HHS Public Access

Author manuscript

Drug Alcohol Depend. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Drug Alcohol Depend. 2015 October 1; 155: 253–259. doi:10.1016/j.drugalcdep.2015.07.001.

Variations in Opioid Receptor Genes in Neonatal Abstinence Syndrome*

Elisha M Wachman[a], **Marie J Hayes**[b], **Richard Sherva**[c], **Mark S Brown**[d], **Jonathan M Davis**[e], **Lindsay A Farrer**[f], and **David A Nielsen**[g]

Elisha M Wachman: Elisha.Wachman@bmc.org; Marie J Hayes: mhayes@maine.edu; Richard Sherva: sherva@bu.edu; Mark S Brown: mbrown@emhs.org; Jonathan M Davis: jdavis@tuftsmedicalcenter.org; Lindsay A Farrer: farrer@bu.edu; David A Nielsen: nielsen@bcm.edu

[a]Pediatrics, Boston Medical Center, 771 Albany Street, Dowling 4109, Boston MA, 02118, US; Phone: 617-414-3690; Fax: 617-414-7297

[b]Graduate School of Biomedical Science & Engineering, University of Maine, Orono ME, 04469, US

[c]Biomedical Genetics, Boston University School of Medicine, 72 East Concord Street, Boston MA, 02118, US

[d]Pediatrics, Eastern Maine Medical Center, 489 State St, Bangor ME, 04401, US

[e]Pediatrics, The Floating Hospital for Children at Tufts Medical Center, 755 Washington Street, Boston MA, 02116, US

[f]Biomedical Genetics, Boston University School of Medicine, 72 E Concord Street, Boston MA, 02118, US

[g]Psychology, Baylor College of Medicine, 1 Baylor Plaza, Houston TX, 77030, US

Abstract

Background—There is significant variability in the severity of neonatal abstinence syndrome (NAS) due to *in-utero* opioid exposure. We wanted to determine if single nucleotide polymorphisms (SNPs) in key candidate genes contribute to this variability.

*Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:…

Contributors

The authors of this manuscript contributed to this research project in the following ways: 1) Initial concept (Dr. Wachman, Dr. Davis, Dr. Hayes, Dr. Brown); 2) Study design (Dr. Wachman, Dr. Sherva, Dr. Farrer, Dr. Nielsen, Dr. Hayes); 3) Subject recruitment, data collection and specimen collection (Dr. Wachman, Dr. Davis, Dr. Hayes, Dr. Brown); 4) Data analysis (Dr. Wachman, Dr. Sherva); 4) Interpretation of data (Dr. Wachman, Dr. Sherva, Dr. Farrer, Dr. Nielsen, Dr. Hayes); 5) Writing of initial draft of the manuscript (Dr. Wachman); 6) Final review and approval of the submitted manuscript (all authors).

Conflict of Interest

We would like to acknowledge the following funding sources for this research: Joel Alpert Foundation Grant to Dr. Wachman; National Institutes of Health (DA024806 to Dr. Hayes, R01DA032889-01A1 to Dr. Davis, DA018197-05 and MD Anderson's Cancer Center Support Grant DA026120 to Dr. Nielsen); Tufts Medical Center's Natalie Zucker and Susan Saltonstall Grants to Dr. Wachman; National Center for Advancing Translational Sciences (UL1 TR000073 through the Tufts Clinical and Translational Science Institute to Dr. Davis); Toomin Family Fund to Dr. Nielsen. The authors have no other conflicts of interest to disclose.

Methods—Full-term opioid-exposed newborns and their mothers (n=86 pairs) were studied. DNA was genotyped for 80 SNPs from 14 genes utilizing a custom designed microarray. The association of each SNP with NAS outcomes was evaluated.

Results—SNPs in two opioid receptor genes in the infants were associated with worse NAS severity: 1) The *PNOC rs732636* A *allele* (OR=3.8, p=0.004) for treatment with 2 medications and a longer hospital stay (LOS) of 5.8 days (p=0.01), and 2) The *OPRK1* rs702764 C allele (OR=4.1, p=0.003) for treatment with 2 medications. The *OPRM1* rs1799971 G allele (β= −6.9 days, p=0.02) and *COMT* rs740603 A allele (β= −5.3 days, p=0.01) were associated with shorter LOS. The *OPRD1* rs204076 A allele in the mothers was associated with a longer LOS by 6.6 days (p=0.008). Results were significant point-wise but did not meet the experiment-wide significance level.

Conclusions—These findings suggest that SNPs in opioid receptor and the *PNOC* genes are associated with NAS severity. However, further testing in a large sample is warranted. This has important implications for prenatal prediction and personalized treatment regimens for infants at highest risk for severe NAS.

Keywords

Neonatal Abstinence Syndrome (NAS); Genetics; single nucleotide polymorphisms (SNPs); opioids

1. INTRODUCTION

In the past decade, there has been a significant increase in both prescribed and illicit opioid use during pregnancy. Up to 1 in 5 women in the US are taking an opioid medication at some point while pregnant, leading to increasing rates of neonatal abstinence syndrome (NAS; Desai et al., 2014; Patrick et al., 2012; USDHHS, 2013). NAS represents a constellation of signs and symptoms due to infant withdrawal from *in utero* opioids and often requires prolonged hospitalization lasting from weeks to over a month, and extensive pharmacological therapy (Jansson et al., 2009). The incidence of NAS has tripled in the past decade, currently estimated between 3.4 – 8.8 per 1000 live births in the US (Hall et al., 2014; Patrick et al., 2012). Infants chronically exposed *in utero* to opioids are monitored in the hospital for 5–7 days for signs of withdrawal; of these, 60–80% typically require pharmacologic treatment with replacement opioids (Jansson et al., 2009). Morphine and methadone are considered the two standard of care options for first-line treatment, with on-going clinical trials to determine best practice and long-term outcomes (Backes et al., 2012; Brown et al., 2014; Hall et al., 2014; Hudak et al., 2012; Lee et al., 2015; Sarkar et al., 2006). Approximately 25% of infants have a particularly severe course of withdrawal, requiring adjunctive medication therapy in addition to first-line opioid replacement – most typically a barbiturate (phenobarbital) or a sympatholytic (clonidine; Jansson et al., 2009; Jones et al., 2010; Logan et al., 2013). Infants with this more severe phenotype of NAS typically have a longer duration of pharmacologic treatment with associated longer inpatient hospitalization (Wachman et al., 2011).

NAS remains a poorly understood syndrome with a variety of clinical factors contributing to its incidence and severity (Jones et al., 2010; Logan et al., 2013; Pritham et al., 2012).

Knowledge of clinical variables such as which opioid medication the mother is prescribed, the dose of maternal opioid, and concurrent psychiatric medication exposure still do not allow us to accurately predict which infants will require treatment, how responsive infants will be to therapy, and which infants will manifest the most severe subtype requiring multi-drug therapy (Jones et al., 2010; Logan et al., 2013; Pritham et al., 2012).

Genetic factors represent an important component in being able to predict NAS severity, clinical course, and outcomes. Single nucleotide polymorphisms (SNPs) in key candidate genes have been identified as important influences on opioid addiction risk, as well as moderators of response to opioid therapy in adults (Goldman et al., 2006; Kreek et al., 2006; Levran et al., 2012; Lotsch et al., 2004). Many of the same genes and pathways, as we have explored in recent studies, are likely involved in NAS. Previously, we identified SNPs in the *mu-opioid receptor* (*OPRM1)* and *catechol-O-methyltransferase* (*COMT)* genes as associated with NAS severity (Wachman et al., 2013). Infants who were carriers of the *OPRM1* rs1799971 G allele (AG or GG genotypes), associated with opioid dependence in adults, had paradoxically less severe withdrawal compared with infants with an AA genotype. Maternal *OPRM1* rs1799971 G-allele carriers (AG/GG genotypes) also were associated with a decreased need for infant treatment. Similarly, the G-allele carriers of the *COMT* rs4680 (AG/GG genotypes), which has been associated with addiction and psychiatric risk, also were associated with a shorter length of hospital stay and less treatment with 2 or more medications compared to those with an AA genotype (Wachman et al., 2013). These two SNPs explained 6% of the variability in infant length of hospitalization. A follow-up study also indicated that epigenetic variation in methylation of the *OPRM1* gene correlated with NAS outcomes (Wachman et al., 2014). These initial pilot studies present proof of principle that genetic factors influence NAS outcomes, but further studies are necessary before this can be clinically applied. In our initial pilot study, five SNPs in three candidate genes were examined (Wachman et al., 2013). It is necessary to examine a much larger panel of SNPs in multiple candidate genes in order to identify more important variants that can eventually be used to create a prediction model to tailor monitoring and treatment of infants with NAS.

Genes in the opioid receptor family represent the most intensely studied for association with opioid addiction (Bauer et al., 2014; Kreek et al., 2006; Levran et al., 2012). Previous studies have examined SNPs in the *mu*-(*OPRM1*), *kappa-(OPRK1*), and *delta-opioid receptor* (*OPRD1*) genes for possible association with substance abuse since these are the primary sites of opioid action (Bauer et al., 2014; Klepstad et al., 2005; Kreek et al., 2006; Levran et al., 2012; Mague and Blendy, 2010). In addition, the endogenous opioid peptides have emerged as key candidate genes modifying opioid addiction risk and response to opioid therapy (Kreek et al., 2006; Levran et al., 2012). Genes related to the hypothalamic pituitary axis (HPA) and associated endogenous stress pathways also play an important role in the neurobiology of opioid addiction and opioid withdrawal (Culpepper-Morgan and Kreek, 1997; Hosak, 2007; Koob and Kreek, 2007; Rakvag et al., 2005; Reyes-Ribby et al., 2007). These include *COMT*, as well as genes related to dopamine, serotonin, and norepinephrine transport (members of the SLC gene family) that previously have been linked to psychiatric disorders, drug abuse, and differences in morphine requirements in adults (Culpepper-

Morgan and Kreek, 1997; Hosak, 2007; Koob and Kreek, 2007; Rakvag et al., 2005; Reyes-Ribby et al., 2007). Large candidate gene microarray and genome-wide association studies also have identified important candidate genes associated with an increased risk for opioid addiction in adults (Gelernter et al., 2014; Hodgkinson et al., 2008; Levran et al., 2008; Li et al., 2011). In these studies, SNPs not only in the opioid receptors and stress pathways, but also in genes related to potassium signaling pathways, were identified as more commonly found in the opioid-dependent individuals compared with controls (Gelernter et al., 2014; Levran et al., 2008).

In the present study, we expand on our previous work related to the genetics of NAS by applying a custom DNA microarray method to identify additional genetic variants in the opioid and stress pathways that may be important predictors of NAS outcomes within opioid-exposed mother-infant pairs. By identifying more key genetic variants, we can begin to develop a prediction model that will guide the care of these infants.

2. MATERIALS AND METHODS

2.1 Participants and Study Design

This was a prospective multi-centered genetic association study for NAS. Eighty-six infants of 36 weeks gestational age or greater and their mothers were enrolled between July, 2011 and July, 2012 from five institutions in Massachusetts and Maine. Inclusion criteria for the study included pregnant mothers who were taking prescribed methadone or buprenorphine for at least 30 days prior to delivery, singleton pregnancies, and infants in stable medical condition as determined by the attending physician. Mothers were approached in the third trimester or postnatally at any point during their infant's initial hospitalization. This study was approved by the institutional review boards of all sites and written informed consent was obtained from all participants.

A DNA sample was collected from cord blood (PAXgene Blood DNA tube, Qiagen, Venlo, The Netherlands), maternal peripheral blood, or a saliva sample (Oragene OG-500 or OG-250 DNA collection kits with CS-1 sponges, DNA Genotek, Kanata, Ontario, Canada) from all participants. This study used the same maternal and infant DNA samples and dataset from a previously published study examining SNPs in the *OPRM1*, *COMT*, and *ABCB1* genes (Wachman et al., 2013).

Baseline characteristics were collected from the infant's chart, including birth demographics, medical diagnoses, and NAS outcome measures. Maternal records were reviewed to obtain information including obstetric complications, use of tobacco and psychotropic medications, and substance abuse treatment during pregnancy. Illicit drug and alcohol histories were collected based on maternal interviews (Maine site), maternal third trimester and Labor and Delivery Admission urine toxicology screening results, and infant meconium and urine toxicology results. Infant breastfeeding status (yes/no) was collected, defined as any amount of mother's milk consumed at any point during the inpatient hospitalization. Race and ethnicity as defined by the maternal participants or electronic medical record were also collected.

Infants were treated according to institutional NAS treatment protocols. All infants were scored every 3 to 4 hours with a modified Finnegan NAS scale that was performed in an identical fashion at all sites. Infants with three consecutive scores >8 or two consecutive scores >10 were started on first-line therapy which was neonatal morphine solution (0.5 – 1.0 mg/kg/day) or methadone (0.5 – 1.0 mg/kg/day). If the infant reached the maximum recommended dose of first-line medication and still had scores >8, then second-line therapy was initiated with phenobarbital or clonazepam. Infants were weaned from morphine, methadone, and clonazepam as inpatients and monitored for 48 hours prior to discharge home. Phenobarbital weaning was completed as an outpatient.

2.2 Microarray Design

A custom microarray was designed containing 80 SNPs in 14 candidate genes (Supplementary Table 1[1]). A minimum minor allele frequency (MAF) based on the Hap Map CEU of 10% was chosen. Genes and SNPs were selected based on identified opioid biological pathways and adult opioid-dependence literature (Gelernter et al., 2014; Goldman et al., 2006; Hodgkinson et al., 2008; Kreek et al., 2006; Levran et al., 2012, 2008; Li et al., 2011; Lotsch et al., 2004). The SNPs selected were from genes of the opioid-receptor family and/or related to the endogenous stress pathway (TABLE 1). Linkage disequilibrium analyses was performed using an R^2 cut-off of <0.7 to narrow down a list of 150 candidate SNPs to the final 80 that were included in the microarray.

2.3 Laboratory Methods

All DNA samples were sent to the Tufts Medical Center Clinical and Translational Research Center Core Laboratory for processing. Blood samples collected in the PAXgene DNA tubes were frozen within 14 days of collection at −70°C until DNA isolation. Salivary samples were stored at room temperature until DNA isolation. DNA was isolated per PAXgene and Oragene protocols and sent as frozen samples to the Boston University Molecular Genetics Core Laboratory for genotyping. SNP genotyping was performed using KASP reagent (LGC Genomics, Beverly, MA). The SNP-specific KASP Assay mix and the universal KASP Master mix were added to DNA samples and a thermal cycling reaction performed, followed by an end-point fluorescent read per manufacture's protocol. All assays were tested on in-house validation DNA prior to being run on project samples. No template controls (NTCs) and 5% of the samples had duplicates included on each plate to enable the detection of contamination or non-specific amplification. Following completion of PCR cycles, all genotyping reaction plates were read on 7900HT Fast Real-Time PCR System (Life Technologies, Grand Island, NY) and analyzed using SDS software version 2.3.

2.4 Statistical Methods

SNPs with call rates less than 90% or genotype frequencies significantly deviating from Hardy-Weinberg equilibrium ($p<0.01$) were excluded. The primary NAS outcome measure was length of hospital stay (LOS), with secondary outcome measures of need for any NAS treatment, and need for treatment with 2 or more medications (yes/no). LOS was chosen as

[1]Supplementary material can be found by accessing the online version of this paper at http://dx.doi.org and by entering doi:…

the primary outcome because it reflects overall severity of NAS, including need for treatment with more medications to control symptoms and longer weaning period. Since LOS highly correlated with total length of opioid treatment (r=0.92, p=0.001), only LOS is reported. An additive genetic model was used to assess the association between each SNP and NAS outcome measures. Potential covariates affecting NAS severity were evaluated in univariate analyses with a minimum α level of 0.05 for inclusion in multivariate models. Multivariate linear and logistic regression models then were created for each of the 80 SNPs including the significant covariates of breastfeeding and study site. Beta coefficients were derived from the linear regression models, representing the difference in LOS with higher minor allele load for each SNP. Adjusted odds ratios of need for treatment and treatment with 2 or more medications were created based on minor allele load. To correct for multiple testing, simulated null distributions were created by randomly permuting the full genetic data for each individual (in order to maintain the linkage disequilibrium across SNPs) to phenotype and covariate data (in order to preserve the correlation structure). Taking the minimum SNP p-value for any trait for each permutation yielded a p-value threshold of 0.00048 for experiment-wide significance. The multiple NAS outcome measures were included in permutation testing. Statistical analyses were performed with R programming (2010) and PLINK software (version 1.90b, 2014). ANOVA models were then created to estimate the percent of the variation in LOS that could be predicted by the SNPs.

3. RESULTS

3.1 Demographics

A total of 140 mother-infant pairs were eligible for this study during the 1 year study period. Of those eligible who were approached for consent, 5 refused consent, and 49 were missed, leading to a total cohort of 86 mother-infant dyads. The vast majority of the participants were non-Hispanic of White race, and most infants were ≥38 weeks gestational age at birth (TABLE 2). Fifty-five (64%) of the mothers were on methadone maintenance therapy during the pregnancy (mean dose at delivery of 106mg (95% CI 81–124mg) and the remainder were on buprenorphine maintenance therapy (mean dose at delivery of 16mg, 95% CI 13–19mg). Eighty-one (94%) of the mothers were on opioid therapy from the first trimester, with 17 (20%) relapsing with any un-prescribed or illicit drug use during the third trimester. The most common co-exposures were marijuana (20%), heroin (13%), and unprescribed opioids (17%).

The average LOS for all infants was 22 days (95% CI 19–26 days); and for treated infants 32 days (95% CI 28–35 days). Fifty-six (65%) of the infants were treated with medication for NAS, with 38% of treated infants also requiring a second agent. A DNA sample was available for all 86 of the infants and 79 of the mothers. Seventy-two percent of the samples were collected from saliva, and the rest from cord blood (infants) or peripheral blood (mothers). Genotype frequencies for the SNPs had a minor allele range of 0.087 to 0.497 with a mean of 0.289 (SD 0.121). All SNPs demonstrated Hardy-Weinberg equilibrium with the exception of rs6090041, rs9332377, rs37022, and rs460000, like due to small sample size. No SNPs were excluded due to low call rate.

Of the potential covariates assessed for association with NAS severity (study site, maternal methadone versus buprenorphine exposure and dose, maternal smoking, benzodiazepine exposure, SSRI exposure, illicit drug exposure in the third trimester, breastfeeding, morphine versus methadone as first-line treatment in the infant), breastfeeding demonstrated a consistent association with all outcome measures, with breastfed infants demonstrating reduced LOS (15.8 vs 27.4 days, p=0.001) and decreased need for any medical treatment for NAS (50% vs 77%, p=0.009). No other variable showed a consistent and significant association with NAS outcomes.

3.2 SNP Associations

The strongest signals were from SNPs in genes from the opioid-receptor family including *prepronociceptin* (*PNOC*), *OPRM1*, *OPRK1*, and O*PRD1*. The strongest signals for infant and maternal SNP associations are shown in TABLE 3. A p-value cut-off of p=0.02 for at least 1 NAS outcome measure was chosen for inclusion in the table.

Among infants, associations were seen with several SNPs in the opioid-receptor family. Within the *PNOC* gene, the minor A allele for rs732636 was associated with increased odds of treatment with 2 medications (OR=3.8, 95% CI 1.5–9.5, p=0.004) and a longer LOS by 5.8 days (95% CI 1.4 – 10.1, p=0.01). Additionally, the minor A allele of rs351776 and the minor A allele of rs2614095 were associated with an increase in need for 2 medications (p=0.01). The minor C allele of rs702764 in the *OPRK1* gene was associated with worse NAS with an increased odds of treatment with 2 medications (OR=4.1, 95% CI 1.6–10.5, p=0.003). There was a similar trend in this SNP for longer LOS (p=0.08), but values did not meet statistical significance. Infants carrying the *COMT* rs740603 minor allele A also demonstrated a decreased need for NAS treatment (OR=0.4, 95% CI 0.2, 0.8, p=0.01) and decreased LOS by 5.3 days (95% CI 1.2–9.5, p=0.01). The minor allele G of *OPRM1* SNP rs1799971 was associated with shortened LOS by 6.9 days (95% CI 1.1–12.7, p=0.02). Need for infant treatment also was lower (OR=0.4, 95% CI 0.2–1.0, p=0.06) and need for 2 medications reduced (OR=0.3, 95% CI 0.1–1.1, p=0.08) among infants with this allele, but these results did not reach statistical significance.

Genetic variation in the mothers also influenced infant NAS outcomes. The minor A allele of rs204076 *OPRD1* in the mothers was associated with longer LOS by 6.6 days (95% CI 1.9–11.3, p=0.008), and an increased need for treatment with 2 medications (p=0.04).

All of these associations remained significant point-wise but after correction for multiple comparison testing they did not meet the experiment-wide significance level. The selected 6 SNPs explained 15% of the variation in infant LOS in an ANOVA model that also accounted for breastfeeding.

4. DISCUSSION

The present study furthered our hypothesis that genetic variation contributes to differences in NAS outcomes, in preparation for future larger genetic association studies in this population. Though none of the results remained significant experiment-wise after correction for multiple testing, notable point-wise associations were found between SNPs in

opioid receptor and stress response genes (*PNOC, OPRM1, OPRK1, OPRD1*, and *COMT*) and NAS outcomes. Identified SNPs explained 15% of the variation seen in length of infant hospitalization, more than the top clinical variable of breastfeeding. These findings suggest that genetic variants in key candidate genes may aid in the prediction of the course of NAS and, ultimately, the development of individualized intervention strategies.

Our prior pilot study of five SNPs within the *OPRM1*, *COMT*, and *ABCB1* genes was performed in the same 86 mother-infant pairs (Wachman et al., 2013). The present exploratory study significantly expands on this prior work by looking at a total of 80 SNPs in 14 candidate genes using microarray technology in order to identify additional genetic variants that will eventually be incorporated into a prediction model to help guide monitoring and treatment of infants with NAS. In our prior study, we used a dominant genetic model which was assumed because of patterns observed in other genetic studies of opioid analgesia for these particular SNPs (Landau et al., 2013; Rakvag et al., 2005; Reyes-Gibby et al., 2007). The present study used a more general additive genetic model, thus results for the *OPRM1* and *COMT* SNPs differ slightly due to the differences in the genetic models.

Genes associated with NAS in this study have known physiologic significance for opioid drug response and withdrawal in adults (Kreek et al., 2006; Levran et al., 2012). In our study, *PNOC* gene variants were associated with worse NAS severity as indicated by longer LOS and increased need for treatment with two or more medications. Prepronociceptin is a precursor for nociception, which is the ligand of the opioid receptor-like receptor *(OPRL1*). It may act as a transmitter in the brain by modulating nociceptive and locomotor behavior (Kreek et al., 2006). Previous studies have demonstrated an association of *PNOC* SNPs with alcohol and other drug abuse and addiction pathways (Kreek et al., 2006; Levran et al., 2012). Specifically, Xuei et al identified association of two SNPs (rs17058952 and rs351779) with alcohol dependence and one SNP (rs4732636) with illicit drug dependence adults (Xuei et al., 2008).

Using an additive genetic model, an association of the *OPRM1* A118G (rs1799971) G alelle with improved NAS outcomes was found in the present study, as was identified by our group using a dominant genetic model (Wachman et al., 2013). This *OPRM1* SNP has been associated with differences in β-endorphin levels and μ-opioid receptor binding, and with differences in pain and withdrawal tolerance (Klepstad, 2005; Mague and Blendy, 2010). As demonstrated in another study in our series, epigenetic mechanisms such as DNA methylation within *OPRM1* after chronic *in utero* opioid exposure also are likely important in modulating the response to post-natal opioid therapy (Wachman et al., 2014).

We found an association with the C allele in rs702764 within the *OPRK1* gene with severe NAS requiring two medications. Kappa-opioid receptors are widely distributed in the central nervous system, particularly within the mesolimbic pathway, and play a key role in pain regulation, addictive behaviors, cardiovascular function, breathing, temperature regulation, feeding behavior, and stress responsivity (Bruchas et al., 2010; Kreek et al., 2006). Many prior studies have identified association of SNPs in *OPRK1* with opioid addiction and severity of opioid withdrawal symptoms (Bauer et al., 2014; Gerra et al., 2007; Levran et al.,

2012, 2008; Wang et al., 2014; Yuferov et al., 2004; Xu et al., 2013). Consistent with our findings, Wang et al identified an association of rs702764 with increased opioid withdrawal symptoms and alcohol use among adult methadone maintenance patients (Wang et al., 2014). Another study found an interaction between *OPRK1* rs702764 and *OPRM1* rs1799971 for risk of heroin addiction (Kumar et al., 2012).

We also found an association with the *OPRD1* rs204076 A allele in opioid-dependent mothers with worse NAS outcomes in the infants. Delta-opioid receptors bind the endogenous ligand enkephalin. These receptors have been implicated in the modulation of addiction, affective state, gastrointestinal function, respiration, pain perception, and analgesia (Kreek et al., 2006; Levran et al., 2012). *OPRD1* SNPs have been associated with risk for opioid addiction as well as differences in the response to opiate treatment and the development of morphine tolerance in adults (Levran et al., 2012; Nelson et al., 2014; Zhang et al., 2008). In a study of 11 *OPRD1* SNPs, three common SNPs (rs2236861, rs2236857 and rs3766951) and a haplotype block composed of SNPs rs204055, rs2236857 and rs2298896 showed association with heroin addiction (Levran et al., 2008). *OPRD1* SNPs rs1042114, rs2234918, and rs581111 have also been linked with opioid addiction (Bauer et al., 2014; Nelson et al., 2014; Zhang et al., 2008).

As in earlier work, we found an association with the A allele in rs740603 within *COMT* with improved NAS outcomes. This SNP has previously been associated with an increased risk of depression in adults as well as nicotine dependence (Beyten et al., 2006; Pap et al., 2012) In addition, rs740603 has been associated with impulsivity, depression, and cocaine-associated paranoia (Ittiwut et al., 2011; Pap et al., 2012). Similarly, our previous study in the same 86 mother-infant dyads found an association within another SNP in *COMT* (rs4680) with NAS severity under a dominant model (Wachman et al., 2013). The minor G allele of this SNP leads to a decrease in COMT enzyme activity suggesting that an increase in circulating catecholamines would lead to improved stress tolerance in infants with NAS (Rakvag et al., 2005).

In addition to genetic factors, clinical variables are also of importance and contribute to differences in NAS outcomes. In our small cohort, breastfeeding was the only clinical variable that significantly affected NAS outcomes, demonstrating a strong protective effect. This has been demonstrated in prior studies and represents an area for focused clinical intervention to improve NAS outcomes (Bagley et al., 2014; Pritham, 2013). We did not see significant differences in NAS outcomes based on maternal opioid substitute, concurrent psychiatric medications, or smoking status likely related to our small sample size.

This study has some limitations. There was variation in the NAS medication protocol used at the five hospitals with some centers using morphine and others methadone as first-line therapy. Though both medications are considered standard of care, there is currently no universal NAS treatment protocol with on-going multi-centered clinical trials attempting to establish best practice (Backes et al., 2012; Brown et al., 2014; Hall et al., 2014; Hudak et al., 2012; Lee et al., 2015; Sarkar et al., 2006). The average length of hospitalization in our study cohort is consistent with the reported national averages from large statewide cohorts which include infants treated with methadone for NAS (Brown et al., 2014; Hall et al., 2014;

Lee et al., 2015; Lind et al., 2015). Some clinical trials such as the MOTHER study have achieved shorter hospitalizations in the range of 10–17 days with the use of a strict study protocol and standardized NAS scoring (Jones et al., 2010). Although the same Finnegan NAS scoring system was used across our study centers, intra-observer variability could be high as no standardized training program was used. In addition, the care models used varied at the five study centers with some centers caring for infants in the NICU and others in nurseries or pediatric wards. In addition, a significant portion of our population were polypharmacy exposed, particularly to nicotine, SSRIs, and un-prescribed opioids. Polypharmacy is common in this patient population; it is difficult to discriminate between signs and symptoms of opioid withdrawal and withdrawal from these concurrent exposures (Jansson et al., 2009; Jones et al., 2010; Wachman et al., 2011). Timing of symptoms manifestation and neurologic symptom predominance may help to distinguish, however available NAS assessment tools do not allow providers to accurately be able to decriminate (Hudak et al., 2012; Jansson et al., 2009; Jones et al., 2010). The determination of illicit drug exposures based on maternal interviews and urine toxicology results also has its limitations. In addition, our results, which were based on a relatively small sample and not significant after correction for multiple comparisons, should be considered tentative. Lastly, 98% of our subjects were White non-Hispanics limiting generalizability as association patterns may vary across populations due to variation in allele frequencies or genetic heterogeneity. However, the use of a racially homogenous population avoids errors due to population stratification.

The identification of genetic markers that correlate with NAS severity can lead to future early noninvasive prenatal genetic testing and individualized treatment regimens designed to improve outcome for these high risk infants. Infants with high-risk genetic profiles can be treated more aggressively from birth therefore shortening length of hospitalization. Infants with low risk profiles may be discharged from the hospital with their mothers without the need for prolonged monitoring for signs of withdrawal, significantly impacting cost of care for this growing population of infants.

These findings suggest that genetic variation in the *PNOC*, *OPRM1*, *OPRK1*, *OPRD1*, and *COMT* genes are associated with differences in NAS severity. Further testing in a large sample is warranted before incorporating genetic testing into clinical practice. The creation of an accurate prediction rule based on clinical and genetic factors would allow us to tailor monitoring and treatment for opioid-exposed infants therefore improving outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Role of Funding Source

Funding for this project was provide by grant support. The specific role of each grant in the project is as follows: 1) DNA collection and isolation (National Institutes of Health DA024806 to Dr. Hayes and R01DA032889-01A1 to Dr. Davis; Tufts Medical Center's Natalie Zucker and Susan Saltonstall grants to Dr. Wachman); 2) Microarray design and genotyping (Joel Alpert Foundation Grant to Dr. Wachman); and 3) Data analysis [NIH DA018197-05

and MD Anderson's Cancer Center Support Grant DA026120 to Dr. Nielsen; National Center for Advancing Translational Sciences (UL1 TR000073 through the Tufts Clinical and Translational Science Institute to Dr. Davis); Toomin Family Fund to Dr. Nielsen]. None of the funding sources were involved in study design, the collection or interpretation of data, the writing of this manuscript, or the decision to submit this manuscript for publication.

We would like to thank the medical and nursing staff of labor and delivery, the mother-infant units, and neonatal units of Tufts Medical Center, Melrose Wakefield Hospital, Brockton Hospital, Lowell General Hospital, and Eastern Maine Medical Center. Specifically, we'd like to acknowledge Irene Simkina from the Boston University Molecular Core Genetic Laboratory for performing the genotyping; Jennifer Curcuru from the Tufts CTRC Core Laboratory for performing the DNA isolation; Karen Harvey-Wilkes, MD, Ozlem Kasaroglu, MD, Mario Cordova, MD, and Teresa Marino, MD from Tufts Medical Center; Hira Shrestha, MA; Nicole Heller, PhD, Beth Logan, PhD, and Deborah Morrison, MA, from the University of Maine for their assistance with enrollment and data collection.

References

Backes CH, Backes CR, Gardner D, Nankervis CA, Giannone PJ, Cordero L. Neonatal abstinence syndrome: transitioning methadone-treated infants from an inpatient to an outpatient setting. J Perinatol. 2012; 32:425–430.10.1038/jp.2011.114 [PubMed: 21852772]

Bagley SM, Wachman EM, Holland E, Brogly SM. Review of the assessment and management of neonatal abstinence syndrome. Addict Sci Clin Pract. 2014; 9:19.10.1186/1940-0640-9-19 [PubMed: 25199822]

Bauer IE, Soares JC, Nielsen DA. The role of opioidergic genes in the treatment outcome of drug addiction pharmacotherapy: a systematic review. Am J Addict. 2015; 24:15–23.10.1111/ajad.12172 [PubMed: 25823631]

Beuten J, Payne TJ, Ma JZ, Li MD. Significant association of catechol-O-methyltransferase (COMT) haplotypes with nicotine dependence in male and female smokers of two ethnic populations. Neuropsychopharmacology. 2006; 31:675–684. [PubMed: 16395295]

Brown MS, Hayes MJ, Thornton LM. Methadone versus morphine for treatment of neonatal abstinence syndrome: a prospective randomized clinical trial. J Perinatol. 2015; 35:278–283.10.1038/jp.2014.194 [PubMed: 25357093]

Bruchas MR, Land BB, Chavkin C. The dynorphin/kappa opioid system as a modulator of stress-induced and pro-addictive behaviors. Brain Res. 2010; 1314:44–45.10.1016/j.brainres.2009.08.062 [PubMed: 19716811]

Culpepper-Morgan JA, Kreek MJ. Hypothalamic–pituitary–adrenal axis hypersensitivity to naloxone in opioid dependence: a case of naloxone-induced withdrawal. Metabolism. 1997; 46:130–134. [PubMed: 9030816]

Desai RJ, Hernandez-Diaz S, Bateman BT, Huybrechts KF. Increase in prescription opioid use during pregnancy among Medicaid-enrolled women. Obstet Gynecol. 2014; 123:997–1002. [PubMed: 24785852]

Gelernter J, Kranzler HR, Sherva R, et al. Genome-wide association study of opioid dependence: multiple associations mapped to calcium and potassium pathways. Biol Psychiatry. 2014; 76:66–74.10.1016/j.biopsych.2013.08.034 [PubMed: 24143882]

Gerra G, Leonardi C, Cortese E, D'Amore A, Lucchini A, Strepparola G, Serio G, Farina G, Magnelli F, Zaimovic A, Mancini A, Turci M, Manfredini M, Donnini C. Human kappa opioid receptor gene (OPRK1) polymorphism is associated with opiate addiction. Am J Med Genet B Neuropsychiatr Genet. 2007; 144:771–777. [PubMed: 17373729]

Goldman D, Oroszi G, Ducci F. The genetics of addiction: uncovering the genes. Focus. 2006; 6:401–415.

Hall ES, Wexelblatt SL, Crowley M, Grow JL, Jasin LR, Klebanoff MA, McClead RE, Meinzen-Derr J, Mohan VK, Stein H, Walsh MC. OCHNAS Consoritum. A multicenter cohort study of treatments and hospital outcomes in Neonatal Abstinence Syndrome. Pediatrics. 2014; 134:e527–534.10.1542/peds.2013-4036 [PubMed: 25070317]

Hodgkinson CA, Qiaoping Y, Xu K, Shen PH, Heinz E, Lobos EA, Binder EB, Cubells J, Ehlers CL, Gelernter J, Mann J, Riley B, Roy A, Tabakoff B, Todd RD, Zhou Z, Godman D. Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. Alcohol Alcohol. 2008; 43:505–515. [PubMed: 18477577]

Hosak L. Role of the *COMT* gene Val158Met polymorphism in mental disorders: a review. Eur. Psychiatry. 2007; 22:276–281.10.1093/alcalc/agn032

Hudak ML, Tan RC. American Academy of Pediatrics, Committee on Fetus and Newborn, Committee on Drugs. Neonatal drug withdrawal. Pediatrics. 2012; 129:e540–560.10.1542/peds.2011-3212 [PubMed: 22291123]

Ittiwut R, Listman JB, Ittiwut C, Cubelis JF, Weiss RD, Brady K, Oslin D, Farrer LA, Kranzler HR, Gelernter J. Association between polymorphisms in catechol-O-methyltransferase (COMT) and cocaine-induced paranoia in European-American and African-American populations. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B:651–660.10.1002/ajmg.b.31205 [PubMed: 21656904]

Lee J, Hulman S, Musci M, Stang E. Neonatal abstinence syndrome: influence of a combined inpatient/outpatient methadone treatment regimen on the average length of stay of a Medicaid NICU population. Popul Health Manag. 2015 Mar.24 (Epub ahead of print).

Jansson LM, Velez M, Harrow C. The opioid-exposed newborn: assessment and pharmacologic management. J Opioid Manag. 2009; 5:47–55. [PubMed: 19344048]

Jones HE, Kaltenbach K, Heil SH, et al. Neonatal abstinence syndrome after methadone or buprenorphine exposure. N Engl J Med. 2010; 363:2320–2331.10.1056/NEJMoa1005359 [PubMed: 21142534]

Klepstad P, Dale O, Skorpen F, Borchgrevink PC, Kaasa S. Genetic variability and clinical efficacy of morphine. Acta Anaesthesiol Scand. 2005; 49:902–908. [PubMed: 16045647]

Koob G, Kreek MJ. Stress, dysregulation of drug reward pathways, and the transition to drug dependence. Am J Psychiatry. 2007; 164:1149–1159. [PubMed: 17671276]

Kreek MJ, Bart G, Lilly C, LaForge KS, Nielsen DA. Pharmacogenetics and human molecular genetics of opiate and cocaine addiction and their treatments. Pharmacol Rev. 2006; 57:1–26. [PubMed: 15734726]

Kumar D, Chakraborty J, Das S. Epistatic effects between variants of kappa-opioid receptor gene and A118G of mu-opioid receptor gene increase susceptibility to addiction in Indian population. Prog Neuropsychopharmacol Biol Psychiatry. 2012; 36:225–230.10.1016/j.pnpbp.2011.10.018 [PubMed: 22138325]

Landau R, Liu SK, Blouin JL, Carvalho B. The effect of OPRM1 and COMT genotypes on the analgesic response to intravenous fentanyl labor analgesia. Anesth Analg. 2013; 116:396–391.10.1213/ANE.0b013e318273f2c7

Levran O, Yuferov V, Kreek MJ. The genetics of the opioid system and specific drug addictions. Hum Genet. 2012; 131:823–842.10.1007/s00439-012-1172-4 [PubMed: 22547174]

Levran O, Londono D, O'Hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M, Kreek MJ. Genetic susceptibility to heroin addiction; a candidate-gene association study. Genes Brain Behav. 2008; 7:720–729.10.1111/j.1601-183X.2008.00410.x [PubMed: 18518925]

Li CY, Zhou WZ, Zhang PW, Johnson C, Wei L, Ulh GR. Meta-analysis and genome-wide interpretation of genetic susceptibility to drug addiction. BMC Genom. 2011; 12:508.10.1186/1471-2164-12-508

Lind JN, Petersen EE, Lederer PA, Phillips-Bell GS, Perrine CG, Li R, Hudak M, Correia JA, Creanga AA, Sappenfield WM, Curran J, Blackmore C, Watkins SM, Anjohrin S. CDC. Infant and maternal characteristics in neonatal abstinence syndrome–selected hospitals in Florida, 2010–2011. MMWR Morb Mortal Wkly Rep. 2015; 64:213–216. [PubMed: 25742381]

Logan BA, Brown MS, Hayes MJ. Neonatal abstinence syndrome: treatment and pediatric outcomes. Clin Obstet Gynecol. 2013; 56:186–192.10.1097/GRF.0b013e31827feea4 [PubMed: 23314720]

Lotsch J, Starke C, Liefhold J, Geisslinger G. Genetic predictors of the clinical response to opioid analgesics: clinical utility and future perspectives. Clin Pharmacokinet. 2004; 43:983–1013. [PubMed: 15530129]

Mague SD, Blendy JA. *OPRM1* SNP (A118G): involvement in disease development, treatment response, and animal models. Drug Alcohol Depend. 2010; 108:172–182.10.1016/j.drugalcdep.2009.12.016 [PubMed: 20074870]

Nelson EC, Lynskey MT, Heath AC, Wray N, Agrawal A, Shand FL, Henders AK, Wallace L, Todorov AA, Schrage AJ, Madden PA, Degenhardt L, Martin NG, Montgomery GW. Association

of OPRD1 polymorphisms with heroin dependence in a large case-control series. Addict Biol. 2014; 19:111–121.10.1111/j.1369-1600.2012.00445.x [PubMed: 22500942]

Pap D, Gonda X, Molnar E, Lazary J, Benko A, Downey D, Thomas E, Chase D, Toth ZG, Mekli K, Platt H, Payton A, Elliott R, Anderson IM, Deakin JF, Bagdy G, Juhasz G. Genetic variants in the catechol-o-methyltransferase gene are associated with impulsivity and executive function: relevance for major depression. Am J Med Genet B Neuropsychiatr Genet. 2012; 159B:928–940.10.1002/ajmg.b.32098 [PubMed: 23008195]

Patrick SW, Schumacher RE, Benneyworth BD, Krans EE, McAllister JM, Davis MM. Neonatal abstinence syndrome and associated health care expenditures: United States, 2000–2009. JAMA. 2012; 307:1934–1940.10.1001/jama.2012.3951 [PubMed: 22546608]

Pritham UA. Breastfeeding promotion for management of neonatal abstinence syndrome. J Obstet Gynecol Neonatal Nurs. 2013; 42:517–526.10.1111/1552-6909.12242

Pritham UA, Paul JA, Hayes MJ. Opioid dependency in pregnancy and length of stay for neonatal abstinence syndrome. J Obstet Gynecol Neonatal Nurs. 2012; 41:180–190.10.1111/j.1552-6909.2011.01330.x

Rakvag TT, Klepstad P, Baar C, Kvarn TM, Dale O, Kaasa S, Krokan HE, Skorpen F. The Val158Met polymorphism of the human catechol-O-methyltransferase (*COMT*) gene may influence morphine requirements in cancer pain patients. Pain. 2005; 116:73–78. [PubMed: 15927391]

Reyes-Gibby CC, Shete S, Rakvag T, Bhat SV, Skorpen F, Bruera E, Kaasa S, Klepstad P. Exploring joint effects of genes and the clinical efficacy of morphine for cancer pain. OPRM1 and COMT gene. Pain. 2007; 130:25–30. [PubMed: 17156920]

Sankar S, Donn SM. Management of neonatal abstinence syndrome in neonatal intensive care units: a national survey. J Perinatol. 2006; 26:15–77. [PubMed: 16355103]

U.S. Department of Health and Human Services, Substance Abuse and Mental Health Administration. Results from the 2013 National Survey on Drug Use and Health: National Findings. 2013. http://www.samhsa.gov/data/sites/default/files/NSDUHresultsPDFWHTML2013/Web/NSDUHresults2013.pdf

Wachman EM, Hayes MJ, Lester BM, Terrin N, Brown MS, Nielsen DA, Davis JM. Epigenetic variation in the mu-opioid receptor gene in infants with Neonatal Abstinence Syndrome. J Pediatrics. 2014; 65:472–478.10.1016/j.jpeds.2014.05.040

Wachman EM, Hayes MJ, Brown MS, Paul J, Harvey-Wilkes K, Terrin N, Huggins GS, Aranda JV, Davis JM. Association of *OPRM1* and *COMT* single nucleotide polymorphisms with hospital length of stay and treatment of neonatal abstinence syndrome. JAMA. 2013; 309:1821–1827.10.1001/jama.2013.3411 [PubMed: 23632726]

Wachman EM, Newby PK, Vreeland J, Byun J, Bonganzi A, Bauchner H, Philipp BL. The relationship between maternal opioid agonists and psychiatric medications on length of hospitalization for neonatal abstinence syndrome. J Addict Med. 2011; 5:293–299.10.1097/ADM.0b013e3182266a3a [PubMed: 21857233]

Wang SC, Tsou HH, Chung RH, Chang YS, Fang CP, Chen CH, Ho IK, Kuo HW, Liu SC, Shih YH, Wu HY, Huang BH, Lin KM, Chen AC, Hsiao CF, Liu YL. The association of genetic polymorphisms in the k-opioid receptor 1 gene with body weight, alcohol use, and withdrawal symptoms in patients with methadone maintenance. J Clin Psychopharm. 2014; 34:205–211.10.1097/JCP.0000000000000082

Xu K, Seo D, Hodgkinson C, Hu Y, Goldman D, Sinha R. A variant on the kappa opioid receptor gene (OPRK1) is associated with stress response and related drug craving, limbic brain activation and cocaine relapse risk. Transl. Psychiatry. 2013; 3:e292.10.1038/tp.2013.62

Xuei X, Flury-Wetherill L, Almasy L, Bierut L, Tischfield J, Schuckit M, Numberger JI Jr, Foroud T, Edenberg HJ. Association analysis of genes encoding the nociceptin receptor (OPRL1) and its endogenous ligand (PNOC) with alcohol or illicit drug dependence. Addict Biol. 2008; 13:80–87. [PubMed: 17910740]

Yuferov V, Fussell D, LaForge KS, Nielsen DA, Gordon D, Ho A, Leal SM, Ott J, Kreek MJ. Redefinition of the human kappa opioid receptor gene (OPRK1) structure and association of haplotypes with opiate addiction. Pharmacogenetics. 2004; 14:793–804. [PubMed: 15608558]

Zhang H, Kranzler HR, Yang BZ, Luo X, Gelernter J. The OPRD1 and OPRK1 loci in alcohol or drug dependence: OPRD1 variation modulates substance dependence risk. Mol Psychiatry. 2008; 13:531–543. [PubMed: 17622222]

Highlights

- We examine genetic variability in neonatal abstinence syndrome (NAS).
- SNPs in opioid receptor and stress response genes are associated with NAS.
- Determining key genetic variants will allow us to better predict NAS outcomes.

TABLE 1

NAS Microarray Genes

Gene Name	Gene Symbol	Chromosome	# SNPs Tested
Mu-opioid receptor	*OPRM1*	6	13
Delta-opioid receptor	*OPRD1*	1	8
Kappa-opioid receptor	*OPRK1*	8	5
Proenkephalin	*PENK*	8	2
Proopiomelanocortin	*POMC*	2	4
Prodynorphin	*PDYN*	20	8
Prepronociceptin	*PNOC*	8	7
Nociceptin/orphanin	*OPRL1*	20	4
Catechol-O-methyltransferase	*COMT*	22	8
Galanin	*GAL*	11	2
Brain-derived neurotrophic factor	*BDNF*	11	4
Solute carrier family 6 (neurotransmitter transporter), member 2, (noradrenaline transporter)	*SLC6A2*	16	7
Solute carrier family 6 (neurotransmitter transporter) member 3, (dopamine transporter)	*SLC6A3*	16	8
Solute carrier family 6 (neurotransmitter transporter) member 4, (serotonin transporter)	*SLC6A4*	16	2

TABLE 2

Demographics of 86 Mother-Infant Pairs

Demographic	Number (%) or Mean (95% CI)
Study Site	
Tufts and affiliates	51 (59%)
Eastern Maine Medical Center	35 (41%)
Gestational Age ≥38 weeks	70 (81%)
White, Non-Hispanic	84 (98%)
Infant Sex	
Male	51 (59%)
Female	35 (41%)
Infant Birth Weight (Kilograms)	3.2 (3.1–3.3)
Maternal Opioid Substitute	
Methadone	55 (64%)
Dose at Delivery (mg/day)	106 (81–124)
Buprenorphine	31 (36%)
Dose at Delivery (mg/day)	16 (13–19)
Breastfed	38 (44%)
Concurrent Exposures	
Cigarette Smoking	67 (78%)
Selective Serotonin Re-uptake Inhibitors	4 (5%)
Benzodiazepines	10 (12%)
Amphetamines	2 (2%)
Barbituates	3 (4%)
Marijuana	17 (20%)
Cocaine	5 (6%)
Heroin	11 (13%)
Other Unprescribed Opioids	15 (17%)
Alcohol	2 (2%)

TABLE 3

SNP associations and NAS outcomes

SNP	Gene	Minor Allele	MAF	NAS Treatment (OR, 95% CI)	p-value	≥ 2 Meds (OR, 95% CI)	p-value	LOS (β in days, 95% CI)	p-value
rs4732636[a]	*PNOC*	A	0.34	2.2 (1.0, 4.8)	0.05	3.8 (1.5, 9.5)	0.004	5.8 (1.4, 10.1)	0.01
rs351776 [a]	*PNOC*	A	0.49	1.5 (0.8, 3.0)	0.26	3.2 (1.3, 7.6)	0.01	4.2 (0, 8.4)	0.05
rs2614095 [a]	*PNOC*	A	0.42	0.6 (0.3, 1.3)	0.22	0.3 (0.1, 0.8)	0.01	−4.7 (−9.1, −0.3)	0.04
rs1799971 [a]	*OPRM1*	G	0.14	0.4 (0.2, 1.0)	0.06	0.3 (0.1, 1.1)	0.08	−6.9 (−12.7, −1.1)	0.02
rs702764 [a]	*OPRK1*	C	0.17	2.0 (0.8, 5.0)	0.16	4.1 (1.6, 10.5)	0.003	4.8 (−0.6, 10.2)	0.08
rs204076 [b]	*OPRD1*	A	0.36	1.7 (0.7, 3.8)	0.23	2.8 (1.0, 7.6)	0.04	6.6 (1.9, 11.3)	0.008
rs740603 [a]	*COMT*	A	0.40	0.4 (0.2, 0.8)	0.01	0.8 (0.4, 1.8)	0.65	−5.3 (−9.5, −1.2)	0.01

Abbreviations: SNP = single nucleotide polymorphisms, MAF = minor allele frequency, LOS = length of hospital stay, Meds = medications, NAS = neonatal abstinence syndrome

[a] Association found with infant SNP

[b] Association found with maternal SNP

Drug Alcohol Depend. Author manuscript; available in PMC 2016 October 01.