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The genetics and epigenetics of Neonatal Abstinence Syndrome

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ABSTRACT

Neonatal abstinence syndrome (NAS) due to in-utero opioid exposure is a growing epidemic with significant variability in clinical presentation and severity. Currently, NAS severity cannot be predicted based on clinical factors alone. To date, small studies have identified genetic variants in opioid receptor and stress response genes that are associated with differences in NAS pharmacologic treatment rates and length of hospitalization. In addition, epigenetic variation in the mu opioid receptor (OPRM1) gene has been associated with differences in NAS hospitalization outcomes. Examination of maternal genetic and epigenetic profiles may assist in prediction of NAS severity. Large-scale genomic studies are needed to elucidate the genetic architecture of and epigenetic modification related to NAS in order to develop more tailored personalized treatments for NAS.

1. Introduction

Neonatal Abstinence Syndrome (NAS), also referred to as Neonatal Opioid Withdrawal Syndrome (NOWS), is an opioid withdrawal syndrome that infants develop after in-utero exposure to opioids [1]. Infants typically experience signs of opioid withdrawal 2–3 days after birth, and are monitored for the need for pharmacologic treatment typically consisting of replacement opioids such as methadone, morphine, or buprenorphine which is weaned during a 1–3 week period [1,2]. Approximately 30–80% of opioid-exposed infants receive pharmacologic treatment, with an associated average length of hospitalization for treated infants of 23.0 days in 2012 [3]. The incidence of NAS increased 5-fold between 2000 and 2012, and has continued to an estimated rate in 2016 of 20 per 1000 live births [3,4]. Most infants with NAS in the U.S. are cared for in intensive care nurseries with associated hospitalization costs of over $93,000 per affected infant [3].

NAS is a highly variable disease that remains very poorly understood despite decades of research. It is impossible to accurately predict which infants will require pharmacologic treatment, necessitating all opioid-exposed infants to monitored in the hospital typically for 4–7 days after birth [1,2]. In addition, a subset of infants, up to 25–30% in some studies, develop a more severe phenotype of NAS that is treated with a multi-drug regimen resulting in a more prolonged course of pharmacotherapy [1,2]. Several clinical variables have been identified that are associated with differences in NAS severity. Specifically, type of maternal opioid agonist medication (methadone versus buprenorphine), concurrent exposures to psychiatric medications and nicotine, illicit drug co-exposures, gestational age, and infant sex have all been associated with differences in NAS inpatient outcomes [5–7]. There is no clear association between the dose of maternal opioid agonist and NAS severity, whereas non-pharmacologic factors including breastfeeding, parental presence, and rooming-in models of care have been associated with lower rates of pharmacologic treatment and significantly shorter hospitalizations [2,5,8]. However, these clinical factors provide limited accuracy in prediction of the need for pharmacotherapy and non-responsiveness to first-line medication treatment for any particular infant.

Genetic factors likely account for much of the unexplained variability in NAS severity. Propensity for opioid addiction is largely heritable according to adult twin studies, with about 50% of risk attributable to a combination of genetic factors, suggesting that the impact of opioids and ability to withdraw are likely genetically influenced [9,10]. In adults, candidate gene studies and genome-wide association studies (GWAS) have identified association of single nucleotide polymorphisms (SNPs) with risk for opioid addiction and response to opioid therapy in both children and adults [9,11,12]. For example, a variant in the mu opioid receptor (OPRM1) gene (SNP 118A > G; rs1799971) has been associated with risk for a number of addictive behaviors and variations

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in response to opioids [9,13]. Associations have also been identified with variants in other opioid receptor and metabolism genes, as well as in genes within the dopamine and endogenous stress pathway [9,11]. Dopamine is the primary neurotransmitter involved in addiction and several drugs including opioids elevate dopamine levels above baseline [14]. The minor allele of SNP rs4680 (158A > G) in the catechol-o-methyltransferase (COMT) gene, which encodes an enzyme that metabolizes dopamine in the central nervous system, has been associated with lower morphine dosing requirements in adults and post-operative patients [15–17]. In addition to DNA sequence variants, there is evidence suggesting that epigenetic factors involving DNA methylation or histone modification may be involved and have been linked in prior adult studies for modifying risk for addiction [18,19]. It is therefore feasible that genetic and epigenetic factors are responsible for the significant inter-patient variability in NAS despite similar maternal exposures and hospital care environments.

The purpose of this paper is to review the studies to date focused on the genetics and epigenetics of NAS, and consider future research aimed at the development and application of personalized medicine approaches that incorporate infant and maternal genetic risk profile into clinical practice.

2. Genetics of NAS

2.1. Opioid receptor and related genes

The mu-opioid receptor gene OPRM1 is the most extensively studied gene in adult opioid addiction [13]. The G allele of SNP rs1799971, which has a frequency of 12–15% in persons of European ancestry (EA), causes an amino acid change (asparagine to aspartic acid) that results in a 3-fold increase in binding with beta-endorphin [20]. Animal studies demonstrated that the G allele is associated with a decrease in opioid receptor binding and SNP genotype influences differences in the affective component of morphine withdrawal [13,20]. The G allele has also been associated with an increased risk for opioid and alcohol addiction in adults [21]. Smith et al. conducted a GWAS of usual daily methadone dose in methadone-treated opioid dependent (OD) adult subjects [12]. In 383 African American OD subjects, they identified a genome-wide significant association between therapeutic methadone dose and rs73568641 ($P = 2.8 \times 10^{-8}$), the nearest gene being OPRM1 [12]. Each minor (C) allele corresponded to an additional 20 mg per day of oral methadone. This association was not evident in the portion of the sample containing persons of European ancestry [12]. In an independent set of 241 opioid-naive African American children treated for surgical pain, the study authors found that rs73568641-C was associated with a higher required dose of morphine ($P = 0.039$) [12].

OPRM1 associations with NAS severity have been examined in several studies. In a study of OPRM1 genotype in 86 opioid-exposed mother-infant dyads, we found that infants with at least one copy of the G allele in rs1799971 had less severe NAS as indicated by an 8.5 day lower length of hospitalization and decrease in pharmacologic treatment [22]. In addition, the maternal G allele was associated with less pharmacologic treatment [22].(Table 1) Paradoxically, infants with the G allele may be protected against experiencing more severe withdrawal symptoms, but have a higher risk for developing an opioid addiction as adults. Mactier et al. also examined rs1799971 in a study of 21 infants exposed to methadone and found no association with NAS pharmacologic treatment [23]. We did not identify any significant associations of NAS inpatient outcomes with 12 additional OPRM1 SNPs in a subsequent study of the same 86 mother-infant dyads as the original study [23].

The delta (OPRD1) and kappa (OPRK1) opioid receptor genes are key opioid action sites in the central nervous system [11,13,21]. Kappa opioid receptors are widely distributed in the CNS, particularly in the mesolimbic pathway, and play a key role in pain regulation, cardiovascular function, breathing, temperature regulation, feeding, and stress response [9]. One study identified an association of the C allele of OPRK1 SNP rs702764 with more severe NAS defined as requiring treatment with two medications [24].(Table 1) This finding is consistent with prior reports of association between with OPRK1 and severity of opioid withdrawal symptoms in adults [11,21,25–27]. Notably, Wang et al. found an association of the rs702764 C allele with increased opioid withdrawal symptoms in adult methadone maintenance patients [26]. The delta opioid receptor encoded by OPRD1 binds the endogenous ligand enkephalin. These receptors have been implicated in the modulation of addiction, gastrointestinal function, respiration, pain perception, and analgesia [11]. OPRD1 SNPs have been associated with differences in response to opioid treatment and development of morphine tolerance in adults [11,28,29]. In the only study to examine the role of OPRD1 in NAS, we found an association with the OPRD1 rs204076 A allele in opioid-dependent mothers with more severe NAS in their infants who required longer hospitalizations and more treatment with adjunctive medications for NAS [24]. (Table 1).

Endogenous opioid peptide genes have also been examined for association with NAS. Prepronociceptin (PNOC) is a precursor of nociception, the ligand of the opioid receptor-like receptor. It is thought to act as a transmitter in the brain by modulating nociceptive and locomotor behavior [9,11]. Nociceptin decreases dopamine transmission and is anti-analgesic, which may explain differences in infant responses to stress and opioid withdrawal [30]. In adults, associations of PNOC SNPs rs351779 and rs4732636 with risk for alcohol and illicit drug dependence have been identified [30]. The minor alleles of one of these SNPs (rs4732636) and two other PNOC SNPs (rs351776 and rs2614095) in infants with NAS were associated with longer LOS and increased need for adjunctive medication treatment [24]. In a subsequent study of an enlarged cohort, rs351776 A genotype in infants and rs4732636 A genotype in mothers were also associated with NAS severity [31]. The maternal rs2614095 A genotype was also associated with NAS severity, but the effect direction was opposite that observed in the previous study [31]. (Table 1).

2.2. Stress response and dopamine genes

Genes related to dopamine and the hypothalamic pituitary axis (HPA) and associated endogenous stress pathways also play an important role in opioid addiction and withdrawal [14–17]. These include the catechol-O-methyltransferase (COMT) gene which is involved in dopamine metabolism, dopamine receptor genes, as well as genes related to dopamine, serotonin, and norepinephrine transport such as SLC6A2, SLC6A3, and SLC6A4. [11,12,14] In a study of three SNPS in the in the gene encoding the dopamine D2 receptor (DRD2) using DNA collected from stored blood spots from newborn testing of 48 drug-exposed (94% opioid-exposed) and 48 control infants, Oei et al. found that the 141ins (rs17997332) allele was more common in opioid-exposed infants who did not require pharmacologic treatment versus those who did (56% vs 22%, $p = 0.02$) [32]. We found that the COMT SNP rs4680 G allele in infants was associated with less need for pharmacologic treatment and shorter LOS by 10.8 days in a cohort of 86 mother-infant dyads [22].(Table 1) The same allele in the mothers was associated with less need for adjunctive medication treatment [31]. In addition, the COMT rs740603 A allele in opioid-exposed infants was associated with shorter hospitalizations and less NAS pharmacologic treatment, but this finding was not sustained in an enlarged cohort [24,31]. However, maternal rs740603 genotype was associated with the amount of adjunctive medication treatment [31]. Mactier examined 4 SNPs in COMT (rs4633, rs4680, rs4818, rs6269) and found no association with NAS pharmacologic treatment rates in a small cohort of 21 opioid-exposed infants [23]. Rs4680 leads to a valine-to-methionine amino acid substitution (158A > G) [16]. The Met-containing enzyme (G allele) demonstrates a 3-fold reduction in COMT enzyme activity with upregulation of the mu-opioid receptor in various brain regions

Table 1
SNP associations with NAS phenotype.

Study	Mother or Infant	Gene	SNP	Allele	NAS Phenotype
Opioid Receptor Genes					
Wachman 2013, 2015 [22,24]	Infant	OPRM1	rs1799971	G	OR = 0.8 Any NAS medication (p = 0.006); LOS β = −8.5 days (p = 0.009)
Wachman 2013 [22]	Mother	OPRM1	rs1799971	G	OR = 0.7 Any NAS medication (p = 0.008)
Wachman 2015 [24]	Infant	OPRK1	rs702764	C	OR = 4.1 Two NAS medications (p = 0.003)
Wachman 2015 [24]	Mother	OPRD1	Rs204076	A	OR = 2.8 Two NAS medications (p = 0.04); LOS β = 6.6 days (p = 0.008)
Wachman 2015 [24]	Infant	PNOC	rs351776	A	OR = 3.2 Two NAS medications (p = 0.01)
Wachman 2017 [31]	Mother	PNOC	rS351776	A	OR = 2.3 Two NAS medications (p = 0.004); LOS β = 2.4 days (p = 0.03)
Wachman 2015 [24]	Infant	PNOC	rs2614095	A	OR = 0.3 Two NAS medications (p = 0.01); LOS β = 0.47 days (p = 0.04)
Wachman 2017 [31]	Mother	PNOC	rs2614095	A	OR = 0.6 Two NAS medications (p = 0.03); LOS β = −2.3 days (p = 0.04)
Wachman 2015 [24]	Infant	PNOC	rs4732636	A	OR = 3.8 Two NAS medications (p = 0.004); LOS β = 5.8 days (p = 0.01)
Wachman 2017 [31]	Mother	PNOC	rs4732636	A	OR = 1.8 Two NAS medications (p = 0.04)
Stress Response Genes					
Wachman 2013 [22]	Infant	COMT	rs4680	G	OR = 0.8 Any NAS medication (p = 0.02); OR = 0.7 Two NAS medications (p = 0.001); LOS β = −10.8 days (p = 0.005)
Wachman 2017 [31]	Mother	COMT	rs4680	G	OR = 0.5 Two NAS medications (p = 0.04)
Wachman 2015 [24]	Infant	COMT	rs740603	A	OR = 0.4 Any NAS treatment (p-0.01); LOS β = −5.3 (p = 0.01)
Wachman 2017 [31]	Infant	COMT	rs740603	A	OR = 1.9 Two NAS medications (p = 0.02)
Wachman 2017 [31]	Mother	COMT	rs740603	A	OR = 0.5 Any NAS medication (p = 0.02)
Oei 2012 [32]	Infant	DRD2	rs1799732	Ins	OR = 0.39 Any NAS medication (p = 0.02)
Metabolism Genes					
Mactier 2017 [23]	Infant	CYP2B6	rs3745274	G	OR = 4.0 Any NAS medication (p = 0.015)
Mactier 2017 [23]	Infant	CYP2B6	rs2279343	A	OR = 3.9 Any NAS medication (p = 0.023)

OR = odds ratio; LOS = length of hospital stay.

[15]. Previous studies in adult cancer patients indicated that individuals with the Met/Met (GG) genotype require less morphine for pain control [16,17]. Infants with NAS with the G allele likely have increased levels of circulating catecholamines resulting in improved stress tolerance and ability to tolerate withdrawal.

2.3. Opioid metabolism genes

Agents that metabolize opioids such as morphine, methadone, and buprenorphine are compelling targets for candidate gene studies. Morphine and methadone are substrates of the P-glycoprotein transporter 170, which is encoded by the ABCB1 gene that regulates opioid absorption, distribution, and elimination [9]. Minor alleles of ABCB1 SNPs rs2032582, rs1128503, and rs1045642 are common (40–50% in persons of European ancestry) and have been associated with higher methadone dose requirements [33,34]. Wachman et al. found no association of the these SNPs with NAS severity in aforementioned cohort of 86 mother-infant opioid-exposed dyads [22]. Another study of 21 opioid-exposed infants also did not find evidence for association of these SNPs with response to NAS pharmacologic treatment, but the sample size was relatively small [23]. Other logical candidate genes include members of the cytochrome P450 (CYP) family, specifically CYP2B6 and CYP2D6 which metabolize methadone, and CYP34A which metabolizes buprenorphine [9,11]. A challenge in interpreting results from studies of these genes in infants is that CYP enzyme levels vary in the neonatal period and, thus, the significance of variant associations is unclear [35]. One small study found a 4-fold increased risk for NAS pharmacologic treatment among methadone-exposed infants who were homozygous for the major alleles of CYP2B6 SNPs rs3745274 (p = 0.015) and rs2279343 (p = 0.023), but no association with two SNPs in CYP2D6.[23] (Table 1).

3. Epigenetics of NAS

In addition to differences in DNA sequence, changes in gene expression due to epigenetic modification may influence NAS-related outcomes. Epigenetic changes are triggered by environmental exposure including in-utero stress and addictive drugs such as opioids; this could theoretically lead to differences in response to pharmacotherapy and alterations in infant withdrawal behaviors after birth [18,36,37]. Cytosine methylation is a common epigenetic change that occurs when a methyl group is added to cytosine residues of cytosine:guanine (CpG) dinucleotides. Chronic opioid exposure is known to alter the methylation status of particular CpG sites within the promoter region of a gene, leading to changes in level of gene expression [18,36].

Studies in adults demonstrated that increased methylation in the OPRM1 promoter region was associated with decreased gene expression of OPRM1 [18]. Hypermethylation in this region has been observed in opioid dependent adults compared with controls [18,19,37]. Transgenerational effects of chronic opioid exposure have been demonstrated in animal models, suggesting that epigenetic changes in mothers triggered by opioid exposure may be transmitted to their infants, thus altering sensitivity to opioids [38]. The heritability of these changes is supported by evidence of examination of hypermethylation in OPRM1 in sperm from opioid addicts compared with controls [39]. It is unknown how this epigenetic variability changes across one's lifespan, and how this may contribute to one's risk to develop opioid addiction. Previous studies in cancer and post-operative patients have demonstrated differences in OPRM1 promoter methylation patterns are associated with the amount of opioid exposure and pain tolerance [40,41]. Wachman et al. found that infants with severe NAS requiring more pharmacologic treatment had higher levels of OPRM1 promoter methylation at specific CpG sites, notably at transcription factor binding sites [42,43].(Table 2) Another study reported an increase in OPRM1 methylation across CpG sites in the opioid-exposed infants compared with controls (8% vs. 3%, p < 0.0005), but no associations of OPRM1

Table 2
Epigenetic findings for NAS in OPRM1.

Study	CpG Site	Mother or Infant	NAS treatment outcome
Wachman 2014 [42]	−10	Infant	Higher methylation with any NAS medication (β = 3.2%, p = 0.03); Higher methylation with two NAS medications (β = 5.0%, p = 0.0005); Correlation with LOS (R = 0.27, p = 0.03)
Wachman 2014 [42]	−14	Infant	Higher methylation with two NAS medications (β = 4.9%, p = 0.003)
Wachman 2014 [42]	+84	Infant	Higher methylation with two NAS medications (β = 3.3%, p = 0.02)
Wachman 2018 [43]	−18	Infant	Higher methylation with any NAS medication (β = 7.0%, p = 0.0001)
Wachman 2018 [43]	−14	Infant	Higher methylation with any NAS medication (β = 22.1%, p = 0.002)
Wachman 2018 [43]	+23	Infant	Higher methylation with any NAS medication (β = 13.4%, p = 0.008)
Wachman 2018 [43]	−169	Mother	Correlation with infant LOS (R = 0.43, p = 0.008)
Wachman 2018 [43]	−152	Mother	Correlation with infant LOS (R = 0.40, p = 0.002)
Wachman 2018 [43]	+84	Mother	Correlation with infant LOS (R = 0.44, p = 0.006)

LOS = length of hospital stay; R = correlation coefficient.

methylation levels with NAS severity [44]. Infants with higher levels of DNA methylation may have down-regulated OPRM1 gene expression leading to reduced levels of mu-opioid receptors. This may in turn lead to an increased need for opioid medications to treat NAS. Studies to date were not able to show causation and did not follow infants prospectively which would allow evaluation at the long-term impact of this epigenetic change on response to opioids and risk for addiction.

As described above, ABCB1 is involved in the transport of opioids such as morphine and methadone, and CYP2D6 in the metabolism of methadone [9,11]. McLaughlin et al. examined buccal cell DNA samples from 21 methadone-exposed neonates, 17 controls exposed to maternal nicotine, and 15 controls without prenatal nicotine or opioid exposure and found that the opioid-exposed infants had higher levels of methylation across ABCB1 (18% vs. 3%, $p < 0.0005$) and CYP2D6 (92% vs. 89%, $p < 0.0005$) compared with opioid-naïve newborns [44]. There was no association of LOS or infant pharmacologic treatment with ABCB1 or CYP2D6 DNA methylation in the opioid-exposed group [44].

4. Challenges and future directions

Studying the genetics and epigenetics of NAS holds significant challenges. First, it is difficult to account for the array of clinical variables that also influence the outcome, including prenatal and postnatal factors. Most notable, there is not a universally accepted standardized NAS phenotype for clinical application of genetic information. Most studies use pharmacologic treatment or LOS as the primary outcome measure for NAS severity, however these outcomes have been shown to be highly influenced by hospital care models such as whether or not rooming-in is available, the NAS assessment tool used, and non-pharmacologic care factors such as breastfeeding, all which can alter the medication treatment rates by up to 60% despite identical prenatal exposure variables [2,8]. Thus, generalizability of genetic and epigenetics data are limited because prediction models would have to account for this vast variability in NAS care practices.

In addition, previous studies were small and lacked replication in independent cohorts, and many of the reported findings were not experiment-wide significant after correction for multiple testing [45]. Much larger samples are needed to have sufficient power to examine many genetic variants in a single study. The requisite sample size will depend on the number of variants (i.e., selected from a group of genes in a pathway versus genome-wide) and anticipated magnitude of effect on the outcome. Recruiting large sample in this population is challenging, particularly in the setting of varying NAS care models. In addition, obtaining genetic material is more challenging in neonates, such that most studies to date have utilized either saliva or buccal cell specimens which yield much less DNA than blood or other tissues. Results from epigenetic studies using DNA extracted from saliva or buccal cells have been questioned because of the limited comparability of methylation patterns in peripheral tissues and brain [46]. Currently, the paucity of human neonatal brain tissue, especially from infants with NAS, hinders studies of genomics in NAS infants and experiments aimed at examining the impact of *in utero* opioid exposure on the brain. Emerging animal models may provide valuable information about these aspects [47].

Another limitation is that most infants are exposed to multiple drugs and other substances, such as nicotine, which are known to alter the epigenetic profile [48]. Lastly, prevalent lifestyle factors in the mothers such as stress and prior exposure to trauma are known to alter DNA methylation and were not examined in studies to date [49,50]. DNA methylation within the placenta of two cortisol response genes, the glucocorticoid receptor (NR3C1), which binds cortisol, and 11-beta hydroxysteroid dehydrogenase (HSD11B2), the enzyme responsible for conversion of cortisol into its inactive form, have been associated with differences in infant stress responses in other populations and have not yet been examined in relation to opioid exposure [49,50]. Maternal stress has been associated with lower levels of placental DNA methylation of HSD11B2 and better stress tolerance [49,50].

There are several directions for future studies of the genetics and epigenetics of NAS to identify clinically actionable information. For example, investigation of the influence of variants in opioid metabolism genes on differential response to medication treatment holds promise for individualized pharmacologic treatment regimens. In addition, there is a need shift our attention away from the initial NAS hospitalization; continued emphasis on factors predicting infant hospital LOS for NAS may not lead to new biological findings with translational impact because LOS is highly influenced by hospital care models and modifiable non-pharmacologic factors. Perhaps more important to focus on is the impact of maternal and infant genetic and epigenetic profiles on the infant's future response to opioids and risk for addiction in young adulthood. While it is known that adults with opioid use disorder have higher levels of DNA methylation in OPRM1 compared with controls, and that higher DNA methylation levels in OPRM1 are also seen in infants with more severe NAS, whether or not these neonatal methylation levels persist throughout young adulthood and contribute to the risk for addiction is current unknown. Animal models designed to investigate the transgenerational effect (including, but not limited to, genetic, epigenetic and *in utero* factors) hold promise for advancing the field. Finally, examining how the maternal and infant epigenetic profile is altered by different maternal treatment regimens represents an area that is potentially modifiable pre-delivery.

5. Conclusions

Genetics and epigenetics of NAS is an innovative area of research that shows promise for improving our understanding of the variability in NAS-related outcomes and individual responses to therapy. Studies to date have been limited by small sample size, lack of replication, and unclear clinical significance of the findings. There is a need for additional research focused on the long-term impact of genetic and epigenetic variation, with a focus on modifiable factors.

6. Practice points

- Neonatal Abstinence Syndrome (NAS) has a highly variable condition which is influenced by a variety of clinical, genetic, and epigenetic factors.
- Genetic variation may explain individualized responses to NAS pharmacotherapy.
- Transgenerational epigenetic modification may also explain initial response to opioid medications and withdrawal in the infant.
- The use of genetic markers to predict NAS outcomes is currently not in clinical practice and requires further research.

7. Research directions

- The development of animal models of NAS examining epigenetics and gene expression in brain tissue after in-utero opioid exposure, as well as the transgenerational impact on risk for addictive behaviors in young adulthood.
- The examination of the various maternal treatments for opioid use disorder in pregnancy, and the impact on epigenetic profiles in the offspring throughout childhood and young adulthood, including the impact on future risk for the development of addiction
- The examination of genes of opioid metabolism in infants with NAS to develop optimal individualized pharmacologic treatment regimens.

Conflicts of interest

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