Title: Maternal prenatal immune activation associated with brain tissue microstructure and metabolite concentrations in newborn infants

Short title: Maternal immune activation and newborn brain development

Marisa N. Spann, PhD, MPH[1,2], Ravi Bansal[*], PhD[3,4], Ezra Aydin[*], PhD[1], Angeliki Pollatou, PhD[1], Kiarra Alleyne, MPH[1], Margaret Bennett, MPH[1], Siddhant Sawardekar, MS[3], Bin Cheng, PhD[2], Seonjoo Lee, PhD[1,2], Catherine Monk, PhD[1,2], Bradley S. Peterson, MD[3,4]

[1]Vagelos College of Physicians and Surgeons, Columbia University, New York, NY
[2]New York State Psychiatric Institute, New York, NY
[3]Children's Hospital Los Angeles, Los Angeles, CA
[4]Department of Psychiatry, Keck School of Medicine, University of Southern California, Los Angeles, CA

[*]Joint second author

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Corresponding Author: Marisa Spann, PhD, MPH, Columbia University Irving Medical Center, 622 West 168th Street, PH Room 1540, New York, NY 10032, Email: mns2125@cumc.columbia.edu, Phone: (646) 774-5824

Key Points

Question: What are the associations of prenatal maternal immune activation (MIA) with newborn brain microstructure, metabolite concentrations, and longitudinal motor development?

Findings: In this longitudinal cohort study we recruited 76 adolescent and young adult pregnant women and assessed maternal interleukin (IL)-6 and C-reactive protein (CRP) levels in the 2nd and 3rd trimesters. These pro-inflammatory markers were significantly associated with brain microstructure and metabolite concentrations in newborns, and longitudinal motor development (prenatally, 4- and 14-months of age).

Meaning: This study suggests that prenatal exposure to MIA has an influence on brain microstructure, metabolite concentration and motor development in offspring.

ABSTRACT

Importance: Few translational human studies have assessed the association of prenatal maternal immune activation with altered brain development and psychiatric risk in newborn offspring.

Objective: To identify the effects of maternal immune activation during the 2nd and 3rd trimesters of pregnancy on newborn brain metabolite concentrations, tissue microstructure, and longitudinal motor development.

Design: Prospective longitudinal cohort study conducted from 2012 – 2017.

Setting: Columbia University Irving Medical Center and Weill Cornell Medical College.

Participants: 76 nulliparous pregnant women, aged 14 to 19 years, were recruited in their 2nd trimester, and their children were followed through 14 months of age.

Exposure: Maternal immune activation indexed by maternal interleukin-6 and C-reactive protein in the 2nd and 3rd trimesters of pregnancy.

Main Outcomes and Measures: The main outcomes included (1) newborn metabolite concentrations, measured as N-acetylaspartate, creatine, and choline using Magnetic Resonance Spectroscopy; (2) newborn fractional anisotropy and mean diffusivity measured using Diffusion Tensor Imaging; and (3) indices of motor development assessed prenatally and postnatally at ages 4- and 14-months.

Results: Maternal interleukin-6 and C-reactive protein levels in the 2nd or 3rd trimester were significantly positively associated with the N-acetylaspartate, creatine, and choline concentrations in the putamen, thalamus, insula, and anterior limb of the internal capsule. Maternal interleukin-6 was associated with fractional anisotropy in the putamen, insula, thalamus, precuneus, and caudate, and with mean diffusivity in the inferior parietal and middle temporal gyrus. C-reactive protein was associated with fractional anisotropy in the thalamus, insula, and putamen. Regional commonalities were found across imaging modalities, though the

direction of the associations differed by immune marker. In addition, a significant positive association was observed between offspring motor development and both maternal interleukin-6 and C-reactive protein (in both trimesters) prenatally and 4- and 14-months of age.

Conclusions and Relevance: Using a healthy sample, these findings demonstrate that levels of maternal immune activation in mid- to late pregnancy associate with tissue characteristics in newborn brain regions primarily supporting motor integration/coordination and behavioral regulation and may lead to alterations in motor development.

Keywords: maternal immune activation, magnetic resonance spectroscopy; diffusion tensor imaging; early brain development

INTRODUCTION

Maternal immune activation (MIA) refers to activation of the innate and adaptive immune system following infection, environmental or psychological stress, and chronic or acute physical illness. MIA is a process that is mediated by activation of inflammatory pathways resulting in increased levels of cytokines that cross the placental and blood-brain barriers[1]– it is the resulting molecular response that alters and shapes the neurodevelopment in the fetus[2-4]. Increasingly, evidence suggests MIA is an important environmental risk factor for neurodevelopmental brain dysfunction – with long-lasting consequences for offspring[5,6]. Existing research (both human and non-human) has focused on long-term changes in brain development and postnatal behavioral outcomes. However, research examining both potential molecular and structural changes that occur in the fetal human brain and relating these potential changes to behavioral markers is limited.

Existing research into the influence of MIA on the developing human brain has largely focused on epidemiological data. These studies report associations between MIA during pregnancy and later neurodevelopment conditions (e.g., schizophrenia[3]). More recently human research studies have shifted to observing both structural and functional influences MIA can have on the developing brain. During activation the immune system releases several classes of proteins to stimulate an immune response. Two of the most commonly assayed immune biomarkers are interleukin (IL)-6, a pro-inflammatory cytokine, and/or C-reactive protein (CRP), an acute phase reactant[5,7], both used to assess the presence and severity of low-grade inflammation. Prior studies found prenatal exposure to IL-6 can influence newborn brain development – a period reflecting prenatal brain development [3,8]. For example, we found maternal IL-6 and CRP levels during the 3rd trimester are associated with altered functional

connectivity of the anterior cingulate and insula with brain regulatory regions, such as the medial prefrontal cortex, in newborns[9]. Others have demonstrated that MIA is associated with an increased strength of anatomical and functional associations across multiple newborn brain networks, including the salience, default mode, and frontoparietal networks[10,11], as well as reduced organization of the uncinate fasciculus, a white matter tract connecting the frontal cortex and amygdala[12]. These findings complement rodent and non-human primate studies suggesting that MIA exposure from immune-activating agents disrupt development of the hippocampus, prefrontal cortex, mid-temporal lobe, parietal lobe, insula, and cingulate cortex[13-15]. At a cellular level, these disruptions largely comprise reduced neuron growth, glial cell proliferation[16-23] and altered cerebellar cytokine and synaptic protein expression[24,25] ultimately resulting in remodeling of the embryonic brain[26].

Existing animal models also highlight potential differential influences of exposure on offspring brain and behavioral development. For example, MIA exposure in early pregnancy (first trimester) has been linked to accelerated increase in brain volume[26] and greater deviations in postnatal neurodevelopmental trajectories in offspring, (e.g., sensorimotor gating and repetitive behaviors[27]). Conversely, MIA exposure later in pregnancy (second trimester) was not associated with behavioral impairments but related to altered developmental trajectories in emotional processing and reward regions of the brain – resulting in decreases in emotional processing ability in adulthood. This demonstrates that MIA during pregnancy can both disrupt and support development of neural systems, in turn this contributes to later offspring capacity for core areas essential for behavioral processes, such as self-regulation. Although pre-clinical findings suggest considerable effects of MIA on the offspring brain, those effects have not been extensively studied in humans[9-11]. Here we aim to provide further insight into low-grade

127 inflammation in healthy pregnancies and its influence on offspring brain development, and in
128 turn, later behavioral outcomes. Given the observed differential effects of MIA exposure *in utero*
129 on the developing prenatal brain in animal models and existing research in this area – it is
130 important to determine (1) whether these differential influences of MIA across gestation can be
131 observed in humans, (2) how these differential brain effects may connect to behavior
132 longitudinally from pre- to postnatal development.

133 One of the earliest brain structures to develop is the cerebellum, emerging from the roof
134 of the rhombencephalon between 4-6 weeks post-conception[28]. This area of the brain is
135 traditionally associated with motor movement and coordination; however, it also connects
136 cortical and subcortical areas. It is via these connections the cerebellum acts as a modulator for
137 many emotional and behavioural functions[29,30] including memory and language. Therefore, we
138 would expect, in humans, to see the influence of low-grade inflammation from the prenatal
139 stages influencing early brain development in offspring, observable via longitudinal behavioral
140 outcomes, particularly in those behaviors associated with the cerebellum (i.e., motor movement).

141 To our knowledge, no studies have assessed the association of MIA with brain metabolite
142 concentrations or tissue microstructure in newborns, which would shed light on the underlying
143 molecular effects of MIA exposure in human newborns. Enhanced understanding of the
144 influence of MIA on the developing brain and later behavioral outcomes will help inform future
145 research and enable researchers and clinicals alike to observe potential deviations. For example,
146 the current pandemic virus, Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2),
147 demonstrated the link between prenatal infections and the cascading inflammatory risk to fetal
148 development [31-37]. This is the first prospective study to assess the association of MIA indices
149 (circulating levels of inflammatory markers IL-6 and CRP) during pregnancy with magnetic

resonance imaging (MRI) measures of microstructural organization and metabolite concentrations in the newborn brain. Given previous associations of MIA with altered newborn brain development[9,11]. We hypothesized that MIA would be associated with increased microstructural organization, as measured by fractional anisotropy (FA) and mean diffusivity (ADC), and elevated concentrations of N-acetylaspartate (NAA), choline (Cho), and creatine (Cr) in prefrontal, temporo-parietal, cingulate, and basal ganglia regions. Furthermore, we hypothesize that these changes in offspring brain organization will be reflected in the longitudinal observations of motor development from pre- to postnatal life.

METHODS

Participants

Nulliparous adolescent and young women aged 14 to 19 years were recruited in the 2nd trimester through the Departments of Obstetrics and Gynecology at Columbia University Irving Medical Center (CUIMC) and Weill Cornell Medical College, and through flyers posted in the CUIMC vicinity as part of a longitudinal study examining adolescent pregnancy behaviors and infant outcomes. Participants reported no major health problems and received routine prenatal care. Approval for the study was given by the Institutional Review Boards of the New York State Psychiatric Institute and of CUIMC. All mothers provided informed written consent. Exclusion criteria included use of recreational drugs, tobacco, alcohol, medications with an effect on cardiovascular function (e.g., beta blockers), or lacking English language fluency. Of the 324 adolescents enrolled during pregnancy, this report includes a sample of 76 infants from whom usable MRI data was obtained (*see supplemental Figure 1 for flow chart of study and data from each timepoint*).

Study Procedures

Immune markers

During their 2nd (24-27 weeks gestation) and 3rd (34-37 weeks gestation) trimesters, women underwent phlebotomy to determine maternal IL-6 and CRP levels. IL-6 was measured using an enzyme-linked immunosorbent assay (ELISA) by R&D systems (Minneapolis, MN). The normal range values for healthy pregnant women during their first trimester is <3.52pg/ml and <4.40pg/ml second and third trimester[38]. CRP was measured using the Cobras Integra 400 Plus (Roche Diagnostics) turbid metric. Normal range values for this population are 0.4-20.3mg/L during the second trimester and 0.4-8.1mg/L in the third[39].

Electronic Health Records

Obstetrical and newborn electronic health records were used to gather information on the participants' pregnancy, labor, and delivery.

Imaging techniques

Infants underwent an imaging protocol within the first weeks of postmenstrual life (mean 42.4, SD 1.6 weeks postmenstrual age at scan). Postmenstrual age (PMA) at the time of the scan was used to subsume variation in gestational age (GA) at birth and time since birth. Infants were assessed with both DTI and MRS. DTI measures both the direction and rate of diffusion water as influenced by tissue microstructure, utilizing both fractional anisotropy (FA) – indexing the degree to which water has a preferential direction of diffusion and average diffusion coefficient

(ADC) – quantifying the overall directionless rate of the diffusion of water in each brain voxel. Infant scans were conducted during natural sleep with no sedation. Foam/wax ear plugs, and ear shields (Natus Medical Inc., San Carlos, CA) were used to damper scanner noise. MRI acquisition, MRS processing and DTI pulse sequence procedures are detailed in the Methods in the supplement.

Longitudinal motor development

Fetal movement (FM) data was collected via a single transabdominal doppler transducer using a Toitu MT 325 fetal echocardiograph (Toitu Co., Ltd, Tokyo, Japan). The Toitu system allows for capturing of fetal heart rate and movement. The received signal is processed through a series of filters, removing frequency components of the Doppler signal that are associated with both fetal and maternal somatic activities allowing for reliable distinction between the two[40]. Postnatal motor development was collected using the BSID-III at 4- and 14-months of age[41]. Scaled (age-standardized) scores were used in statistical analyses.

Statistical Analyses

Analysis was conducted through SAS (Version 9.4) and R (Version 4.0.1). IL-6 and CRP were log-transformed due to their skewed distributions. Spearman correlations and ANOVA F-tests were used to assess the associations of MIA measures with continuous and categorical demographic variables, respectively. Spearman correlations were also used to assess the associations of MIA and brain measures with standardized scores for motor development (gross and fine) on the BSID-III. All continuous variables were standardized by subtracting their means and being divided by their standardized deviations. We applied multiple linear regression at each voxel in template space within each MRI modality to test our hypothesis that MIA would be

associated with motor development in the newborn brain. For DTI analyses, the dependent measure was either FA or ADC, and for MPCSI analyses, it was NAA, Cr, or Cho concentration. The independent variable (MIA) was either prenatal CRP or IL-6. Covariates in all analyses were sex and infant PMA at the time of scan.

We used the topological false discovery rate (FDR) procedure[42] at an FDR=0.05 to control for false positives when conducting multiple hypotheses testing across all voxels in the brain; p-values that survived the FDR correction were color-coded and mapped onto the T1-template.–Furthermore, to account for the correlation in test statistics in MRI due to limited spatial resolution and data smoothing, we applied both a simple conservative modification of the FDR procedure. For additional post hoc analysis, see Methods in the Supplement.

RESULTS

Demographic characteristics

Maternal and newborn demographic characteristics are summarized in *Table 1*. The study included 76 infants (birthweight: M=3224.6g, SD=463.2, GA at birth: M=39.3, SD=1.3 weeks) and were scanned at an average of 42.4 (SD=1.6) weeks PMA. 63.5% of infants were male.

Confounding variables: All participant characteristic variables (inclusive of maternal, newborn and pregnancy) and our primary variables of interest were analyzed to observe any significant relationships. Pregnancy complications (i.e., infections such as chorioamnionitis) were significantly associated with CRP during the 3rd trimester (p=0.0003). In the newborn characteristics, there was a significant association between CRP during the 3rd trimester and GA

at time of birth (r=-0.34, p=0.04). No other significant associations were found between immune markers and demographic variables.

Maternal Immune Marker levels: IL-6 values during the second trimester, mean=1.3pg/ml (SD:0.7), and third mean=1.7pg/ml (SD:0.9). CRP in second trimester mean=5.3mg/L (SD:3.0) and mean=5.4mg/L in the third (SD:3.4). The correlation between IL-6 and CRP was not significant (R=0.39, p=0.08, DF=19)

[Table 1]

Diffusion Tensor Imaging

CRP in the 2nd and 3rd trimester was significantly and inversely associated with newborn FA values in the putamen, ALIC, and thalamus (p<.0001) (*top panel of Figure 1 and Supplemental Figure 2*); 2nd trimester CRP levels were positively associated with ADC values in the orbital gyrus (p values varying between <.001 to <.05).

IL-6 in the 2nd and 3rd trimester was positively associated with FA in the thalamus, insula, and putamen (p<.0001). In addition, 3rd trimester IL-6 was positively associated with FA in the caudate and precuneus (p<.0001). Lastly, IL-6 also demonstrated significant positive correlation with ADC in both the 2nd and 3rd trimester in the inferior parietal and middle temporal gyrus (p values varying between <.001 to <.05) (*see bottom panel of Figure 1 and Supplemental Figure 2*).

[Figure 1]

Magnetic Resonance Spectroscopy

CRP in the 2nd and 3rd trimesters was positively associated with newborn Cho, Cr, and NAA concentrations in regions identified using DTI, specifically the thalamus, insula, caudate,

and precuneus (*Figure 2 and Supplemental Figure 3*). CRP during the 3rd trimester was also positively associated with metabolite concentration in the anterior and posterior limb of the internal capsule (ALIC, PLIC) (*Figure 2 and Supplemental Figure 3*).

[Figure 2]

IL-6 in the 2nd and 3rd trimesters was inversely associated with newborn Cho, Cr, and NAA in several regions, with additional inverse associations in the 2nd trimester seen in the thalamus, putamen, insula and ALIC. IL-6 in the 3rd trimester was inversely associated with Chlo and Cr in the thalamus, and with Cr in the ALIC and putamen (*Figure 3 and scatterplots in Supplemental Figure 3*), and positively with NAA in the putamen and PLIC.

[Figure 3]

Comparison of Associations in 2nd and 3rd Trimesters

When we compared the strength of association between the immune markers and brain microstructural organization and biochemical levels (*see Supplemental Figure 4*), we found that the strength of associations was generally greater with 3rd trimester immune markers and FA, and 2nd trimester immune markers and brain metabolite levels. The differences across the two trimesters for the strength of association of immune markers with MRI measures were generally small (<0.2) (*Supplemental Figure 4 for values*).

Associations of MIA with Longitudinal Motor Development

Prenatal motor development: During the 3rd trimester both IL-6 (R=0.34, p=0.03) and CRP (R=0.41, p=0.01) showed a positive correlation with fetal movement. In the 2nd trimester a significant association was only observed with CRP (R=0.41, p=0.01).

Postnatal motor development: IL-6 during the 2nd trimester was significantly and positively associated with 4-month gross motor scores (R=0.34, p<0.04) and 14-month fine motor movement (R=0.48, p<0.00). IL-6 in the 3rd trimester was positively associated with both fine motor (R=0.49, p<0.003) and gross motor scores (R=-0.37, p=0.03) at 14-months. CRP during the 2nd trimester was significantly associated with fine motor scores (R=0.35, p=0.03) and overall (composite of fine and gross) motor scores (R=0.33, p=0.04) at 4-months of age.

Mediation Analyses

The left thalamus significantly mediated the effect of maternal immune activation (maternal 2nd trimester IL-6) on gross motor movement at 4-months of age (NIE=0.517, p = 0.021). The NDE was estimated as -0.290 (95% bootstrap confidence interval: [-0.896, 0.526]) and the NIE was estimated as 0.517 (95% bootstrap confidence interval: [0.172, 1.344]), and the percentage of effect mediated was 228% of the mediation. In addition, the left PLIC mediated the effect of maternal immune activation (maternal 2nd trimester IL-6) on fine motor movement at 14-months of age (NIE=0.795, p = 0.036). The NIE was estimated as 0.220 (95% bootstrap confidence interval: [-0.667, 2.280]) and the NIE was estimated as 0.795 (95% bootstrap confidence interval: [-0.225, 2.093]), and the percentage of effect mediated was 78.3% of the mediation (*see Figure 4*). No other significant mediations were observed.

[Figure 4]

DISCUSSION

In this prospective longitudinal study, we detected significant associations of prenatal MIA (indexed using CRP and IL-6) with microstructural measures of newborn brain tissue (FA and ADC) and metabolite concentrations (NAA, Cr, and Cho). These associations were generally

consistent across the 2nd and 3rd trimesters, with the direction of associations differing with the specific MIA exposure measure and were centered on subcortical gray matter regions (e.g., thalamus, putamen). In addition, a significant association was observed between prenatal MIA (CRP and IL-6) and offspring motor behaviors. CRP concentration in the 2nd trimester and both IL-6 and CRP concentrations during the 3rd trimester were significantly associated with prenatal movement. Similarly, an association between offspring motor movement at 4- and 14-months of age was significantly associated with both immune markers during both 2nd and 3rd trimesters. Thus, demonstrating maternal inflammation during pregnancy (indexed using CRP and IL-6 concentrations) appears to have implications for the developing newborn brain that is reflected in observations of pre- and postnatal motor development.

The pathogenic importance of the timing of MIA exposure for brain development has been well documented in preclinical studies but has not been well characterized in human studies. Our findings suggest MIA during both 2nd and 3rd trimesters have comparable strength of associations with newborn brain measures and in similar regions across all modalities. During the 2nd and 3rd trimesters of pregnancy the fetal brain experiences rapid growth characterized by dendritic arborization, synaptogenesis and glial cell proliferation[43]. The observed significant inverse association between CRP and FA values putamen, insula, and thalamus during both trimesters, and a positive association of CRP with ADC values in the orbital gyrus during only the 2nd trimester, are suggestive of reduced neuronal integrity and connectivity in the context of low-grade inflammation. Overall demonstrating regardless of the degree, immune activation during pregnancy, has an influence on the developing fetal brain.

In addition, we demonstrate a significant association between MIA markers and newborn microstructural indices and metabolite concentrations, primarily in subcortical brain regions

linked to the cortico-striato-thalamic-cortical (CSTC) circuit, including the insula and precuneus. These findings compliment those from extensive rodent and non-human primate studies, and limited human studies, suggesting that prenatal MIA disrupts the development of widespread brain regions that include the hippocampus, prefrontal cortex, mid-temporal lobe, parietal lobe, insula, and cingulate cortex[13-15]. To date, one other study has considered newborn brain microstructure, also identifying an association with maternal IL-6, however this study was limited to only a single tract, the uncinate fasciculus[12], and to our knowledge, none have considered brain metabolites.

In adults, the CSTC pathway controls important physiological functions, such as control of movement execution, and is a major site of synaptic dysfunction related to behavioral reinforcement and reward[44]. NAA is present primarily in the mitochondria of neurons and therefore is considered a good surrogate marker for neuronal health[45]. It contributes to signaling between neurons and oligodendrocytes and participates in myelin synthesis by oligodendrocytes[46]. Cho is involved in membrane synthesis and degradation, and therefore used as a marker of the structural integrity and turnover of cell membranes[47]. Lastly Cr is mainly involved in the storage and transfer of energy in metabolically active tissues (e.g., the brain). The observed CRP related elevations in NAA, Cho and Cr suggest the presence of greater neuronal density and higher cell membrane turnover in the cingulate gray matter and associated white matter pathways of CSTC circuits. Conversely, in IL-6, inverse associations (at both trimesters) were observed in relation to NAA, Cho and Cr concentrations suggesting a degradation of neuronal density and cell membrane turnover in these areas of the CSTC circuit. These contrasting findings suggest there may be counter effects of different immune markers, some being protective, supporting the developing fetal brain and others being disruptive, negatively

356 impacting the early formation of neuronal pathways and structural growth. However, further
357 studies are necessary with more extensive immune profiles to further elucidate these early
358 influences. While further study is needed to distinguish between these differing directions of
359 association and the implications – our findings suggest that both immune markers (CRP and IL-
360 6) play an important role in supporting the early development of the fetal brain. These results
361 demonstrate a relationship between low-grade inflammation and the developing fetal brain in
362 uncomplicated, healthy pregnancies, supporting the need to further explore this interplay
363 between the maternal immune environment and the fetal brain.

364 Lastly, many of the regions implicated within this study have structural and functional
365 connections with one another and support coordinated higher-order cognition, motor functions
366 and behavioral regulation. For example, basal ganglia, thalamus, and insula are involved in
367 motor coordination, cognitive control, and emotional processing[48]. The anterior and posterior
368 limbs of the internal capsule have short- and long-range fibers that interconnect widespread
369 cortical regions with the basal ganglia, thalamus, and brainstem[49]. Prior studies have reported
370 that prenatal MIA is associated with offspring behavioral disturbances, including increased
371 behavioral reactivity or disinhibition, and deficits in emotion regulation, attention, cognition and
372 memory[11,12,50-54]. Our findings uniquely contribute to the existing literature by demonstrating the
373 longitudinal associations between MIA and motor development from *in utero*. Results highlight
374 the potential associations between MIA markers, prenatal motor movement, newborn CSTC
375 circuit connectivity and later infant motor coordination. Furthermore, we saw the additional
376 influence of MIA to several brain regions consistent with their roles in motor coordination - the
377 basal ganglia, thalamus, and ALIC[12,55]. Our results are consistent with those of a recent study
378 that reported maternal IL-6 at 26 weeks gestation in HIV-exposed but uninfected toddlers was

379 associated with poorer motor skills at 24 to 28 months of age[55]. IL-6 is associated with a lower
380 number of inhibitory synapses and altered morphology of dendritic spines[56]. In contrast, CRP
381 influences synaptic pruning, among other brain development processes.[57] Therefore, alterations
382 maternal CRP or IL-6 during pregnancy may lead to deviations in neuronal development and
383 reduced connectivity across short and long-range brain networks, leading to these observed
384 disruptions in longitudinal motor development.

385 *Strengths and Limitations*

386 To our knowledge, this is one of the few infant neuroimaging studies in humans to
387 consider associations with more than one prenatal immune marker and longitudinal motor
388 development, prior to postnatal influences. Interestingly, while directionality of associations
389 sometimes differed between the immune markers, we saw consistency in influence of these
390 markers (CRP and IL-6) across brain regions. In particular in those involved in CSTC pathways.
391 It is also important to note this study has several limitations.

392 Challenges with follow-up assessments through toddler age led to a reduced sample with
393 behavioral outcome data (*see Figure 1*), limiting statistical power to assess the potential brain-
394 based mediation of associations of MIA with infant motor outcomes. As the original study was
395 interested in stress and nutrition in the context of adolescent pregnancy, the majority of
396 participants were adolescent women and of Hispanic descent. As such, the results may not be
397 generalizable to a more diverse population. Our sample had very few major maternal infections
398 during pregnancy, as reflected in the low rate of obstetrical complications. While it is possible
399 that our measures of MIA reflect inflammation due to infection or other physical health
400 conditions, the findings more likely reflect normal variation of immune levels in the context of
401 adolescent pregnancy and minor infectious illnesses during pregnancy, thus only assessing low

level inflammation. Results in the context of significant MIA may differ. Lastly, there was a limited sample of cortical mantel due to the need for saturation bands that overlap portions of the cortex and the low number of directions for DTI due to our relatively young (newborn) sample.

Conclusion

Given neuroinflammation is consistently identified as playing a role in multiple neuropsychiatric and neurodevelopmental conditions it is important for researchers and clinicians alike to understand the prenatal influence on the next generation from low-grade inflammation during uncomplicated, healthy pregnancies. Future studies with MIA measures that can isolate the time course of innate and adaptive immune pathways could have great utility in establishing whether metabolic (e.g., inflammatory cytokines) or environmental (e.g., microbial, particulate matter) stressors are the primary drivers of the effects of MIA on early brain development.

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Table 1. Maternal and Newborn Demographics

Variables	n	Mean (SD) or %
Maternal		
Age at delivery, years	63	18.2 (1.4)
Pre-pregnancy Body Mass Index (BMI)	63	25.1 (6.2)
Years of Education		
8th grade	1	1.6%
9th grade	7	11.1%
10th grade	8	12.7%
11th grade	22	34.9%
12th grade or higher	25	39.7%
Ethnicity		
Not Hispanic/Latina	6	9.5%
Hispanic/Latina	57	90.5%
Type of Delivery		
Vaginal spontaneous	25	43.1%
Assisted vaginal	23	39.7%
Emergent Cesarean section	10	17.2%
Pregnancy Complications		
None	47	85.5%
Complications (infection)	8	14.5%
Newborn		
Gestational Age at Birth, wks	63	39.3 (1.3)
Birth Weight, gms	63	3224.6 (463.2)
Birth Head Circumference, cms	52	33.9 (1.3)
Apgar 1 minute	56	8.5 (1.2)
Apgar 5 minute	56	8.5 (1.2)
Postmenstrual age at scan	60	42.4 (1.6)
Sex		
Female	23	36.5%
Male	40	63.5%

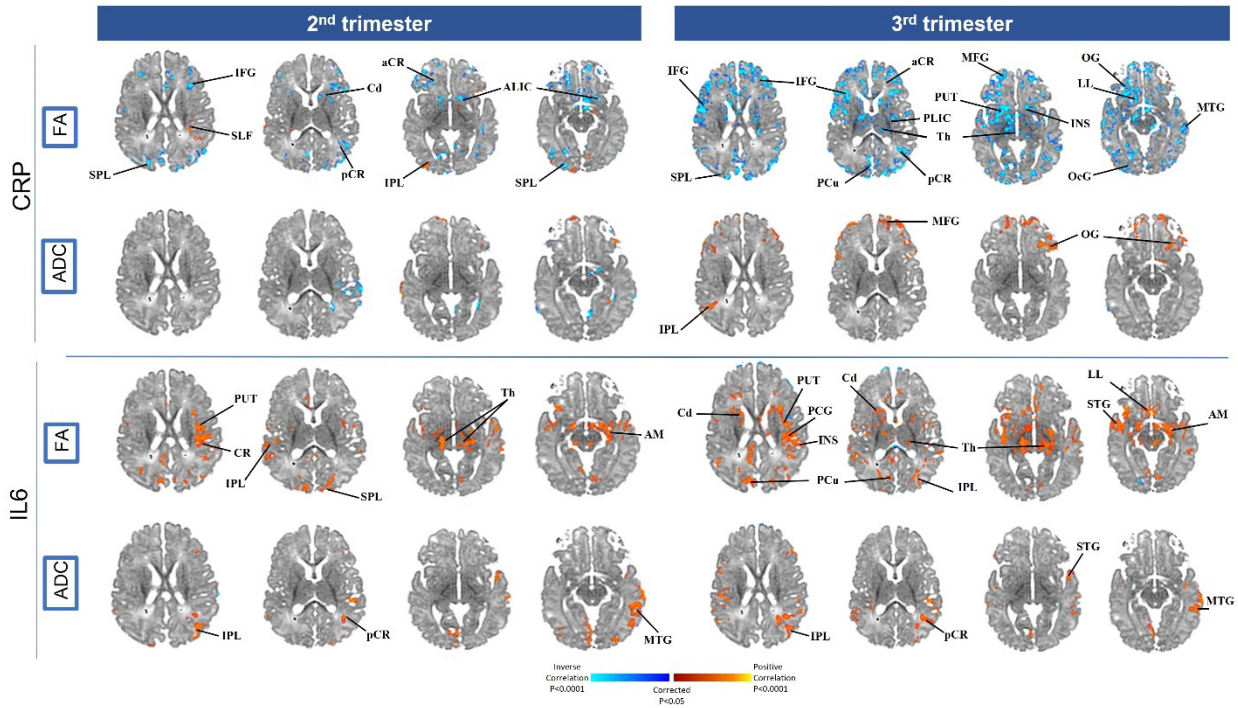

Figure 1: Association between diffusion tensor imaging (DTI), fractional anisotropy (FA) and mean diffusivity (ADC), and maternal immune activation through IL-6 and CRP during the 2nd and 3rd trimester. The red/yellow (positive) and purple/blue (inverse) areas show locations where maternal immune markers are associated with FA and ADC. aCR, anterior region of corona radiata; ALIC, Anterior limb of the internal capsule; Am, Amygdala; Cd, Caudate; CG, cingulum; CR, corona radiata; CC, corpus callosum; Cu, Cuneus; IFG, inferior frontal gyrus; IFO, inferior fronto-occipital; ITG, inferior temporal gyrus; Ins, Insula; IPL, inferior parietal lobule; LL, limbic lobe; MFG, medial frontal gyrus; MB, midbrain; MTG, middle temporal gyrus; OcG, occipital gyrus; OG, orbital gyrus; pCR, posterior region of corona radiata; PCG, precental gyrus; Pcu, Precuneus; PUT, Putamen; ST, stria terminalis; SFG, superior frontal gyrus; SFO, superior fronto-occipital; SLF, superior longitudinal fasciculus; SCR, superior region of corona radiata; SPL, superior parietal lobule; STG, superior temporal gyrus; Th, Thalamus; PLIC, posterior limb internal capsule.

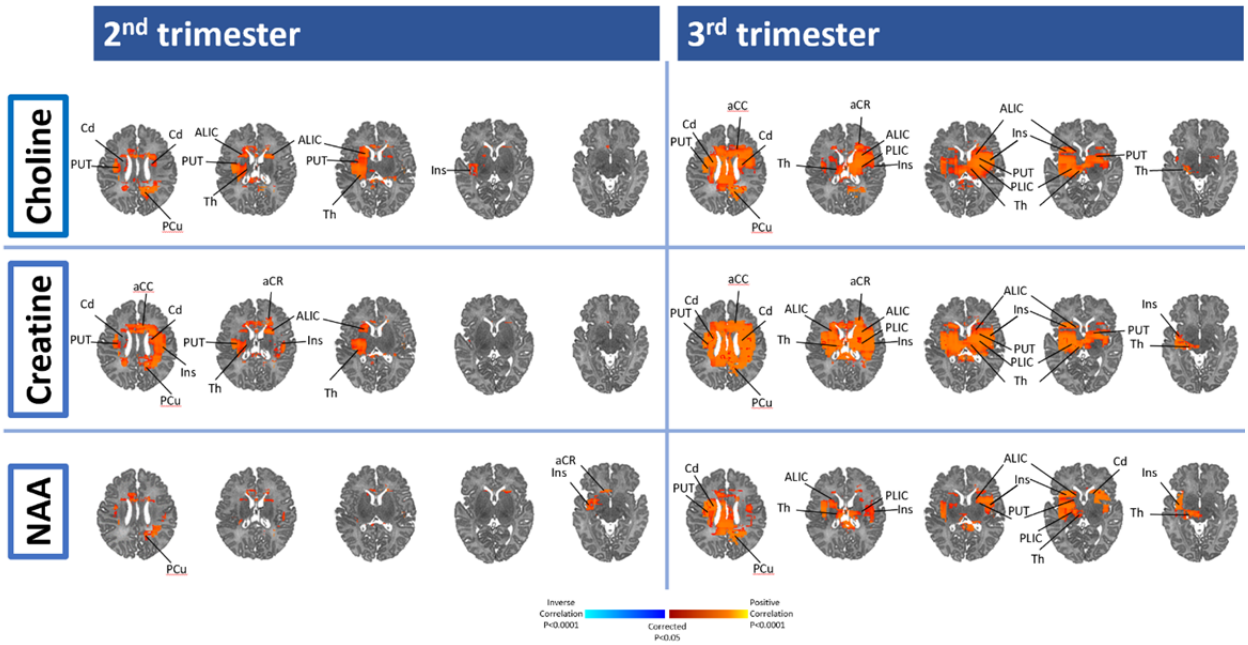

Figure 2: 2nd and 3rd trimester maternal immune marker (CRP) association with neonatal brain metabolites. Using the whole brain, MRS, red/yellow (positive) and purple/blue (inverse) areas show locations where maternal immune markers are associated with Choline, Creatine, and *N*-acetylaspartate. aCC, anterior cingulate gyrus; ALIC indicates anterior limb internal capsule; aCR, anterior region of corona radiata; Cd, Caudate; CR, corona radiata; CC, corpus callosum; Cu, Cuneus; Ins, Insula; MFG, medial frontal gyrus; MB, midbrain; MTG, middle temporal gyrus; pCR, posterior region of corona radiata; PCu, Precuneus; PUT, Putamen; SFG : superior frontal gyrus, STG, superior temporal gyrus; Th, Thalamus; PLIC, posterior limb internal capsule.

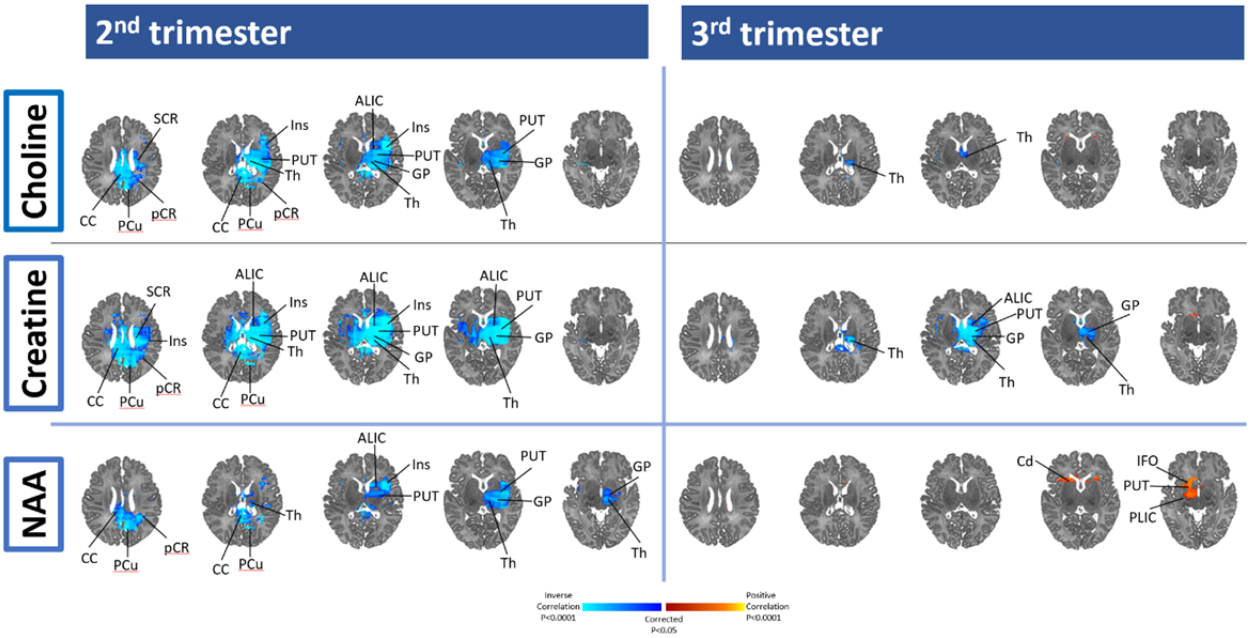

Figure 3: 2nd and 3rd trimester maternal immune marker (IL-6) association with Neonatal brain metabolites. Using the whole brain, MRS, red/yellow (positive) and purple/blue (inverse) areas show locations where maternal immune markers are associated with Choline, Creatine, and *N*-acetylaspartate. ALIC indicates anterior limb internal capsule; aCR, anterior region of corona radiata; Cd, Caudate; CC: cingulate cortex; CR, corona radiata; CC, corpus callosum; Cu, Cuneus; GP, Globus Pallidus; IFO, inferior fronto-occipital; ITG, inferior temporal gyrus; Ins, Insula; MFG, medial frontal gyrus; MB, midbrain; MTG, middle temporal gyrus; pCR, posterior region of corona radiata; PUT, Putamen; SFG, superior frontal gyrus; SFO, superior fronto-occipital; SLF, superior longitudinal fasciculus; SCR, superior region of corona radiata; STG, superior temporal gyrus; Th, Thalamus; PLIC, posterior limb internal capsule.

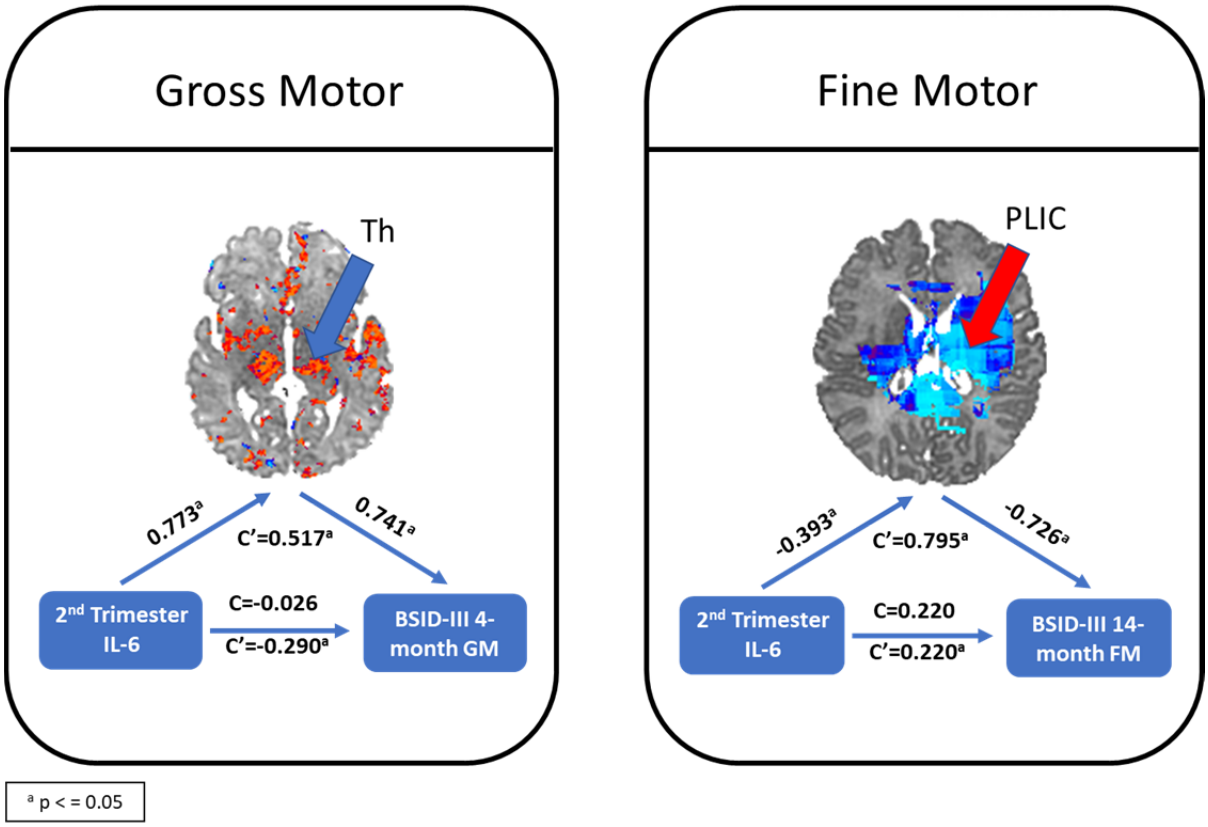

Figure 4: Mediation Model with 2nd trimester IL-6, infant connectivity and BSID-III indices. *Left panel:* mediating effect of 2nd trimester IL-6 to gross motor movement at 4-months of age through the thalamus. *Right panel:* mediating effect of 2nd trimester IL-6 to fine motor movement at 14-months of age through the posterior limb internal capsule. These two data points had significant mediation in our study, but no other significant mediation was observed. C is the direct effect of the exposure on the outcome controlling for the mediator, while C' the mediation effect, that the exposure changed the outcome through the mediator.