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Associations between maternal cytokine levels during gestation and measures of child cognitive abilities and executive functioning

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Abstract

Preclinical studies demonstrate that environmentally-induced alterations in inflammatory cytokines generated by the maternal and fetal immune system can significantly impact fetal brain development. Yet, the relationship between maternal cytokines during gestation and later cognitive ability and executive function remains understudied. Children ($n = 246$) were born of mothers enrolled in the Newborn Epigenetic Study – a prospective pre-birth cohort in the Southeastern US. We characterized seven cytokines [$IL - 1\beta$, $IL - 4$, $IL - 6$, $IL - 12p70$, $IL - 17A$, tumor necrosis factor- α ($TNF\alpha$), and interferon- γ ($IFN\gamma$)] and one chemokine ($IL - 8$) from maternal plasma collected during pregnancy. We assessed children's cognitive abilities and executive functioning at a mean age of 4.5 ($SD = 1.1$) years. Children's DAS-II and NIH toolbox scores were regressed on cytokines and the chemokine, controlling for maternal age, race, education, body mass index, IQ,

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Appendix A. Supplementary data

Supplementary information is available at MD's website: <http://mdozmorov.github.io/>. Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.bbi.2018.03.029>.

Reported Conflicts of Interest.

The authors declare no conflict of interest.

parity, smoking status, delivery type, gestational weeks, and child birth weight and sex. Higher IL-12p70 ($\beta_{IL-12p70} = 4.26$, $p = 0.023$) and IL-17A ($\beta_{IL-17A} = 3.70$, $p = 0.042$) levels were related to higher DAS-II GCA score, whereas higher IL-1 β ($\beta_{IL-1\beta} = -6.07$, $p = 0.003$) was related to lower GCA score. Higher IL-12p70 was related to higher performance on NIH toolbox measures of executive functions related to inhibitory control and attention ($\beta_{IL-12p70} = 5.20$, $p = 0.046$) and cognitive flexibility ($\beta_{IL-12p70} = 5.10$, $p = 0.047$). Results suggest that dysregulation in gestational immune activity are associated with child cognitive ability and executive functioning.

Keywords

Proinflammatory cytokines; Chemokine; Cognitive abilities; NIH toolbox; Executive function

1. Introduction

Neurodevelopmental impairments affect an estimated 15% of children aged 2 to 8 years in the United States (Bitsko et al., 2016). Throughout the life course, childhood neurodevelopmental delays and cognitive impairments extend to poor academic achievement, higher engagement in health risk behaviors, increased risk for chronic disease and mental health problems, and lack of economic productivity in adulthood (Engle et al., 2007; Walker et al., 2011; Heckman, 2008; Heckman and Raut, 2016; Shonkoff, 2016). Previous studies indicate that both genetic factors (Sniekers et al., 2017; Gialluisi et al., 2014; Hansell et al., 2015) and the prenatal milieu profoundly affect brain development and neurodevelopmental abilities later in childhood (Park et al., 2016; von Ehrenstein et al., 2012; Singer et al., 2016). A better understanding of the prenatal factors contributing to suboptimal cognitive abilities and neurodevelopmental outcomes could be useful to developing prevention strategies to promote optimal development in these areas.

Environmental exposures, such as toxins, stress, nutrition, and infection during pregnancy have all been linked with behavioral, cognitive and neurodevelopmental impairments in offspring (Lee et al., 2016). Emerging evidence suggests that some of these exposures exert their influence via maternal immune regulation (Calderon-Garciduenas et al., 2009; Wright et al., 2010; Gilman et al., 2017). Several immune molecules produced in response to microglial activation within the central nervous system (CNS) are implicated in neurogenesis, synaptic maturation, generation of neural networks and other important processes of brain development (McAfoose and Baune, 2009; Bilbo and Schwarz, 2009). For example, cytokines are common immune and neuronal cell signaling proteins, with the ability to pass through the blood-brain barrier directly through active transport mechanisms, or indirectly through other means. Thus, if maternal cytokines mediate bidirectional brain-immune communication across the placental and blood-brain barriers during fetal development, it is possible that they also influence measurable differences in children's cognitive and neurodevelopmental abilities (von Ehrenstein et al., 2012). Determining the degree to which gestational immune activity relates to cognitive ability and executive functioning in children could help elucidate potential pathways by which the prenatal environment influences brain development, cognitive and neurodevelopmental abilities.

There is a growing, yet limited, set of studies that have investigated the role of gestational immune markers and subsequent neurodevelopmental outcomes in children (McAfoose and Baune, 2009; Hohlfeld et al., 2007). Previously conducted studies using cell cultures and model organisms have already demonstrated the critical role that prenatal circulating cytokines and chemokines play in brain development (Deverman and Patterson, 2009). In humans, developmental neuropsychiatric conditions, such as schizophrenia in adulthood and autism in childhood, have been linked with elevated gestational levels of cytokines and chemokines (Brown et al., 2004; Jones et al., 2017). Further, lower gestational levels of IL-8 have been associated with physician assessed neurological abnormalities at 1 year of age (Gilman et al., 2017), and at least one study has shown that umbilical cord blood concentrations of inflammatory cytokines are associated with child cognitive intellectual abilities (i.e., intellectual quotient (IQ)) (von Ehrenstein et al., 2012). These studies highlight the association between gestational immune activity and broad-based cognitive abilities, but less is known regarding the extent to which gestational immune activity relates to other domains of functioning, such as those related to executive functioning. Executive functions are neurocognitive processes involved with the execution of goal directed behaviors and optimization of cognitive resources in contexts with competing or unexpected demands. Deficits in executive functions are related to a number of childhood neurodevelopmental problems (Rosenthal et al., 2013; Snyder et al., 2015; Willcutt et al., 2005). Yet, no study to date has examined the extent to which gestational immune activity specifically relates to executive functioning.

The present study contributes novel features to the growing body of literature examining gestational cytokines in relation to child cognitive and neurodevelopment in a variety of ways. First, while previous studies have examined broad-based neurodevelopmental outcomes, and one study has examined intellectual ability (IQ) as an outcome (Mansouri et al., 2009), this study includes assessment of both cognitive ability and executive functioning. This allows for the extension of previous research on intellectual abilities, while gaining further insight into the specific cognitive domains that may be linked to gestational immune functioning. Second, we included in our assessment the significance and direction of the effect of seven cytokines *IL* – 1 β , *IL* – 4, *IL* – 6, *IL* – 12p70, *IL* – 17A, tumor necrosis factor- α (*TNF α*), and interferon- γ (*IFN γ*) and one chemokine (*IL* – 8). This panel was custom built based on existing pre-clinical and clinical literature (i.e., analytes with a putative role in normal neurodevelopment (Bilbo and Schwarz, 2009), previously associated with either neuroprotection or adverse neurodevelopmental outcomes (e.g., *IL*-1 β , *IL* - 6) in model organism or human studies (von Ehrenstein et al., 2012; Gilman et al., 2017; Bilbo and Schwarz, 2009; Jones et al., 2017), while balancing feasibility in number of analytes assessed. Finally, we included a number of covariates in our models, including the assessment of maternal cognitive ability and executive function, thereby enabling the assessment of the magnitude of the effect of gestational cytokines and chemokines beyond the potential genetic or environmental contributions of maternal cognitive ability and executive functioning.

2. Materials and methods

2.1. Participants

Participants were part of the Newborn Epigenetic Study (NEST), a Southeastern United States pre-birth cohort initiated in 2005. The Institutional Review Board approved studies involving these participants, and informed written consent was obtained from all participants. Participant identification and enrollment procedures are described elsewhere (Liu et al., 2012; Hoyo et al., 2011). Briefly, 2595 pregnant women were recruited from prenatal clinics serving Duke University Hospital and Durham Regional Hospital Obstetrics facilities from April 2005 to June 2011. Eligibility criteria were as follows: aged 18 years, pregnant, and had intentions to use one of the two obstetrics facilities enabling access to labor and birth outcome data for the index pregnancy. Maternal blood specimens were collected along with survey data on health, nutrition, stress, and lifestyle behaviors during the enrollment period, which occurred during the first trimester for most participants.

In 2014, women with live births and who had agreed to be recontacted were recruited for a follow-up study examining prenatal smoke exposure, epigenetics, childhood Attention Deficit Hyperactivity Disorder (ADHD) symptoms, cognitive abilities, and executive functioning development. Eligibility criteria required women participants to speak English and have had stored biological samples collected during the prenatal phase and at birth (maternal plasma samples and cord blood samples). Analyses were conducted on 246 mother-child pairs from this study for whom we had cytokine data already assayed and for whom we had assessments of child cognitive ability and executive functioning. Children had to be at least 3 years of age to allow for executive functioning testing. Compared to the overall NEST sample, the analysis sample included a higher percentage of women who were college graduates (41.3% vs 32.3%, $\chi^2 = 9.70$, $p = 0.02$) and a greater percentage of women who were African American (58.5% vs 42.0%, $\chi^2 = 45.13$, $p < 0.001$). There were no differences with respect to factors potentially related to gestational immune functioning: self-reported smoking status in the analysis group was generally similar to the full sample (19.1% vs 24.6%, $\chi^2 = 3.66$, $p = 0.06$), Cesarean delivery was similar (34.4% vs 37.9%, $\chi^2 = 2.33$, $p = 0.31$), prevalence of gestational diabetes was similar (6.7% vs 7.1%, $\chi^2 = 4.83$, $p = 0.18$), and the mean pre-pregnancy Body Mass Index (BMI) was similar (28.7 vs 28.0, $p = 0.24$).

2.2. Assessment of child cognitive ability and executive functioning

Eligible mothers in the larger NEST cohort were contacted via recruitment letters mailed from the study team and/or were recruited during a well-child clinical visit. Mothers who agreed to participate were scheduled for a three-hour visit at study clinic offices where they provided informed consent, were asked to complete survey measures on their child's health and behaviors, and were administered assessments for cognitive ability and executive functioning. Meanwhile, another staff member administered assessments for cognitive ability and executive functioning to their child. A licensed clinical psychologist trained staff in standardized administration of these assessments.

Child cognitive ability was assessed using the Differential Abilities Scale, second edition (DAS-II) (Elliot, 2007). The DAS-II yields a General Conceptual Abilities (GCA) score, which is obtained from a battery of 4–6 tests (depending on age) measuring verbal, non-verbal, and spatial reasoning. The DAS-II is appropriate for diverse populations and is a validated measure with strong support for its convergent validity with other measures of IQ (Farmer et al., 2016; Kuuriakose, 2014).

The computer-based NIH toolbox was used to assess executive functioning in children (Miyake et al., 2000). The “Early Childhood Battery” cognitive domain instruments for children 3 to 6 years of age include the: Flanker, Dimensional Change Card Sort, Picture Sequence Memory, and Picture Vocabulary, which assess inhibitory control and attention, cognitive flexibility, episodic memory, and language development respectively. In addition to producing scores for each cognitive domain, a “composite score” is calculated from sub-test scores. The instrument was validated in a diverse sample and demonstrates good test-retest and convergent validity (Miyake et al., 2000).

2.3. Assessment of maternal characteristics

Trained research staff abstracted parturition data from medical records after delivery, including maternal diabetes status, preeclampsia status, parity, mode of delivery, birth weight, gestational age at birth (weeks), and sex of the child. Other characteristics, such as race and pre-pregnancy BMI were obtained from maternal self-report, collected from the first trimester enrollment survey. For both mothers and children, age was calculated from the date of follow-up assessment and birthdays. Smoking during pregnancy was based on maternal self-report and dichotomized as non-smoking or current smoker (at least one cigarette each day, in the past 30 days). Maternal cognitive intellectual ability was assessed using the widely used and reliable Wechsler Abbreviated Scale of Intelligence (WASI - II), which provides a composite IQ score derived from four subtests: Block Design, Vocabulary, Matrix Reasoning, and Similarities (Wechsler, 2011).

2.4. Assessment of cytokine/chemokine concentrations

Procedures for specimen collection and handling have been previously described (Liu et al., 2013). Briefly, 10 ml of peripheral blood was drawn at enrollment, which occurred between the first and second trimesters of pregnancy (average time of blood draw = 12.5 weeks of gestation). Blood was collected using EDTA vacutainer tubes and processed to obtain plasma from which cytokines were measured. Plasma was stored in 200 µl aliquots to reduce degradation from subsequent freeze-thaw cycles. The following cytokines and one chemokine were measured: *IL* – 1 β , *IL* – 4, *IL* – 6, *IL* – 12p70, *IL* – 17A, tumor necrosis factor- α (*TNF α*), interferon- γ (*IFN γ*), and *IL* – 8. *IL*-4 was later excluded from analyses, due to missingness of several cases that had values below the limit of detection in this sample. Correlations between inflammatory markers are displayed in Fig. 1.

To measure these cytokines, a custom high-sensitivity human cytokine 8-plex MAP® kit from EMD Millipore was used. Twenty-five µl samples were run in duplicate according to the manufacturer’s recommendations. Plates were read on a Luminex® platform by the Duke University Human Vaccine Institute Core Facility. Data were analyzed using Milliplex

Analyst® version 5.1. Limits of detection for each cytokine were as follows (pg/ml): IFN γ : 0.87; IL-1 β : 0.40; IL-12p70: 0.30; IL-17A: 0.56; IL-4: 0.94; TNF α : 0.43; IL-8: 0.34; IL-6: 0.24. The intra-assay coefficient of variation for all analytes was 3.71%, and the inter-assay CV was 14.89%.

2.5. Statistical analysis

To be included in the analysis, child participants had to have data on the DAS-II or NIH Toolbox, covariates, and cytokine or chemokine concentrations. Disproportionally small racial/ethnic groups (e.g., “Hispanics”, “Others”) were excluded from analyses. Because cytokine or chemokine concentrations were right-skewed, they were analyzed on a natural log scale. A total of 246 subjects had at least four cytokine or chemokine concentrations in measurable ranges. Of the cytokines or chemokine measured, one subject had four missing, three had two missing, and sixteen had one missing. No imputations were conducted for cytokines below LOD. For the full models, child’s scores were regressed on cytokine or chemokine concentrations with adjustment for several maternal characteristics (age, race, IQ, BMI, diabetes status, smoking status, week of blood draw, parity), delivery characteristics (gestational age, delivery type, preeclampsia status), and child’s characteristics (sex, weight). Covariates were selected on the basis that they were associated with at least one of the cytokines/chemokine or measures of childhood cognitive ability or executive functioning. A priori, we included maternal IQ because we wanted to evaluate what effect cytokines have on the outcomes of interest, after taking into account the known strong correlation between maternal IQ and child IQ.

Given the large number of covariates tested, it is important to focus on a subset of covariates most significantly explaining the variable of interest. Selection of the most significant variables was performed using the variable selection analysis. The most important covariates were selected from the final multivariate linear model using the Elastic Net algorithm implemented in the R package glmnet (Friedman et al., 2010). Elastic net regression is a combination of traditional Lasso and ridge regression methods that emphasizes model sparsity, while appropriately balancing the contribution of correlated variables. Ridge regression is a technique for thwarting overfitting by penalizing the Euclidean distance (L2 norm) of the coefficient vector that results in the “shrinking” of the beta coefficient, with the penalty controlled by the parameter, lambda. Lasso regression is related, in that it penalizes the Manhattan distance (L1 norm) of the coefficient vector. Since lasso regression uses the L1 norm, some coefficients will shrink to zero, as lambda increases. Thus, lasso regression is a feature selection technique, in addition to a shrinkage method. Elastic net regression is a hybrid of these two approaches in that it blends penalization of L2 and L1 norms; so, at worst, it performs as well as either the lasso or ridge regression methods, and at best, it outperforms both. The objective function that elastic net minimizes is RSS (residual sum of

squares) $RSS = \sum_{j=1}^N \left(y_j - \beta_0 - \sum_{i=1}^p X_{ij}\beta_i \right)^2$. Elastic net algorithm constrains this function as $(1 - \alpha)\|\beta\|_1 + \alpha\|\beta\|_2 \leq t$, where $\|\beta\|_1 = \sum_{i=1}^p |\beta_i|$ and $\|\beta\|_2 = \sqrt{\sum_{i=1}^p \beta_i^2}$, for $\alpha \in [0, 1]$ and some t . The first constraint is the L1-norm (LASSO-type) that forces the coefficients to shrink to 0, favoring sparsity in the final model. The second is the L2-norm (Ridge type) that forces

similar values for the coefficients, thus avoiding picking one variable over the other if both are redundant. The α parameter, set to 0.5 in our study, specifies the contribution of each constant. Optimal regularization parameters were estimated via 10-fold cross-validation.

3. Results

Descriptive characteristics of the study population are shown in Table 1. The study sample included fewer Whites than African Americans (34.2% vs. 58.5%, respectively) with an average age of 28 (SD = 5.6) years at enrollment. Approximately 41% of mothers reported being college educated, and 19.1% reported active smoking on the prenatal survey. In terms of pregnancy characteristics, more than one-third (34.4%) had a Cesarean delivery, none experienced infection during pregnancy, and less than 10% experienced gestational diabetes or preeclampsia. Children were on average aged 4.5 (SD = 1.1) at the time of the follow-up assessment.

3.1. Univariate associations between sample characteristics and child IQ and NIH Toolbox performance

Child IQ (i.e. as measured by DAS-II GCA score) ranged from 61 to 152, with mean 101 (SD = 16.1) and median 100. Baby's birth weight ($\beta_{birth\ weight} = 0.01$, $p = 4.98 \times 10^{-4}$) was positively associated with child IQ. Children born to Caucasian ($\beta_{White} = 15.23$, $p = 3.19 \times 10^{-3}$) and college educated mothers ($\beta_{college\ graduate} = 23.00$, $p = 1.16 \times 10^{-14}$) had the highest IQ scores. Relative to no other pregnancies, having three or more pregnancies was negatively associated with IQ ($\beta_{parity\ 3} = -9.09$, $p = 0.02$; $\beta_{parity\ 4} = -12.95$, $p = 4.66 \times 10^{-3}$), as was pre-pregnancy BMI ($\beta_{maternal\ BMI} = -0.30$, $p = 0.02$), and maternal age ($\beta_{maternal\ age} = 0.96$, $p = 2.79 \times 10^{-7}$).

The composite NIH Toolbox score was moderately significantly correlated with the child's IQ ($r = 0.13$, $p < 0.0001$) indicating shared variability between these assessment tasks, despite assessing different cognitive aptitudes. Like IQ, better performance on the NIH Toolbox was significantly related to both child and maternal characteristics. Specifically, the NIH Toolbox score was higher for children who were older ($\beta_{age\ at\ assessment} = 5.47$, $p = 1.31 \times 10^{-12}$), greater birth weights ($\beta_{birth\ weight} = 0.01$, $p = 0.03$). As for maternal characteristics, higher NIH Toolbox score was associated with Caucasian race ($\beta_{White} = 7.61$, $p = 1.16 \times 10^{-3}$), higher maternal educational attainment ($\beta_{college\ graduate} = 8.42$, $p = 0.02$), pre-pregnancy BMI ($\beta_{maternal\ BMI} = -0.34$, $p = 0.01$), older maternal age at delivery ($\beta_{maternal\ age} = 0.42$, $p = 0.04$).

3.2. Univariate associations between inflammatory markers and child IQ and NIH Toolbox performance

The *IL* - 12p70 cytokine was positively associated with child IQ ($\beta_{IL-12p70} = 4.18$, $p = 2.80 \times 10^{-3}$). *IL* - 6 showed a non-significant trend towards higher child IQ ($\beta_{IL-6} = 2.64$, $p = 0.09$). None of the inflammatory markers were associated with NIH toolbox performance in univariate models (Supplementary Table 1).

3.3. Multivariate analyses of inflammatory markers and child IQ and NIH toolbox performance

Regressing child IQ on all cytokines in a single model with covariates showed significant positive associations for $IL-12p70$ and $IL-17A$ ($\beta_{IL-12p70} = 4.26$, $p\text{-value} = 0.023$; $\beta_{IL-17A} = 3.70$, $p\text{-value} = 0.042$). An association between higher $IL-1\beta$ and lower child IQ was also found ($\beta_{IL-1\beta} = -6.07$, $p\text{-value} = 0.003$). The variable selection with elastic net regression confirmed significant effects of higher maternal education, higher maternal IQ, Caucasian race, $IL-12p70$, and $IL-17A$ with higher child IQ scores and higher $IL-1\beta$ with lower child IQ scores. The final selected model accounted for 47% of the variance in child IQ, with the combined effects of the inflammatory markers contributing 7%. For the sub-components of IQ (verbal, nonverbal, and spatial abilities), $IL-12p70$ was positively associated with verbal ($\beta_{IL-12p70} = 4.56$, $p\text{-value} = 0.022$) and non-verbal ($\beta_{IL-12p70} = 4.19$, $p\text{-value} = 0.036$) abilities. $IL-1\beta$ was negatively associated with nonverbal ($\beta_{IL-1\beta} = -5.72$, $p = 0.008$) and spatial abilities ($\beta_{IL-1\beta} = -7.26$, $p = 0.004$), and $IL-8$ was positively associated with verbal abilities ($\beta_{IL-8} = 4.75$, $p = 0.046$) and negatively associated with child spatial abilities ($\beta_{IL-8} = -6.89$, $p = 0.012$) (Table 2).

None of the inflammatory markers were associated with the NIH toolbox composite score. With regard to the subscales, significant positive associations between $IL-12p70$ and performance on the Flanker inhibitory control task assessing inhibitory control and attention ($\beta_{IL-12p70} = 5.20$, $p = 0.046$) and the dimensional change card sort assessing cognitive flexibility ($\beta_{IL-12p70} = 5.10$, $p = 0.047$) was observed. $IFN\gamma$ was negatively associated with cognitive flexibility, as measured by the dimensional change card sort ($IFN\gamma = -6.24$, $p = 0.003$) (Table 3).

4. Discussion

The primary purpose of this study was to examine the extent to which gestational inflammatory markers relate to childhood measures of cognitive ability and executive functioning. Results support a possible link between maternal gestational immune activity and offspring IQ and executive functions related to cognitive flexibility and inhibitory control and attention. Higher levels of $IL-12p70$ and $IL-17A$ were associated with a higher child IQ score, whereas higher levels of $IL-1\beta$ were associated with a lower child IQ score. We further observed that higher levels of $IL-12p70$ were associated with higher performance on executive function tasks measuring cognitive flexibility and inhibitory control and attention, domains impaired in children with ADHD and autism (Zelazo et al., 2002; Mulas et al., 2006). Also, lower levels of $IFN\gamma$ were associated with poorer performance on the task measuring cognitive flexibility. This is one of the first studies to examine gestational immune markers assayed from maternal blood in relation to early childhood measures of cognitive ability and executive functioning in a population-based sample.

The other study most similar to ours by von Ehrenstein et al (von Ehrenstein et al., 2012) examined inflammatory markers assayed from umbilical cord blood in relation to child verbal and performance IQ at 5 years of age (von Ehrenstein et al., 2012). Performance-based tasks measure non-verbal abilities related to inductive and deductive reasoning,

whereas, verbal abilities reflect language development. In the study by von Ehrenstein et al, higher levels of *IFN γ* and *IL – 12p70* were both significantly related to better performance-based skills (reduced odds of having a performance IQ < 70), but not to verbal abilities (von Ehrenstein et al., 2012). Similarly, our findings corroborate this positive association between cytokine *IL – 12p70* and child measures of non-verbal abilities, but we also found significant associations between this cytokine and overall IQ, verbal abilities, and executive functions related to cognitive flexibility and inhibitory control and attention. Unlike von Ehrenstein et al, we did not observe find *IFN γ* to be related to cognitive abilities; however, we did observe that higher levels of *IFN γ* was associated with worse performance on the DCCS measuring cognitive flexibility. Cognitive flexibility is not captured by the IQ assessments used in the von Ehrenstein et al study. The added novelty of the current study is found in the significant associations observed between inflammatory markers not previously evaluated within the von Ehrenstein et al study (*e.g.* *IL – 1 β* , *IL – 17A*, and *IL - 8*) and measures of child cognitive ability and executive functioning. Specifically we found the following associations: higher *IL – 1 β* with lower child IQ scores, higher *IL – 12p70* levels with higher child IQ scores and higher performance on executive function tasks measuring cognitive flexibility and inhibitory control and attention, and higher *IL-8* and higher levels of verbal abilities and lower levels of spatial ability.

The observation in our data showing higher *IL-8* to be positively related to verbal abilities, but negatively associated with spatial abilities was unexpected. Since there are subsets of children that display differences in verbal and non-verbal abilities (verbal abilities are stronger than non-verbal or vice versa), it could be possible that prenatal exposure to *IL-8* has effects on neurodevelopmental process that underlie these learning differences. The positive association with verbal abilities may also be a false positive finding, whereas higher *IL-8* may be more primarily linked with spatial abilities. Further studies examining how inflammatory cytokines related to learning differences could help shed light on some of the associations observed here.

A unique feature of this study is that it examines gestational cytokine or chemokines in relation to measures of both child cognitive ability and executive functioning – of which there are currently few studies. Two studies have shown that across several cytokines, higher mid-gestational levels are associated with an increased risk of autism and autism with intellectual disability or developmental delay relative to controls (Jones et al., 2017; Abdallah et al., 2013). In another recent study utilizing a historical cohort ascertained between 1959 and 1966, investigators found lower *IL- 8* assayed from maternal serum obtained in early in the third trimester of pregnancy to be related to a higher risk of physician assessed neurological abnormalities at 4 months and 1 year of age (Gilman et al., 2017). Generally, it is difficult to compare our findings to these studies since the populations and outcomes being assessed across these studies differ materially. Ideally, continued research employing both clinical psychiatric populations and children in the general population are needed to more clearly elucidate the role of gestational cytokines on cognitive and neurodevelopment in children.

The findings here should be interpreted within the context of limitations. First, the sample is of moderate size and a larger sample size when examining prenatal factors in relation to later

childhood outcomes would allow for more precise estimation of effects. Nevertheless, we were able to detect significant associations with some inflammatory markers which were consistent with previous findings. However, in general, a larger sample size can help guard against false positive effects especially if the true associations are small in magnitude. Second, although the prospective observational design is a strength over other study design choices, causation cannot be established with certainty using such designs. Third, given the natural course of both inflammation over pregnancy, as well as fetal brain maturation there may be trimester-specific effects which we were unable to analyze in this study. Notably, however, there is less variability in this study relative to others, in terms of time of collection. This is a strength of the current study, and highlights the importance of the end of the first trimester as potentially important time to investigate the relationship between gestational inflammatory signaling and subsequent child neurodevelopment. Finally, it may be that immune profiles differ depending on the biological matrix used for assessing cytokines. Maternal blood samples during pregnancy were used in the current study, which may differ from the profile assayed from placental or amniotic samples (Djuardi et al., 2009). Future studies might want to investigate: how cytokine and chemokine profiles vary during gestation, what effect these changes may have on child cognitive development, and whether any of these changes are influenced by genetic or environmental factors.

Some of the strengths of this study include the use of robust statistical methodologies to maximally utilize data information content by using continuous measures, where possible. This was done intentionally, as many studies often ignore potential confounding effects, bin continuous variables into discrete categories, or combine categorical variables into similar categories (Altman and Royston, 2006). Further, we confirmed the reported effects using Elastic Net algorithm for variable selection, thereby emphasizing the importance of accounting for covariates in analyses. Finally, we evaluate potential the associations controlling for a large set of variables, including maternal education, IQ and executive functioning. Previous studies have not included a robust assessment of maternal cognitive capacities in analyses. Maternal education, IQ and executive functioning are strong correlates of child neurodevelopment that may represent both genetic and post-natal environmental effects. Thus, the findings here represent the association of inflammatory markers on child neurodevelopmental outcomes holding these factors constant. It would be useful for future studies to examine how such social-biological factors might disrupt (e.g. buffer or exacerbate) the gestational immune-child neurodevelopmental relationship.

5. Conclusions

In sum, mounting evidence supports the connection between immune activity and brain development and functioning. Consistent with our findings among children, experimental studies of spinal cord injury in mice suggest *IL – 12p70* activates recovery and neurogenesis (Yaguchi et al., 2008), suggesting that *IL – 12p70* has a protective role on brain development and functioning. Also, consistent with our findings, elevated levels of *IL – 1β* during the perinatal period is implicated in adverse cognitive outcomes in rats (Williamson et al., 2011) and mice (Bolton et al., 2013), and has been shown to have a negative effect on neurogenesis in both animal models (Koo and Duman, 2008; Crampton et al., 2012) and humans (Yoon et al., 1997). Continued work across species will be useful in clarifying the role of specific

inflammatory markers on child cognitive ability and executive functioning. Studies that elucidate the down-stream factors contributing to prenatal inflammation are needed, as well as studies examining potential mechanisms [e.g., epigenetic alterations (McCullough et al., (2017))] that may help explain the link between immune activity and measures of cognitive ability and executive functioning. To the extent that early child cognitive and executive functioning abilities are a harbinger for physical and mental health outcomes over the life-course, understanding the role of maternal gestational immune activity could be useful for early identification and remediation of long-term deleterious outcomes with high societal impact.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Abdallah MW, Larsen N, Grove J, Norgaard-Pedersen B, Thorsen P, Mortensen EL, et al., 2013 Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders PubMed PMID: World J. Biol. Psychiatry 14 (7), 528–538. 10.3109/15622975.2011.639803. [PubMed: 22175527]
- Altman DG, Royston P, 2006 The cost of dichotomising continuous variables. Brit. Med. J. 332 (7549), 1080 10.1136/bmj.332.7549.1080 PubMed PMID: [PubMed: 16675816]
- Bilbo SD, Schwarz JM, 2009 Early-life programming of later-life brain and behavior: a critical role for the immune system. Front. Behav. Neurosci. 3, 14 10.3389/neuro.08.014.2009 PubMed PMID: [PubMed: 19738918]
- Bitsko RH, Holbrook JR, Robinson LR, Kaminski JW, Ghandour R, Smith C, et al., 2016 Health care, family, and community factors associated with mental, behavioral, and developmental disorders in early childhood – United States, 2011–2012 PubMed PMID: MMWR Morb Mortal Wkly Rep. 65 (9), 221–226. 10.15585/mmwr.mm6509a1. [PubMed: 26963052]
- Bolton JL, Huff NC, Smith SH, Mason SN, Foster WM, Auten RL, et al., 2013 Maternal stress and effects of prenatal air pollution on offspring mental health outcomes in mice. Environ. Health Perspect. 121 (9), 1075–1082. 10.1289/ehp.1306560 PubMed PMID: [PubMed: 23823752]
- Brown AS, Hooton J, Schaefer CA, Zhang H, Petkova E, Babulas V, et al., 2004 Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring PubMed PMID: Am. J. Psychiatry 161 (5), 889–895. 10.1176/appi.ajp.161.5.889. [PubMed: 15121655]
- Calderon-Garciduenas L, Macias-Parra M, Hoffmann HJ, Valencia-Salazar G, Henriquez-Roldan C, Osnaya N, et al., 2009 Immunotoxicity and environment: immunodysregulation and systemic

inflammation in children PubMed PMID: Toxicol. Pathol. 37 (2), 161–169.
10.1177/0192623308329340. [PubMed: 19171930]

- Crampton SJ, Collins LM, Toulouse A, Nolan YM, O’Keeffe GW, 2012 Exposure of foetal neural progenitor cells to IL-1 β impairs their proliferation and alters their differentiation - a role for maternal inflammation? PubMed PMID: J Neurochem. 120 (6), 964–973. 10.1111/j.1471-4159.2011.07634.x. [PubMed: 22192001]
- Deverman BE, Patterson PH, 2009 Cytokines and CNS development PubMed PMID: Neuron 64 (1), 61–78. 10.1016/j.neuron.2009.09.002. [PubMed: 19840550]
- Djuardi Y, Wibowo H, Supali T, Ariawan I, Bredius RG, Yazdanbakhsh M, et al., 2009 Determinants of the relationship between cytokine production in pregnant women and their infants. PLoS ONE 4 (11), e7711 10.1371/journal.pone.0007711 PubMed PMID: ; [PubMed: 19898617]
- Elliot CD. Differential Ability Scales-Second Edition. 2 ed. San Antonio, TX 2007.
- Engle PL, Black MM, Behrman JR, Cabral de Mello M., Gertler PJ, Kapiriri L, et al., 2007 Strategies to avoid the loss of developmental potential in more than 200 million children in the developing world PubMed PMID: Lancet 369 (9557), 229–242. 10.1016/S0140-6736(07)60112-3. [PubMed: 17240290]
- Farmer C, Golden C, Thurm A, 2016 Concurrent validity of the differential ability scales, second edition with the Mullen Scales of Early Learning in young children with and without neurodevelopmental disorders PubMed PMID: Child Neuropsychol. 22 (5), 556–569. 10.1080/09297049.2015.1020775. [PubMed: 25833070]
- Friedman J, Hastie T, Tibshirani R, 2010 Regularization paths for generalized linear models via coordinate descent PubMed PMID: ; PubMed Central PMCID: PMC2929880 J. Stat. Software 33 (1), 1–22. [PubMed: 20808728]
- Gialluisi A, Newbury DF, Wilcutt EG, Olson RK, DeFries JC, Brandler WM, et al., 2014 Genome-wide screening for DNA variants associated with reading and language traits. Genes, Brain Behav. 13 (7), 686–701. 10.1111/gbb.12158 PubMed PMID: ; [PubMed: 25065397]
- Gilman SE, Hornig M, Ghassabian A, Hahn J, Cherkerzian S, Albert PS, et al., 2017 Socioeconomic disadvantage, gestational immune activity, and neurodevelopment in early childhood. Proc. Natl. Acad. Sci. U.S.A. 114 (26), 6728–6733. 10.1073/pnas.1617698114 PubMed PMID: ; [PubMed: 28607066]
- Hansell NK, Halford GS, Andrews G, Shum DH, Harris SE, Davies G, et al., 2015 Genetic basis of a cognitive complexity metric PubMed PMID: ; PubMed Central PMCID: PMC4393228 PLoS ONE 10 (4), e0123886 10.1371/journal.pone.0123886. [PubMed: 25860228]
- Heckman JJ, 2008 Schools, skills, and synapses PubMed PMID: ; PubMed Central PMCID: PMC2812935 Econ. Inq. 46 (3), 289. [PubMed: 20119503]
- Heckman JJ, Raut LK, 2016 Intergenerational long-term effects of preschool - structural estimates from a discrete dynamic programming model PubMed PMID: ; PubMed Central PMCID: PMC4689204 J. Econ. 191 (1), 164–175. 10.1016/j.jeconom.2015.10.001. [PubMed: 26709326]
- Hohlfeld R, Kerschensteiner M, Meinel E, 2007 Dual role of inflammation in CNS disease discussion S91–6 PubMed PMID: Neurology 68 (22 Suppl 3), S58–S63. 10.1212/01.wnl.0000275234.43506.9b. [PubMed: 17548571]
- Hoyo C, Murtha AP, Schildkraut JM, Forman MR, Calingaert B, Demark-Wahnefried W, et al., 2011 Folic acid supplementation before and during pregnancy in the Newborn Epigenetics Study (NEST). BMC Public Health 11 (1), 46 10.1186/1471-2458-11-46 PubMed PMID: ; [PubMed: 21255390]
- Jones KL, Croen LA, Yoshida CK, Heuer L, Hansen R, Zerbo O, et al., 2017 Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Mol. Psychiatry 22 (2), 273–279. 10.1038/mp.2016.77 PubMed PMID: ; [PubMed: 27217154]
- Koo JW, Duman RS, 2008 IL-1 β is an essential mediator of the antineurogenic and anhedonic effects of stress PubMed PMID: ; PubMed Central PMCID: PMC2206608 Proc. Natl. Acad. Sci. U.S.A. 105 (2), 751–756. 10.1073/pnas.0708092105. [PubMed: 18178625]

- Kuuriakose S, 2014 Concurrent validity of the WISC-IV and DAS-II in children with autism spectrum disorder. *J. Psychoeduc. Assess.* 32 (4), 282–294.
- Lee GO, Richard SA, Kang G, Hout ER, Seidman JC, Pendergast LL, et al., 2016 A Comparison of diarrheal severity scores in the MAL-ED multisite community-based cohort study. *J. Pediatr. Gastroenterol. Nutr.* 63 (5), 466–473. 10.1097/MPG.0000000000001286 PubMed PMID: ; [PubMed: 27347723]
- Liu Y, Murphy SK, Murtha AP, Fuemmeler BF, Schildkraut J, Huang Z, et al., 2012 Depression in pregnancy, infant birth weight and DNA methylation of imprint regulatory elements. *Epigenet. Off. J. DNA Methylation Soc.* 7 (7), 735–746. 10.4161/epi.20734 PubMed PMID: ; [PubMed: 22677950]
- Liu Y, Hoyo C, Murphy S, Huang Z, Overcash F, Thompson J, et al., 2013 DNA methylation at imprint regulatory regions in preterm birth and infection e1– 7PubMed PMID: ; PubMed Central PMCID: PMCPMC3838789 *Am. J. Obstetrics Gynecol.* 208 (5), 395 10.1016/j.ajog.2013.02.006. [PubMed: 23477525]
- Mansouri FA, Tanaka K, Buckley MJ, 2009 Conflict-induced behavioural adjustment: a clue to the executive functions of the prefrontal cortex Epub 2009/01/21PubMed PMID: *Nat. Rev. Neurosci.* 10 (2), 141–152. 10.1038/nrn2538. [PubMed: 19153577]
- McAfoose J, Baune BT, 2009 Evidence for a cytokine model of cognitive function PubMed PMID: *Neurosci. Biobehav. Rev.* 33 (3), 355–366. 10.1016/j.neubiorev.2008.10.005. [PubMed: 18996146]
- McCullough LE, Miller EE, Calderwood LE, Shivappa N, Steck SE, Forman MR, et al., 2017 Maternal inflammatory diet and adverse pregnancy outcomes: Circulating cytokines and genomic imprinting as potential regulators? PubMed PMID: *Epigenet Off. J. DNA Methylation Soc.* 0 10.1080/15592294.2017.1347241. [PubMed: 28678596]
- Miyake A, Friedman NP, Emerson MJ, Witzki AH, Howerter A, Wager TD, 2000 The unity and diversity of executive functions and their contributions to complex “Frontal Lobe” tasks: a latent variable analysis Epub 2000/08/18PubMed PMID: *Cogn. Psychol.* 41 (1), 49–100. 10.1006/cogp.1999.0734. [PubMed: 10945922]
- Mulas F, Capilla A, Fernandez S, Etchepareborda MC, Campo P, Maestu F, et al., 2006 Shifting-related brain magnetic activity in attention-deficit/hyperactivity disorder PubMed PMID: *Biol. Psychiatry* 59 (4), 373–379. 10.1016/j.biopsych.2005.06.031. [PubMed: 16154541]
- Park S, Bellinger DC, Adamo M, Bennett B, Choi NK, Baltazar PI, et al., 2016 Mechanistic pathways from early gestation through infancy and neurodevelopment PubMed PMID: ; PubMed Central PMCID: PMCPMC5127069 conflicts of interest to disclose *Pediatrics* 138 (6). 10.1542/peds.2016-1843. [PubMed: 27940699]
- Rosenthal M, Wallace GL, Lawson R, Wills MC, Dixon E, Yerys BE, et al., 2013 Impairments in real-world executive function increase from childhood to adolescence in autism spectrum disorders. *Neuropsychology* 27 (1), 13–18. 10.1037/a0031299 PubMed PMID: ; [PubMed: 23356593]
- Shonkoff JP, 2016 Capitalizing on advances in science to reduce the health consequences of early childhood adversity PubMed PMID: *JAMA Pediatr.* 170 (10), 1003–1007. 10.1001/jamapediatrics.2016.1559. [PubMed: 27548291]
- Singer AB, Windham GC, Croen LA, Daniels JL, Lee BK, Qian Y, et al., 2016 Maternal exposure to occupational asthmagens during pregnancy and autism spectrum disorder in the study to explore early development. *J. Autism Dev. Disord.* 46 (11), 3458–3468. 10.1007/s10803-016-2882-6 PubMed PMID: ; [PubMed: 27511194]
- Snickers S, Stringer S, Watanabe K, Jansen PR, Coleman JRI, Krapohl E, et al., 2017 Genome-wide association meta-analysis of 78,308 individuals identifies new loci and genes influencing human intelligence PubMed PMID: *Nat. Genet.* 10.1038/ng.3869. [PubMed: 28530673]
- Snyder HR, Miyake A, Hankin BL, 2015 Advancing understanding of executive function impairments and psychopathology: bridging the gap between clinical and cognitive approaches. *Front. Psychol.* 6, 328 10.3389/fpsyg.2015.00328 PubMed PMID: ; [PubMed: 25859234]
- von Ehrenstein OS, Neta GI, Andrews W, Goldenberg R, Goepfert A, Zhang J, 2012 Child intellectual development in relation to cytokine levels in umbilical cord blood. *Am.J. Epidemiol.* 175(11), 1191–1199. 10.1093/aje/kwr393 PubMed PMID: ; [PubMed: 22508393]

- Walker SP, Wachs TD, Grantham-McGregor S, Black MM, Nelson CA, Huffman SL, et al., 2011 Inequality in early childhood: risk and protective factors for early child development PubMed PMID: Lancet 378 (9799), 1325–1338. 10.1016/S0140-6736(11)60555-2. [PubMed: 21944375]
- Wechsler D, 2011 Wechsler Abbreviated Scale of Intelligence. Psychological Corporation, San Antonio, TX.
- Willcutt EG, Doyle AE, Nigg JT, Faraone SV, Pennington BF, 2005 Validity of the executive function theory of attention-deficit/hyperactivity disorder: a meta-analytic review PubMed PMID: Biol. Psychiatry 57 (11), 1336–1346. <https://doi.org/10.1016/j.biopsych.2005.02.006>. [PubMed: 15950006]
- Williamson LL, Sholar PW, Mistry RS, Smith SH, Bilbo SD, 2011 Microglia and memory: modulation by early-life infection. J. Neurosci. Off. J. Soc. Neurosci. 31 (43), 15511–15521. 10.1523/JNEUROSCI.3688-11.2011 PubMed PMID: ; [PubMed: 22031897]
- Wright RJ, Visness CM, Calatroni A, Grayson MH, Gold DR, Sandel MT, et al., 2010 Prenatal maternal stress and cord blood innate and adaptive cytokine responses in an inner-city cohort PubMed PMID: ; PubMed Central PMCID: PMC2902757 Am. J. Respiratory Critical Care Med. 182 (1), 25–33. 10.1164/rccm.200904-0637OC. [PubMed: 20194818]
- Yaguchi M, Ohta S, Toyama Y, Kawakami Y, Toda M, 2008 Functional recovery after spinal cord injury in mice through activation of microglia and dendritic cells after IL-12 administration PubMed PMID: J. Neurosci. Res. 86 (9), 1972–1980. 10.1002/jnr.21658. [PubMed: 18438913]
- Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, et al., 1997 Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. Am. J. Obstetrics Gynecol. 177 (1), 19–26. PubMed PMID: . [PubMed: 9240577]
- Zelazo PD, Jacques S, Burack J, Frye D, 2002 The relation between theory of mind and rule use: evidence from persons with autism-spectrum disorders. Inf. Child. Dev. Special Issue 11 (2), 171–195.

Fig. 1.
Correlogram of Inflammatory Markers.

Table 1

Sample Characteristics.

	Total	No. of Subjects	%	Mean (SD)
<i>Child characteristics</i>				
Gestational age, weeks	240			39.3 (1.3)
Birth weight, grams	244			3280.0 (524.0)
Child age at follow-up assessment	246			4.5 (1.1)
Sex, female	246	125	50.8	
<i>Race/ethnicity</i>				
White	246	84	34.2	
Black/African-American	246	144	58.5	
Hispanic	246	9	3.7	
Other	246	9	3.7	
<i>Maternal characteristics</i>				
Age, years	246			28.0 (5.6)
<i>Maternal Education</i>				
Less than high school	240	38	15.8	
High school diploma	240	51	21.3	
Some college	240	52	21.7	
College graduate	240	99	41.3	
<i>Parity (# of live births)</i>				
None	245	82	33.5	
One	245	78	31.8	
Two	245	49	20.0	
Three or more	245	22	5.7	
Maternal smoking during pregnancy	241	14	19.1	
Pre-pregnancy Body Mass Index (BMI)	239			28.7 (8.5)
<i>Pregnancy characteristics</i>				
Caesarian delivery	244	84	34.4	
Maternal Cytokines				Mean (SD)
IFNy				2.77 (0.75)

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	Total	No. of Subjects	%	Mean (SD)
IL-6				0.98 (0.70)
IL-8				1.44 (0.56)
TNF α				1.56 (0.47)
IL-17A				1.78 (0.87)
IL-12p70				1.48 (0.77)
β -171				1.37 (0.66)

Table 2

Associations between Inflammatory Markers and Child Cognitive Abilities.

	General Conceptual Ability		Verbal Abilities		Nonverbal Ability		Spatial Ability	
	beta (95% CI)	p-value	beta (95% CI)	p-value	beta (95% CI)	p-value	beta (95% CI)	p-value
IFN γ	-3.40 (-6.89, 0.09)	0.111	-2.21 (-5.93, 1.51)	0.329	-2.29 (-6.04, 1.46)	0.317	-3.92 (-8.10, 0.27)	0.126
IL-1 β	-6.07 (-9.34, -2.79)	0.003	-2.64 (-6.14, 0.85)	0.215	-5.72 (-9.24, -2.19)	0.008	-7.26 (-11.32, -3.20)	0.004
IL-12p70	4.26 (1.22, 7.29)	0.023	4.56 (1.33, 7.80)	0.022	4.19 (0.92, 7.45)	0.036	2.38 (-1.33, 6.08)	0.293
IL-17A	3.70 (0.73, 6.67)	0.042	1.26 (-1.90, 4.43)	0.512	3.52 (0.33, 6.71)	0.071	3.24 (-0.34, 6.82)	0.139
TNF α	-1.36 (-5.82, 3.10)	0.617	-4.14 (-8.90, 0.61)	0.154	-1.19 (-5.99, 3.61)	0.683	3.37 (-2.33, 9.07)	0.332
IL-8	-0.08 (-3.73, 3.66)	0.971	4.75 (0.86, 8.63)	0.046	-0.01 (-3.93, 3.91)	0.997	-6.89 (-11.35, -2.43)	0.012
IL-6	0.45 (-2.53, 3.44)	0.804	-0.89 (-4.08, 2.29)	0.645	-1.03 (-4.24, 2.18)	0.597	3.20 (-0.35, 6.75)	0.141

Note: This table shows four separate regression analyses (with rows indicating predictors, and columns indicating outcomes). Each of these four separate regression models are adjusted for the following covariates: child gestational age (in weeks) and birth weight (grams), child age at assessment, maternal pre-pregnancy BMI, race/ethnicity, IQ, education, active smoker status, parity, delivery type, and days of blood draw. **Bold values** indicate statistically significant associations found at the p-value threshold < 0.05.

Table 3

Association between inflammatory markers and NIH Toolbox Performance.

	Overall Child Cognition		Flanker Inhibitory Control		Dimensional Change Card Sort		Picture Sequence Memory		Picture Vocabulary	
	beta (95% CI)	p-value	beta (95% CI)	p-value	beta (95% CI)	p-value	beta (95% CI)	p-value	beta (95% CI)	p-value
IFN γ	-2.67 (-6.61, 2.48)	0.505	-5.10 (-9.95, -0.24)	0.086	-6.24 (-11.02, -1.46)	0.003	-0.39 (-4.42, 3.64)	0.874	-0.04 (-1.86, 1.78)	0.972
IL-1 β	1.27 (-3.04, 5.58)	0.486	-1.08 (-5.65, 3.50)	0.699	-3.02 (-7.54, 1.50)	0.274	0.81 (-3.01, 4.63)	0.727	-0.23 (-1.93, 1.48)	0.827
IL-12p70	1.69 (-2.38, 5.77)	0.688	5.20 (0.96, 9.45)	0.046	5.10 (0.91, 9.28)	0.047	0.63 (-2.97, 4.23)	0.773	0.69 (-0.89, 2.26)	0.474
IL-17A	0.01 (-3.94, 3.96)	0.986	-0.20 (-4.38, 3.98)	0.939	0.44 (-3.67, 4.56)	0.859	-1.28 (-4.78, 2.22)	0.549	-0.14 (-1.68, 1.41)	0.886
TNF α	-0.48 (-6.61, 5.64)	0.985	-5.84 (-12.40, 0.72)	0.145	-4.00 (-10.44, 2.45)	0.309	0.08 (-5.35, 5.50)	0.982	-1.76 (-4.08, 0.56)	0.214
IL-8	-4.03 (-8.86, 0.81)	0.176	-0.83 (-5.96, 4.30)	0.791	3.06 (-1.98, 8.11)	0.320	-4.77 (-9.05, -0.48)	0.069	1.57 (-0.36, 3.49)	0.183
IL-6	2.48 (-1.42, 6.37)	0.338	3.01 (-1.10, 7.13)	0.230	0.49 (-3.57, 4.54)	0.844	3.93 (0.48, 7.38)	0.063	0.20 (-1.35, 1.74)	0.836

Note: This table shows five separate regression analyses (with rows indicating predictors, and columns indicating outcomes). Each of these five separate regression models are adjusted for the following covariates: child gestational age (in weeks) and birth weight (grams), child age at assessment, maternal pre-pregnancy BMI, race/ethnicity, maternal NIH Toolbox measures, education, active smoker status, parity, delivery type, and days of blood draw. **Bold values** indicate statistically significant associations found at the p-value threshold < 0.05.