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The long-term impact of elevated C-reactive protein levels during pregnancy on brain morphology in late childhood

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ABSTRACT

Importance: Animal studies show that Maternal Immune Activation (MIA) may have detrimental effects on fetal brain development. Clinical studies provide evidence for structural brain abnormalities in human neonates following MIA, but no study has investigated the long-term effects of MIA (as measured with biomarkers) on human brain morphology ten years after the exposure.

Objective: Our aim was to evaluate the long-term impact of MIA on brain morphology in 10-year-old children, including the possible mediating role of gestational age at birth.

Design: We leveraged data from Generation R, a large-scale prospective pregnancy cohort study. Pregnant women were included between 2002 and 2006, and their children were invited to participate in the MRI study between 2013 and 2015. To be included, mother-child dyads had to have data on maternal C-reactive protein levels during gestation and a good quality MRI-scan of the child's brain at age 10 years. Of the 3,992 children scanned, a total of 2,053 10-year-old children were included in this study.

Exposure: Maternal C-reactive protein was measured in the first 18 weeks of gestation. For the analyses we used both a continuous approach as well as a categorical approach based on clinical cut-offs to determine if there was a dose-response relationship.

Main outcomes and measures: High-resolution MRI brain morphology measures were used as the primary outcome. Gestational age at birth, established using ultrasound, was included as a mediator using a causal mediation analysis. Corrections were made for relevant confounders and multiple comparisons. Biological sex was investigated as moderator.

Results: We found a direct association between continuous MIA and lower cerebellar volume. In girls, we demonstrated a negative indirect association between continuous MIA and total brain volume, through the mediator gestational age at birth. We observed no associations with categorical MIA after multiple testing correction.

Conclusion and relevance: Our results suggest sex-specific long-term effects in brain morphology after MIA. Categorical analyses suggest that this association might be driven by acute infections or other sources of severe inflammation, which is of clinical relevance given that the COVID-19 pandemic is currently affecting millions of pregnant women worldwide.

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1. Introduction

Prenatal life is a period of rapid neurodevelopment in which the brain is susceptible to environmental exposures, such as maternal immune activation (MIA) (Bergdolt and Dunaevsky, 2019; Knuesel et al., 2014). The mother's immune system can be triggered during pregnancy by infections or inflammatory triggers, such as stress, obesity, and mental health problems (Rieder et al., 2017; Cox et al., 2015). What we know from animal studies is that MIA can lead to an inflammatory response in the fetal circulation and brain within hours after the inflammatory stimulus (Bergdolt and Dunaevsky, 2019; Knuesel et al., 2014; Malek et al., 2006; Bronson and Bale, 2014; Ozaki et al., 2020). CRP, an acute-phase reactant, is a reliable and widely used marker to measure infection and inflammation (Ablij and Meinders, 2002), and here we used CRP as a proxy for MIA (Gabay and Kushner, 1999; Pepys and Hirschfield, 2003). During typical fetal brain development, microglia act as primary immune mediators of neurodevelopment. They also regulate important neurodevelopmental processes from neuronal differentiation to neural circuit formation (Han et al., 2021). Thus, alterations in microglia function due to MIA could play a pivotal role in the development of atypical brain structure and function (Knuesel et al., 2014; Gumusoglu and Stevens, 2019; Bilbo et al., 2018). This is of particular importance in the COVID-19 pandemic (Villar et al., 2021).

Animal studies demonstrate that MIA is associated with reduced volumes of the fetal hippocampus and amygdala (Crum et al., 2017; Antonson et al., 2018); and increased white matter volume (Crum et al., 2017; Duchatel et al., 2016). To date, human neuroimaging studies of MIA have shown altered brain structure and function in neonates and toddlers (Rasmussen et al., 2019; Graham et al., 2018; Rudolph et al., 2018). These studies were small ($N \leq 86$) and did not include potential mediators, such as gestational age at birth or potential moderators such as biological sex. Preterm delivery is associated with both elevated CRP levels in pregnant women and atypical child brain morphology (Ernst et al., 2011; Lohsoonthorn et al., 2007; El Marroun et al., 2020). Moreover, the male fetal placental-unit has been shown to be more sensitive to MIA compared to females (Hunter et al., 2019), and spontaneous preterm delivery occurs more often in males (Peelen et al., 2016), which may be due to the generally higher expression of inflammatory markers in male placentas (Goldenberg et al., 2006; Clifton, 2010). Further, considering the plasticity of the human brain, it's unclear if these effects persist longer-term. Thus, studies with larger cohorts and longer follow-up are warranted. Here, our aim was to evaluate the long-term impact of MIA on brain morphology in 10-year-old children, including gestational age at birth as a possible mediator. Based on animal studies demonstrating long-term effects, our hypothesis was that MIA during pregnancy has long-term effects on brain morphology, and specifically volumetric differences in white matter, the hippocampus, and the amygdala. Moreover, we expected to find a mediating effect of gestational age at birth, specifically a moderated-mediating effect in the direction of preterm birth, and a stronger effect in males compared to females.

2. Methods

2.1. Study selection and participants

This study leveraged the Generation R Study, a large prospective population-based cohort investigating the development of child health from fetal life onwards (Jaddoe et al., 2012). The inclusion criteria for the Generation R Study were as follows: women needed to i) be pregnant, ii) live in Rotterdam, the Netherlands, and iii) have a delivery date between April 2002 and January 2006. There were no additional exclusion criteria. The response rate at baseline for the Generation R Study was 61%, resulting in a sample of 9,778 mothers. Postnatally, these mothers and their children have been assessed in multiple follow-up waves. For the research visit at 9–12 years of age, 8,548 children

were invited to participate (Jaddoe et al., 2012; White et al., 2018). During this visit, 3,992 children were scanned between March 2013 and November 2015 using Magnetic Resonance Imaging (MRI) (White et al., 2018). The Medical Ethics Committee of the Erasmus Medical Centre approved all study procedures. All parents provided written informed consent and children provided assent (White et al., 2018).

We included mother–child pairs with measures of maternal CRP during pregnancy and MRI data in offspring aged 9–12 years. Participants were excluded if they were scanned under a different scanning sequence; had unusable MRI data due to poor image quality, dental braces, or incidental findings that significantly altered brain morphology. In case of twins or siblings, one was randomly excluded.

2.2. MIA assessment

Venous blood samples were collected in EDTA tubes during a research visit in early pregnancy (≤ 18 weeks of gestation). Plasma samples were processed and stored at -80°C (-112°F) (Jaddoe et al., 2007; Kooijman et al., 2016) by the regional laboratory (Star-MDC, Rotterdam, The Netherlands). High-sensitivity (hs)-CRP was measured at the Department of Clinical Chemistry of the Erasmus MC in 2009. CRP concentrations were measured in EDTA plasma samples using an immunoturbidimetric assay on the Architect System (Abbot Diagnostics B.V., Hoofddorp, The Netherlands). The interassay variation at 12.9 mg/L and 39.9 mg/L was 0.9% and 1.3%, respectively. The lowest possible level of detection was 0.2 mg/L (Ernst et al., 2011; de Jonge et al., 2011).

2.3. Gestational age at birth assessment

Gestational age was established via ultrasound examinations during prenatal visits at the research center (El Marroun et al., 2020). Details of the standardized method for fetal ultrasonographic measurements can be found elsewhere (Verburg et al., 2008).

2.4. Image acquisition

Before scanning, the children underwent a mock session for acustomization with the scanning environment. Structural MRI was performed in the Erasmus MC Radiology Department with a 3-Tesla General Electric 750w Discovery MR System (General Electric Healthcare, Milwaukee, WI, USA) with an 8-channel head coil (White et al., 2018). The structural MRI images were obtained with high resolution T_1 -weighted 3D coronal inversion recovery fast spoiled gradient recalled (IR-FSPGR, BRAVO) sequence (White et al., 2018). This sequence had the following parameters: repetition time = 8.77 ms, echo-time = 3.4 ms, inversion-time = 600 ms, number of excitations = 1, flipangle = 10° , readout bandwidth = 25.0 kHz, acquisition matrix size = 220×220 , acceleration factor = 2, number of slices = 230, slice thickness = 1 mm and an in-plane resolution of 1.0 mm^2 (White et al., 2018).

2.5. Image processing

To process images, the FreeSurfer version 6.0 analysis suite was used to conduct a standard cortical reconstruction (see Supplement) (White et al., 2018; Fischl, 2012; Desikan et al., 2006; Muetzel et al., 2018; Muetzel et al., 2019). The volumes (in mm^3) of the following brain measures were used as the outcome variables: total brain, cortical gray matter, total white matter, subcortical gray matter, cerebellum cortex, cerebrospinal fluid, hippocampus, and amygdala. Because left and right hemisphere volumes are highly correlated (cerebellum: $r = 0.97$, $p < 2.2 \times 10^{-16}$; hippocampus: $r = 0.84$, $p < 2.2 \times 10^{-16}$; amygdala: $r = 0.74$, $p < 2.2 \times 10^{-16}$), and because we had no hypothesis regarding lateralized effects, we created global measures by summing the right and left structures. Our measure of cerebrospinal fluid (CSF) refers to all CSF within the intracranial volume. This includes the lateral ventricles, third

ventricle, fourth ventricle and surface CSF.

2.6. Confounders/covariates

Confounders and covariates were selected based on literature (Gegenhuber and Tollkuhn, 2020; Damoiseaux, 2017; Knickmeyer et al., 1991, 2017; Hair et al., 2015; El Marroun et al., 2016; Cantacors et al., 2018; Lautarescu et al., 2020; Soneji and Beltrán-Sánchez, 2019; Weile et al., 2020; Lee et al., 2012; Wang et al., 2010; Henshaw et al., 2021; Lahti-Pulkkinen et al., 2020). See Supplement for more information on the measurement method on each covariate and confounder. Information on the child's biological sex and birthweight was obtained from midwives and hospital registries. Information on inflammatory comorbidities of pregnancy was retrieved from birth records after delivery. The age at MRI was calculated based on the child's date of birth and the date of the MRI appointment. Information on maternal national background (categories based on previous literature) (Kooijman et al., 2016), maternal age, maternal psychotropic medication use, maternal smoking, alcohol, and psychoactive substance use during pregnancy was assessed with a questionnaire at enrollment. Prenatal maternal psychopathology was measured with a validated self-reported questionnaire (Brief Symptom Inventory). From this 53-item questionnaire (de Beurs, 2004), a Global Severity Index was calculated that served as a continuous score with higher scores indicating more problems. Information on maternal education and household income was obtained via questionnaires when the children were 5–8 years old. See Supplement for more information on the measurement method and selection procedure of the covariates and confounders.

2.7. Statistical analyses

All analyses were conducted in R (version 3.6.1) (R-project. <https://www.r-project.org>). For all analyses, the mediator and the outcomes were used as continuous variables. To establish whether the effect of maternal CRP on brain morphology had a linear relationship or a relationship above an a priori cut-off, all analyses were performed with CRP used as both continuous and categorical exposures. The following clinical cut-offs were used for the categorical CRP variable: <1 mg/L (no MIA), 1–3 mg/L (low-grade MIA), 3–10 mg/L (moderate MIA) and >10 mg/L (high MIA). The 'no MIA' (CRP <1 mg/L group) was used as the reference group (Póvoa et al., 2005; Pearson et al., 2013; Mihu et al., 2008; Xu et al., 2021; Nehring et al., 2021).

Causal mediation analyses (VanderWeele, 2016; Imai et al., 2011) were conducted for all eight child brain morphology outcomes with continuous gestational age at birth as mediator in the mediation package in R (R mediation package. <https://cran.r-project.org/web/packages/mediation/mediation.pdf>; Imai et al., 2010). The natural direct effect (NDE) is the effect of CRP on child brain morphology if the effect of gestational age at birth was what it would have been if all mothers were to have low CRP. The natural indirect effect (NIE) is the effect of CRP on child brain morphology if all mothers were to have high CRP, in which gestational age at birth is set to what it would have been with low compared to high CRP. The total effect (TE) denotes the overall effect of the exposure on the outcome (NDE + NIE). The proportion mediated (PM) refers to alterations in brain morphology that are mediated by gestational age at birth and is calculated as the ratio of NIE over TE.

Analyses were conducted using a three-step approach. Model 1 was minimally adjusted for child's sex, child's age at MRI and, due to the gradual increase in inflammation during pregnancy, gestational age at which CRP was measured. Model 2 was further adjusted for maternal national origin, maternal education, household income and maternal age. Model 3 was additionally adjusted for psychoactive substances usage, smoking, and drinking during pregnancy, and maternal psychopathology. Model 4 was further adjusted for total brain volume for all the regional brain volume outcomes. Maternal BMI was not included because it may be on the causal pathway (Howell and Powel, 2017;

Minakova and Warner, 2018). Moreover, a moderation analysis (and subsequent stratification in case of significance) was conducted with biological sex. Further, we also explored the mediatory role of continuous birth weight in the association between continuous CRP and child brain morphology.

Then, we conducted four sensitivity analyses. First, we conducted a sensitivity analysis repeating model 4 without the covariate 'maternal psychopathology'. Second, we conducted a sensitivity analysis excluding the mothers who used any type of medication two months before or during pregnancy, and third, we performed a sensitivity analysis excluding the mothers who used psychotropic medication during pregnancy. Lastly, to investigate whether inflammatory comorbidities of pregnancy impacted our results, we conducted a sensitivity analysis excluding mothers with pregnancy induced hypertension, pre-eclampsia, pre-existing hypertension based on medical records, superimposed preeclampsia/HELLP (hemolysis, elevated liver enzymes, and low platelets), and diabetes gravidarum. Because we hypothesized the mediating effect of gestational age at birth to be in the direction of preterm birth, an interaction between the exposure and mediator was examined and was added to all models in cases of significance.

To correct for multiple testing, Bonferroni correction was applied for eight tests, the alpha level was set to 0.006. Missing data for confounders and covariates were imputed in R with multiple imputation using chained equations (R mice package. <https://cran.r-project.org/web/packages/mice/mice.pdf>). The maximum of missingness for a confounder was 15.9%. To assess differences in participant characteristics between the mother-child pairs in the study population and the excluded sample, a non-response analysis was conducted using chi-square tests and t-tests (see Supplement). We compared all demographic variables mentioned in Table 1.

3. Results

3.1. Demographics participants

A total of 3,968 T₁-weighted MRI scans were available. Following exclusion of children scanned under a different scanning sequence (n = 22), those with incidental findings (n = 25), braces (n = 88), no or unusable FreeSurfer reconstruction (n = 647), no maternal serum CRP available (n = 1,044), and random exclusion of one twin/sibling (n = 89), a total of 2,053 children were included (Fig. 1). The participant characteristics are shown in Table 1.

3.2. Child brain morphology

Table 2 shows the direct associations between continuous measures of CRP and child brain morphology, and the indirect effect via gestational age at birth for model 4. Results for the other models are shown in Supplementary Table 1. In model 4, we found a negative association between CRP levels and child cerebellar volume ($\beta = -0.054$; 95%CI = -0.087, -0.020; p = 0.001) (Fig. 2). The relationship between MIA and child cerebellar volume remained significant after multiple testing correction. These associations were not mediated by gestational age at birth (Table 2) nor by birth weight (Table 4). Results of categorical measures of CRP can be found in Table 3 and Supplementary Table 2. Child cerebellar volume was found to be negatively associated with MIA in model 4 of CRP > 10 mg/L compared to CRP < 1 mg/L ($\beta = -0.215$; 95%CI = -0.368, -0.060; p = 0.008) (Fig. 2). However, this association was no longer significant after multiple testing correction.

3.2.1. Sex-specific effects

Biological sex was found to be an effect-modifier in some models (Supplementary Tables 3, 4). In analyses stratified by sex, we observed a negative indirect association in females between MIA and total brain volume via the mediator gestational age at birth. We observed no direct effects in females, nor did we observe any direct or indirect effects in

Table 1
Baseline characteristics participants.

MOTHERS		MRI sample (n = 2,053)	Males (n = 1,012)	Females (n = 1,041)
General characteristics				
	Age at enrollment (mean, SD)	30.8 ± 4.7	31.0 ± 4.7	30.6 ± 4.6
	Pre-pregnancy BMI (kg/m ²) (mean, SD)	23.4 ± 4.1	23.3 ± 4.0	23.6 ± 4.1
National background				
	Dutch (%)	1203 (58.6)	604 (59.7)	599 (57.5)
	Other Western (%)	245 (11.9)	119 (11.8)	126 (12.1)
	Non-Western (%)	557 (27.1)	280 (27.7)	297 (28.5)
	Missing (%)	28 (1.4)	9 (0.9)	19 (1.8)
Education				
	Low (no/primary education) (%)	40 (1.9)	18 (1.8)	22 (2.1)
	Intermediate (secondary school, lower vocational training) (%)	621 (30.2)	286 (28.3)	335 (32.2)
	High (higher vocational training, university) (%)	1143 (55.7)	588 (58.1)	555 (53.3)
	Missing (%)	249 (12.1)	120 (11.9)	129 (12.4)
Household income				
	< €2000 (%)	6 (0.3)	6 (0.6)	0 (0.0)
	> €2000 (%)	1720 (83.8)	852 (84.2)	868 (83.4)
	Missing (%)	327 (15.9)	154 (15.2)	173 (16.6)
Psychoactive drug use				
	No (%)	1790 (87.2)	874 (86.4)	916 (88.0)
	Yes, until pregnancy (%)	38 (1.9)	23 (2.3)	15 (1.4)
	Yes, continued during pregnancy (%)	0.5 (0.5)	7 (0.7)	3 (0.3)
	Missing (%)	215 (10.5)	108 (10.7)	107 (10.3)
Smoking habits				
	Never smoked during pregnancy (%)	1414 (68.9)	690 (68.2)	724 (69.5)
	Smoked until pregnancy was known (%)	178 (8.7)	78 (7.7)	100 (9.6)
	Continued smoking in pregnancy (%)	261 (12.7)	138 (13.6)	123 (11.8)
	Missing (%)	200 (9.7)	106 (0.5)	94 (9.0)
Alcohol consumption				
	Never drank in pregnancy (%)	712 (34.7)	324 (32.0)	388 (37.3)
	Drank until pregnancy was known (%)	293 (14.3)	141 (13.9)	152 (14.6)
	Continued drinking occasionally (%)	733 (35.7)	393 (38.8)	340 (32.7)
	Continued drinking frequently (1 or more glass/week for at least 2 trimesters) (%)	179 (8.7)	82 (8.1)	97 (9.3)
	Missing (%)	136 (6.6)	72 (7.1)	64 (6.1)
Comorbidities				
	Psychopathology (GSI)	0.15 (0.06 – 0.31)	0.13 (0.06 – 0.31)	0.15 (0.06 – 0.29)
Exposure				
	CRP <1 mg/L (n)	177		
	CRP 1–3 mg/L (n)	605		
	CRP 3–10 mg/L (n)	991		
	CRP >10 mg/L (n)	369		
	CRP (mg/L) (median, IQR)	4.30 (2.20 – 7.90)	4.30 (2.30 – 7.90)	4.40 (2.20 – 7.90)
	Time of blood sampling in pregnancy (weeks) (mean, SD)	13.3 ± 1.9	13.4 ± 1.9	13.3 ± 1.9
CHILDREN				
General characteristics				
	Biological sex, male (%)	1012 (49.3)		
	Birthweight (grams) (mean, SD)	3436 ± 559	3513.2 ± 548.8	3360.6 ± 558.3
	Gestational age at birth (weeks) (mean, SD)	40.0 ± 1.8	40.0 ± 1.7	39.9 ± 1.8
	Age MRI (years) (mean, SD)	10.1 ± 0.6	10.2 ± 0.6	10.1 ± 0.6
	Preterm birth, <37 weeks (%)	93 (4.5)	42 (4.2)	51 (4.9)
	Low birthweight, <2500 g (%)	95 (4.6)	39 (3.9)	56 (5.4)
Outcome				
	Total brain volume (mm ³) (mean, SD)	1214903 ± 109306	1270400 ± 98819	1160951 ± 90392

[†]When continuous variables were normally distributed the values are presented mean ± SD and when skewness was observed the values are presented as median (interquartile range).

males.

3.2.2. Sensitivity analyses

The results did not change after omitting maternal psychopathology as covariate in model 4 (see [Supplementary Table 5](#)). Further, the results did not change after excluding the mothers who used any type of medication two months before or during pregnancy (see [Supplementary Table 6](#)), nor did the results change after excluding the mothers who used psychotropic medication during pregnancy (see [Supplementary](#)

[Table 7](#)). And lastly, the results remained unchanged after excluding mothers with inflammatory comorbidities of pregnancy (see [Supplementary Table 8](#)).

3.3. Exposure and mediator interaction

None of the interaction terms between maternal CRP and gestational age at birth were significant ([Supplementary Table 9](#)). There were no moderated-mediating effects, only pure mediating effects.

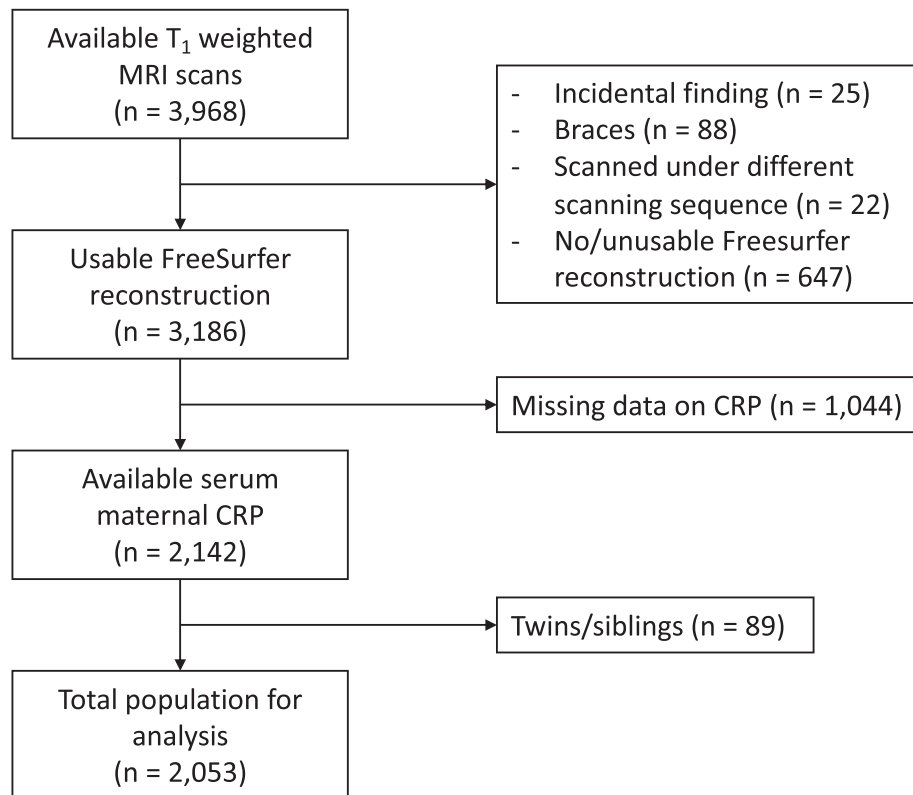


Fig. 1. Flowchart study population.

Table 2

Association between continuous CRP levels and child brain morphology at 9–12 years (model 4).

	Total effect		Natural direct effect		Natural indirect effect		Proportion mediated (%)**
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	
Total brain volume	-0.003 (-0.037, 0.030)	0.9	0.001 (-0.033, 0.040)	0.9	-0.004 (-0.009, 0.000)	0.06	-
Cortical gray matter volume	0.002 (-0.011, 0.010)	0.8	0.002 (-0.010, 0.010)	0.8	-0.002 (-0.001, 0.000)	0.7	-
Total white matter volume	0.001 (0.000, 0.020)	0.1	0.001 (0.000, 0.020)	0.1	0.000 (0.000, 0.000)	0.1	0
Subcortical gray matter volume	-0.010 (-0.033, 0.010)	0.4	-0.008 (-0.031, 0.020)	0.5	-0.002 (-0.003, 0.000)	0.09	20.0
Cerebellum cortex volume	-0.055 (-0.088, -0.020)	0.001*	-0.054 (-0.087, -0.020)	0.001*	-0.001 (-0.003, 0.000)	0.3	1.8
Cerebrospinal fluid volume	-0.029 (-0.071, 0.010)	0.2	-0.030 (-0.071, 0.010)	0.2	0.000 (-0.002, 0.000)	0.3	0
Hippocampus volume	0.019 (-0.013, 0.050)	0.2	0.019 (-0.013, 0.050)	0.2	0.000 (-0.002, 0.000)	0.5	0
Amygdala volume	0.021 (-0.010, 0.050)	0.2	0.020 (-0.011, 0.050)	0.2	0.000 (0.000, 0.000)	0.2	0

†Significant results are presented in bold.

† Model 1 was corrected for sex, child age at assessment, gestational age at which CRP was measured, model 2 was additionally corrected for maternal national background, maternal education, household income and maternal age, and model 3 was additionally corrected for psychoactive substances, smoking, alcohol, and maternal psychopathology. Model 4 was further adjusted for total brain volume for all the regional brain volume outcomes.

†For easier interpretation of multiple outcomes with different ranges, the exposure, mediator and the outcome were all standardized to enable comparison.

†The TE, NDE and NIE (with corresponding p-values and 95% confidence intervals) were calculated with quasi-Bayesian methods (sampling $n = 10,000$). To compute the confidence intervals the bias-corrected and accelerated (BCa) intervals were chosen.

*Represents significant p-values after Bonferroni correction.

**The proportion mediated is only displayed when it could be calculated, i.e., when the NDE and NIE were in the same direction.

4. Discussion

Leveraging a large population-based prospective cohort, our study demonstrates long-term associations between MIA during pregnancy and child brain morphology in humans. Specifically, we found that children of mothers with elevated CRP levels during early pregnancy showed smaller cerebellar volumes. Sex-specific indirect associations among females via gestational age at birth were found for total brain

volume. There was no indirect effect of gestational age at birth nor birth weight in the full sample, nor did we find a moderated-mediating effect (i.e., preterm birth was not a mediator) in any of the models.

4.1. MIA and child brain morphology

There has been increasing interest in the role of the cerebellum, not only in disorders of movement, but also in disorders of behavior and

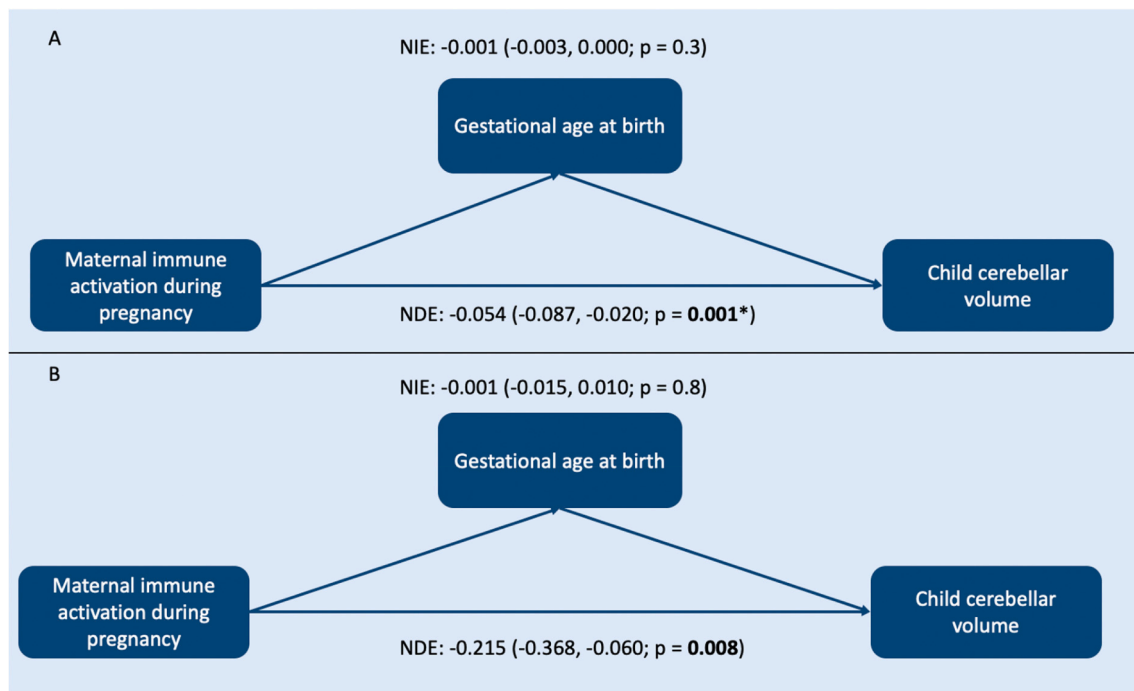


Fig. 2. Mediation analysis. The exposure is ‘maternal immune activation during pregnancy’, the mediator ‘gestational age at birth’ and the outcome ‘child cerebellar volume’. P-values in bold represent $p < 0.05$ and an asterisk represents significant after correcting for multiple testing ($p < 0.006$). Fig. 2A represents the natural direct effect (NDE) and natural indirect effect (NIE) for continuous CRP as measure of MIA. Fig. 2B represents the NDE and NIE for categorical CRP as measure of high MIA (>10 mg/L compared to the reference group < 1 mg/L).

(social) cognition (Stoodley, 2016). The cerebellum has been shown to entail unique microglial activity different from other brain regions (Ayata et al., 2018; Kana et al., 2019). Animal studies have found that microglial priming, which may occur in MIA, impacts the cerebellum, resulting in reduced volume (Childs et al., 2021). Hence, microglial priming may be a mechanism underlying our finding between MIA and reduced child cerebellar volume. Moreover, mouse studies investigating MIA have shown a reduction in GABA-ergic cells and specifically Purkinje cells in the cerebellum (Bergdolt and Dunaevsky, 2019; Pendyala et al., 2017; Wang and Kriegstein, 2009). The reduction in these cells could contribute to synaptic impairment, but how this reduction translates to volumetric changes is unknown (Pendyala et al., 2017). Both GABA-ergic and Purkinje cells are some of the earliest active neurons in utero (Wang and Kriegstein, 2009; Dusart and Flamant, 2012).

Interestingly, the developmental trajectory of the cerebellum is unique compared to the cerebral cortex (Tiemeier et al., 2010). The contribution of specifically the cerebellum to typical neurodevelopment involves optimization of the cerebro-cerebellar circuits, which is related to multiple domains of optimal neurodevelopment. If this process is disrupted in its structure or function, long-term alterations in the cerebro-cerebellar circuit can consequently significantly impact behavior (Stoodley, 2016). As such, a reduced cerebellar volume may be related to adverse neurodevelopment, such as autism spectrum disorder (ASD), ADHD, developmental dyslexia, and atypical social-behavioral development (Stoodley, 2016; Bruchhage et al., 2018; Steinlin, 2008; Kelly et al., 2020; Stoodley and Limperopoulos, 2016). In studies investigating brain morphometrics and ASD, the cerebellum is one of the most consistently observed regions (D’Mello and Stoodley, 2015). Moreover, cerebellar damage is linked to an increased risk of ASD (Okada et al., 2021). Dysfunction in the previously mentioned cerebro-cerebellar circuits, which are related to cortical regions in the cerebrum, contain functions connected to language and social processing, which may underlie ASD symptoms such as deficits in social interaction and showing repetitive behavior (D’Mello and Stoodley, 2015).

Contrary to the literature, we found no association of MIA on

volumetric measures of white matter, the hippocampus, or the amygdala. One positive explanation is that protective or neuroplastic mechanisms either prevent or reverse long-term alterations resulting MIA. Previous research demonstrating long-term effects were carried out in animals, which differ greatly from humans regarding brain development (Oberheim et al., 2009; Kaas, 2013). Another difference is the severity of inflammation and infection caused by a inflammatory triggers in lab-based animals compared to human population-based studies, where the former has much greater control over the level of MIA and confounding variables and where the latter has a wide spectrum of the severity of MIA. This emphasizes the importance of long-term follow-up in human studies. So far, human literature on the effects MIA on structural brain abnormalities has been limited to a follow-up of two years (Graham et al., 2018; Rudolph et al., 2018). Considering the inherent plasticity of the developing brain, early effects of MIA may be corrected via downstream developmental processes, even though our earlier work suggests limitations in postnatal alterations resulting from prenatal events (Ars et al., 2019; Zou et al., 2021). One study in rats found that volumetric reduction in both the hippocampus and the amygdala only occurred at postnatal day 50 but not 100 (Crum et al., 2017). Similarly, structural differences in the amygdala in relation to MIA observed at two years of age (Graham et al., 2018) may no longer be evident in late childhood in humans. Alternatively, the time period could be an explanation. We defined MIA as elevated CRP levels in the first eighteen weeks. However, it is possible that MIA impacts developmental processes occurring later in pregnancy. An example of such processes is myelination, which is also affected by an overactivation of microglial cells and initiates around the 29th week of gestation (Knuesel et al., 2014; Gumusoglu and Stevens, 2019; Antonson et al., 2018; Meyer et al., 2006). Earlier work in animals studies have shown impairments in myelination to be related to hippocampal and white matter deficits (Knuesel et al., 2014; Antonson et al., 2018).

Table 3
Association between categorical CRP levels and child brain morphology at 9–12 years (model 4).

	Total effect		Natural direct effect		Natural indirect effect		Proportion mediated (%)**
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	
Total brain volume							
CRP 1–3 mg/L	−0.040 (−0.186, 0.100)	0.58	−0.037 (−0.182, 0.100)	0.62	−0.004 (−0.021, 0.010)	0.59	10.0
CRP 3–10 mg/L	−0.008 (−0.157, 0.140)	0.92	−0.008 (−0.139, 0.150)	0.91	−0.016 (−0.042, 0.010)	0.18	66.7
CRP > 10 mg/L	−0.054 (−0.222, 0.110)	0.53	−0.035 (−0.201, 0.130)	0.68	−0.020 (−0.054, 0.010)	0.17	37.0
Cortical gray matter volume							
CRP 1–3 mg/L	0.007 (−0.047, 0.060)	0.81	0.007 (−0.047, 0.060)	0.81	0.000 (−0.003, 0.000)	0.98	0
CRP 3–10 mg/L	0.040 (−0.011, 0.090)	0.12	0.040 (−0.010, 0.090)	0.12	0.000 (−0.003, 0.000)	0.93	0
CRP > 10 mg/L	0.031 (−0.028, 0.090)	0.29	0.034 (−0.026, 0.090)	0.27	−0.002 (−0.009, 0.000)	0.37	–
Total white matter volume							
CRP 1–3 mg/L	0.001 (−0.059, 0.060)	0.99	−0.001 (−0.061, 0.060)	0.97	0.002 (−0.003, 0.010)	0.48	–
CRP 3–10 mg/L	−0.013 (−0.070, 0.040)	0.64	−0.014 (−0.071, 0.040)	0.62	0.001 (−0.002, 0.010)	0.57	–
CRP > 10 mg/L	0.021 (−0.044, 0.090)	0.51	0.018 (−0.047, 0.080)	0.59	0.004 (−0.002, 0.010)	0.27	19.0
Subcortical gray matter volume							
CRP 1–3 mg/L	−0.055 (−0.155, 0.050)	0.29	−0.048 (−0.146, 0.050)	0.35	−0.007 (−0.027, 0.010)	0.45	12.7
CRP 3–10 mg/L	−0.064 (−0.161, 0.030)	0.20	−0.058 (−0.156, 0.040)	0.24	−0.006 (−0.016, 0.000)	0.15	9.4
CRP > 10 mg/L	−0.083 (−0.120, 0.030)	0.15	−0.079 (−0.192, 0.040)	0.17	−0.004 (−0.018, 0.000)	0.36	4.8
Cerebellum cortex volume							
CRP 1–3 mg/L	−0.029 (−0.167, 0.110)	0.67	−0.026 (−0.164, 0.110)	0.70	−0.003 (−0.015, 0.010)	0.55	10.3
CRP 3–10 mg/L	−0.119 (−0.256, 0.020)	0.09	−0.119 (−0.256, 0.020)	0.09	0.000 (−0.008, 0.010)	0.01	–
CRP > 10 mg/L	−0.216 (−0.369, −0.060)	0.007	−0.215 (−0.368, −0.060)	0.008	−0.001 (−0.015, 0.010)	0.79	0.5
Cerebrospinal fluid volume							
CRP 1–3 mg/L	0.143 (−0.041, 0.330)	0.13	0.142 (−0.042, 0.330)	0.13	0.001 (−0.009, 0.010)	0.93	0.7
CRP 3–10 mg/L	0.082 (−0.087, 0.250)	0.34	0.083 (−0.085, 0.250)	0.33	−0.011 (−0.012, 0.010)	0.81	–
CRP > 10 mg/L	0.051 (−0.134, 0.240)	0.59	0.053 (−0.133, 0.240)	0.57	−0.002 (−0.019, 0.010)	0.76	–
Hippocampus volume							
CRP 1–3 mg/L	−0.054 (−0.196, 0.090)	0.45	−0.049 (−0.190, 0.090)	0.49	−0.005 (−0.022, 0.010)	0.46	9.3
CRP 3–10 mg/L	0.013 (−0.124, 0.150)	0.86	0.014 (−0.122, 0.150)	0.84	−0.001 (−0.011, 0.010)	0.70	–
CRP > 10 mg/L	−0.008 (−0.017, 0.150)	0.91	−0.010 (−0.017, 0.150)	0.90	0.002 (−0.001, 0.020)	0.79	–
Amygdala volume							
CRP 1–3 mg/L	−0.036 (−0.166, 0.010)	0.58	−0.036 (−0.165, 0.010)	0.58	−0.001 (−0.008, 0.010)	0.85	2.8
CRP 3–10 mg/L	−0.007 (−0.137, 0.120)	0.91	−0.010 (−0.140, 0.120)	0.87	0.003 (−0.003, 0.010)	0.38	–
CRP > 10 mg/L	0.052 (−0.097, 0.200)	0.49	0.047 (−0.102, 0.200)	0.53	0.005 (−0.006, 0.020)	0.45	–

†The reference group is CRP < 1 mg/L.

‡ Model 1 was corrected for sex, child age at assessment, gestational age at which CRP was measured, model 2 was additionally corrected for maternal national background, maternal education, household income and maternal age, and model 3 was additionally corrected for psychoactive substances, smoking, alcohol, and maternal psychopathology. Model 4 was further adjusted for total brain volume for all the regional brain volume outcomes.

§Significant results are presented in bold.

¶For easier interpretation of multiple outcomes with different ranges, the exposure, mediator and the outcome were all standardized to enable comparison.

‡‡The TE, NDE and NIE (with corresponding p-values and 95% confidence intervals) were calculated with quasi-Bayesian methods (sampling n = 10,000). To compute the confidence intervals the bias-corrected and accelerated (BCa) intervals were chosen.

*Represents significant p-values after Bonferroni correction.

**The proportion mediated is only displayed when it could be calculated, i.e., when the NDE and NIE were in the same direction.

4.2. Exposure-response relationship

We found a nominal relationship between high MIA (CRP >10 mg/L) in women and child brain morphology. An acute infection has probably been the cause for increased CRP levels above 10 mg/L in most women, although for some women CRP levels will have been increased by other causes. No relationship was observed between low-grade inflammation (CRP between 1 and 10 mg/L) and child brain morphology compared to the no MIA group (CRP <1 mg/L). Our continuous analyses showed a significant relationship between MIA and child cerebellar volume, which may be driven by acute infections or other sources of more severe

inflammation.

4.3. Gestational age at birth

In contrast to the literature (Ernst et al., 2011; Lohsoonthorn et al., 2007; El Marroun et al., 2020), we found no moderated-mediating effect of gestational age at birth. Earlier work in baboons found that preterm birth was related to alterations in the cerebellum; however, only when preterm birth was followed by a two week Neonatal Intensive Care Unit experience (Barron and Kim, 2020). The low prevalence of preterm birth in this cohort (~4.5%) may limit our ability to detect effects.

Table 4
Investigating mediatory effect of birth weight between continuous CRP levels and child brain morphology at 9–12 (model 4).

	Total effect		Natural direct effect		Natural indirect effect		Proportion mediated (%)**
	β (95% CI)	P-value	β (95% CI)	P-value	β (95% CI)	P-value	
Total brain volume	0.002 (−0.032, 0.030)	0.9	0.005 (−0.027, 0.040)	0.8	−0.003 (−0.012, 0.010)	0.5	0
Cortical gray matter volume	0.002 (−0.010, 0.010)	0.8	0.002 (−0.010, 0.010)	0.8	0.000 (0.000, 0.000)	0.7	0
Total white matter volume	0.011 (−0.002, 0.020)	0.1	0.011 (−0.002, 0.020)	0.1	0.000 (0.000, 0.000)	0.7	0
Subcortical gray matter volume	−0.010 (−0.033, 0.010)	0.4	−0.00 (−0.032, 0.010)	0.4	0.000 (−0.002, 0.000)	0.5	0
Cerebellum cortex volume	−0.055 (−0.087, −0.020)	<0.001*	−0.055 (−0.087, −0.020)	<0.001*	0.000 (−0.002, 0.000)	0.7	0
Cerebrospinal fluid volume	−0.029 (−0.069, 0.010)	0.2	−0.029 (−0.069, 0.010)	0.2	0.000 (−0.001, 0.000)	1.0	0
Hippocampus volume	0.020 (−0.012, 0.050)	0.2	0.020 (0.013, 0.040)	0.2	0.000 (−0.001, 0.000)	0.9	0
Amygdala volume	0.021 (−0.009, 0.050)	0.2	0.021 (−0.009, 0.050)	0.2	0.001 (−0.002, 0.000)	0.5	0

†Significant results are presented in bold.

‡Model 1 was corrected for sex, child age at assessment, gestational age at which CRP was measured, model 2 was additionally corrected for maternal national background, maternal education, household income and maternal age, and model 3 was additionally corrected for psychoactive substances, smoking, alcohol, and maternal psychopathology. Model 4 was further adjusted for total brain volume for all the regional brain volume outcomes.

§For easier interpretation of multiple outcomes with different ranges, the exposure, mediator and the outcome were all standardized to enable comparison.

††The TE, NDE and NIE (with corresponding p-values and 95% confidence intervals) were calculated with quasi-Bayesian methods (sampling $n = 10,000$). To compute the confidence intervals the bias-corrected and accelerated (BCa) intervals were chosen.

*Represents significant p-values after Bonferroni correction.

**The proportion mediated is only displayed when it could be calculated, i.e., when the NDE and NIE were in the same direction.

4.4. Sex-specific effects

Interestingly, we observed a sex-specific indirect effect of MIA on total brain volume in females mediated by gestational age. Prior literature generally stated that males are more sensitive to MIA (Hunter et al., 2019), a finding that may be driven by the higher incidence of preterm birth in males (Peelen et al., 2016). The low incidence of preterm birth in our cohort may have limited our ability to show a mediation effect of preterm birth in males on MIA and child brain morphology. This still does not explain the global effects of MIA on total brain volume in females specifically, which is puzzling and needs replication in other clinical cohorts.

4.5. Strengths and limitations

The major strengths of our study are the large population-based cohort with neuroimaging in children, the inclusion of relevant confounders, including maternal psychopathology and socioeconomic factors, the use of biological measures for MIA, and the use of an objective measure (ultrasound) for gestational age. Our study also has several limitations. First, the non-response analysis showed that some demographic variables differed significantly between those included versus the excluded group. Although this may influence the generalizability of this study, our population included a large sample of ethnically and socially diverse participants and we controlled for these variables in our analyses. Second, our study included a spectrum of inflammation in the general population rather than severe infections or major medical problems that result in hospitalization. Third, we were limited to the use of a single marker to define our exposure variable. Although CRP is a reliable biomarker for infection and inflammation and widely used in clinical practice (Gabay and Kushner, 1999; Pepys and Hirschfield, 2003), use of a single biomarker is a limitation of our study. There are advantages in using multiple biomarkers such as cytokines (or a compound measurement) or numbers and functions of various immune cells, but these data are not currently available in this cohort.

5. Conclusion

The results of this study implicate possible long-term findings of MIA on child brain morphology. Specifically, we found direct effects for

cerebellar volume, and sex-specific indirect effects for total brain volume. Our study is particularly relevant considering the COVID-19 pandemic with record numbers of infections and inflammation in pregnant women. Moreover, our study provides the foundation for future research to investigate how atypical cerebellum development due to MIA translates into downstream behavioral effects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author contributions

T.W. and V.B. conceptualized the study. A.J.S., E.B., and T.W. developed the research plan together. A.J.S. wrote the initial draft of the paper and conducted the statistical analyses. E.B., M.D., A.S.R., L.D.W., V.W.V.J., V.B., and T.W. critically reviewed the paper and edited later iterations of the paper. V.B. and T.W. supervised the project. All authors

approved the final submission.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bbi.2022.03.018>.

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