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Variations in opioid receptor genes in neonatal abstinence syndrome☆

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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.
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 ($\beta = -6.9$ days, $p = 0.02$) and *COMT* rs740603 A allele ($\beta = -5.3$ 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.

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 and 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., 2015; Hall et al., 2014; Hudak et al., 2012; Lee et al., 2015; Sankar and Donn, 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).

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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., 2015; 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., 2015; 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-Gibby 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-Gibby 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 h with a modified Finnegan NAS scale that was performed in an identical fashion at all sites. Infants with three consecutive scores $\geq$8 or two consecutive scores $\geq$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 $\geq$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 h prior to discharge home. Phenobarbital weaning was completed as an outpatient.