Cellular aging of the placenta due to maternal preconception adversity is influenced by mRNA expression of the pro-inflammatory chemokine, *CCL2*

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Background

Maternal life-course adversity, including both preconception adversity and prenatal stress, impacts infant development and can influence health trajectories.[1-4] Evidence suggests that alterations to placental function contribute to the transmission of maternal life-course adversity across generations.[5,6] Furthermore, there appear to be differential effects of maternal preconception adversity and prenatal maternal stress (PNMS) on placental function.[5,7] We have shown accelerated placental aging is one pathway through which maternal exposure to preconception adversity impacts her offspring, wherein maternal adverse childhood experiences (ACE)[8], and not PNMS, is associated with shorter placental telomere length (TL).[5] To further understand mechanisms associated with accelerated placental aging, we examined whether pro-inflammatory processes in the placenta were associated with shorter placental TL due to two primary empirical relationships, which are (1) altered immune regulation during pregnancy in mothers exposed to preconception adversity[9] and, (2) inflammation can induce telomere shortening.[10] We measured telomere length and mRNA expression of the *CCL2* gene, a proinflammatory chemokine, in the villus tissue of the placenta. *CCL2* encodes the C-C motif chemokine ligand 2 protein and was selected a priori based on its role in placental development and function[11], as well as associations with early life adversity[12] and PNMS.[13]

Objectives

The overarching objective was to examine whether proinflammatory processes in the placenta contribute to accelerated placental aging due to maternal adverse childhood experiences, as well as the moderation by sex. The stepwise hypothesis testing was as follows:

A. Independent effects of maternal ACE score and PNMS on placental *CCL2* mRNA expression
B. The association between placental TL and *CCL2* mRNA expression
C. The moderation of ACE score and placental TL by *CCL2* mRNA expression
D. Sex-specific effects of maternal ACE score and PNMS on *CCL2* mRNA expression

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Results

Quality control of RT-qPCR gene expression data

- RNA integrity number (RIN) averaged 6.20 ± 1.31 (Range: 1.9 - 9.0). Lower RIN values were associated with higher *CCL2* mRNA expression (Figure 1A; r=-0.41; P=0.0007). Excluding RIN values less than 3, indicating poor quality, RIN was not associated with *CCL2* mRNA expression (r=-0.07; P=0.59).
- Five villus samples no did not pass quality control due to amplification of the noRT controls and were therefore excluded from analyses.

Figure 1. mRNA expression quality control

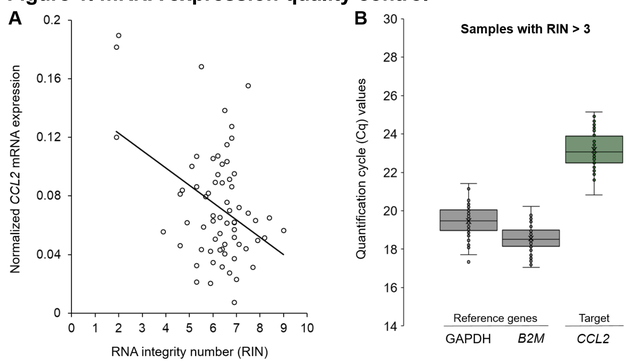


Table 1. Sample characteristics

Demographic outcome, mean (SD)	Total (N=67)
ACE score	2.4 (2.4)
Maternal conception age (yrs)	27.9 (6.1)
Gestational age (wks)	39.2 (1.3)
SES index	2.7 (2.0)
Infant birth weight (kg)	3.3 (0.6)
Duration to sample collection (h)	0.8 (0.6)
Parity	1.3 (1.4)
PNMS score (≥3 exposures)	% (n)
Low	77.6 (52)
High	22.4 (15)
Maternal race	
Black	58.2 (39)
White	31.3 (21)
Other	10.5 (7)
Infant sex	
Male	55.2 (37)
Female	44.8 (30)
Delivery mode	
Vaginal	52.2 (35)
C-section	47.8 (32)
Pregnancy complications	
No	79.1 (53)
Yes	20.9 (14)

Associations between placental TL and *CCL2* mRNA expression with sample characteristics

- TL was not associated with demographic or pregnancy-related outcomes.
- *CCL2* mRNA expression was not associated with TL (r=-0.05; P=0.71) or with any other covariate, with the exception of infant sex (r=0.31; P=0.012); females had higher *CCL2* mRNA expression relative to males.

The effect of maternal ACE score and PNMS on placental TL

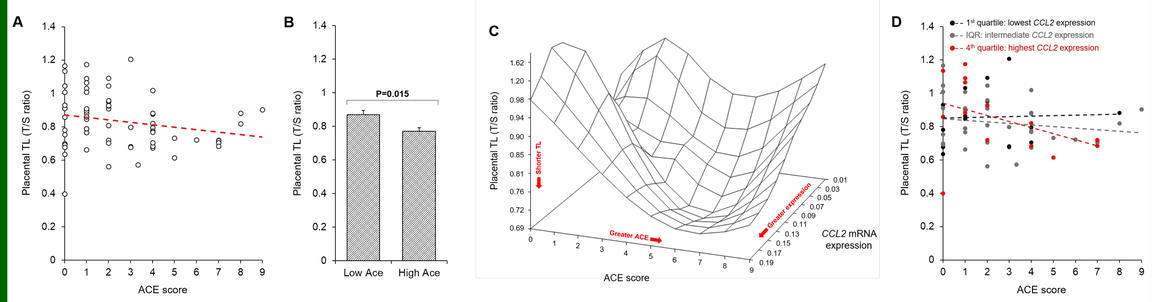
- Higher maternal ACE score was associated with shorter TL (Figure 2A; β=-0.018, P=0.023), while PNMS was not associated with TL. Mothers reporting four or more ACEs also had shorter TL (Figure 2B; β=-0.103, P=0.015).

The effect of maternal ACE score and PNMS on placental *CCL2* mRNA expression

- *CCL2* mRNA expression was not associated with maternal ACE score (β=0.003, P=0.14) or PNMS (β=-0.001, P=0.88).

Implicating proinflammatory processes in placental aging

Figure 2. The effects of maternal ACE score on placental TL and *CCL2* mRNA expression



The moderation of maternal ACE score and placental TL by *CCL2* mRNA expression

- *CCL2* mRNA did not moderate the relationship between maternal ACE score and TL (Figure 2C; β=-0.201, P=0.29). Given that there may be a threshold of inflammation that triggers TL erosion, we categorized *CCL2* mRNA expression by quartile and tested whether placentas with the highest *CCL2* mRNA expression differed from those with lower *CCL2* mRNA expression. Placentas with the highest *CCL2* mRNA expression (4th quartile) had shorter TL with higher maternal ACE score (Figure 2D; β=-0.048; P=0.0498).

Examining sex differences

- Sex differences were not observed in the relationship between maternal ACE score and TL.
- Given that females exhibited higher *CCL2* mRNA expression, we conducted sex-stratified analyses. In females, higher maternal ACE score was associated with higher *CCL2* mRNA expression (β=0.009; P=0.009), while higher PNMS was associated with lower *CCL2* mRNA expression (β=-0.044; P=0.034). No associations were observed in males.
- The moderation of maternal ACE score and TL by quartiles of *CCL2* mRNA expression was significant in females (β=-0.068; P=0.030) but not in males.

Methods

Participants

- N=76 mothers were recruited at any point during pregnancy. Of these, n=72 completed the prenatal survey and had placental tissue collection.

Maternal ACE collection

- The ACE survey was collected prenatally and assessed mothers prior to the age of 17.[8]

Composite prenatal maternal stress (PNMS) measurement

- Measurements of PNMS were collected prenatally through self-report. The PNMS predictor was derived using factor analysis and included five scales: Pregnancy Related Anxiety Scale, the Chronic Strain Questionnaire, Prenatal Life Events Scale – Revised, Perceived Stress Scale, and Edinburgh Depression Scale. A potential score of five represented positive values on all scales.[3]

Demographic and pregnancy-related data collection

- Demographic data were collected by maternal report at the prenatal interview and pregnancy-related data were abstracted from medical records.

Placental sample collection

- Fetal villus tissue samples were collected below the chorionic plate within 4cm from the cord insertion site. Tissue samples were washed in PBS, flash frozen in liquid nitrogen, and then stored at -80ºC.

DNA isolation and quality control

- DNA & RNA were extracted from the same placental sample.
- DNA was isolated using QIAamp DNA mini kit protocol for tissues according to manufacturer protocols (Qiagen, Valencia, CA). DNA samples were evaluated for integrity and concentration with Qubit and for purity with Nanodrop-2000.

RNA isolation and quality control

- RNA was isolated using a modified QIAzol® reagent protocol[15] with a phenol-chloroform phase separation and an RNeasy cleanup (Qiagen, Valencia, CA).
- RNA concentration was measured by NanoDrop-2000 and RNA integrity was evaluated by RNA integrity number (RIN) via microchip electrophoresis (Agilent 2100 Bioanalyzer).

Telomere length (TL) measurement

- Average relative TL was determined as the telomere repeat to single gene (albumin) copy number (T/S) ratio using monochrome multiplex quantitative real-time polymerase chain reaction (PCR) and standard methods in our laboratory.[5,14]
- The coefficient of variance (CV) for placental TL measurements was 3.10% for all plates and n=3 samples with an unacceptably high CV were repeated.

***CCL2* mRNA expression**

- mRNA expression was measured via RT-qPCR following MIQE guidelines.[16]
- cDNA was synthesized using iScript™ Advanced cDNA Synthesis Kit (Bio-Rad, Hercules, CA). cDNA concentration was measured via Qubit ssDNA Assay Kit (Invitrogen, Carlsbad, CA).
- The qPCR step used SYBR green detection (SsoAdvanced™ Universal SYBR® Green Supermix; Bio-Rad, Hercules, CA) with noRT and negative controls on each plate.
- *GAPDH* and *B2M* were selected as reference genes.
- TaqMan® primers were used for *CCL2* (Hs00234140_m1), *GAPDH* (Hs99999905_m1) and *B2M* (Hs00187842_m1).
- Target gene relative expression was evaluated via the ΔΔCt method using the geometric mean of both reference genes and corrected for standard curve amplification efficiency.

Analytic approach

- Generalized linear regression models, adjusted for sex, race, and gestational age, were used to test the study hypotheses. Sex-stratified analyses were also evaluated with generalized linear models.

Conclusions

The study findings suggest that proinflammatory processes, as indexed by *CCL2* mRNA expression, are contributors to shorter placental TL due to higher maternal ACE score. There also appear to be sex-specific placental changes as a function of maternal life-course exposures, where females exhibit more changes relative to males. The differential effects of maternal preconception adversity and PNMS on placental gene expression suggest that future multi-generational studies examining the pathways through which maternal life-course adversity is transmitted to the next generation need to consider both preconception and prenatal exposures.