Sex-Dependent Gene Expression in Infants with Neonatal Opioid Withdrawal Syndrome

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Objectives To evaluate salivary biomarkers that elucidate the molecular mechanisms by which in utero opioid exposure exerts sex-specific effects on select hypothalamic and reward genes driving hyperphagia, a hallmark symptom of infants suffering from neonatal opioid withdrawal syndrome (NOWS).

Study design We prospectively collected saliva from 50 newborns born at ≥34 weeks of gestational age with prenatal opioid exposure and 50 sex- and gestational age-matched infants without exposure. Saliva underwent transcriptomic analysis for 4 select genes involved in homeostatic and hedonic feeding regulation (neuropeptide Y2 receptor [*NPY2R*], proopiomelanocortin [*POMC*], leptin receptor [*LEPR*], dopamine type 2 receptor [*DRD2*]). Normalized gene expression data were stratified based on sex and correlated with feeding volume on day of life 7 and length of stay in infants with NOWS requiring pharmacotherapy.

Results Expression of *DRD2*, a hedonistic/reward regulator, was significantly higher in male newborns compared with female newborns with NOWS (Δ threshold cycle 10.8 ± 3.8 vs 13.9 ± 3.7, $P = .01$). In NOWS requiring pharmacotherapy expression of leptin receptor, an appetite suppressor, was higher in male subjects than female subjects (Δ threshold cycle 8.4 ± 2.5 vs 12.4 ± 5.1, $P = .05$), *DRD2* expression significantly correlated with intake volume on day of life 7 ($r = 0.58$, $P = .02$), and expression of *NPY2R*, an appetite regulator, negatively correlated with length of stay ($r = -0.24$, $P = .05$).

Conclusions Prenatal opioid exposure exerts sex-dependent effects on hypothalamic feeding regulatory genes with clinical correlations. Neonatal salivary gene expression analyses may predict hyperphagia, severity of withdrawal state, and length of stay in infants with NOWS. *(J Pediatr 2019;214:60-5)*.

In the era of the current opioid epidemic, an infant is born with neonatal opioid withdrawal syndrome (NOWS) every 15 minutes.[1] NOWS, also known as neonatal abstinence syndrome, is a constellation of withdrawal symptoms following termination of maternal opioid supply at birth. Male sex is a risk factor for the development of NOWS requiring pharmacotherapy.[2] A hallmark manifestation of NOWS is hyperphagia.[3] Hyperphagia occurs in 26%-56% of infants and is linked to a more severe withdrawal course requiring pharmacotherapy and results in excessive weight gain in the neonatal period.[4,5] Hyperphagia in NOWS has been attributed to an increased metabolic demand associated with withdrawal symptoms (eg, irritability),[5] however, the molecular basis for this behavior remains unproven. Our objective was to evaluate the gene expression profiles of select hypothalamic and reward signaling pathways in infants with and without NOWS, and relate these profiles with their feeding behavior.

The hypothalamus contains anorexigenic and orexigenic peptides that inhibit and stimulate feeding, respectively.[6-8] Other areas of the brain, such as the mesolimbic area, provide hedonic or reward-based signaling. Animal studies have shown that opioids alter the balance of feeding regulation, resulting in a predominance of reward signaling and food-seeking behavior, particularly highly palatable fatty and carbohydrate-rich foods.[9-13] The proximity of feeding receptors to the receptors for drugs (eg, opioids, cocaine, cannabinoids) in the brain may result in developmentally aberrant

Ct	Threshold cycle	NOWS-Pharm	NOWS requiring pharmacotherapy
DOL	Day of life		
DRD2	Dopamine type 2 receptor	NOWS-No-Pharm	NOWS not requiring pharmacotherapy
LEPR	Leptin receptor	NPY2R	Neuropeptide Y2 receptor
LOS	Length of stay		
MWH	Melrose-Wakefield Hospital	PCR	Polymerase chain reaction
NOWS	Neonatal opioid withdrawal syndrome	TMC	Tufts Medical Center

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signaling pathways and the subsequent hyperphagia seen in infants with NOWS. Sex differences in feeding behavior may also impact the severity of NOWS.[14]

We hypothesized that in infants with NOWS, hyperphagia could be a compensatory behavior replacing the reward signaling following the termination of opioid exposure. Using validated salivary transcriptomic methods,[15,16] we analyzed three genes involved in homeostatic feeding regulation (neuropeptide Y2 receptor [NPY2R], leptin receptor [LEPR], proopiomelanocortin [POMC]), and 1 gene at the epicenter of hedonic regulation (dopamine receptor type 2 [DRD2]) (**Table I**; available at www.jpeds.com).[17-20] We hypothesized that prenatal opioid exposure dysregulates homeostatic and hedonistic signaling in the hypothalamus, and distinct salivary gene expression profiles in infants with NOWS are linked to their clinical feeding behavior in a sex-dependent manner.

Methods

In this prospective pilot study, 100 subjects between 34 and 42 weeks of gestation were recruited and enrolled within 48 hours of birth. Recruitment took place at the Floating Hospital for Children at Tufts Medical Center (TMC), Boston, Massachusetts and Melrose-Wakefield Hospital (MWH), Melrose, Massachusetts between September 2016 and July 2018. Informed consent was obtained from mothers, and the study was approved by the institutional review boards at both TMC and MWH. The study population consisted of 50 infants with NOWS with positive maternal toxicology screen test for opioids during pregnancy. Control group consisted of 50 sex- and gestational age-matched infants (within 1 week) born to mothers without a history of opioid use during gestation. We excluded infants of mothers with diabetes as these infants may have immature sucking patterns or poor oral intake,[21] infants whose mothers were treated with antidepressants because these psychotropic medications are known to affect the hypothalamic-pituitary-adrenal axis,[22] and infants in the custody of Department of Children and Families. Infants with NOWS were further categorized as those requiring pharmacotherapy (NOWS-Pharm) and those not requiring pharmacotherapy (NOWS-No-Pharm) based on Finnegan Scoring.[23,24]

Demographic data collected from medical records included gestational age, birth weight, sex, mode of delivery, diet (breast milk and/or formula), and Apgar scores. In the infants with NOWS, additional data collected included use of maternal medications, maternal urine toxicology, infant's toxicology (urine and/or meconium toxicology), and pharmacotherapy. For infants with NOWS-Pharm, length of stay (LOS), type and maximum dose of pharmacotherapy, dietary intake in the first week of life (intake volume by day of life [DOL] 7), and time to regain birth weight were also obtained. We chose to record volume intake by DOL 7, as most late-preterm and term infants will have established a mature feeding pattern by the end of the first week of life. LOS encompassed the length of hospital course that was strictly related to the pharmacotherapy treatment (normally equates to a total length of treatment plus an additional 1-2 days for observation of infants' clinical symptoms following the discontinuation of pharmacotherapy, as per the Finnegan protocol). If any LOS surpassed this defined duration, it was further analyzed and adjusted.

Saliva samples were collected for all subjects within 48 hours of birth according to our established techniques.[25] To minimize breast milk and associated maternal RNA contamination, saliva was collected prior to feeding. For infants with NOWS, saliva collection was performed prior to the start of pharmacotherapy so as not to reflect gene expression changes associated with postdelivery medication administration. RNA extraction was performed using RNeasy Micro Kit (Qiagen, Hilden, Germany) per manufacturer's instructions. On column DNase treatment was performed for each sample using RNase-free DNase I Set (Qiagen) to minimize DNA contamination. Once extracted, total RNA was stored at −80°C pending gene expression analysis.

Following total RNA extraction, samples were converted to complementary DNA (cDNA) using SuperScript VILO cDNA Synthesis Kit (Invitrogen, Carlsbad, California) according to the manufacturer's protocols. Samples subsequently underwent a targeted preamplification using TaqMan PreAmp Master Mix Kit (Applied Biosystems, Foster City, California) based upon the inventoried amplicons of 4 targeted genes of interest and 3 reference genes. This targeted preamplification was utilized to limit amplification bias inherent in a whole transcriptomic amplification approach. The preamplification was run on the Mastercycler pro S Thermal Cycler (Eppendorf, Hamburg, Germany) at 95°C for 10 minutes for enzyme activation, followed by 14 cycles of amplification at 95°C for 15 seconds and 60°C for 4 minutes. Preamplified samples of cDNA were stored at −20°C until quantitative polymerase chain reaction (PCR) amplification.

The preamplified cDNA was diluted 1:5 with RNase-free water (Qiagen) prior to quantitative PCR amplification. Diluted samples were mixed with TaqMan Fast Advanced Master Mix (×2) (Applied Biosystems) and RNase-free water (Qiagen). Sample mix was plated on Custom TaqMan Array 96-Well Plate (Applied Biosystems). The 96-well plate was customized for singleplex PCR for expression of 4 target genes and 3 reference genes. The custom-made plate also included ribosomal 18S per company standard as an additional internal control gene. This gene was not used in our analysis. Preamplified samples were run on QuantStudio 7 Flex Real-Time PCR (Applied Biosystems, Thermo Fisher Scientific, Carlsbad, California) platform with the following thermal cycle profile: incubation at 50°C for 2 minutes followed by 40 cycles of denaturation at 95°C for 1 second, and annealing and extension at 60°C for 20 seconds. All samples were run in duplicate.

To ensure plate functionality and plate-to-plate consistency, water was used as a negative control. An independent saliva sample procured from an infant of a nonopioid

exposed pregnancy was used as a positive control. This sample was not part of the control cohort. In addition, a nonpreamplified control sample was run as a comparison to ensure uniform amplification across all gene targets. The positive control was plated in the same column for each plate. Ribosomal 18S expression of this positive control was used to measure the variance across the plates.

Because of the inherent degraded nature of RNA found in saliva and to properly normalize for varying starting total mRNA input across samples, we used 3 reference genes that have been previously shown in our laboratory to remain stable across sex and postconceptional age.[26] These genes were *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), *YWHAZ* (tyrosine 3-mono-oxygenase/tryptophan 5-monooxygenase activation protein, zeta polypeptide), and *HPRT1* (hypoxanthine phosphoribosyl transferase 1). Amplification of all 3 reference genes was a prerequisite for inclusion in the final analysis.

Statistical Analyses

Data analysis was conducted using SAS Enterprise Guide v 7.15 (SAS Institute, Cary, North Carolina). Threshold cycle (Ct) values for genes of interest and reference genes were detected using QuantStudio 7 Flex Real-Time PCR machine. Normalized ΔCt values were obtained by subtracting the mean Ct values of each target gene (samples run in duplicate) from the geometrical mean Ct values of the reference genes. Normalized gene expression levels were analyzed using the student *t* test; categorical data were analyzed using χ^2 tests of statistical significance. Data were further analyzed based on the newborn's sex; 2-factor ANOVA was used to explore interactions between study group and sex for the gene expression profiles. Pearson correlation coefficient was used to evaluate the correlation between gene expression levels and intake volume, as well as with LOS. Statistical significance was set at $P \leq .05$.

Results

Subject recruitment and sample collection are depicted in **Figure 1** (available at www.jpeds.com). Only a lower 1-minute Apgar score was statistically significant in infants with NOWS compared with the control group (**Table II**). The majority of enrolled infants with NOWS were exposed to buprenorphine prenatally. Sixteen (32%) infants with NOWS required pharmacotherapy for their withdrawal symptoms, two-thirds of whom were male. Stratification based on exposure (without prenatal opioid exposure vs NOWS) and types of feeding (breast milk [alone or in combination with formula] vs formula) showed that 82% of infants without prenatal exposure were fed breast milk compared with 54% of infants with NOWS ($P = .003$). Among infants with NOWS, 38% of the 16 infants requiring pharmacotherapy were fed breast milk compared with 62% of the 34 infants not requiring pharmacotherapy ($P = .108$).

Maternal methadone and other opioid use were linked to a higher percentage of infants with NOWS-Pharm compared with maternal buprenorphine use (50% vs 24%; OR 3.14; 95% CI 0.76-12.94 for methadone vs buprenorphine; and 33.3% vs 24%; OR 1.57; 95% CI 0.31-7.99 for other opioids vs buprenorphine). Median LOS for NOWS-Pharm cohort was 18 days (IQR 14-25 days). There was a statistically significant relationship between LOS and maternal methadone use compared with the other maternal opioid medications (methadone: median 18 days [IQR 6-26 days], buprenorphine: 6 days [IQR 5-14 days], other opioids: 4 days [IQR 4-6 days], global $P = .01$).

There was no gene amplification in the negative control wells. The independent sample that served as a positive control had similar ΔCt for ribosomal 18S across all plates, with percentage variance of 16.8% (IQR 6.5%-23.7%). There was no significant difference in the gene expression levels in infants with NOWS compared with healthy infants (**Table III**; available at www.jpeds.com). However, stratification on the basis of sex demonstrated that male infants with NOWS had a significantly higher *DRD2* expression than female infants in the same cohort (**Table IV**; available at www.jpeds.com). Furthermore, there was a significant interaction between sex, prenatal drug exposure, and postnatal pharmacotherapy requirement for the expression of the reward gene *DRD2* (**Figure 2**). Compared with the controls, prenatal opioid exposure had an opposite effect on the *DRD2* expression across sex (ie, an upregulation in male infants and a downregulation in female infants). **Table V** shows that although *DRD2* expression was significantly higher in male infants with NOWS—irrespective of the need for pharmacotherapy—compared with their female counterparts, *LEPR* expression was significantly higher only in male infants with NOWS who needed pharmacotherapy.

Average intake volume of infants with NOWS on DOL 7 was 174.1 ± 29.9 mL/kg/day (female: 154.6 ± 29.8 mL/kg/day vs male: 183.0 ± 26.7 mL/kg/day, $P = .11$). Pearson coefficient correlation in **Figure 3**, A revealed that in infants with NOWS-Pharm (n = 16), *DRD2*, and *POMC* expression had a

Table II. Study population characteristics

Demographic data	NOWS (n = 50)	Control (n = 50)
Gestation age (wk)	38.4 ± 1.5	38.4 ± 1.4
Birth weight (g)	3145.0 ± 595.1	3242.5 ± 509.5
Cesarean delivery (%)	52	38
Apgar at 1 min (median)*	8.5	9
Apgar at 5 min (median)	9	9
Male (n, %)	27 (54)	27 (54)
Maternal medication		
Methadone (n, %)	12 (24)	
Buprenorphine (n, %)	29 (58)	N/A
Other drugs (n, %)	9 (18)	
Maternal smoking (n, %)	16 (33.3)	N/A

Control refers to sex- and gestational age-matched infants without prenatal opioid exposure. Counts and percentages are presented for categorical variables; means and SDs for continuous variables, Apgar scores in median.
*$P = .01$, other P values >.05.

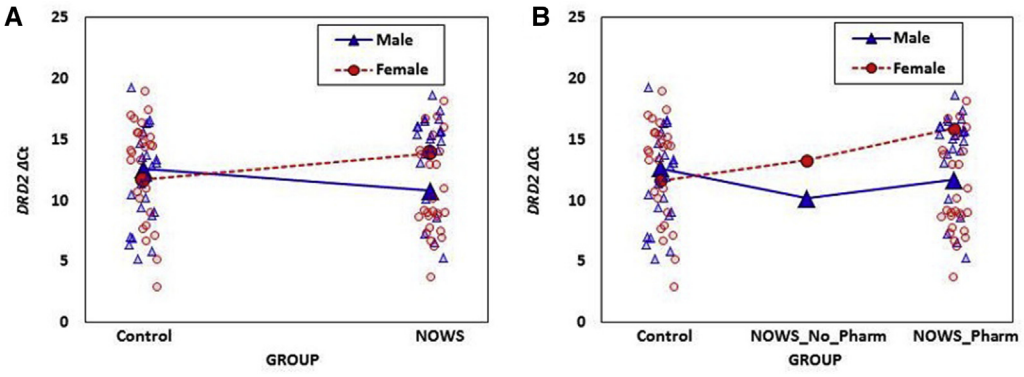

Figure 2. Two-way ANOVA looking at *DRD2* expression in infants with NOWS and interaction with **A,** sex ($P = .01$), and **B,** sex and pharmacotherapy requirement ($P = .02$). ΔCt values are inversely proportional to the levels of gene expression. Interaction with sex **A,** showed that male infants with NOWS had a significantly higher *DRD2* expression than female infants with NOWS. Interaction with sex and pharmacotherapy requirement **B,** also showed that *DRD2* expression was significantly higher in male infants with and without pharmacotherapy than in their corresponding female counterparts.

significantly positive correlation with intake volume on day 2, with persistently significant correlation for *DRD2* expression when assessed on day 7. Infants' intake volumes on day 7 also significantly correlated with intake volume on the day of discharge (r = 0.73, $P = .01$). On the day of discharge, average intake volume was 201.0 ± 40.9 mL/kg/day (female: 180.3 ± 28.6 mL/kg/day vs male: 210.2 ± 43.6 mL/kg/day, $P = .18$). In addition, there was a positive trend correlating *DRD2* expression with percent weight change at discharge (**Figure 3**, B). In **Figure 4** (available at www.jpeds.com) Pearson coefficient correlation only revealed a significant negative correlation between *NPY2R* expression and LOS in infants with NOWS.

Discussion

In our study, we demonstrate the feasibility to detect genes that are reflective of hypothalamic regulation of feeding behavior in infants undergoing opioid withdrawal. Using saliva, we were able to assess ongoing developmental patterns in newborns with NOWS and to work toward the development of a potential predictive tool to assess withdrawal severity and feeding aberrance in this vulnerable population. Our research was based on mechanistic data derived from animal studies, which led us to selectively study targeted hypothalamic genes involved in anorexigenic, orexigenic, and reward pathways of feeding regulation. The expression of these genes was objectively quantified to further characterize infants with NOWS who are at increased risk to develop feeding aberrance and a more severe withdrawal course.

Table V. Gene expression levels in the NOWS cohort as stratified on the basis of pharmacotherapy and sex

NOWS cohort	Gene names	Male	Female	*P* values
NOWS-No-Pharm	n = 34	n = 16	n = 18	.57
	NPY2R	8.6 ± 4.8	7.6 ± 4.8	**.03**
	DRD2	10.2 ± 4.1	13.3 ± 4.0	.75
	LEPR	8.4 ± 3.3	8.1 ± 2.8	.86
	POMC	10.7 ± 4.5	10.5 ± 4.0	
NOWS-Pharm	n = 16	n = 11	n = 5	.94
	NPY2R	10.1 ± 5.0	9.9 ± 6.3	**.02**
	DRD2	11.7 ± 3.4	15.9 ± 0.9	**.05**
	LEPR	8.4 ± 2.5	12.4 ± 5.1	.81
	POMC	11.8 ± 4.2	12.4 ± 4.3	

ΔCt values are in mean ± SD; bolded values indicate significance.

Our study identifies a potential modulating effect of sex on the expression of hypothalamic and reward genes that govern feeding behavior in infants with NOWS. As shown in **Table IV**, male infants with NOWS had significantly higher *DRD2* expression than female infants with NOWS. This higher reward signaling may explain the higher proportion of male infants with NOWS who require pharmacotherapy,[2] and why men are epidemiologically more prone to substance use disorders.[27]

To better understand hyperphagia in infants with NOWS-Pharm, we correlated initial gene expression levels with feeding volume in the first week of life. We demonstrated a strong and significant association between early *DRD2* expression and infants' feeding volumes between DOL 2 up to DOL 7, at which time a mature feeding pattern is usually established. In general, the average food consumption of newborns at 1 week of age is between 120 mL/kg/day and 150 mL/kg/day.[28] Infants with NOWS-Pharm consumed at least 20% above this amount, averaging 174 mL/kg/day. Salivary *DRD2* may have utility in predicting excessive feeding and a worse withdrawal state in infants with NOWS. In addition, the significant correlation between feeding volume on DOL 7 and at discharge may indicate a lasting impact of opioid exposure on infants' feeding behavior, further supporting the potentially predictive utility of *DRD2* as an informative biomarker in infants with NOWS.

In our study, all male subjects tended to have lower *NPY2R* expression levels at baseline compared with female subjects.

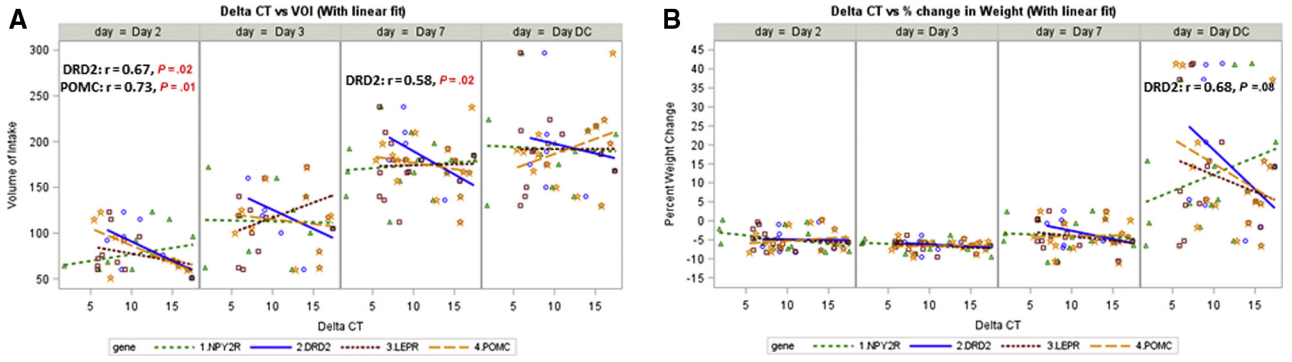

Figure 3. Correlation between gene expression levels in infants with NOWS requiring pharmacotherapy and **A,** intake volume, and **B,** percent weight change. Columns represent timing of measurement (days 2, 3, 7, and discharge [DC]). Given the inverse relationship between ΔCt values and gene expression levels, negative slopes represent positive correlations and vice versa (*NPY2R* [*green*]; *DRD2* [*blue*]; *LEPR* [*red*]; *POMC* [*orange*]).

Prenatal opioid exposure further lowered the *NPY2R* expression in both sexes. As an anorexigenic modulator, lower *NPY2R* expression will stimulate hunger signaling and drive feeding,[17,29] suggesting that the higher intake volume in infants with NOWS (especially those who required pharmacotherapy) was driven, in part, by a lower expression of *NPY2R*. The significant inverse correlation between *NPY2R* expression with LOS supports our hypothesis that prenatal opioid perturbs the normal biological response in feeding regulation.

In the NOWS-Pharm cohort, male infants consumed an average of 180 mL/kg/day by day 7 and 210 mL/kg/day on the day of discharge, approximately 15% above their female counterparts. Similarly, at the molecular level, the expression of *DRD2* and *LEPR* was also higher in male infants with NOWS-Pharm compared with female infants with NOWS-Pharm. *LEPR* provides satiety in the context of energy surplus,[18] and *DRD2* triggers feeding based on reward signaling.[20] Our results suggest that in this male cohort, reward signaling superseded the impact of *LEPR* on feeding behavior. These data support our hypothesis that food replaces the reward signaling once made available by maternal opioid use and parallel animal studies where opioid exposure results in the predominance of reward signaling and increased efforts to obtain food.[9-13] In addition, our findings complement prior addiction studies in both adults and animals, where deviation in *DRD2* expression has been shown to affect the amount of substance consumption.[30,31]

From a clinical standpoint, the trend of higher breast milk use in the NOWS-No-Pharm compared with NOWS-Pharm cohort may support the previously reported protective effect of breastfeeding or breast milk use in reducing LOS and the need for pharmacotherapy.[32,33] Furthermore, our results validate prior studies where methadone exposure has been linked to increased need for pharmacotherapy and LOS. Compared with buprenorphine, methadone exposure led to more severe withdrawal symptoms and worse neurobehavioral scores.[34] Altogether, our findings raise further awareness among providers in identifying factors that may be linked to worse withdrawal course and poorer outcomes.

Our study carries several strengths. The noninvasive method of sample collection limited the possible confounding effect of discomfort or pain on hypothalamic gene expression. In addition, our samples were matched for sex and gestational age, thereby minimizing potentially confounding effects of these variables on gene expression levels. The combined approach of salivary gene analysis and assessment of clinical feeding behavior provided an opportunity to objectively and safely evaluate biologically plausible mechanisms to further our understanding of the developmentally detrimental effects of prenatal opioid exposure on the newborn brain.

Limitations of our study included the small sample size and the small number of female infants with NOWS-Pharm. As such, our study may not be generalizable. These limitations are inherent to human translational studies. Importantly, our pilot study did not lend itself to a statistical exploration of the relationships of multiple covariates (including sex, delivery type, gestational age, smoking, Apgar scores) and gene expression with the outcome because of the risk of overfitting models to the available data. Rather, we presented the unadjusted associations, along with unadjusted *P* values, to allow for observation of effect sizes. Finally, we had performed a single-point salivary analysis, restricting our ability to understand the long-term impact of prenatal opioid exposure on infants' reward and hypothalamic signaling related to their feeding behavior. Nevertheless, our pilot study lays the foundation for future observational studies where serial salivary samples may be analyzed and correlated to feeding intake in infants with NOWS during their withdrawal course and beyond.

In summary, salivary gene expression profiles are a promising tool to objectively evaluate the sex-dependent adverse effects of prenatal opioid exposure on a transcriptomic level and predict the development of feeding aberrance in infants with NOWS. This objective evidence has great potential to

advance the field and enable personalized care surrounding education about food and other reward-seeking behaviors in this vulnerable population. ■

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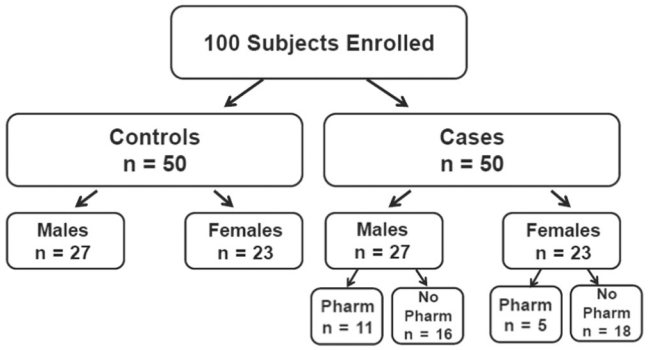

Figure 1. Flowchart of subject recruitment and analysis. Cases are infants with NOWS, controls are sex- and gestational age-matched healthy infants.

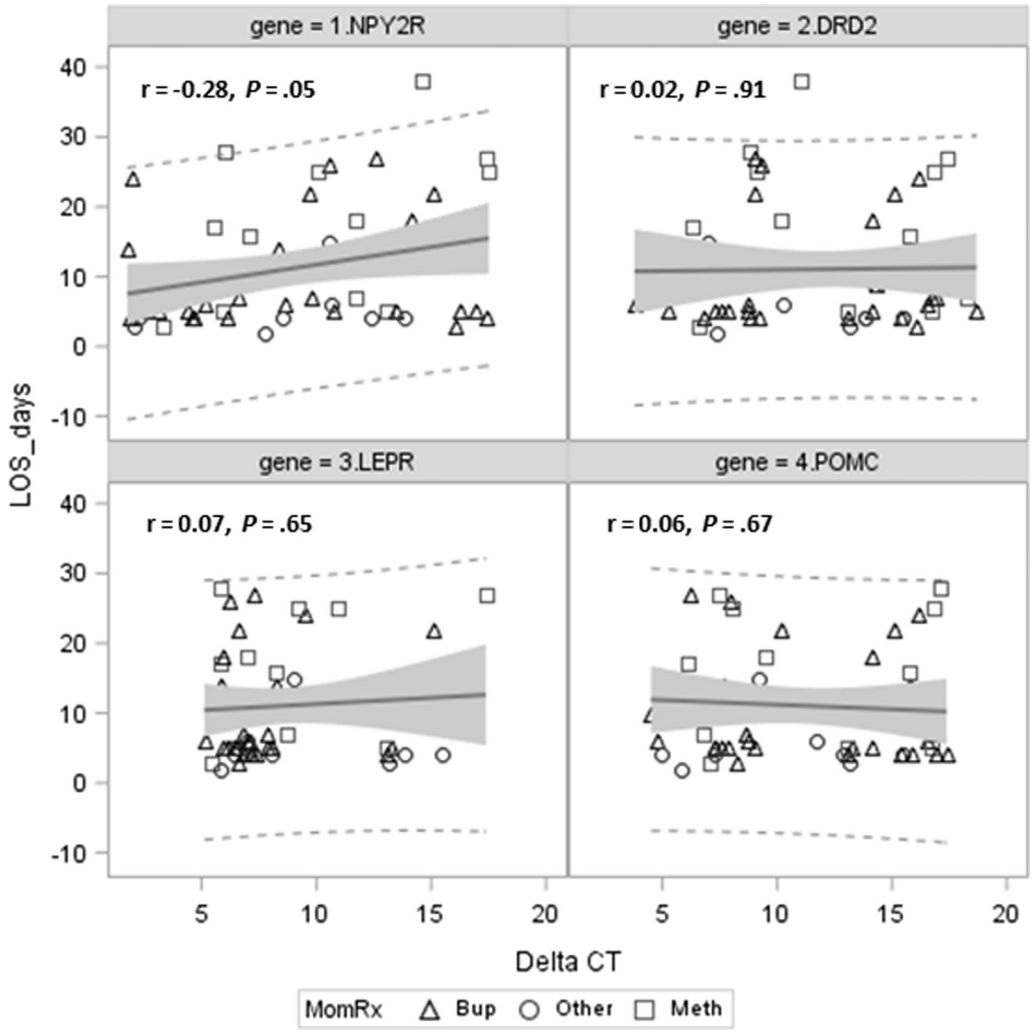

Figure 4. Correlation between gene expression and LOS in infants with NOWS. Among select genes, *NPY2R* gene expression had a positive, significant correlation with LOS (P = .05).

Table I. Select hypothalamic and reward genes involved in feeding regulation, their tissue expression profiles, and functions

Gene names	Chromosome	Locations in the brain*	Functions
NPY2R	4q32.1	Hypothalamus, hippocampus, nucleus accumbens, cerebellum, amygdala	A modulator of hunger and anorexigenic signaling.[17]
LEPR	1p31.3	Pituitary gland, hypothalamus, cerebral cortex, hippocampus, caudate, cerebellum	An enhancer of satiety and anorexigenic signaling.[18]
POMC	2p23.3	Hypothalamus, hippocampus, pituitary gland, thalamus, cerebellum	A mediator of satiety signaling.[19]
DRD2	11q23.2	Basal ganglia (caudate, putamen, nucleus accumbens), pituitary, cerebellum	Key regulator of reward pathways.[20]

*Sources: GeneCards (https://www.genecards.org); GTEx Portal (https://gtexportal.org).

Table III. Gene expression levels stratified on the basis of prenatal opioid exposure

Gene names	NOWS (n = 50)	Control (n = 50)	*P* values
NPY2R	8.7 ± 4.9	7.9 ± 4.1	.38
DRD2	12.2 ± 4.0	12.2 ± 4.0	.98
LEPR	8.7 ± 3.3	9.0 ± 3.5	.64
POMC	11.0 ± 4.2	12.1 ± 4.3	.22

ΔCt values (in mean ± SD) are inversely proportional to the level of gene expression (ie, the higher the ΔCt values, the lower the gene expression).

Table IV. Gene expression levels stratified on the basis of sex

Exposure cohort	Gene names	Male	Female	*P* values
NOWS cohort	n = 50	n = 27	n = 23	.45
	NPY2R	9.2 ± 4.8	8.1 ± 5.1	**.01**
	DRD2	10.8 ± 3.8	13.9 ± 3.7	.51
	LEPR	8.4 ± 3.0	9.0 ± 3.8	.80
	POMC	11.2 ± 4.3	10.9 ± 4.1	
Control cohort	n = 50	n = 27	n = 23	.28
	NPY2R	8.5 ± 4.5	7.2 ± 3.5	.41
	DRD2	12.6 ± 4.2	11.7 ± 3.9	.70
	LEPR	9.2 ± 3.8	8.8 ± 3.3	.23
	POMC	12.8 ± 4.0	11.3 ± 4.6	

ΔCt values are in mean ± SD; bolded value indicates significance.