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Association of maternal and infant variants in *PNOC* and *COMT* genes with Neonatal Abstinence Syndrome severity

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Abstract

Background and Objectives—There is significant variability in severity of neonatal abstinence syndrome (NAS) due to *in-utero* opioid exposure. Our previous study identified single nucleotide polymorphisms (SNPs) in the *prepronociceptin* (*PNOC*) and *catechol-O-methyltransferase* (*COMT*) genes that were associated with differences in NAS outcomes. This study looks at the same SNPs in *PNOC* and *COMT* in an independent cohort in an attempt to replicate previous findings.

Methods—For the replication cohort, full-term opioid-exposed newborns and their mothers (n=113 pairs) were studied. A DNA sample was obtained and genotyped for 5 SNPs in the *PNOC* and *COMT* genes. The association of each SNP with NAS outcomes (length of hospitalization, need for pharmacologic treatment, and total opioid days) was evaluated, with an experiment-wise significance level set at $\alpha<0.003$ and point-wise level of $\alpha<0.05$.. SNP associations in a combined cohort of n=199 pairs (replication cohort plus 86 pairs previously reported), were also examined.

Results—In the replication cohort, mothers with the *COMT* rs4680 G allele had infants with a reduced risk for treatment with 2 medications for NAS (adjusted OR=0.5, p=0.04), meeting point-wise significance. In the combined cohort, infants with the *PNOC* rs4732636 A allele had a reduced need for medication treatment (adjusted OR 2.0, p=0.04); mothers with the *PNOC* rs351776 A allele had infants who were treated more often with 2 medications (adjusted OR 2.3, p=0.004) with longer hospitalization by 3.3 days (p=0.01). Mothers with the *COMT* rs740603 A

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DECLARATIONS OF INTERESTS

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allele had infants who were less often treated with any medication (adjusted OR 0.5, p=0.02). Though all SNP associations all met point wise and clinical significance, they did not meet the experiment-wise significance threshold.

Conclusions and Scientific Significance—We found differences in NAS outcomes depending on *PNOC* and *COMT* SNP genotype. Further testing in a larger sample is warranted. This has important implications for prenatal prediction and personalized treatment regimens for infants with NAS.

INTRODUCTION

Neonatal Abstinence Syndrome (NAS) due to *in-utero* opioid exposure has increased 5-fold in the United States over the past decade, now affecting 5 per 1000 live births.[1] NAS is a syndrome of neonatal opioid withdrawal affecting the neurologic, gastrointestinal, and respiratory systems, and associated with prolonged hospital stays in the range of 3–8 weeks and totaling an average of $93,000 for affected infants.[1–2] Infants chronically exposed *in-utero* to opioids are monitored in the hospital for 4–7 days for signs of withdrawal; of these, 30–80% typically require pharmacologic treatment with replacement opioids such as morphine or methadone, with currently, an inability to predict which infants will require treatment.[2] A subset of infants have a severe phenotype requiring adjunctive medication therapy, typically with phenobarbital or clonidine, with associated longer hospitalizations.[2–3] There are a number of clinical variables that contribute to NAS severity including maternal opioid exposure details, concurrent exposures to psychiatric medications, illicit drugs and nicotine, gestational age, infant feeding method, and hospital care model.[4–8] However, NAS remains poorly understood and it cannot accurately be predicted which infants will require medication and which will have the severe phenotype which is unresponsive to first-line therapy.

Genetic factors likely are responsible for this significant inter-patient variability in NAS phenotype despite similar maternal exposures. Research in adults has indicated that 50% of one's risk for opioid addiction is due to a combination of genetic factors.[9–13] 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.[9–13] While genes in the opioid receptor family represent the most intensely studied for opioid addiction and withdrawal, the endogenous opioid peptides such as prepronociceptin (PNOC) may be involved, and modify responses to opioid therapy.[10–11] PNOC acts as a transmitter in the brain by modulating nociceptive and locomotor behavior. Genetic variants in the *PNOC* gene have been linked with risk for alcohol and other drug abuse in adults.[10–11,14]

Genes related to dopamine and the endogenous stress pathway are appropriate candidate genes for opioid withdrawal as well.[9–23] Dopamine is the primary neurotransmitter involved in addiction as addictive drugs such as opioids elevate dopamine levels above baseline.[24] Genes whose products are involved in the dopamine pathway are important for understanding the differential response to opioids. Catechol-*O*-methyltransferase (COMT) is the key enzyme that metabolizes dopamine in the central nervous system. Single nucleotide

polymorphisms (SNPs) such as rs4680 (158A>G) in *COMT* result in a 3–4 fold reduction in the amount of this enzyme and higher circulating dopamine levels. Individuals carrying the rs4680 minor G allele have been shown to require less morphine for pain control in adult cancer patients.[18–19, 22–23]

Variants in the *PNOC* and *COMT* genes may be involved in process of neonatal opioid withdrawal phenotypes. Our previous studies of the genetic contributors to NAS examined common variants in the μ-opioid receptor, *PNOC*, and *COMT* genes in a cohort of 86 opioid-exposed mother-infant pairs.[25–26] The strongest associations in that study were found with variants in the *PNOC* and *COMT* genes. Within *PNOC*, we found that the minor alleles in rs4732636 and rs351776 in the infants were associated with more severe NAS.[26] Conversely, the minor A allele in *PNOC* rs2614095 in the infants was associated with less severe NAS. Minor variants in *COMT* rs4680 and rs750603 in the infants were associated with improved NAS outcomes.[25=26] These initial pilot studies indicated that genetic variation in these two genes influenced NAS outcomes. However, further studies including replication studies are necessary. In the present study, we expand on our previous work by examining genetic variations in the *PNOC* and *COMT* genes in an independent cohort of opioid-exposed mother infant pairs. We also examine SNP associations in the combined cohort of this replication and the previously studied cohorts.

METHODS

Original Cohort

The original cohort consisted of 86 infants ≥36 weeks gestational age and their mothers from 5 institutions enrolled between 2011–2012.[25–26] The mothers were on prescribed methadone or buprenorphine during the pregnancy. DNA samples were collected from cord blood, maternal blood, or saliva from all mother-infant pairs and genotyped for 80 SNPs in 14 candidate genes using a custom designed microarray. Infants were treated according to institutional NAS treatment protocol.

Replication Cohort and Study Design

This was a prospective multi-centered genetic association study for NAS that enrolled 113 mother-infant dyads from Boston Medical Center (BMC) and Eastern Maine Medical Center (EMMC) between 2013 and 2015. Inclusion criteria for the study included pregnant mothers who were taking prescribed methadone or buprenorphine for at least 30 days prior to delivery, able to provide informed consent, had singleton pregnancies of gestational age ≥36 weeks at birth, and with infants in stable medical condition. Mothers were approached in the second or third trimester, or postnatally at any point during their infant's initial hospitalization for consent. This study was approved by the institutional review boards of both sites and written informed consent was obtained from all participants.

A DNA sample was collected from saliva (Oragene OG-500 or OG-250 DNA collection kits with CS-1 sponges, DNA Genotek, Kanata, Ontario, Canada) or from buccal cells (Isohelix SK-1 swabs with Dri-capsules, Boca Scientific, Boca Raton, Florida) once from all participants. 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 psychiatric medications, and substance use disorder treatment during the pregnancy. Illicit drug and alcohol histories were collected based on maternal interviews (EMMC), 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. At all participating institutions, all infants are treated first with non-pharmacologic care, which includes promotion of breastfeeding for eligible mothers, rooming-in if available, swaddling, skin-to-skin contact, and reduction of excessive environmental stimuli. All infants were scored every 3 to 4 hours with the original Finnegan opioid withdrawal scale. Infants with 2 consecutive scores ≥8 or 1 score ≥12 were started on first-line therapy which was neonatal morphine solution (0.3 – 0.9 mg/kg/day divided q4 hours) at BMC or methadone (0.2 – 0.8 mg/kg/day divided q6 hours) at EMMC. 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, clonidine, or clonazepam. Infants were weaned from the opioids, clonidine, and clonazepam as inpatients and monitored for 24–48 hours prior to discharge home. Phenobarbital weaning was completed as an outpatient.

Laboratory Methods

All DNA samples were sent to the Boston University Molecular Genetics Core Laboratory for processing. Salivary and buccal cell samples were stored at room temperature until DNA isolation. DNA was isolated per Oragene and Isohelix protocols.

A microarray platform was used containing three SNPs in the *PNOC* (rs4732636, rs351776, and rs2614095), and two SNPs in the *COMT* (rs740603, rs4680) gene, selected based on prior published data of findings of top genetic associations with NAS severity in genes other than the primarily studied opioid receptor genes.[25–26] A minimum minor allele frequency (MAF) based on the Hap Map CEU of 10% was chosen. 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 according to the manufacturer's protocol. All assays were tested on in-house validation DNA prior to being run on project samples. No template controls 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. All assays had over 90% call rates.

Statistical Methods

The primary NAS outcome measure was length of hospital stay (LOS), with secondary outcome measures of need for any NAS pharmacologic treatment, need for treatment with two or more medications (yes/no), and total opioid days. 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 were created for each of the five SNPs including the significant covariates of breastfeeding, study site, maternal opioid agonist, and infant medication treatment. Beta coefficients were derived from the linear regression models, representing the difference in LOS and opioid days with higher minor allele load for each SNP. Adjusted odds ratios for need for pharmacologic treatment, and treatment with two or more medications were created based on minor allele load. SNP associations were tested first in the replication cohort, and then in the combined cohort. To correct for multiple SNP testing of 5 SNPs for 4 outcomes, a p-value threshold of 0.003 was used for experiment-wise significance. Point-wise significance for SNP associations was a p-value <0.05. Statistical analyses were performed with R programming (2010) and PLINK software (version 1.90b, 2014).

RESULTS

Replication Cohort Characteristics

A total of 131 mother-infant pairs were eligible for this study during the 2 year study period, representing 64% of all opioid-exposed infants during the period. Reasons for ineligibility included preterm birth (40%), infant not in maternal custody (40%), no opioid maintenance therapy (10%), maternal severe psychiatric illness compromising ability to consent (5%), and twin gestation (5%). Of those eligible who were approached for consent, 113 (86%) mother-infant dyads were consented and included in the study. There were 99 (87.6%) participants from BMC and 14 (12.3%) from EMMC. The vast majority of the participants were non-Hispanic of White race, with a mean gestational age at birth of 39.0 weeks. (TABLE 1) Sixty eight (60%) of the mothers were on methadone-maintenance therapy during the pregnancy (mean dose at delivery of 88.1mg) and the remainder were on buprenorphine-maintenance therapy (mean dose at delivery of 12.6mg). The most common co-exposures were cigarette smoking, marijuana, benzodiazepines, and un-prescribed or illicit opioids. Cigarette smoking rates were not significantly different between mothers who were prescribed methadone (76%) or buprenorphine (77%). Alcohol was denied during pregnancy, although in interview (EMMC) was reported in all peri-conceptual estimates. The average LOS for all infants was 18.9 days (95% CI 17.0, 20.8), corresponding to an average of 17.8 opioid treatment days (95% CI 16.2, 19.4) for pharmacologically treated infants. Ninety four (83%) of the infants were treated with medication for NAS, with 29 infants (26%) requiring a second agent.

When compared with the original cohort, notable demographic differences included a lower percentage of White Non-Hispanics in the replication cohort (88% vs 98%, p=0.02), fewer marijuana and un-prescribed opioid co-exposures, and more infants pharmacologically treated for NAS (83% vs 65%, p<0.001).[25–26](TABLE 1)

Of the potential covariates assessed for association with NAS severity, breastfeeding demonstrated the strongest association with NAS outcomes with breastfed infants demonstrating reduced LOS by 7.5 days in the replication cohort (p<0.001).(TABLE 2) The majority (90%) of the infants in the breastfed group were fed a mix of formula and breastmilk. In addition, there were differences in NAS pharmacologic treatment rates between the two sites, with 85.8% of the infants from BMC and 64.2% from EMMC treated with medication, with corresponding differences in mean LOS. Buprenorphine-exposed infants had a shorter LOS compared with methadone-exposed infants [16.4 (95% CI 13.5, 19.3) vs 20.5 (95% CI 17.9, 22.9), p=0.04], and infants with maternal smoking co-exposure had longer LOS by 4.2 days (p=0.03). These co-variate associations were strengthened when examined in the combined cohort due to increased subject numbers. There were no significant associations between psychiatric medication and illicit drug co-exposures with NAS outcomes in our replication or combined cohorts.

SNP Associations

A DNA sample was available for all 113 of the infants and 91 of the mothers in the replication cohort. Eighty-seven percent of the samples were collected from buccal cells, and the remaining from saliva. Genotype frequencies for the SNPs had a minor allele frequency of 0.29 – 0.49. Linkage disequilibrium (LD) analysis revealed that the 2 *COMT* variants were moderately correlated $r^2 = 0.42$). LD among the three *PNOC* SNPs was modest to moderate ($r^2 = 0.17–0.65$). None of the SNPs deviated from Hardy Weinberg equilibrium.

SNP association results for infants and mothers in the replication cohort are shown in TABLE 3. Mothers with the *COMT* rs4680 *G* allele had infants with a 50% reduced odds of requiring treatment with two medications (p=0.04). The rs4680 SNP association did not meet the experiment wide significance level after correction for multiple testing. SNP associations from the combined cohort of 199 mother-infant pairs are shown in TABLE 4. Infants with the *PNOC* rs4732636 *A* allele had a two-fold increased need for medication treatment for NAS (p=0.04). Mothers with the *PNOC* rs351776 *A* mothers had infants with two-fold increased risk for treatment with 2 medications (p=0.004) and longer LOS by 3.3 days (p=0.01). Mothers with the *COMT* rs740603 *A* allele had infants with a 50% reduced need for medication treatment (p=0.02). All of the combined cohort SNP associations remained significant point-wise, but did not meet experiment-wide significance after multiple comparison testing correction.

DISCUSSION

The present study is the largest study to date of genetic association with NAS severity, and furthered our hypothesis that genetic variation contributes to differences observed in the NAS phenotype. Specifically, we found differences in NAS outcomes depending on maternal and infant variants within the *PNOC* and *COMT* genes, expanding on our findings from our prior studies, and implicating dopamine, stress and pain pathways in NAS. All of our SNP associations met point-wise significance, though they did not meet experiment-wise significance after correction for multiple SNP and outcome testing.

The *PNOC* maternal SNP associations identified in the enlarged combined cohort are similar to associations found in the infants in our original cohort.[26] Specifically, the A allele in PNOC rs351776 in the mothers was correlated with worse NAS severity, and the rs2614095 *A* allele with improved NAS outcomes, though did not meet experiment wise significance. We also found the same findings of more severe NAS with the rs4732636 A allele in the infants as found in our original cohort. PNOC is a precursor of nociceptin, the ligand of the opioid receptor-like receptor. PNOC is thought to act as a transmitter in the brain by modulating nociceptive and locomotive behavior. Nociceptin also decreases dopamine transmission via activation of GABAergic activity and is anti-analgesic, which may explain the differences in infant response to the stressful process of opioid withdrawal.[24] Previous studies in adults have found associations within the same SNPs in *PNOC* (rs351779 and rs4732636) with risk for alcohol and illicit drug dependence.[14]

The findings with the *COMT* variants in this study have known physiologic significance for opioid drug response in adults.[10–11] The *COMT* rs4680 minor G allele was found to be associated with decreased need for morphine in adult cancer patients.[22–23] *COMT* variants have previously been associated with an increased risk of depression in adults, nicotine dependence, and cocaine-associated paranoia.[17,19,21] Our findings in our replication cohort are similar to prior findings in our original cohort in that G allele carriers of *COMT* rs4680 had improved NAS outcomes.[25] We found this point-wise association in the mothers in our replication cohort, while the association was observed in the infants in the original cohort. Since the minor G allele of rs4680 leads to a 3–4 fold decrease in *COMT* enzyme activity, an increase in circulating catecholamines may lead to improved stress tolerance in opioid-exposed infants.[22] Previously, our group found that the *COMT* rs740603 A allele in opioid-exposed infants was associated with shorter hospital stay.[26] We had a similar finding in the present study with rs740603 in the mothers in the combined cohort.

Products of both the *PNOC* and the *COMT* genes are involved in the dopamine system, and variants in these genes have been linked with risk for opioid addiction and the endogenous stress response.[24] Stress response has been demonstrated as a key element explaining differences in NAS phenotype.[27–28] Prenatal maternal stress can compromise the intrauterine environment and can be transmitted to the infant, with associated elevations in cortisol levels and impaired maternal-infant interaction, all of which can impact infant neurobehavior.[29–31] Maternal stress and elevated cortisol levels may impact the development of the fetal hypothalamic-pituitary-adrenal (HPA)-axis, a main regulator of the stress response. Infants of mothers with opioid use disorders are exposed to high levels of maternal psychosocial stress during the pregnancy that may be transmitted to the infants and contribute to withdrawal severity. Future studies should evaluate genetic variants in additional stress response genes for association with NAS outcomes.

In terms of clinical co-variates associated with NAS severity, we found a strong association between breastfeeding and improved NAS outcomes. This is consistent with prior findings from other studies that have demonstrated less severe withdrawal for infants who are breastfed to any extent, likely representing an increased maternal presence and involvement in infant care, particularly given that the vast majority of infants were given a mix of formula and breastmilk in our cohort.[6] Also, as previously reported in the MOTHER study and in

meta-analysis, we found that buprenorphine-exposed infants had a less severe NAS course than methadone-exposed infants; this may reflect differences in addiction severity and other confounding variables between these two groups of women.[5,7] Lastly, we found an association between co-exposure to nicotine during the third trimester with more severe NAS, consistent with previously published findings.[35] Signs and symptoms of nicotine withdrawal can often mimic opioid withdrawal leading to increased medication treatment of these infants.[35]

This study has some limitations. There was variation in the NAS medication protocol used at the hospital systems with some centers using morphine and others using 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.[2,32] 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.[33] In addition, the care models varied at the centers with some infants cared for in newborn intensive care units, and some within pediatric inpatient wards. The differences seen in medication treatment rates and opioid treatment days between the two cohorts is most likely explained by these institutional differences in NAS care practices, necessitating for the adjustment for study site and medication treatment in all of our regression models. In addition, a significant portion of our population were polypharmacy exposed. It is often difficult to determine which signs and symptoms on the Finnegan scale are due to opioid withdrawal versus withdrawal from these concurrent exposures.[3,7,34] Lastly, our results were based on a relatively small sample and our findings did not reach statistical significance after correction for multiple comparisons. The lack of replication and experiment-wise significance for some of our SNP associations suggests that much larger sample sizes will be needed to optimize the evaluation of the effects of genetic influences on NAS outcomes.

In conclusion, our findings suggest that genetic variation of the *PNOC* and *COMT* genes within opioid-exposed mother infant pairs are associated NAS severity. Further testing in a large sample is warranted before incorporating genetic testing into clinical practice. The identification of a panel of key genetic markers that can assist with prediction of NAS outcomes would allow for more effective monitoring and treatment protocols for opioid-exposed infants in the future.

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TABLE 1

Demographics and NAS Outcomes Replication and Original Cohorts

Demographic	Replication Cohort (n=113) N (%) or Mean (95% CI)	Original Cohort (n=86) N (%) or Mean (95% CI)	P-Value
Study Site			
Tufts Medical Center & Affiliates		51 (59.0%)	
Eastern Maine Medical Center	14 (12.4%)	35 (41.0%)	
Boston Medical Center	99 (87.6%)		
Gestational Age (weeks)	39.0 (38.7, 39.3)	38.7 (38.2, 39.3)	NS
White, Non-Hispanic	99 (88%)	84 (98%)	0.02
Infant Sex – Male	41 (45%)	51 (59%)	0.05
Infant Birth Weight (grams)	3108 (3015–3199)	3207 (3105–3309)	NS
Maternal Opioid Agonist Medication			
Methadone	68 (60%)	55 (64%)	NS
Dose at Delivery (mg/day)	88.1 (78.5, 97.7)	106.0 (81.0, 124.0)	NS
Buprenorphine	45 (40%)	31 (36%)	NS
Dose at Delivery (mg/day)	12.6 (10.6, 14.6)	16.0 (13.0, 19.0)	NS
Breastfed	59 (52%)	38 (44%)	NS
Concurrent Exposures			
Cigarette Smoking	85 (75%)	67 (78%)	NS
SSRI	11 (10%)	4 (5%)	NS
Benzodiazepines	6 (5%)	10 (12%)	NS
Marijuana	13 (12%)	17(20%)	0.02
Cocaine	7 (6%)	5 (6%)	NS
Heroin	9 (8%)	11 (13%)	NS
Other Un-prescribed Opioids	10 (9%)	15 (17%)	0.04
Alcohol	8 (7%)	2 (2%)	NS
NAS Outcomes			
Infant Treated Pharmacologically	94 (83%)	56 (65%)	<0.001
Treated with Two Medications	29 (26%)	38 (44%)	NS
Length of Hospitalization (days)	18.9 (17.0, 20.8)	22.0 (19.0, 26.0)	NS
Length of Opioid Therapy (days)	17.8 (16.2, 19.4)	30.5 (26.2, 34.0)	<0.001

Abbreviations: SSRI = Selective serotonin re-uptake inhibitor; NS = non-significant p-value >0.05; mg = milligrams

TABLE 2

Clinical Variables and Associations with Length of Hospital Stay

Clinical Variable	Replication Cohort (n=113) Length of Stay (days-Mean, 95% CI	P-value	Combined Cohort (n=199) Length of Stay (days)-Mean, 95% CI	P-value
Study Site				
BMC (n=99)	20.1 (18.1, 22.1)	<0.001	20.1 (18.1, 22.1)	NS
EMMC (n=49)	10.3 (5.8, 14.8)		19.0 (13.3, 24.7)	
Tufts (n=51)			26.0 (17.3, 34.7)	
Breastfed – Yes vs No	15.3 (13.1, 17.5) vs 22.8 (19.8, 25.6)	<0.001	15.3 (13.2, 17.4) vs 24.8 (22.0, 27.6)	<0.001
Methadone vs Buprenorphine	20.5 (17.9, 22.9) vs 16.4 (13.5, 19.3)	0.04	22.3 (19.8, 24.8) vs 17.3 (14.5, 20.1)	0.009
Smoking – No vs Yes	15.7 (12.6, 18.8) vs 19.9 (17.6, 22.2)	0.03	16.8 (13.5, 20.1) vs 21.3 (19.0, 23.6)	0.02
Psych Meds – No vs Yes				
Benzodiazepines	18.9 (16.9, 20.7) vs 19.0 (6.0, 32.0)	NS	19.6 (17.7, 21.5) vs 25.2 (14.8, 35.6)	NS
SSRIs	18.2 (16.4, 20.0) vs 24.5 (14.9, 34.1)	NS	20.1 (18.1, 22.1) vs 20.3 (12.3, 28.3)	NS
Other Psych Meds *	18.3 (16.4, 20.2) vs 21.9 (15.3, 28.5)	NS	19.8 (17.8, 21.8) vs 22.3 (16.5, 28.1)	NS

Abbreviations: Psych Meds = Psychiatric medications; SSRIs = selective serotonin reuptake inhibitors; BMC = Boston Medical Center; EMMC = Eastern Maine Medical Center; NS = non-significant p-value >0.05;

* Other psychiatric medications = Gabapentin, Clonidine, Wellbutrin, Neurontin, Amitriptyline

TABLE 3

SNP Associations in Replication Cohort (n=113)

SNP (Allele)	Gene	Mom or Infant	LOS (β in days, 95% CI)	p-value	NAS Medication Treatment (OR, 95% CI)	p-value	≥ 2 Meds (OR, 95% CI)	p-value	Opioid Days	P-value
rs4732636 (A)	PNOC	Infant	−0.7 (−3.6, 2.3)	NS	1.8 (0.7, 4.4)	NS	1.0 (0.5, 2.0)	NS	−1.4 (−3.9, 1.1)	NS
rs4732636 (A)	PNOC	Mom	−0.7 (−3.6, 2.3)	NS	0.9 (0.4, 2.3)	NS	0.9 (0.4, 2.1)	NS	−1.2 (−3.9, 1.6)	NS
rs351776 (A)	PNOC	Infant	1.8 (−1.2, 4.7)	NS	1.3 (0.6, 3.1)	NS	0.7 (0.3, 1.5)	NS	1.3 (−1.3, 3.9)	NS
rs351776 (A)	PNOC	Mom	1.8 (−1.2, 4.7)	NS	0.6 (0.2, 1.4)	NS	1.6 (0.8, 3.3)	NS	2.0 (−0.6, 4.6)	NS
rs2614095 (A)	PNOC	Infant	−0.8 (−3.3, 1.8)	NS	0.5 (0.2, 1.0)	NS	1.3 (0.7, 2.5)	NS	0.3 (−1.9, 2.5)	NS
rs2614095 (A)	PNOC	Mom	−0.8 (−3.3, 1.8)	NS	1.3 (0.5, 3.4)	NS	0.8 (0.4, 1.7)	NS	−1.3 (−3.7, 1.2)	NS
rs740603 (A)	COMT	Infant	1.7 (−1.2, 4.5)	NS	1.8 (0.8, 3.9)	NS	1.8 (0.9, 3.6)	NS	0.5 (−2.1, 3.1)	NS
rs740603 (A)	COMT	Mom	−2.9 (−5.8, 0)	NS	0.5 (0.2, 1.3)	NS	0.7 (0.4, 1.4)	NS	−2.0, (−.4.5, 0.6)	NS
rs4680 (G)	COMT	Infant	−0.8 (−3.3, 1.6)	NS	1.4 (0.7, 2.7)	NS	1.0 (0.6, 2.6)	NS	−0.4 (−2.6, 1.9)	NS
rs4680 (G)	COMT	Mom	0.8 (−1.6, 3.3)	NS	0.6 (0.25, 1.7)	NS	0.5 (0.2, 1.0)	**0.04**	2.3 (−0.1, 4.8)	NS

Results are adjusted for breastfeeding, study site, and methadone vs buprenorphine exposure

Abbreviations: SNP = single nucleotide polymorphisms; LOS = length of hospital stay; Meds = medications; NAS = neonatal abstinence syndrome; NS = non-significant p-value ≥0.05.

TABLE 4

SNP Association in Combined Cohort (n=199 pairs)

SNP (Allele)	Gene	Mom or Infant	LOS (β in days, 95% CI)	p-value	NAS Medication Treatment (OR, 95% CI)	p-value	≥ 2 Meds (OR, 95% CI)	p-value	Opioid Days	P-value
rs4732636 (A)	PNOC	Infant	1.0 (−1.3, 3.2)	NS	2.0 (1.0, 38)	**0.04**	1.5 (0.9, 2.7)	NS	0.5 (−2.2, 3.2)	NS
rs4732636 (A)	PNOC	Mom	1.0 (−1.2, 3.3)	NS	1.5 (0.8, 2.7)	NS	1.8 (1.0, 3.0)	**0.04**	1.0 (−1.8, 3.8)	NS
rs351776 (A)	PNOC	Infant	1.5 (−0.5, 3.5)	NS	1.0 (0.6, 1.7)	NS	0.9 (0.6, 1.6)	NS	2.1 (−0.4, 4.6)	NS
rs351776 (A)	PNOC	Mom	2.4 (0.3, 4.4)	**0.03**	1.1 (0.6, 1.9)	NS	2.3 (1.3, 3.9)	**0.004**	3.3 (0.7, 5.8)	**0.01**
rs2614095 (A)	PNOC	Infant	−0.2 (−2.1, 1.7)	NS	0.7 (0.4, 1.1)	NS	1.0 (0.6, 1.6)	NS	−0.3 (−2.5, 2.0)	NS
rs2614095 (A)	PNOC	Mom	−2.3 (−4.4, −0.2)	**0.04**	0.8 (0.5, 1.5)	NS	0.6 (0.3, 0.9)	**0.03**	−3.2 (−5.8,−0.6)	**0.02**
rs740603 (A)	COMT	Infant	0.1 (−1.9, 2.1)	NS	1.4 (0.9, 2.4)	NS	1.9 (1.1, 3.1)	**0.02**	0.8 (−1.8, 3.4)	NS
rs740603 (A)	COMT	Mom	−1.7 (−3.9, 0.4)	NS	0.5 (0.3, 0.9)	**0.02**	0.9 (0.5, 1.3)	NS	−2.2 (−4.8, 0.4)	NS
rs4680 (G)	COMT	Infant	−0.8 (−2.8, 1.2)	NS	1.3 (0.8, 2.3)	NS	1.1 (0.6, 1.8)	NS	0.4 (−2.2, 2.9)	NS
rs4680 (G)	COMT	Mom	0.7 (−1.5, 2.9)	NS	1.0 (0.6, 1.9)	NS	1.4 (0.8, 2.3)	NS	1.2 (−1.5, 3.8)	NS

Results are adjusted for breastfeeding, study site, meth vs bph. LOS models also adjusted for infant treatment.

Abbreviations: SNP = single nucleotide polymorphisms; LOS = length of hospital stay; Meds = medications, NAS = neonatal abstinence syndrome; NS = non-significant p-value ≥0.05.