



Published in final edited form as:

*Brain Behav Immun.* 2019 February ; 76: 97–103. doi:10.1016/j.bbi.2018.11.009.

## Elevated pro-inflammatory gene expression in the third trimester of pregnancy in mothers who experienced stressful life events

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### Abstract

**Background.**—Stress exposure is associated with risk for adverse pregnancy outcomes, potentially in part through dysregulated immune and inflammatory activity. Evidence suggests that stress during pregnancy is associated with inflammation during pregnancy, consistent with risk for preterm birth. However, research has not tested whether complementary changes are reflected in immune cell gene expression, or upstream regulation of inflammation. The purpose of this study was to test associations between preconception and prenatal stress exposure and third trimester immune cell gene expression, focusing specifically on sets of genes previously linked to stress in non-pregnant samples: Pro-inflammatory genes, and antiviral and antibody genes.

**Methods.**—A sample of 116 low-income, diverse women was recruited from 5 U.S. sites by the Community Child and Health Network at the birth of a child. This study is of the subgroup of women who became pregnant again over the two-year follow-up period, and provided information on stressful life events that occurred both preconception and during the third trimester of the subsequent pregnancy. Dried blood spots (DBS) were collected in the third trimester of pregnancy, and used for gene expression analysis.

**Results.**—Women with more prenatal stressful life events had higher expression of pro-inflammatory genes when compared to those with fewer life events, and the effect was driven by increased activation of pro-inflammatory transcription factors, NF- $\kappa$ B and AP-1. Preconception stressful life event exposure was not associated with gene expression profiles. When entered into models simultaneously, only prenatal stressful life events were associated with up-regulation of pro-inflammatory genes. No differences between high or low stress groups emerged for antiviral or antibody genes.

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**Conclusions.**—Prenatal stress exposure was associated with up-regulated pro-inflammatory gene expression during pregnancy, and increased activity of NF- $\kappa$ B and AP-1. In contrast, stress occurring preconception was not associated with gene expression. These results are consistent with the hypothesis that stress-induced activation of pro-inflammatory transcriptional pathways in pregnancy, but not earlier, may increase risk for inflammation-driven adverse pregnancy outcomes.

### Keywords

Preconception; pregnancy; gene expression; stressful life events; inflammation

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## 1. Introduction

Altered or dysregulated immune and inflammatory activity during pregnancy increases risk for adverse pregnancy outcomes, like preterm birth (Christian, 2012b; Romero et al., 2006). The immune system plays a key regulatory role across pregnancy (Christian, 2012b; Cunningham et al., 2014), and is characterized by unique, pregnancy-specific adaptations that protect the developing fetus from the maternal immune system and support the tissue remodeling necessary to pregnancy (Mor and Cardenas, 2010). Factors that derail these pregnancy immunological adaptations towards the end of the third trimester of pregnancy, such as infections or low-grade inflammation caused by obesity or smoking, can prematurely activate inflammatory labor and delivery cascades, resulting in premature birth and lower birth weight (Bowen et al., 2002; Christian, 2012b; Denison et al., 2010; Dizon-Townson, 2001; Greig et al., 1997; Holt et al., 2011; Murtha et al., 2007; Park et al., 2005; Romero et al., 2006). Indeed, low-grade inflammation, as indexed by elevated levels of peripheral pro-inflammatory markers, e.g. interleukin (IL) 6 and IL1 $\beta$ , has been observed in women who went on to have preterm labor compared to those who did not (Ferguson et al., 2014; Gargano et al., 2008; Greig et al., 1997). As such, understanding the factors that alter late pregnancy immune or inflammatory activity has implications for understanding preterm birth risk.

Stress exposure during pregnancy has been associated with increased risk for adverse pregnancy outcomes, including preterm birth (Coussons-Read, 2012; Dunkel Schetter, 2011). Stress-induced changes in pregnancy immune and inflammatory activity are one proposed pathway linking prenatal stress and preterm birth (Christian, 2015; Coussons-Read et al., 2012). Higher prenatal stress is associated with increased circulating levels of pro-inflammatory markers IL6 and tumor necrosis factor (TNF) $\alpha$ , and lower anti-inflammatory marker IL10 during pregnancy (Coussons-Read et al., 2005), elevated production of pro-inflammatory cytokines IL6 and IL1 $\beta$  following lipopolysaccharide (LPS) activation of peripheral blood mononuclear cells obtained during the third trimester (Coussons-Read et al., 2007), and greater pregnancy Epstein-Barr virus re-activation, or reduced cellular immune competence (Christian, 2012b). Although these effects are believed to be due to stress-driven changes in immune cell function, published research has not directly tested the intracellular inflammatory profile using gene expression profiling, nor the related transcription control pathways that may mediate such effects in the context of pregnancy.

In non-pregnant adults, stress exposure activates neuroendocrine pathways, i.e. hypothalamic-pituitary-adrenal (HPA) axis and the sympathoadrenal system, that release glucocorticoids and catecholamines, respectively, into the circulation. Glucocorticoids and catecholamines, in turn, act on immune cell glucocorticoid and adrenergic receptors to promote expression of pro-inflammatory genes, and thus change immune cell function (Cacioppo et al., 2000; Charmandari et al., 2005; Irwin and Cole, 2011; Sapolsky et al., 2000). Specifically, in healthy adults, stress exposure can induce a gene expression program known as the conserved transcriptional response to adversity (CTRA), which involves up-regulated expression of pro-inflammatory genes, e.g. *IL1 $\beta$* , *IL8*, *PTGS2*, and down-regulated expression of antiviral, e.g., Type I interferon innate response gene families, and antibody-related genes, e.g. *IGL*, *IGH*, *IGG* (Cole, 2013, 2014). Given that the CTRA profile indicates an immune cell regulation program skewed towards pro-inflammatory activity, it is possible that activation of the CTRA gene expression profile in the context of pregnancy could be a mechanism through which stress exposure links to immune-related adverse birth outcomes. However, neuroendocrine axis and immune system activity and physiological responses to stress exposure during pregnancy are in many ways different from those observed in non-pregnant individuals (Christian, 2012a; Mor and Cardenas, 2010). For example, evidence suggests that pro-inflammatory activity is down-regulated mid-pregnancy, but increasingly up-regulated from mid- to late-pregnancy. In contrast, innate viral immunity, e.g. interferon family of cytokines, is up-regulated mid-pregnancy, but down-regulated from mid- to late-pregnancy (e.g., Ross et al., 2016). Given that both of these processes are important components of the CTRA, it is important to understand how CTRA dynamics unfold in the context of pregnancy, and whether stress exposure during pregnancy elicits a CTRA gene expression profile in a manner similar to non-pregnant adults.

In addition, the role of stress exposure *before* pregnancy is understudied, with most studies focusing on the effects of stress exposure *during* pregnancy (Coussons-Read, 2012; Dunkel Schetter, 2011). The role of preconception stress exposure in pregnancy immune and inflammatory activity is, in large part, understudied due to the difficulty and expense in collecting prospective samples prior to conception. A few epidemiology studies have explored the role of bereavement, experienced either preconception or prenatally, on infant mortality risk in Swedish and Danish populations (Class et al., 2013; Class et al., 2015). In both studies, death of a first degree relative experienced 6 months prior to conception, but not during the pregnancy, was associated with increased infant mortality, possibly through altered risk for adverse pregnancy outcomes, such as preterm birth. In contrast, another study compared the effect of preconception chronic stressors, such as socioeconomic status, and preconception experience of stressful life events, and found that chronic stressors before pregnancy, but not stressful life events during pregnancy, predicted birth weight (Strutz et al., 2014). These studies collectively suggest a potential role of preconception stress in predicting adverse pregnancy outcomes. Given that stressors experienced in early life, e.g. during childhood, are associated with adult immune cell gene expression (Chen et al., 2011; Cole et al., 2012; Miller et al., 2009), it is conceivable that stressors experienced prior to conception could also affect immune cell gene expression during pregnancy. To date, no studies have tested the associations between preconception stress and pregnancy immune

parameters, such as immune cell gene expression, or compared the independent contributions of preconception and prenatal stress exposure to inflammatory or immune activity during pregnancy.

In sum, although stress exposure could increase risk for preterm birth through altered immune function, no studies have explored the transcriptional control pathways or programs, i.e. the CTRA, that could mediate these effects, or assessed whether timing of stress before or during pregnancy affects these processes. As such, the purpose of this study was to explore links between prenatal and preconception stress exposure and immune cell gene expression during the third trimester of pregnancy using data from a prospective-study of stress and postpartum outcomes conducted by the Community Child Health Network (CCHN). A sample of diverse, low-income women was recruited following the birth of a child at five sites across the US. A subset of women in this cohort became pregnant again over the two-year study period, and were followed through their subsequent pregnancy. Consequently, both preconception and prenatal data on stress exposure are available for this sub-sample. Dried blood spots (DBS) were collected during the third trimester of the subsequent pregnancy, and were assayed for immune cell gene expression. We hypothesized that 1) greater stress exposure *during* pregnancy would be associated with up-regulated pro-inflammatory gene expression; 2) greater stress exposure *prior* to conception would also be associated with up-regulated pro-inflammatory gene expression; and 3) preconception and prenatal exposure to stressful life events would be independent predictors of the CTRA. Given alterations in antiviral immunity during pregnancy, exploratory analyses also examined whether greater prenatal stress exposure was associated with the other major component of the CTRA gene expression profile, that is, lower expression of Type I interferon-related antiviral genes and antibody-related genes (Cole, 2013, 2014).

## 2. Methods

### 2.1. Participants

The data were collected by the Community Child Health Network (CCHN; 2008-2010). The full cohort consists of 2,510 low-income women recruited from five sites across the US (Washington, DC; Baltimore, MD; Los Angeles, CA; Lake County, IL; eastern North Carolina). Women were recruited following the birth of a child, as per eligibility criteria and recruitment procedures that are reported elsewhere (Dunkel Schetter et al., 2013; Ramey et al., 2015). By study design, the sample is Black, Latina and non-Hispanic White only, and they were residing in diverse and mostly low-income areas. At some point over the follow-up period, 416 participants (20%) indicated that they had become pregnant again. Of these, 343 (82%) consented to participate in the subsequent pregnancy follow-up study. The sample in the present research consists of 116 women who had banked dried blood spots (DBS) and stressful life event data. Participants provided informed, signed consent to participate, and all protocols followed Declaration of Helsinki procedures. Study procedures and protocols were reviewed and approved by the Institutional Review Boards of all community and academic institutions associated with CCHN (Ramey et al., 2015).

## 2.2. Procedure

Trained interviewers conducted structured interviews in English or Spanish in participants' homes at 1 month, 6 months, and 12 months after the birth of the index child (which was the preconception period for the subsequent pregnancy), and during the second and third trimester of the subsequent pregnancy. Dried blood spots (DBS) were collected during the third trimester of the subsequent pregnancy, and assayed for genome-wide transcriptional profiles using RNA sequencing (RNAseq). Stressful life events in the past year were assessed at 1 month and 12 months after the birth of the index child (preconception), and during the third trimester of the subsequent pregnancy (prenatal). Because women became pregnant at varying times over the preconception follow-up period, and to ensure that all women had preconception stressful life events reported over a consistent 12 month period, preconception stressful life events were taken from *either* the 1-month or 12-months postpartum assessment, depending on which *immediately preceded the pregnancy*.

## 2.3. Stressful Life Events

Participants reported stressful life events using an adapted version of the 25-item Life Events Inventory (Dominguez et al., 2005). Items included major changes in work or home responsibilities (including job loss), serious illness or injuries, accidents or violent crimes, death of close friends or family, and natural disasters. Although most events were episodic, some represent chronic stressors (e.g. interpersonal problems), and events themselves are known to have chronic aftermaths. At each assessment, participants reported only events that had occurred over the past year with yes (1) or no (0). Number of stressful life events reported preconception and during pregnancy were significantly correlated,  $r = .443$ , suggesting that women who experienced more stressful life events preconception were likely to experience more stressful life events during pregnancy, but that variability in stressful life experiences between the two time points was also evident (80% unshared variance). To facilitate interpretation of gene expression results in terms of fold-differences, women were classified into one of two groups for both the preconception and prenatal assessments, based on a cut-off that produced an equivalent distribution of participants among groups for both the preconception and prenatal periods: Those who had < 3 stressful life events over the reporting period (low stress; 0), or those who had  $\geq 3$  stressful life events over the reporting period (high stress; 1). To ensure that this dichotomization did not distort results, ancillary analyses also treated stressful events as a continuous variable.

## 2.4. Inflammatory gene expression

A non-fasting blood sample was collected onto a DBS card and assayed for genome-wide transcriptional profiles using RNAseq. Briefly, a finger was lanced and capillary blood collected on filter paper. DBS cards were allowed to dry for 30 minutes, and then stored at  $-30^{\circ}\text{C}$  until a subset of each sample was assayed for metabolic markers (data not reported here). Remaining DBS were stored at  $-80^{\circ}\text{C}$  and shipped to the UCLA Social Genomics Core Laboratory for RNA extraction as previously described (McDade et al., 2016). RNA samples were converted to cDNA libraries using the QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen Inc. Greenland, NH) and sequenced using an Illumina HiSeq4000 instrument (Illumina Inc., San Diego CA) in the UCLA Neuroscience Genomics

Core Laboratory, following the manufacturers' standard protocol. The QuantSeq assay is highly sensitive and produces reliable results even for moderately degraded samples (valid down to RNA Integrity Number value of 3), and is thus particularly well suited for assay of DBS-derived RNA samples. DBS samples generally yield insufficient RNA for quantification of RNA integrity prior to assay (e.g., by Agilent capillary electrophoresis), so this study used standard endpoint quality control metrics that assess sample validity by testing for an expected high cDNA yield (i.e., sufficient for > 10 million sequencing reads), expected high rates of read alignment to the human genome sequence (which does not happen if samples are highly degraded), and a high correlation of gene expression values with the average expression level derived from all other samples (i.e., a correlogram internal consistency metric) (McDade et al., 2016). All samples passed multiple endpoint quality assurance thresholds for DBS RNAseq samples, including >10 million single-strand 65-nucleotide reads per sample, >90% of reads aligning to the reference human genome, and a correlogram average profile  $r = .80$ . Gene expression values were normalized to transcript counts per million total transcripts and  $\log_2$ -transformed for analysis by linear statistical models as outlined below.

## 2.5. Covariates

We considered several covariates for analyses: Maternal age (years) recorded during the third trimester of pregnancy; maternal self-identified race/ethnicity (Black vs. Latina and White, Latina vs. Black and White); alcohol consumption, smoking; pre-pregnancy body mass index (BMI) taken at last preconception assessment; gestational age at the third trimester assessment based on all available information (e.g. ultrasounds, reported conception date, and medical charts); and maternal infection reported or treated over the pregnancy (present/absent). Height and weight measurements were taken at 1 month, 6 months and 12 months postpartum, and were used to calculate BMI ( $\text{kg/m}^2$ ) = weight / height<sup>2</sup>. Infections during pregnancy were obtained from medical risks and conditions questions administered during both the second and third trimester interviews. Questions included whether a health care provider had told the participant they had, for example, a bladder infection, or if they were "given any medications to treat infections, such as vaginal, bladder or kidney infections, during this pregnancy." If women responded 'yes' to any of these questions, at either the second or third trimester assessment, they were coded as having had an infection over the pregnancy. Few women reported any alcohol consumption ( $N = 1$ ) or any cigarette use ( $N = 5$ , 4%) at any point over the pregnancy. As such, these variables were not included as covariates.

There is evidence that peripheral inflammation across pregnancy varies as a function of gestational age at assessment (e.g., Ross et al., 2016). However, data on gestational age at the prenatal assessment were available for only 89 participants (77%). Since having missing data on gestational age was not associated with number of stressful life events experienced over pregnancy,  $r = -.159$ ,  $p = .090$ , or preconception,  $r = .006$ ,  $p = .951$ , this variable was not included in analyses. Thus, final covariates included in models were age, race/ethnicity, pre-pregnancy BMI, and infection reported over pregnancy.

## 2.6. Statistical analyses

Gene expression data were analyzed using standard methods for targeted hypothesis testing in genome-wide transcriptional profiles (Cole et al., 2003; Cole et al., 2005; Fredrickson et al., 2013; Miller et al., 2008). Gene expression data were normalized “gene transcripts per million total transcripts” (TPM), that were floored at 1 TPM,  $\log_2$ -transformed, and screened to exclude transcripts that varied by  $< 0.5 \log_2$  units across participants (to remove genes that were generally undetectable or showed no appreciable variation in expression levels across samples). Remaining data were analyzed using linear mixed models to predict differences in gene expression profiles from stressful life event group, controlling for age, race/ethnicity, pre-pregnancy BMI, and recent infection. Where indicated, ancillary analyses additionally controlled for variations in leukocyte subset prevalence by including as covariates the abundance of mRNAs encoding CD3, CD4, CD8, CD19, CD14, CD16, and CD56.

Primary analyses examined relationships between stress exposures and average expression of an a priori-specified set of 19 pro-inflammatory gene transcripts utilized in previous CTRA research (e.g., *IL1B*, *IL6*, *IL8/CXCL8*, *COX2/PTGS2*, *TNF*, etc.; Fredrickson et al., 2013). Exploratory analyses also examined the average expression of 34 antiviral- and antibody-related gene transcripts that comprise the inverse component of the CTRA profile (e.g., *IFNB*, *IRF7*, *IFI27*, *MX1*, *OAS1*, etc.; Fredrickson et al., 2013), although we had no a priori hypothesis about this gene set in the context of pregnancy. Secondary analyses used empirical stress-associated differences in genome-wide transcriptional profiles to test for increased activity of pro-inflammatory transcription factors (NF- $\kappa$ B and AP-1). These analyses took as input all genes that showed  $\geq 1.5$ -fold difference in average expression across groups (high stress or low stress), or across the range from 2 SD below the average of a given stress measure to 2 SD above the average (i.e., over the 4-SD range of normal variation). Gene lists were analyzed using a 2-sample variant of the Transcription Element Listening System (TELiS; Cole et al., 2005) quantifying the prevalence of NF- $\kappa$ B and AP-1 transcription factor-binding motifs (TFBMs) within the promoters of differentially expressed genes using TFBMs derived from the TRANSFAC database (V\$CREL\_01 and V\$AP1\_Q6, respectively). Results were averaged across 9 alternative technical specifications involving variations of promoter length (–300 bp, –600 bp, and –1000 bp to +200 bp) and TFBM detection stringency (MatSim .80, .90, .95). In all analyses, statistical testing was based on standard errors derived from bootstrap resampling of linear model residual vectors (to account for correlation among genes).

## 3. Results

### 3.1. Sample characteristics

Sample characteristics are presented in Table 1. The sample was approximately half Latina, one quarter White, and one quarter Black, and participants were on average 27.6  $\pm$  5.24 years old during the third trimester of pregnancy. On average, the preconception assessment occurred 6.53  $\pm$  4.54 months before conception.

### 3.2. Stressful Life Events

Number of stressful life events reported at the preconception and pregnancy assessments were significantly different,  $t(109) = 3.70$ ,  $p < .001$ , with participants reporting fewer stressful life events during their pregnancy ( $M = 2.66$ ,  $SD = 2.65$ ) compared to the year preceding the preconception assessment ( $M = 4.02$ ,  $SD = 3.29$ ). Using 3 or more stressful life events as a cut-point, 43% of participants were exposed to high levels of stressful events during pregnancy ( $n = 50$ ) and 60% were exposed to high levels of stressful events in the period prior to pregnancy ( $n = 67$ ). These are unusually high levels of stress compared to other samples but not unexpected given the nature of this sample (Dominguez et al., 2005). Over pregnancy, the most commonly reported stressful life events reported were partner relationship problems (28%), arguments with a specific person (26%), change in living arrangements (23%), serious illness or injury (20%), and death of a close friend or family member (17%). A similar pattern emerged for preconception stressful life events, with the addition of problems at work or school (25%) and loss of job, either for someone close or for self (44%).

### 3.3. Pregnancy stressful life events and gene expression.

In analyses controlling for age, race/ethnicity, pre-pregnancy BMI, and infection during pregnancy, prenatal blood samples from women who were exposed to high levels of stressful life events during pregnancy showed a significantly higher expression of pro-inflammatory genes than did samples from women exposed to low levels of stress,  $b = .168 \log_2 \text{mRNA} \pm \text{SE} .065$ , corresponding to a 13% increase on average,  $p = .019$  (see Figure 1). Similar results emerged in analyses controlling for the prevalence of major leukocyte subsets ( $b = .204 \pm .058$ , corresponding to 15% difference,  $p = .003$ ). No differences in expression of antiviral- or antibody-related genes as a function of stressful life events during pregnancy were found,  $b = .098 \pm .076$ ,  $p = .206$ .

Secondary analyses of 387 gene transcripts showing > 1.5-fold difference in expression in blood samples from women with high- vs. low-stressful event exposures implicated increased activity of the pro-inflammatory transcription factors, NF- $\kappa$ B and AP-1, in shaping the observed differences in gene expression (ratio of TFBMs in promoters of up- vs. down-regulated genes = 2.28-fold  $\pm$  .15-fold for NF- $\kappa$ B,  $p < .001$ ; and 1.30-fold  $\pm$  .13 for AP-1,  $p = .040$ ; see Figure 2). To determine whether these results might be due solely to dichotomization of stressful event exposures, we repeated these analyses utilizing stressful event exposure as a continuous metric and found similar results (ratio of TFBMs in promoters of genes up-regulated > 2-fold over a 4-SD range of stressful event exposures vs. down-regulated = 1.28-fold  $\pm$  .11-fold for NF- $\kappa$ B,  $p = .018$ ; and 1.51-fold  $\pm$  .12 for AP-1,  $p < .001$ ).

### 3.4. Preconception stressful life events and gene expression.

In contrast to stressful life event exposures during pregnancy, high levels of stressful life events during the preconception period showed no significant association with pro-inflammatory gene expression in later pregnancy,  $b = .006 \pm .072$ , corresponding to a 0.3% increase on average,  $p = .934$ ; see Figure 1. Associations between preconception stressful life events and pregnancy gene expression remained non-significant even after controlling

for time between preconception and pregnancy assessments, or a time x stress interaction term. Similar non-significant results also emerged in analyses controlling for the prevalence of major leukocyte subsets,  $b = -.046 \pm .066$ ,  $p = .491$ . A non-significant trend toward elevated expression of antiviral- or antibody-related genes as a function of preconception stress exposure was found,  $b = .124 \pm .067$ ,  $p = .073$ . Secondary analyses also found no indication of differential NF- $\kappa$ B or AP-1 transcription factor activity,  $p = .436$  and  $.792$ , respectively.

### 3.5. Differential effects of stressful life event timing.

To test whether prenatal life events had an effect independent of any effect of preconception events, we conducted analyses examining both risk factors simultaneously. We continued to find an association between elevated pro-inflammatory gene expression and stressful life events during pregnancy,  $b = .182 \pm .070$ ,  $p = .018$ , but not stressful life events preconception,  $b = -.048 \pm .070$ ,  $p = .506$ . To further highlight the importance of the prenatal period compared to the preconception, the difference between stressful event exposure levels during preconception as compared to during pregnancy was associated with greater expression of pro-inflammatory genes,  $b = .115 \pm .053$ ,  $p = .044$ . However, the average level of stressful life event exposure at both time points was not a significant predictor of pro-inflammatory gene expression,  $b = .134 \pm .078$ ,  $p = .101$ .

## 4. Discussion

This study examined the associations of preconception and prenatal stressful life events with immune cell gene expression during the third trimester of pregnancy. Our results indicate that prenatal, but not preconception, stress exposure is associated with increased pro-inflammatory gene expression during the third trimester of pregnancy. Compared to women with fewer stressful life events during pregnancy, those exposed to high stressful life events during pregnancy showed up-regulated expression of a pre-specified composite of pro-inflammatory genes. In addition, bioinformatics analyses implicated the pro-inflammatory transcription factors, NF- $\kappa$ B and AP-1, in driving stress-related transcriptional differences observed across the genome as a whole. In contrast, neither stressful life events experienced prior to pregnancy, either in separate models or tested independently of prenatal stressful life events, nor average preconception and prenatal stressful life events were associated with any differences in pro-inflammatory gene expression in late pregnancy.

These findings are consistent with a small amount of published research linking prenatal stress to altered immune function and increased expression of inflammatory biomarkers during pregnancy (Christian, 2015; Coussons-Read, 2012; Coussons-Read et al., 2012; Coussons-Read et al., 2007; Coussons-Read et al., 2005). Specifically, prenatal stress activates specific immune cell transcription control pathways, i.e. NF- $\kappa$ B and AP-1, which results in increased pro-inflammatory gene expression, and in turn drives increased prenatal peripheral inflammation and altered immune function, in a manner consistent with increased risk for preterm birth. These shed new light on the pathways through which stressful life circumstances may affect the risk for inflammation-related adverse pregnancy outcomes, such as preterm birth, through activation of pro-inflammatory immune cell gene

transcription control pathways. Future research should expand this work by testing whether stress-associated changes in pregnancy pro-inflammatory gene expression are, in turn, reflected in peripheral inflammatory markers during pregnancy, are tied to activity in other physiological systems (e.g. HPA axis activity or immune cell resistance to glucocorticoids), or are associated with adverse pregnancy outcomes, such as preterm birth or low birthweight.

The present results are consistent with previous research linking adverse life circumstances to activation of the CTRA gene expression program in circulating immune cells (Cole, 2013, 2014). However, the stress-related transcriptional alterations observed here deviate from the classical CTRA profile in one notable respect: They did not involve any complementary reduction in antiviral and antibody-related gene expression, which is typically observed in consort with up-regulation of pro-inflammatory genes in the context of psychosocial adversity (Cole, 2013, 2014). It is possible that limited statistical power available in this study or use of a “nosier” measure of gene expression (dried blood spots versus venipuncture) undermined the detection of any decrement in antiviral and antibody-related gene expression. However, it is also possible that pregnancy represents a unique regulatory environment within the immune system, and that the stress-induced suppression of antiviral gene expression observed under non-pregnant conditions may be disrupted during pregnancy, e.g., as part of the broader set of immunologic adaptations required to initiate and maintain pregnancy (Mor and Cardenas, 2010). To address that hypothesis directly, future research will need to compare the transcriptional correlates of stress exposure in pregnant and non-pregnant women in parallel.

Preconception stressful life events were not associated with third trimester gene expression, either in separate models or in models including the effects of prenatal stressful life events. As such, we did not observe any evidence for a “programming” effect of preconception stressful life event exposure. This result is consistent with patterns observed by Strutz et al. (2014), but not by Class et al. (2015). This is of interest given that, on average, women reported more stressful life events experienced over the preconception period than over the pregnancy itself, and that women may become less emotionally reactive to negative life events, and thus stressful life events, during later pregnancy (Glynn et al., 2004). However, there are several possible reasons for why effects of preconception stressful life events were not detected for gene expression in the prenatal period. Most likely, the stressful life events occurring in the preconception period did not impact gene expression in our sample due to variability in the timing of the preconception year reported, and so for some of our participants preconception life events could have occurred long before conception. Thus, it remains possible that preconception stressors do exert an impact on gene regulation at the time of the stressor, but these effects are attenuated and not detectable here because other, more contemporaneous and proximal factors, i.e. prenatal stressful life events, came to dominate the physiological signaling dynamics that control gene transcription. Nonetheless, this distinction between the relevance of preconception vs. prenatal stress has significant implications for prevention strategies and whether intervention is needed prior to conception, as opposed to prenatally.

Stress can be conceptualized in many ways. In this investigation, we chose to focus on life events due to timing of the assessments of life events. Now that a general relationship between life event exposure and gene regulation has been established in pregnancy, future research should extend these findings by taking a more comprehensive approach to stress. For example, this study treated all stressful life events equally, but certain kinds of stressors, such as those related to the social environment, may be more physiologically impactful than others. Furthermore, life events measures include both episodic and chronic stressors, and even episodic stressors have chronic aftermaths. For this reason, interview approaches to the measurement of stress have become the gold standard in which objective judges can categorize type and duration of stress (Brown and Harris, 1978; Hammen, 1991). Future research should take a more nuanced approach to measuring and conceptualizing stress, to better understand both acute and chronic stress and their independent and joint roles in pregnancy immune and inflammatory activity and function.

There are several limitations to consider. First, our sample size is relatively small compared to other studies in this research area. However, it consists of diverse, low-income women who are traditionally underrepresented in health research, which contributes to the value of our findings. Furthermore, we were able to take advantage of a rare prospective cohort that had both preconception and prenatal information on stressful life experiences. Second, gene expression data was derived from banked DBS, which tends to yield “noisier” transcriptome profiles than those derived from the larger blood volumes available from traditional venipuncture (McDade et al., 2016). However, this “noise” implies that any true differences will be harder to detect, which underscores the strength of the particular pro-inflammatory signal observed here and suggests that additional patterns may potentially be detected in future studies that use venipuncture blood samples. Third, this study is correlational, so the causal relationships among stress and pro-inflammatory gene expression remain to be defined in future intervention research (for a relevant example, see Miller et al., 2017). Fourth, this set of analyses was planned to test a limited number of a priori-specified genomic hypotheses; it is not powered to discover statistically significant associations involving individual genes, or associations involving any gene sets other than the CTRA profile. Future research using larger samples is required to support genome-wide hypothesis-free discovery analyses, and it is likely that other genomic correlates of stress exist besides those identified here. Finally, we explored links between preconception and prenatal stressful life events with pregnancy immune cell gene expression. However, early childhood trauma and stress, e.g. childhood abuse, is also associated with elevated markers of systemic inflammation (i.e., C-reactive protein) during pregnancy (Mitchell et al., 2018). As such, future research should also consider whether childhood stress is associated with pregnancy immune cell gene expression.

#### 4.1. Conclusions.

In this sample of ethnically and racially diverse, low-income women, those who experienced higher stressful life events during pregnancy had third trimester immune cell gene expression patterns indicating up-regulation of pro-inflammatory genes and increased activity of NF- $\kappa$ B and AP-1. These results are consistent with the hypothesis that stress-

induced activation of pro-inflammatory transcriptional pathways may increase risk for inflammation-driven adverse pregnancy outcomes.

## Acknowledgements

This study was conducted by Community Child Health Network (CCHN) through cooperative agreements with the Eunice Kennedy Shriver National Institute of Child Health and Human Development [UHD44207, U HD44219, U HD44226, U HD44245, U HD44253, U HD54791, U HD54019, U HD44226-05S1, U HD44245-06S1, R03 HD59584] and the National Institute for Nursing Research [U NR008929]. This project received support from the Cousins Center for Psychoneuroimmunology, UCLA Semel Institute. Dried blood spot RNAseq assays was supported by the USC-UCLA Biodemography Center [NIH P30 AG017265]. K. Ross and C. Dunkel Schetter were supported through the National Institute for Health [R01 HD073491] and Eunice Kennedy Shriver National Institute of Child Health and Human Development [R01 HD072021-01A1]. J. Carroll was supported by the National Institute on Aging [K01 AG044462 NIA] and NICHD [R01 HD 072021]

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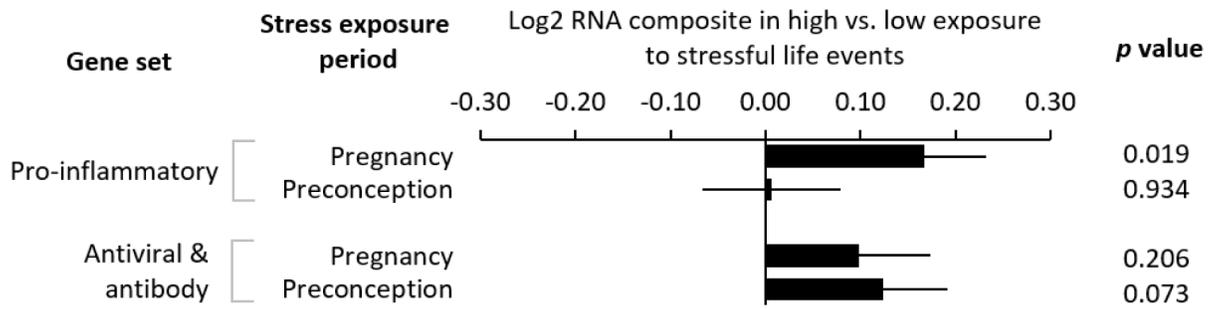
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**Highlights**

- Prenatal life stress predicted third trimester pro-inflammatory gene expression
- Pro-inflammatory gene expression patterns were regulated by NF-kB and AP-1
- Preconception life stress was not associated with third trimester gene expression



**Figure 1.**

During pregnancy, women exposed to high levels of stressful life events had higher pro-inflammatory gene expression compared to women exposed to low levels of stress. For the preconception period, however, no significant differences in third trimester pro-inflammatory gene expression emerged by stressful life event exposure. No differences in antiviral and antibody gene expression were detected by stressful life event exposure, either preconception or during pregnancy.

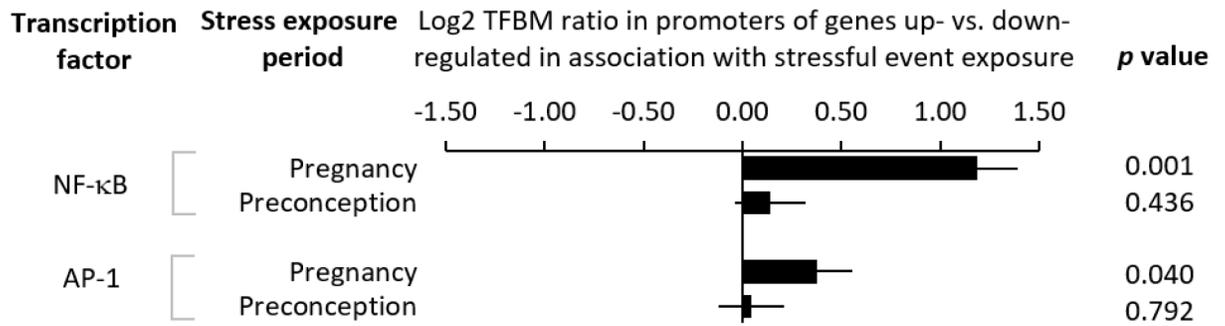
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**Figure 2.**

Women who experienced three or more stressful life events during pregnancy, but not preconception, had up-regulated NK-κB and AP-1 transcriptional pathways, compared to women who experienced 2 or fewer prenatal stressful life events. Transcription factor binding motif (TFBM) ratios > 1 indicate up-regulation of a specified transcriptional pathway in high-stressful life events women.

**Table 1.**

Sample characteristics (N = 116).

Variable	Mn +/- SD or % (N)
Age (years)	27.6 +/- 5.24
Race/ethnicity	
Black	30 (35)
Latina	46 (53)
White	24 (28)
Pre-pregnancy BMI (kg/m <sup>2</sup> )	29.5 +/- 6.25
Infection over pregnancy (yes)	28 (32)
Third trimester gestational age (weeks)	32.9 +/- 3.69
Days between Preconception and Pregnancy Assessments	410 +/- 147
# Stressful Life Events	
Preconception	4.02 +/- 3.29
Third trimester of Pregnancy	2.66 +/- 2.65
3 Stressful Life Events (High Group)	
Preconception	67 (60)
Third trimester of Pregnancy	44 (50)