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Intra-amniotic inflammation in the mid-trimester of pregnancy is a risk factor for neuropsychological disorders in childhood

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Abstract

Objectives: Intra-amniotic inflammation is a subclinical condition frequently caused by either microbial invasion of the amniotic cavity or sterile inflammatory stimuli, e.g., alarmins. An accumulating body of evidence supports a role for maternal immune activation in the genesis of fetal neuroinflammation and the occurrence of neurodevelopmental disorders such as cerebral palsy, schizophrenia, and autism. The objective of this study was to determine whether fetal exposure to mid-trimester intra-amniotic inflammation is associated with neurodevelopmental disorders in children eight to 12 years of age.

Methods: This is a retrospective case-control study comprising 20 children with evidence of prenatal exposure to intra-amniotic inflammation in the mid-trimester and 20 controls matched for gestational age at amniocentesis and at delivery. Amniotic fluid samples were tested for concentrations of interleukin-6 and C-X-C motif chemokine ligand 10, for bacteria by culture and molecular microbiologic methods as well as by polymerase chain reaction for eight viruses. Neuropsychological testing of children, performed by two experienced psychologists, assessed cognitive and behavioral domains. Neuropsychological dysfunction was defined as the presence of an abnormal score (<2 standard deviations) on at least two cognitive tasks.

Results: Neuropsychological dysfunction was present in 45% (9/20) of children exposed to intra-amniotic inflammation but in only 10% (2/20) of those in the control group (p=0.03). The relative risk (RR) of neuropsychological dysfunction conferred by amniotic fluid inflammation remained significant after adjusting for gestational age at delivery [aRR=4.5 (1.07–16.7)]. Of the 11 children diagnosed with neuropsychological dysfunction, nine were delivered at term and eight of them had mothers with intra-amniotic inflammation. Children exposed to intra-amniotic inflammation were found to have abnormalities in neuropsychological tasks evaluating complex skills, e.g., auditory attention, executive functions, and social skills, whereas the domains of reasoning, language, and memory were not affected in the cases and controls.

Conclusions: Asymptomatic sterile intra-amniotic inflammation in the mid-trimester of pregnancy, followed by a term birth, can still confer to the offspring a substantial risk for neurodevelopmental disorders in childhood. Early recognition and treatment of maternal immune activation in pregnancy may be a strategy for the prevention of subsequent neurodevelopmental disorders in offspring.

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Keywords: amniocentesis; amniotic fluid; behavioral testing; C-X-C motif chemokine ligand 10 (CXCL-10); cognitive testing; cytokine; infection; interferon-γ-inducible protein 10 (IP-10); interleukin 6 (IL-6); neurodevelopment; neuroinflammatory.

Introduction

Intra-amniotic inflammation, i.e., the presence of an inflammatory signature in the amniotic fluid, is recognized by either an excessive number of immune cells [1, 2], such as neutrophils [3–10] or lymphocytes [11–17], or inflammatory cytokines [14, 18–29]. The inflammatory process can be caused by microorganisms (bacteria, viruses, fungi, and protozoa) [30–34] or by alarmins [35–39] released by host cells under conditions of stress or cell death. Intra-amniotic inflammation has been recognized in patients who have obstetrical complications such as preterm labor [1, 2, 8, 18, 30, 40–59], preterm prelabor rupture of the membranes [60–71], cervical insufficiency [72–75], idiopathic vaginal bleeding [76], and a sonographic short cervix [77, 78]; however, in some cases, intra-amniotic inflammation is present in the absence of any complication of pregnancy and can be incidentally detected at the time of mid-trimester amniocentesis by measuring inflammatory cytokines [79].

Previous studies indicated that intra-amniotic inflammation is a heterogeneous condition and that perturbations of the cytokine network can occur in intra-amniotic infection as well as in sterile intra-amniotic inflammation [28, 80–83]. In the mid-trimester of pregnancy, two subtypes of intra-amniotic inflammation have been described: one characterized by an elevation of interleukin (IL)-6 and another by an elevated concentration of C-X-C motif chemokine ligand 10 (CXCL-10), also known as interferon-γ-inducible protein 10 (IP-10) [79]. Of interest, patients with high amniotic fluid IL-6 concentrations are at risk for early preterm delivery, and their placentas typically show histological evidence of acute histologic chorioamnionitis [20, 24]. By contrast, those with elevated CXCL-10 are at risk for late spontaneous preterm delivery and show evidence of chronic placental inflammatory lesions, e.g., chronic chorioamnionitis, villitis of unknown etiology, and deciduitis of the basal plate [14, 84].

Based upon epidemiologic studies, fetal exposure to inflammation and infection during pregnancy has been implicated in the origin of mental illnesses, including cerebral palsy [85–101], autism spectrum disorder [102–110], schizophrenia [102, 103, 111–117], bipolar disorder [109], and depression [109, 118–121]. A body of experimental evidence also supports the concept that infection or inflammation during pregnancy can induce fetal neuroinflammation, impair neurodevelopment, and predispose to adverse outcomes such as neurologic and mental disorders [110, 122, 123]. Although the literature sometimes refers to exposure to a maternal inflammatory response, it is likely that maternal inflammation operates through the induction of fetal neuroinflammation.

Intra-amniotic inflammation can be diagnosed as early as the mid-trimester, often occurs in the absence of a detectable clinical or subclinical maternal inflammatory response [79], and is frequently associated with delivery at term [79]. It is unknown if this condition increases the risk for adverse neurodevelopmental outcome. Therefore, the purpose of this study was to determine whether fetuses exposed to intra-amniotic inflammation in the mid-trimester of pregnancy are at risk for long-term neuropsychological disorders. We addressed this question by examining the outcomes of children whose mothers underwent a mid-trimester amniocentesis for clinical indications and in whom amniotic fluid analysis permitted a diagnosis of subclinical intra-amniotic inflammation.

Material and methods

Study design

This retrospective nested case-control study was conducted to determine the likelihood of neurodevelopmental disorders in children exposed to intra-amniotic inflammation in the mid-trimester of pregnancy. The study is based on a cohort of 847 consecutive mothers who underwent a diagnostic mid-trimester amniocentesis for clinical indications. Exclusion criteria for this study were chromosomal anomalies and confirmed fetal anomalies. Amniotic fluid cultures for aerobic and anaerobic microorganisms, polymerase chain reaction (PCR) testing for eight viruses, white blood cell counts, IL-6 and CXCL-10 concentrations, and obstetrical outcomes of this cohort were previously reported and the specific assays for IL-6 and CXCL-10 described [79, 124].

Women had previously consented to donate samples of amniotic fluid and maternal blood for research purposes and extended their consent to the performance of neuropsychological testing in their children under a protocol approved by the Institutional Review Boards of the participating institutions (Azienda Ospedaliera Treviso, Azienda Ospedale/Universita' Padova, Veneto Region, Italy). All patients provided written informed consent prior to the collection of samples. The hospitals have a Federal Wide Assurance with the Department of Health and Human Services of the United States.

Definition of intra-amniotic inflammation

Intra-amniotic inflammation was defined as either an IL-6 or a CXCL-10 amniotic fluid concentration above the 95th percentile of the normal

values for a study population with uncomplicated pregnancies and term deliveries [79]. The cut-off values for IL-6 and CXCL-10 concentrations were 2,935 and 2,200 pg/mL, respectively. In the parent cohort, the prevalence of elevated amniotic fluid IL-6 was 6.3% (50/796) and the prevalence of elevated amniotic fluid CXCL-10 was 5.8% (45/770).

Selection of cases and controls

We selected as "cases" the children of the first 20 consecutive women with evidence of exposure to intra-amniotic inflammation in the mid-trimester according to the criteria outlined above. Twenty children of mothers without evidence of intra-amniotic inflammation, matched for gestational age at amniocentesis and at delivery (≤3 weeks), were selected as controls. Matching did not address the sex of the child. The sample size was set arbitrarily and therefore represents a sample of convenience. All amniotic fluid samples were considered negative for bacteria after testing by culture and molecular microbiologic methods (16 s broad primers). Moreover, samples were PCR-negative for eight viruses (adenoviruses, herpes simplex virus, varicella-zoster virus, human herpesvirus 6, parvovirus B19, human cytomegalovirus, enteroviruses, and Epstein-Barr virus).

Neurodevelopmental testing: Neurodevelopmental performance was assessed with a battery of tests that analyzed the cognitive and behavioral domains (Table 1). Neuropsychological testing was performed by two experienced psychologists who were masked to the results of the amniocenteses. Tests took approximately 90 min per child and involved both psychologists for each child. Neuropsychological dysfunction was defined as the presence of an abnormal score (<2 standard deviations) on at least two cognitive tasks. This approach provides dichotomous results, and it is considered useful to assess a wide range of impairments, which are then summarized in a single score. Table 1 provides a list of the neuropsychological tests [125–132] and behavioral questionnaires [133–135] administered. Neuropsychological scores were transformed into age-corrected standardized scores by using published normative data.

Table 1: The battery of tests performed to assess neuropsychological status and behavior.

Domain tested	Specific instrument used for evaluation
Abstract reasoning	Raven's Coloured Progressive Matrices™ [125]
Language	Naming test [126]
Attention	Visual and auditory attention tests of the NEPSY-II[a] [127, 128]
Memory	
Short-term verbal and visual-spatial memory	Digit span test [126] Corsi block-tapping test [126]
Long-term verbal memory	Word's list [126] List recall [126]
Executive function	Phonemic verbal fluency test [126] Tower of London test [129] Coding test of the WISC-IV [130] Stroop test [131] Backward digit span test [126]
Visual-motor function	Visual-motor integration test [132]
Social skills	Theory of mind and emotion, a sub-test of NEPSY-II[a] [127, 128]
Behavior	
	Child behavior checklist (CBCL) [133] Strengths and difficulties questionnaire (SDQ) [134] Conners' rating scales-revised (CRS-R) [135]

[a]A developmental NEuroPSYchological assessment. WISC-IV, Wechsler Intelligence Score for Children.

Statistical analysis

Descriptive statistics for continuous variables are displayed as medians and quartiles as well as mean and standard deviations (Table 2). Results of the neuropsychological and behavioral tests were age-corrected and converted to the following scores: z, equivalent, T, and standard as appropriate, by using published normative data [125, 126, 128–132]. The z score indicates the deviation from the mean population score, which is set to 0, standard deviation 1. A z score of −2 (or less) comprises 2.5% of the normal distribution and is considered significantly lower than average. Equivalent scores are a 5-point scale standardized after adjustment for age and education. An equivalent score of 0 is considered significantly lower than average. A T score indicates the deviation from the mean population score, which is set to 50, standard deviation 10. A T score of 70 (or more) is considered significantly higher than average. Scaled scores indicate the deviation from the mean population score, which is set to 10 (standard deviation 3). A scaled score of 4 (or less) is considered significantly lower than average. Scores were also converted in dichotomized outcomes (0: normal, 1: altered). Abnormal scores were those <5th percentile or 2 standard deviations. Pearson's chi-square (X^2) and Fisher's exact tests were used for the dichotomous variable. All tests were 2-tailed, and a p-value <0.05 was considered statistically significant. Statistical analysis was performed with SPSS 20.0 (Chicago, IL). In addition, the effect of the amniotic fluid inflammation on the rate of neuropsychological dysfunction was also assessed by utilizing robust Poisson regression models and the *geepack* package in the R statistical language and environment (www.r-project.org).

Results

Characteristics of the study population

Demographic, clinical, and laboratory characteristics of the study population are displayed in Table 2. There were no significant differences between cases and controls for all of the examined variables except for IL-6 and CXCL-10, which, by design, were used to define cases and controls and for age at evaluation, which was solved by converting neuropsychological scores into age-correlated standardized scores by using published normative data. The adequacy of matching was evaluated with the standardized

Table 2: Demographic, clinical, and laboratory characteristics of the study population.

Characteristics	Controls (n=20)	Intraamniotic inflammation (n=20)	p-Value
Maternal age, years	36 (35–37.2)	35.5 (34–37)	0.262
Nulliparity	35% (n=7)	30% (n=6)	1.0
Body mass index, kg/m^2	22.8 (20.9–25.3)	23.5 (21.1–25.2)	0.818
Gestational age at amniocentesis, weeks	16.7 (16.2–17.2)	16.2 (15.9–18.1)	0.416
Amniotic fluid interleukin-6, pg/mL	465.3 (362.7–1182.2)	5690.4 (4707.5–8106.4)	<0.001
Amniotic fluid CXCL-10, pg/mL	616.1 (430.9–978.7)	3536.9 (2411.1–5194.3)	<0.001
Maternal blood interleukin-6, pg/mL	0.69 (0.56–0.89)	0.69 (0.64–0.90)	0.23
Maternal blood CXCL-10, pg/mL	88.31 (65.4–123)	92.39 (72.1–117.1)	0.69
Gestational age at test, weeks	16.5 (16.1–17.2)	16.15 (15.6–18.1)	0.768
Gestational age at delivery, weeks	39.6 (38.1–40.3)	38.6 (37.5–39.3)	0.123
Cesarean delivery	20% (n=4)	50% (n=10)	0.096
Birthweight, grams	3377.5 (3,135–3472.5)	3,145 (2,575–3,685)	0.417
Birthweight percentile for gestational age	55 (50–75)	50 (25–75)	0.509
Small for gestational age (birthweight below 10th centile)	0% (n=0)	5% (n=1)	1.0
Male neonate	40% (n=8)	70% (n=14)	0.111
Apgar score at 1 min<7	9 (9–9)	9 (9–9)	1.0
Apgar score at 5 min<7	10 (10–10)	10 (10–10)	0.979
Duration of hospitalization of the newborn, days	3 (3–4)	3 (3–4)	0.371

Data presented as median (interquartile range) and percentage (n/N); CXCL-10, C-X-C motif chemokine ligand 10.

mean differences between the intra-amniotic inflammation and control groups, and there were no differences between them. Of the 20 cases with intra-amniotic inflammation, only four delivered preterm (<37 weeks of gestation). Apgar scores were above 6 in all cases and controls except for one child in the control group who was born at 28 weeks, had a birthweight of 850 g, and had Apgar scores of 2 (1 min) and 6 (5 min).

Intra-amniotic inflammation and neuropsychological dysfunction

Table 3 describes the clinical and laboratory characteristics of the women included in the study as well as pregnancy outcomes and results of neuropsychological tests of their children. Neuropsychological dysfunction was present in 45% (9/20) of children exposed to intra-amniotic inflammation but in only 10% (2/20) in the control group (p=0.03) (Table 4). Of the 11 children with neuropsychological dysfunction, nine were term deliveries, and eight of them had mothers with intra-amniotic inflammation. The relative risk (RR) of neuropsychological dysfunction conferred by amniotic fluid inflammation remained significant [aRR=4.5 (1.07–16.7)] after adjusting for gestational age at delivery. Some cognitive domains, such as reasoning, language, and memory, were not affected in the cases and controls. Neuropsychological tasks evaluating complex skills such as auditory attention, executive functions, and social skills were abnormal in children exposed to intra-amniotic inflammation.

Of the cases with intra-amniotic inflammation, 12 (12/20) had elevated amniotic fluid IL-6 and eight (8/19) had elevated amniotic fluid CXCL-10 (Table 3). Of the cases with neuropsychological dysfunction, 5/11 had elevated amniotic fluid IL-6, 4/11 had elevated CXCL-10, and 2/11 had both IL-6 and CXCL-10 within normal range at the time of mid-trimester amniocentesis (Table 3). Of note, of the 11 children who tested positive for a neuropsychological dysfunction, nine were male, and seven were delivered by cesarean section. The outcome of behavioral testing, as reflected by interviews of the parents, showed that behavioral modifications were observed in 30% (6/20) of the cases and in 20% (4/20) of controls (p-value>0.05).

Discussion

Principal findings of the study

The main conclusion of our study is that exposure to subclinical mid-trimester intra-amniotic inflammation is a risk factor for abnormal neurodevelopmental outcomes in children 8–12 years of age and that most pregnancies affected by intra-amniotic inflammation were delivered at term.

Table 3: Clinical and laboratory characteristics of the study participants, pregnancy outcomes, and neuropsychological tests results.

		Gestational age at amniocentesis, weeks	Amniotic fluid IL-6, pg/mL	Amniotic fluid CXCL-10, pg/mL	Spontaneous preterm labor with intact membranes or PPROM	Birth weight, grams	Birthweight percentile	Gestational age at birth, weeks	Gestational age at evaluation, years	Neuropsychological index	Neuropsychological domains affected
1	Case	18.2	4930.42	612.592	No	3,120	25	40 + 5	9.2	0	
	Control	20.3	402.671	1584.97	No	3,350	25	41 + 3	9.5	0	
2	Case	16.2	841.313	9100.3	No	2,600	25	36 + 2	11.3	0	
	Control	16.5	125.62	366.846	No	2,800	10	41 + 0	11.5	0	
3	Case	22.3	984.547	2303.03	No	3,800	75	40 + 4	9.5	0	
	Control	22.1	456.444	1933.06	No	3,440	50	39 + 3	10.3	0	
4	Case	15.5	4855.84	447.826	No	2,300	<3	38 + 6	9.1	1	Executive and social skills, attention
	Control	16.6	70.844	815.295	No	3,140	50	38 + 1	9.4	0	
5	Case	15.2	6360.99	307.433	No	3,105	50	37 + 1	10.7	0	
	Control	15.4	482.061	652.659	No	3,120	50	38 + 1	10.8	0	
6	Case	15.6	6909.96	334.995	No	980	30	28 + 0	10.7	1	Executive functions, attention
	Control	17.6	172.11	534.117	No	850	25	28 + 4	8.8	0	
7	Case	16.5	13142.7	1570.45	No	3,110	25	39 + 2	8.8	0	
	Control	16.4	421.461	1233.48	No	3,330	30	40 + 5	9.2	0	
8	Case	20.2	414.103	5152.2	No	3,170	50	38 + 2	9.1	1	Executive functions, reasoning
	Control	15.5	576.301	212.972	No	3,400	75	38 + 4	11.2	0	
9	Case	15.6	3163.88	541.281	No	4,240	>95	39 + 2	10.4	1	Attention
	Control	16.2	242.731	404.357	No	3,390	75	38 + 6	11.4	0	
10	Case	15.6	439.111	4105.66	No	3,680	75	38 + 2	9.11	0	
	Control	15.5	425.053	538.109	No	3,300	50	39 + 5	10.9	0	
11	Case	16.1	3183.68	667.341	No	3,560	75	40 + 0	10.8	0	
	Control	16.5	1458.75	436.925	No	3,365	75	37 + 4	10.11	0	
12	Case	15.3	110.547	2413.12	No	3,270	40	39 + 0	10	1	Executive and social skills
	Control	16.1	709.132	693.992	No	3,560	60	39 + 6	10.2	0	
13	Case	15.6	7806.19	1057.8	No	2,610	10	39 + 1	11.2	0	
	Control	16.2	2287.26	518.54	No	3,480	50	40 + 4	12	0	
14	Case	18	528.102	2404.89	PPROM	2,360	25	35 + 4	9	0	
	Control	17.1	690.765	1278.08	No	2,800	50	36 + 1	9.7	0	
15	Case	16	5019.86	622.452	No	3,870	>90	38 + 0	11	1	Executive functions and attention
	Control	17.3	448.5	1649.09	No	3,470	60	39 + 6	11.7	0	
16	Case	15.5	9007.19	N/A	No	2,410	10	37 + 4	11.3	0	
	Control	16.4	2605.82	376.591	No	3,860	90	40 + 3	12.2	0	

Table 3: (continued)

		Gestational age at amniocentesis, weeks	Amniotic fluid IL-6, pg/mL	Amniotic fluid CXCL-10, pg/mL	Spontaneous preterm labor with intact membranes or PPROM	Birth weight, grams	Birthweight percentile	Gestational age at birth, weeks	Gestational age at evaluation, years	Neuropsychological index	Neuropsychological domains affected
17	Case	16.6	4262.65	872.118	No	3,720	75	39 + 3	10.1	0	
	Control	16.5	761.038	719.676	No	4,265	>90	40 + 2	10.5	0	
18	Case	25.5	522.934	5320.62	No	3,700	90	39 + 3	11.2	1	Executive functions and attention
	Control	20.1	1423.45	878.888	No	3,680	75	40 + 1	10.8	1	Social skills
19	Case	19.4	293.863	2968.17	Spontaneous preterm labor with intact membranes	2,500	70	34 + 0	9.6	1	Social skills, abnormal lateralization
	Control	16.6	1745.63	452.248	PPROM	2000	75	31 + 6	9.4	1	Executive and social skills
20	Case	16.3	14008.8	2146.28	No	3,225	75	37 + 5	12.11	1	Executive functions
	Control	16	1089.98	387.847	No	3,450	75	39 + 0	12.6	0	

IL-6, interleukin-6; CXCL-10, C-X-C motif chemokine ligand 10; PPROM, preterm prelabor rupture of the membranes; Neuropsychological index; 0, absence of neuropsychological dysfunction; 1, presence of neuropsychological dysfunction.

Table 4: Frequency of neuropsychological dysfunction in children exposed and unexposed to intra-amniotic inflammation.

	Neuropsychological dysfunction		
	Absent	**Present**	**Total**
Controls	18	2	20
Intra-amniotic inflammation	11	9	20
Total	29	11	40

Fisher's exact test, p=0.03; Intra-amniotic inflammation was defined as an elevated IL-6 or CXCL-10 concentration in amniotic fluid.

Results in the context of what is known

Intra-amniotic inflammation is observed in 5–6% of asymptomatic patients in the mid-trimester of pregnancy, and it is a risk factor for spontaneous preterm delivery [79]. However, most patients with this condition deliver at term after a pregnancy without known complications [79, 136, 137]. Herein, we report that exposure to sterile intra-amniotic inflammation during fetal life confers risk for abnormal neurodevelopmental outcomes in childhood.

Maternal immune activation due to infection or sterile inflammation has been implicated in the genesis of neurodevelopmental disorders [110,138–152], e.g., cerebral palsy [85–101], schizophrenia [102, 103, 111–117], depression [109, 118–121], autism spectrum disorder [102–110], attention deficit hyperactivity disorder [110, 153–155], and Tourette syndrome [110]. Moreover, the spectrum of disorders may extend to learning disabilities [156–158] without demonstrable neurologic deficits.

The initial observations linking maternal infection and the subsequent occurrence of neurodevelopmental disorders in the offspring were made by Mednick et al. who reported that individuals diagnosed with schizophrenia were more likely to have been exposed to influenza during fetal life (ascertained by the diagnosis of maternal infection) [159]. This association was subsequently confirmed in prospective studies, and Brown et al. estimated that 14–21% of cases with schizophrenia were attributable to maternal infection [115, 160, 161]. The association was not specific to influenza and was observed with other viral infections such as rubella [162–166]. Since that time, a large body of epidemiological evidence has accumulated, indicating that maternal bacterial and viral infections during pregnancy can be associated with neurodevelopmental disorders [108].

Experimental studies provide strong evidence for the hypothesis that infections during pregnancy cause maternal and/or fetal immune activation, and this, in turn, can

damage the developing brain [102, 142, 167–173]. During pregnancy, maternal exposure to bacterial endotoxin or lipopolysaccharide (LPS) or polyinosinic:polycytidylic acid (Poly I:C), a synthetic analog of double-stranded RNA that mimics some viral infections [172, 174, 175], can induce brain injury. These effects are mediated through an inflammatory response elicited by these microbial products and microorganisms. Further, there is evidence that fetal exposure to maternal sterile inflammation in autoimmune disorders [176–182], asthma [183–185], and obesity [186] can increase the risk of neurodevelopmental disorders.

Since maternal immune activation is associated with elevation in the concentrations of proinflammatory cytokines, these molecules have been studied and found to mediate the effects of maternal immune activation on fetal brain development [151, 161, 168, 187–189]. Specifically, IL-6 has been identified as a mediator of fetal brain injury, based on observations in pregnant mice in whom a single dose of IL-6 administered to the mother (on gestational day 12.5) led to behavioral deficits in the offspring, which can be prevented with the administration of anti-IL-6 [190]. Similar observations have been made with other cytokines [145, 172, 187, 188, 191–198]. Recent studies in humans indicated a relationship between maternal IL-6 concentrations during pregnancy and neonatal functional connectivity [199–201], as assessed by magnetic resonance imaging (MRI), as well as infant cognitive development [199, 202].

Although a major emphasis has been the relationship between cytokine concentrations in the maternal compartment and neurodevelopmental outcome, it is possible and indeed likely that these effects are mediated through cytokines in the fetus. Indeed, amniotic fluid cytokine concentrations are thought to reflect fetal rather than purely maternal immune activation [203]. A series of studies using the Danish Historical Birth Cohort reported that individuals diagnosed with autism spectrum disorder had a higher mid-trimester amniotic fluid concentration of several analytes, e.g., monocyte chemoattractant protein-1 (MCP-1) [204], IL-4 [104], IL-10 [104], tumor necrosis factor-alpha (TNF-α) [104], tumor necrosis factor-beta [104], and matrix metalloproteinase-9 [205]. These observations are consistent with our report that individuals exposed to an inflammatory state during fetal life have a greater risk for abnormal neuropsychological testing in the future.

The observations in the present study are based on the results of the analysis of amniotic fluid obtained in the mid-trimester when genetic amniocenteses were performed. However, similar observations have been made between amniotic fluid concentrations of cytokines in the third trimester and the subsequent development of neurodevelopmental disorders. We reported that infants who develop white matter injury, diagnosed by ultrasound in the neonatal period, had higher amniotic fluid concentrations of IL-6, IL-1β, and TNF-α, and a subset of these infants developed cerebral palsy [86]. The same association was found between the umbilical cord concentrations of cytokines (reflecting fetal systemic inflammation) and the occurrence of white matter lesions [85]. Similar observations were reported by other investigators [93, 206–208]. Indeed, Lu et al. studied a group of patients who had an episode of premature labor and underwent amniocentesis <34 weeks of gestation. The amniotic fluid concentrations of IL-1β, IL-6, IL-8, TNF-α, granulocyte-colony stimulating factor, MCP-1, soluble intercellular adhesion molecule-1, and activin were significantly higher in cases subsequently diagnosed with brain injury by neurosonography or MRI. Similar findings were observed when examining umbilical cord blood, and this argues for the importance of fetal inflammation [209]. Indeed, there was high correlation between amniotic fluid and umbilical cord concentrations of IL-1β, IL-6, and TNF-α [209].

What is the relationship between a maternal immune response and a concentration of cytokines in amniotic fluid? Although the amniotic fluid and the maternal circulatory compartment are considered to be separate, further evidence indicated that systemic administration of LPS to pregnant rats results in an elevation in amniotic fluid concentrations of cytokines [210, 211] and also in a fetal inflammatory response, as reflected by the cytokine response in the brain as well as by changes in the fetal brain metabolome [212]. These observations are important because potential interventions aimed at maternal immune activation may target fetal inflammation and prevent brain injury. For example, the administration of N-acetyl-cysteine to pregnant rats prevents amniotic fluid [213, 214] and placental inflammation [213] and reduces cytokines in the maternal blood [213, 215], fetal blood [215, 216], and fetal brain [217]. Magnesium sulfate, currently used for neuroprotection, appears to exert its effects by down-regulation of fetal inflammation [211], as demonstrated by the decrease in cytokine concentrations in the fetal brain after maternal exposure to endotoxin [218, 219]. After birth, treatment with minocycline [220–222], a microglia modulator, cyclooxygenase-2 (COX-2) inhibitors [223, 224], or N-acetyl-cysteine in dendrimers [225] can down-regulate brain inflammation and improve neurologic or behavioral responses, suggesting that early treatment of neuroinflammation is possible.

Neurodevelopmental abnormalities

Among the cognitive domains, executive functions, attention, and social skills were the most affected in our cases. These high-order cognitive functions are strictly interrelated and overlapping. Executive functions have been strongly associated with academic functioning in childhood [226] and, in many cases, they are a better predictor of adult outcomes than intelligence quotient (IQ) and socio-economic-status [227]. Executive functions in childhood also predict the successful transition to kindergarten [228], school performance, and social competence in adolescence [229], and fewer drug-related problems and criminal convictions in adulthood [227]. Furthermore, abnormal social skills, attention, and executive functions are thought to be a core problem in several neurodevelopmental disorders, e.g., autism spectrum disorder [230], obsessive-compulsive disorder [231], and attention-deficit hyperactivity disorder (ADHD) [232].

These cognitive functions and some neurodevelopmental disorders, such as autism spectrum disorder, are considered disturbances of neuro-connectivity and are highly correlated to the integrity of the white matter [233–235]. The pathophysiologic mechanisms underlying white matter's contribution to abnormal cognition and behavior are only partially understood but most probably involve the generation of reactive oxygen species that disrupt oligodendrocytes' maturation and, ultimately, myelin formation [95, 167, 202, 236]. Given the importance of white matter in coordinating neuronal assemblies firing through myelination, even mild alterations in white matter structure may interfere with the cognitive performance [236, 237].

Clinical and research implications

The main clinical implication of this study is that a fraction of neurodevelopmental disorders appears to result from exposure to subclinical intra-amniotic inflammation in early pregnancy. The etiology, natural history, and consequences of mid-trimester intra-amniotic inflammation are unknown. Microbial invasion of the intra-amniotic cavity is a cause of intra-amniotic inflammation; however, it was not demonstrated in any of our cases. Sterile intra-amniotic inflammation has been attributed to alarmins, which are released under conditions of cellular stress [35–39, 71, 238]. Further work is required to determine if alarmins are elevated in the subset of patients with sterile intra-amniotic inflammation. Important clinical questions include whether it is possible to develop non-invasive methods to identify women with subclinical intra-amniotic inflammation as well as a method of treatment. Recent observations suggest that antimicrobial agents can be used to eradicate intra-amniotic inflammation even in the absence of intra-amniotic infection [239, 240].

Strengths and limitations

The strengths of this study include the ascertainment of intra-amniotic inflammation, which was objectively defined by the presence of an elevated concentration of inflammatory markers and by the performance of bacterial cultures and molecular tests to exclude bacterial infection as well as a subset of viruses. We also assessed two cytokines, IL-6, a biomarker of acute intra-amniotic inflammation, and CXCL-10, which reflects chronic inflammatory processes associated with maternal anti-fetal rejection. Another strength of this study is the long-term follow-up of children (8–12 years of age). The weakness is the limited sample size; however, this type of study is extremely difficult to conduct given that amniocentesis is less frequently performed now because of the preference for non-invasive prenatal testing for antenatal diagnosis.

Conclusions

Fetal exposure to mid-trimester intra-amniotic inflammation increases the risk of neurodevelopmental disorders even in infants born at term.

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References

1. Romero R, Quintero R, Nores J, Avila C, Mazor M, Hanaoka S, et al. Amniotic fluid white blood cell count: a rapid and simple test to diagnose microbial invasion of the amniotic cavity and predict preterm delivery. Am J Obstet Gynecol 1991;165: 821–30.
2. Romero R, Yoon BH, Mazor M, Gomez R, Diamond MP, Kenney JS, et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and Gram stain in patients with preterm labor and intact membranes. Am J Obstet Gynecol 1993;169:805–16.
3. Kacerovsky M, Musilova I, Andrys C, Hornychova H, Pliskova L, Kostal M, et al. Prelabor rupture of membranes between 34 and 37 weeks: the intraamniotic inflammatory response and neonatal outcomes. Am J Obstet Gynecol 2014;210: 325.e1–10.
4. Gomez-Lopez N, Romero R, Xu Y, Leng Y, Garcia-Flores V, Miller D, et al. Are amniotic fluid neutrophils in women with intraamniotic infection and/or inflammation of fetal or maternal origin? Am J Obstet Gynecol 2017;217:693.e1–6.
5. Gomez-Lopez N, Romero R, Garcia-Flores V, Xu Y, Leng Y, Alhousseini A, et al. Amniotic fluid neutrophils can phagocytize bacteria: a mechanism for microbial killing in the amniotic cavity. Am J Reprod Immunol 2017;78:e12723.
6. Gomez-Lopez N, Romero R, Xu Y, Miller D, Unkel R, Shaman M, et al. Neutrophil extracellular traps in the amniotic cavity of women with intra-amniotic infection: a new mechanism of host defense. Reprod Sci 2017;24:1139–53.
7. Martinez-Varea A, Romero R, Xu Y, Miller D, Ahmed AI, Chaemsaithong P, et al. Clinical chorioamnionitis at term VII: the amniotic fluid cellular immune response. J Perinat Med 2017; 45:523–38.
8. Gomez-Lopez N, Romero R, Galaz J, Xu Y, Panaitescu B, Slutsky R, et al. Cellular immune responses in amniotic fluid of women with preterm labor and intra-amniotic infection or intra-amniotic inflammation. Am J Reprod Immunol 2019;82: e13171.
9. Galaz J, Romero R, Slutsky R, Xu Y, Motomura K, Para R, et al. Cellular immune responses in amniotic fluid of women with preterm prelabor rupture of membranes. J Perinat Med 2020;48: 222–33.
10. Gomez-Lopez N, Romero R, Varrey A, Leng Y, Miller D, Done B, et al. RNA sequencing reveals diverse functions of amniotic fluid neutrophils and monocytes/macrophages in intra-amniotic infection. J Innate Immun 2021;13:63–82.
11. Gersell DJ, Phillips NJ, Beckerman K. Chronic chorioamnionitis: a clinicopathologic study of 17 cases. Int J Gynecol Pathol 1991; 10:217–29.
12. Gersell DJ. Chronic villitis, chronic chorioamnionitis, and maternal floor infarction. Semin Diagn Pathol 1993;10:251–66.
13. Jacques SM, Qureshi F. Chronic chorioamnionitis: a clinicopathologic and immunohistochemical study. Hum Pathol 1998;29:1457–61.
14. Kim CJ, Romero R, Kusanovic JP, Yoo W, Dong Z, Topping V, et al. The frequency, clinical significance, and pathological features of chronic chorioamnionitis: a lesion associated with spontaneous preterm birth. Mod Pathol 2010;23:1000–11.
15. Xu Y, Tarquini F, Romero R, Kim CJ, Tarca AL, Bhatti G, et al. Peripheral CD300a+CD8+ T lymphocytes with a distinct cytotoxic molecular signature increase in pregnant women with chronic chorioamnionitis. Am J Reprod Immunol 2012;67:184–97.
16. Gomez-Lopez N, Romero R, Xu Y, Miller D, Leng Y, Panaitescu B, et al. The immunophenotype of amniotic fluid leukocytes in normal and complicated pregnancies. Am J Reprod Immunol 2018;79:e12827.
17. Gomez-Lopez N, Romero R, Xu Y, Miller D, Arenas-Hernandez M, Garcia-Flores V, et al. Fetal T cell activation in the amniotic cavity during preterm labor: a potential mechanism for a subset of idiopathic preterm birth. J Immunol 2019;203:1793–807.
18. Romero R, Avila C, Santhanam U, Sehgal PB. Amniotic fluid interleukin 6 in preterm labor. Association with infection. J Clin Investig 1990;85:1392–400.
19. Romero R, Yoon BH, Kenney JS, Gomez R, Allison AC, Sehgal PB. Amniotic fluid interleukin-6 determinations are of diagnostic and prognostic value in preterm labor. Am J Reprod Immunol 1993;30:167–83.
20. Hillier SL, Witkin SS, Krohn MA, Watts DH, Kiviat NB, Eschenbach DA. The relationship of amniotic fluid cytokines and preterm delivery, amniotic fluid infection, histologic chorioamnionitis, and chorioamnion infection. Obstet Gynecol 1993;81:941–8.
21. Coultrip LL, Lien JM, Gomez R, Kapernick P, Khoury A, Grossman JH. The value of amniotic fluid interleukin-6 determination in patients with preterm labor and intact membranes in the detection of microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1994;171:901–11.
22. Greig PC, Herbert WN, Robinette BL, Teot LA. Amniotic fluid interleukin-10 concentrations increase through pregnancy and are elevated in patients with preterm labor associated with intrauterine infection. Am J Obstet Gynecol 1995;173:1223–7.
23. Dudley DJ, Hunter C, Varner MW, Mitchell MD. Elevation of amniotic fluid interleukin-4 concentrations in women with preterm labor and chorioamnionitis. Am J Perinatol 1996;13:443–7.
24. Baud O, Emilie D, Pelletier E, Lacaze-Masmonteil T, Zupan V, Fernandez H, et al. Amniotic fluid concentrations of interleukin-1beta, interleukin-6 and TNF-alpha in chorioamnionitis before 32 weeks of gestation: histological associations and neonatal outcome. Br J Obstet Gynaecol 1999;106:72–7.
25. Perni SC, Vardhana S, Korneeva I, Tuttle SL, Paraskevas LR, Chasen ST, et al. Mycoplasma hominis and ureaplasma urealyticum in midtrimester amniotic fluid: association with

amniotic fluid cytokine levels and pregnancy outcome. Am J Obstet Gynecol 2004;191:1382–6.
26. Oggé G, Romero R, Lee DC, Gotsch F, Than NG, Lee J, et al. Chronic chorioamnionitis displays distinct alterations of the amniotic fluid proteome. J Pathol 2011;223:553–65.
27. Combs CA, Gravett M, Garite TJ, Hickok DE, Lapidus J, Porreco R, et al. Amniotic fluid infection, inflammation, and colonization in preterm labor with intact membranes. Am J Obstet Gynecol 2014;210:125.e1–5.
28. Bhatti G, Romero R, Rice GE, Fitzgerald W, Pacora P, Gomez-Lopez N, et al. Compartmentalized profiling of amniotic fluid cytokines in women with preterm labor. PLoS One 2020;15: e0227881.
29. Gershater M, Romero R, Arenas-Hernandez M, Galaz J, Motomura K, Tao L, et al. IL-22 plays a dual role in the amniotic cavity: tissue injury and host defense against microbes in preterm labor. J Immunol 2022;208:1595–615.
30. Romero R, Sirtori M, Oyarzun E, Avila C, Mazor M, Callahan R, et al. Infection and labor. V. Prevalence, microbiology, and clinical significance of intraamniotic infection in women with preterm labor and intact membranes. Am J Obstet Gynecol 1989; 161:817–24.
31. Gibbs RS, Romero R, Hillier SL, Eschenbach DA, Sweet RL. A review of premature birth and subclinical infection. Am J Obstet Gynecol 1992;166:1515–28.
32. Romero R, Gomez-Lopez N, Winters AD, Jung E, Shaman M, Bieda J, et al. Evidence that intra-amniotic infections are often the result of an ascending invasion – a molecular microbiological study. J Perinat Med 2019;47:915–31.
33. Winters AD, Romero R, Graffice E, Gomez-Lopez N, Jung E, Kanninen T, et al. Optimization and validation of two multiplex qPCR assays for the rapid detection of microorganisms commonly invading the amniotic cavity. J Reprod Immunol 2022; 149:103460.
34. Kacerovsky M, Stranik J, Matulova J, Chalupska M, Mls J, Faist T, et al. Clinical characteristics of colonization of the amniotic cavity in women with preterm prelabor rupture of membranes, a retrospective study. Sci Rep 2022;12:5062.
35. Romero R, Chaiworapongsa T, Savasan ZA, Xu Y, Hussein Y, Dong Z, et al. Damage-associated molecular patterns (DAMPs) in preterm labor with intact membranes and preterm PROM: a study of the alarmin HMGB1. J Matern Fetal Neonatal Med 2011; 24:1444–55.
36. Romero R, Chaiworapongsa T, Savasan ZA, Hussein Y, Dong Z, Kusanovic JP, et al. Clinical chorioamnionitis is characterized by changes in the expression of the alarmin HMGB1 and one of its receptors, sRAGE. J Matern Fetal Neonatal Med 2012;25:558–67.
37. Motomura K, Romero R, Garcia-Flores V, Leng Y, Xu Y, Galaz J, et al. The alarmin interleukin-1α causes preterm birth through the NLRP3 inflammasome. Mol Hum Reprod 2020;26:712–26.
38. Motomura K, Romero R, Plazyo O, Garcia-Flores V, Gershater M, Galaz J, et al. The alarmin S100A12 causes sterile inflammation of the human chorioamniotic membranes as well as preterm birth and neonatal mortality in mice†. Biol Reprod 2021;105: 1494–509.
39. Schwenkel G, Romero R, Slutsky R, Motomura K, Hsu CD, Gomez-Lopez N. HSP70: an alarmin that does not induce high rates of preterm birth but does cause adverse neonatal outcomes. J Matern Fetal Neonatal Med 2021;34:4110–8.
40. Miller JM Jr, Pupkin MJ, Hill GB. Bacterial colonization of amniotic fluid from intact fetal membranes. Am J Obstet Gynecol 1980;136:796–804.
41. Bobitt JR, Hayslip CC, Damato JD. Amniotic fluid infection as determined by transabdominal amniocentesis in patients with intact membranes in premature labor. Am J Obstet Gynecol 1981;140:947–52.
42. Wallace RL, Herrick CN. Amniocentesis in the evaluation of premature labor. Obstet Gynecol 1981;57:483–6.
43. Wahbeh CJ, Hill GB, Eden RD, Gall SA. Intra-amniotic bacterial colonization in premature labor. Am J Obstet Gynecol 1984;148: 739–43.
44. Hameed C, Tejani N, Verma UL, Archbald F. Silent chorioamnionitis as a cause of preterm labor refractory to tocolytic therapy. Am J Obstet Gynecol 1984;149:726–30.
45. Leigh J, Garite TJ. Amniocentesis and the management of premature labor. Obstet Gynecol 1986;67:500–6.
46. Gravett MG, Hummel D, Eschenbach DA, Holmes KK. Preterm labor associated with subclinical amniotic fluid infection and with bacterial vaginosis. Obstet Gynecol 1986;67:229–37.
47. Romero R, Emamian M, Quintero R, Wan M, Hobbins JC, Mazor M, et al. The value and limitations of the Gram stain examination in the diagnosis of intraamniotic infection. Am J Obstet Gynecol 1988;159:114–9.
48. Skoll MA, Moretti ML, Sibai BM. The incidence of positive amniotic fluid cultures in patients preterm labor with intact membranes. Am J Obstet Gynecol 1989;161:813–6.
49. Romero R, Jimenez C, Lohda AK, Nores J, Hanaoka S, Avila C, et al. Amniotic fluid glucose concentration: a rapid and simple method for the detection of intraamniotic infection in preterm labor. Am J Obstet Gynecol 1990;163:968–74.
50. Gauthier DW, Meyer WJ, Bieniarz A. Correlation of amniotic fluid glucose concentration and intraamniotic infection in patients with preterm labor or premature rupture of membranes. Am J Obstet Gynecol 1991;165:1105–10.
51. Coultrip LL, Grossman JH. Evaluation of rapid diagnostic tests in the detection of microbial invasion of the amniotic cavity. Am J Obstet Gynecol 1992;167:1231–42.
52. Watts DH, Krohn MA, Hillier SL, Eschenbach DA. The association of occult amniotic fluid infection with gestational age and neonatal outcome among women in preterm labor. Obstet Gynecol 1992;79:351–7.
53. Gomez R, Romero R, Ghezzi F, Yoon BH, Mazor M, Berry SM. The fetal inflammatory response syndrome. Am J Obstet Gynecol 1998;179:194–202.
54. Hussey MJ, Levy ES, Pombar X, Meyer P, Strassner HT. Evaluating rapid diagnostic tests of intra-amniotic infection: gram stain, amniotic fluid glucose level, and amniotic fluid to serum glucose level ratio. Am J Obstet Gynecol 1998;179:650–6.
55. Rizzo G, Capponi A, Vlachopoulou A, Angelini E, Grassi C, Romanini C. Ultrasonographic assessment of the uterine cervix and interleukin-8 concentrations in cervical secretions predict intrauterine infection in patients with preterm labor and intact membranes. Ultrasound Obstet Gynecol 1998;12:86–92.
56. Yoon BH, Chang JW, Romero R. Isolation of ureaplasma urealyticum from the amniotic cavity and adverse outcome in preterm labor. Obstet Gynecol 1998;92:77–82.
57. Elimian A, Figueroa R, Canterino J, Verma U, Aguero-Rosenfeld M, Tejani N. Amniotic fluid complement C3 as a

marker of intra-amniotic infection. Obstet Gynecol 1998;92: 72–6.
58. Oyarzún E, Yamamoto M, Kato S, Gómez R, Lizama L, Moenne A. Specific detection of 16 micro-organisms in amniotic fluid by polymerase chain reaction and its correlation with preterm delivery occurrence. Am J Obstet Gynecol 1998;179:1115–9.
59. Yoon BH, Romero R, Moon JB, Shim SS, Kim M, Kim G, et al. Clinical significance of intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Obstet Gynecol 2001;185:1130–6.
60. Garite TJ, Freeman RK. Chorioamnionitis in the preterm gestation. Obstet Gynecol 1982;59:539–45.
61. Cotton DB, Hill LM, Strassner HT, Platt LD, Ledger WJ. Use of amniocentesis in preterm gestation with ruptured membranes. Obstet Gynecol 1984;63:38–43.
62. Zlatnik FJ, Cruikshank DP, Petzold CR, Galask RP. Amniocentesis in the identification of inapparent infection in preterm patients with premature rupture of the membranes.J Reprod Med 1984; 29:656–60.
63. Broekhuizen FF, Gilman M, Hamilton PR. Amniocentesis for Gram stain and culture in preterm premature rupture of the membranes. Obstet Gynecol 1985;66:316–21.
64. Feinstein SJ, Vintzileos AM, Lodeiro JG, Campbell WA, Weinbaum PJ, Nochimson DJ. Amniocentesis with premature rupture of membranes. Obstet Gynecol 1986;68:147–52.
65. Romero R, Emamian M, Wan M, Yarkoni S, McCormack W, Mazor M, et al. The value of the leukocyte esterase test in diagnosing intra-amniotic infection. Am J Perinatol 1988;5: 64–9.
66. Dudley J, Malcolm G, Ellwood D. Amniocentesis in the management of preterm premature rupture of the membranes. Aust N Z J Obstet Gynaecol 1991;31:331–6.
67. Romero R, Yoon BH, Mazor M, Gomez R, Gonzalez R, Diamond MP, et al. A comparative study of the diagnostic performance of amniotic fluid glucose, white blood cell count, interleukin-6, and Gram stain in the detection of microbial invasion in patients with preterm premature rupture of membranes. Am J Obstet Gynecol 1993;169:839–51.
68. Yoon BH, Jun JK, Park KH, Syn HC, Gomez R, Romero R. Serum C-reactive protein, white blood cell count, and amniotic fluid white blood cell count in women with preterm premature rupture of membranes. Obstet Gynecol 1996;88:1034–40.
69. Blackwell SC, Berry SM. Role of amniocentesis for the diagnosis of subclinical intra-amniotic infection in preterm premature rupture of the membranes. Curr Opin Obstet Gynecol 1999;11: 541–7.
70. Shim SS, Romero R, Hong JS, Park CW, Jun JK, Kim BI, et al. Clinical significance of intra-amniotic inflammation in patients with preterm premature rupture of membranes. Am J Obstet Gynecol 2004;191:1339–45.
71. Romero R, Miranda J, Chaemsaithong P, Chaiworapongsa T, Kusanovic JP, Dong Z, et al. Sterile and microbial-associated intra-amniotic inflammation in preterm prelabor rupture of membranes. J Matern Fetal Neonatal Med 2015;28: 1394–409.
72. Romero R, Gonzalez R, Sepulveda W, Brandt F, Ramirez M, Sorokin Y, et al. Infection and labor. VIII. Microbial invasion of the amniotic cavity in patients with suspected cervical incompetence: prevalence and clinical significance. Am J Obstet Gynecol 1992;167:1086–91.
73. Mays JK, Figueroa R, Shah J, Khakoo H, Kaminsky S, Tejani N. Amniocentesis for selection before rescue cerclage. Obstet Gynecol 2000;95:652–5.
74. Lee SE, Romero R, Park CW, Jun JK, Yoon BH. The frequency and significance of intraamniotic inflammation in patients with cervical insufficiency. Am J Obstet Gynecol 2008;198:633.e1–8.
75. Bujold E, Morency AM, Rallu F, Ferland S, Tétu A, Duperron L, et al. Bacteriology of amniotic fluid in women with suspected cervical insufficiency. J Obstet Gynaecol Can 2008;30:882–7.
76. Gómez R, Romero R, Nien JK, Medina L, Carstens M, Kim YM, et al. Idiopathic vaginal bleeding during pregnancy as the only clinical manifestation of intrauterine infection. J Matern Fetal Neonatal Med: Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies 2005;18: 31–7.
77. Hassan S, Romero R, Hendler I, Gomez R, Khalek N, Espinoza J, et al. A sonographic short cervix as the only clinical manifestation of intra-amniotic infection. J Perinat Med 2006;34:13–9.
78. Romero R, Miranda J, Chaiworapongsa T, Chaemsaithong P, Gotsch F, Dong Z, et al. Sterile intra-amniotic inflammation in asymptomatic patients with a sonographic short cervix: prevalence and clinical significance. J Matern Fetal Neonatal Med 2015;28:1343–59.
79. Gervasi MT, Romero R, Bracalente G, Erez O, Dong Z, Hassan SS, et al. Midtrimester amniotic fluid concentrations of interleukin-6 and interferon-gamma-inducible protein-10: evidence for heterogeneity of intra-amniotic inflammation and associations with spontaneous early (<32 weeks) and late (>32 weeks) preterm delivery. J Perinat Med 2012;40:329–43.
80. Keelan JA, Wang K, Chaiworapongsa T, Romero R, Mitchell MD, Sato TA, et al. Macrophage inhibitory cytokine 1 in fetal membranes and amniotic fluid from pregnancies with and without preterm labour and premature rupture of membranes. Mol Hum Reprod 2003;9:535–40.
81. Romero R, Erez O, Espinoza J. Intrauterine infection, preterm labor, and cytokines. J Soc Gynecol Invest 2005;12:463–5.
82. Romero R, Grivel JC, Tarca AL, Chaemsaithong P, Xu Z, Fitzgerald W, et al. Evidence of perturbations of the cytokine network in preterm labor. Am J Obstet Gynecol 2015;213:836 e1–8.
83. Tarca AL, Fitzgerald W, Chaemsaithong P, Xu Z, Hassan SS, Grivel JC, et al. The cytokine network in women with an asymptomatic short cervix and the risk of preterm delivery. Am J Reprod Immunol 2017;78:e12686.
84. Kim MJ, Romero R, Kim CJ, Tarca AL, Chhauy S, LaJeunesse C, et al. Villitis of unknown etiology is associated with a distinct pattern of chemokine up-regulation in the feto-maternal and placental compartments: implications for conjoint maternal allograft rejection and maternal anti-fetal graft-versus-host disease. J Immunol 2009;182:3919–27.
85. Yoon BH, Romero R, Yang SH, Jun JK, Kim IO, Choi JH, et al. Interleukin-6 concentrations in umbilical cord plasma are elevated in neonates with white matter lesions associated with periventricular leukomalacia. Am J Obstet Gynecol 1996;174: 1433–40.
86. Yoon BH, Jun JK, Romero R, Park KH, Gomez R, Choi JH, et al. Amniotic fluid inflammatory cytokines (interleukin-6, interleukin-1beta, and tumor necrosis factor-alpha), neonatal brain white matter lesions, and cerebral palsy. Am J Obstet Gynecol 1997;177:19–26.

87. Grether JK, Nelson KB. Maternal infection and cerebral palsy in infants of normal birth weight. JAMA 1997;278:207–11.
88. Dammann O, Leviton A. Maternal intrauterine infection, cytokines, and brain damage in the preterm newborn. Pediatr Res 1997;42:1–8.
89. Sävman K, Blennow M, Gustafson K, Tarkowski E, Hagberg H. Cytokine response in cerebrospinal fluid after birth asphyxia. Pediatr Res 1998;43:746–51.
90. Nelson KB, Dambrosia JM, Grether JK, Phillips TM. Neonatal cytokines and coagulation factors in children with cerebral palsy. Ann Neurol 1998;44:665–75.
91. Yoon BH, Romero R, Park JS, Kim CJ, Kim SH, Choi JH, et al. Fetal exposure to an intra-amniotic inflammation and the development of cerebral palsy at the age of three years. Am J Obstet Gynecol 2000;182:675–81.
92. Wu YW, Colford JM Jr. Chorioamnionitis as a risk factor for cerebral palsy: a meta-analysis. JAMA 2000;284:1417–24.
93. Dammann O, Leviton A. Role of the fetus in perinatal infection and neonatal brain damage. Curr Opin Pediatr 2000;12:99–104.
94. Moon JB, Kim JC, Yoon BH, Romero R, Kim G, Oh SY, et al. Amniotic fluid matrix metalloproteinase-8 and the development of cerebral palsy. J Perinat Med 2002;30:301–6.
95. Leviton A, Dammann O. Coagulation, inflammation, and the risk of neonatal white matter damage. Pediatr Res 2004;55:541–5.
96. Jacobsson B, Hagberg G. Antenatal risk factors for cerebral palsy. Best Pract Res Clin Obstet Gynaecol 2004;18:425–36.
97. Bartha AI, Foster-Barber A, Miller SP, Vigneron DB, Glidden DV, Barkovich AJ, et al. Neonatal encephalopathy: association of cytokines with MR spectroscopy and outcome. Pediatr Res 2004;56:960–6.
98. Hagberg H, Mallard C, Jacobsson B. Role of cytokines in preterm labour and brain injury. BJOG An Int J Obstet Gynaecol 2005;112: 16–8.
99. Leviton A, Allred EN, Kuban KC, Hecht JL, Onderdonk AB, O'Shea TM, et al. Microbiologic and histologic characteristics of the extremely preterm infant's placenta predict white matter damage and later cerebral palsy. the ELGAN study. Pediatr Res 2010;67:95–101.
100. Fleiss B, Gressens P. Tertiary mechanisms of brain damage: a new hope for treatment of cerebral palsy? Lancet Neurol 2012; 11:556–66.
101. Lawrence SM, Wynn JL. Chorioamnionitis, IL-17A, and fetal origins of neurologic disease. Am J Reprod Immunol 79:2018: e12803.
102. Patterson PH. Immune involvement in schizophrenia and autism: etiology, pathology and animal models. Behav Brain Res 2009;204:313–21.
103. Meyer U, Feldon J, Dammann O. Schizophrenia and autism: both shared and disorder-specific pathogenesis via perinatal inflammation? Pediatr Res 2011;69:26r–33r.
104. Abdallah MW, Larsen N, Grove J, Nørgaard-Pedersen B, Thorsen P, Mortensen EL, et al. Amniotic fluid inflammatory cytokines: potential markers of immunologic dysfunction in autism spectrum disorders. World J Biol Psychiatry 2013;14: 528–38.
105. Zerbo O, Iosif AM, Walker C, Ozonoff S, Hansen RL, Hertz-Picciotto I. Is maternal influenza or fever during pregnancy associated with autism or developmental delays? Results from the CHARGE (CHildhood autism risks from genetics and environment) study. J Autism Dev Disord 2013;43:25–33.
106. Lee BK, Magnusson C, Gardner RM, Blomström Å, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. Brain Behav Immun 2015;44:100–5.
107. Madore C, Leyrolle Q, Lacabanne C, Benmamar-Badel A, Joffre C, Nadjar A, et al. Neuroinflammation in autism: plausible role of maternal inflammation, dietary omega 3, and microbiota. Neural Plast 2016;2016:3597209.
108. Jiang HY, Xu LL, Shao L, Xia RM, Yu ZH, Ling ZX, et al. Maternal infection during pregnancy and risk of autism spectrum disorders: a systematic review and meta-analysis. Brain Behav Immun 2016;58:165–72.
109. al-Haddad BJS, Jacobsson B, Chabra S, Modzelewska D, Olson EM, Bernier R, et al. Long-term risk of neuropsychiatric disease after exposure to infection in utero. JAMA Psychiatr 2019;76:594–602.
110. Han VX, Patel S, Jones HF, Nielsen TC, Mohammad SS, Hofer MJ, et al. Maternal acute and chronic inflammation in pregnancy is associated with common neurodevelopmental disorders: a systematic review. Transl Psychiatry 2021;11:71.
111. Brown AS, Susser ES. In utero infection and adult schizophrenia. Ment Retard Dev Disabil Res Rev 2002;8:51–7.
112. Gilmore JH, Fredrik Jarskog L, Vadlamudi S, Lauder JM. Prenatal infection and risk for schizophrenia: IL-1beta, IL-6, and TNFalpha inhibit cortical neuron dendrite development. Neuropsychopharmacology 2004;29:1221–9.
113. Nawa H, Takei N. Recent progress in animal modeling of immune inflammatory processes in schizophrenia: implication of specific cytokines. Neurosci Res 2006;56:2–13.
114. Brown AS, Vinogradov S, Kremen WS, Poole JH, Deicken RF, Penner JD, et al. Prenatal exposure to maternal infection and executive dysfunction in adult schizophrenia. Am J Psychiatr 2009;166:683–90.
115. Brown AS, Derkits EJ. Prenatal infection and schizophrenia: a review of epidemiologic and translational studies. Am J Psychiatr 2010;167:261–80.
116. Aguilar-Valles A, Rodrigue B, Matta-Camacho E. Maternal immune activation and the development of dopaminergic neurotransmission of the offspring: relevance for schizophrenia and other psychoses. Front Psychiatr 2020;11:852.
117. Mongan D, Ramesar M, Föcking M, Cannon M, Cotter D. Role of inflammation in the pathogenesis of schizophrenia: a review of the evidence, proposed mechanisms and implications for treatment. Early Interv Psychiatry 2020;14:385–97.
118. Khan D, Fernando P, Cicvaric A, Berger A, Pollak A, Monje FJ, et al. Long-term effects of maternal immune activation on depression-like behavior in the mouse. Transl Psychiatry 2014; 4:e363.
119. Majidi-Zolbanin J, Doosti MH, Kosari-Nasab M, Salari AA. Prenatal maternal immune activation increases anxiety- and depressive-like behaviors in offspring with experimental autoimmune encephalomyelitis. Neuroscience 2015;294: 69–81.
120. Depino AM. Early prenatal exposure to LPS results in anxiety- and depression-related behaviors in adulthood. Neuroscience 2015;299:56–65.
121. Ronovsky M, Berger S, Zambon A, Reisinger SN, Horvath O, Pollak A, et al. Maternal immune activation transgenerationally modulates maternal care and offspring depression-like behavior. Brain Behav Immun 2017;63:127–36.

122. Jin C, Londono I, Mallard C, Lodygensky GA. New means to assess neonatal inflammatory brain injury. J Neuroinflammation 2015;12:180.
123. Han VX, Patel S, Jones HF, Dale RC. Maternal immune activation and neuroinflammation in human neurodevelopmental disorders. Nat Rev Neurol 2021;17:564–79.
124. Gervasi MT, Romero R, Bracalente G, Chaiworapongsa T, Erez O, Dong Z, et al. Viral invasion of the amniotic cavity (VIAC) in the midtrimester of pregnancy. J Matern Fetal Neonatal Med 2012; 25:2002–13.
125. Raven JC, Court JH, Raven J. Manual for raven's progressive matrices. Oxford, UK: Oxford Psychologists Press; 1992.
126. Bisiacchi PS, Cendron M, Gugliotta M, Tressoldi P, Vio C. Batteria di valutazione neuropsicologica per l'età evolutiva. Centro studi Erickson Trento; 2005.
127. Korkman M, Kirk U, Kemp S. NEPSY-II: a developmental neuropsychological assessment, 2nd ed. San Antonio, TX: Psychological Corporation; 2007.
128. Urgesi C, Campanella F, Fabbro F. NEPSY-II. In: Second Edition contributo alla taratura italiana firenze: Giunti OS Organizzazioni Speciali; 2011.
129. Facello GS, Vio C, Cianchetti C. TOL torre di Londra, test ti valutazione delle funzioni esecutive (pianificazione e problem solving). Trento, Italy: Erickson; 2006.
130. Orsini A, Pezzuti L, Picone L. WISC-IV. Contributo alla taratura italiana. Firenze: Giunti OS Organizzazioni Speciali; 2012.
131. Marzocchi GM, Re A, Cornoldi C. BIA - Batteria italiana per l'ADHD per la valutazione dei bambini con deficit di attenzione/iperattività. Trento: Erickson; 2010.
132. Beery KE, Beery NA. The Beery-Buktenica developmental test of visual motor integration: administration, scoring, and teaching manual, 5th ed. Cleveland, OH: Modern Curriculum Press; 2004.
133. Achenbach TM, Rescorla L. Manual for the ASEBA school-age forms & profiles: an integrated system of multi-informant assessment. Burlington, VTYouth, &Families: University of Vermont, Research Center for Children; 2001.
134. Goodman R, Meltzer H, Bailey V. The strengths and difficulties questionnaire: a pilot study on the validity of the self-report version. Eur Child Adolesc Psychiatry 1998;7:125–30.
135. CK C. Conners' rating scales-revised. New York: Multi-Health Systems; 2000.
136. Malamitsi-Puchner A, Vrachnis N, Samoli E, Baka S, Iliodromiti Z, Puchner KP, et al. Possible early prediction of preterm birth by determination of novel proinflammatory factors in midtrimester amniotic fluid. Ann N Y Acad Sci 2006;1092: 440–9.
137. Puchner K, Iavazzo C, Gourgiotis D, Boutsikou M, Baka S, Hassiakos D, et al. Mid-trimester amniotic fluid interleukins (IL-1β, IL-10 and IL-18) as possible predictors of preterm delivery. In Vivo 2011;25:141–8.
138. Garbett KA, Hsiao EY, Kálmán S, Patterson PH, Mirnics K. Effects of maternal immune activation on gene expression patterns in the fetal brain. Transl Psychiatry 2012;2:e98.
139. Hagberg H, Gressens P, Mallard C. Inflammation during fetal and neonatal life: implications for neurologic and neuropsychiatric disease in children and adults. Ann Neurol 2012;71:444–57.
140. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. Nat Rev Neurol 2014; 10:643–60.
141. Van Steenwinckel J, Schang AL, Sigaut S, Chhor V, Degos V, Hagberg H, et al. Brain damage of the preterm infant: new insights into the role of inflammation. Biochem Soc Trans 2014; 42:557–63.
142. Bauman MD, Iosif AM, Smith SE, Bregere C, Amaral DG, Patterson PH. Activation of the maternal immune system during pregnancy alters behavioral development of rhesus monkey offspring. Biol Psychiatr 2014;75:332–41.
143. Hagberg H, Mallard C, Ferriero DM, Vannucci SJ, Levison SW, Vexler ZS, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol 2015;11:192–208.
144. Armstrong-Wells J, Donnelly M, Post MD, Manco-Johnson MJ, Winn VD, Sébire G. Inflammatory predictors of neurologic disability after preterm premature rupture of membranes. Am J Obstet Gynecol 2015;212:212.e1–9.
145. Estes ML, McAllister AK. Maternal immune activation: implications for neuropsychiatric disorders. Science 2016;353: 772–7.
146. Boulanger-Bertolus J, Pancaro C, Mashour GA. Increasing role of maternal immune activation in neurodevelopmental disorders. Front Behav Neurosci 2018;12:230.
147. Gumusoglu SB, Stevens HE. Maternal inflammation and neurodevelopmental programming: a review of preclinical outcomes and implications for translational psychiatry. Biol Psychiatr 2019;85:107–21.
148. Al-Haddad BJS, Oler E, Armistead B, Elsayed NA, Weinberger DR, Bernier R, et al. The fetal origins of mental illness. Am J Obstet Gynecol 2019;221:549–62.
149. Furman D, Campisi J, Verdin E, Carrera-Bastos P, Targ S, Franceschi C, et al. Chronic inflammation in the etiology of disease across the life span. Nat Med 2019;25: 1822–32.
150. Prieto-Villalobos J, Alvear TF, Liberona A, Lucero CM, Martínez-Araya CJ, Balmazabal J, et al. Astroglial hemichannels and pannexons: the hidden link between maternal inflammation and neurological disorders. Int J Mol Sci 2021;22:9503.
151. Zawadzka A, Cieślik M, Adamczyk A. The role of maternal immune activation in the pathogenesis of autism: a review of the evidence, proposed mechanisms and implications for treatment. Int J Mol Sci 2021;22. https://doi.org/10.3390/ijms222111516.
152. Kwon HK, Choi GB, Huh JR. Maternal inflammation and its ramifications on fetal neurodevelopment. Trends Immunol 2022;43:230–44.
153. Werenberg Dreier J, Nybo Andersen AM, Hvolby A, Garne E, Kragh Andersen P, Berg-Beckhoff G. Fever and infections in pregnancy and risk of attention deficit/hyperactivity disorder in the offspring. J Child Psychol Psychiatry Allied Discip 2016;57: 540–8.
154. Dunn GA, Nigg JT, Sullivan EL. Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacology Biochem Behav 2019;182:22–34.
155. Ginsberg Y, D'Onofrio BM, Rickert ME, Class QA, Rosenqvist MA, Almqvist C, et al. Maternal infection requiring hospitalization during pregnancy and attention-deficit hyperactivity disorder in offspring: a quasi-experimental family-based study. J Child Psychol Psychiatry Allied Discip 2019;60:160–8.

156. Ellman LM, Yolken RH, Buka SL, Torrey EF, Cannon TD. Cognitive functioning prior to the onset of psychosis: the role of fetal exposure to serologically determined influenza infection. Biol Psychiatr 2009;65:1040–7.
157. Brown AS, Vinogradov S, Kremen WS, Poole JH, Bao Y, Kern D, et al. Association of maternal genital and reproductive infections with verbal memory and motor deficits in adult schizophrenia. Psychiatr Res 2011;188:179–86.
158. Thangarajan R, Tantradi RR, Rai KS, Gopalakrishnan S, Perumal V. Inflammation during gestation induced spatial memory and learning deficits: attenuated by physical exercise in juvenile rats. J Clin Diagn Res 2015;9:Cf01–4.
159. Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult schizophrenia following prenatal exposure to an influenza epidemic. Arch Gen Psychiatr 1988;45:189–92.
160. Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. Arch Gen Psychiatr 2004;61:774–80.
161. Brown AS, Hooton J, Schaefer CA, Zhang H, Petkova E, Babulas V, et al. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. Am J Psychiatr 2004;161:889–95.
162. Watson CG, Kucala T, Tilleskjor C, Jacobs L. Schizophrenic birth seasonality in relation to the incidence of infectious diseases and temperature extremes. Arch Gen Psychiatr 1984;41:85–90.
163. O'Callaghan E, Sham PC, Takei N, Murray G, Glover G, Hare EH, et al. The relationship of schizophrenic births to 16 infectious diseases. Br J Psychiatry 1994;165:353–6.
164. Brown AS, Cohen P, Harkavy-Friedman J, Babulas V, Malaspina D, Gorman JM, et al. A.E. Bennett research award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. Biol Psychiatr 2001;49:473–86.
165. Fatemi SH, Earle J, Kanodia R, Kist D, Emamian ES, Patterson PH, et al. Prenatal viral infection leads to pyramidal cell atrophy and macrocephaly in adulthood: implications for genesis of autism and schizophrenia. Cell Mol Neurobiol 2002;22:25–33.
166. Gordon-Lipkin E, Hoon A, Pardo CA. Prenatal cytomegalovirus, rubella, and Zika virus infections associated with developmental disabilities: past, present, and future. Dev Med Child Neurol 2021;63:135–43.
167. Dammann O, Kuban KC, Leviton A. Perinatal infection, fetal inflammatory response, white matter damage, and cognitive limitations in children born preterm. Ment Retard Dev Disabil Res Rev 2002;8:46–50.
168. Elovitz MA, Mrinalini C, Sammel MD. Elucidating the early signal transduction pathways leading to fetal brain injury in preterm birth. Pediatr Res 2006;59:50–5.
169. Burd I, Chai J, Gonzalez J, Ofori E, Monnerie H, Le Roux PD, et al. Beyond white matter damage: fetal neuronal injury in a mouse model of preterm birth. Am J Obstet Gynecol 2009;201:279.e1–8.
170. Patterson PH. Maternal infection and immune involvement in autism. Trends Mol Med 2011;17:389–94.
171. Elovitz MA, Brown AG, Breen K, Anton L, Maubert M, Burd I. Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury. Int J Dev Neurosci 2011;29:663–71.
172. Meyer U. Prenatal poly(i:C) exposure and other developmental immune activation models in rodent systems. Biol Psychiatr 2014;75:307–15.
173. Yim YS, Park A, Berrios J, Lafourcade M, Pascual LM, Soares N, et al. Reversing behavioural abnormalities in mice exposed to maternal inflammation. Nature 2017;549:482–7.
174. Cai Z, Pan ZL, Pang Y, Evans OB, Rhodes PG. Cytokine induction in fetal rat brains and brain injury in neonatal rats after maternal lipopolysaccharide administration. Pediatr Res 2000;47:64–72.
175. Zuckerman L, Weiner I. Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. J Psychiatr Res 2005;39:311–23.
176. Dalsgaard S, Waltoft BL, Leckman JF, Mortensen PB. Maternal history of autoimmune disease and later development of tourette syndrome in offspring. J Am Acad Child Adolesc Psychiatry 2015;54:495–501.e1.
177. Chen SW, Zhong XS, Jiang LN, Zheng XY, Xiong YQ, Ma SJ, et al. Maternal autoimmune diseases and the risk of autism spectrum disorders in offspring: a systematic review and meta-analysis. Behav Brain Res 2016;296:61–9.
178. Instanes JT, Halmøy A, Engeland A, Haavik J, Furu K, Klungsøyr K. Attention-deficit/hyperactivity disorder in offspring of mothers with inflammatory and immune system diseases. Biol Psychiatr 2017;81:452–9.
179. Mataix-Cols D, Frans E, Pérez-Vigil A, Kuja-Halkola R, Gromark C, Isomura K, et al. A total-population multigenerational family clustering study of autoimmune diseases in obsessive-compulsive disorder and Tourette's/chronic tic disorders. Mol Psychiatr 2018;23:1652–8.
180. Zhao L, Li X, Liu G, Han B, Wang J, Jiang X. The association of maternal diabetes with attention deficit and hyperactivity disorder in offspring: a meta-analysis. Neuropsychiatric Dis Treat 2019;15:675–84.
181. Zeng Y, Tang Y, Yue Y, Li W, Qiu X, Hu P, et al. Cumulative evidence for association of parental diabetes mellitus and attention-deficit/hyperactivity disorder. Neurosci Biobehav Rev 2020;117:129–39.
182. Nielsen TC, Nassar N, Shand AW, Jones H, Guastella AJ, Dale RC, et al. Association of maternal autoimmune disease with attention-deficit/hyperactivity disorder in children. JAMA Pediatr 2021;175:e205487.
183. Croen LA, Grether JK, Yoshida CK, Odouli R, Van de Water J. Maternal autoimmune diseases, asthma and allergies, and childhood autism spectrum disorders: a case-control study. Arch Pediatr Adolesc Med 2005;159:151–7.
184. Gong T, Lundholm C, Rejnö G, Bölte S, Larsson H, D'Onofrio BM, et al. Parental asthma and risk of autism spectrum disorder in offspring: a population and family-based case-control study. Clin Exp Allergy 2019;49:883–91.
185. Liu X, Dalsgaard S, Munk-Olsen T, Li J, Wright RJ, Momen NC. Parental asthma occurrence, exacerbations and risk of attention-deficit/hyperactivity disorder. Brain Behav Immun 2019;82:302–8.
186. Jenabi E, Bashirian S, Khazaei S, Basiri Z. The maternal prepregnancy body mass index and the risk of attention deficit hyperactivity disorder among children and adolescents: a systematic review and meta-analysis. Korean J Pediatr 2019;62:374–9.
187. Ponzio NM, Servatius R, Beck K, Marzouk A, Kreider T. Cytokine levels during pregnancy influence immunological profiles and neurobehavioral patterns of the offspring. Ann N Y Acad Sci 2007;1107:118–28.

188. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science 2016;351:933–9.
189. Krakowiak P, Goines PE, Tancredi DJ, Ashwood P, Hansen RL, Hertz-Picciotto I, et al. Neonatal cytokine profiles associated with autism spectrum disorder. Biol Psychiatr 2017;81:442–51.
190. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. J Neurosci 2007;27:10695–702.
191. Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Wagner RL, Yolken RH. Maternal cytokine levels during pregnancy and adult psychosis. Brain Behav Immun 2001;15:411–20.
192. Boksa P. Effects of prenatal infection on brain development and behavior: a review of findings from animal models. Brain Behav Immun 2010;24:881–97.
193. Abdallah MW, Larsen N, Mortensen EL, Atladóttir H, Nørgaard-Pedersen B, Bonefeld-Jørgensen EC, et al. Neonatal levels of cytokines and risk of autism spectrum disorders: an exploratory register-based historic birth cohort study utilizing the danish newborn screening biobank. J Neuroimmunol 2012; 252:75–82.
194. Abdallah MW, Larsen N, Grove J, Bonefeld-Jørgensen EC, Nørgaard-Pedersen B, Hougaard DM, et al. Neonatal chemokine levels and risk of autism spectrum disorders: findings from a Danish historic birth cohort follow-up study. Cytokine 2013;61: 370–6.
195. Zerbo O, Yoshida C, Grether JK, Van de Water J, Ashwood P, Delorenze GN, et al. Neonatal cytokines and chemokines and risk of autism spectrum disorder: the early markers for autism (EMA) study: a case-control study. J Neuroinflammation 2014;11: 113.
196. Jones KL, Croen LA, Yoshida CK, Heuer L, Hansen R, Zerbo O, et al. Autism with intellectual disability is associated with increased levels of maternal cytokines and chemokines during gestation. Mol Psychiatr 2017;22:273–9.
197. Kirsten TB, Casarin RC, Bernardi MM, Felicio LF. Pioglitazone abolishes autistic-like behaviors via the IL-6 pathway. PLoS One 2018;13:e0197060.
198. Heuer LS, Croen LA, Jones KL, Yoshida CK, Hansen RL, Yolken R, et al. An exploratory examination of neonatal cytokines and chemokines as predictors of autism risk: the early markers for autism study. Biol Psychiatr 2019;86:255–64.
199. Spann MN, Monk C, Scheinost D, Peterson BS. Maternal immune activation during the third trimester is associated with neonatal functional connectivity of the salience network and fetal to toddler behavior. J Neurosci 2018;38:2877–86.
200. Rudolph MD, Graham AM, Feczko E, Miranda-Dominguez O, Rasmussen JM, Nardos R, et al. Maternal IL-6 during pregnancy can be estimated from newborn brain connectivity and predicts future working memory in offspring. Nat Neurosci 2018;21: 765–72.
201. Graham AM, Rasmussen JM, Rudolph MD, Heim CM, Gilmore JH, Styner M, et al. Maternal systemic interleukin-6 during pregnancy is associated with newborn amygdala phenotypes and subsequent behavior at 2 Years of age. Biol Psychiatr 2018; 83:109–19.
202. Rasmussen JM, Graham AM, Entringer S, Gilmore JH, Styner M, Fair DA, et al. Maternal Interleukin-6 concentration during pregnancy is associated with variation in frontolimbic white matter and cognitive development in early life. Neuroimage 2019;185:825–35.
203. Chow SS, Craig ME, Jones CA, Hall B, Catteau J, Lloyd AR, et al. Differences in amniotic fluid and maternal serum cytokine levels in early midtrimester women without evidence of infection. Cytokine 2008;44:78–84.
204. Abdallah MW, Larsen N, Grove J, Nørgaard-Pedersen B, Thorsen P, Mortensen EL, et al. Amniotic fluid chemokines and autism spectrum disorders: an exploratory study utilizing a danish historic birth cohort. Brain Behav Immun 2012;26: 170–6.
205. Abdallah MW, Pearce BD, Larsen N, Greaves-Lord K, Nørgaard-Pedersen B, Hougaard DM, et al. Amniotic fluid MMP-9 and neurotrophins in autism spectrum disorders: an exploratory study. Autism Res 2012;5:428–33.
206. Dammann O, Phillips TM, Allred EN, O'Shea TM, Paneth N, Van Marter LJ, et al. Mediators of fetal inflammation in extremely low gestational age newborns. Cytokine 2001;13:234–9.
207. Leviton A, Hecht JL, Allred EN, Yamamoto H, Fichorova RN, Dammann O. Persistence after birth of systemic inflammation associated with umbilical cord inflammation. J Reprod Immunol 2011;90:235–43.
208. Leviton A, Joseph RM, Fichorova RN, Allred EN, Gerry Taylor H, Michael O'Shea T, et al. Executive dysfunction early postnatal biomarkers among children born extremely preterm. J Neuroimmune Pharmacol 2019;14:188–99.
209. Lu H, Huang W, Chen X, Wang Q, Zhang Q, Chang M. Relationship between premature brain injury and multiple biomarkers in cord blood and amniotic fluid. J Matern Fetal Neonatal Med 2018;31:2898–904.
210. Gayle DA, Beloosesky R, Desai M, Amidi F, Nuñez SE, Ross MG. Maternal LPS induces cytokines in the amniotic fluid and corticotropin releasing hormone in the fetal rat brain. Am J Physiol Regul Integr Comp Physiol 2004;286:R1024–9.
211. Reicher L, Dabaja H, Ginsberg Y, Khatib N, Fouks Y, Attali E, et al. Fetal neuroprotective mechanism of maternal magnesium sulfate: proteomic analysis. J Mol Neurosci 2022;72:626–32.
212. Brown AG, Tulina NM, Barila GO, Hester MS, Elovitz MA. Exposure to intrauterine inflammation alters metabolomic profiles in the amniotic fluid, fetal and neonatal brain in the mouse. PLoS One 2017;12:e0186656.
213. Beloosesky R, Gayle DA, Amidi F, Nunez SE, Babu J, Desai M, et al. N-acetyl-cysteine suppresses amniotic fluid and placenta inflammatory cytokine responses to lipopolysaccharide in rats. Am J Obstet Gynecol 2006;194:268–73.
214. Awad N, Khatib N, Ginsberg Y, Weiner Z, Maravi N, Thaler I, et al. N-acetyl-cysteine (NAC) attenuates LPS-induced maternal and amniotic fluid oxidative stress and inflammatory responses in the preterm gestation. Am J Obstet Gynecol 2011;204: 450.e15–20.
215. Beloosesky R, Weiner Z, Khativ N, Maravi N, Mandel R, Boles J, et al. Prophylactic maternal n-acetylcysteine before lipopolysaccharide suppresses fetal inflammatory cytokine responses. Am J Obstet Gynecol 2009;200:665.e1–5.
216. Beloosesky R, Gayle DA, Ross MG. Maternal N-acetylcysteine suppresses fetal inflammatory cytokine responses to maternal lipopolysaccharide. Am J Obstet Gynecol 2006;195:1053–7.
217. Beloosesky R, Weiner Z, Ginsberg Y, Ross MG. Maternal N-acetyl-cysteine (NAC) protects the rat fetal brain from

inflammatory cytokine responses to lipopolysaccharide (LPS). J Matern Fetal Neonatal Med 2012;25:1324–8.
218. Tam Tam HB, Dowling O, Xue X, Lewis D, Rochelson B, Metz CN. Magnesium sulfate ameliorates maternal and fetal inflammation in a rat model of maternal infection. Am J Obstet Gynecol 2011;204:364.e1–8.
219. Khatib N, Ginsberg Y, Shalom-Paz E, Dabaja H, Gutzeit O, Zmora O, et al. Fetal neuroprotective mechanism of maternal magnesium sulfate for late gestation inflammation: in a rodent model. J Matern Fetal Neonatal Med 2020;33:3732–9.
220. Carty ML, Wixey JA, Colditz PB, Buller KM. Post-insult minocycline treatment attenuates hypoxia-ischemia-induced neuroinflammation and white matter injury in the neonatal rat: a comparison of two different dose regimens. Int J Dev Neurosci 2008;26:477–85.
221. Tang J, Chen Q, Guo J, Yang L, Tao Y, Li L, et al. Minocycline attenuates neonatal germinal-matrix-hemorrhage-induced neuroinflammation and brain edema by activating cannabinoid receptor 2. Mol Neurobiol 2016;53:1935–48.
222. Giovanoli S, Engler H, Engler A, Richetto J, Feldon J, Riva MA, et al. Preventive effects of minocycline in a neurodevelopmental two-hit model with relevance to schizophrenia. Transl Psychiatry 2016;6:e772.
223. Fan LW, Kaizaki A, Tien LT, Pang Y, Tanaka S, Numazawa S, et al. Celecoxib attenuates systemic lipopolysaccharide-induced brain inflammation and white matter injury in the neonatal rats. Neuroscience 2013;240:27–38.
224. Kaizaki A, Tien LT, Pang Y, Cai Z, Tanaka S, Numazawa S, et al. Celecoxib reduces brain dopaminergic neuronaldysfunction, and improves sensorimotor behavioral performance in neonatal rats exposed to systemic lipopolysaccharide. J Neuroinflammation 2013;10:45.
225. Kannan S, Dai H, Navath RS, Balakrishnan B, Jyoti A, Janisse J, et al. Dendrimer-based postnatal therapy for neuroinflammation and cerebral palsy in a rabbit model. Sci Transl Med 2012;4:130ra46.
226. Allan NP, Hume LE, Allan DM, Farrington AL, Lonigan CJ. Relations between inhibitory control and the development of academic skills in preschool and kindergarten: a meta-analysis. Dev Psychol 2014;50:2368–79.
227. Moffitt TE, Arseneault L, Belsky D, Dickson N, Hancox RJ, Harrington H, et al. A gradient of childhood self-control predicts health, wealth, and public safety. Proc Natl Acad Sci U S A 2011; 108:2693–8.
228. Blair C, Razza RP. Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. Child Dev 2007;78:647–63.
229. Mischel W, Shoda Y, Rodriguez MI. Delay of gratification in children. Science 1989;244:933–8.
230. O'Hearn K, Asato M, Ordaz S, Luna B. Neurodevelopment and executive function in autism. Dev Psychopathol 2008;20:1103–32.
231. Pietrefesa AS, Evans DW. Affective and neuropsychological correlates of children's rituals and compulsive-like behaviors: continuities and discontinuities with obsessive-compulsive disorder. Brain Cognit 2007;65:36–46.
232. Castellanos FX, Sonuga-Barke EJ, Milham MP, Tannock R. Characterizing cognition in ADHD: beyond executive dysfunction. Trends Cognit Sci 2006;10:117–23.
233. Ameis SH, Catani M. Altered white matter connectivity as a neural substrate for social impairment in autism spectrum disorder. Cortex 2015;62:158–81.
234. Bettcher BM, Mungas D, Patel N, Elofson J, Dutt S, Wynn M, et al. Neuroanatomical substrates of executive functions: beyond prefrontal structures. Neuropsychologia 2016;85:100–9.
235. Wang Y, Metoki A, Alm KH, Olson IR. White matter pathways and social cognition. Neurosci Biobehav Rev 2018;90:350–70.
236. Bells S, Lefebvre J, Prescott SA, Dockstader C, Bouffet E, Skocic J, et al. Changes in white matter microstructure impact cognition by disrupting the ability of neural assemblies to synchronize. J Neurosci 2017;37:8227–38.
237. Cainelli E, Arrigoni F, Vedovelli L. White matter injury and neurodevelopmental disabilities: a cross-disease (dis) connection. Prog Neurobiol 2020;193:101845.
238. Romero R, Miranda J, Chaiworapongsa T, Korzeniewski SJ, Chaemsaithong P, Gotsch F, et al. Prevalence and clinical significance of sterile intra-amniotic inflammation in patients with preterm labor and intact membranes. Am J Reprod Immunol 2014;72:458–74. 5.
239. Yoon BH, Romero R, Park JY, Oh KJ, Lee J, Conde-Agudelo A, et al. Antibiotic administration can eradicate intra-amniotic infection or intra-amniotic inflammation in a subset of patients with preterm labor and intact membranes. Am J Obstet Gynecol 2019; 221:142.e1–22.
240. Kacerovsky M, Romero R, Stepan M, Stranik J, Maly J, Pliskova L, et al. Antibiotic administration reduces the rate of intraamniotic inflammation in preterm prelabor rupture of the membranes. Am J Obstet Gynecol 2020;223. https://doi.org/10.1016/j.ajog.2020.01.043.