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The impact of drug metabolizing enzyme polymorphisms on outcomes after antenatal corticosteroid use

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Abstract

Objective—To determine the impact of maternal and fetal single nucleotide polymorphisms (SNPs) in key betamethasone (BMZ) pathways on neonatal outcomes.

Study design—DNA was obtained from women given BMZ and their infants. Samples were genotyped for 73 exploratory drug metabolism and glucocorticoid pathway SNPs. Clinical variables and neonatal outcomes were obtained. Logistic regression analysis using relevant clinical variables and genotypes to model for associations with neonatal respiratory distress syndrome (RDS) was performed.

Results—109 women delivering 117 babies were analyzed. Sixty-four babies (49%) developed RDS. Multivariable analysis revealed that RDS was associated with maternal SNPs in *CYP3A5* (OR 1.63, 95%CI 1.16–2.30) and the glucocorticoid receptor (OR 0.28, 95%CI 0.08–0.95) and fetal SNPs in *ADCY9* (OR 0.17, 95%CI 0.03–0.80) and *CYP3A7*1E* (rs28451617, OR 23.68, 95%CI 1.33–420.6).

Conclusion—Maternal and fetal genotypes are independently associated with neonatal RDS after treatment with BMZ for preterm labor.

Keywords

betamethasone; neonatal respiratory distress syndrome; preterm birth; pharmacogenetics

Introduction

One of the most important interventions developed for improving neonatal outcomes in preterm birth is the administration of antenatal corticosteroids.¹ Decreasing preterm neonatal mortality and morbidity, antenatal corticosteroids are routinely administered to women with anticipated/threatened preterm delivery. However, not all neonates receive the same benefit. Differences in neonatal respiratory outcomes are seen in different ethnic groups,

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independent of gestational age, weight, and other socio-demographic factors.²⁻⁴ Additionally, respiratory distress syndrome (RDS) related mortality has a racial disparity that cannot be explained by demographic characteristics.³

Pharmacogenetics attempts to clarify differences in response to drug therapy. Various ethnic groups have different rates of genetic polymorphisms in genes that play a major role in day-to-day functions within the body.⁵ Drug metabolizing enzymes, transporters, and receptors contain genetic variations that can explain differences in response and side effects to many drugs, notably cyclosporine, warfarin, and clopidogrel.⁶⁻⁸ In these cases and others, single nucleotide polymorphisms (SNPs) in key drug metabolizing enzymes and receptor pathways can have a profound impact on drug concentrations and response.

Glucocorticoids like betamethasone (BMZ) and dexamethasone are metabolized by the cytochrome P450 (CYP) 3A family of enzymes.⁹ SNPs in this enzyme family are identified that impact the drug concentrations of many compounds. Additionally, SNP variants in the glucocorticoid receptor pathway have been associated with treatment differences in response to corticosteroids in asthmatics.¹⁰⁻¹² We are unaware of any study on the pharmacogenetic impact of SNP variants in the outcomes from BMZ use in pregnancy.

The objective of this project was to determine the impact of genetic polymorphisms in the drug metabolism and glucocorticoid pathways of BMZ on neonatal outcomes. Additionally, as pregnancy is a unique environment where both the maternal and fetal/placental compartments play a role in drug metabolism, we sought to determine the impact on these outcomes analyzing both the maternal and fetal genotypes.

Materials and Methods

Study Population

Women who were admitted to the hospital with threatened preterm labor and who received at least one dose of BMZ were recruited for this study. Informed consent was obtained from all subjects. Subjects were recruited at any of the hospitals associated with the Indiana University School of Medicine Department of OB/GYN. Women had to be at least 18 years old and at least 23 weeks gestation. Women were excluded if they had a known fetal anomaly or were unable to give consent. Standard neonatal resuscitation program protocols were followed by the neonatal provider teams at delivery. The neonatal outcome of RDS was diagnosed by the pediatricians following standard NICHD Neonatal Research Network criteria.¹³ The project was approved by the governing IRB.

Sample Collection and Processing

Blood Samples—Maternal whole blood was obtained on admission or during her hospital course. If she was unable to give a blood sample for some reason, we obtained a saliva sample as below for DNA isolation. Umbilical cord blood samples were obtained immediately following delivery in K₂EDTA vacutainers by trained cord blood collectors. All whole blood samples were mixed by inversion 8–10 times after being drawn and then stored at –80°C until DNA isolation.

Saliva Samples—If maternal blood was not obtained, she was asked to provide a saliva sample using the Oragene® saliva kit collection. If umbilical cord blood was not acquired at the time of delivery because of an off-hour delivery when the study personnel were not available, a trained member of the research team obtained buccal swabs from the neonate. For buccal cell collection we used Oragene® saliva kit collection with cotton swabs. Oragene® kits are shown to reduce microbial contamination and provide immediate

stabilization of samples, allowing it to be stored at room temperature for years without processing or DNA degradation. Oragene® “Saliva collection with cotton swabs or buccal brushes” protocol was followed and 5 swabs were obtained with each collection. Kits were then stored at room temperature. Prior to purification the Oragene saliva sample kits were briefly mixed by gentle inversion and incubated for a minimum of 2 hours in an air incubator set to 50° C. To remove saliva from buccal swabs manufacturer protocols for “DNA Recovery from Saliva Sponges” were followed.

DNA Isolation

DNA was extracted from blood samples using the QIAamp® DNA mini kits (Qiagen Inc., Valencia, CA). Manufacturer spin protocol instructions were followed for all kits. When manufacturer protocols listed steps for highly concentrated DNA those steps were followed. Isolated DNA was transferred into cryovials and all samples were stored at –80° C until quantification.

DNA was purified from saliva and buccal swab samples using the Oragene® Kit (DNA Genotek) manufacturer recommendations. DNA isolation was done according to manufacturer instructions. Samples were frozen at –80° C until quantification.

DNA Quantification

Concentration of double-stranded DNA in our samples was determined using a Quant-iT dsDNA Broad Range or High Sensitivity assay Kit and Qubit Fluorometer (Invitrogen, Carlsbad, California.) This system utilizes a fluorescent nucleic acid stain to accurately and specifically measure dsDNA at highly sensitive levels. Maternal DNA yields were similar for blood and saliva. Neonatal DNA yields from buccal swabs were less than from umbilical cord blood but were still adequate for the analysis (data not shown).¹⁴

Genotyping

Seventy-three SNPs were genotyped using a combination of methods specific to the desired SNP. Genotypic designations assigned from assayed SNPs in *CYP3A4*, *CYP3A5*, *CYP3A7*, Sulfotransferase (*SULT*), Multi-Drug Resistance gene-1 (*ABCB1*), Glucocorticoid Receptor (GR) and associated pathway gene assays are listed in Table 1. The SNPs were selected based on known metabolism pathways of glucocorticoids and prior work on relevant SNPs for glucocorticoid response in asthma.^{10–12, 15, 16} The individuals were genotyped for the majority of SNPs using the OpenArray™ Taqman™ genotyping platform (Applied Biosystems, Foster City, CA, USA). A high throughput genotyping 32 SNP chip was utilized for some of the SNPs. For SNPs that did not have valid OpenArray™ assays, predesigned and commercially available Taqman or fragment analysis real-time polymerase chain reaction (PCR) assays were used following manufacturer published methods (Applied Biosystems, Foster City, CA, USA). For other SNPs that did not have predesigned assays (namely *CYP3A7* and GR), Sanger dideoxy-DNA bidirectional sequencing using high throughput capillary sequencing instrumentation was performed (<http://polymorphicdna.com/reeqvardisc.html>). *SULT1A1* copy number variation was determined using semi-quantitative PCR followed by fragment analysis. For each SNP, once a platform was chosen, all samples were genotyped using the same platform.

Statistical Data Analysis

Maternal clinical variables of age, weight, BMI, number of doses of BMZ, time from BMZ to delivery, status of membranes at admission, and the number of fetuses were recorded as potential cofactors. The gestational age at delivery, birth weight, and Apgars were recorded. Neonatal outcomes of RDS, details of any respiratory or oxygen support after birth,

surfactant use, bronchopulmonary dysplasia (BPD), necrotizing enterocolitis (NEC), intraventricular hemorrhage (IVH), and death were all recorded. The primary outcome of interest was RDS.

While assessing the associations between the clinical and genetic predictors and phenotypes, we usually start from their one-on-one relationships, because the analysis can be quick and efficient. This univariate analysis is routinely performed and reported in pharmacogenetic research. For the categorical phenotypes, i.e. RDS, chi-square or Fisher's exact test were employed to test the phenotype-genotype associations. Results are presented in percentage incidence, Odds Ratios (OR) and 95% confidence intervals (CI). Since many clinical variables may influence the development of outcomes such as RDS, we rigorously tested our genetic hypothesis through multivariate analysis. Demographic and delivery information listed above were analyzed as independent variables in a multivariate analysis. The variables included in the model were: specific SNP genotype, maternal age, maternal race, estimated gestational age (EGA) at delivery, birth weight, infant gender, cesarean delivery (vs. vaginal delivery), EGA at first dose of BMZ, and the presence of chorioamnionitis. This allowed for a calculation of how much of the outcome was explained by the genotype allele when controlling for clinical.

Genotype was analyzed in the model in different ways. Allelic models assay for each of the 3 possible allele combinations separately. The dominant model assumes that one or two copies of the risk allele (variant) lead to increased RDS risk. The recessive model assumes that the two copies of risk variant develop risk of RDS. The trend model (or additive model) looked at an "allele-dose" effect where the more variant alleles that were present were tested for associations with risk of disease. A CYP3A5 genotype score was calculated based on the individual SNPs to categorize the mothers as either expressing or not expressing CYP3A5. Women who possess two $*3$ allele do not express CYP3A5 where as those who are $CYP3A5^{*1/*1}$, $*1/*3$, $*1/*6$, or $*1/*7$ do demonstrate some CYP3A5 activity.¹⁷

Receiver-Operator Curves (ROC) were generated based on the clinical predictors of RDS used in the logistic regression model. This was performed independently for the mother and infant. The clinical predictors used in the model were the same ones controlled for in the multivariable model. The relative additions of the genotypes to each model were assessed based on the change in the ROC curve with the addition of any statistically significantly associated SNP.

Statistical power

RDS incidence in treated preterm infants and CYP3A5 variant alleles are known to vary by race. Baseline incidence of RDS is estimated to be 75% in preterm Caucasian infants. We assume RDS incidence in African American infants reflects 40% observed in African infants. We further assumed the patient recruitment racial profile would be representative of the community serviced by IUSM; we expect to recruit 76% Caucasians and 24% African Americans.

Our power calculations were based on *CYP3A5* $*1$, $*3$, and $*6$ alleles, with assumed frequencies of 10%, 90%, and 0% in Caucasians and 35%, 50%, and 15% in African Americans and genotypes in Hardy-Weinberg proportions. A sample size of 76 Caucasian infants would provide 83% power to demonstrate a baseline RDS incidence difference of 34.7% between infants carrying at least one $*1$ allele and infants homozygous or heterozygous for $*3$ or $*6$ variants. For African American infants, a sample size of 24 will have 86.8% power to demonstrate a baseline RDS incidence difference of 66.7% using the same comparison. These sample size calculations are based on allele incidence rates above and the ethnic diversity of our population. Power analysis was conducted with S-Plus

version 6.2 (Insightful Corp, Seattle, WA). Comparisons are based on the *CYP3A5* *1 allele, which expresses large amounts of CYP3A5, resulting in increased enzymatic activity. This would hypothetically lead to a lower amount of drug at the target tissue and a higher rate of RDS. The association of genetic variants in *CYP3A7*, *MDR-1*, *SULT* enzymes, and the GR pathway genes were also tested but the *a priori* power calculation was based on the more fully studied CYP3A5 enzymes. We planned to recruit 150 women to the study, anticipating that we would be unable to obtain complete maternal-child pairs of samples on all subjects and to ensure that we would have at least 100 complete pairs with adequate racial representation.

Results

General clinical population outcomes

Of the 150 women consented for the study, 32 subjects did not have any samples collected due to hospital discharge with later delivery at a different facility, later subject withdrawal of consent to obtain infant samples, or inability to obtain infant samples for DNA. Two subjects withdrew consent after initially agreeing to participate. This left 116 women in the cohort who delivered 131 babies (14 twins, 1 triplet pregnancy). Seven subjects were missing maternal DNA and 14 were missing neonatal DNA in adequate quantities, leaving 109 women with adequate samples and 117 complete maternal-neonatal pairs for complete analysis.

The mean maternal age was 26.5 ± 5.7 years. The mean gravidity was 3.0 ± 2.0 and mean parity was 1.5 ± 1.5 . Nineteen women (16.4%) were Hispanic ethnicity. Racial distribution was white ($n=58$, 54%), black (36, 33%), other (11, 10%), mixed (2, 2%), and Indian (1, 1%). Paternal ethnicity was Hispanic for 17 (16%) of subjects. Paternal race was white (46, 41%), unknown (29, 26%), black (27, 24%), other (9, 8%), and Indian (1, 1%). Thirteen women had gestational diabetes (11%). The gestational age at receiving the first dose of BMZ was 28.8 ± 3.3 weeks gestation and the mean gestational age at delivery was 32.2 ± 3.9 weeks. The admission diagnoses were preterm labor (37%), preterm premature ruptured membranes (35%), preeclampsia (15%), other (10%), and placenta previa (3%). Tocolytic medications were given to 35% of the women, with nifedipine and magnesium sulfate being the most commonly used (45% and 42% respectively of those receiving tocolytics). “Rescue” doses of BMZ were used in 8 women. The mean number of days from first dose of BMZ to delivery was 23.4 ± 22.2 . Sixty-four (55%) women delivered babies vaginally.

The mean birth weight of the babies was 1913 ± 785 grams. Half of the babies were male (51%). Median 1 and 5 minute Apgars were 7 and 8 respectively. Sixty-four babies were diagnosed with RDS (49%) and subsequently 19 were diagnosed with BPD (14.5%). Necrotizing enterocolitis developed in 9 (7%) babies, IVH in 10 (7.6%), and neonatal death occurred in six (4.6%) of the babies. The gestational age at birth of the babies who died ranged from 24–29 weeks. Neonatal sepsis occurred in 23 (17.6%) of the babies. 39 babies (30%) were intubated, 70 (53.4%) placed on CPAP, 34 (26%) on vapotherm, and 38 (29%) treated with nasal cannula room air for respiratory support. Surfactant was given to 36 (27.5%) of the babies. Mean total hospital days was 40 ± 39 and mean total days of respiratory support in hospital was 19 ± 33 .

Pharmacogenetic results for BMZ

All SNPs assayed were in Hardy-Weinberg equilibrium ($p=0.00083$) after correction for multiple comparison. SNP allele frequencies are listed in Table 1. For *SULT1A1* copy number variants, 27.9% of the mothers and 22.9% of the babies had 3 or more copies. The only single maternal SNP associated with the outcome of neonatal RDS was maternal

NR3C1 (GR, rs41423247, OR 0.28, $p=0.03$ in a dominant model, $p=0.04$ for a gene-dose effect model). Neonatal *ADCY9* and *CYP3A7*1E* were associated with RDS (*ADCY9*, rs2230739, OR 0.17, $p=0.02$ dominant model; *CYP3A7*1E*, rs28451617, OR 23.68, $p=0.03$). *MDR1* (*ABCB1*) haplotype analysis for exons 26/21/12 demonstrated that the respective variant/wild-type/variant haplotype was associated with a protective effect against RDS (maternal haplotype OR 0.41, $p=0.03$; fetal haplotype OR 0.29, $p=0.01$).

Controlling for the EGA at delivery, infant gender, birth weight, having a cesarean delivery, race, chorioamnionitis, and EGA at first dose of BMZ, Table 2 displays the SNPs that are significantly associated with RDS. Maternal *CYP3A5* activity (OR 1.63, 95% CI 1.16–2.30) and GR (rs41423247, OR 0.28, 95% CI 0.08–0.95) were both significantly independently associated with RDS. SNPs in the *IPO13* gene (rs2486014, OR 4.15, $p=0.06$), *CRHR1* (rs242941, OR 0.34, $p=0.08$), and *CYP3A7*1E* (rs28451617, OR 6.16, $p=0.09$) all demonstrated trends toward associations with RDS. Neonatal SNP genotypes of *ADCY9* (rs2230739, OR 0.17, 95% CI 0.03–0.80) and *CYP3A7*1E* (rs28451617, OR 23.68, 95% CI 1.33–420.6) were associated with RDS in the multivariable model.

Adding SNPs to clinical predictors of RDS did not improve the area under the ROC curve for maternal SNPs (Figure 1). Maternal clinical predictors accounted for 92.3% of RDS cases. Adding the significant maternal SNPs did not increase the area under the curve (AUC) significantly (clinical + GR=92.8%, clinical+*CYP3A5*=93.3%). However, adding neonatal genotype to the clinical predictive model for RDS did improve the ability to predict RDS in the infant (clinical=90.2%, clinical+*ADCY9*=92.3%, $p<0.05$, clinical + *CYP3A7*1E*=92.2%, $p<0.05$).

Comment

This study reveals that genetic variants in key BMZ pathway genes are associated with the incidence of RDS in the neonates. The large odds ratios indicate that drug metabolism of BMZ by both the mother and placenta (represented by the fetal genotype) may play a large role in the response to BMZ therapy. Thus, these SNPs may be responsible for some of the outcome differences seen in other larger trials. As providers search for ways to improve therapy for anticipated preterm delivery, understanding the role of pharmacogenetics in pregnancy therapeutics is important.

The *CYP3A* family of enzymes is responsible for the majority of drug metabolism in humans.^{18, 19} This enzyme activity is increased somewhat in pregnancy.^{20, 21} Some *CYP3A7* SNPs allow the enzyme to persist into adulthood.²² This increased activity of *CYP3A7* would further increase the metabolic action of the placenta on BMZ given to pregnant women, possibly reducing the amount available to get through the placenta to the fetus. Thus, less would be available to stimulate lung maturity, as evidence by the higher rate of RDS seen in this study for subjects with the *CYP3A7*1E* polymorphism. While we know that *CYP3A7* is expressed more in fetal life,²³ it is interesting that the *1E variant in the fetus was so strongly associated with RDS with an OR of 23.68. As we are unaware of any prior study analyzing the impact of fetal *CYP3A7*1E* genotype on the fetal-placental metabolism of drugs such as BMZ, this finding may open the door to further study aimed at understanding the role of fetal genotype on the metabolism of drugs given to mothers.

The *ABCB1* gene that encodes for p-glycoprotein efflux transporter was found to be protective against RDS. The haplotype analysis for the three *ABCB1* exons has been performed for other drugs with mixed associations.^{8, 24, 25} In general, the more variant alleles, the less p-glycoprotein activity.²⁵ Thus haplotypes with more variant alleles would have diminished activity of the placental efflux transporter. In turn, more of the drug would

be allowed to cross the placenta to act on the fetus to stimulate lung maturation. While this may be somewhat oversimplified, it has physiologic plausibility and should be further studied.

The glucocorticoid receptor and pathways have been well characterized in the asthma literature.^{11, 12, 16} We are unaware of any other investigation into the genetics of this pathway and BMZ response from antenatal corticosteroids. Variants associated with improved glucocorticoid response in asthma treatment were also found to be associated with lower rates of RDS in this study population. Variants in the GR (NR3C1) have been found to show hyper-responsiveness to glucocorticoid binding.²⁶ Thus, for those with the variant who receive BMZ, there may be an improved fetal response, leading to improved lung function and less RDS in the neonate. The adenylate cyclase polymorphism identified as significantly protective against RDS, as well as the *IPO13* and *CRHR1* SNPs, have been associated with improved lung function after steroid treatment in asthmatics.^{11, 15, 27} Polymorphisms in both the maternal glucocorticoid receptor and in steroid pathway genes in the fetus and placenta that lead to improved response to antenatal corticosteroids may aid in the individualizing of antenatal corticosteroid therapy. There are questions about the optimal dose and schedule of BMZ.^{28, 29} This current study extends the findings in the complex respiratory disease of asthma to another complex respiratory disease in RDS. Further investigations into the genetic factors that may impact RDS incidence and severity are warranted.

As an initial investigation into pharmacogenetic impact on BMZ therapy, this study has some limitations. While the sample size was large enough to demonstrate significant genotype effects in the multivariate analysis, the p values would not stand up to adjustments for multiple comparisons with the number of genotypes analyzed. However, controlling for the most important clinical predictors of RDS and other adverse outcomes allows for isolation of the genotype effect. Coupled with the biological plausibility of the results, the significant associations will be utilized in a larger trial designed to overcome this limitation. A larger study would also allow for more precise analysis of the less frequent outcomes. While the addition of genotype to clinical predictors in Figure 1 significantly improved the area under the ROC curve, the small improvement may have limited clinical utility. We do not have pharmacokinetic data on all of these subjects. As most of them delivered >48 hours after the BMZ was given, the concentrations of BMZ would be expected to be undetectable.³⁰ An analysis of the genetic implications on outcomes when delivery occurs shortly after initiating BMZ therapy that will incorporate pharmacokinetic data is ongoing. In addition, it is possible that the polymorphisms identified in the glucocorticoid pathway as being protective against RDS exert an effect on this outcome independent of BMZ administration. As an independent genetic association with RDS has not been reported in the literature to our knowledge, replication or analysis of this question in an existing data set is needed. There was also no control group of preterm deliveries for women who did not receive BMZ. As very few women deliver at less than 34 weeks gestation without receiving BMZ in our institution, obtaining this control group would be difficult and it would be unethical to withhold BMZ from these women.

Pharmacogenetics in pregnancy therapeutics is in its infancy. As has been shown in other fields, however, we believe there is tremendous potential in obstetrics to utilize genotype to help guide and individualize therapy. For instance, knowing the maternal genotype for *CYP3A7*1E* may prompt the use of higher doses of BMZ or an alternative strategy for fetal lung maturation. While this pharmacogenetics-informed therapy is still far off in obstetrics, sophisticated dosage modeling techniques are established to help take data like those presented here and make recommendations for individualized pharmacotherapy.³¹

In conclusion, this initial investigation into the pharmacogenetics of BMZ therapy demonstrated that genotypes of drug metabolizing enzymes and the glucocorticoid pathway play independent roles in neonatal RDS. Knowing the patient genotypes may improve the ability to optimize both maternal and neonatal outcomes. Obstetricians must be aware of the progress of research in this field and more therapeutic studies in pregnancy should report genotype data. Only through an integrated translational research effort can we unlock the full potential of pharmacogenetics in pregnancy therapeutics.

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References

1. ACOG practice bulletin. Management of preterm labor. Number 43, May 2003. *Obstet Gynecol.* 2003; 101:1039–47. [PubMed: 12738177]
2. Frisbie WP, Forbes D, Pullum SG. Compromised birth outcomes and infant mortality among racial and ethnic groups. *Demography.* 1996; 33:469–81. [PubMed: 8939419]
3. Frisbie WP, Song SE, Powers DA, Street JA. The increasing racial disparity in infant mortality: respiratory distress syndrome and other causes. *Demography.* 2004; 41:773–800. [PubMed: 15622954]
4. Kavvadia V, Greenough A, Dimitriou G, Hooper R. Influence of ethnic origin on respiratory distress syndrome in very premature infants. *Arch Dis Child Fetal Neonatal Ed.* 1998; 78:F25–8. [PubMed: 9536836]
5. Ensom MH, Chang TK, Patel P. Pharmacogenetics: the therapeutic drug monitoring of the future? *Clin Pharmacokinet.* 2001; 40:783–802. [PubMed: 11735602]
6. Scott SA, Sangkuhl K, Gardner EE, et al. Clinical Pharmacogenetics Implementation Consortium Guidelines for Cytochrome P450-2C19 (CYP2C19) Genotype and Clopidogrel Therapy. *Clin Pharmacol Ther.* 2011; 90:328–32. [PubMed: 21716271]
7. Grossniklaus D. Testing of VKORC1 and CYP2C9 alleles to guide warfarin dosing. Test category: pharmacogenomic (treatment). *PLoS Curr.* 2010; 2
8. Crettol S, Venetz JP, Fontana M, Aubert JD, Pascual M, Eap CB. CYP3A7, CYP3A5, CYP3A4, and ABCB1 genetic polymorphisms, cyclosporine concentration, and dose requirement in transplant recipients. *Ther Drug Monit.* 2008; 30:689–99. [PubMed: 18978522]
9. Perloff MD, von Moltke LL, Cotreau MM, Greenblatt DJ. Unchanged cytochrome P450 3A (CYP3A) expression and metabolism of midazolam, triazolam, and dexamethasone in mdr(–/–) mouse liver microsomes. *Biochem Pharmacol.* 1999; 57:1227–32. [PubMed: 10230766]
10. Hawkins GA, Lazarus R, Smith RS, et al. The glucocorticoid receptor heterocomplex gene STIP1 is associated with improved lung function in asthmatic subjects treated with inhaled corticosteroids. *J Allergy Clin Immunol.* 2009; 123:1376–83. e7. [PubMed: 19254810]
11. Tantisira KG, Lake S, Silverman ES, et al. Corticosteroid pharmacogenetics: association of sequence variants in CRHR1 with improved lung function in asthmatics treated with inhaled corticosteroids. *Hum Mol Genet.* 2004; 13:1353–9. [PubMed: 15128701]
12. Weiss ST, Lake SL, Silverman ES, et al. Asthma steroid pharmacogenetics: a study strategy to identify replicated treatment responses. *Proc Am Thorac Soc.* 2004; 1:364–7. [PubMed: 16113459]
13. Stoll BJ, Hansen NI, Bell EF, et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. *Pediatrics.* 2010; 126:443–56. [PubMed: 20732945]
14. Lehmann AS, Haas DM, McCormick CL, Skaar TC, Renbarger JL. Collection of human genomic DNA from neonates: a comparison between umbilical cord blood and buccal swabs. *Am J Obstet Gynecol.* 2011; 204:362, e1–6. [PubMed: 21324432]

15. Raby BA, Van Steen K, Lasky-Su J, et al. Importin-13 genetic variation is associated with improved airway responsiveness in childhood asthma. *Respir Res.* 2009; 10:67. [PubMed: 19619331]
16. Tantisira K, Weiss S, Tantisira K, Weiss S. The pharmacogenetics of asthma treatment. *Curr Allergy Asthma Rep.* 2009; 9:10–7. [PubMed: 19063819]
17. Lamba JK, Lin YS, Schuetz EG, Thummel KE. Genetic contribution to variable human CYP3A-mediated metabolism. *Adv Drug Deliv Rev.* 2002; 54:1271–94. [PubMed: 12406645]
18. Cholerton S, Daly AK, Idle JR. The role of individual human cytochromes P450 in drug metabolism and clinical response. *Trends Pharmacol Sci.* 1992; 13:434–9. [PubMed: 1293869]
19. Thummel KE, Wilkinson GR. In vitro and in vivo drug interactions involving human CYP3A. *Annu Rev Pharmacol Toxicol.* 1998; 38:389–430. [PubMed: 9597161]
20. Hebert MF, Easterling TR, Kirby B, et al. Effects of pregnancy on CYP3A and P-glycoprotein activities as measured by disposition of midazolam and digoxin: a University of Washington specialized center of research study. *Clin Pharmacol Ther.* 2008; 84:248–53. [PubMed: 18288078]
21. Tracy TS, Venkataramanan R, Glover DD, Caritis SN. Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy. *Am J Obstet Gynecol.* 2005; 192:633–9. [PubMed: 15696014]
22. Smit P, van Schaik RH, van der Werf M, et al. A common polymorphism in the CYP3A7 gene is associated with a nearly 50% reduction in serum dehydroepiandrosterone sulfate levels. *J Clin Endocrinol Metab.* 2005; 90:5313–6. [PubMed: 15985487]
23. Leeder JS, Gaedigk R, Marcucci KA, et al. Variability of CYP3A7 expression in human fetal liver. *J Pharmacol Exp Ther.* 2005; 314:626–35. [PubMed: 15845858]
24. Kalabis GM, Petropoulos S, Gibb W, Matthews SG. Multidrug resistance phosphoglycoprotein (ABCB1) expression in the guinea pig placenta: developmental changes and regulation by betamethasone. *Canadian Journal of Physiology & Pharmacology.* 2009; 87:973–8. [PubMed: 19935905]
25. Leschziner GD, Andrew T, Pirmohamed M, Johnson MR. ABCB1 genotype and PGP expression, function and therapeutic drug response: a critical review and recommendations for future research. *Pharmacogenomics J.* 2006; 7:154–79. [PubMed: 16969364]
26. van Rossum EF, van den Akker EL. Glucocorticoid resistance. *Endocr Dev.* 2011; 20:127–36. [PubMed: 21164266]
27. Tantisira KG, Small KM, Litonjua AA, Weiss ST, Liggett SB. Molecular properties and pharmacogenetics of a polymorphism of adenylyl cyclase type 9 in asthma: interaction between beta-agonist and corticosteroid pathways. *Hum Mol Genet.* 2005; 14:1671–7. [PubMed: 15879435]
28. Jobe AH, Soll RF. Choice and dose of corticosteroid for antenatal treatments. *Am J Obstet Gynecol.* 2004; 190:878–81. [PubMed: 15118606]
29. Haas DM, McCullough W, Olsen CH, et al. Neonatal outcomes with different betamethasone dosing regimens: a comparison. *J Reprod Med.* 2005; 50:915–22. [PubMed: 16444891]
30. Ballard PL, Granberg P, Ballard RA. Glucocorticoid levels in maternal and cord serum after prenatal betamethasone therapy to prevent respiratory distress syndrome. *J Clin Invest.* 1975; 56:1548–54. [PubMed: 1202085]
31. Hebert MF, Ma X, Naraharisetti SB, et al. Are we optimizing gestational diabetes treatment with glyburide? The pharmacologic basis for better clinical practice. *Clin Pharmacol Ther.* 2009; 85:607–14. [PubMed: 19295505]

A.

B.

Figure 1.

ROC curves for maternal and fetal predictors of RDS

ROC- Receiver-operator curves. A= maternal variables and genotype. B=clinical variables and fetal genotype

Table 1

Genotypic designations assigned from assayed SNP and CNV assays, their characteristic mutations, and method of genotyping

Gene & Allele Designation*	RSID#	SNP Type	Location	BP Change	SNP allele frequency**
<i>OpenArray® platform</i>					
ABCB1 Exon 12	rs1128503	Synonymous	Chr. 7- 87179601	C>T	0.367/0.317
ABCB1 Exon 21	rs2032582	Nonsynonymous	Chr. 7- 87179616	G>T/A	0.548/0.505
ABCB1 Exon 26	rs1045642	Synonymous	Chr. 7- 87138645	C>T	0.450/0.409
ADCY9	rs2250739	Nonsynonymous	Chr. 16-4033436	A>G	0.278/0.297
CRH	rs12721511	Nonsynonymous	Chr. 8- 67089709	C>G	†
CRHR1	rs1876828	Intron	Chr. 17-43911525	A>G	0.126/0.161
CRHR1	rs242941	Intron	Chr. 17-43892520	G>T	0.402/0.383
CYP3A4	rs4987159	Synonymous	Chr. 7-99366068	C>T	0.016/0.027
CYP3A5* ₃	rs776746	Intron	Chr. 7-99270539	A>G	0.303/0.328
CYP3A7	rs2687133	Intron	Chr. 7-99332083	A>G	0.258/0.255
CYP3A7* ₂	rs2257401	Nonsynonymous	Chr. 7-99306685	C>G	0.269/0.218
GLCCII	rs37973	nearGene-5'	Chr. 7-8007876	A>G	0.376/0.349
IPO13	rs7412307	nearGene-5'	Chr. 1-44433864	C>G	0.409/0.389
IPO13	rs2301992	Intron	Chr. 1-44426823	A>G	†
IPO13	rs2301993	Intron	Chr. 1-44426025	C>T	0.400/0.400
IPO13	rs2428953	Intron	Chr. 1-44443459	C>T	0.238/0.234
IPO13	rs4448553	nearGene-5'	Chr. 1-4441189	A>G	0.369/0.359
IPO13	rs2486014	Intron	Chr. 1-44419546	A>G	0.116/0.107
IPO13	rs2240447	Synonymous	Chr. 1-44415414	A>G	0.438/0.417
IPO13	rs1990150	Intron	Chr. 1-44414127	C>G	0.244/0.212
IPO13	rs6671164	nearGene-5'	Chr. 1-44403489	G/T	0.433/0.406
IPO13	rs1636879	Intron	Chr. 1-44428680	C>G	†
NR3C1	rs41423247	Intron	Chr. 5-142778575	C>G	0.325/0.310
OLR1	rs3736233	Intron	Chr. 12-10313265	A>G	0.440/0.427

Gene & Allele Designation *	RSID#	SNP Type	Location	BP Change	SNP allele frequency **
STIP1/FERMT3	rs2236647	Intron	Chr. 11-63964605	C>T	0.482/0.500
SULT1A2	rs1136703	Nonsynonymous	Chr. 16-28607232	T>C	‡
TBX21	rs2240017	Nonsynonymous	Chr. 17-45810919	C>G	‡
TRAPPC5, FCER2	rs28364072	Intron	Chr. 19-7755285	A>G	0.315/0.280
DNA sequencing					
CRHR1	rs4564621	Intron	Chr. 17-43895501	C>G	0.124/0.168
CRHR1	rs2316763	Intron	Chr. 17-43895530	C>T	0.124/0.168
CRHR1	N/A	Intron	Chr. 17-43895531	G>A	0.000/0.000
CRHR1	rs242939	Intron	Chr. 17-43895579	A>G	0.149/0.143
CRHR1	rs2316764	Intron	Chr. 17-43895602	G>T	0.124/0.168
CRHR1	rs4277389	Intron	Chr. 17-43895653	A>G	0.124/0.168
CRHR1	rs66586071	Intron	Chr. 17-43893780	G>-	0.284/0.347
CRHR1	N/A	Intron	Chr. 17-43895671	T>-	0.000/0.010
CRHR1	rs80249281 A KA rs57839112	Intron	Chr. 17-43893790	-/A	0.000/0.000
CRHR1	rs11316767	Intron	Chr. 17-43893791	-/A	0.000/0.000
CRHR1	rs116593005	Intron	Chr. 17-4393810	C>T	0.008/0.010
CRHR1	rs149144271	Intron	Chr. 17-9167967	A>G	0.008/0.015
CRHR1	rs8077279	Intron	Chr. 17-43893884	C>T	0.012/0.000
CRHR1	rs16940655	Intron	Chr. 17-43893886	C>T	0.012/0.005
CYP3A7	rs45496695	nearGene-5'	Chr. 7-99332867	A>C	0.000/0.000
CYP3A7	rs45446698	nearGene-5'	Chr. 7-99332948	A>C	0.029/0.031
CYP3A7	rs113874418	nearGene-5'	Chr. 7-99332955	A>G	0.004/0.000
CYP3A7	rs11568826	nearGene-5'	Chr. 7-99332978	A>T	0.029/0.031
CYP3A7	rs45467892	nearGene-5'	Chr. 7-99332997	A>T	0.029/0.031
CYP3A7	rs45575938	nearGene-5'	Chr. 7-99332998	C>T	0.029/0.031
CYP3A7	rs45494802	nearGene-5'	Chr. 7-99333000	A>T	0.029/0.031
CYP3A7	rs11568824	nearGene-5'	Chr. 7-9933007	G>T	0.029/0.031
CYP3A7 *1B	rs45465393	nearGene-5'	Chr. 7-9933030	C>T	0.000/0.005

Gene & Allele Designation*	RSID#	SNP Type	Location	BP Change	SNP allele frequency**
CYP3A7*1C	rs11568825	nearGene-5'	Chr. 7-99332986	G>T	0.029/0.031
CYP3A7*1D	rs55798860	5'UTR	Chr. 7-99332807	C>T	0.000/0.000
CYP3A7*1E	rs28451617	5'UTR	Chr. 7-99332765	C>T	0.033/0.026
NR3C1	rs6189	Synonymous	Chr. 5-142780338	A>G	0.012/0.010
NR3C1	rs6190	Nonsynonymous	Chr. 5-142780337	A>G	0.012/0.010
NR3C1	rs72542738	Synonymous	Chr. 5-142780332	A>G	0.000/0.000
NR3C1	rs72481829	Nonsynonymous	Chr. 5-142780331	A>G	0.000/0.000
NR3C1	rs61759025	Synonymous	Chr. 5-142780264	A>G	0.000/0.000
NR3C1	rs79138720	Nonsynonymous	Chr. 5-14278056	A>C	0.000/0.000
NR3C1	rs6192	Nonsynonymous	Chr. 5-142780212	G>T	0.012/0.010
NR3C1	rs72558023	Synonymous	Chr. 5-142780206	A>G	0.000/0.000
NR3C1	rs72542745	Nonsynonymous	Chr. 5-142779432	A>G	0.000/0.000
NR3C1	rs72558022	Nonsynonymous	Chr. 5-142779369	C>G	0.000/0.000
NR3C1	rs13306589	Synonymous	Chr. 5-142779340	A>G	0.000/0.000
NR3C1	rs56149945	Nonsynonymous	Chr. 5-142779317	A>G>T	0.025/0.015
NR3C1	rs1800445	Nonsynonymous	Chr. 5-142779311	A>G	0.000/0.000
Custom PCR assay					
CYP3A4*1B	rs2740574	nearGene-5'	Chr. 7-99382096	A>G	0.211/0.259
CYP3A5*6	rs10264272	Synonymous	Chr. 7-99262835	C>T	0.041/0.040
CYP3A5*7	rs41303343	Frameshift	Chr. 7-99250393	A>T	0.074/0.045
SULT1A1*2	rs9282861	Nonsynonymous	Chr. 16-286171514	A>G	0.847/0.942
SULT1A1	rs3760091	5'UTR	Chr. 16-28620800	C>G>T	0.609/0.709
SULT1A1	rs750155	5'UTR	Chr. 16-28620572	C>T	0.726/0.674

SNP= single nucleotide polymorphism, N/A=not available, BP= base pair, PCR= polymerase chain reaction

ADCY9= adenylate cyclase 9, CRHR1= corticotropin releasing hormone receptor 1, CRH= corticotropin releasing hormone, CYP= cytochrome P450, GCC11= glucocorticoid induced transcript 1, IPO13= importin 13, NR3C1= nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor), OLR1= oxidized low density lipoprotein (lectin-like) receptor 1, STIP1/FERMT3= stress-induced-phosphoprotein 1/fermitin family member 3, SULT= sulfotransferase, TBX21= T-box 21, TRAPPC5/FCE2= Trafficking protein particle complex 5/Fc fragment of IgE, low affinity II receptor

* If applicable, location done according to genome build 37.1

SNP variant minor allele frequency in mothers/babies

^zThese assays failed on the Open Array® and were not assayed with custom PCR assays

Table 2

Multivariable analysis of genetic predictors of neonatal RDS

Genetic SNP	Rate of RDS (%) in wild type	Rate of RDS (%) in variant	OR(95%CI)	P value
<u>Maternal</u> *CYP3A5 (activity score)	0.0	51.3	1.63 (1.16–2.30)	0.005
*NR3C1 (rs41423247)	60.4	39.4	0.28 (0.08–0.95)	0.04
IPO13 (rs2486014)	42.7	60.0	4.15 (0.92–18.76)	0.06
CRHR1 (rs242941)	58.7	42.2	0.34 (0.10–1.15)	0.08
CYP3A7 *1E (rs28451617)	47.8	71.4	6.16 (0.71–53.24)	0.09
<u>Fetal</u> *ADCY9 (rs2230739)	58.3	46.5	0.17 (0.03–0.80)	0.02
*CYP3A7 *1E (rs28451617)	50.5	80.0	23.68 (1.33–420.6)	0.03
CYP3A5 *6 (rs10264272)	52.9	55.5	5.22 (0.73–37.44)	0.10

RDS= respiratory distress syndrome; CYP= cytochrome P450, OR= Odds Ratio, CI= confidence interval Multivariable analysis controlling for maternal age, maternal and paternal race, estimated gestational age (EGA) at delivery, birth weight, infant gender, cesarean delivery (vs. vaginal delivery), EGA at first dose of BMZ, and the presence of chorioamnionitis. Only those SNPs with a p value ≤ 0.10 are listed in the Table. The dominant genetic model was used for these calculations.

Data represent the rates of RDS in subjects with either homozygous wild type compared to the presence of a variant allele.

* statistically significant association