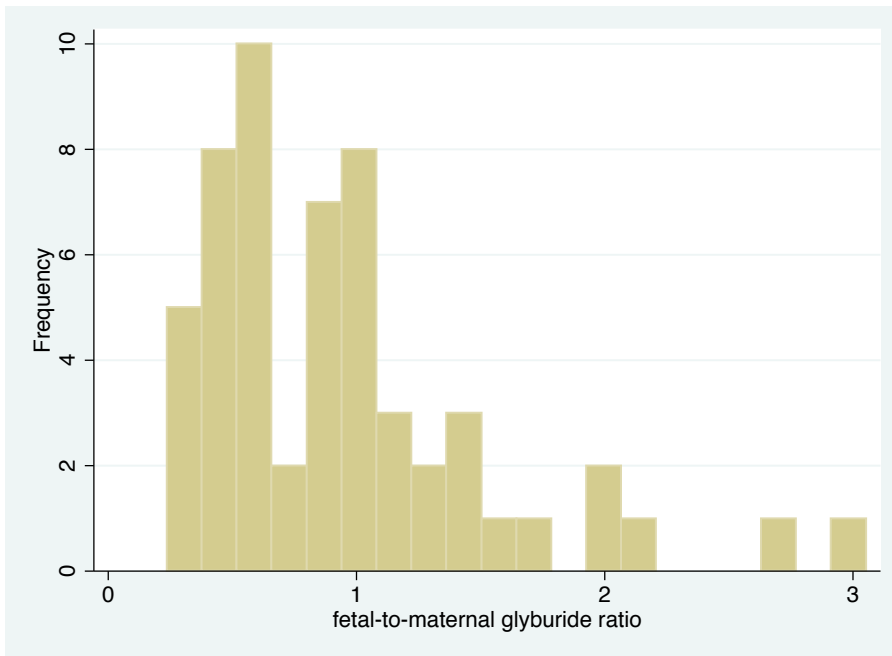
all mother-infant pairs: $\mu=1.00\pm 0.61$ N=58



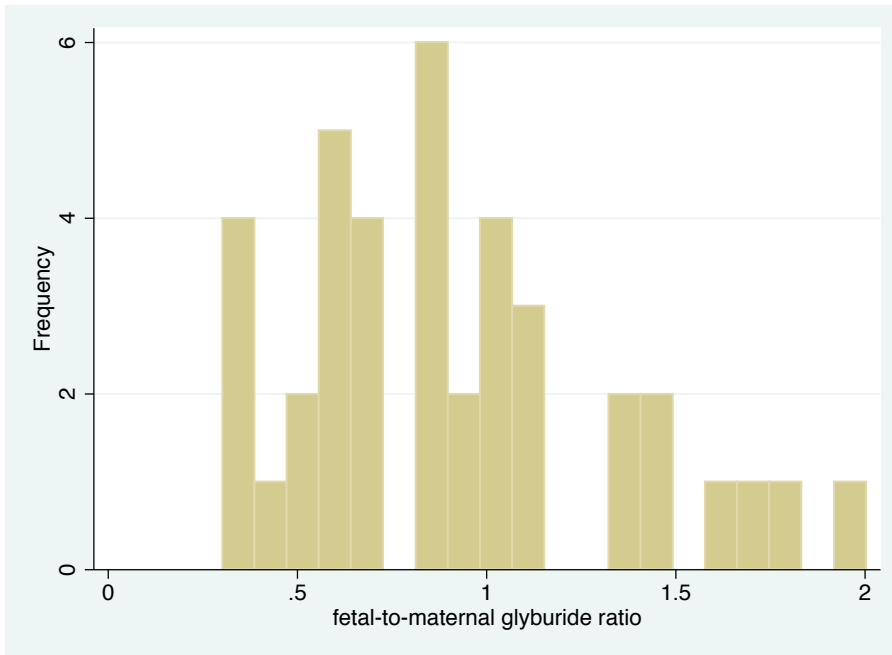
excluding LOD < fetal gly < LOQ, sampling interval < 1h
 $\mu=1.01\pm 0.61$ N=57



excluding fetal gly <1ng/ml, sampling interval <1h
 $\mu=0.91\pm0.42$ N=39

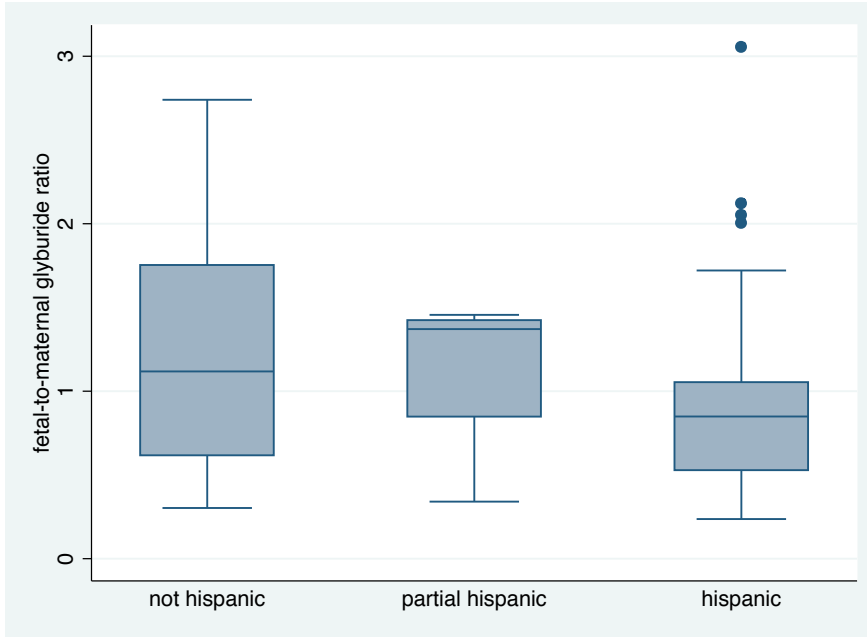


excluding sample interval >60m, sampling interval <1h
 $\mu=0.95\pm0.59$ N=55

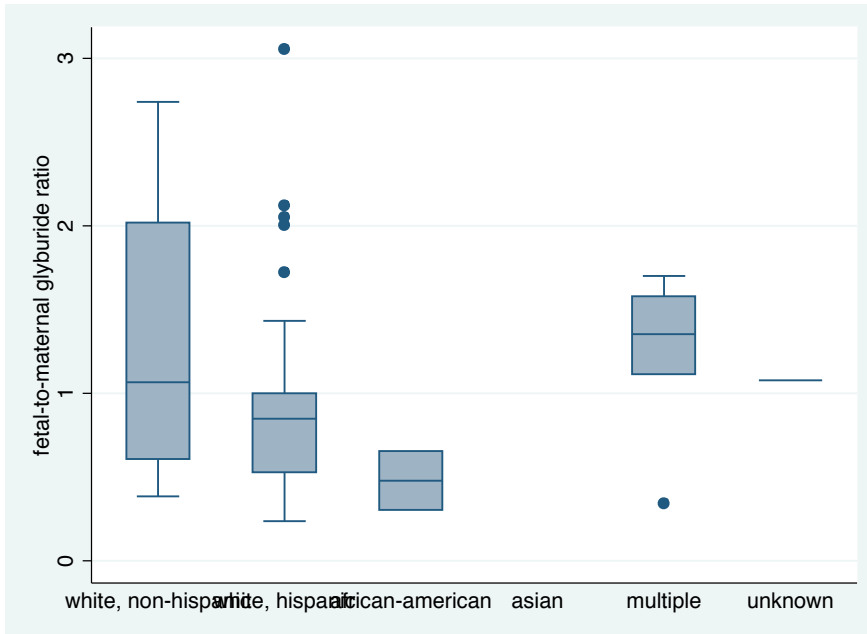


excluding time since dose >24h, sampling interval <1h
 $\mu=0.91\pm 0.43$ N=39

FIGURE 10: GLYBURIDE RATIO BY INFANT RACE & ETHNICITY

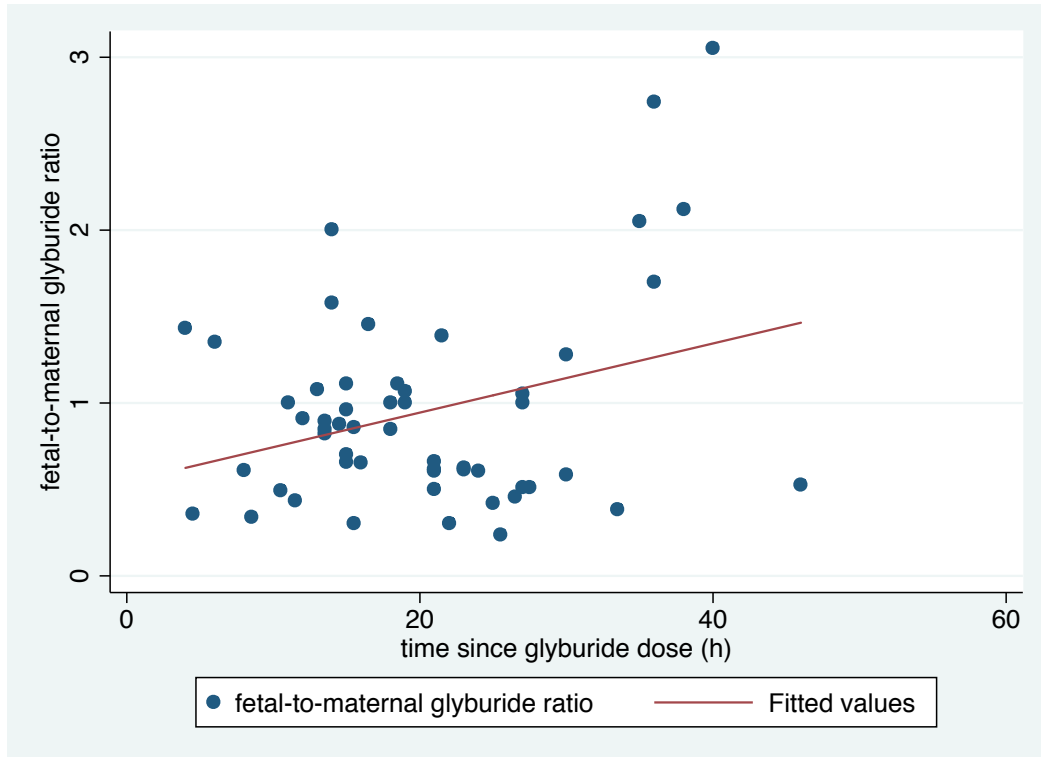


Not Hispanic: $\mu=1.22\pm0.75$ N=12
 Partial Hispanic: $\mu=1.13\pm0.53$ N=4
 Hispanic: $\mu=0.92\pm0.56$ N=42
 p=0.124



White, non-Hispanic: $\mu= 01.32\pm0.88$, N=7
 White, Hispanic: $\mu= 0.92\pm0.57$, N=41
 African American: $\mu= 0.48\pm0.25$, N=2
 Multiple: $\mu= 1.24\pm0.45$, N=7
 Unknown: $\mu= 1.08$, N=1

FIGURE 11: GLYBURIDE RATIO BY TIME



$R^2=0.098$

N=54

p=0.021

FIGURE 12: HARDY-WEINBERG EQUILIBRIUM

| | GG | Gt | tt |
|----------|-----------|-----------|-----------|
| f | 46 | 21 | 1 |
| m | 52 | 14 | 2 |
| a | 98 | 35 | 3 |

| ALL | expected | observed | p=0.9519 |
|----------------------|-----------------|-----------------|-----------------|
| p² | 0.721250541 | 0.720588235 | |
| 2pq | 0.25602833 | 0.257352941 | |
| q² | 0.022721129 | 0.022058824 | |

| FETAL | expected | observed | p=0.4148 |
|----------------------|-----------------|-----------------|-----------------|
| p² | 0.690365484 | 0.676470588 | |
| 2pq | 0.281033737 | 0.308823529 | |
| q² | 0.028600779 | 0.014705882 | |

| MATERNAL | expected | observed | p=0.393 |
|----------------------|-----------------|-----------------|----------------|
| p² | 0.752811419 | 0.764705882 | |
| 2pq | 0.22967128 | 0.205882353 | |
| q² | 0.017517301 | 0.029411765 | |

FIGURE 13: ABCG2 Q141K GENOTYPES

| | GG | Gt | tt |
|----------|-----------|-----------|-----------|
| f | 46 | 21 | 1 |
| m | 52 | 14 | 2 |
| a | 98 | 35 | 3 |

Detectable maternal and fetal gly and >1h between maternal and umbilical blood draws:

| | GG | Gt | tt |
|----------|-----------|-----------|-----------|
| f | 37 | 17 | 1 |

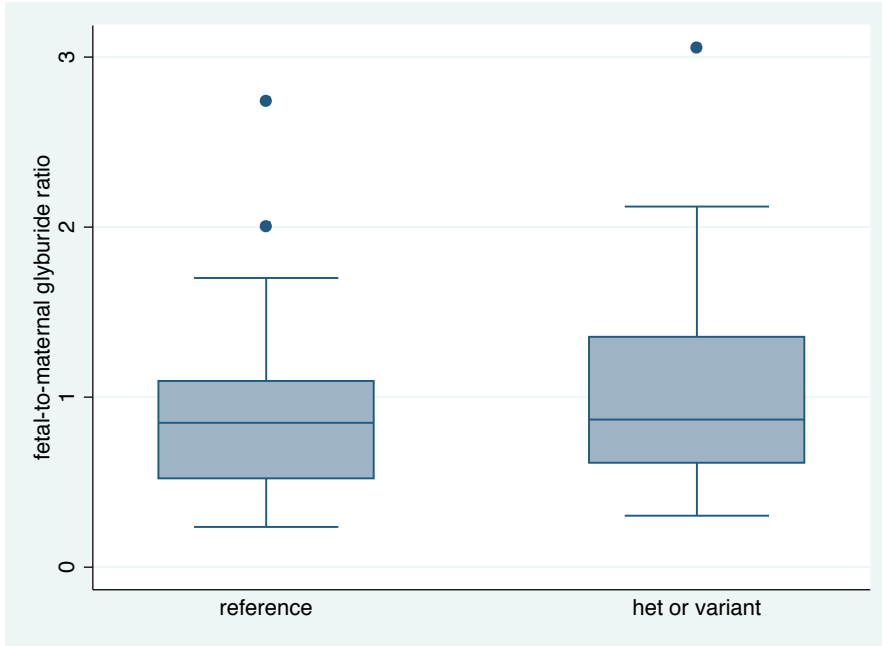
Variant Allele Frequency: 1,000 Genomes, Phase 3

| | |
|-----------------|------|
| All Populations | 0.12 |
| American-ALL | 0.14 |
| CEU | 0.12 |
| Mexican | 0.2 |
| Puerto Rican | 0.11 |

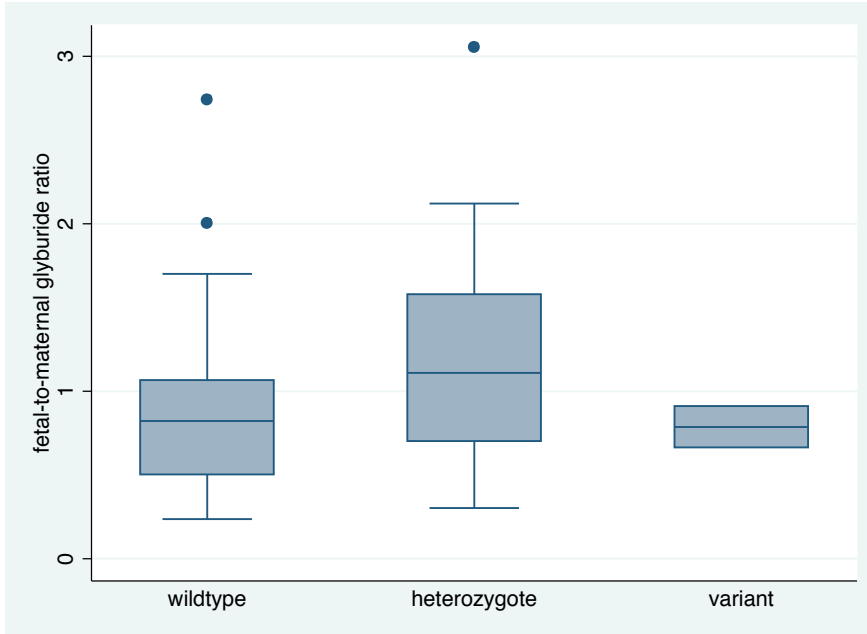
Variant Allele Frequency: Observed

| | |
|---------------------|------|
| All Participants | 0.15 |
| Infants | 0.17 |
| Mothers | 0.13 |
| White, non-Hispanic | 0.04 |
| White, Hispanic | 0.19 |

FIGURE 14: GLYBURIDE RATIO BY ABCG2 Q141K GENOTYPE

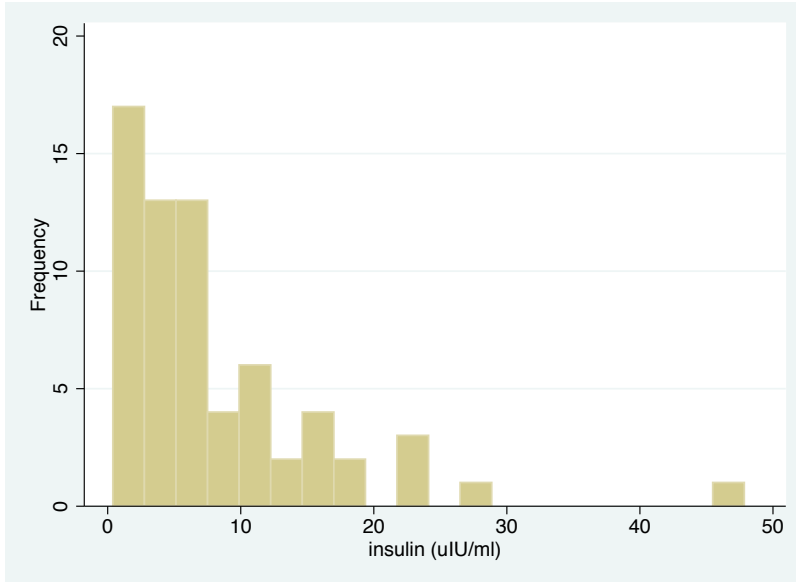


Reference: $\mu=0.91\pm0.53$, N=36
Heterozygous or Variant: $\mu=1.07\pm0.71$, N=18
p=0.345

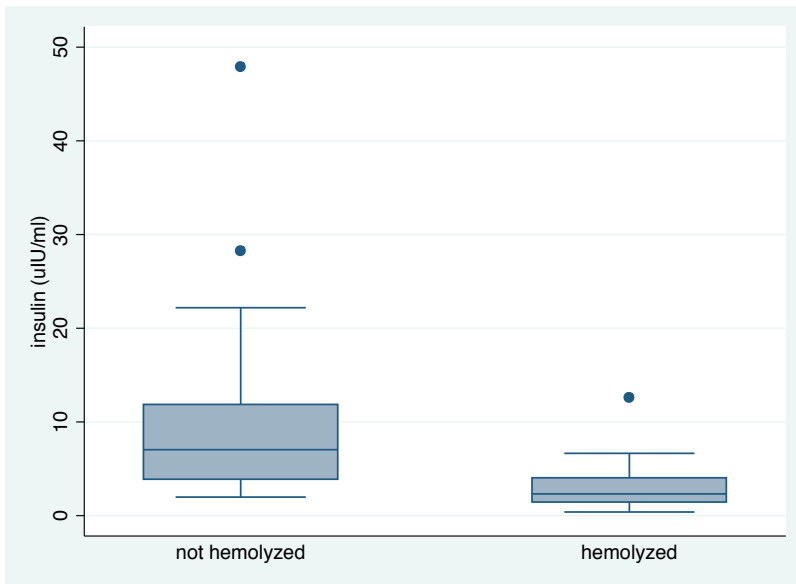


Reference: $\mu=0.85\pm0.50$, N=39
Heterozygous: $\mu=1.31\pm0.76$, N=13
Variant: $\mu=0.79\pm0.18$ N=2
p=0.103

FIGURE 15: INSULIN AND C-PEPTIDE



Note: excludes one outlier (226.75)

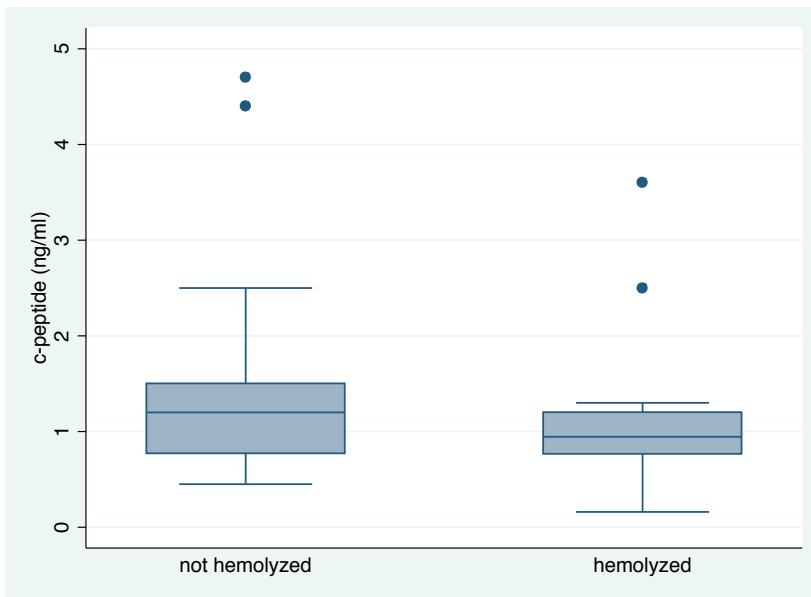
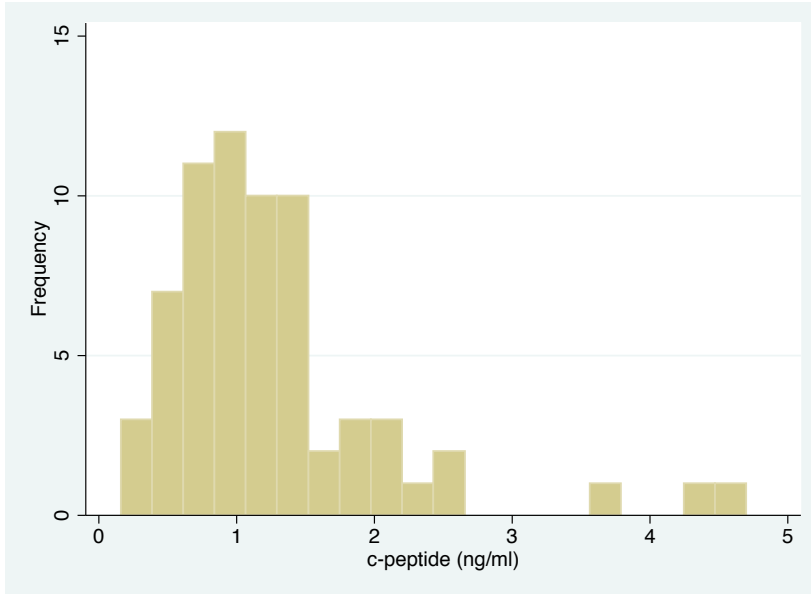


Non-hemolyzed: $\mu=9.60\pm 8.38$, N=50

Hemolyzed: $\mu=3.25\pm 3.03$, N=16

p=0.004

Note: Hemolysis determined by RTS. Hemolysis degrades insulin



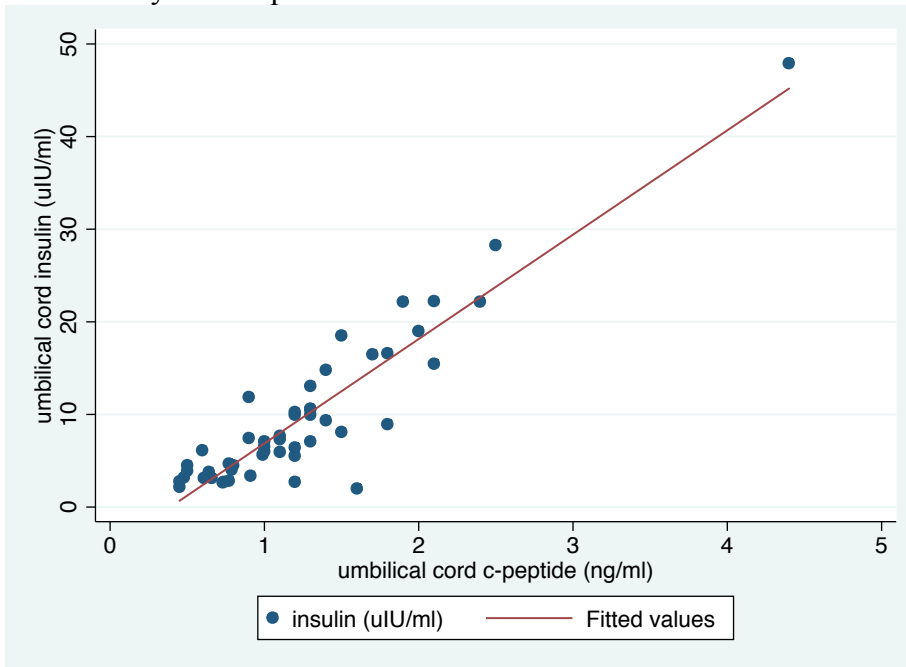
Non-hemolyzed: $\mu=1.31\pm 0.83$, N=51

Hemolyzed: $\mu=1.11\pm 0.85$, N=16

p=0.416

Hemolysis not known to affect c-peptide

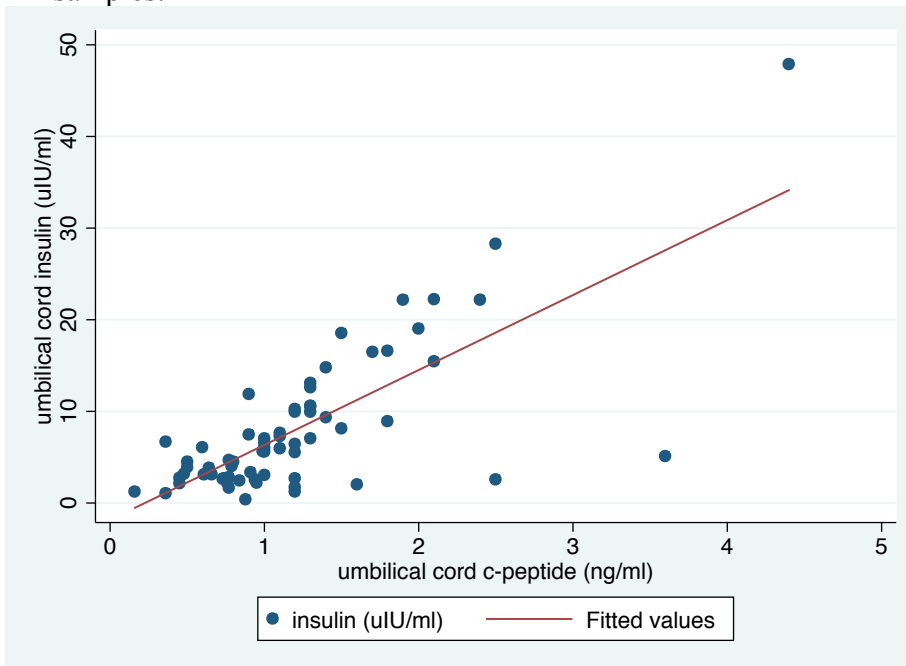
Non-hemolyzed samples:



$R^2=0.848$, $N=50$

$p<0.001$

All samples:

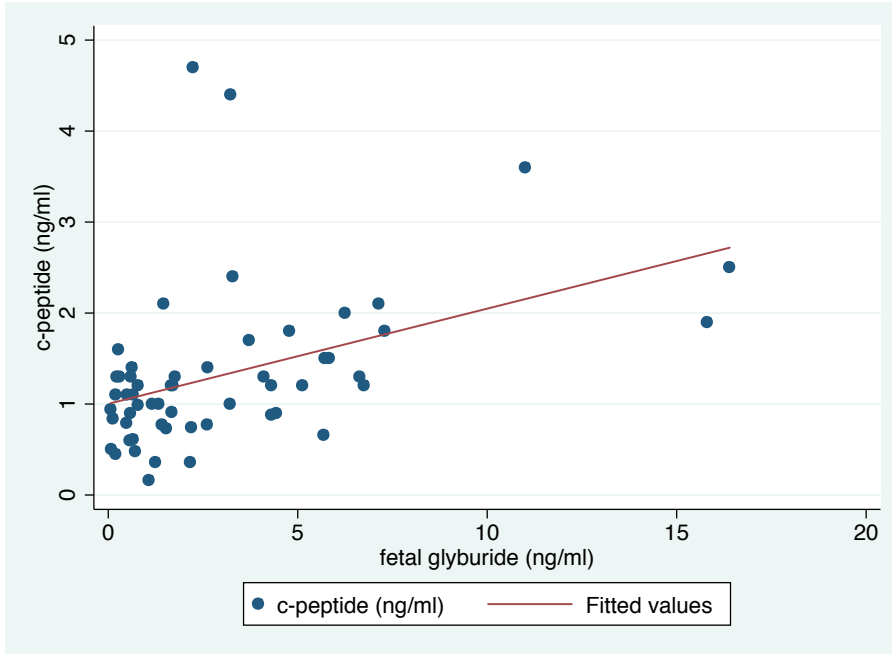


$R^2=0.557$, $N=66$

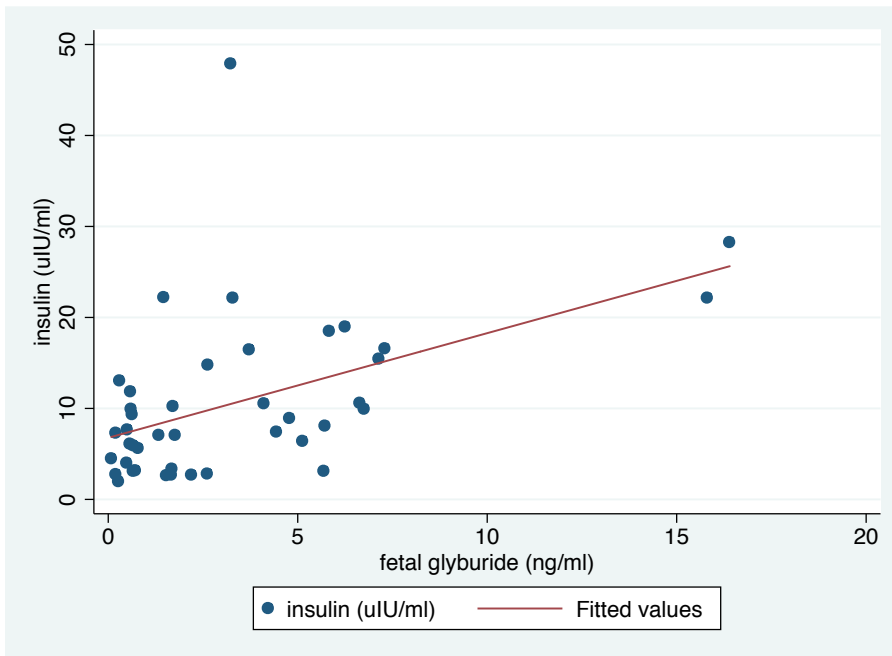
$p<0.001$

Correlation stronger when restricted to non-hemolyzed samples ($t=16.38$) as compared to all samples ($t=8.96$).

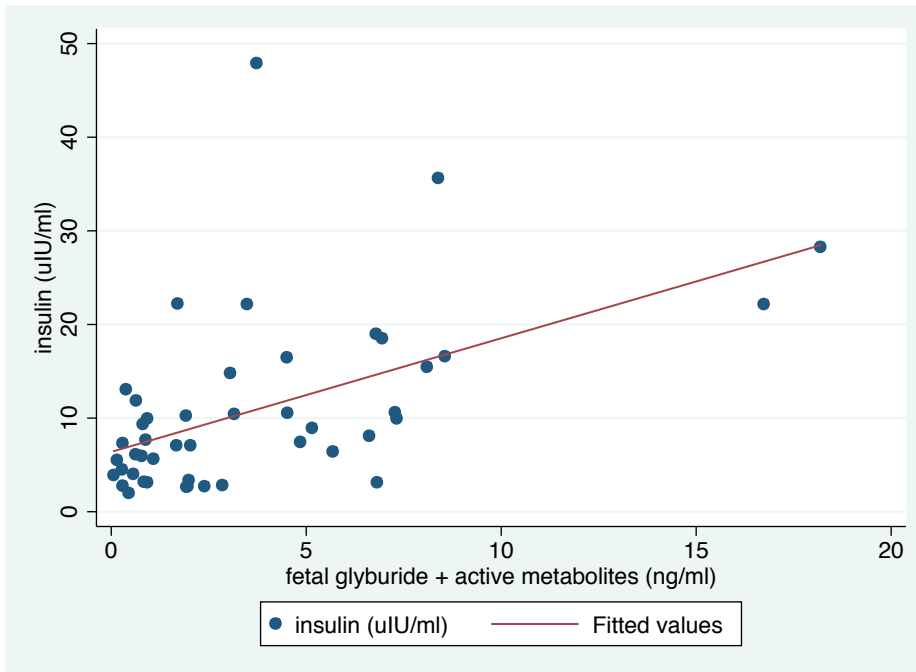
FIGURE 16: INSULIN, C-PEPTIDE & FETAL GLYBURIDE



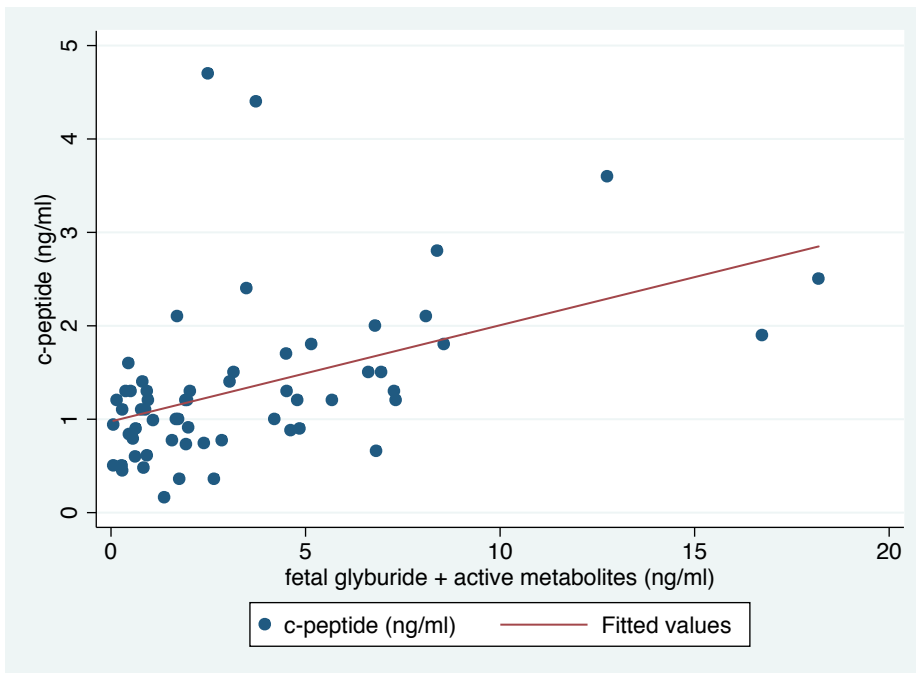
$R^2=0.177$, $N=56$
 $p=0.001$



$R^2=0.231$, $N=42$ (non-hemolyzed samples only)
 $p=0.001$



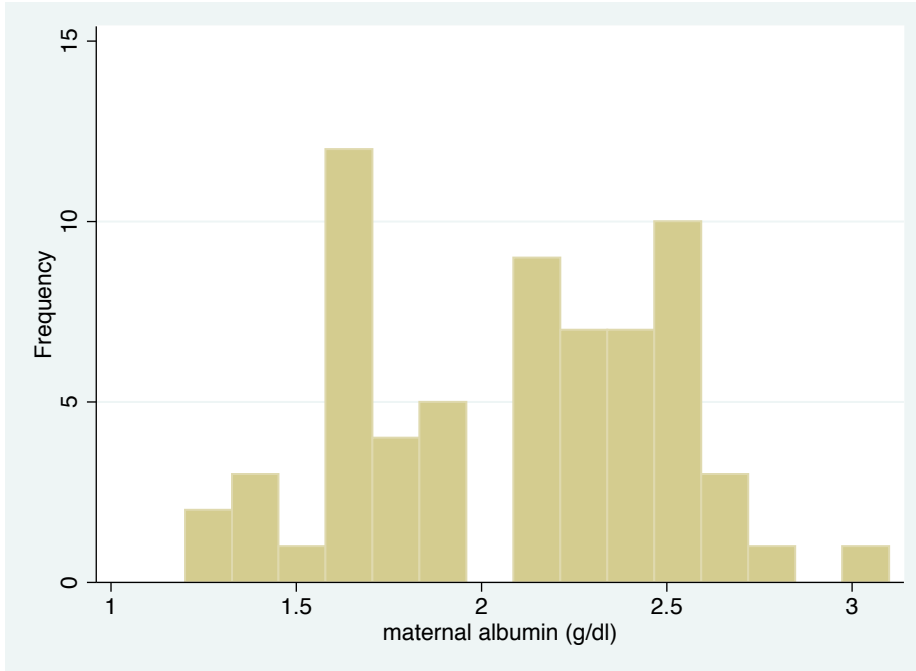
$R^2=0.269$, $N=46$
 $p<0.001$



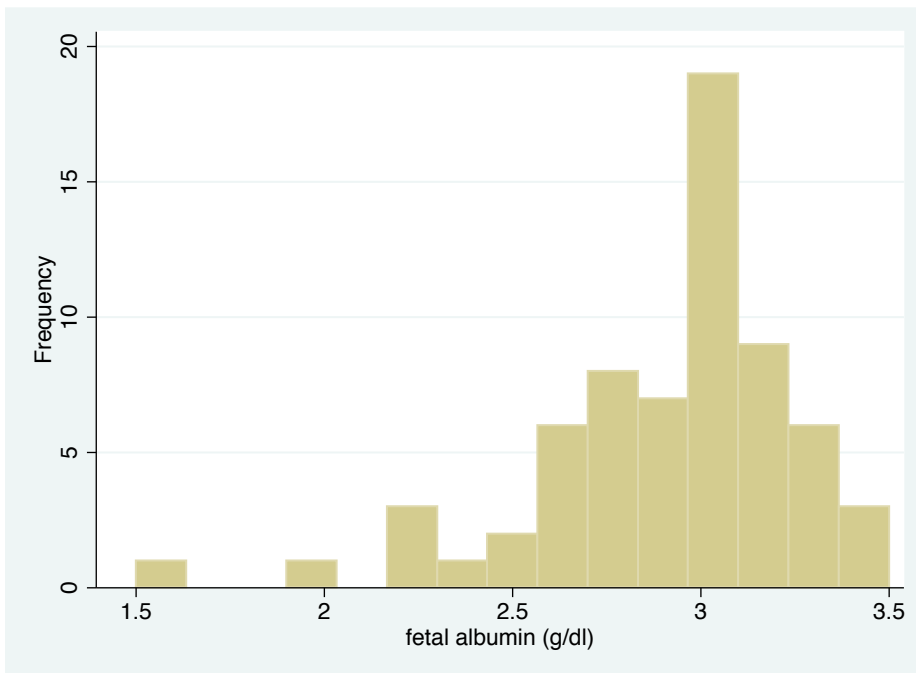
$R^2=0.204$, $N=60$
 $p<0.001$

More highly significant correlation when potency-adjusted metabolites included, as compared to parent drug only.

FIGURE 17: ALBUMIN

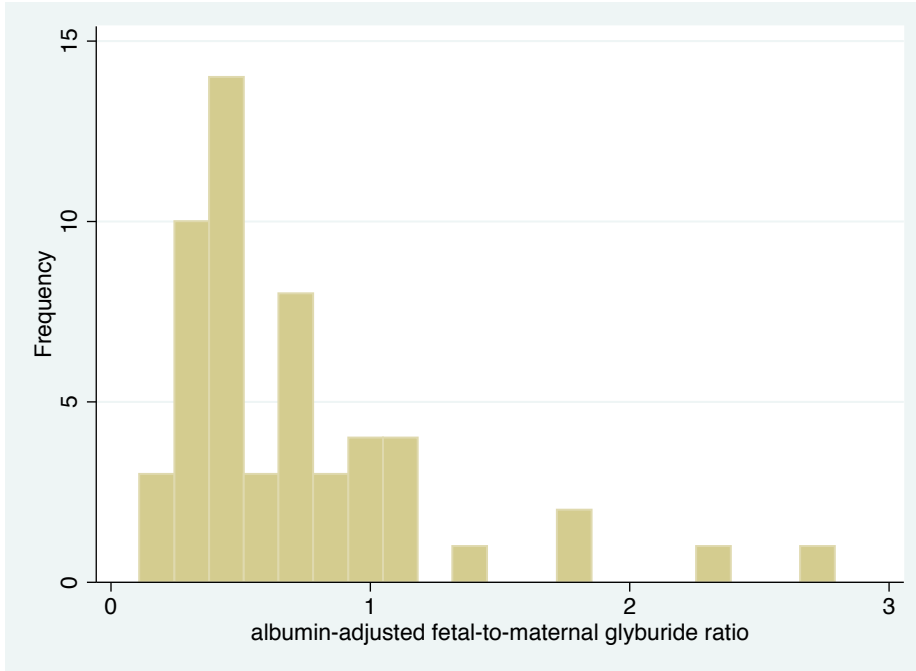


$\mu=2.10\pm0.42$
N=65

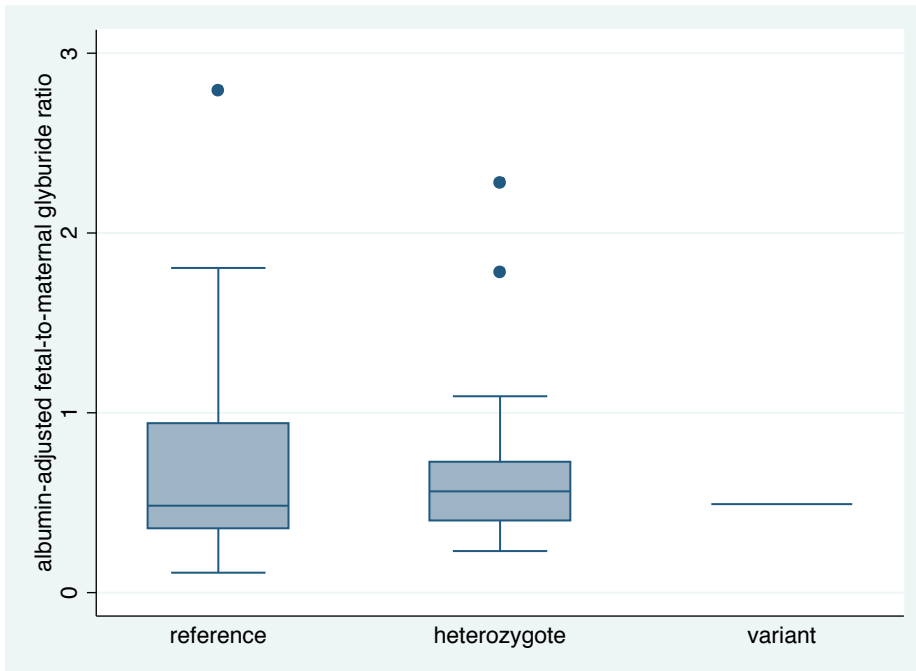


$\mu=2.91\pm0.36$
N=66

FIGURE 18: ALBUMIN-ADJUSTED GLYBURIDE RATIO



$\mu=0.70\pm0.51$
N=54



Reference: $\mu=0.70\pm0.52$, N=35
Heterozygote: $\mu=0.73\pm0.55$, N=17
Variant: $\mu=0.49$, N=1
p=0.976

FIGURE 19: FETAL OUTCOMES

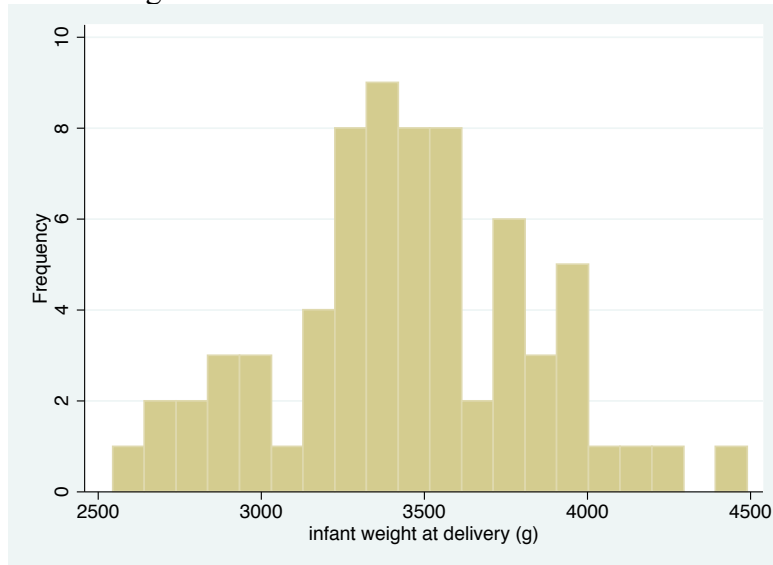
Neonatal Hypoglycemia

| | All Infants | Detectable Fetal Gly |
|------------------------------|-------------|----------------------|
| euglycemic | 57 | 47 |
| clinical (40-60mg/dl) | 9 | 9 |
| severe (<40mg/dl) | 4 | 3 |

Shoulder Dystocia

| | All Infants | Detectable Fetal Gly |
|-----------------------------|-------------|----------------------|
| no shoulder dystocia | 67 | 56 |
| shoulder dystocia | 3 | 3 |

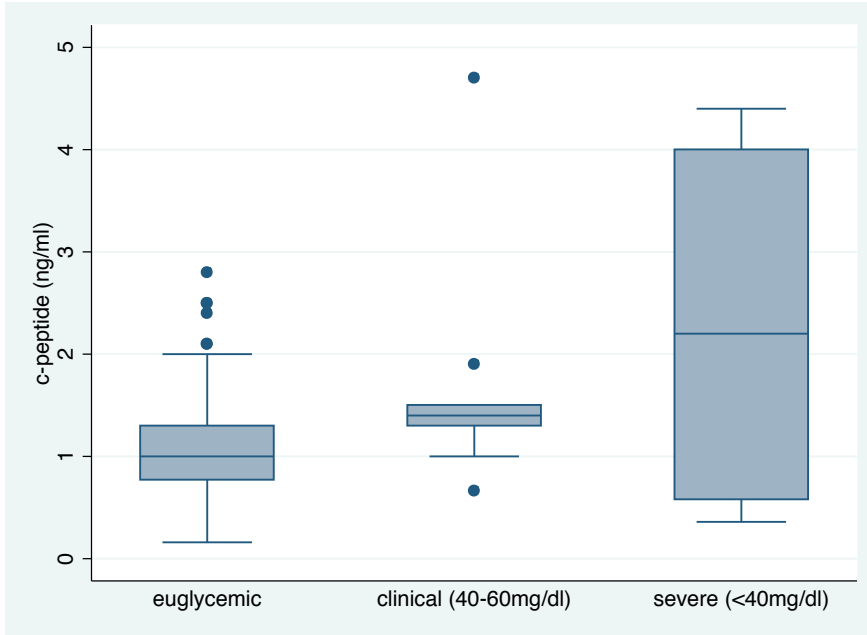
Infant Weight



$\mu=3445.1\pm385.8g$

N=69

FIGURE 20: NEONATAL HYPOGLYCEMIA

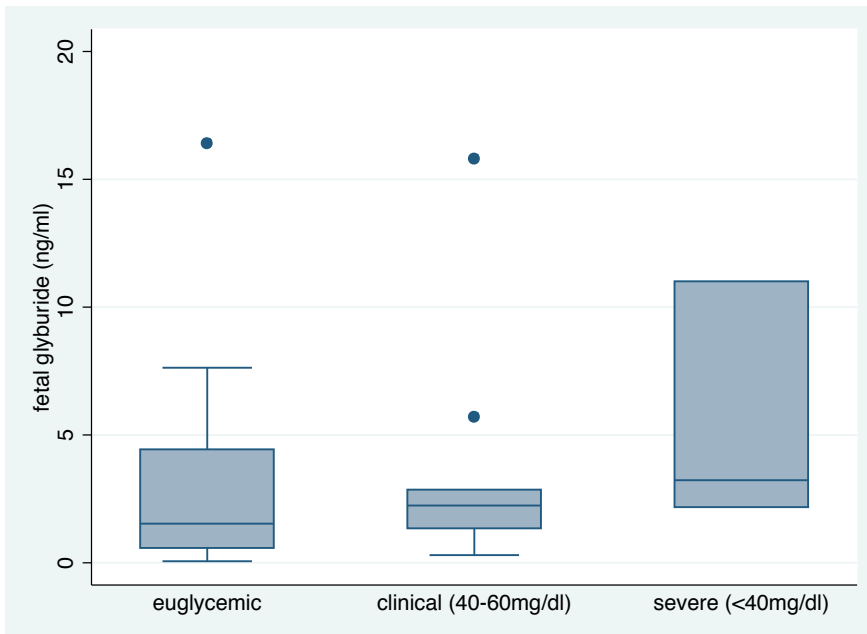


Euglycemic: $\mu=1.15\pm0.58$, N=56

Clinical: $\mu=1.68\pm1.18$, N=9

Severe: $\mu=2.29\pm2.01$, N=4

p=0.002

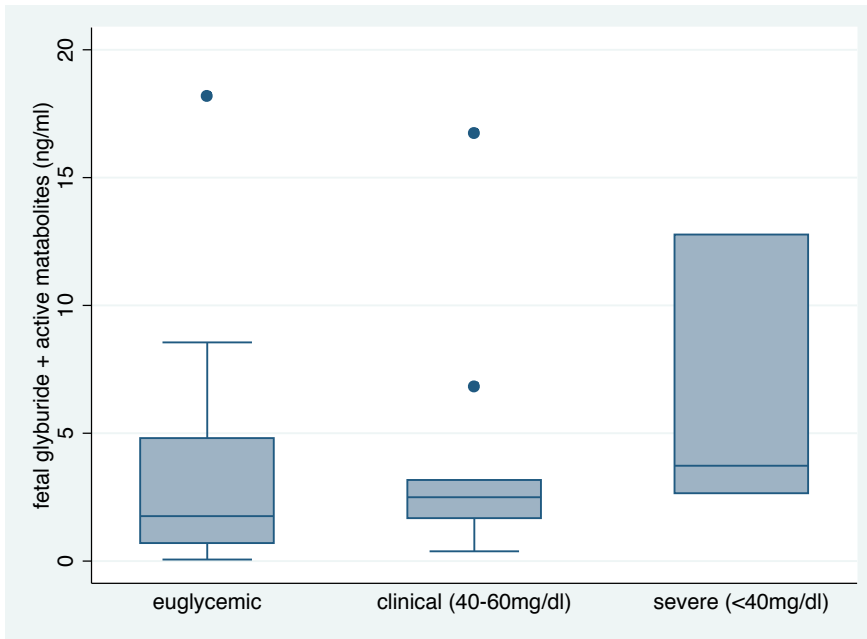


Euglycemic: $\mu=2.82\pm3.11$ ng/ml, N=47

Clinical: $\mu=3.69\pm4.81$ ng/ml, N=9

Severe: $\mu=5.46\pm4.82$ ng/ml, N=4

p=0.174



Euglycemic: $\mu=3.12\pm 3.41$ ng/ml, N=49

Clinical: $\mu=4.13\pm 5.08$ ng/ml, N=9

Severe: $\mu=6.38\pm 5.55$ ng/ml, N=3

p=0.128

CHAPTER 4: CONCLUSIONS

Interdisciplinary training involves exposure to multiple academic disciplines and their attendant methodologies and perspectives. This ideally results in a superior ability to understand and address a defined problem as compared to working exclusively within an individual discipline. Students in the Institute for Public Health Genetics receive training in the legal, ethical, regulatory, economic and cultural aspects of genomics, as well as the traditional coursework in epidemiology, biostatistics, pharmacogenetics and clinical genetics that are included in most graduate programs in public health and genomics. There is an emphasis on identification of issues and groups that are excluded or inadequately represented in traditional disciplinary approaches and structures, and disparities in health outcomes and the distribution of risks and benefits in biomedical research. All of this training was the genesis for the research in this dissertation, and the results underscore the importance and value of an interdisciplinary approach.

There has been extensive recent attention and progress in the fields of prenatal genetic testing and pharmacogenetics. For the former, this includes scrutiny of the nature and implications of an expanded scope of prenatal genetic testing enabled by the technical feasibility of non-invasive fetal sequencing. For the latter, there is a growing number of medications with pharmacogenetic information on drug labels as well as available clinical pharmacogenetic tests. By conferring information about medication efficacy, safety and dosing requirements, pharmacogenetic tests are fairly straightforward in their ability to offer clinical utility, particularly when compared to other types of genomic data

such as carrier status for Mendelian diseases and variants associated with disease susceptibility. It was therefore surprising to discover that pharmacogenomic applications had no presence in the many discussions taking place regarding the implications and applications of non-invasive prenatal genomic sequencing of a developing fetus.

As described in Chapter 2, a broad assessment of possible pharmacogenetic applications of prenatal genetic testing identified three distinct ways in which the fetal genome likely influences the extent and nature of fetal exposure to maternally administered medications and other chemical exposures. Placental drug transport, which is influenced by fetal-origin efflux and uptake transport proteins present at the interface between maternal and fetal circulation, can influence drug concentrations in fetal circulation. Placental and fetal drug metabolism, although minor overall relative to maternal metabolism and unlikely to affect total pharmacokinetics, can influence fetal exposure to toxic and reactive metabolites. There is a growing body of evidence from experimental work with non-human animals, placental expression (RNA and protein) studies, and clinical pharmacokinetic investigations in non-pregnant adults that supports the hypothesis that fetal genotype can predict fetal exposure to some medications and other chemicals. While further research is needed to demonstrate specific instances of polymorphisms in genes for transport proteins and drug metabolizing enzymes significantly affecting fetal exposures and outcomes in humans, there is clearly sufficient cause to include pharmacogenetic applications in discussions of responsible innovation in prenatal genetic testing.

Furthermore, for medications that are routinely prescribed to pregnant women and used through delivery, it is possible to do opportunistic, minimal risk pharmacokinetic studies assessing relative and absolute fetal exposure at the time of delivery and evaluate the impact of gene polymorphisms on these parameters. An example of such a study is presented in Chapter 3. Glyburide is an oral hypoglycemic agent that is used to treat gestational diabetes as an alternative to insulin. Fetal exposure to the medication seemed likely to be influenced by fetal genotype for the gene *ABCG2*, which encodes the placental drug transporter breast cancer resistance protein (BCRP). By simultaneously collecting blood from the mother and the umbilical cord just after delivery in women receiving glyburide for gestational diabetes, we were able to measure the parent drug and metabolites in both maternal and fetal circulation, and calculate the fetal-to-maternal concentration ratio. Contrary to expectation, regression analysis did not identify fetal genotype as a statistically significant predictor of absolute or relative fetal glyburide concentration. Average fetal concentrations equaled those of the mother. While the presence of therapeutic drug concentrations in the fetus raise concerns about drug-induced neonatal hypoglycemia, our data also suggested that maternal glucose control plays a greater role in the risk of this neonatal adverse event than fetal glyburide concentration. This finding warrants further follow up given the increasing use of glyburide to treat gestational diabetes.

This study generated data that will be useful in directing additional research to optimize the use of glyburide in the treatment of gestational diabetes, and also serves as an important reminder of the limitations of generalizing data from non-human animals and

non-pregnant adults. As is described in Chapter 1, while we can and should learn as much as possible before doing any interventional trials in pregnant women, the only truly predictive and reliable model of human pregnancy is human pregnancy. The unfortunate reality is that the majority of medications have never been evaluated for safety or efficacy in this population. There are numerous barriers to conducting research in this patient population, and as detailed in Chapter 1, when they are actually conducted, obstetric pharmacology trials encounter unique challenges that are reflected in study metrics available through ClinicalTrials.gov. Awareness and appreciation of these factors is particularly important given the recent surge of interest in the prenatal environment and possible fetal origins of adolescent- and adult-onset disease. The increased research activity and funding focused on fetal exposures will be able to be used most effectively and efficiently if these unique factors that affect the research process are better understood and incorporated into program planning and funding mechanisms.

While researching the plausibility of prenatal pharmacogenetic testing, it became abundantly clear that while there is strong biological plausibility and some evidence supporting contributions of fetal genetic variation to inter-individual differences in drug disposition during pregnancy, there are much more fundamental aspects of pharmacokinetics and pharmacodynamics that remain unknown in this population. Prenatal pharmacogenetic testing opportunities merit further study, but they are most appropriately contextualized into the broad knowledge deficits that exist in obstetric pharmacology. Efforts that will help improve the quantity and quality of clinical research

on the safety and efficacy of medication use in pregnancy will make a greater impact on achieving healthier pregnancies than will focusing on the opportunities in genomics.