Variations in Infant CYP2B6 Genotype Associated with the Need for Pharmacological Treatment for Neonatal Abstinence Syndrome in Infants of Methadone-Maintained Opioid-Dependent Mothers

Helen Mactier, MBChB, MD¹ Poppy McLaughlin, PhD² Cheryl Gillis, MBChB¹ Michael David Osselton, PhD²

¹Neonatal Unit, Princess Royal Maternity, Glasgow, United Kingdom
²Department of Anthropology and Forensic Science, Bournemouth University, Fern Barrow, Poole, United Kingdom


Abstract

Background Neonatal abstinence syndrome (NAS) in infants of methadone-maintained opioid-dependent (MMOD) mothers cannot be predicted in individual cases. We investigated whether variation in infant genotype is associated with severity of NAS.

Methods This is a pilot observational cohort study of 21 MMOD mothers and their newborns. Infant buccal swabs were obtained soon after delivery, together with a maternal blood sample for the determination of maternal plasma methadone concentration. Genomic variation in five opioid-related genes (ABCB1, COMT, CYP2B6, CYP2D6, and OPRM1) was ascertained from infant buccal swabs and related to need for pharmacological treatment of NAS.

Results Out of 21 infants, 11 (52%) required treatment for NAS. Mothers of treated infants tended to have been prescribed higher doses of methadone, but plasma methadone concentrations did not differ between mothers of treated or untreated babies. Treated and untreated babies did not differ in terms of method of feeding. Treated infants were more likely to carry the normal (homozygous) allele at 516 and 785 regions of CYP2B6 gene (p = 0.015 and 0.023, respectively). There were no differences in any other genes between infants who did or did not require treatment for NAS.

Conclusion Genomic variation in CYP2B6 may explain, at least in part, severity of NAS.

Variation in opioid metabolism is well recognized in adults. The CYP2B6 gene encodes a cytochrome P450 mono-oxygenase enzyme involved in the metabolism of methadone. Seventy-five percent of the Caucasian population carries the homozygous, or wild-type, alleles at CYP2B6 516 G>T and 785 A>G, associated with normal enzyme function. Single-nucleotide polymorphisms (SNPs) in CYP2B6 are associated with variation in methadone response and carriers of the CYP2B6*6 genotype demonstrate slower metabolism and higher trough plasma concentrations of methadone.
methadone compared with noncarriers.\textsuperscript{6–8} SNPs in \textit{OPRM1} and \textit{COMT} opioid-related genes in the infant are associated with NAS treatment and length of hospital stay,\textsuperscript{8} but to date the association between SNPs in infant \textit{CYP2B6} and NAS has not been reported.

Methods

All methadone-maintained opioid-dependent (MMOD) mothers delivering after 36 weeks' gestation in a single maternity unit in Glasgow were eligible to participate in this study if they were being accommodated with their baby in the postnatal ward. Potential participants were identified in the postnatal wards within 48 hours of delivery. Mothers had been managed within a multidisciplinary service for women with substance misuse problems; methadone maintenance was provided in collaboration with social work and addiction services, tailored to symptoms. Sufficient methadone was prescribed to eliminate physical withdrawals, with the aim of reducing to the lowest acceptable dose of methadone prior to delivery.

Following parental consent, a buccal swab (Catch-All; Cambio Ltd., Cambridge) was obtained from the baby and a venous blood sample obtained from the mother for estimation of trough plasma methadone concentration.

Demographic data included maternal age and prescribed dose of methadone at delivery, infant gestation, birth weight, feeding method, and duration of hospital stay. Local policy was to nurse all babies of MMOD mothers in the postnatal ward with their mothers, regardless of the need for NAS treatment unless the baby had another medical or social indication for admission to the neonatal unit. NAS was managed according to protocol using a local version of the Lipsitz system for scoring.\textsuperscript{10} Infants who scored 5 or more on two consecutive occasions and/or with poor feeding or ongoing weight loss after 5 days were commenced on oral morphine at 60 µg/kg six times daily. Dose was increased to 80 µg/kg per dose if the baby remained symptomatic; otherwise morphine was weaned daily by 10 µg/kg/dose. If NAS symptoms were not controlled by oral morphine, phenobarbital was given in addition. Regardless of treatment, all infants remained in the postnatal ward with their mother for a minimum of 5 days. Length of stay for treated babies was determined by success of weaning of morphine; for treatment periods longer than 10 to 12 days, the mother was discharged from hospital and baby admitted to the neonatal unit. Following weaning of oral morphine, phenobarbital treatment could be continued as an outpatient. Breast-feeding was encouraged for all babies. The research team was not involved in any decision to treat an infant.

Buccal DNA was extracted from swabs by immersion in QuickExtract DNA solution (Cambio Ltd., Cambridge). Extracted DNA was analyzed for genomic variants in five genes; \textit{ABCB1, COMT, CYP2B6, CYP2D6,} and \textit{OPRM1} by LGC Genomics using Kompetitive Allele Specific Polymerase chain reaction (KASP). SNPs analyzed included ABCB1 1236 C>T, 2677 G>T, and 3435 C>T; COMT –98 A>G, 186 C>T, 408 C>G, and 472 A>G; CYP2B6 516 G>T and 785 A>G; CYP2D6 1707 T>delT; and OPRM1 118 A>G. The number of functioning \textit{CYP2D6} alleles was ascertained using a Hy-Beacon assay (LGC Genomics, Teddington). Maternal plasma samples were analyzed for methadone using liquid–liquid extraction and subsequent gas chromatography mass spectrometry.

The influence of gestation, birth weight, methadone dose, and plasma methadone concentration on NAS treatment and length of hospital stay was determined using independent sample t-tests (parametric) or Mann–Whitney U-test (nonparametric data). Fisher’s exact test was used to compare genotype distribution between infants who did or did not require treatment for NAS. The study was approved by West of Scotland Research Ethics Committee, and written informed consent obtained from all participating mothers.

Results

Twenty-one of 22 mothers approached agreed to participate; samples were obtained from all 21 mother–infant pairs. Gestation ranged from 36 \textsuperscript{1} to 41 \textsuperscript{0} (median 39 \textsuperscript{0}) weeks and birth weight from 2,198 to 3,400 (median: 2,746) g. Median maternal age at delivery was 32 (range: 25–42) years. Median prescribed dose of methadone at delivery was 55 (range: 2–100) mg; all mothers reported cigarette smoking. Maternal plasma methadone concentration ranged from 20 to 720 (median: 200) ng/mL.

Eleven of 21 (52\%) infants required treatment with oral morphine, five of whom additionally received phenobarbital. Total duration of treatment varied from 8 to 134 (median: 44) days. There was no difference between babies who did or did not receive treatment in terms of gestation, birth weight, or method of feeding. Treated babies' length of hospital stay was longer (median: 20 [range: 10–42] vs. 7 [range: 5–11] days; \(p < 0.005\)). Mothers of treated infants were older (\(p = 0.02\)) and tended to be prescribed higher doses of methadone; plasma concentrations of methadone tended to be higher for mothers of treated infants, but the difference was not significant (\(\text{Table 1}\)). All mothers and their partners were of Caucasian origin.

Methadone-exposed infants who required treatment for NAS were more likely to carry the normal (homozygous) allele at 516 (\(n = 8\) [treated] vs. 1 [untreated]; \(p = 0.015\)) and 785 (\(n = 7\) [treated] vs. 2 [untreated]; \(p = 0.023\)) regions of the \textit{CYP2B6} gene (\(\text{Table 2}\)). There were no differences between treated and untreated babies in any other genes studied.

Discussion

NAS is a common condition which accounts for a significant and increasing proportion of neonatal care worldwide.\textsuperscript{11} NAS is poorly predicted, resulting in many babies and their mothers having a prolonged postnatal stay for observation of developing NAS. Being able to predict NAS would reduce unnecessary hospital stay and its associated costs, and also afford an opportunity to investigate preemptive treatment for babies likely to develop NAS.
The CYP2B6*6 genotype is commonly reported in Caucasian populations and is associated with slower metabolism of methadone. Within our infant population, the overall frequency of the CYP2B6*6 genotype was 25%, consistent with the general Caucasian population, but untreated infants were much more likely to carry alleles associated with decreased enzyme function at CYP2B6 516GG and SNPs in this gene are unrelated to opioid addiction in adults. A previous study reported more than 20 years ago that severity of NAS was related to the rate of decline of the infant’s plasma methadone concentration over the first 4 days of life. Our findings are consistent with these findings, with slower metabolism of methadone in the infant less likely to result in abrupt withdrawal from transplacentally acquired methadone and hence less severe NAS. Mothers of treated babies tended to have been on higher doses of methadone at delivery, but their plasma concentrations of methadone were not significantly higher, suggesting faster maternal metabolism of methadone, consistent with their infants. A genomic variant of CYP2B6 common to mother and baby would explain both larger dosages of methadone required by the mother to control cravings for opioid and faster decline in neonatal plasma methadone concentration reflected in more severe neonatal withdrawal. Genetic variation may also explain, at least in part, why there is differing opinion as to whether maternal methadone dose is reflected in the likelihood of the baby developing NAS.

The weaknesses of this study are the small number of patients, lack of toxicology to confirm additional substance misuse during pregnancy, and absence of maternal genetic data. Mean plasma methadone concentration was almost twice as high in mothers of treated babies, but the range of values was very similar for both groups; small study numbers may have contributed to a type II error. Additional substance misuse is notoriously difficult to ascertain from maternal history, but within the population attending our service polydrug use is common and positively correlated with the prescribed dose of methadone. A previous study demonstrated ongoing illicit drug use in 90% of our MMOD mothers, predominantly benzodiazepines and heroin, and all of the mothers included in this study reported cigarette smoking. It is possible that both nicotine and additional substances of abuse contributed to the development of NAS.

ABCBI affects drug deposition rather than metabolism and SNPs in this gene are unrelated to opioid addiction in adults. Consistent with published literature, we did not see differences in ABCBI associated with the likelihood of the baby requiring treatment for NAS. Maternal CYP2D6 genotype has been associated with higher than predicted conversion of codeine to morphine and fatal respiratory depression in a breast-fed baby, but the latter genotype is uncommon in the Caucasian population. We did not see any association between NAS and either of the two CYP2D6 genotypes tested.
in this study. Small study numbers may explain our failure to replicate the findings of Wachman et al, in whose study SNPs in both OPRM1 and COMT predicted NAS.  

The strengths of this small pilot study are that mothers and babies were cared for in one facility, with a practiced and consistent approach to management of NAS. Persons caring for the babies were unaware of the infant’s genotype. Even within a small number of patients, genomic variation in CYP2B6 between treated and untreated babies was significant. 

Future studies should consider maternal genotype and its relationship to infant genotype and/or the prediction of NAS as well as associated maternal polydrug use.

**Conclusion**

Genomic variation in CYP2B6 in the newborn is associated with severity of NAS; this has not previously been reported. Better understanding of the role of pharmacogenetics in the etiology of NAS may result in improved care for mother and baby.

**Note**

This study was undertaken as part of a PhD cosponsored by Bournemouth University and Randox. Royal Bournemouth Hospital donated the space for sample analysis.

**Authors’ Contributions**

Helen Mactier was involved in study concept and sample collection and wrote the manuscript. Poppy McLaughlin analyzed samples and data, contributed to manuscript revisions, and approved the final version. Cheryl Gillis recruited patients and collected samples, contributed to manuscript revisions, and approved the final version. Michael David Osselton conceived the study, oversaw analysis of samples, contributed to manuscript revisions, and approved the final version.

**Conflict of Interest**

None.

**References**