Chapter 4

Is poor neonatal adaptation after exposure to antidepressants related to fetal cortisol levels? An explorative study

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Under review
ABSTRACT

Objective
As a marker for poor neonatal adaptation (PNA) is lacking, the diagnostic process is difficult and includes invasive additional testing. In order to develop a marker, it is essential to gain insight into the etiology of PNA. We hypothesized that the fetal cortisol level may play a role in this etiology.

Methods
In this non-randomized, prospective controlled study we examined hair cortisol levels of infants exposed and not exposed to selective antidepressants (SADs) during pregnancy. These cortisol levels represent the mean cortisol level during the last trimester of pregnancy. Infants exposed to SADs who developed PNA according to the pediatrician (PNA+, n=25), infants exposed to SADs who did not develop PNA (PNA-, n=40) and infants not exposed to SADs (controls, n=105) were compared.

Results
In infants with PNA, hair cortisol levels were higher compared to infants without PNA. However this difference was only statistically significant in female infants (girls B-0.25, p=0.08, boys B-0.25, p=0.12). There was no correlation between nonspecific distress, measured by the Finnegan score and fetal hair cortisol levels (B-0.15, p=0.30). All analyses were adjusted for type of delivery and gestational age.

Conclusions
Hair cortisol levels of female infants with PNA were higher compared to female infants without PNA, while there was no significant difference in boys. This suggests that the hypothalamic pituitary adrenal (HPA) axis activity may play a sex-specific role in the development of PNA. In contrast, we did not find a correlation between fetal hair cortisol levels and nonspecific distress in infants. As PNA is most likely of a multifactorial origin, it would be interesting to examine other factors possibly involved in the etiology of PNA in future studies, such as (epi) genetics.
INTRODUCTION

Use of antidepressants during pregnancy has increased during the last decade; approximately 2-9% of Western pregnant women use selective serotonin reuptake inhibitors (SSRIs) during pregnancy.\(^1,2\) After exposure to SSRIs and other selective antidepressants (SADs) in fetal life, 20-30% of infants develop poor neonatal adaptation (PNA).\(^3,5\) This syndrome consists of symptoms of restlessness, such as tremors and sleeping difficulties that are mostly mild and self-limiting.\(^3,5-7\) As a marker for PNA is not available, the pediatrician has to establish this diagnosis based on the moment of onset and type of symptoms, which is mostly between eight and 48 hours postpartum.\(^3-6\) This diagnostic process can be difficult, as symptoms are nonspecific and can also represent other, more severe neonatal pathology, such as perinatal infection.\(^3,6-8\) In order to exclude these other diseases, invasive additional tests are frequently performed, which can be harmful and stressful for the infant and parents.

A marker for PNA would be of additional value for the diagnostic process. In order to develop such a marker, it is essential to gain insight into the etiology of PNA. This etiology is most likely multifactorial as several other factors seem to play a role in the development of PNA apart from SAD-exposure itself. However, research into the etiology of PNA is limited. Factors that have been suggested are (epi-) genetic alterations, neurotransmitters, maternal psychiatric symptoms and hormones.\(^9-14\)

We hypothesized that fetal cortisol levels may play a role in the etiology of PNA. As the hypothalamic pituitary adrenal (HPA)-axis and serotonergic system are interrelated, SAD-exposure might alter maternal and fetal cortisol levels.\(^10,15,16\) Furthermore, results of previous studies indicate that elevated maternal cortisol levels during fetal life result in elevated cortisol levels in infants and impaired neonatal stress regulation.\(^10,16\) This led to our hypothesis that cortisol levels may be elevated in SAD-exposed infants compared to non-exposed infants and even more elevated in infants with PNA compared to infants without PNA.

In order to examine if this hypothesis is correct, we examined neonatal hair cortisol levels, representing the mean cortisol level during the last trimester or fetal life, in three groups of infants: infants exposed to SADs who developed PNA according to the pediatrician (PNA+), infants exposed to SADs who did not develop PNA (PNA-) and infants not exposed to SADs (controls).

As PNA consists of nonspecific symptoms of restlessness, we were interested whether the fetal cortisol level was specifically associated with PNA, or also with nonspecific distress in infants. Therefore, we examined if there was a significant correlation between the fetal cortisol level and Finnegan score in all infants (SAD-
exposed and non-exposed). The Finnegan score is used as an observational tool for PNA, however lacks specificity.\(^{17}\) Therefore, infants with symptoms of distress due several other causes, apart from PNA, may also have an elevated score.

**METHODS**

**Setting**

We conducted a non-randomized, prospective controlled study in a teaching hospital in Amsterdam, the Netherlands. The psychiatric, obstetric, pediatric (POP) clinic of this hospital is a center of expertise for pregnancy and psychiatric disorders and advises women with a psychiatric disorder before, during and after pregnancy. Approximately fifty percent of all pregnant women who visit the POP clinic live in our catchment area and therefore deliver in this hospital. Within eight hours after delivery these women are admitted to the maternity ward together with their infants for an observation period of at least 72 hours. Infants who need more surveillance due to severe PNA or other neonatal problems are admitted to the neonatal care unit (NCU). Trained nurses observe infants for PNA by means of the Finnegan scoring list three times a day. This observation tool was originally designed to assess PNA after exposure to opiates, but has been widely used for the observation of PNA after exposure to SADs.\(^ {18}\) A validated observational tool does not exist. As the Finnegan score has a high sensitivity, however low specificity., all infants are examined by a pediatrician on a daily basis. At the end of the observation period the pediatrician in charge concludes if PNA has been present or absent. This decision is made upon evaluation of all completed Finnegan scoring lists, the moment of onset and course of symptoms, the physical examination and exclusion of other neonatal pathology.

**Participants**

From February 2012 to August 2013, infants were included (n=178). The patient group consisted of infants admitted for observation of possible PNA, whose mothers used one or more SAD during at least the last two weeks of pregnancy. Two weeks is the maximum time for SADs to reach their effective dosage. SADs were defined as SSRIs, serotonin norepinephrine reuptake inhibitors (SNRIs) or noradrenergic and specific serotonergic antidepressants (NaSSAs). If the mother also used another type of psychotropic drug the infant was excluded, because exposure to other psychotropic drugs might also cause PNA.

Infants were included in the control group if the mother did not use psychotropic medication during pregnancy and if the infant was admitted to the maternity ward.
Cortisol and poor neonatal adaptation

or NCU for another neonatal or maternal reason with an expected hospital stay of at least 72 hours.

Exclusion criteria for both groups were insufficient knowledge of the Dutch or English language, mental retardation of one or both parents, multiple pregnancy, use of illicit drugs or regular alcohol use (>2 units per week) during the last trimester of pregnancy, use of systemic corticosteroids during pregnancy or if counselling or participation in this study would interfere with the clinical course of infant or mother. If possible, parents were informed on the study prior to delivery. Otherwise, parents were informed within 24 hours after delivery. All subsequent eligible patients were included.

The study was approved by the medical ethics committees of the OLVG west Hospital and VU Medical Center in Amsterdam, the Netherlands. The authoritative parent(s) of all infants signed an informed consent form.

Determinants
Exposure to SADs in utero was determined by medical interview during pregnancy at the POP expert center and verified during admittance to the maternity ward. The mother was asked to complete a questionnaire on the first day after delivery with respect to demographic characteristics, medication, alcohol and illicit drug use during pregnancy. The pediatrician established PNA at the end of the observation period as described earlier. A total of five pediatricians participated and were blinded to the cortisol levels of infants.

Outcome measure
The outcome measure was the cortisol level in neonatal hair. Analysis of hair cortisol is a noninvasive and validated procedure. Hair cortisol has previously been shown to be significantly correlated with cortisol in 24-hours urine and saliva, however, disturbing factors as the circadian rhythm do not acutely influence hair cortisol levels. It can serve as a biomarker for long-term cortisol.

Hair collection
On the first day after delivery, neonatal hair was collected. A tuft of hair was cut of the posterior vertex as close as possible to the scalp, as this region of the scalp shows the least variance between different strands. The total length of fetal hair was analyzed. As fetal hair starts growing during the third trimester of pregnancy this represents the mean cortisol levels during the last trimester of fetal life.
Hair cortisol analysis

Cortisol levels were measured in scalp hair as described previously.\textsuperscript{23} Briefly, cortisol was extracted using LC-grade methanol at 25°C for 18h in the presence of deuterium labeled glucocorticoids as internal standard. The extracts were then centrifuged and cleaned using solid phase extraction, after which the cortisol concentration was quantified by liquid chromatography - tandem mass spectrometry (LC-MS/MS) (Waters XEVO-TQ-S system, Waters Corporation, Milford, MA, USA), with positive electrospray ionization. Cortisol concentrations were reported as pg per mg hair. A minimum of 1.25mg of hair was needed for a reliable measurement.

Data analysis

Statistical analyses were performed with SPSS version 21 (IBM, New York, USA). Baseline characteristics were compared between PNA+, PNA- and control infants using analysis of variance (one-way ANOVA) for normally distributed variables. In case of a skewed distribution, the Kruskal-Wallis test was used. Categorical variables were analyzed by means of the chi square test. In cases where more than 20% of the expected cell counts were less than five, the Fisher exact test was performed. As hair cortisol levels have a skewed distribution, logistic transformation was applied.

To examine if there is a difference in fetal cortisol levels between PNA+, PNA- and control infants, we performed univariate linear regression analysis whereby a regression coefficient (B) and p-value were estimated. Subsequently, multiple linear regression analysis was performed in order to adjust for confounding variables (mentioned below). Variables that were significantly correlated with the fetal cortisol level were selected and added to the multivariate model by means of forward selection. The number of variables were restricted by the number of patients whereby a maximum of one variable was added for every 20 patients. Furthermore, as earlier studies indicate that hair cortisol values might differ between boys and girls,\textsuperscript{24} we examined if neonatal gender modified the relation between group (PNA+/PNA- and controls) and the fetal cortisol level. In case of a statistically significant interaction term between gender and group, results were stratified.

Furthermore, we were interested if fetal cortisol levels were correlated with the highest Finnegan score of each infant, which was assessed in all infants (SAD-exposed and non-exposed). The correlation was calculated by means of linear regression analysis and adjusted for the same confounders as in the previous multivariate analysis. A p-value of <0.05 was considered significant.
Potential confounders
Variables examined for their potential confounding effect included complications during pregnancy (such as hypertension or pre-eclampsia), the type and dosage of antidepressant, type of delivery, elevated maternal psychological distress during the third trimester of pregnancy, neonatal gender, neonatal stress, gestational age and birth weight. The type of antidepressant was divided in SSRIs, SNRIs, NaSSA or a combination of SADs. Dosage was defined as low in case of a dosage less than the minimal effective dosage, normal in case of the minimal effective dosage and high if the dosage exceeded the minimal effective dosage. If two types of SADs were used, the dosage of the SAD with the highest dose was taken into account. Type of delivery was categorized as vaginal delivery, planned- or emergency caesarean section. Maternal psychological distress was assessed by the hospital anxiety and depression scale (HADS). This instrument is validated in an antenatal population and consists of 14 questions, seven for anxiety and seven for depression. An anxiety and/or depression score of eight or higher indicates depression and anxiety and elevated psychological distress. In mothers of the patient group, the HADS score during the third trimester of pregnancy was used. In mothers of the patient group who did not visit our POP expert center during the last trimester of pregnancy (n=11) and in mothers of the control group, the HADS score at the first day postpartum was used as a proxy for psychological distress during the third trimester of pregnancy. Neonatal stress was defined as small for gestational age (birth weight of <10th percentile according to the Dutch perinatal registration based on ethnicity), prematurity (gestational age <37 weeks) or complications during or after birth, such as infection.

RESULTS

Inclusion of patients
Of infants exposed to SADs, 97 met the inclusion criteria. Parents of nineteen infants (20%) did not give consent and 12 infants (12%) fulfilled exclusion criteria (Figure 1). Of the remaining 66 infants (68%), hair cortisol was successfully analyzed in 65 infants (98%).

Of infants of the control group (not exposed to SADs), 296 infants met the inclusion criteria. Parents of 80 infants (27%) did not give consent and 104 infants (35%) fulfilled exclusion criteria (Figure 1). Of the remaining 112 infants (38%), hair cortisol was successfully analyzed in 105 infants (94%).
Baseline characteristics

In Table 1, the baseline characteristics of SAD-exposed and non-exposed infants are presented, further stratified into infants with (n=25, 38%) and without PNA according to the pediatrician (n=40, 62%). None of the infants with PNA needed pharmacological treatment.
Table 1. Baseline characteristics of infants exposed to selective antidepressants (SADs) with poor neonatal adaptation (PNA, n=25), infants exposed to SADs without PNA (n=40) and infants of the control group (n=105).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Control group Infants not exposed to SADs (n=105)</th>
<th>Patient group Infants exposed to SADs with PNA (n=25)</th>
<th>Patient group Infants exposed to SADs without PNA (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complications during pregnancy*, n(%)</td>
<td>18 (17.1)</td>
<td>10 (40.0)</td>
<td>6 (15.0)</td>
<td>0.03</td>
</tr>
<tr>
<td>Type of antidepressant, n(%)</td>
<td></td>
<td></td>
<td></td>
<td>0.69</td>
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<tr>
<td>SSRIb</td>
<td>-</td>
<td>19 (76.0)</td>
<td>25 (62.5)</td>
<td></td>
</tr>
<tr>
<td>SNRIc</td>
<td>-</td>
<td>2 (8.0)</td>
<td>5 (12.5)</td>
<td></td>
</tr>
<tr>
<td>NaSSAd</td>
<td>-</td>
<td>2 (8.0)</td>
<td>7 (17.5)</td>
<td></td>
</tr>
<tr>
<td>Combination of antidepressants</td>
<td>-</td>
<td>2 (8.0)</td>
<td>3 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Dosage of antidepressant, n(%)</td>
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<td></td>
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<tr>
<td>Low</td>
<td>-</td>
<td>0 (0.0)</td>
<td>5 (12.5)</td>
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<tr>
<td>Normal</td>
<td>-</td>
<td>7 (28.0)</td>
<td>15 (37.5)</td>
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<tr>
<td>High</td>
<td>-</td>
<td>18 (72.0)</td>
<td>20 (50.0)</td>
<td></td>
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<td>Type of delivery</td>
<td></td>
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<td></td>
<td>&lt;0.001</td>
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<td>Vaginal</td>
<td>34 (32.4)</td>
<td>21 (84.0)</td>
<td>30 (75.0)</td>
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<td>Planned caesarean section</td>
<td>35 (33.3)</td>
<td>2 (8.0)</td>
<td>3 (7.5)</td>
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<td>Emergency caesarean section</td>
<td>36 (34.4)</td>
<td>2 (8.0)</td>
<td>7 (17.5)</td>
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<td>HADSª score, n(%)</td>
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<td>Anxiety and/or depression score elevated (≥8)</td>
<td>17 (16.8)</td>
<td>7 (28.0)</td>
<td>17 (42.5)</td>
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<td>Unknown</td>
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<td>0</td>
<td>0</td>
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<td>Gender male, n(%)</td>
<td>60 (57.1)</td>
<td>11 (44.0)</td>
<td>22 (55.0)</td>
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<tr>
<td>Prematurity</td>
<td>52 (49.5)</td>
<td>5 (20.0)</td>
<td>16 (40.0)</td>
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<td>Small for gestational age</td>
<td>10 (9.5)</td>
<td>0 (0.0)</td>
<td>5 (12.5)</td>
<td></td>
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<tr>
<td>Birth complications³</td>
<td>7 (6.7)</td>
<td>2 (8.0)</td>
<td>2 (5.0)</td>
<td></td>
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<tr>
<td>Neonatal complications during hospital stay³</td>
<td>6 (5.7)</td>
<td>0 (0.0)</td>
<td>4 (10.0)</td>
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<td>Gestational age days median (range)</td>
<td></td>
<td></td>
<td></td>
<td>0.13</td>
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<tr>
<td>Birth weight gram mean (SD)</td>
<td>3512.1 (625.3)</td>
<td>3295.3 (336.9)</td>
<td>3384.5 (588.1)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

a. complications during pregnancy: hypertension, preeclampsia, cholestasis, hyper- and hypothyroidism, diabetes b. SSRI: selective serotonin reuptake inhibitor c. SNRI: serotonin and norepinephrine reuptake inhibitor d. NaSSA: noradrenergic and specific serotonergic antidepressant e. HADS: hospital anxiety and depression scale f. More than one cause of neonatal stress was possible. g. birth complications: 5 minute Apgar score <7, shoulder dystocia h. neonatal complications during hospital stay: infection requiring antibiotics, hyperbilirubinemia, hypoglycaemia.

Differences in fetal hair cortisol levels between groups

In Figure 2, the comparison of hair cortisol levels between PNA+ infants, PNA- infants and infants of the control group is presented. There were two significant confounders, which were gestational age and type of birth. There was no difference
in the fetal cortisol level between infants with and without PNA (B0.17, p=0.32 unadjusted, B0.20, p=0.13 adjusted) neither between infants with PNA and infants of the control group (B0.03, p=0.84, unadjusted, B-0.04, p=0.72 adjusted). In infants without PNA, the fetal cortisol level was lower compared to infants of the control group (B-0.14, p=0.27 unadjusted, B-0.25, p=0.02 adjusted).

Gender appeared to be a significant effect modifier, which implies that the association between group and fetal cortisol level differed for boys and girls. Therefore we stratified our data according to gender. In girls (n=77), the cortisol level in infants with PNA was higher compared to infants without PNA (B0.33, p=0.12 unadjusted, B0.33, p=0.04 adjusted). In boys (n=93), there was no difference in the fetal cortisol level between infants with and without PNA (B-0.04, p=0.89 unadjusted, B0.05, p=0.82 adjusted). In both boys and girls, there was no difference between infants with PNA and infants of the control group (girls B0.32, p=0.08 unadjusted, B0.08 p=0.59 adjusted, boys B-0.27, p=0.25 unadjusted, B-0.19, p=0.34 adjusted). The difference between infants without PNA and infants of the control group remained stable, however statistical significance disappeared after stratification (girls B-0.01, p=0.95 unadjusted, B-0.25, p=0.08 adjusted, boys B-0.23, p=0.19 unadjusted, B-0.25, p=0.12 adjusted).

Figure 2. Hair cortisol levels (logarithmic transformed) of infants exposed to selective antidepressants (SADs) with poor neonatal adaptation (PNA), compared to infants exposed to SADs without PNA and to non-exposed infants. Mean, and 95% confidence interval are presented. All comparisons are adjusted for type of delivery and gestational age.
Correlation between Finnegan score and fetal hair cortisol level

The median of the highest Finnegan score of every infant was 4 (range 1 to 16). There was no statistically significant correlation between the Finnegan score and fetal cortisol levels (B=-0.02, p=0.61). SAD-exposure and gender did not modify this correlation. After adjustment for the known confounders gestational age and type of delivery, the correlation was still not statistically significant (B=-0.15, p=0.30).

DISCUSSION

This is the first study on fetal hair cortisol in relation to development of PNA in SAD-exposed infants. We showed that in infants with PNA, hair cortisol levels were higher compared to infants without PNA. However, this difference was only statistically significant in female infants. This suggests that the HPA axis activity may play a sex-specific role in the development of PNA. It is possible that the lack of statistical significance in the total group of infants is caused by the limited sample size and broad range of cortisol levels. Unfortunately, there are no earlier studies for comparison.

We found no significant correlation between fetal hair cortisol levels and the Finnegan score in infants, which implies that there might not be a relationship between fetal hair cortisol levels and nonspecific distress in infants. There is one other study that examined the relationship between the neonatal cortisol level, measured in umbilical cord blood, and the Finnegan score. This study described a positive correlation. The contrast in findings might be explained by the fact that we measured long-term cortisol in hair, while this study examined short-term cortisol in umbilical cord blood. Other studies examined the correlation between maternal cortisol levels during pregnancy and symptoms of neonatal restlessness and temperament in newborn. Some reported a positive correlation, others did not report a significant correlation.

In infants of the control group, not exposed to SADs, cortisol was significantly higher compared to infants without PNA. It is possible that SAD-exposure leads to a decreased fetal cortisol level, which can explain our findings. Earlier studies showed that SAD-exposure or the underlying psychiatric disorder leads to inadequate maturation of the HPA axis and in turn a lower fetal cortisol level. However, it is also possible that SAD-exposure results in a delayed decline in 11-beta-hydroxysteroid dehydrogenase type 2 (11ß-HSD-2). This enzyme serves as a cortisol barrier during pregnancy. About 10-20% of cortisol passes the placenta until, just before delivery, the enzyme-function decreases. A delayed decline might result in a decreased fetal cortisol level. Although there are no previous
studies on hair cortisol levels of SAD-exposed infants, there are three studies that examined short-term cortisol levels in SSRI-exposed and unexposed infants.\textsuperscript{10,15,16} Davidson et al. and Pawluski et al. examined cortisol levels in umbilical cord blood of SSRI-exposed and non-exposed infants.\textsuperscript{10,16} Davidson revealed lower cortisol levels in SSRI-exposed infants, while Pawluski showed no difference. Oberlander examined salivary cortisol levels of three-month old SSRI-exposed infants and described lower cortisol levels compared to non-exposed infants.\textsuperscript{15}

Strengths of this study are the non-invasive measurement of cortisol in hair, the prospective controlled design and the measurement and adjustment of a broad range of confounders. This study also has several limitations. First of all, the power of this study might have been too limited in order to identify a difference between PNA and no PNA. This is partly due to the fact that we did not exclude outliers, leading to a broad range in cortisol levels. This decision was based on the fact that normal values of fetal hair cortisol are not available. Also, a power analysis was not possible due to this lack of normal values. Another limitation is that SAD-use was established based on the outpatient visits and a questionnaire and not verified by blood testing, which might have led to misclassification. Furthermore, the comparison between the patient and control group might be hampered as in all infants of the control group there was a (maternal or neonatal) indication for clinical observation while in most infants of the patient group SAD-exposure was the only indication for hospital stay. However, we analyzed all indications for potential confounding and did not find a significant relation with the fetal cortisol level.

In conclusion, we showed that in infants with PNA, hair cortisol levels were higher compared to infants without PNA. As this difference was only statistically significant in female infants, this suggests that the HPA axis activity may play a sex-specific role in the development of PNA. In contrast, we did not find a correlation between fetal hair cortisol levels and nonspecific distress in infants. Furthermore, we found a lower cortisol level in SAD-exposed infants without PNA compared to non-exposed infants, which might be explained by a delayed decline in 11ß-HSD-2 or inadequate maturation of the HPA axis due to SAD-exposure. As PNA is most likely of a multifactorial origin, it would be interesting to examine other factors possibly involved in the etiology of PNA in future studies, such as (epi) genetics. Also, analysis of hair cortisol in SAD-exposed infants of three months of age, might provide insight into the long-term effects of SAD exposure and PNA.

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