Timing of prenatal exposure to trauma and altered placental expressions of hypothalamic-pituitary-adrenal axis genes and genes driving neurodevelopment

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Prenatal maternal stress increases the risk for negative developmental outcomes in offspring; however, the underlying biological mechanisms remain largely unexplored. In the present study, alterations in placental gene expression associated with maternal stress were examined to clarify the potential underlying epi/genetic mechanisms. Expression levels of 40 selected genes involved in regulating foetal hypothalamic-pituitary-adrenal axis and neurodevelopment were profiled in placental tissues collected from a birth cohort established around the time of Superstorm Sandy. Objective prenatal traumatic stress was defined as whether mothers were exposed to Superstorm Sandy during pregnancy. Among the 275 mother-infant dyads, 181 dyads were delivered before Superstorm Sandy (ie, Control), 66 dyads were exposed to Superstorm Sandy during the first trimester (ie, Early Exposure) and 28 were exposed to Superstorm Sandy during the second or third trimester (ie, Mid-Late Exposure). Across all trimesters, expression of HSD11B2, MAOA, ZNF507 and DYRK1A was down-regulated among those exposed to Superstorm Sandy during pregnancy. Furthermore, trimester-specific differences were also observed: exposure during early gestation was associated with down-regulation of HSD11B1 and MAOB and up-regulation of CRHBP; exposure during mid-late gestation was associated with up-regulation of SRD5A3. The findings of the present study suggest that placental gene expression may be altered in response to traumatic stress exposure during pregnancy, and the susceptibility of these genes is dependent on the time of the exposure during pregnancy. Further studies should aim to clarify the biological mechanisms that underlie trimester-specific exposure by evaluating the differential impact on offspring neurodevelopment later in childhood.

KEYWORDS
mRNA, placenta, pregnancy, prenatal stress
INTRODUCTION

During critical periods in pregnancy, the foetus has a heightened susceptibility to prenatal maternal stress (PNMS). Animal research shows that PNMS is associated with altered development of foetal neurobiological systems, particularly the central nervous system (CNS) and the hypothalamic-pituitary-adrenal (HPA) axis, which subsequently leads to impairment of health, cognition, affect and behaviour. Human population studies also have demonstrated associations between PNMS, foetal CNS, HPA axis development, and offspring long-term neurobehavioural and neurodevelopmental aberrations. However, these human studies are not yet able to causally link PNMS and CNS/HPA development as a result of possible confounds in recalling stress during pregnancy. Although random assignment to reduce the impact of confounds is possible in animal research, a controlled experiment with random assignment of stress is not ethically feasible using a human population. Alternatively, a stressor with substantial negative valence, such as war or natural disaster, where individuals in the same region are independently and randomly exposed regardless of their demographic, genetic and psychosocial characteristics, can be leveraged to study its effects on subsequent changes and health consequences. Indeed, studies have already shown that PNMS as a result of acute trauma, natural and man-made disasters increased the risks for emotional, cognitive, behavioural and physical problems in offspring.

In an attempt to understand the underlying biological mechanisms that influence sub/optimal development of the foetus as a result of PNMS, changes in placental gene expression have attracted interest in the research community in recent years. The human placenta is the major interface between the mother and foetus, and is the critical organ that regulates foetal homeostasis, growth and development. The disruption of the maternal milieu by stress can program vital aspects of placental functioning in response, including the expression of placental genes. In addition, because the placenta is derived from the extra-embryonic layer of the blastocyst, the placenta shares genetic and epigenetic characteristics of the developing embryo/foetus. PNMS has been linked to foetal growth and development, which may be partially explained by changes in placental functioning and its underlying genomics. These programming processes have been studied in greater detail for the genes encoding 11β-hydroxysteroid dehydrogenase enzymes (HSD11B2), glucocorticoid and mineralocorticoid receptors (NR3C1 and NR3C2), and monooamine oxidase A (MAOA). However, the types of PNMS investigated also vary, from prenatal depression and prenatal anxiety, to prenatal perceived stress. For example, prenatal depression was associated with up-regulation of NR3C1 and/or NR3C2. On the other hand, prenatal depression was also associated with down-regulation of MAOA. In addition, decreased HSD11B2 expression was found to be associated with prenatal anxiety but not with prenatal depression, whereas increased HSD11B2 expression was associated with perceived prenatal stress and negative health-related stress. The types and degree of PNMS may affect distinct molecular pathways/placental processes, and the role of placental gene expression in relaying the effects of PNMS from disaster or trauma exposure remains elusive.

Furthermore, investigations aiming to clarify the role of PNMS exposure timing on placental functioning are lacking. Animal studies (eg, mice, rats, guinea pigs) suggest that the programming effects of PNMS on offspring outcomes are subject to the time of exposure. For example, one study has found that only rats exposed to PNMS during the first trimester suffered behavioural and physiological deficits. Male mice exposed to stress in early gestation showed increased stress reactivity (eg, elevated levels of corticotrophin-releasing factors, reduced hippocampal glucocorticoid receptor expression), cognitive deficits in learning and memory and anxiety-related behaviours. Stress early in pregnancy was also associated with up-regulation of placental peroxisome proliferator-activated receptor α (PPARα), insulin-like growth factor binding protein 1 (IGFBP-1), hypoxia-inducible factor 3a (HIF3a) and glucose transporter 4 (GLUT4) gene expression in male mice. These studies broadly suggest that early pregnancy is a sensitive period for development. Stress exposure, especially during the first trimester, may disrupt developmental programming and potentially increase the risk of long-term neurodevelopmental disorders in offspring. Similarly, human studies provide evidence suggesting that PNMS exposure during early pregnancy may bring about the most devastating consequences. One study reported that women exposed to an earthquake in their first trimester experienced the highest level of stress and had infants with lower gestational ages at birth than women exposed during later trimesters. King and Laplante found that exposure to a natural disaster in early and mid-pregnancy was associated with lower mental development scores. In addition, many prior studies show that increased risk for schizophrenia is associated with extreme stress in early pregnancy. For example, Khaskan et al. found that pregnant women who experienced a familial death during the first trimester of pregnancy had children who were at a higher risk for schizophrenia and related disorders later in life. Another study also linked a higher risk for schizophrenia to first trimester exposure to the Dutch famine of 1944-1945. However, there is also evidence that the risks for other outcomes, such as autism, are associated with stress experienced in mid- or late-pregnancy. For example, Beversdorf et al. reported the PNMS during the second and third trimesters (but not the first trimester) was associated with greater risk for autism. Similarly, a study that investigated the effect of PNMS from a tropical storm or hurricane found that storm exposure during mid (5-6 months) and late (9-10 months) pregnancy predicted an increased risk for autism. Taken together, these studies highlight the importance of examining whether early trimester has specific noxious influences on developing organisms and, if so, through what molecular mechanisms.

Studies specifically investigating stress exposure on placental changes also observe differences based on the timing of exposure. For example, Reynolds et al. found that higher prenatal depression throughout pregnancy was associated with up-regulated placental NR3C1 and NR3C2 expression and that these effects were particularly significant for symptoms experienced in the third trimester for...
NR3C1 and in the second trimester for NR3C2, although their study focused on a small subset of genes.

To date, differences in the timing of the exposure to PNMS on gene expression in the placenta have not yet been systematically investigated. Uncovering the biological mechanisms that are associated with earlier or later stress exposure and its subsequent influence on developmental and mental health outcomes could further explain the somewhat inconsistent findings and move our understanding forward.

In the present study, we aimed to evaluate acute PNMS experienced earlier and later in pregnancy by virtue of a devastating natural disaster, Superstorm Sandy. Superstorm Sandy was one of the worst natural disasters on record in the USA and was the second costliest cyclone to hit the USA since 1900. The New York metropolitan area was severely affected by the storm in October 2012.28 Superstorm Sandy drove extensive storm surge, waves, rainfall and flooding into the New York coastlines, where residences, businesses, cars and other property was heavily damaged. In New York, over 300,000 homes were severely destroyed primarily as a result of the storm. Significant damage also occurred to public transportation, particularly the subway system, resulting in suspensions of services, which ranged from a few hours to as long as several weeks. Other significant effects included widespread and prolonged power outages and a gasoline shortage. There were 117 deaths total (53 deaths in New York) attributed to Superstorm Sandy.29 Because of its magnitude in size and impact, Superstorm Sandy brought to the population residing in the affected area both economical and psychological damages as a result of the destruction, providing us with a unique opportunity to conduct a quasi-experimental study. The quasi-experiment allows us to understand whether PNMS as a result of a natural disaster and its gestational timing may lead to dysregulation of the placental genome, particularly for 40 candidate genes known to be associated with HPA axis functioning and neurodevelopment (see Supporting information, Table S1).

2 | MATERIALS AND METHODS

2.1 | Study population

The Stress in Pregnancy (SIP) Study is an ongoing longitudinal study that enrols and follows mothers throughout pregnancy and their offspring after their birth. All women were recruited as part of the SIP Study from the prenatal obstetrics and gynecological clinics at Mount Sinai Medical Center and New York Presbyterian Queens in New York City. The unexposed participants are comprised mainly of women who reside in Manhattan and received obstetric care at Mount Sinai Hospital, whereas the Sandy exposed participants are comprised of women who reside in regions of Queens and Long Island devastated by the storm. Participants were excluded if positive for HIV infection, maternal psychosis, maternal age <15 years, life-threatening medical complications related to the mother, and congenital or chromosomal abnormalities in the foetus. A detailed description of the study population is provided elsewhere.14,30

Demographic information, such as mother’s race, marital status, education, age, smoking behaviour during pregnancy and prenatal normative psychosocial stress measures, were collected during the second trimester. Data on mode of delivery, gestational age (weeks) at birth, infant sex and birth weight (g) were recorded at birth.

A total of 328 placental tissues collected from mothers who were pregnant before or during Superstorm Sandy were included in the present study. Preterm infants born before 34 weeks (n = 10) were not included as a result of higher risks of developing severe health and developmental problems.31,32 An additional 43 cases were excluded because of missing normative psychosocial stress measures, resulting in a final sample of 275 in the present study. Table 1 shows the demographic characteristics of the sample used in the current study. Included (N = 275) and excluded participants (N = 43) did not differ with respect to major demographic characteristics, such as infant sex, gestational age at birth, birthweight, maternal age, race or education. Missing education (n = 1), marital status (n = 1) and mode of delivery data (n = 7) have been imputed.

All participants provided their written informed consent before any assessment or data collection. All procedures involving human subjects in this study were approved by the Institutional Review Boards at the City University of New York, New York Presbyterian/Queens, and the Icahn School of Medicine at Mount Sinai.

2.2 | Timing of trauma exposure during pregnancy

Among the 275 mother-infant dyads, 181 mothers included in the present study gave birth before Superstorm Sandy (Control) and 94 mothers were pregnant during Superstorm Sandy. Among these 94 mothers, 66 were exposed to Superstorm Sandy during the first trimester (Early Exposure) and 28 were exposed to Superstorm Sandy during the second or the third trimester (Mid-Late Exposure).

2.3 | Selected genes known to modulate HPA axis and neurodevelopment

The 40 candidate genes were identified a priori for their involvement in HPA axis functioning and neurodevelopment, as based on extensive literature search and the Ingenuity Knowledge Base (http://www.ingenuity.com). Among the 20 HPA axis functioning genes, 14 genes were expressed in the placenta and six genes were not sufficiently expressed. Among the remaining 20 genes associated with neurodevelopment, 13 genes were sufficiently expressed in the placenta and seven genes were not sufficiently expressed. Details regarding candidate genes can be found in the Supporting information (Table S1).

2.4 | Placenta collection and gene expression profiling

Biopsies, free of maternal decidua, were collected from each placenta quadrant midway between the cord insertion and the placenta rim, within 1 hour of delivery to prevent RNA degradation. The collected tissues were first snap-frozen in liquid nitrogen and then stored at –80°C.
| TABLE 1 | Characteristics of the study population in total and by stress groups (control, early exposure and mid-late exposure) |
|------------------|------------------|------------------|------------------|------------------|
|                  | Total (N = 275)  | Control (n = 181) | Early exposure (n = 66) | Mid-Late exposure (n = 28) | P value a |
| Infant sex       |                  |                  |                  |                  |          |
| Males, N (%)     | 150 (54.5)       | 98 (54.1)        | 40 (60.6)        | 12 (42.9)        | .282     |
| Females, N (%)   | 125 (45.5)       | 83 (45.9)        | 26 (39.4)        | 16 (57.1)        |          |
| Gestational age, weeks, mean ± SD | 39.31 ± 1.47 | 39.30 ± 1.49 | 39.31 ± 1.47 | 39.33 ± 1.38 | .997     |
| Birthweight, N (%) |                  |                  |                  |                  |          |
| < 2500 g         | 15 (5.5)         | 13 (7.2)         | 1 (1.5)          | 1 (3.6)          | .196     |
| ≥ 2500 g         | 258 (93.8)       | 166 (91.7)       | 65 (98.5)        | 27 (96.4)        |          |
| Missing           | 2 (0.7)          | 2 (1.1)          |                |                |          |
| Mode of delivery, N (%) |            |                  |                  |                  |          |
| C-section         | 93 (33.8)        | 65 (35.9)        | 19 (28.8)        | 9 (32.1)         | .389     |
| Vaginal           | 175 (63.6)       | 114 (63)         | 45 (68.2)        | 16 (57.1)        |          |
| Missing           | 7 (2.5)          | 2 (1.1)          | 2 (3)            | 3 (10.7)         |          |
| Maternal age, years, mean ± SD | 27.76 ± 5.90 | 27.65 ± 6.18 | 27.70 ± 5.34 | 28.68 ± 5.35 | .688     |
| Mother's race, N (%) |                  |                  |                  |                  |          |
| White             | 27 (9.8)         | 13 (7.2)         | 10 (15.2)        | 3 (10.7)         | .219     |
| Non-White         | 248 (90.2)       | 168 (92.8)       | 56 (84.8)        | 25 (89.3)        |          |
| Black             | 68 (24.7)        | 53 (29.3)        | 12 (18.2)        | 3 (10.7)         |          |
| Hispanic/Latino   | 145 (52.7)       | 98 (54.1)        | 29 (43.9)        | 19 (67.9)        |          |
| Asian             | 21 (7.6)         | 7 (3.9)          | 12 (18.2)        | 2 (7.1)          |          |
| Others            | 14 (5.1)         | 10 (5.5)         | 3 (4.5)          | 1 (3.6)          |          |
| Maternal education, N (%) |            |                  |                  |                  |          |
| Less than high school | 53 (19.3)       | 46 (25.4)        | 5 (7.6)          | 2 (7.1)          | .003     |
| High school graduate | 62 (22.5)       | 44 (24.3)        | 13 (19.7)        | 5 (17.9)         |          |
| Some college      | 108 (39.3)       | 69 (38.1)        | 27 (40.9)        | 12 (42.9)        |          |
| College graduate  | 30 (10.9)        | 13 (7.2)         | 11 (16.7)        | 6 (21.4)         |          |
| Graduate degree   | 21 (7.6)         | 9 (5)            | 9 (13.6)         | 3 (10.7)         |          |
| Missing           | 1 (0.4)          | 1 (1.5)          |                |                |          |
| Mother's marital status, N (%) |            |                  |                  |                  | <.001    |
| Married           | 90 (32.7)        | 39 (21.5)        | 35 (53)          | 16 (57.1)        |          |
| Common law        | 20 (7.3)         | 13 (7.2)         | 6 (9.1)          | 1 (3.6)          |          |
| Single            | 159 (57.8)       | 126 (69.6)       | 22 (33.3)        | 11 (39.3)        |          |
| Divorced/separated/ widowed | 5 (1.8) | 3 (1.7) | 2 (3.0) |                |          |
| Missing           | 1 (0.4)          | 1 (1.5)          |                |                |          |
| Smoking during pregnancy, N (%) |            |                  |                  |                  | .246     |
| No                | 238 (86.5)       | 154 (85.1)       | 57 (86.4)        | 27 (96.4)        |          |
| Yes               | 35 (12.7)        | 27 (14.9)        | 7 (10.6)         | 1 (3.6)          |          |
| Missing           | 2 (0.7)          | 2 (3)            |                |                |          |
| Prenatal depression, mean ± SD | 7.35 ± 5.4 | 7.26 ± 5.36 | 7.62 ± 5.37 | 7.36 ± 5.88 | .902     |
| Prenatal related anxiety, mean ± SD | 5.87 ± 2.3 | 5.89 ± 2.28 | 6.04 ± 2.34 | 5.36 ± 2.32 | .423     |
| Perceived stress during pregnancy, mean ± SD | 36.20 ± 7.43 | 36.37 ± 7.47 | 35.58 ± 7.58 | 36.49 ± 6.96 | .748     |

(Continues)
TABLE 1 (Continued)

<table>
<thead>
<tr>
<th></th>
<th>Total (N = 275)</th>
<th>Control (n = 181)</th>
<th>Early exposure (n = 66)</th>
<th>Mid-Late exposure (n = 28)</th>
<th>P value *</th>
</tr>
</thead>
<tbody>
<tr>
<td>State anxiety, mean ± SD</td>
<td>38.00 ± 11.50</td>
<td>37.87 ± 11.35</td>
<td>38.58 ± 12.62</td>
<td>37.57 ± 10.11</td>
<td>.896</td>
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<tr>
<td>Trait anxiety, mean ± SD</td>
<td>38.43 ± 10.75</td>
<td>38.46 ± 10.83</td>
<td>38.35 ± 10.31</td>
<td>38.50 ± 11.61</td>
<td>.997</td>
</tr>
<tr>
<td>Negative stressful events, Mean (SD)</td>
<td>1.57 ± 1.97</td>
<td>1.55 ± 1.93</td>
<td>1.62 ± 2.04</td>
<td>1.57 ± 2.10</td>
<td>.974</td>
</tr>
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</table>

Normative psychosocial stress group N (%)

| Low         | 104 (37.8) | 69 (38.1) | 24 (36.4) | 11 (39.3) | .744 |
| Moderate    | 127 (46.2) | 82 (45.3) | 34 (51.5) | 11 (39.3) |
| High        | 44 (16)    | 30 (16.6) | 8 (12.1)  | 6 (21.4)  |

*p values for the test for the differences among the 3 groups: ANOVA for continuous variables and Chi-square/Fisher’s exact tests for categorical variables.

RNA was extracted using Maxwell 16 automated DNA/RNA extraction equipment (Promega, Madison, WI, USA) in accordance with the manufacturer’s instructions. RNA was quantified with a Nanodrop spectrophotometer (Thermo Electron North America, Madison, WI, USA). Placental RNA expression was profiled using the nCounter platform (nanoString Technologies, Seattle, WA, USA) as described previously. The nanoString Norm package was used to normalise data. Differences in sample content were accounted for by normalising the data against the geometric mean of the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosomal protein L19 (RPL19), and ribosomal protein lateral stalk subunit P0 (RPLP0). Genes where more than 50% of the samples fell below the limit of detection were considered unexpressed. After filtering out unexpressed genes, a total of 27 genes remained in the final analysis.

2.5 | Covariates

2.5.1 | Demographic variables

Various maternal and child demographic and health characteristics were included as covariates. Maternal characteristics included: maternal age, race (white, non-white), education, marital status (married/common law, single, divorced/separated/widowed), and smoking behaviour during pregnancy (smoking, nonsmoking). Infant characteristics included infant sex (male, female), gestational age and mode of delivery (C-section, vaginal).

2.5.2 | Normative psychosocial stress measures

Normative psychosocial stress during pregnancy was defined as a composite of prenatal depression, pregnancy related anxiety, perceived stress, state and trait anxiety, and negative stressful events. The co-experience of multiple types of normative psychosocial stress during pregnancy is relatively commonplace, capturing various domains of stress that mothers experience during pregnancy, and using an aggregate measure of stress would increase the validity and reliability of the normative prenatal stress measure, as opposed to relying on only a single stressor. These variables were measured using maternal self-report scales completed during the second trimester of the pregnancy and were used as a covariate when investigating the relationship between effects of prenatal trauma exposure and gene expression in the placenta. Prenatal depression was measured using the Edinburgh Postnatal Depression Scale. Mothers were asked to report how they felt during the past 7 days on a 4-point Likert scale based on severity. This inventory is well-validated in several languages and has acceptable reliability ranging from 0.79 to 0.86. Pregnancy related anxiety was measured using the Pregnancy Related Anxieties Questionnaire-revised (PRAQ-R), which measures pregnancy related fears and worries. Perceived stress during pregnancy was measured using the Perceived Stress Scale (PSS-14), which assessed the degree to which the rater appraises situations as stressful. The PSS-14 has good reliability and validity. State and trait anxiety during pregnancy was measured using the State-Trait Anxiety Inventory (STAI), which assessed temporary “state anxiety” and long-standing, characterological “trait anxiety.” Each of the 2 subscales consists of 20 items rated on a 4-point Likert scale. A meta-analysis of 45 articles reporting Cronbach’s α for internal consistency for this inventory determined the mean to be 0.92. Negative stressful events during pregnancy was measured using the Psychiatric Epidemiology Research Interview Life Events Scale (LES), which assessed the occurrence of stressful events in 5 major areas of life: relationships, health, legal matters, work and financials, and friendships. This measure is widely used, has been shown to have good validity with narrative reports of life events, and has low intra-category variability. The measures of normative psychosocial stress above were categorised to create a composite latent measure created by latent profile analysis (LPA). Model fits were assessed by Bayesian Information Criteria (BIC), adjusted BIC, Lo-Mendell-Rubin test P values, and the entropy values for the 2-4 class models. LPA was performed using the full maximum likelihood estimation in mplus, version 6. Methodological details on the extraction of the latent confounding variable are provided in the Supporting information (Methods S1). Overall, all stress variables were significantly correlated (see Supporting information, Table S2). LPA indicated that the 3-class solution provided the best solution (see Supporting information,
Table S3). The composite latent measure was categorised into 3 values from (0) low normative stress, (1) medium normative stress to (2) high normative stress. In total, 104 individuals were labelled as “low normative stress”, 127 individuals were labelled as “medium normative stress” and 44 were labelled as “high normative stress” (see Supporting information, Table S4).

2.6 Statistical analysis

ANOVA for continuous variables and chi-square/Fisher’s exact tests for categorical variables were conducted to examine the differences among groups (Control, Early Exposure and Mid-Late Exposure) across demographic and psychosocial factors. A generalised linear model was used to evaluate the effects of acute PNMS on gene expression by comparing group differences on the placental expression of each gene, adjusting for covariates determined a priori. Significance of main effects (significance $P < .05$) was further examined using the sequential Bonferroni (Holm) multiple comparison tests. All main statistics were conducted using SPSS, version 19 (IBM Corp., Armonk, NY, USA), whereas LPA was conducted using Mplus, version 6 (https://www.statmodel.com).

3 RESULTS

3.1 Characteristics of the study population

The distribution of the demographic characteristics of the 275 dyads included in the present study is shown in Table 1. The population consisted of infants (mean age at gestation of 39.31 weeks), with approximately equivalent numbers of males and females (females: 45.5%). The SIP study consists of an urban, ethnically diverse cohort, with over half of the population reported to be of Hispanic/Latino descent (52.7%). Enrolled mothers were largely single (57.8%) and of mixed educational background, ranging from no high school degree (19.3%) to post/college degree (18.5%).

Except for significant differences in maternal education ($P = .003$) and marital status ($P < .001$), with relatively more educated and married women in the exposed groups as opposed to the control, no significant group differences were observed for other demographic or psychosocial factors (Table 1).

3.2 Timing of Superstorm Sandy exposure and gene expression in placental HPA axis genes

Figure 1 and Table 2 show the results for the overall group differences and follow-up pairwise comparisons with Holm correction for multiple testing. There are significant overall group differences in CRHB, DYRK1A, HSD11B1 and HSD11B2. When adjusted for multiple comparisons, CRHB gene expression level was up-regulated in those exposed in early gestation compared to the unexposed controls ($P = .030$). DYRK1A gene expression level was down-regulated in those exposed in mid-late gestation compared to the unexposed controls ($P = .005$). HSD11B1 gene expression level was down-regulated in those exposed in early gestation compared to unexposed controls ($P = .038$) and those exposed in mid-late gestation ($P = .038$). HSD11B2 gene expression level was down-regulated in...
those exposed in early gestation \((P = .043)\) and mid-late gestation \((P < .001)\) compared to the unexposed controls.

### 3.3 Timing of Superstorm Sandy exposure and gene expression in placental neurodevelopment genes

Table 2 shows significant group differences in neurodevelopment genes, including MAOA, MAOB, MECP2, SRD5A3 and ZNF507. As indicated in Figure 1, when adjusted for multiple comparisons, MAOA gene expression level was down-regulated in those exposed in early \((P = .039)\) and mid-late gestation \((P = .011)\) compared to unexposed controls. MAOB gene expression level was down-regulated in those exposed in early gestation compared to unexposed controls \((P < .001)\). SRD5A3 gene expression level was up-regulated in those exposed in mid-late gestation compared to unexposed controls \((P = .019)\) and those exposed in early gestation \((P = .019)\). ZNF507 gene expression level was down-regulated in those exposed in early \((P = .005)\) and mid-late gestations \((P = .001)\) compared to unexposed controls.

### 4 DISCUSSION

Accumulating evidence from animal and human research suggests that PNMS exposure exerts long-term impacts on foetal...
programming by altering placental function, which may be reflected in the gene expression profile in the placenta. Given the predominant foetal origin of the placenta, the findings of the present study offer interesting insights into the impacts of acute PNMS on offspring.

Our results show that PNMS, as a result of exposure to a natural disaster, at different stages of pregnancy was associated with down-regulation of HSD11B2, MAOA and ZNF507 genes. The trend of down-regulation of DYRK1A across pregnancy was also observed, whereas the effect was significant for mid-late gestation, it was marginally significant for early gestation \( (P = .084) \). Overall, many of these down-regulated genes across trimesters are vital for placental function and foetal development.

The placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) enzyme (encoded by the HSD11B2 gene), which converts active cortisol into inactive cortisone, acts as a barrier regulating the transfer of the maternal cortisol to the foetus. \(^{53}\) Cortisol is essential to foetal growth but may be harmful to the foetus when in high concentrations. \(^{54}\) Under normal circumstances, 11β-HSD2 largely converts cortisol into cortisone, thereby protecting the foetus from excessive glucocorticoid exposure. \(^{53}\) This is supported by the finding that foetal blood has 13-fold lower cortisol concentrations than maternal blood. \(^{55}\) Studies demonstrate that stressed mothers commonly secrete greater amounts of glucocorticoids, \(^{56,57}\) despite contradictory evidence. \(^{58,59}\) Consequently, elevated levels of glucocorticoids may enter the foetal circulation and influence foetal HPA axis development. Our results suggest that when exposed to acute PNMS, the protective effect of placental HSD11B2 can be overwhelmed. Indeed, prior research suggested that reduced placental HSD11B2 may be associated with poor infant outcomes, including decreased infant movement quality and lower muscle tone. \(^{60,61}\)

Our findings are inconsistent with some of the previous literature regarding stress-related effects on HSD11B2 gene expression. For example, prenatal anxiety, but not depression, has been associated with lower HSD11B2 expression. \(^{15}\) The distinction may be explained by a lack of evolutionary benefit for prenatal depression compared to prenatal anxiety, such that a depressed mother may not perceive danger, and therefore depression may not play a role in affecting foetal and child development in future dangerous and stressful situations. \(^{62}\) Furthermore, prenatal perceived stress and health-related stress were reported to be positively associated with HSD11B2. \(^{11}\) It has also been suggested that mild to moderate levels of PNMS may not decrease (and indeed may enhance) development. For example, mid-level PNMS, such as nonspecific stress and prenatal depressive symptoms, were found to be positively associated with mental and motor development in younger children. \(^{63}\) The present study is the first to have associated acute PNMS as a result of a natural disaster and decreased HSD11B2 expression and therefore requires further replication. Nevertheless, this finding advocates that different types of stressors may exert differential impacts on gene expression that in turn program distinct foetal and child outcomes. \(^{64}\)

In addition to maternal glucocorticoids, serotonin is an important stress-related neurotransmitter \(^{65}\) which is synthesised in the placental and foetal compartments and is vital for foetal brain development. MAOA metabolises serotonin, dopamine and norepinephrine. Maternal blood serotonin can cross the placenta and enter the foetal circulation; overexposure to serotonin disrupts foetal brain development. \(^{66}\) Recent research suggests that PNMS is associated with elevated levels of serotonin \(^{67}\) and a reduction in MAOA gene expression. \(^{60,67}\) Mutations in MAOA have been linked to disordered neurodevelopment and behaviours, including autism-like disorders and antisocial behaviours. \(^{68,69}\)

Little is known about how the expression levels of placental genes, such as DYRK1A and ZNF507, influence the development of brain function and behaviour in typically developing children. DYRK1A is involved in cell proliferation and has been implicated in Down’s syndrome. \(^{70}\) ZNF507 modulates transcriptional regulation and reduced expression of ZNF507 has been related to schizophrenia. \(^{71}\) Stress induced down-regulation of these genes may also have an impact on placental function, intrauterine homeostasis and foetal growth.

Furthermore, these down-regulated genes are more markedly altered among women exposed during mid-late gestation, which suggests that the impact of PNMS may be exaggerated as women advance throughout pregnancy, although this warrants further investigation. Our group comparison results reflect no significant statistical differences between early and mid-late gestation \( (P = .053; \text{MAOA}, P = 0.270, \text{ZNF507}, P = .168 \text{and DYRK1A}, P = .124) \), which may be attributed to the relatively small sample of exposed participants during mid-late gestation.

Prior animal and human research suggests the timing of exposure appears to be crucial when considering the effect of PNMS on offspring outcomes. \(^{67}\) PNMS is considered to be associated with adverse outcomes, particularly in cases of early gestation exposure. Our findings are partially consistent with this line of research. Specifically, our results show that up-regulation of CRHBP and down-regulation of HSD11B1 and MAOB were observed among those exposed to Superstorm Sandy in early pregnancy. Because of the relatively small group size with respect to mid-late gestation, we did not observe significant differences between early and mid-late gestation exposure for CRHBP \( (P = .697) \) and MAOB \( (P = .055) \) expression.

CRHBP encodes the corticotrophin-releasing hormone (CRH)-binding protein, which inactivates CRH that stimulates the production of adrenocorticotropic hormone and cortisol throughout pregnancy in the maternal and the foetal compartments. \(^{72,73}\) Increased circulating maternal CRH concentrations have been associated with lower concentrations of CRHBP. \(^{73}\) In a normal human pregnancy, maternal CRH, as derived from the placenta, provides information on the length of gestation. \(^{74,75}\) Circulating maternal CRH concentrations rise over the course of gestation, correlating with increased placental CRH mRNA expression. \(^{73}\) Although an elevation in circulating maternal CRH concentrations increases risks for foetal growth restriction during early gestation \(^{76}\), an increase in these concentrations during the last few weeks of pregnancy accompanied by a fall in the concentrations of CRH-binding proteins allows for the preparation of events leading to parturition. \(^{77}\) It has been suggested...
that exposure to stress, especially during early gestation, is associated with an increase in placental CRH concentrations in plasma. The results of the present study suggest that, for individuals exposed to Superstorm Sandy in early pregnancy, a rise in CRH may lead to up-regulation of CRHBP, which can produce prolonged excessive CRH-binding proteins that prevent inappropriate pituitary-adrenal stimulation but disrupts the developmental increase of maternal CRH concentrations. In adults, CRHBP dysfunctionality is associated with post-traumatic stress and depression symptoms.89,90

Comparedly, the expression and activity of HSD11B1, which is primarily involved in reactivation of cortisol from cortisone, increases during normal pregnancy. Decreased HSD11B1 has been associated with increased risks for newborns with intrauterine growth restriction (ie, small-for-gestational-age), in part due to reduced cortisol regeneration.83,84 Offspring exposed to the traumatic event in early gestation may be more vulnerable to these disruptions as the consequences of deficient HSD11B1 expression.

Similar to MAOA, MAOB plays a critical role in regulating dopamine metabolism and dietary amines including phenylethylamine.82 The placental tissue contains a small amount of MAOB.83,84 MAOB activity increases with ageing in humans and is associated with neurodegenerative diseases such as Parkinson’s and Alzheimer’s diseases.85,86 The role of MAOB gene expression in the placenta has not been well described. Lower MAOB platelet activity has been linked to mood disorders, alcoholism, sensation seeking and impulsivity.87 Down-regulation of placental MAOB may increase risks for neuropsychiatric and behaviour disorders in offspring exposed to PNMS in early gestation.

Finally, up-regulation of SRD5A3 was observed among those exposed to Superstorm Sandy in mid-late pregnancy. SRD5A3 plays an important role in protein glycosylation, is widely expressed in the human brain tissues and body organs (eg, retina, skin, kidney), and plays a crucial role in brain development.89 Mutations in SRD5A3 have been linked to a congenital defect in dolichol metabolism.89,90 Animal research has found that placental SRD5A3 is altered by tri-closan (an antimicrobial agent often used in personal care products) exposure.91 The 3 human 5α-reductases are encoded by the SRD5A1, SRD5A2, and SRD5A3 genes. During pregnancy, the 5α-reductases in the placenta provide precursors for the synthesis of allopregnanolone, a neurosteroid that may exert neuroprotective effects on foetal brain development.92,93 Therefore, it is essential to further investigate the prenatal risk factors such as maternal stress that may influence the allopregnanolone synthesis pathway. One recent investigation found that maternal plasma allopregnanolone concentrations were not related to the genotypes of SRD5A1 and SRD5A2 and maternal depressive symptoms during pregnancy.94 However, little is known about the role of SRD5A3 gene in the human placenta and its relation to the prenatal stress influences.

Our findings suggest that trauma exposure may uniquely impact developmental processes through changes in expression of genes which foster distinct developmental processes. Furthermore, it is likely that some of the genes we identified are fully developed and begin functioning during early pregnancy, whereas others only begin functioning during mid-late pregnancy. Although we were able to identify changes in gene expression as a result of placental development, the underlying molecular mechanisms by which this occurs requires further exploration; thus, replication is needed. Expression of placental genes likely varies across gestation to accommodate the dynamically changing needs of the developing foetus, although the molecular basis of placental development has yet to be fully uncovered.95 Our findings suggest that several genes may be more vulnerable to maternal trauma exposure depending on the timing of exposure during gestation.

We acknowledge several limitations of the present study. Although we observed associations between PNMS and differences in gene expression, the implications of these findings on neurodevelopmental outcomes in childhood and adulthood remain unknown. Because we see significant observations between CRH binding proteins and maternal stress in the present study, follow-up studies will include further characterisation of the response of the corticotrophin signaling pathway, HPA axis functioning and maternal stress, by evaluating additional components of the pathway, including placental levels of CRH, ACTH and CORT. Furthermore, the RNA integrity and quality were not assessed in the present study, whereas they should have been evaluated for each extraction, especially because placental tissues contain high levels of RNase. Our opportunistic sample was relatively small, especially once divided it into groups by windows of exposure, requiring that our conclusions be corroborated by future studies with larger sample sizes. The small size of our groups also supported the combining of mid- and late-trimester groups into one.

This grouping is justifiable given that results of prior animal studies show that the first trimester is when the foetus might be most vulnerable to PNMS; however, it may have been more informative to have retained each trimester as a separate group. Furthermore, although prior research has shown that sex is likely a significant moderator of the effect of PNMS, our small sample size did not provide us with sufficient power to evaluate potential sex-specific effects.96 It should also be noted that the control and exposed groups were different with regard to marital status and education. Prior research has associated socio-economic status (ie, education level) and altered placental gene expression levels.97 Although these differences could have happened by chance alone, the control group was composed of mainly women residing in Manhattan who received obstetric care at Mount Sinai Hospital, whereas the exposed group was mainly composed of women residing in storm devastated regions, Queens and Long Island as a result of the study design. As such, although our findings were independent of a range of covariates, statistical control may not have been fully adequate in addressing group differences. Additionally, investigating the associations between gene expression and the covariates was outside the scope of the present study, and exploring such relationships may be worth pursuing in future studies. Finally, stress is a subjective experience and we did not include measures of how subjectively stressful the experience of Superstorm Sandy was for each of the mothers.
Despite these shortcomings, to our knowledge, the present study is the first to present an analysis of a list of candidate genes in HPA axis regulation and neurodevelopment in a functional organ (placenta) by exposure to a traumatic event during pregnancy. In comparison with previous research, we were able to investigate how the timing of trauma exposure impacts placental gene expression. Our observations suggest that PNMS from trauma across trimesters down-regulates placental expression of DYRK1A, HSD11B2, MAOA, and ZNF507. However, traumatic stress exposure in early gestation is associated with up-regulation of CRHBP and down-regulation of HSD11B1 and MAOB, whereas exposure in mid-late gestation is associated with up-regulation of SRD5A3. Our findings also demonstrated the importance of corroborating and extending the results of animal research in human populations. Longitudinal follow-up studies are needed to investigate how the alterations in the expression of these genes affect the neurobehavioural and neurodevelopmental outcomes in the offspring.

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REFERENCES


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