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# Timing of Prenatal Exposure to Trauma and Altered Placental Expressions of HPA-Axis Genes and Genes Driving Neurodevelopment

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## Abstract

Prenatal maternal stress increases the risk for negative developmental outcomes in offspring, however the underlying biological mechanisms remain largely unexplored. In this study, alterations in placental gene expression associated with maternal stress were examined to elucidate potential underlying epi/genetic mechanisms. Expression levels of 40 selected genes involved in regulating fetal HPA-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 (i.e., Control), 66 dyads were exposed to Superstorm Sandy during the first trimester (i.e., Early Exposure) and 28 were exposed to Superstorm Sandy during the second or third trimester (i.e., Mid-Late Exposure). Across all trimesters, expression of *HSD11B2*, *MAOA*, *ZNF507,* and *DYRK1A* was downregulated among those exposed to Superstorm Sandy during pregnancy. Furthermore, trimester specific differences were also observed: exposure during early gestation was associated with downregulation of *HSD11B1* and *MAOB,* and upregulation of *CRHBP*; exposure during mid-late gestation was associated with upregulation of *SRD5A3*. Our findings suggest that

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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 can elucidate the biological mechanisms that underlie trimester-specific exposure by evaluating the differential impact on offspring neurodevelopment later in childhood.

**Keywords**

pregnancy; prenatal stress; mRNA; placenta

## Introduction

During critical periods in pregnancy, the fetus has a heightened susceptibility to prenatal maternal stress (PNMS) [1,2]. Animal research shows that PNMS is associated with altered development of fetal 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 behavior [3]. Human population studies also have demonstrated associations between PNMS, fetal CNS, HPA-axis development [4,5] and offspring's long-term neurobehavioral and neurodevelopmental aberrations [6]. However, these human studies are not yet able to causally link PNMS and CNS/HPA development due to possible confounds in recalling stress during pregnancy. While 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 risks for emotional, cognitive, behavioral, and physical problems in offspring [7–9].

In an attempt to understand the underlying biological mechanisms that influence sub/optimal development of the fetus due to PNMS, changes in placental gene expression have attracted interest in the research community in recent years [10–13]. The human placenta is the major interface between the mother and fetus, and is the critical organ that regulates fetal 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, since the placenta is derived from the extraembryonic layer of the blastocyst, the placenta shares genetic and epigenetic characteristics of the developing embryo/fetus [14]. PNMS has been linked to fetal 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 11-beta hydroxysteroid dehydrogenase enzymes (*HSD11B2*) [11,12,15], glucocorticoid and mineralocorticoid receptors (*NR3C1* and *NR3C2*) [11–13,16], and monoamine oxidase A (*MAOA*)[10]. 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 upregulation of *NR3C1* and/or *NR3C2* [13,16]. Prenatal depression was also

associated with downregulation of *MAOA* [10]. In addition, decreased *HSD11B2* expression was found to be associated with prenatal anxiety but not with prenatal depression [15]; while increased *HSD11B2* expression was associated with perceived prenatal stress and negative health related stress [11]. 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, examinations aimed at elucidating the role of PNMS exposure timing on placental functioning are underexplored. Animal studies (e.g., mice, rats, guinea pigs) suggest that the programming effects of PNMS on offspring outcomes are subject to the time of exposure [17–20]. For example, one study has found that only rats exposed to PNMS during the first trimester suffered behavioral and physiological deficits [20]. Male mice exposed to stress in early or mid-gestation showed increased stress reactivity (e.g., elevated levels of corticotrophin releasing factors, reduced hippocampal glucocorticoid receptor expression), cognitive deficits in learning and memory and anxiety-related behaviors [18]. Stress early in pregnancy was also associated with upregulation of placental peroxisome proliferator-activated receptor alpha (*PPARa*), insulin-like growth factor binding protein 1 (*IGFBP-1*), hypoxia-inducible factor 3a *(HIF3a*), and glucose transporter 4 (*GLUT4*) gene expression in male mice [18]. 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 [17]. 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 [21]. King and Laplante (2005) found that exposure to a natural disaster in early and mid-pregnancy was associated with lower mental development scores [8]. In addition, many prior studies show that increased risk for schizophrenia is associated with extreme stress in early pregnancy [22–25]. For example, Khashan et al. (2008) 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 [22]. Another study also linked higher risk for schizophrenia to first trimester exposure to the Dutch famine of 1944–1945 [24]. 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 instance, Beversdorf et al. (2005) reported the PNMS during the second and third trimesters, but not the first trimester, was associated with greater risk for autism [26]. 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 [27]. Taken together, it is of importance to examine 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. (2015) found that higher prenatal depression throughout pregnancy was associated with upregulated placental *NR3C1* and *NR3C2* expression, and these effects were particularly significant for symptoms

experienced in the third trimester for *NR3C1* and in the second trimester for *NR3C2* [16]. However, this 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 this 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 United States and was the second costliest cyclone to hit the U.S. 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 due to 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 (Supplementary Table 1).

## Materials and Methods

### Study Population

The Stress in Pregnancy (SIP) Study is an on-going longitudinal study that enrolls 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, while 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 fetus. A detailed description of the study population can be found elsewhere [14,30]. Demographic information, such as mother's race, marital status, education, age, smoking behavior during pregnancy and prenatal normative psychosocial stress measures were collected during the second trimester. Data on mode of delivery, gestational age (in weeks) at birth, infant sex, and birth weight (grams) 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 current study. Preterm infants born before 34 weeks (n = 10) were not included due to higher risks of developing severe health and developmental problems [31,32]. An additional 43 cases were excluded due to missing normative psychosocial stress measures, resulting in a final sample of 275 in this current 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 on 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 gave 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.

## Timing of Trauma Exposure during Pregnancy

Among the 275 mother-infant dyads, 181 mothers included in this study gave birth before Superstorm Sandy (Control) and 94 mothers were pregnant during Superstorm Sandy. Among these 94, 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).

## 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 Data. Among the 20 HPA-axis functioning genes, 14 genes were expressed in the placenta and 6 genes were not sufficiently expressed. Among the remaining 20 genes associated with neurodevelopment, 13 genes were sufficiently expressed in the placenta and 7 genes were not sufficiently expressed. Details regarding candidate genes can be found in Supplementary Table 1.

## 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 one hour of delivery to prevent RNA degradation. The collected tissues were first snap-frozen in liquid nitrogen and then stored at −80°C. RNA was extracted with the Maxwell 16 automated DNA/RNA extraction equipment (Promega – Madison, WI, USA) using the proprietary extraction kits following the manufacturer's protocol. RNA was quantified with Nanodrop spectrophotometer (Thermo Electron North America – Madison, WI, USA).

Placental RNA expression was profiled using the nCounter platform (nanoString Technologies, Seattle, WA) as previously described [33]. The nanoString Norm package [34] was used to normalize data. Differences in sample content were accounted for by normalizing 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.

## Covariates

**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 behavior during pregnancy (smoking, non-smoking). Infant characteristics included infant sex (male, female), gestational age, and mode of delivery (C-section, vaginal).

**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 [35–37]. 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 by the Edinburgh Postnatal Depression Scale (EPDS) [38]. Mothers were asked to report how they felt during the past seven 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 [39–42]. *Pregnancy related anxiety* was measured by the Pregnancy Related Anxieties Questionnaire-revised (PRAQ-R) [43], which measures pregnancy related fears and worries. *Perceived stress during pregnancy* was measured by the Perceived Stress Scale (PSS-14) [44], which assessed the degree to which the rater appraises situations as stressful. The PSS-14 has good reliability and validity [44]. *State and trait anxiety during pregnancy* was measured by the State-Trait Anxiety Inventory (STAI) [45], which assessed temporary “state anxiety” and long-standing, characterological “trait anxiety.” Each of the two subscales consists of 20 items rated on a 4-point Likert scale. A meta-analysis of 45 articles reporting Cronbach’s alpha for internal consistency for this inventory determined the mean to be 0.92 [46]. *Negative stressful events during pregnancy* was measured by the Psychiatric Epidemiology Research Interview Life Events Scale (LES) [47], assessed the occurrence of stressful events in five 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 [48].

The measures of normative psychosocial stress above were categorized to create a composite latent measure created by latent profile analysis (LPA). Model fits were assessed by Bayesian Information Criteria (BIC) [49], adjusted BIC (ABIC) [50], Lo-Mendell-Rubin (L-M-R) test [51] $p$ values, and the entropy values for the two to four class models. LPA was

performed using the full maximum likelihood estimation using Mplus version 6 [52]. Methodological details on the extraction of the latent confounding variable are provided in the Supplementary Methods. Overall, all stress variables were significantly correlated (Supplementary Table 2). LPA indicated that the three-class solution provided the best solution (Supplementary Table 3). The composite latent measure was categorized into three values from (0) low normative stress, (1) medium normative stress, to (2) high normative stress. 104 individuals were labeled as “low normative stress”, 127 individuals as “medium normative stress”, and 44 as “high normative stress” (Supplementary Table 4).

### Main Statistical Analysis

Analyses of variance (ANOVAs) 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. Generalized linear model (GLM) 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 $p < 0.05$) was further examined using the sequential Bonferroni (Holm) multiple comparison tests. All main statistics were conducted using SPSS version 19; while LPA was done using Mplus version 6.

## Results

### Characteristics of 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 = 39.31 weeks), with roughly 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 ($p = 0.003$) and marital status ($p < 0.001$), 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).

### Timing of Superstorm Sandy Exposure and Gene Expression in Placental HPA-axis Genes

Table 2 and Figure 1 show results for the overall group differences and follow-up pairwise comparisons with Holm correction for multiple testing. There are significant overall group differences in *CRHBP*, *DYRK1A*, *HSD11B1*, and *HSD11B2.* When adjusted for multiple comparisons, *CRHBP* gene expression level was upregulated in those exposed in early gestation as compared to the unexposed controls ($p = 0.030$). *DYRK1A* gene expression level was downregulated in those exposed in mid-late gestation as compared to the unexposed controls ($p = 0.005$). *HSD11B1* gene expression level was downregulated in those exposed in early gestation when compared with the unexposed controls ($p = 0.038$)

and those exposed in mid-late gestation ($p = 0.038$). *HSD11B2* gene expression level was downregulated in those exposed in early gestation ($p = 0.043$) and mid-late gestation ($p < 0.001$) as compared to the unexposed controls.

### Timing of Superstorm Sandy Exposure and Gene Expression in Placental Neurodevelopment Genes

The bottom half of 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 downregulated in those exposed in early ($p = 0.039$) and mid-late gestation ($p = 0.011$) compared to the unexposed controls. *MAOB* gene expression level was downregulated in those exposed in early gestation compared to the unexposed controls ($p < 0.001$). *SRD5A3* gene expression level was upregulated in those exposed in mid-late gestation when compared with the unexposed controls ($p = 0.019$) and those exposed in early gestation ($p = 0.019$). *ZNF507* gene expression level was downregulated in those exposed in early ($p = 0.005$) and mid-late gestations ($p = 0.001$) when compared to the unexposed controls.

## Discussion

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

Our results showed that PNMS, as a result of exposure to a natural disaster, at different stages of pregnancy was associated with downregulation of *HSD11B2*, *MAOA* and *ZNF507* genes. The trend of downregulation of *DYRK1A* across pregnancy was also observed, while the effect was significant for mid-late gestation, it was marginally significant for early gestation ($p = 0.084$). Overall, many of these downregulated genes across trimesters are vital for placental function and fetal development.

The placental 11$\beta$-hydroxysteroid dehydrogenase type 2 (11$\beta$-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 fetus [53]. Cortisol is essential to fetal growth but, may be harmful to the fetus when in high concentrations [54]. Under normal circumstances, 11$\beta$-HSD2 largely converts cortisol into cortisone, thereby protecting the fetus from excessive glucocorticoid exposure [53]. This is supported by the finding that fetal 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]. As a consequence, elevated levels of glucocorticoids may enter the fetal circulation and influence fetal HPA-axis development. Our results suggest that when exposed to acute PNMS, the protective effect of placental *HSD11B2* can be overwhelmed. Indeed, lower placental *HSD11B2* has been 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 as compared to prenatal anxiety, such that a depressed mother may not perceive danger, and therefore it may not play a role in affecting fetal 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, but in fact 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]. Our study is the first to have associated acute PNMS due to 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 fetal and child outcomes [64].

In addition to maternal glucocorticoids, serotonin is an important stress related neurotransmitter [65] which is synthesized in the placental and fetal compartments and is vital for fetal brain development. *MAOA* metabolizes serotonin, dopamine and norepinephrine. Maternal blood serotonin can cross the placenta and enter the fetal circulation; overexposure to serotonin disrupts fetal brain development [66]. Recent research suggests that PNMS is associated with elevated levels of serotonin [67] and a reduction in *MAOA* gene expression [10,67]. Mutations in *MAOA* have been linked to disordered neurodevelopment and behaviors, including autism-like disorders and antisocial behaviors [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 behavior in typically developing children. *DYRK1A* is involved in cell proliferation and has been implicated in Down syndrome [70]. *ZNF507* modulates transcriptional regulation; reduced expression of *ZNF507* has been related to schizophrenia [71]. Stress induced downregulation of these genes may also have an impact on placental function, intrauterine homeostasis and fetal growth.

Furthermore, these downregulated 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, yet this warrants further investigation. Our group comparison results reflect no significant statistical differences between early and mid-late gestation (*HSD11B2*, $p = 0.053$; *MAOA*, $p = 0.270$, *ZNF507*, $p = 0.168$ and *DYRK1A*, $p = 0.124$), 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 believed 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 upregulation of *CRHBP* and downregulation of *HSD11B1* and *MAOB* were observed among those exposed to Superstorm Sandy in early pregnancy. Due to a relatively small group of mid-late gestation, we did not observe significant differences between early and mid-late gestation exposure for *CRHBP* ($p = 0.697$) and *MAOB* ($p = 0.055$) expression.

*CRHBP* encodes the corticotrophin-releasing hormone (CRH)-binding protein, which inactivates CRH that stimulates the production of adrenocorticotropic hormone (ACTH) and cortisol throughout pregnancy in the maternal and the fetal compartments [72,73]. Increased circulating maternal CRH concentrations have been associated with lower concentrations of CRHBP [73]. In a normal human pregnancy, maternal CRH, 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]. While an elevation in circulating maternal CRH concentrations increases risks for fetal 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 [78]. Our results suggest that for individuals exposed to Superstorm Sandy in early pregnancy, a rise in CRH may lead to upregulation of *CRHBP,* which can produce prolong 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 posttraumatic stress and depression symptoms [79,80].

Comparably, 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 reduced cortisol regeneration and increased risks for newborns with intrauterine growth restriction, i.e., small-for-gestational-age [81]. 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 aging 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]. Downregulation of placental *MAOB* may increase risks for neuropsychiatric and behavior disorders in offspring exposed to PNMS in early gestation.

Finally, upregulation of *SRD5A3* was observed among those exposed to Superstorm Sandy in mid-late pregnancy. *SRD5A3* plays an important role in protein glycosylation [88], is widely expressed in the human brain tissues and body organs (e.g., 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 Triclosan (an antimicrobial agent often used in personal care products) exposure [91]. The three 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 fetal 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 fetus, 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 this study. Although we observed associations between PNMS and differences in gene expression, implications of these findings on neurodevelopmental outcomes in childhood and adulthood remain unknown. As we see significant observations between CRH binding proteins and maternal stress in the current study, follow up studies will include further characterizing 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, while they should have been evaluated for each extraction especially since 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 fetus might be most vulnerable to PNMS; however, it may have been more informative to have kept each trimester as a separate group. Furthermore, while 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]. Readers should also be reminded that the control and exposed groups were different with regards to marital status and education. Prior research has associated socio-economic status (i.e., education level) and altered placental gene expression levels [97]. While 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, while the exposed group was mainly composed of women residing in storm devastated regions, Queens and Long Island due to the study design. As such, while 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 current 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 each mother's experience of Superstorm Sandy was.

Despite these shortcomings, this is the first study, to our knowledge, 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 study how the timing of trauma exposure impacts placental gene expression. Our observations suggest that PNMS from trauma across trimesters downregulates placental expression of *DYRK1A*, *HSD11B2, MAOA,* and *ZNF507.* However, traumatic stress exposure in early gestation is associated with upregulation of *CRHBP* and downregulation of *HSD11B1* and *MAOB*, while exposure in mid-late gestation is associated with upregulation 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 neurobehavioral and neurodevelopmental outcomes in the offspring.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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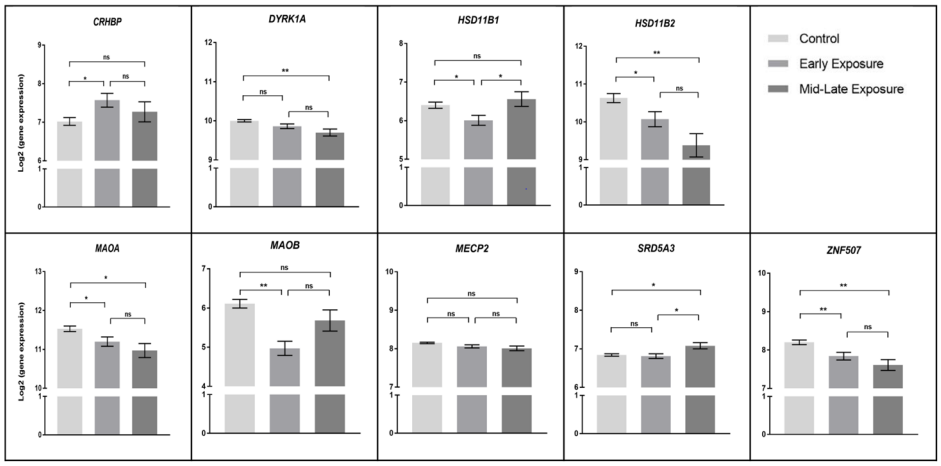


**Figure 1.**

Timing of trauma exposure and gene expression in placenta. Only significant overall differences in gene expression related to trauma exposure are reported. The bar represents the average expression level of each gene using housekeeping genes for normalization and the whisker the standard error. The pairwise comparisons were adjusted for multiple comparisons using sequential Bonferroni procedure. ns, nonsignificant; * $p < 0.05$; ** $p < 0.01$.

**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 | | | | | | .282 |
| Males | N (%) | 150 (54.5) | 98 (54.1) | 40 (60.6) | 12 (42.9) | |
| 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 | | | | | | .196 |
| < 2500 g | N (%) | 15 (5.5) | 13 (7.2) | 1 (1.5) | 1 (3.6) | |
| ≥ 2500 g | N (%) | 258 (93.8) | 166 (91.7) | 65 (98.5) | 27 (96.4) | |
| Missing | N (%) | 2 (.7) | 2 (1.1) | | | |
| Mode of delivery | | | | | | .389 |
| C-section | N (%) | 93 (33.8) | 65 (36) | 19 (28.7) | 9 (32.1) | |
| Vaginal | N (%) | 175 (63.6) | 114 (63) | 45 (68.2) | 16 (57.1) | |
| Missing | N (%) | 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 | | | | | | .219 |
| White | N (%) | 27 (9.8) | 13 (7.2) | 10 (15.2) | 3 (10.7) | |
| Non-White | N (%) | 248 (90.2) | 168 (92.8) | 56 (84.8) | 25 (89.3) | |
| *Black* | N (%) | 68 (24.7) | 53 (29.3) | 12 (18.2) | 3 (10.7) | |
| *Hispanic/Latino* | N (%) | 145 (52.7) | 98 (54.1) | 29 (43.9) | 19 (67.9) | |
| *Asian* | N (%) | 21 (7.76) | 7 (3.9) | 12 (18.2) | 2 (7.1) | |
| *Others* | N (%) | 14 (5.1) | 10 (5.5) | 3 (4.5) | 1 (3.6) | |

| | | Total(N = 275) | Control(n = 181) | Early Exposure (n = 66) | Mid-Late Exposure(n = 28) | *p* value [a] |
|---|---|---|---|---|---|---|
| Maternal education | | | | | | .003 |
| Less than high school | N (%) | 53 (19.3) | 46 (25.4) | 5 (7.6) | 2 (7.1) | |
| High school graduate | N (%) | 62 (22.5) | 44 (24.3) | 13 (19.7) | 5 (17.9) | |
| Some college | N (%) | 108 (39.2) | 69 (38.1) | 27 (40.9) | 12 (42.9) | |
| College graduate | N (%) | 30 (10.9) | 13 (7.2) | 11 (16.7) | 6 (21.4) | |
| Graduate degree | N (%) | 21 (7.6) | 9 (5) | 9 (13.6) | 3 (10.7) | |
| Missing | N (%) | 1 (.4) | | 1 (1.5) | | |
| Mother's marital status | | | | | | < .001 |
| Married | N (%) | 90 (32.7) | 39 (21.5) | 35 (53) | 16 (60.7) | |
| Common law | N (%) | 20 (7.3) | 13 (7.2) | 6 (9.1) | 1 (3.6) | |
| Single | N (%) | 159 (57.8) | 126 (69.6) | 22 (33.3) | 11 (39.3) | |
| Divorced/separated/widowed | N (%) | 5 (1.8) | 3 (1.7) | 2 (3.0) | | |
| Missing | N (%) | 1 (.4) | | 1 (1.5) | | |
| Smoking during pregnancy | | | | | | .246 |
| No | N (%) | 238 (86.5) | 154 (85.1) | 57 (86.4) | 27 (96.4) | |
| Yes | N (%) | 35 (12.7) | 27 (14.9) | 7 (10.6) | 1 (3.6) | |
| Missing | N (%) | 2 (.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 |
| State anxiety | Mean (SD) | 38.00 (11.50) | 37.87 (11.35) | 38.58 (12.62) | 37.57 (10.11) | .896 |
| Trait anxiety | Mean (SD) | 38.43 (10.75) | 38.46 (10.83) | 38.35 (10.31) | 38.50 (11.61) | .997 |

| | | Total(N = 275) | Control(n = 181) | Early Exposure (n = 66) | Mid-Late Exposure(n = 28) | *p* value [a] |
|---|---|---|---|---|---|---|
| Negative stressful events | Mean (SD) | 1.57 (1.97) | 1.55 (1.93) | 1.62 (2.04) | 1.57 (2.10) | .974 |
| Normative psychosocial stress group | | | | | .744 | |
| Low | N (%) | 104 (37.8) | 69 (38.1) | 24 (36.4) | 11 (39.3) | |
| Moderate | N (%) | 127 (46.18) | 82 (45.3) | 34 (51.5) | 11 (39.3) | |
| High | N (%) | 44 (16) | 30 (16.6) | 8 (12.1) | 6 (21.4) | |

NB.

[a] *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.

**Table 2**

Gene expression differences among stress groups (Control, Early Exposure, and Mid-Late Exposure). There are significant overall group differences in HPA-axis genes (*CRHBP*, *DYRK1A*, *HSD11B1*, and *HSD11B2*) and genes driving neurodevelopment (*MAOA*, *MAOB*, *MECP2*, *SRD5A3*, and *ZNF507*). Mean value denotes the average expression level of each gene, using housekeeping genes for normalization. SE represents standard error.

| Group | Control (n = 181) | Early Exposure (n = 66) | Mid-Late Exposure (n = 28) | |
|---|---|---|---|---|
| | Mean (*SE*) | Mean (*SE*) | Mean (*SE*) | Overall *p* value |
| HPA-Axis | | | | |
| *AVPR1B* | 3.80 (.06) | 3.85 (.09) | 4.00 (.14) | .453 |
| *CFL1* | 12.13 (.02) | 12.06 (.03) | 12.07 (.05) | .109 |
| *CREB1* | 9.23 (.03) | 9.32 (.05) | 9.25 (.07) | .282 |
| *CREBBP* | 4.33 (.05) | 4.42 (.08) | 4.38 (.12) | .599 |
| *CRHBP* | 7.02 (.10) | 7.57 (.18) | 7.27 (.26) | .035 |
| *DYRK1A* | 10.00 (.03) | 9.86 (.06) | 9.70 (.09) | .003 |
| *HSD11B1* | 6.40 (.08) | 6.01 (.13) | 6.56 (.19) | .016 |
| *HSD11B2* | 10.63 (.12) | 10.07 (.20) | 9.38 (.31) | <.001 |
| *NCOR1* | 10.39 (.03) | 10.33 (.05) | 10.25 (.07) | .121 |
| *NCOR2* | 9.35 (.04) | 9.38 (.06) | 9.45 (.10) | .664 |
| *NR3C1* | 10.85 (.03) | 10.94 (.05) | 10.76 (.08) | .107 |
| *NR3C2* | 7.22 (.05) | 7.33 (.08) | 7.00 (.12) | .078 |
| *NR4A1* | 7.53 (.07) | 7.19 (.13) | 7.60 (.19) | .053 |
| *POMC* | 3.62 (.06) | 3.59 (.10) | 3.68 (.14) | .869 |
| Neurodevelopment | | | | |
| *ADRA2A* | 5.95 (.06) | 5.82 (.10) | 5.86 (.16) | .538 |

| Group | Control (n = 181) | Early Exposure (n = 66) | Mid-Late Exposure (n = 28) | |
|---|---|---|---|---|
| | Mean (*SE*) | Mean (*SE*) | Mean (*SE*) | Overall *p* value |
| *CDKL5* | 7.43 (.04) | 7.43 (.06) | 7.28 (.09) | .285 |
| *DBH* | 3.80 (.07) | 3.96 (.11) | 4.11 (.17) | .176 |
| *FOXP1* | 6.49 (.04) | 6.42 (.07) | 6.51 (.10) | .644 |
| *HTR1B* | 5.35 (.06) | 5.30 (.10) | 5.29 (.16) | .886 |
| *MAOA* | 11.53 (.07) | 11.20 (.12) | 10.97 (.18) | .004 |
| *MAOB* | 6.11 (.11) | 4.97 (.18) | 5.68 (.27) | <.001 |
| *MECP2* | 8.15 (.02) | 8.06 (.04) | 8.01 (.06) | .044 |
| *PON3* | 4.68 (.06) | 4.89 (.09) | 4.89 (.14) | .107 |
| *SNAP25* | 3.61 (.06) | 3.66 (.10) | 3.74 (.14) | .701 |
| *SRD5A3* | 6.84 (.03) | 6.81 (.06) | 7.08 (.08) | .017 |
| *ZNF507* | 8.20 (.06) | 7.84 (.10) | 7.61 (.14) | <.001 |
| *ZNHIT6* | 7.91 (.03) | 7.89 (.05) | 7.85 (.08) | .792 |

NB: *p* values are calculated based on generalized linear models controlling for maternal age, race, education, marital status, smoking behavior during pregnancy, infant sex, gestational age, mode of delivery, and normative psychosocial stress.