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Genetic Determinants of Fetal Opiate Exposure and Risk of Neonatal Abstinence Syndrome: Knowledge Deficits and Prospects for Future Research

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Introduction

Opiate dependent pregnant women receive opiate maintenance medications to prevent illicit use and withdrawal. Fetal opiate exposure causes central nervous system alterations which manifest as postnatal physical withdrawal. The extensive variability in Neonatal Abstinence Syndrome phenotype remains unexplained and may be related to variability in fetal exposure and response. Improved understanding of functionally significant genetic variants in pathways influencing placental opiate transfer and fetal response can lead to personalized maternal therapy and optimized neonatal outcomes.

Background

Neonatal Abstinence Syndrome (NAS) is the physical manifestation of opiate dependence in a newborn who was chronically exposed to opiates during gestation via maternal use. Infants at risk for NAS must be monitored in the inpatient setting after birth, and a fraction require prolonged hospitalization for pharmacologic therapy and weaning. The most severely affected infants cannot orally feed, fail to gain weight, and have seizure activity related to opiate withdrawal. There is also long-term neurodevelopmental morbidity associated with *in utero* opiate exposure. When compared to controls, children who were *in utero* opiate exposed have deficits in language, motor and cognitive development¹.

The burden of NAS is increasing^{2,3}, in part due to improved recognition and enrollment of opiate dependent women into treatment programs. There has also been a sharp rise in prescription opiate dependence among adults of childbearing age. In 2006, approximately

5.2 million individuals in the United States reported using prescription analgesics nonmedically in the prior month. In 2007, 23% of pregnant women in a large cohort of Medicaid-insured patients filled a prescription for opiates during their pregnancy⁴, and although many women have a short exposure for acute pain, it clear that some portion of women become dependent and addicted to these medications during pregnancy. Similar increases in prescription opiate use have been observed in other populations⁵, including a commercially insured cohort in which 2.2% of pregnant women were dispensed opiates three or more times during gestation⁶. In all large studies looking at prescription opiate use, hydrocodone preparations are the most prevalent, followed by codeine and oxycodone. Among pregnant women who are prescribed opiates, morphine and fentanyl are more often prescribed in a chronic fashion (>2 months) as compared to hydrocodone and oxycodone⁶. A higher proportion of pregnant women in opiate treatment programs used prescription drugs prior to pregnancy than those in the general population⁷.

The current standard of care for pregnant women with opiate addiction or dependence is not to attempt to wean them off their medications or illicit drugs, but instead to provide excellent prenatal care with the adjunct of a maintenance opiate program. The two maintenance medications most commonly used are methadone and buprenorphine. According to the Substance Abuse and Mental Health Services Administration, there were 1,739 programs in the United States offering addiction treatment to pregnant women in 2011, comprising 12.7% of all treatment programs. There are no current data about absolute numbers of pregnant women admitted to methadone and buprenorphine treatment programs, but around 20% of pregnant women admitted to drug treatment programs report opiates as their primary substance of abuse⁸. There have been many attempts to correlate maternal exposure, as measured by dose, to neonatal outcomes. A recent systematic review confirms the results of many smaller studies, namely that there is no currently known relationship between the maternal dose of methadone and the incidence or severity of NAS⁹. This lack of association is likely because our current thinking on the maternal – fetal – neonatal transfer and effect of opiate medications is oversimplified and does not account for the potential impact of pharmacokinetics, pharmacodynamics and pharmacogenetics and how these are affected by gestational changes in maternal physiology. In alternate terms, a central issue is the lack of knowledge to explain the relationship between maternal dose and systemic fetal exposure at the level of the individual mother-infant dyad.

Pharmacologic factors can affect the amount of free drug in the maternal circulation available for fetal transfer at any given time. The factors include both physiologic changes in drug disposition during gestation (ontogeny), maternal co-medication and cigarette use (environmental), and genetic variability in key maternal drug metabolizing pathways, both of which affect the quantities of parent opiate and active metabolites available for placental transfer. In addition to maternal dose-exposure variability, the efficiency of placental metabolizing enzymes and placental transport proteins such as Multi-drug Resistant Protein 1 (MDR1)**¹ and Breast Cancer Resistance Protein (BCRP) may alter the ratio of maternal to fetal drug exposures. Lastly, ontogeny and genetic variation in fetal drug disposition and

¹**The *ABCB1* gene encodes Multi-Drug Resistant Protein 1, otherwise known as Permeable-glycoprotein (P-gp). For the purpose of this review article, we will refer to the official gene name *ABCB1*, and the protein will be referred to as MDR1.

response (mu-opioid receptor) may influence the risk and severity of NAS in the newborn to a given extent of exposure to maternal medications.

In addition to gestational and ontogenic changes in these pharmacologic factors, genetic variation potentially adds an additional layer of complexity to placental opiate transfer and fetal response (Figure 1). Population-based maternal opiate maintenance treatment paradigms are not optimal for Neonatal Abstinence Syndrome prevention or minimization because they fail to recognize the individual factors that influence placental opiate transfer and fetal / neonatal response. . The aim of this article is to review the pertinent literature addressing the effects of changing physiology during pregnancy and pharmacogenetic variation as sources of inter-individual variation in the maternal dose-exposure relationship, placental metabolism and transport, and fetal / neonatal opiate disposition and response.

Pharmacogenetics of Maternal Dose-Exposure Relationship

Variability in the amount of active maternal opiate available for transfer to the fetus at any given time is the first potential source of inter-individual difference in fetal opiate exposure. If all women are treated with similar opiate doses, but the maternal (and thus placental) exposure based on these doses is highly variable, then we must understand how maternal dose relates to exposure to better understand the fetal risk of Neonatal Abstinence Syndrome

Methadone

Methadone is the major drug used for opioid addiction therapy in pregnant women. However, methadone is a difficult drug to use effectively for a number of reasons. The drug is administered as a racemic mixture, with *R*-methadone being the active enantiomer at the μ -receptor with approximately 10 times higher binding affinity. *S*-methadone is approximately 50 times less potent and there is some evidence to suggest that this enantiomer is involved in methadone toxicity¹⁰. Methadone displays stereoselective pharmacokinetic profile, with plasma concentrations of the *R*-enantiomer typically circulating at greater concentrations than its antipode. However, in a group of methadone maintenance treatment patients, *R/S*-methadone ratios varied from 0.63 – 2.4¹¹. This range would suggest that therapeutic effects could vary from efficacious to toxic, depending on the enantiomeric ratio. Methadone is primarily N-demethylated to the inactive metabolite, 2-ethyl-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) in the liver, predominantly by Cytochrome P450 2B6 (CYP2B6). CYP2B6 exhibits stereoselectivity towards the *S*-enantiomer of methadone and this is consistent with the pharmacokinetic profile for the drug¹²⁻¹⁴. There are approximately 40 *CYP2B6* allelic variants described, which may partially explain the differences in methadone clearance observed¹⁵.

The most frequently occurring and commonly studied allelic variants of *CYP2B6* are: *CYP2B6**4 (785 A>G; K262R; rs2279343), *CYP2B6**5 (1459 C>T; R487C; rs3211371), and *CYP2B6**6 (785 A>G, 516 G>T; K262R, Q172H; rs2279343, rs3745274)¹⁵. The frequency of the variants differs among ethnic groups and of the allelic variants, *CYP2B6**6 occurs with the highest frequency in the population¹⁶. *In vitro* studies have assessed altered CYP2B6 enzyme function leading to changes in metabolite formation as compared to wild type enzyme. Gadel et. al evaluated recombinantly expressed CYP2B6.6 N-demethylation

rates of methadone and found activity was reduced to 25-33% of wild-type enzyme¹⁷. An *in vivo* study involving 336 patients undergoing methadone maintenance therapy showed an association between CYP2B6 haplotypes containing both intronic and exonic single-nucleotide polymorphisms (SNPs) and plasma concentrations of methadone and the concentration to dose ratio¹⁸. A re-analysis of phenotypic extremes in a different cohort of methadone maintenance patients has shown that certain CYP2B6 SNPs are more or less common in patients with high and low methadone concentrations, strengthening the etiologic link between genetic changes in the metabolizing enzyme and differences in methadone plasma concentrations¹⁹. In a systematic review, it was shown that patients homozygous for the *CYP2B6**6 allele have higher trough *R*- and *S*- methadone plasma concentrations, suggesting that methadone metabolism is significantly slower in *6 homozygous carriers²⁰. Genetic polymorphisms in *CYP2B6* are potential predictors of methadone clearance and maternal concentrations and could prospectively be used to understand which fetuses are going to be exposed to more parent methadone.

In addition to CYP2B6, there are a number of other CYP isoforms that are also implicated in the oxidation of methadone, including: CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP19 (aromatase)^{12,13,21-24}. Given the polymorphic nature of the *CYP2B6* gene, alternate pathways of methadone metabolism may become prominent with variable and/or decreasing CYP2B6 activity. Polytherapy during pregnancy also confounds methadone clearance, with CYP2B6 being an enzyme that is subject to both inhibition and induction. The variability of methadone disposition is compounded further with the introduction of biochemical and physiologic changes that occur during gestation. The fluctuation in endocrine levels throughout the course of pregnancy can lead to variable CYP enzyme activity, particularly isoforms that are partially regulated by hormone concentration. *In vitro* experiments conducted in single donor primary human hepatocytes have shown that estradiol and progesterone at typical concentrations observed in pregnancy induce a number P450s involved in methadone metabolism²⁵. However, the extent of induction for each P450 studied was different; and there was considerable interindividual variability of fold-induction for each isoform studied²⁵. Certain P450s, such as CYP1A2, are actually down-regulated during pregnancy²⁶. The placenta contains P450s involved in steroidogenesis which are also capable of xenobiotic metabolism. Methadone is oxidized by CYP19 in human placenta tissue²⁷. *In vivo* studies in healthy postmenopausal assessing coadministration of methadone and letrozole, a potent CYP19 inhibitor, show significantly reduced methadone clearance²⁴. Altogether, methadone use during pregnancy is subject to great interindividual variability. The interaction of genetic polymorphisms and physiological changes of pregnancy could help explain the differences in methadone pharmacokinetics, pharmacodynamics, effectiveness, and toxicity that have been documented in both mother and newborn^{28,29}.

Oxycodone

Despite its low binding potential at the μ -opioid receptor and resultant G-protein activation, oxycodone exhibits potent analgesic activity^{30,31}. *In vitro* studies conducted in rat brain homogenates showed that certain oxycodone metabolites had higher binding affinity compared to parent. This would implicate a role for active metabolites to oxycodone activity³⁰. However, an *in vivo* study in healthy human volunteers showed that the

pharmacodynamics of oxycodone was most strongly associated with parent drug³¹. This result was further supported by calculations using oxycodone pharmacokinetic parameters and opioid receptor affinity³². Because of these conflicting results, it is difficult to gauge the role of genetic modifiers to oxycodone therapeutic efficacy. What is presented here is instead a review of variability in oxycodone metabolism and how these differences can be magnified during pregnancy.

The major P450s involved in oxidative metabolism of oxycodone are CYP3A and CYP2D6³³. The molecule is N-demethylated by CYP3A to form the major circulating metabolite, noroxycodone. The drug can also be O-demethylated by CYP2D6 to form oxymorphone. Noroxycodone and oxymorphone can also then be sequentially metabolized to form noroxymorphone, the didemethylated metabolite. Oxycodone also undergoes 6-ketoreduction to form α - and β - oxycodol and all metabolites in which there is a hydroxyl group are potential substrates for glucuronidation. Binding affinities for the μ -opioid receptor are in decreasing order: oxymorphone > noroxymorphone > oxycodone > noroxycodone³⁰. There is currently no binding affinity data for either isomer of oxycodol and glucuronidated metabolites. Because the CYP3A enzymes are important in converting oxycodone to the relatively inactive metabolite, variable CYP3A activity could be a major source of differences in oxycodone exposure. CYP3A4 is the isoform that is expressed to the greatest degree in liver for most individuals in the population; however, the variability is > 100-fold³⁴. Unfortunately, most CYP3A4 genetic variants are rare and lack a phenotypic effect. Perhaps the allelic variant with the most clinical relevance is *CYP3A4*22* (15389 C>T, rs35599367), an intronic SNP that is associated with decreased expression of CYP3A4^{15,34}. Variability of CYP3A4 activity also occurs due to both inhibition and induction. CYP3A5, CYP3A7, and CYP3A43 are typically expressed at lower concentrations compared to CYP3A4. Allelic frequency of *CYP3A5*1* varies amongst ethnic groups and has been shown to have an impact on drug metabolism³⁴. It is still unclear how the minor isoforms of CYP3A, when present, affect oxycodone clearance.

CYP2D6 is the most polymorphic member of the cytochrome P450 super-family with over 100 described allelic variants¹⁵. These variants include SNPs, copy number variations, and hybridization of neighboring pseudogenes CYP2D7 and CYP2D8. For ease of analysis, each variant is typically assigned an activity score, with 0 being no activity, 0.5 being partial activity, and 1 being fully functional activity. An individual's phenotype is a sum of the activity score (AS) for each copy of CYP2D6 present. There are four phenotypic groups based on CYP2D6 activity: poor metabolizers (abbreviated as PM, sum AS = 0), intermediate metabolizers (abbreviated as IM, sum AS = 0.5), extensive metabolizers (abbreviated as EM, sum AS = 1-2), and ultrarapid metabolizers (abbreviated as UM, sum AS = 2+)³⁵. Samer et. al conducted an *in vivo* study to assess differential metabolic profile of oxycodone in individuals with differing CYP2D6 genotype. With decreasing CYP2D6 activity, more of the metabolic oxidation was shunted to the CYP3A pathway³⁶. In a follow-up study conducted by Samer et. al, 10 healthy individuals with differing CYP2D6 genotype were enrolled in a randomized five-arm double-blind placebo-controlled study (placebo, oxycodone, oxycodone + CYP2D6 inhibitor, oxycodone + CYP3A4 inhibitor, and oxycodone + CYP2D6 inhibitor + CYP3A4 inhibitor). Pharmacodynamic effects such as cold pressor test and pupil size was associated with increasing CYP2D6 activity. These

markers were also associated positively with CYP3A4 inhibition³⁷. This study suggests that lack of conversion to the inactive metabolite and increased formation of the active metabolite are associated with therapeutic activity of oxycodone. A larger study involving 121 adult post-operative patients showed that ratios of oxymorphone to oxycodone significantly differed by CYP2D6 genotype and that PMs had the highest oxycodone consumption. This suggests a clinical consequence of poor metabolism to the active form of the drug³⁸.

As previously mentioned in the methadone metabolism section, pregnancy can result in profound changes in expression level of drug metabolizing enzymes. Both CYP3A and CYP2D6 appear to be induced during pregnancy but not to the same extent²⁶. This is most likely due to differences in mechanism of induction. CYP3A induction is partially regulated by increased estradiol and progesterone levels²⁵. Information for CYP2D6 induction during pregnancy is sparse, though it does not appear to be mediated by either estradiol or progesterone²⁵. The fold induction during pregnancy with relation to CYP2D6 genotype is also as yet unknown. The interaction and contribution of CYP3A and CYP2D6 in metabolism of oxycodone during pregnancy is difficult to predict given uncertainty in expression levels of each enzymes during pregnancy. This uncertainty, along with differential metabolism to either oxymorphone or noroxycodone in the maternal system, can alter the concentrations of active drug or metabolite available for placental transfer.

Buprenorphine

Buprenorphine, a structural analog of thebaine and partial μ -opioid receptor agonist, is used primarily for narcotic abuse maintenance / cessation and pain relief. Low doses of the drug are used for pain control, whereas higher exposures are required for effective opioid replacement therapy. Buprenorphine is extensively metabolized when taken orally and has low bioavailability. Sublingual administration has been shown to achieve adequate exposure when using the drug for opioid replacement therapy. When used sublingually, buprenorphine pharmacokinetic parameters are highly variable with time to peak plasma concentration between 40 minutes and 3.5 hours with values for terminal half-life between 3 – 44 hours. The extent of variability in buprenorphine pharmacokinetics must be considered as one of the influential factors for variability in NAS incidence with this medicine's use³⁹.

Variability in buprenorphine pharmacokinetics may be partially explained by extensive metabolism via CYP3A and CYP2C8. The major metabolite formed is norbuprenorphine, through loss of the cyclopropylmethyl side chain. Norbuprenorphine also exhibits partial agonist activity at the μ -opioid receptor and is considered to be a potent agonist⁴⁰. Other minor metabolites of buprenorphine and subsequent metabolism of norbuprenorphine have been observed both *in vivo* and *in vitro*. These metabolites involve hydroxylation of the aromatic ring and the hydroxy-dimethyl-butanyl side chain. These metabolites also appear to be CYP3A mediated⁴⁰. Both buprenorphine and norbuprenorphine both undergo phase II metabolism through glucuronidation by UGTs. The glucuronidated metabolites are considered to be inactive. Parent drug is glucuronidated mainly by UGTs 1A1 and 2B7, with some contribution from UGTs 1A3 and 2B17. Norbuprenorphine is glucuronidated by UGTs

1A1 and 1A3. The conjugated species are mainly excreted in the bile and may be subject to enterohepatic circulation.

The variability seen with buprenorphine and norbuprenorphine exposure has the potential to be heightened during pregnancy. Variable CYP3A activity in the population and changes during pregnancy were discussed previously in the oxycodone section and these same considerations are applicable to buprenorphine, given that CYP3A is the main isoform involved in the metabolism of buprenorphine. CYP2C8 accounts for metabolism of approximately 5% of drugs on the market⁴¹. Recognition of this isoform in contribution to xenobiotic metabolism has increased in recent years. However data describing effects of polymorphisms in this gene are lacking. While there are over 450 SNPs reported for CYP2C8, most of information available is with regards to the genetic variation within the coding region. The most commonly occurring variants are *CYP2C8*2* (805 A>T; I269F; rs11572103), *CYP2C8*3* (416 A>G, 1196 A>G; R139K, K399R; rs11572080, rs10509681), and *CYP2C8*4* (792 C>G; I264M; rs1058930)⁴¹. Most genetic variants studied resulted in change in activity, but this was substrate dependent⁴¹. There is limited data detailing the changes of CYP2C8 activity during pregnancy²⁶. However, it has been suggested that CYP2C8 is one of the most inducible P450s. Nuclear receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), and the glucocorticoid receptor are involved in transcriptional activation of the gene⁴¹. It is probable, but unconfirmed, that CYP2C8 activity would increase during pregnancy leading to decreased parent drug. Currently, information for UGT activity changes during the pregnancy is only available for a few isoforms. There is no *in vivo* data for changes in the UGT 1A1, 1A3, and 2B17 during pregnancy. UGT2B7 does not display change in activity during gestation²⁶. Interestingly, the potential for increased metabolite formation without corresponding increased conjugation may result in increase fetus exposure to norbuprenorphine.

Genetic Variation in Placental Opiate Transfer

The placenta is the primary line of defense between the fetus and maternal opiate exposure due to its potential to metabolize active opiates to inactive metabolites and actively transport opiate medication from the fetal compartment back to the maternal circulation. Inter-individual variability in the placental function of these roles can influence fetal opiate exposure and resultant risk of Neonatal Abstinence Syndrome.

General Considerations of Placental Effects on Drug Disposition

The placenta is a major line of defense for the developing fetus against pathogens and potentially harmful endogenous and exogenous substances in the maternal system. Placental transporters are known to modulate fetal exposure to maternal medications and other environmental exposures. Multi drug resistant protein-1 (MDR1) is encoded by *ABCB1*. Breast cancer resistance protein (BCRP) is encoded by *ABCG2*. Both of these transporters are part of a larger subgroup of ATP binding cassette (ABC) transporters known to control efflux of drug across the placenta. There are comprehensive reviews about transporters and the placenta^{42,43}. Briefly, *in vitro* studies have demonstrated that inhibition of BCRP cause significant changes in fetal-to-maternal concentrations of antidiabetic medications⁴⁴ and

inhibition of MDR1 significantly increases fetal exposure to drugs such as dexamethasone and ritonavir⁴⁵. In animal studies, Mdr1 deficient progeny and progeny of dams treated with Mdr1 inhibitors have significantly higher fetal-to-maternal drug concentration ratios of digoxin, saquinavir and paclitaxel (Figure 2 – black bars)⁴⁶. Additionally, animals who carry one or two null copies of Mdr1 are at an allelic dose-dependent increased risk of congenital anomalies after maternal treatment with ivermectin⁴⁷.

In addition to the studies aforementioned which demonstrate the importance of transporters in modulating fetal drug exposure, the ontogeny of placental transporters has been investigated using human placental tissue. In a study of homogenized placental tissue comparing 13-14 weeks obtained via chorionic villus sampling and 38-41 weeks obtained via vaginal and c-section deliveries, it was shown that immunoreactive MDR1 protein was twice as high in early placentas as compared to late (p-value 0.0004)⁴⁸. A second study confirmed these findings by comparing *ABCB1* mRNA levels and MDR1 expression by immunoblot between 6-13 week placentas from therapeutic terminations, 24-35 week placentas from preterm births, and term placentas. Results (seen in Figure 3) show that there is statistically significantly more mRNA in early placentas when compared to term⁴⁹.

Placental Opiate Metabolism

The placental disposition of opiates has been reviewed prior⁵⁰, but this review will highlight genetic variation in placental metabolism and transport that could affect the fetal development of NAS. Due largely to the work of the Obstetric-Fetal Pharmacology Research Unit at University of Texas, we have *in vivo* and *in vitro* data for placental opiate metabolism and transport. Studies in placental microsomal preparations have given insight into the placental metabolism of methadone both in preterm²⁷ and term placentas⁵¹. Using placentas from term healthy pregnancies, microsomal fractions of trophoblast tissue were studied using selective inhibitors for different metabolizing enzymes and biotransformation of methadone to EDDP was quantified. Aromatase chemical inhibitors and neutralizing monoclonal antibodies were found to have the most profound effect on methadone metabolism, with 70-88% reduction in EDDP formation. To further describe aromatase expression during pregnancy, similar kinetic experiments were conducted in placenta tissue collected from earlier in gestation, late second trimester, early third trimester, and late third trimester. The apparent *K_m* values for the different gestations were similar (see Supplementary Table 1) but the catalytic efficiency increased with gestation showing a statistically significant doubling of intrinsic clearance between late second trimester and late third trimester, most likely due to increased expression of placental aromatase. Notably, there is four to six fold interindividual variability in metabolizing enzyme activity within the same gestational age. This suggests that aromatase function could be one of the important factors in fetal exposure to active methadone and resultant NAS development.

Buprenorphine is also metabolized by aromatase in the placenta and, similar to methadone, placental microsome studies have confirmed that enzyme activity in biotransformation of buprenorphine to norbuprenorphine (norBUP) increases with gestational age (Supplementary Table 1)⁵².

Genetic variation in *CYP19* could greatly influence fetal exposure to methadone and buprenorphine. *CYP19* spans 123 kilobases and includes an extensive regulatory region with at least ten identified promoters which facilitate tissue specific expression. Estrogen-related receptor γ (ERR γ) plays an obligatory role in the oxygen dependent increase in *CYP19* expression in syncytiotrophoblasts via a nuclear receptor element within placenta-specific promoter I.1⁵³. In addition to the estrogen mediated increase in maternal hepatic *CYP2B6* expression during gestation⁵⁴, it is possible that increasing aromatase activity within the placenta contributes to decreased maternal methadone concentrations.

Placental Opiate Transport

MDR1 is a known transporter for methadone. Using a single layer of syncytiotrophoblasts lineage cells in a dual perfusion model, Nanovskaya and colleagues showed that methadone transfer to the fetal circuit was increased by 30% by different MDR1 inhibitors⁵⁵. The authors concluded based on this experiment that the concentration of methadone in the fetal circulation is likely affected by the expression and activity of MDR1.

Data from *ex vivo* experiments in a dually perfused placental model provides evidence that buprenorphine transport across the placenta is not mediated by MDR1⁵⁶, but rather via passive diffusion. Buprenorphine crosses placental cells into the fetal circuit to a lesser degree than methadone, with only 8.6 +/- 1.3% of initial maternal circuit concentrations detected in the fetal circuit after a four hour equilibration⁵⁷. This decreased transfer of buprenorphine is thought to be secondary to its highly lipophilic nature and significant tissue accumulation within the placenta as compared to both the maternal and fetal compartments. Of note, these *ex vivo* studies assess short time frames of placental pharmacokinetics and the physiology of chronic buprenorphine dosing in human pregnancies may not follow similar patterns.

In addition to ontogenic changes discussed prior, there is evidence that placental transporter expression can be induced by exposure to certain illicit substances, potentially impacting the maternal-fetal balance of methadone exposure. In 24 term placentas tested in a dual perfusion model, experiments showed that the addition of methadone to control medium for two hours significantly increased the expression of MDR1 (as measured by western blot analysis) by 49 +/- 41% (p-value 0.03). Addition of cocaine and methadone, or heroine and methadone, increased the MDR1 expression by 75 +/- 63% (p-value 0.01) and 59 +/- 51% (p-value 0.03) respectively⁵⁸. Given that MDR1 is known to transport methadone, this *ex vivo* evidence suggests that methadone-mediated induction of MDR1 expression potentially adds to the mechanisms of protecting the fetus from high opiate exposures.

Genetic variation in transporter genes is another potential source of inter-individual variability in fetal drug exposure following maternal administration. Three common single nucleotide polymorphisms within the protein coding region of *ABCB1* – rs1128503 (1236T>C, Gly412Gly), rs2032582 (2677T>G/A, Ser893Ala), and rs1045642 (3435T>C, Ile1145Ile)⁵⁹ - have been extensively investigated in multiple populations. C3435T, occurring in 62% of European Americans and 13% of African Americans, is in linkage disequilibrium with G2677T and C1236T, and has been assigned haplotype *ABCB1**2 and associated with enhanced *in vivo* MDR activity⁶⁰. There are conflicting human trials

regarding the genotype-phenotype correlation for SNPs in *ABCB1* in regards to MDR1 function, drug pharmacokinetics and clinical drug response⁶¹. Potential limitations of current studies, discussed in a comprehensive review article⁶¹, include unmeasured ascertainment bias introduced by creation of cohorts and definition of phenotype retrospectively, low samples sizes and power, lack of controlling for multiple testing, no reporting of genotyping errors and confounding from lack of ethnic stratification in analysis.

Human placental *in vitro* studies into the effects of genetic variation are many, and have shown that genetic polymorphisms in genes that encode placental transporters have an effect on mRNA, protein expression and activity⁶². The biggest concerns in this field of literature are the generally small number of placentas under study, the substrate specific effects of genetic changes, and the inability to fully control for environmental factors that can alter transporter expression and function. A comprehensive review of the genetic implications for placental transport published in 2014 by Dr Daud and colleagues proposes grouping the SNPs in placental proteins by similar functional *in vitro* effect or suspected phenotype in order to move away from individual allele frequency analysis. This group proposes that by grouping SNPs in placental transporters by their functionality, a “cumulative phenotype frequency” can be used instead of allele frequencies of individual SNPs⁶³. The potential for fetal teratogenicity has been studied in mothers carrying the *ABCB1* C3435T SNP, and there is increased risk of cleft lip and palate⁶⁴ and congenital heart disease⁶⁵ in infants of mothers with the minor allele.

The two most common SNPs in *ABCG2* in a racially diverse cohort include C421A (35.5%) and G34A (18%)⁶⁶. In 100 human placentas from Japanese patients, homozygotes for the A421 allele had significantly lower BCRP protein levels than those for the C421 allele, and heterozygotes had intermediate values⁶⁶. The clinical implications of SNPs in *ABCG2* have not yet been investigated in pregnancy.

Pharmacogenetics of Fetal / Neonatal Opiate Disposition and Response

There are known important genetic modifiers of opiate metabolism. In addition to genetic modifiers of pharmacokinetic properties, there are data suggesting that variants in *OPRM1*, which encodes the mu opioid receptor, and catecholamine-O-methyltransferase (COMT), the enzyme which metabolizes CNS dopamine, are associated with incidence and severity of NAS⁶⁷. Genetic variation in fetal and neonatal opiate disposition and response may play a role in an infant's propensity to develop NAS given a similar fetal opiate exposure. Additionally, postnatal opiate disposition will affect response to opiate therapy for NAS.

Fetal / Neonatal Opiate Disposition

In order for morphine to be metabolized and cleared, it must first be transported from the plasma to the hepatocyte and then metabolized via glucuronidation. OCT1 facilitates hepatic morphine uptake⁶⁸. In humans, carriers of a loss of function allele in *OCT1* had a mean AUC of morphine 56% higher than non-carriers⁶⁸. Race appears to correlate with clinical response to morphine for pain, adverse events and morphine clearance⁶⁹. Because this correlation is not fully explained by SNPs in drug metabolizing enzymes, liver uptake proteins including OCT1 are being investigated. Morphine clearance was compared

among 146 post-operative children, stratified by haplotype for loss-of-function *OCT1* variants. Using population modeling and *post hoc* Bayesian estimates, morphine clearance was 17% lower in homozygotes for loss-of-function alleles⁷⁰. In this population, it was confirmed that the genetic contribution of *UGT2B7*-900A>G to morphine clearance was low compared to the contribution of *OCT1* genetic variability. The OCT1 transporter may not only affect PK of postnatally administered opiates, but also the clearance of maternally acquired drug in the first few days after delivery. The rapidity of clearance of maternally acquired opiate may impact onset and severity of NAS symptoms.

In addition to hepatic transport, the ontogeny of *UGT2B7* and the associated effects on morphine metabolism in neonates is well described. It is known that neonates have little glucuronidation capacity in utero and at birth, but that glucuronidation matures rapidly in the first weeks of life. A review article by Allegaert and colleagues⁷¹ highlights the extensive interindividual variability in glucuronidation among neonates, even after accounting for covariates such as postnatal and postmenstrual age. SNPs in *CYP2B6* are known to correlate with clearance of methadone in adults, and potentially have similar effects in infants, influencing the rate of clearance of maternal methadone after birth.

Fetal / Neonatal Drug Effector Compartment

In addition to the genetic influences on PK, an important aspect of development of NAS and response to clinical treatment postnatally is the effector site. For NAS to develop in utero, opiate must move across the blood brain barrier and act upon the opiate receptor. MDR1 is functional at the blood brain barrier. There is animal data linking genetic variation in this gene to opiate induced hyperalgesia, tolerance and dependence⁷². The observations in this mouse study were especially powerful because the investigators were able to measure opiate concentrations in the brain and further validate the underlying physiology of genetic modifiers of blood-brain barrier transport function. In human adults, SNPs in *ABCB1* are correlated with need for rescue medication in chronic pain therapy and opiate consumption in immediate post-operative pain. Genetic variability in blood-brain barrier penetrance of opiate medications during fetal life could influence the development of NAS.

OPRM1 encodes the mu-opioid receptor 1 which is the main receptor target for opiate exposure in utero and morphine therapy in treatment of NAS. The *OPRM1* 118A>G SNP is associated with reduced need for pharmacologic intervention and shorter length of treatment in infants with *in utero* acquired NAS⁶⁷. Although data about MOR1 variability in NAS is limited, there is extensive recent adult literature which shows that common variants in *OPRM1* are associated with post-operative opiate response to pain, clinical severity of opiate drug overdose and tolerance to experimentally induced pain. Given the similar opiate receptor target for pain treatment, fetal response to in utero opiate exposure and postnatal NAS treatment, suffice to say that genetic variability in *OPRM1* may contribute to NAS severity and clinical response to neonatal opiate therapy for NAS. Combination genotypes on *ABCB1* and *OPRM1* (i.e. wild type for both vs. mutant for either or both) are associated with oxycodone clinical effects and adverse drug reactions in adult humans. In addition to single gene effects, the combination effect of genetic variability in blood brain barrier

transport and opiate receptor function may be synergistic or additive in a fetus' risk of developing NAS given a similar in utero opiate exposure.

Conclusion

In summary, there are knowledge gaps in how maternal opiate maintenance therapy relates to fetal opiate exposure and fetal response. If we can more fully understand how maternal dose relates to maternal exposure, how the placenta regulates the maternal exposure to the fetus, and how the fetus and neonate are variable in their pharmacodynamic effects, therapy for these mother-infant dyads could be more tailored than current. (See Table 1 for representative examples). Of note, the ontogenic and genetic influence on opiate PK and PD are only one piece of this complex clinical issue of Neonatal Abstinence Syndrome. There are maternal risk factors including polypharmacy with benzodiazepines and psychiatric medications during pregnancy, prenatal cigarette and alcohol use, that all can influence the development of NAS in an infant. In addition, the postnatal child rearing environment can have effects on developmental outcomes that confounds the causative association with prenatal opiate exposure. Although these issues are important and must be accounted for in clinical research, this review is meant to highlight the genetic influences on prenatal opiate effects, the knowledge of which can allow the influences of these other pathways to be more fully understood.

The gestational changes in drug metabolizing enzyme function are not adequately understood. Although there are current PBPK models for pregnant women, certain enzymatic pathways require more data to understand the complete picture of drug disposition. The interplay of relative upregulation and downregulation of multiple hepatic elimination pathways, and how these interact with increased renal clearance must be taken into account. Secondly, we need further research to understand the influence of genetic variants on placental transporter and enzyme expression and function with regards to fetal opiate exposure. It is possible that with improved knowledge of genotype-phenotype correlation, the most at risk fetuses could be identified prenatally and appropriate maternal medication adjustments could be made. Lastly, interindividual variability in fetal likelihood to develop NAS given a similar exposure and the response to postnatal treatment is multifactorial. The intensity of in utero exposure, fetal / neonatal opiate disposition, the dynamic postnatal clearance and pharmacodynamic effects in the central nervous system all play a role in an individual infant's risk of NAS and the response to postnatal opiate therapy. As discussed in a recent commentary²⁶ on changes in drug disposition during pregnancy, the most promising path forward is to integrate findings from in vitro studies, animal studies, and in vivo human clinical studies to create a more complete understanding of maternal drug disposition and fetal exposure (Figure 4).

Neonatal Abstinence Syndrome carries long term morbidity, and the current empiric approaches used to choose maternal drug and dose, decisions to wean, identifying the infants most at risk, and postnatal treatment paradigms could all use improvements towards more personalized therapies in order to potentially mitigate these morbidities. A recent article compares the early neurodevelopmental outcomes of infants exposed to heroine, methadone and other opiates *in utero* with age matched Bayley-III standard controls. Mean scores for

language (82.12 vs. 100), motor (96.25 vs. 100) and cognition (90.18 vs. 100) were all statistically significantly lower in the opiate exposed group¹. In addition, infants exposed prenatally to opiates are shown to have alterations in attention⁷³ and increased childhood behavioral problems⁷⁴. Infants of opiate dependent women are exposed to varying levels of drug during a critical window of brain development, and a more tailored understanding of placental opiate transfer and a more individualized approach to maternal treatment founded in known genetic risk factors may improve long term outcomes for these infants.

One example of the promise of this knowledge includes the ability to prenatally genotype a fetus, and thus the placenta, and identify genetically high risk placental factors or fetal factors that would prompt a change in the maternal care. There are recent and rapidly progressing advances in non-invasive fetal genotyping⁷⁵, and the fetal genotype largely encodes the placenta. This technology has potential to change the field of Obstetric and Perinatal Pharmacology. If we can understand genetic alterations in placental metabolism and transport, and this understanding is complemented with the ability to know a placental genotype early in pregnancy, maternal medications can be modified to minimize fetal exposure to harmful exogenous compounds.

Prenatal opiate exposure is increasing and the genetic underpinnings of placental opiate transfer and neonatal susceptibility to NAS and response to therapy for NAS are currently very poorly understood. In order to move the field of maternal opiate therapy and medical management of NAS forward, a deeper understanding of the variability in propensity to develop NAS and its resultant severity are necessary. The mechanisms which underlie maternal dose-exposure correlations, placental opiate metabolism and transport, and fetal / neonatal opiate disposition and response likely all play some role in the complex process of an infant withdrawing from opiates in the first weeks of life after gestational opiate exposure. Given the evidence presented in this review of the known clinically significant polymorphisms in the genes involved, understanding the genetic underpinnings of these mechanisms is an important next step in clinical research for the field of NAS and perinatal opiate pharmacology. In the future, all of these factors could be combined into a comprehensive systems-based model to predict risk and individualize treatment of NAS in affected newborns.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Factors in maternal to fetal opiate transfer with known genetic variability

Figure 2. Ratio of fetal concentration to maternal plasma concentrations by MDR1 genotype
 Smit JW, Huisman MT, van Tellinghen O, Wiltshire HR, Schinkel AH. Absence or pharmacological blocking of placental P-glycoprotein profoundly increases fetal drug exposure. *The Journal of Clinical Investigation*. Nov 1999;104(10):1441-1447.

Figure 3. Changes in MDR1 mRNA with increasing gestation in human placenta

Sun M, Kingdom J, Baczyk D, Lye SJ, Matthews SG, Gibb W. Expression of the multidrug resistance P-glycoprotein, (ABCB1 glycoprotein) in the human placenta decreases with advancing gestation. *Placenta*. Jun-Jul 2006;27(67):602-609.

In vivo human clinical studies

- Probe studies to address enzyme specific changes
- PBPK modeling of changes in probe and clinically relevant drug disposition
- Analysis of endogenous signaling molecules and their concentrations throughout gestation
- PBPK models of organ specific mechanisms

Elaboration of mechanisms

Quantitative and qualitative prediction of pregnancy DMPK

Safety studies, rationalization of changes

Correlation of *in vivo* changes and integration of mechanisms

In vitro evaluation

cell lines, hepatocytes and other tissue preparations

- Enzyme specific studies of regulation and existence of induction/down-regulation
- Potency and efficacy of hormones and other regulators
- Enzyme specific mechanisms

Confirmation of mechanisms, and integration with pregnancy

Mechanistic hypotheses

Animal studies

- Determine the effect of pregnancy on specific enzyme and transporter activity/expression
- In vivo mechanistic studies of gene regulation by hormones and other compounds
- Fetal exposure studies and toxicology

Figure 4. Overview of the integration of in vitro and in vivo data into understanding of drug disposition during pregnancy

Isoherranen N, Thummel KE. Drug metabolism and transport during pregnancy: How does drug disposition change during pregnancy and what are the mechanisms that cause such changes? *Drug metabolism and disposition: the biological fate of chemicals*. 2013; 41(2): 256-262.

Table 1
Example Genetic Considerations for Drugs Commonly Implicated in Neonatal Abstinence Syndrome

(A) Methadone	Example Gene(s) Involved	Common Genetic Variants Associated and/or Effect on Pharmacokinetics	Potential Therapeutic Implications
Maternal Drug Exposure	<i>CYP2B6</i>	<i>CYP2B6</i> *6 <ul style="list-style-type: none"> Decreased CYP2B6 expression Higher methadone exposure in mother and fetus 	<ul style="list-style-type: none"> Lower maternal dose of methadone
Pregnancy and Placenta	<i>ABCB1</i> <i>CYP19</i>	<i>ABCB1</i> *2 <ul style="list-style-type: none"> Increased MDR1 efflux activity Lower drug exposure to the fetus <i>CYP19</i> <ul style="list-style-type: none"> Prominent expression in placenta Metabolism decreases exposure to parent drug in mother and fetus 	<ul style="list-style-type: none"> Infant at lower risk of NAS Methadone may induce MDR1, complicates genetic influence May need increased dose with gestational age to achieve therapeutic benefit for mother
Fetal/Neonatal Effect	<i>OPRM1</i>	<i>OPRM1</i> 118 A>G <ul style="list-style-type: none"> Reduced need for pharmacological treatment of NAS 	<ul style="list-style-type: none"> Communicate prognosis with parents
(B) Oxycodone	Example Gene(s) Involved	Common Genetic Variants Associated and/or Effect to Pharmacokinetics	Potential Therapeutic Considerations
Maternal Drug Exposure	<i>CYP3A</i> <i>CYP2D6</i>	<i>CYP3A4</i> *22 <ul style="list-style-type: none"> Decreased expression and activity Higher exposure of parent drug to mother and fetus <i>CYP2D6</i> <ul style="list-style-type: none"> Numerous variants Poor metabolizers with highest exposure of parent drug, while ultra-rapid metabolizers have the highest exposure to metabolites. 	<ul style="list-style-type: none"> Maternal genotyping predictive of fetal exposure and risk of NAS

(A) Methadone	Example Gene(s) Involved	Common Genetic Variants Associated and/or Effect on Pharmacokinetics	Potential Therapeutic Implications
Pregnancy and Placenta	<i>CYP3A</i> <i>CYP2D6</i>	<ul style="list-style-type: none"> Maternal CYP3A and CYP2D6 expression and activity increase during pregnancy 	<ul style="list-style-type: none"> Induction of CYP3A and CYP2D6 during pregnancy can alter parent-to-metabolite ratio exposure
Fetal/Neonatal Effect	<i>OPRM1</i>	<i>OPRM1</i> 118 A>G <ul style="list-style-type: none"> Reduced need for pharmacological treatment of NAS 	<ul style="list-style-type: none"> Communicate prognosis with parents
(C) Buprenorphine	Example Gene(s) Involved	Common Genetic Variants Associated and/or Effect to Pharmacokinetics	Therapeutic Considerations
Maternal Drug Exposure	<i>CYP3A</i> <i>CYP2C8</i>	<i>CYP3A4</i> *22 <ul style="list-style-type: none"> Decreased expression and activity Higher exposure of parent drug to mother and fetus <i>CYP2C8</i> *2, <i>CYP2C8</i> *3, <i>CYP2C8</i> *4 <ul style="list-style-type: none"> Most variants have equal or lower activity compared to wild-type 	<ul style="list-style-type: none"> Maternal genotyping predictive of fetal exposure and risk of NAS Maternal genotyping predictive of fetal exposure and risk of NAS
Pregnancy and Placenta	<i>CYP19</i> <i>UGT2B7</i>	<i>CYP19</i> <ul style="list-style-type: none"> Prominent expression in placenta Metabolism decreases exposure to parent drug in mother and fetus <i>UGT2B7</i> <ul style="list-style-type: none"> Activity does not appear to change 	<ul style="list-style-type: none"> May need increased dose with gestational age to achieve therapeutic benefit for mother Less phase II metabolism relative to phase I may result in increased active metabolite exposure
Fetal/Neonatal Effects	<i>OPRM1</i> <i>UGT2B7</i>	<i>OPRM1</i> 118 A>G <ul style="list-style-type: none"> Reduced need for pharmacological treatment of NAS <i>UGT2B7</i> <ul style="list-style-type: none"> Expression and activity not fully developed in fetus / neonate Exposure of parent and metabolite will be higher at younger post-natal age 	<ul style="list-style-type: none"> Communicate prognosis with parents Ontogeny of enzyme function will dictate clearance of active metabolite