UNIVERSITY OF TEXAS, ARLINGTON

Prenatal Exposure to Per- and Polyfluoroalkyl Substances and Child Neurodevelopment

DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in concentration on Environmental Science

by

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Dedicated to my husband, baby, and my parents

TABLE OF CONTENTS

LIST OF FIGURES

Page

LIST OF TABLES

Page

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1. **Oh J**, Bennett DH, Tancredi D, Calafat A, Schmidt RJ, Hertz-Picciotto I, Shin HM. (under review). Longitudinal changes in Maternal Serum Concentrations of Per- and Polyfluoroalkyl Substances from Pregnancy to Two Years Postpartum.
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[Poster Presentation]

1. **Oh J**, Choi JY, Ahn YA, Kim SK. 2017. Pharmacokinetics of bisphenol S in humans after a single oral administration. *SETAC-Europe*, Brussels, Belgium, May 2017.
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ABSTRACTS OF THE DISSERTATION

Prenatal Exposure to Per- and Polyfluoroalkyl Substances and
Child Neurodevelopment

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Pregnant women are exposed to per- and polyfluoroalkyl substances (PFAS) primarily via ingestion of contaminated food, water, and house dust. PFAS in pregnant women can be transferred to their child through the placenta during pregnancy. Many long-chain PFAS have been frequently detected in blood of pregnant women, amniotic fluid, and umbilical cord blood for the last few decades. Prenatal exposure to PFAS has shown potential to adversely affect offspring's neurodevelopment in laboratory animals. However, epidemiological evidence on associations between prenatal PFAS exposure and child neurodevelopment remains inconsistent.

My dissertation research consists of four sub-studies whose objectives are (1) to examine associations between prenatal maternal serum PFAS concentrations and the risk of autism spectrum disorder (ASD), (2) to examine associations between prenatal exposure to PFAS and cognitive development in infancy and toddlerhood, (3) to examine associations between cord blood PFAS concentrations and cognitive development in infancy and toddlerhood, and (4) to investigate longitudinal changes in maternal PFAS concentrations from pregnancy to two years

postpartum. The first, second, and fourth studies used data from the MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns), a longitudinal cohort of children with a first degree relative who was diagnosed with ASD. The MARBLES children were administered to Mullen Scales of Early Learning (MSEL) for cognitive functions at 6, 12, 24, and 36 months of age and clinically diagnosed with ASD at 36 months of age. Nine PFAS were quantified in maternal serum collected during the 1st, 2nd, and 3rd trimester of pregnancy and at 3, 6, and 24 months after delivery.

In the first study, I performed Poisson regression analyses to examine associations of ASD risk with individual PFAS as well as combined PFAS using a principal component analysis (PCA). I observed that prenatal maternal serum concentrations of perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) were associated with increased risk of child ASD. In the second study, I used multiple linear regression models to examine cross-sectional associations of PFAS with the MSEL Composite and four subscale scores at each time point and generalized estimating equations to examine associations between PFAS and longitudinal changes in the MSEL scores over the four assessment time points. I also classified trajectories of the MSEL Composite scores into low- and high-score groups and fit Poisson regression models to estimate relative risk (RR) of a high-score group versus a low-score group. I observed that prenatal maternal serum PFOA concentrations were inversely associated with child MSEL Composite scores at 24 and 36 months of age. When assessing longitudinal changes in the scores over the four time points, PFOA was associated with a negative slope for Composite scores and all four subscales. When examining RR between low- and high-score groups, PFOA was associated with increased risk of having lower and/or decreasing Composite scores.

In the third study, I used a Japanese population to confirm the findings from the second study. I used the same statistical models to examine the cross-sectional and longitudinal associations between cord blood PFOA and PFOS concentrations and MSEL Composite and subscale scores assessed at 4, 6, 10, 14, 18, 24, 32, and 40 months of age. MSEL Composite scores were inversely associated with cord blood PFOA at 18 months of age, but not at other ages. When accounting for changes in scores from 4 to 40 months of age, PFOA and PFOS were inversely associated with Fine Motor scores and positively associated with Receptive Language scores.

In the fourth study, I fit separate linear mixed models during pregnancy, early postpartum (from delivery to 6 months postpartum) and late postpartum (from 6 to 24 months postpartum) to estimate percent changes for each sub-period. During pregnancy, linear and branched perfluorooctane sulfonate (n- and Sm-PFOS), linear PFOA (n-PFOA), and PFNA concentrations changed -4% to -3% per month. During early postpartum, perfluorohexane sulfonate and n-PFOA concentrations changed -6% and -4%, respectively, per month, and Sm-PFOS and PFNA concentrations changed -1% per month. During late postpartum, n-PFOS, Sm-PFOS, and PFNA concentrations changed -1% per month.

CHAPTER 1: INTRODUCTION

1.1. Background

1.1.1. Per- and polyfluoroalkyl substances

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals containing carbon-fluorine bonds. They have both hydrophilic properties in the carboxylate or sulfonate head group and hydrophobic properties in the fluorocarbon tail group (3M 1999). Because of their surfactant properties, PFAS have been widely applied in the manufacture of consumer and industrial products, such as non-stick cookware, food contact materials, water- and stain-resistant fabrics, carpets, and fire-fighting foams (ATSDR 2018). Many of the long-alkyl chain PFAS, including perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS), are persistent in the environment and are resistant to photolysis, hydrolysis, biodegradation, and oxidative and reductive processes owing to their strong carbon-fluorine bonds (Buck et al. 2011; Kissa 2001). Among numerous families of PFAS, nine common PFAS discussed in this study are summarized in Table 1.1.

Table 1.1 Nine per- and polyfluoroalkyl substances (PFAS) discussed in this study.

Class / Group	Acronym	Chemical name	Chemical formula
Perfluoroalkane carboxylates (PFCAs)	PFOA	Perfluorooctanoate	$C_8H_{15}O_2$
	PFNA	Perfluorononanoate	$C_9H_{17}O_2$
	PFDA	Perfluorodecanoate	$C_{10}H_{19}O_2$
	PFUnDA	Perfluoroundecanoate	$C_{11}H_{17}O_2$
	PFDoDA	Perfluorododecanoate	$C_{12}H_{23}O_2$
Perfluoroalkane sulfonates (PFSAs)	PFOS	Perfluorooctane sulfonate	$C_8H_{17}O_3S$
	PFHxS	Perfluorohexane sulfonate	$C_6H_{13}O_3S$
Perfluoroalkane sulfonamido substances	MeFOSAA	2-(N-methyl-perfluorooctane sulfonamido) acetate	$C_{11}H_6F_{17}NO_4S$
	EtFOSAA	2-(N-ethyl-perfluorooctane sulfonamido) acetate	$C_{12}H_8F_{17}NO_4S$

Human exposure

PFAS are directly or indirectly released to air, water, and soil from the industrial facilities, disposal sites of consumer products, and firefighting areas (Paul et al. 2009; Prevedouros et al. 2006; Shin et al. 2011). Common long-alkyl chain PFAS are widely detected in drinking water across the United States (U.S.) (Hu et al. 2016). From 1999 to 2017, the number of sites contaminated by PFAS has increased, suggesting continued exposure of the general population to these compounds (Sunderland et al. 2019). The major exposure pathways of PFAS for adults and toddlers is via ingestion of contaminated food and water (Trudel et al. 2008). In addition, non-dietary dust ingestion, hand-to-mouth transfer from treated carpets, migration into food from food contact materials, inhalation of indoor air, and dermal contact of treated clothes can also contribute to PFAS exposure (Begley et al. 2008; Begley et al. 2005; Björklund et al. 2009; Haug et al. 2011; Trudel et al. 2008; Yuan et al. 2016).

Half-life

Half-lives of PFAS in human serum are on the order of years. The elimination half-lives of PFOA ranged from 2.1 to 3.8 years (Bartell et al. 2010; Li et al. 2018; Olsen et al. 2007; Russell et al. 2015; Worley et al. 2017). One study reported that the half-lives of PFOA differed by exposure level: 2.9 years for low-exposure populations and 8.5 years for high-exposure populations (Seals et al. 2011). The half-lives of PFOS ranged from 3.3 to 5.4 years, and those of perfluorohexane sulfonate (PFHxS) ranged from 5.3 to 15.5 years (Li et al. 2018; Olsen et al. 2007; Wong et al. 2014; Worley et al. 2017). Due to the long half-lives, PFAS concentrations in blood are considered a standard biomarker of PFAS exposure over the past months or years.

Biomonitoring

Longitudinal biomonitoring studies reported that serum or plasma concentrations of PFOA, PFOS, PFHxS, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), and perfluoroundecanoate (PFUnDA) in the general population generally increased from 1980s to 1990s (Berg et al. 2021; Göckener et al. 2020; Nøst et al. 2014). In early 2000s, there were regulatory and voluntary phase-out of PFOA, PFOS, and related compounds by global manufacturers due to their toxic, persistent, and bioaccumulative properties (Lindstrom et al. 2011). Accordingly, serum or plasma concentrations of PFOA and PFOS have decreased since 2000s (Berg et al. 2021; Ding et al. 2020; Göckener et al. 2020; Nøst et al. 2014; Olsen et al. 2017). Other PFAS concentrations, including PFHxS, PFNA, PFDA, and PFUnDA showed mixed trends depending on country and population. According to the U.S. National Health and Nutrition Examination Survey (NHANES), several PFAS have been frequently detected in the serum of the U.S. general population for the last 20 years (CDC 2019).

Adverse health effects in children

PFAS are shown to have liver toxicity (Liu et al. 1996; Seacat et al. 2002), metabolic toxicity (Kudo et al. 1999), reproductive and developmental toxicity (Butenhoff et al. 2004; Das et al. 2015; Lau et al. 2004; Lau et al. 2003; Sato et al. 2009; Wang et al. 2021; Yahia et al. 2010), neurotoxicity (Austin et al. 2003; Johansson et al. 2008), and immunotoxicity (DeWitt et al. 2012) in laboratory animals. Prenatal exposure to PFAS during the critical periods of development is of particular concern because numerous epidemiological studies reported its associations with adverse health outcomes in children. Prenatal PFAS exposure was associated with reduced fetal or infant growth (Chen et al. 2021; Jin et al. 2020; Kashino et al. 2020),

immune dysfunction (Liew et al. 2018; Okada et al. 2012), neurodevelopmental disorders (Hoffman et al. 2010; Lenters et al. 2019; Lien et al. 2016; Oh et al. 2021a; Oh et al. 2021b; Vuong et al. 2021; Vuong et al. 2016), and thyroid disruption (Kim et al. 2011a; Preston et al. 2020).

1.1.2. Case Studies

Prenatal PFAS exposure and neurodevelopment

Animal studies reported that prenatal exposure to PFAS has neurotoxic and behavioral effects on offspring (Mariussen 2012). Male mice that were prenatally exposed to PFOA showed higher exploratory behaviors than female mice and increased activity in home cage (Onishchenko et al. 2011). Mice that were prenatally exposed to PFOS showed delayed development of neuromotor skills (Fuentes et al. 2007) and decreased exploratory activity and increased number of inactive periods only among males (Onishchenko et al. 2011). In male rats, prenatal exposure to PFOS led to increased locomotor activity and decreased habituation on postnatal day (PND) 17 but not on PND 13, 21, and 61(Butenhoff et al. 2009).

Throughout pregnancy, a number of different biological processes concerning brain development (e.g., neurogenesis, neural migration, synaptogenesis, and neural network formation) occur with overlapping timing (Hertz-Picciotto et al. 2018a); disruption of such processes by PFAS can adversely affect child neurodevelopment (Braun 2017; Kalkbrenner et al. 2014; Lyall et al. 2014). For example, PFAS are reported to alter the levels of neuroprotein critical for brain growth and synaptogenesis (Johansson et al. 2009; Lee and Viberg 2013) and induce neuronal cell apoptosis and oxidative stress (Berntsen et al. 2017; Lee et al. 2013; Reistad et al. 2013). PFAS are also shown to interfere with thyroid hormone homeostasis of pregnant

women, (Berg et al. 2015; Wang et al. 2014), which are critical for fetal brain development (Zoeller and Rovet 2004). Subtle changes in maternal thyroxine levels during pregnancy were associated with increased risk of child's delayed mental and motor development (Berbel et al. 2009; Henrichs et al. 2010; Pop et al. 2003; Pop et al. 1999), attention-deficit/hyperactivity disorder (ADHD) symptoms (Modesto et al. 2015), and autistic behaviors (Román et al. 2013) in infancy and early childhood.

Gestational transfer of PFAS

During pregnancy, PFAS are known to be transported from mother to fetus through the placenta (Ma et al. 2021). Long-alkyl chain PFAS are commonly detected in blood of pregnant women, amniotic fluid, and umbilical cord blood (Bjerregaard-Olesen et al. 2016b; Inoue et al. 2004; Kato et al. 2014; Kim et al. 2011a; Kim et al. 2011b; Liu et al. 2011; Long et al. 2019; Manzano-Salgado et al. 2015; Monroy et al. 2008; Ode et al. 2013; Tsai et al. 2018). Certain PFAS concentrations in prenatal maternal blood are moderately to highly correlated with those in matched amniotic fluid (r [Pearson or Spearman correlation coefficient] = 0.64 to 0.80 for PFOA, PFOS and PFHxS) (Stein et al. 2012) and cord blood (r = 0.63 to 0.94 for PFOA, r = 0.50 to 89 for PFOS, r = 0.59 to 0.89 for PFHxS, r = 0.51 to 0.82 for PFNA, r = 0.82 to 0.84 for PFDA, r = 0.55 to 0.70 for PFUnDA), supporting the placental transfer of PFAS (Beesoon et al. 2011; Fromme et al. 2010; Gützkow et al. 2012; Kim et al. 2011b; Liu et al. 2011; Manzano-Salgado et al. 2015; Monroy et al. 2008; Ode et al. 2013). In these studies, individual PFAS showed different transplacental transfer efficiencies, and the transfer efficiencies tended to decrease with increasing carbon chain length (Beesoon et al. 2011; Gützkow et al. 2012; Kim et al. 2011b). The transplacental mechanisms of PFAS are not fully understood, but proposed

mechanisms include passive diffusion and active transport through binding to transporter proteins in the placenta (Ma et al. 2021).

1.2. Rationale for the dissertation

Prenatal PFAS exposure and autism spectrum disorder

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by deficits in social communication and interaction and by the presence of restricted interests, repetitive behaviors, and sensory sensitivities (American Psychiatric Association 2013). In the U.S., 1 in 54 children are affected by ASD, with an increasing number of ASD diagnoses for the last three decades (Maenner et al. 2020). The prevalence of ASD is approximately 4.3 times higher in boys than in girls. Twin studies revealed that environmental factors play a significant role in the development of ASD (Frazier et al. 2014; Hallmayer et al. 2011; Sandin et al. 2014; Tordjman et al. 2014). A growing body of epidemiological studies suggests that prenatal exposure to environmental neurotoxicants is associated with increased risk of ASD (Hertz-Picciotto et al. 2018a).

Five epidemiological studies that examined associations between prenatal exposure to PFAS and ASD risk showed mixed results (Table 1.2). For example, Liew et al. and Shin et al. reported increased risk of ASD in association with prenatal maternal concentrations of PFHxS and PFOS and PFHxS, respectively (Liew et al. 2014; Shin et al. 2020). On the contrary, Braun et al. and Lyall et al. observed decreased risk of ASD in association with prenatal maternal concentrations of PFOA and PFOS and PFOA, respectively (Braun et al. 2014; Lyall et al. 2018). Long et al. also reported that PFOS concentrations in amniotic fluid were association with decreased risk of ASD (Long et al. 2019). Although pregnant women are exposed to the mixture

of correlated PFAS, rather than a single compound, these studies examined associations for individual PFAS, which may explain the inconsistent findings. Therefore, studying the effect of individual PFAS as well as the potential combined effect of exposure to a PFAS mixture on ASD risk can help improve understanding of these associations.

Table 1.2 Previous epidemiological studies examining the associations between prenatal PFAS exposure and the risk of autism spectrum disorder.

Papers	Study population (study period)	Sample collection	Results
Braun et al., 2014	U.S. Cincinnati birth cohort (2003-2006)	Maternal blood at 16-26 weeks' gestation	PFOA: less autistic behaviors in children at 4-5 years (↓)
Liew et al., 2015	Danish National Birth Cohort (1996-2002)	Maternal blood at 1st or 2nd trimester of pregnancy	PFHxS: some evidence of increased risk of autism (↑)
Lyall et al., 2018	U.S. California autism cohort (2000-2003)	Maternal blood at 15-19 weeks' gestation	PFOA, PFOS: decreased risk of ASD (↓)
Long et al., 2019	Denmark Historic Birth Cohort (1995-1999)	Amniotic fluid at 2nd trimester of pregnancy	PFOS: decreased risk of ASD (↓)
Shin et al., 2020	U.S. California autism cohort (2009-2017)	Modeled from the maternal blood samples collected when children are 2-5 years	PFOS, PFHxS: increased risk of ASD (↑)

Abbreviation: autism spectrum disorder (ASD), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS)

Prenatal PFAS exposure and cognitive development in infancy and toddlerhood

Four epidemiological studies examining the associations between prenatal exposure to PFAS and cognitive development in infancy and toddlerhood (before or at 36 months of age) showed mixed results (Table 1.3). These studies assessed cognitive abilities at different ages using various assessment tools. For example, Donauer et al. observed that prenatal maternal PFOA concentrations were associated with hypotonic response assessed using Neonatal Intensive Care Unit Network Neurobehavioral Scale at 5 weeks of age (Donauer et al. 2015).

Goudarzi et al. reported that prenatal maternal PFOA concentrations were associated with lower mental developmental index assessed using Bayley Scales of Infant Development among 6-month-old female infants (Goudarzi et al. 2016). Chen et al. observed that cord blood PFOS concentrations were associated with lower developmental quotients assessed using Comprehensive Developmental Inventory for Infants and Toddlers at 24 months of age (Chen et al. 2013). Spratlen et al. repeatedly assessed cognitive development using Bayley Scales at 12, 24, and 36 months of age and observed that cord blood PFOA and PFHxS concentrations were associated with higher mental developmental index at 3 years of age, but not at 1 and 2 years (Spratlen et al. 2020). These studies investigated cross-sectional associations between prenatal PFAS exposure and cognitive development, but little is known about longitudinal changes or trajectories of child's cognitive abilities in associations with prenatal PFAS exposure. Because cognitive abilities fluctuate during infancy and toddlerhood, studying longitudinal changes or trajectories of cognitive functions in infancy and toddlerhood may improve understanding of their associations with prenatal PFAS exposure.

Table 1.3 Previous epidemiological studies examining the associations between prenatal PFAS exposure and child's cognitive development in infancy and toddlerhood.

Papers	Study population (study period)	Assessment age / tool	Sample collection	Results
Donauer et al., 2015	U.S. Cincinnati birth cohort (2003-2006)	5 weeks / Neonatal Intensive Care Unit Network Neuro-behavioral Scale	Maternal blood at 16 weeks' gestation	PFOA: hypotonic response (↓)
Goudarzi et al., 2016	Japan Hokkaido birth cohort (2002-2005)	6 and 18 months / Bayley Scales of Infant Development	Maternal blood after 2nd trimester	PFOA: lower mental developmental index among 6-month-old females (↓)
Chen et al., 2013	Taiwan Birth Panel Study (2004-2005)	24 months / Comprehensive Developmental Inventory for Infants and Toddlers	Cord blood at delivery	PFOS: lower developmental quotients (↓)
Spratlen et al., 2020	U.S. New York birth cohort (2001-2002)	12, 24, and 36 months / Bayley Scales of Infant Development	Cord blood or maternal blood at delivery	PFOA, PFHxS: higher mental developmental index at 3 years (↑), but not at 1 and 2 years (-)

Longitudinal changes in maternal PFAS concentrations from pregnancy to postnatal periods

Changes in maternal PFAS concentrations from pregnancy to postnatal periods are related to placental or lactational transfer rates from mother to child. Four previous studies quantified PFAS concentrations in serial blood samples collected during pregnancy or postnatal periods and investigated concentration changes (Table 1.4). Buck Louis et al. and Chen et al. collected maternal blood samples at the 1st, 2nd, and 3rd trimesters of pregnancy and observed decreases in PFOA, PFOS, PFNA, PFDA, PFUnDA, and perfluorododecanoate (PFDoDA) concentrations, but PFHxS concentrations increased or did not change (Buck Louis et al. 2019; Chen et al. 2021). Kato et al. collected maternal blood samples at the 2nd trimester of pregnancy and delivery and observed decreases in PFOA, PFOS, PFHxS, and PFNA concentrations (Kato

et al. 2014). Glynn et al. collected maternal blood samples at the 1st and 3rd trimesters of pregnancy and 3 weeks and 3 months after delivery (Glynn et al. 2012). They observed decreases in PFOA, PFOS, and PFNA concentrations during pregnancy and declines in PFOA concentrations from 3 weeks to 3 months after delivery. However, little is known about changes in maternal PFAS concentrations during postnatal periods when mothers cease exclusive breastfeeding. After the start of the weaning period, PFAS are less likely to be transported from mother to child compared to the earlier periods (i.e., pregnancy, lactation). Therefore, changes in maternal PFAS concentrations during pregnancy and early postpartum compared to those during late postpartum may help understand gestational and lactational transfers of PFAS.

Table 1.4 Previous studies investigating changes in maternal PFAS concentrations during pregnancy and postnatal periods.

Papers	Study population (study period)	Sample collection	Results
Buck Louis et al., 2019	50 pregnant women in the U.S. (1992-1995)	Maternal blood samples at the 1st, 2nd, and 3rd trimesters of pregnancy	During pregnancy PFOA, PFOS, PFNA, PFDA, PFUnDA, PFDoDA: decreasing (↓), PFHxS: increasing (↑)
Chen et al., 2021	214 pregnant women in China (2013-2015)	Maternal blood samples at the 1st, 2nd, and 3rd trimesters of pregnancy	During pregnancy PFOA, PFOS, PFNA, PFDA, PFUnDA, PFDoDA: decreasing (↓), PFHxS: no change (-)
Kato et al., 2014	71 pregnant women in the U.S. (2003-2006)	Maternal blood samples at 16 weeks' gestation and at delivery	During pregnancy PFOA, PFOS, PFHxS, PFNA: decreasing (↓)
Glynn et al., 2012	19 pregnant women in Sweden (1996-1999)	Maternal blood samples at the 1st and 3rd trimesters of pregnancy, 3 weeks, and 3 months after delivery	During pregnancy PFOA, PFOS, PFNA: decreasing (↓) 3 weeks to 3 months postpartum PFOA: decreasing (↓), PFOS, PFNA: no change (-)

Abbreviation: perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA)

1.3. Research objectives and hypotheses

The main objectives of my dissertation research were (1) to examine associations of prenatal maternal serum PFAS concentrations with child ASD and cognitive development and (2) to investigate longitudinal changes in maternal serum PFAS concentrations from pregnancy to postnatal periods. Specific research objectives and their corresponding hypotheses are outlined below:

1) Examine associations between ASD risk and prenatal maternal serum PFAS concentrations and a PFAS mixture.
    a) H_0: Prenatal maternal serum PFAS concentrations are ***not*** associated with increased risk of ASD.
    b) H_1: Prenatal maternal serum PFAS concentrations are associated with the risk of ASD.

2) Examine cross-sectional and longitudinal associations between prenatal maternal serum PFAS concentrations and child cognitive development in infancy and toddlerhood.
    a) H_0: Prenatal maternal serum PFAS concentrations are ***not*** associated with cognitive development in infancy and toddlerhood.
    b) H_1: Prenatal maternal serum PFAS concentrations are associated with cognitive development in infancy and toddlerhood.

3) Investigate longitudinal changes in maternal serum PFAS concentrations during pregnancy and early and late postpartum. Identify the potential determinants of the changes for each sub-period.
    a) H_0: The changes in maternal serum PFAS concentrations are the same for all compounds during pregnancy and early and late postpartum.
    b) H_1: The changes in maternal serum PFAS concentrations are different for any two of the study compounds during pregnancy and early and late postpartum.

1.4. Overview of dissertation

The first phase of my research is epidemiological studies to examine associations between prenatal maternal PFAS exposure and child ASD risk and cognitive development in infancy and toddlerhood. The second phase is to investigate longitudinal changes in maternal serum PFAS concentrations from pregnancy to two years postpartum.

In Chapter 2, I examined associations between prenatal maternal serum PFAS concentrations and the risk of child ASD assessed at 3 years of age. I used Poisson regression models to estimate the relative risks (RRs) of ASD compared to typical development. I performed a principal component analysis (PCA) to examine the combined effect of exposure to a PFAS mixture on ASD risk. As effect modification analyses by child's sex and maternal age at delivery, I compared stratum-specific estimates and evaluated *p*-values for the interaction terms between PFAS and potential effect modifiers.

In Chapter 3, I examined associations between prenatal maternal serum PFAS concentrations and child cognitive development assessed using Mullen Scales of Early Learning (MSEL) at 6, 12, 24, and 36 months of age. For cross-sectional associations, I used multiple

linear regression models to estimate regression coefficients at each assessment time point. I also used generalized estimating equations models to examine longitudinal changes in MSEL scores over the four assessment time points in association with prenatal maternal serum PFAS concentrations. I performed a trajectory analysis to identify the two groups with distinct trajectories of MSEL scores over the four assessment time points and used Poisson regression models to estimate the RRs of the low-score group compared to the high-score group in association with prenatal maternal serum PFAS concentrations.

In Chapter 4, to support the findings from Chapter 3, I investigated associations between cord blood PFOA and PFOS concentrations and cognitive development in a Japanese population that is representative of the general population. Child cognitive development was assessed at 4, 6, 10, 14, 18, 24, 32, and 40 months of age using MSEL. I used the same statistical models to examine the cross-sectional and longitudinal associations.

In Chapter 5, I grouped maternal blood samples into three sub-periods: 1st, 2nd, and 3rd trimesters of pregnancy (pregnancy), 3rd trimester and 3 and 6 months postpartum (early postpartum), and 6 and 24 months postpartum (late postpartum). Then I used linear mixed models to estimate the percent changes in maternal serum PFAS concentrations per one-unit increase in time for each sub-period. From the linear mixed models, I also identified determinants for the maternal serum PFAS concentration changes.

In Chapter 6, I summarized the results from Chapters 2 through 5 and described the conclusions of my dissertation.

CHAPTER 2: PRENATAL EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES IN ASSOCIATION WITH AUTISM SPECTRUM DISORDER IN THE MARBLES STUDY

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Oh J, Bennett DH, Calafat AM, Tancredi D, Roa DL, Schmidt RJ, Hertz-Picciotto I, Shin HM. 2021. Prenatal exposure to per-and polyfluoroalkyl substances in association with autism spectrum disorder in the MARBLES study. *Environment International* 147, 106328.

2.1. Introduction

2.1.1. Background

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made fluorinated chemicals (Olsen et al. 2017). Because of their water- and stain-resistant properties, PFAS have been widely used in the manufacture of consumer and industrial products, including cookware non-stick coatings, food contact materials, stain- and water-resistant fabric coatings, industrial surfactants, and fire-fighting foams (Benford et al. 2008). Due to their wide range of applications and persistence in the environment, several PFAS have been frequently detected in the serum of the general U.S. population for the last 20 years (CDC 2019; Kato et al. 2011b).

Exposure to PFAS during pregnancy is of concern. PFAS can cross placenta and be transported to the fetus (Gützkow et al. 2012), and a number of PFAS have been detected in cord blood samples (Fisher et al. 2016; Kato et al. 2014). PFAS may adversely affect child's brain development. In animal studies, mice that were prenatally exposed to perfluorooctane sulfonate (PFOS) exhibited delays in developmental landmarks and neuromotor maturation (Fuentes et al. 2007). Prenatal exposure to perfluorooctanoate (PFOA) resulted in alterations in exploratory behaviors in male and female mice (Onishchenko et al. 2011). In humans, there is evidence that PFAS disrupt thyroid hormone homeostasis of pregnant women (Berg et al. 2015; Wang et al. 2014), which may adversely affect fetal brain development (Morreale de Escobar et al. 2000). Several prospective birth cohort studies showed that maternal thyroid hormone deficiency during pregnancy is associated with increased risk of child neurodevelopmental delay, attention-deficit/hyperactivity disorder (ADHD) symptoms, and autistic behaviors in infancy and childhood (Modesto et al. 2015; Pop et al. 2003; Román et al. 2013). However, previous

epidemiological findings on the associations between prenatal exposure to PFAS and the risk of neurodevelopmental concerns or autistic behaviors have been inconclusive (Liew et al. 2018).

2.1.2. Statement of research problem

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impairments in social communication and interaction, restricted interests and repetitive behaviors, and sensory sensitivities. A growing body of literature suggests that exposure to environmental neurotoxicants during pregnancy can contribute to the development of ASD (Hertz-Picciotto et al. 2018a). Five epidemiologic studies examined the associations between prenatal PFAS exposure and the risk of ASD. A prospective cohort study observed that higher PFOA, but not other PFAS, in 16-week prenatal maternal serum was associated with less autistic behaviors in 4- and 5-year-old children (Braun et al. 2014). A nested case-control study did not find convincing evidence of associations between maternal PFAS plasma concentrations collected during the first or second trimester of pregnancy and increased risk of childhood autism (Liew et al. 2014). Two case-control studies reported that higher PFOA and PFOS in 15- to 19-week prenatal maternal serum (Lyall et al. 2018) or PFOS in amniotic fluid collected during the second trimester (Long et al. 2019) were associated with decreased risk of child ASD, while other PFAS were not associated. Another case-control study observed that modeled prenatal maternal serum PFOS and perfluorohexane sulfonate (PFHxS), but not other PFAS, were associated with increased risk of child ASD (Shin et al. 2020). Because PFAS are weakly or moderately correlated each other and one PFAS may confound another PFAS, consideration of a single compound in the model may explain, at least in part, the inconsistent findings from these studies.

2.1.3. Objectives

In this study, we used maternal serum samples prospectively collected during pregnancy in a high-risk ASD cohort to examine whether prenatal maternal serum PFAS concentrations were associated with increased risk of child ASD. We also examined the potential combined effect of exposure to a mixture of PFAS on ASD risk.

2.2. Methods

2.2.1. Study population

MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns) is a prospective cohort study, which began in 2006, that enrolls women who are pregnant with a child who has a first degree relative with ASD and thereby is at elevated risk (~ 20%) for developing ASD (Hertz-Picciotto et al. 2018b; Ozonoff et al. 2011). Participants are primarily recruited from families receiving services for children with ASD through the California Department of Developmental Services. Inclusion criteria of MARBLES are: 1) mother or father has a child or other first degree relative with ASD; 2) mother is 18 years old or older; 3) mother is pregnant; 4) mother speaks, reads, and understands English; and 5) mother resides within 2.5 hours of the Davis/Sacramento region at the time of enrollment. For families who consent to participate in the MARBLES study, demographic information, medical records, and biological specimens are prospectively collected. Detailed information on study design, study population, inclusion criteria, recruitment and data collection is described elsewhere (Hertz-Picciotto et al. 2018b).

For the present study, we included 193 mothers who provided blood samples during pregnancy between 2009 and 2015. One mother who did not have a previous child with ASD at

enrollment was included in this study because she had three siblings with ASD. Among 193 mothers, eight mothers participated in the study for two pregnancies and three mothers delivered twins. Thus, 204 mother-child pairs comprised the study population (Figure 2.1).

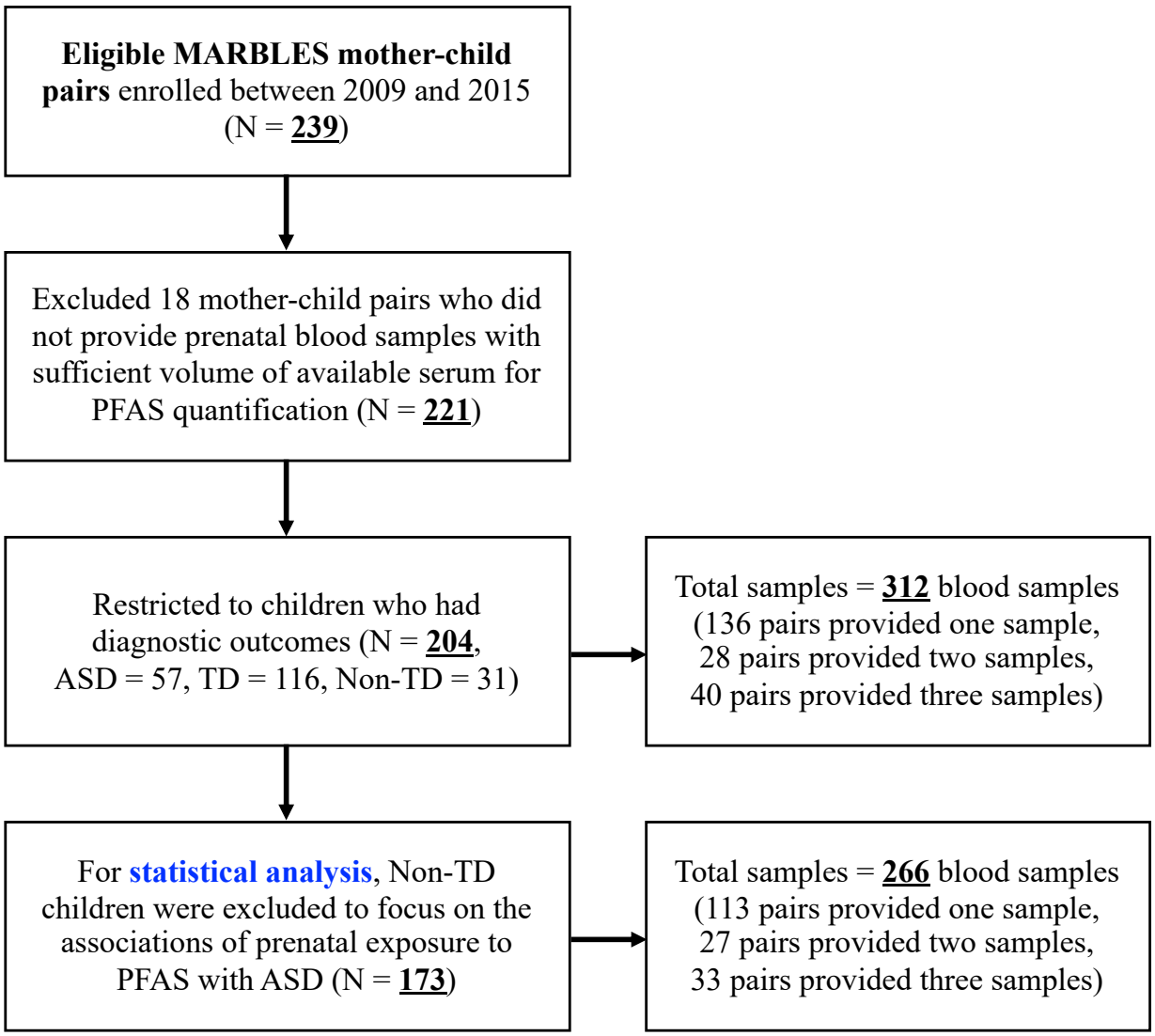

Figure 2.1 Study flow and blood sample collection from MARBLES participants to examine the associations between prenatal exposure to PFAS and the risk of ASD.

2.2.2. Serum sample collection and PFAS quantification

A total of 312 maternal blood samples were collected during pregnancy from 193 mothers. Each mother provided a varying number of blood samples during pregnancy (mean = 1.5; standard deviation (SD) = 0.8), with most samples (79%) being provided in the second and third trimesters. Of 312 samples, 67 were collected in the first trimester, 142 in the second trimester, and 103 in the third trimester. Of 204 mother-child pairs, 136 pairs provided one sample, 28 pairs provided two samples, and 40 pairs provided three samples (Figure 2.1). Whole blood was centrifuged, and separated serum was stored at -80 °C within 24 hours of blood draw.

Quantification of PFAS in maternal sera was performed at the Centers for Disease Control and Prevention (CDC) using online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–isotope dilution tandem mass spectrometry. Detailed analytical methods for quantification of PFAS are described elsewhere (Kato et al. 2011a). For quality control, blank samples and two quality control materials (low and high concentrations) were included in each batch. For quality assurance, we additionally analyzed 25 blind duplicate samples along with the study samples. The median coefficient of variation for these duplicate pairs of samples varied from 0% to 11%, depending on the analyte.

Nine PFAS were quantified in serum: PFOA, PFOS, PFHxS, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA). The limit of detection (LOD) for all nine PFAS was 0.1 ng/mL. For PFAS concentrations below the LOD, we used instrument-observed values as there seems to be less bias with this approach than using an imputation method that

assigns the same value (e.g., LOD/2) to all non-detected concentrations (Lubin et al. 2004; Richardson and Ciampi 2003).

2.2.3. Child neurodevelopmental assessment

At approximately 36 months of age, children were administered the Autism Diagnostic Observation Schedule (ADOS), which is a semi-structured, standardized diagnostic assessment of ASD conducted by a trained psychologist (Ozonoff et al. 2005). Raw ADOS scores were further converted into calibrated severity scores (CSS) ranging from 1 to 10 (CSS ≥ 4 represents ASD classification) (Esler et al. 2015). Children were also assessed for cognitive development using Mullen Scales of Early Learning (MSEL) which has scores for four subscales (i.e., visual reception, fine motor, receptive language, and expressive language) (Mullen 1997). Based on ADOS and MSEL scores, we defined the neurodevelopmental outcome of each child and classified 204 children into three diagnostic groups using a previously published algorithmic method with some modifications (Esler et al. 2015; Ozonoff et al. 2014). Children who met *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (DSM-5) criteria for ASD and had ADOS CSS ≥ 4 were classified into ASD (n = 57). Children who did not meet DSM-5 criteria for ASD but had ADOS CSS ≥ 3 and/or lower MSEL scores (> 1.5 SD below the mean in two or more MSEL subscales or > 2 SD below the mean in at least one MSEL subscales) were classified into non-typical development (Non-TD; n = 31). The rest were classified into typically developing children (TD; n = 116) (Hertz-Picciotto et al. 2018b). The children with Non-TD diagnosis were excluded from the current study, as our focus was on ASD specifically. Therefore, 173 mother-child pairs were used in the statistical analyses.

2.2.4. Statistical analysis

We computed univariate statistics and compared population characteristics of ASD and TD groups using the Pearson's chi-squared test for categorical variables and the Wilcoxon rank-sum test for a continuous variable. We compared median PFAS concentrations between the two diagnostic groups using the Wilcoxon rank-sum test. We also compared median PFAS concentrations among groups of population characteristics using the Wilcoxon rank-sum test for binary variables and the Kruskal-Wallis test for other categorical variables. Further, we compared our median PFAS concentrations with those of the pregnant women (n = 61) reported in the National Health and Nutrition Examination Survey (NHANES) during the same study period (2009-2014). We combined PFAS concentration data from three NHANES cycles (i.e., 2009-2010, 2011-2012, 2013-2014) and computed median PFAS concentrations restricted to the pregnant women in the NHANES population (CDC 2019).

We used Poisson regression models with robust error variance to examine the associations between prenatal PFAS concentrations and the relative risks (RRs) of ASD, compared with TD (Chen et al. 2018). We constructed a directed acyclic graph (DAG) including a priori chosen potential confounders and risk factors for ASD identified in the previous studies (Figure 2.2). We evaluated percent changes of the beta estimate by excluding each covariate from the DAG full model and selected only those covariates which changed the estimate by greater than 10% (Weng et al. 2009). In the final model, we included child's sex (female, male), birth year (2009-2010, 2011-2013, 2014-2015), maternal vitamin intake in the first month of pregnancy (yes, no), and two variables as proxies of socioeconomic status: maternal education (high school or some college credit, bachelor's degree, graduate or professional degree) and homeownership (owner, non-owner). For mothers who delivered twins or were enrolled for two

children from different pregnancies, we adjusted for within-family correlations by participant identification numbers in the regression models by using clustered sandwich variance estimators (Zou 2004). Because there were missing values in two covariates, we performed multiple imputation by chained equations, which included all exposure and outcome variables as well as the covariates (White et al. 2011). Ten imputed datasets were used in the regression models.

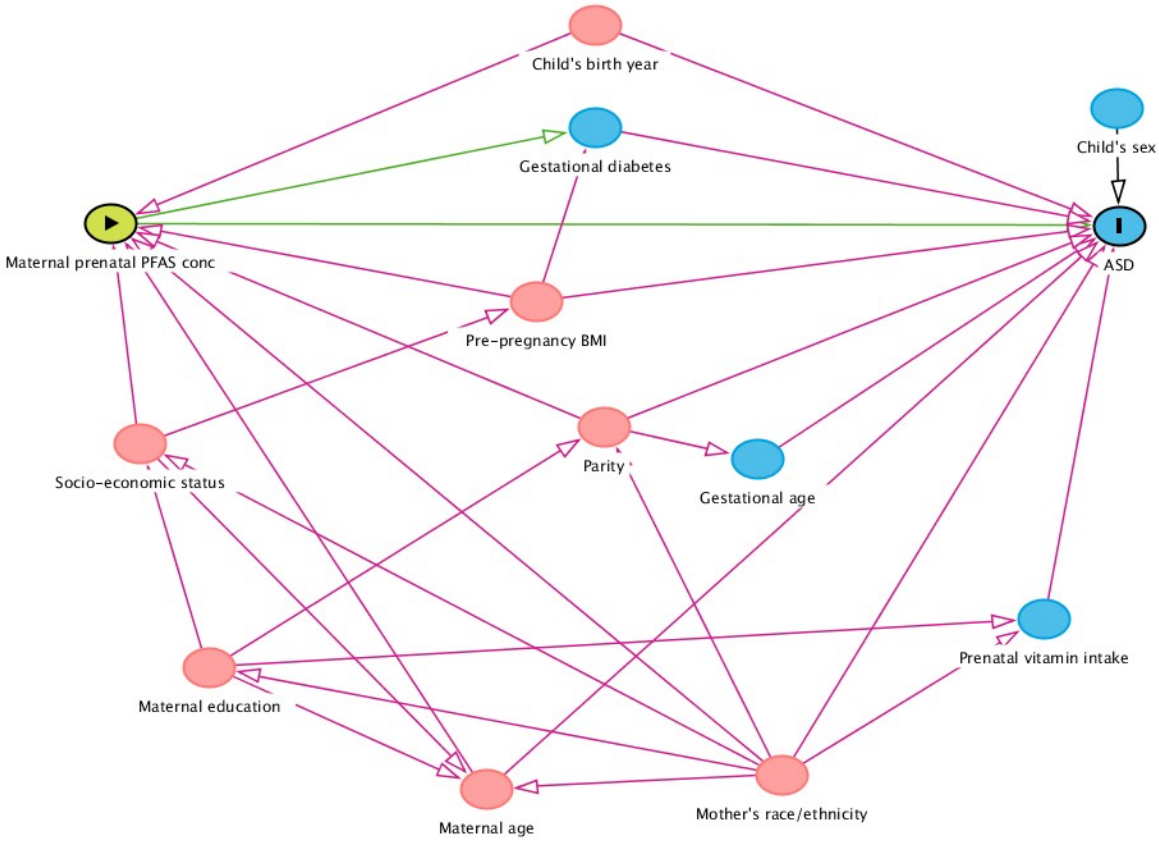

Figure 2.2 Directed acyclic graph constructed to identify potential confounders of association between prenatal PFAS concentrations and ASD. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome.

Using the final Poisson regression models, we estimated adjusted RRs and 95% confidence intervals (CIs) for four PFAS which were detected in more than 99% of the study samples (i.e., PFOA, PFOS, PFHxS, and PFNA). We fit two different models for each of the individual PFAS using (1) log 2-transformed (or binary log-transformed) PFAS concentrations, describing the two-fold increase in the log of the RR in relation to the PFAS concentrations (to lessen the influence of outlying measurements from the skewed untransformed distributions) and (2) PFAS concentrations without transformation, describing the linear increase in the log of the RR in relation to the PFAS concentrations (because a previous study suggested a linear dose-response relationship between the risk of ASD and PFAS concentrations) (Shin et al. 2020). For mothers who provided multiple samples during pregnancy, we used average PFAS concentrations in the regression models. In order to examine the combined effect of the four PFAS concentrations on ASD, we performed a principal component analysis which reduces a set of correlated variables into a smaller number of principal components (PCs). We selected a PC that had eigenvalue greater than one based on the Kaiser's criterion (Kaiser 1960) and used its scores in the regression models.

As a sensitivity analysis, we excluded the top 2.5 percentiles of individual PFAS concentrations and ran the regression models to examine the effect of extreme values. We also ran the regression models by additionally adjusting for a set of potential confounders identified from a DAG: maternal pre-pregnancy body mass index (BMI) (normal/underweight, overweight, obese), parity (≤ 1, > 1), maternal age at delivery (< 35 years, ≥ 35 years), and maternal race/ethnicity (non-Hispanic white, Hispanic, other). For mothers who provided more than one sample during pregnancy, we additionally ran the same final models using the highest concentration or a randomly selected concentration among multiple samples instead of their

average. To evaluate effect modification of the associations between PFAS and ASD risk, we examined child's sex (female, male) and maternal age at delivery (< 35 years, ≥ 35 years) as potential effect modifiers on the grounds of biological plausibility (Braun et al. 2014; Janecka et al. 2017; Jeddy et al. 2017; Werling and Geschwind 2013). We compared stratum-specific estimates and examined *p*-values for the interaction terms. Statistical analyses were performed using STATA/IC version 15.1 (StataCorp LLC, College Station, TX, USA).

2.3. Results

2.3.1. Population characteristics

In this study, we observed differences in several population characteristics between ASD and TD groups (Table 2.1). As expected, the male to female ratio of children with ASD (2.35) was higher than TD children (1.15). Mothers of TD children were more likely to have a bachelor's degree or higher (53.4%), compared to those of children with ASD (36.8%) and more likely to own a home (60.3%) than those of ASD children (45.6%). Fewer mothers who had children with ASD took prenatal vitamins during the first month of pregnancy (40.4%), compared to those who had TD children (56.0%).

Table 2.1 Characteristics of the study participants by diagnostic group. g

Characteristics [a]	All (n = 173)	ASD (n = 57)		TD (n = 116)		p-value [b]
	n	n	%	n	%	
Child's sex						0.04
Female	71	17	29.8	54	46.6	
Male	102	40	70.2	62	53.4	
Child's birth year						0.53
2009-2010	61	19	33.3	42	36.2	
2011-2013	57	22	38.6	35	30.2	
2014-2015	55	16	28.1	39	33.6	
Gestational age at delivery						0.37
≤ 37 weeks	10	2	3.5	8	6.9	
> 37 weeks	163	55	96.5	108	93.1	
Maternal age at delivery						0.91
< 35 years	93	31	54.4	62	53.4	
≥ 35 years	80	26	45.6	54	46.6	
Maternal BMI at pre-pregnancy						0.37
Normal/underweight	83	23	40.4	60	51.7	
Overweight	50	19	33.3	31	26.7	
Obese	40	15	26.3	25	21.6	
Gestational diabetes						0.56
Yes	32	12	21.1	20	17.2	
No	140	45	78.9	95	81.9	
Maternal race/ethnicity						0.95
Non-Hispanic white	94	30	52.6	64	55.2	
Hispanic	41	14	24.6	27	23.3	
Other [c]	38	13	22.8	25	21.6	
Maternal education						0.09
High school, some college	90	36	63.2	54	46.6	
Bachelor's degree	50	11	19.3	39	33.6	
Graduate or professional	33	10	17.5	23	19.8	
Homeownership						0.11
Yes	96	26	45.6	70	60.3	
No	75	29	50.9	46	39.7	
Parity						0.47
≤ 1	72	21	36.8	51	44.0	
> 1	99	34	59.6	65	56.0	
Maternal vitamin intake in the first month of pregnancy						0.07
Yes	88	23	40.4	65	56.0	
No	84	33	57.9	51	44.0	
Breastfeeding duration (months)						
		Mean (SD)		**Mean (SD)**		
		11.5 (10.3)		11.7 (9.5)		0.61

[a] Missing information (n): gestational diabetes (1), homeownership (2), parity (2), maternal vitamin intake in the first month of pregnancy (1), breastfeeding duration (10).

[b] p-value from the Pearson's chi-squared test for categorical variables and the Mann-Whitney test for continuous variables.

[c] Includes Black/African American (3%), Asian (16%), and multiracial (3%).

2.3.2. Prenatal maternal PFAS serum concentrations and principal component

The detection frequency of PFOA, PFOS, PFHxS, and PFNA was 100%, 100%, 99%, and 99.6%, respectively, and five other PFAS were detected in less than 85% of the samples (Table 2.2). We observed the highest median for PFOS (3.0 ng/mL), followed by PFOA (0.9 ng/mL), PFNA (0.5 ng/mL), and PFHxS (0.4 ng/mL). The median PFOA of the ASD group was higher than that of the TD group (p-value = 0.02). There was no statistically significant difference in the medians of other PFAS between the two diagnostic groups. The medians of PFOA, PFOS, PFHxS, and PFNA in our study were slightly lower than those of pregnant women reported in NHANES, while those of other five PFAS concentrations were similar. At least one of the four PFAS detected in more than 99% of the samples (i.e., PFOA, PFOS, PFHxS, and PFNA) differed by child's sex and birth year, maternal age at delivery, maternal pre-pregnancy BMI, gestational age at delivery, homeownership, parity, and prenatal vitamin intake (Table S2.1).

Among 40 mothers who provided samples during all three trimesters, geometric mean of PFOA, PFOS, and PFNA was the highest in the first trimester and slightly decreased over time (Table S2.2). The correlation coefficients between the pairs of trimesters for PFOA, PFOS, and PFNA were greater than 0.85, indicating the stability of these three PFAS concentrations during pregnancy (Table S2.3). For the same four PFAS, concentrations were weakly or moderately correlated each other (r = 0.36 to 0.65) (Table S2.4). From the principal component analysis, the first principal component (PC-1) was individually selected based on the log 2-transformed and untransformed PFAS, accounting for approximately 67% and 55% of the variance, respectively (Table 2.3). The two PC-1s had moderate positive loadings on all four PFAS (weight = 0.36 to

0.57). The scores for PC-1 between the two groups were not statistically different (p-value = 0.15 for log 2-transformed PFAS; p-value = 0.12 for untransformed PFAS).

Table 2.2 Distribution of nine PFAS concentrations (ng/mL) in 266 maternal serum samples collected from 173 mother-child pairs.

PFAS [a]	LOD (ng/mL)	% detect	All children (*n* = 173)			ASD (*n* = 57)			TD (*n* = 116)			*p*-value [a]	NHANES pregnant women median [b]
			Percentiles			Percentiles			Percentiles				
			5th	50th	95th	5th	50th	95th	5th	50th	95th		
PFOA	0.1	100	0.3	0.9	2.3	0.4	1.1	2.4	0.3	0.9	2.2	0.02	1.3
PFOS	0.1	100	1.1	3.0	6.8	1.0	3.2	7.0	1.2	2.9	6.7	0.23	3.7
PFHxS	0.1	99	0.2	0.4	1.6	0.2	0.4	1.7	0.2	0.4	1.5	0.45	0.6
PFNA	0.1	99.6	0.2	0.5	1.0	0.2	0.5	1.0	0.2	0.5	1.1	0.32	0.6
PFDA	0.1	83	<LOD	0.1	0.4	<LOD	0.1	0.4	<LOD	0.1	0.3	0.78	0.2
PFUnDA	0.1	60	<LOD	0.1	0.3	<LOD	0.1	0.4	<LOD	0.1	0.3	0.46	<LOD
PFDoDA	0.1	34	<LOD	<LOD	0.1	<LOD	<LOD	0.1	<LOD	<LOD	0.1	0.64	<LOD
MeFOSAA	0.1	57	<LOD	0.1	0.8	<LOD	0.1	1.1	<LOD	0.1	0.7	0.58	0.1
EtFOSAA	0.1	4	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	0.46	<LOD

[a] *p*-value from the Wilcoxon rank-sum test comparing ASD and TD groups.

[b] Median PFAS concentrations of pregnant women from 2009-2010, 2011-2012, 2013-2014 NHANES (*n* = 61).

Abbreviation: autism spectrum disorder (ASD), limit of detection (LOD), National Health and Nutrition Examination Survey (NHANES), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-ethyl-perfluoro-octane sulfonamido) acetate (EtFOSAA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), typical development (TD)

Table 2.3 Selected principal component and its component-loading weights for PFAS.

PC-1	Log 2-transformed PFAS	Untransformed PFAS
Proportion of variance	0.67	0.55
Weight for PFOA	0.52	0.56
Weight for PFOS	0.53	0.48
Weight for PFHxS	0.43	0.36
Weight for PFNA	0.52	0.57

2.3.3. Associations between prenatal maternal PFAS serum concentrations and ASD risk

After adjusting for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy, using log 2-transformed exposure measures mostly produced null associations between ASD risk and maternal prenatal PFAS serum concentrations, including PC-1 scores (Table 2.4). PFOA and PFNA showed positive associations with increased ASD risk, though the associations were slightly above the null (per 2 nanogram per millimeter increase: RR = 1.20, 95% CI: 0.90, 1.61 for PFOA; RR = 1.24, 95% CI: 0.91, 1.69 for PFNA), while PFHxS showed a negative association with increased ASD risk (RR = 0.88, 95% CI: 0.77, 1.01). When using PFAS concentrations without transformation, RRs for PFOA, PFOS, and PC-1 increased with narrower CIs. ASD risk was increased with higher PFOA (per nanogram per millimeter increase: RR = 1.31, 95% CI: 1.04, 1.65), PFNA (RR = 1.79, 95% CI: 1.13, 2.85), and PC-1 (RR = 1.10, 95% CI: 0.97, 1.25), while the association of ASD risk with PFHxS moved toward the null (RR = 0.97, 95% CI: 0.54, 1.51).

When excluding the top 2.5 percentiles of individual PFAS concentrations, results of the models with log 2-transformed PFAS were similar, while the models with untransformed PFAS showed broader CIs (Table S2.5). When additionally adjusting for potential confounders (i.e., maternal pre-pregnancy BMI, parity, maternal age at delivery, and maternal race/ethnicity) or when using the highest concentration or a randomly selected PFAS concentration instead of the average concentration, similar results were observed in models with concentrations for both log 2-transformed PFAS and untransformed PFAS concentrations (Table S2.6).

Table 2.4 Adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (n = 57) versus TD (n = 116) in association with prenatal maternal PFAS concentrations and principal component scores.

PFAS (ng/mL) [a] or **principal component score**	**Log 2-transformed PFAS RR** [b] (95% CI)	**Untransformed PFAS RR** [b] (95% CI)
PFOA	1.20 (0.90, 1.61)	1.31 (1.04, 1.65)
PFOS	0.97 (0.74, 1.28)	0.99 (0.90, 1.08)
PFHxS	0.88 (0.77, 1.01)	0.90 (0.54, 1.51)
PFNA	1.24 (0.91, 1.69)	1.79 (1.13, 2.85)
PC-1 [c]	1.03 (0.87, 1.22)	1.10 (0.97, 1.25)

[a] Four PFAS detected in more than 99% of the study samples were individually included in the Poisson regression model.
[b] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.
[c] Selected from the principal component analysis because its eigenvalue was higher than 1.

When analyses were stratified by maternal age at delivery (<35, ≥ 35 years old), we observed effect modification of associations between ASD risk and all four PFAS concentrations as well as PC-1 scores (p-value for interaction ≤ 0.10) (Table 2.5). Among mothers who were 35 years old or older, ASD risk was increased with higher PFOA (RR = 2.29, 95% CI: 1.30, 4.04), PFOS (RR = 1.67, 95% CI: 1.03, 2.71), PFNA (RR = 2.16, 95% CI: 1.23, 3.78), and PC-1 (RR = 1.61, 95% CI: 1.16, 2.23). On the other hand, among mothers who were younger than 35 years old, ASD risk was decreased with higher PFOS (RR = 0.65, 95% CI: 0.44, 0.98), PFHxS (RR = 0.72, 95% CI: 0.59, 0.89), and PC-1 (RR = 0.83, 95% CI: 0.68, 1.01). When analyses were stratified by child's sex, we did not find sex-specific associations of any PFAS compounds with ASD risk (p-value for interaction > 0.30), and effect size estimates and 95% CI were broadly consistent across strata. When untransformed PFAS concentrations were used in the stratified analyses, the effect modification by maternal age at delivery were similarly found and sex-specific associations were not observed for any PFAS compounds (Table S2.7).

Table 2.5 Adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (versus TD) in association with prenatal maternal PFAS concentrations, with log 2-transformation, stratified by child's sex and maternal age at delivery.

Log 2-transformed PFAS (ng/mL) or **principal component score**	**RR** (95% CI)		***p*-value** [a]
Child's sex [b]	**Females** [c]	**Males** [d]	
PFOA	1.20 (0.79, 1.80)	1.25 (0.87, 1.81)	0.91
PFOS	0.99 (0.64, 1.54)	1.07 (0.77, 1.48)	0.86
PFHxS	0.80 (0.68, 0.95)	1.04 (0.81, 1.33)	0.31
PFNA	1.25 (0.76, 2.06)	1.20 (0.82, 1.76)	0.66
PC-1	1.00 (0.77, 1.31)	1.10 (0.90, 1.35)	0.80
Maternal age at delivery [e]	**< 35 years** [f]	**≥ 35 years** [g]	
PFOA	0.91 (0.61, 1.35)	2.29 (1.30, 4.04)	0.04
PFOS	0.65 (0.44, 0.98)	1.67 (1.03, 2.71)	0.02
PFHxS	0.72 (0.59, 0.89)	1.30 (0.90, 1.89)	0.08
PFNA	0.90 (0.58, 1.39)	2.16 (1.23, 3.78)	0.05
PC-1	0.83 (0.68, 1.01)	1.61 (1.16, 2.23)	0.01

[a] *p*-value for interaction term of PFAS with each potential effect modifier (potential effect modifiers and an interaction term were added as additional terms in the regression model, along with other covariates).

[b] Adjusted for child's birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[c] Females (n = 71; TD = 54, ASD = 17), [d] Males (n = 102; TD = 62, ASD = 40).

[e] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[f] < 35 years (n = 93; TD = 62, ASD = 31), [g] ≥ 35 years (n = 80; TD = 54, ASD = 26).

2.4. Discussion

In the present study, we examined whether prenatal exposure to PFAS was associated with increased risk of ASD using maternal serum samples collected during pregnancy in a high-risk ASD cohort. When using log 2-transformed PFAS concentrations, we found evidence of positive associations of ASD risk with prenatal PFOA and PFNA and inverse association with PFHxS. When the analyses were stratified by maternal age at delivery, each of the individual PFAS was associated with increased risk of ASD among mothers who were 35 years old or older and decreased risk of ASD among mothers who were younger than 35 years old. When untransformed PFAS concentrations were used in the models, we observed that higher PFOA, PFNA, and PC-1 (i.e., the first principal component with the largest variance from PCA) were associated with increased risk of ASD and the negative associations of PFHxS with ASD risk moved toward the null. The models with untransformed PFAS resulted in smaller standard errors of RRs for PFOA and PFOS compared to those with log 2-transformed PFAS. However, after excluding the extreme values (i.e., top 2.5 percentiles), the RRs and confidence intervals from the models with untransformed concentrations were relatively unstable compared to those from the models with log-transformed concentrations. Therefore, we cannot exclude the possibility that the models with untransformed concentrations may have been driven more by the influence of outliers and the results of the untransformed PFAS models should be interpreted with caution.

This current study did not show consistent results with previous studies that examined associations between prenatal PFAS exposure and the risk of ASD. Braun et al. observed that higher PFOA was associated with lower Social Responsive Scale scores, indicating fewer autistic behaviors in children, while PFOS, PFHxS, and PFNA showed null associations (Braun et al. 2014). They used continuous scales, rather than a clinical diagnosis, to assess autistic-like behaviors in a general population sample, limiting direct comparison of their results with our

study. Liew et al. found evidence that higher PFHxS was associated with increased risk of childhood autism but other PFAS did not show apparent associations (Liew et al. 2014). Lyall et al. observed that most of the PFAS were not associated with the risk of ASD, with the exception that PFOA and PFOS showed associations with decreased ASD risk (Lyall et al. 2018). Compared to our study, these two studies (Liew et al. 2014; Lyall et al. 2018) had higher maternal plasma or serum concentrations of PFOA, PFOS, and PFHxS, while their PFNA concentrations were similar. Long et al. found that ASD risk was decreased with both higher PFOS and a principal component that was dominated by PFAS congeners in amniotic fluid (Long et al. 2019). However, their results may not be directly comparable to ours because PFOS was only detected in fewer than 50% of amniotic fluid samples and correlations between maternal serum and amniotic fluid concentrations varied considerably by PFAS (Stein et al. 2012). In a case-control study, Shin et al. used modeled prenatal maternal PFAS concentrations from maternal samples collected when their child was 2-5 years old and separately fit three different models using ln-transformed PFAS concentrations, PFAS concentrations with no transformation, and categorized PFAS concentrations (Shin et al. 2020). They observed that prenatal ln-transformed PFHxS was associated with increased risk of ASD, and additionally found that PFOS was borderline associated with the ASD risk when using PFAS concentrations without transformation.

Advanced maternal age (mostly considered to be 35 years old or older) was shown to contribute to child's neurodevelopmental disorders, including ASD (Janecka et al. 2017; Shelton et al. 2010). From the stratified analyses by maternal age at delivery, we observed that prenatal exposure to PFOA, PFOS, PFHxS, and PFNA as well as the combined PFAS was consistently associated with increased risk of having a child with ASD among mothers who delivered their

child at 35 years old or older. A previous study also observed that prenatal maternal serum concentrations of PFOA and PFOS were associated with decreased communication development scores of 38-month-old females among mothers who were over 30 years old at delivery (Jeddy et al. 2017). One potential mechanism for the effect modification is age-induced epigenetic changes. During aging, epigenetic changes influenced by environmental exposures are accumulated and thus may lead to alterations in various gene expression pathways critical for fetal development, resulting in the development of ASD (Banik et al. 2017; Fraga and Esteller 2007). Changes in maternal hormone levels during pregnancy, which are altered by advanced maternal age, can also affect fetal brain development (Barrett et al. 2019; Miranda and Sousa 2018).

Strengths of this study include clinically confirmed ASD diagnosis by trained psychologists using gold standard diagnostic instruments. Furthermore, our multiple maternal serum samples collected during pregnancy may better represent fetal exposure to PFAS during critical time windows of neurodevelopment than individual samples. However, several limitations should be noted. A relatively small sample size limited the statistical power of our analysis. In addition, as chance findings may not be excluded, further studies should be conducted with a large sample size. There might be residual confounding by unmeasured variables such as lifestyle, behavior, or socioeconomic status because the primary exposure sources of PFAS are diet, drinking water, or dust ingestion (Jian et al. 2017). In this study, we did not include children who did not complete the study for final diagnosis or mothers who did not provide blood samples during pregnancy. As some mothers may have dropped out the study after having girls due to the lower ASD rates in females, there might be potential selection bias in this study. Because MARBLES children have increased genetic susceptibility, null associations for

most PFAS in the current study could indicate that genetic factors may have contributed to the development of ASD in this study population more than environmental factors such as exposure to PFAS during pregnancy. Except for two mothers, all children included in the current study had at least one older sibling. Thus, our results should be interpreted with caution and may not extrapolate to the general population such as those children without ASD siblings or who were delivered from first pregnancies. Furthermore, as a growing body of literature suggests environmental factors can interact with genetic factors in the development of ASD (Chaste and Leboyer 2012; Tordjman et al. 2014), further studies using integrated approaches including gene and environment interactions are warranted.

CHAPTER 3: PRENATAL EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES AND COGNITIVE DEVELOPMENT IN INFANCY AND TODDLERHOOD

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3.1. Introduction

3.1.1. Background

Per- and polyfluoroalkyl substances (PFAS) are a group of synthetic fluorine-containing compounds that have been widely used in consumer and industrial applications, including non-stick cookware, food packaging materials, and stain- and water-repellent fabrics (ATSDR 2018). The widespread applications of PFAS in consumer products resulted in ubiquitous detection of several PFAS in serum of the general U.S. population (CDC 2019; Kato et al. 2011b; Olsen et al. 2017). PFAS have also been detected in blood of pregnant women as well as in amniotic fluid, umbilical cord blood, and breast milk (Bjerregaard-Olesen et al. 2016b; Inoue et al. 2004; Kato et al. 2014; Kim et al. 2011b; Liu et al. 2011; Long et al. 2019; Monroy et al. 2008; Tsai et al. 2018; von Ehrenstein et al. 2009). In addition, concentrations of commonly-detected PFAS, including perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), in prenatal maternal blood are moderately to highly correlated with corresponding measurements in amniotic fluid and cord blood (Beesoon et al. 2011; Gützkow et al. 2012; Stein et al. 2012), supporting the transfer of PFAS across the placenta.

Prenatal exposure to PFAS is of concern for young child cognitive development because PFAS have shown neurobehavioral toxicity in prenatally-exposed offspring of laboratory animals (Mariussen 2012). In mice, gestational exposure to PFOS resulted in delayed neuromotor maturation (Fuentes et al. 2007) and decreased locomotion (Onishchenko et al. 2011). Among mice prenatally exposed to PFOA, males showed more active exploratory behaviors, while females displayed decreased activity (Onishchenko et al. 2011). In rats, gestational exposure to PFOS increased motor activity and reduced habituation in male offspring on postnatal day (PND) 17 but not on PND 13, 21, and 61 (Butenhoff et al. 2009), suggesting

that PFOS may have neurobehavioral effects at a certain life stage of offspring. Collectively, these findings highlight the relevance of examining the potential effect of prenatal maternal exposure to PFAS on child cognitive development.

3.1.2. Statement of research problem

Epidemiological studies have examined associations of prenatal PFAS exposure with child's neurobehaviors at different assessment time points in infancy and toddlerhood (Chen et al. 2013; Donauer et al. 2015; Goudarzi et al. 2016; Spratlen et al. 2020). Birth cohort studies in the United States and Japan observed that prenatal serum PFOA was associated with hypotonic response in 5-week-old infants and lower mental developmental index among 6-month-old females, respectively (Donauer et al. 2015; Goudarzi et al. 2016). In the Taiwanese Birth Panel Study, PFOS in cord blood was inversely associated with whole-test developmental quotients among children at 2 years of age (Chen et al. 2013). A recent study in the United States reported that prenatal exposures to PFOA and perfluorohexane sulfonate (PFHxS) were positively associated with mental developmental index in children at 3 years of age, but not at 1 and 2 years (Spratlen et al. 2020). Except for Spratlen et al., children in the previous studies were assessed for neurodevelopment at one or two time points in infancy or toddlerhood (before or at 36 months of age). In addition, little is known about trajectories of child' neurodevelopment over time or longitudinal changes in associations between prenatal maternal PFAS exposure and child neurodevelopment.

3.1.3. Objectives

The present study aimed to investigate associations between prenatal maternal serum

PFAS concentrations and child cognitive development assessed at four time points in infancy and toddlerhood (i.e., 6, 12, 24, 36 months of age). We also examined longitudinal changes in child cognitive developmental scores as well as trajectories of the developmental scores over the four time points in association with prenatal maternal serum PFAS concentrations.

3.2. Methods

3.2.1. Study population

The present study included participants from MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns) (Hertz-Picciotto et al. 2018b). Launched in 2006, the MARBLES study is an ongoing cohort that follows pregnant women who previously had a child diagnosed with autism spectrum disorder (ASD) and are thus at high risk (~28%) of having a child with delays or deficits in areas of development or behavior (Ozonoff et al. 2014). Participants are mostly recruited from the lists of families receiving state-funded services for a child with ASD. MARBLES eligibility criteria at enrollment include: a) mother or father has a child or other first degree relative with ASD; b) mother is 18 years old or older; c) mother is pregnant; d) mother speaks, reads, and understands English; e) mother resides within 2.5 hours of the Davis/Sacramento region. Informed consent is obtained from participants before collecting any data, and the protocols were approved by the institutional review boards for the State of California and the University of California Davis (UC Davis). The analysis of coded samples at the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects' research. Details of study design, study population, inclusion criteria, recruitment, and data collection are available elsewhere (Hertz-Picciotto et al. 2018b).

Enrollment of MARBLES began in 2006; in 2009, MARBLES started collecting serum.

Therefore, we firstly selected 218 mother-child pairs (MARBLES baseline population) (1) who provided prenatal blood samples with sufficient volume for PFAS quantification since 2009 and conceived their babies by 2014 and (2) whose child has ever been assessed for cognitive development at any time point. We further restricted our study population to 140 children who were repeatedly evaluated for cognitive development at all four time points, i.e., at 6, 12, 24, and 36 months of age (Figure 3.1).

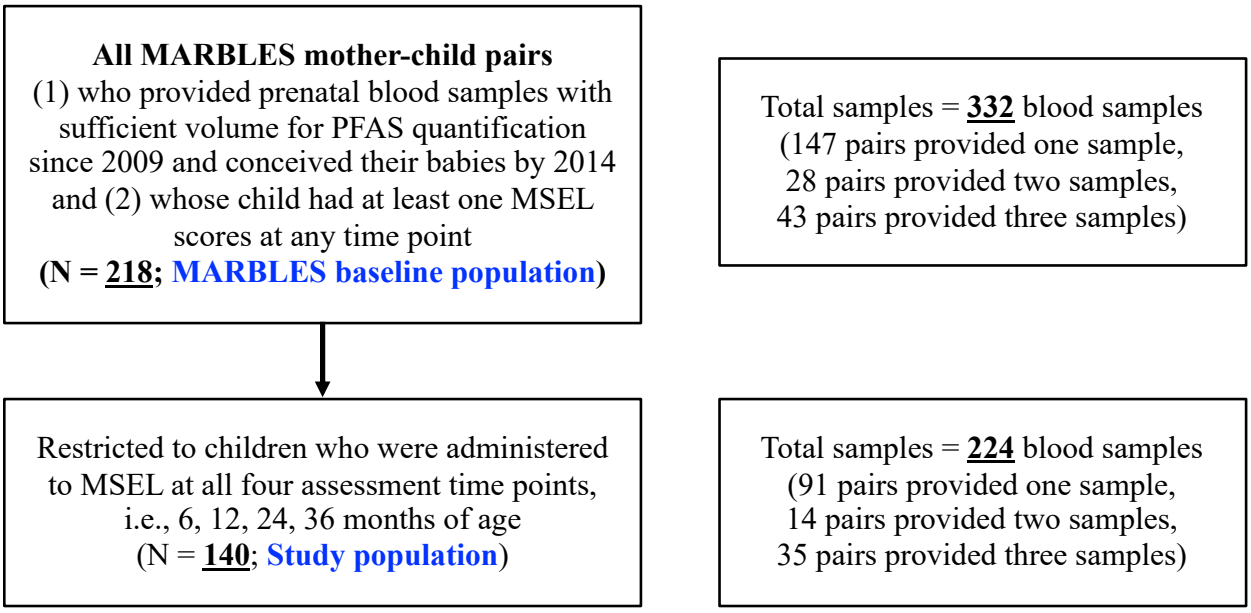

Figure 3.1 Study flow and blood sample collection from MARBLES participants to examine the associations between prenatal exposure to PFAS and child's cognitive development.

3.2.2. Serum sample collection and PFAS quantification

Each mother included in the present study provided one to three blood samples during pregnancy. Of 140 mother-child pairs, 91 pairs provided one sample, 14 pairs two samples, and 35 pairs three samples. Thus, a total of 224 blood samples were used in this study. Whole blood was centrifuged, and separated serum was stored at -80 °C at the UC Davis.

At the CDC, we quantified PFAS in maternal serum using online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography–isotope dilution tandem mass spectrometry. Analytical methods for PFAS quantification are described elsewhere (Kato et al. 2011a). To ensure reproducibility, quality control samples spiked at low and high concentrations of PFAS were included in each batch. To assess the reliability of the data, 25 blind duplicate samples were analyzed with the study samples. The median coefficient of variation for these duplicate pairs of samples ranged from 0 to 11%, depending on the analyte.

We quantified nine PFAS: PFOA, PFOS, PFHxS, perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA). The limit of detection (LOD) was 0.1 ng/mL for all PFAS. For concentrations below the LOD, we used instrument-observed values to reduce bias (Lubin et al. 2004; Richardson and Ciampi 2003).

3.2.3. Assessment of child cognitive development

Licensed clinical psychologists at the UC Davis Medical Investigations of Neurodevelopmental Disorders (MIND) Institute assessed all children included in this study for cognitive development. MARBLES staff who were trained by senior clinicians assessed child

cognitive development during home visits at 6 and 12 months of age and during participants' visits to the UC Davis MIND Institute at 24 and 36 months of age, which were then reviewed by senior clinicians (Hertz-Picciotto et al. 2018b). Cognitive development in infancy and toddlerhood was assessed using Mullen Scales of Early Learning (MSEL), a performance-based measure of cognitive functions or abilities designed to evaluate infants and young children up to 68 months of age (Burns et al. 2013; Mullen 1997). Child's cognitive functions are scored on four subscales: Fine Motor, Visual Reception, Receptive Language, and Expressive Language. Raw scores of the four subscales are converted to age-standardized T-scores with a mean of 50 and a standard deviation (SD) of 10. The T-scores of four subscales are combined to yield an Early Learning Composite (Composite), which is a standardized score of overall cognitive development with a mean of 100 and an SD of 15 (Swineford et al. 2015; Turner-Brown et al. 2013). A higher score on Composite and each subscale indicates better cognitive functions.

When children became approximately 36 months of age, they were classified into three diagnostic groups, including ASD, non-typical development (Non-TD), and typical development (TD), using an algorithmic method based on Autism Diagnostic Observation Schedule and MSEL scores. The algorithmic method is described elsewhere (Oh et al. 2021a).

3.2.4. Statistical analysis

All statistical analyses were conducted using STATA/IC version 15.1 (StataCorp LLC, College Station, TX, USA). At each assessment time point, we compared mean MSEL Composite scores with respect to demographic characteristics using the Wilcoxon rank-sum test or the Kruskal-Wallis test. Separately for the MSEL Composite scores and each of the subscale T-scores, we tested for whether mean scores differed across the four time points by fitting a

panel data regression model with fixed effects for child and timepoint (as a categorical variable) and then assessing the Wald test for the omnibus null hypothesis associated with timepoint, using robust sandwich variance estimators to account for heteroskedasticity and Bonferroni correction for multiple comparison (Bland and Altman 1995).

To investigate prenatal maternal serum PFAS concentrations in association with MSEL Composite scores and T-scores of each subscale at each assessment time point, we constructed multiple linear regression models and estimated regression coefficients (βs) and 95% confidence intervals (CIs). Six PFAS with detection frequency greater than 60% were individually included in the regression models: PFOA, PFOS, PFHxS, PFNA, PFDA, and PFUnDA. Three PFAS that were detected in less than 60% of the samples were excluded from the further analyses. For mothers who provided multiple blood samples during different trimesters, PFAS concentrations were averaged per participant. Because most PFAS concentrations showed right-skewed distributions, we used log 2-transformed PFAS concentrations to reduce the impact driven by outliers and centered each log 2-transformed PFAS concentration by subtracting its median value, to facilitate the interpretability of models with interactions terms. We selected a priori potential confounders using a directed acyclic graph (Figure 3.2) (Hernán et al. 2004). We retained the covariates that were associated with MSEL Composite scores at least one time point ($p < 0.10$) in the regression models. Covariates adjusted in the final models included: child's sex (female, male), parity (≤ 1, > 1), maternal pre-pregnancy body mass index (BMI) (normal/underweight, overweight, obese), gestational diabetes (yes, no), maternal education (high school or some college credit, bachelor's degree, graduate or professional degree), and breastfeeding duration (< 12 months, ≥ 12 months). Because two mothers participated in the study with twins, we applied clustered sandwich variance estimators to the regression models to

adjust for within-family correlations. We imputed missing covariates with chained equations by including all exposures, outcomes, and covariates (White et al. 2011).

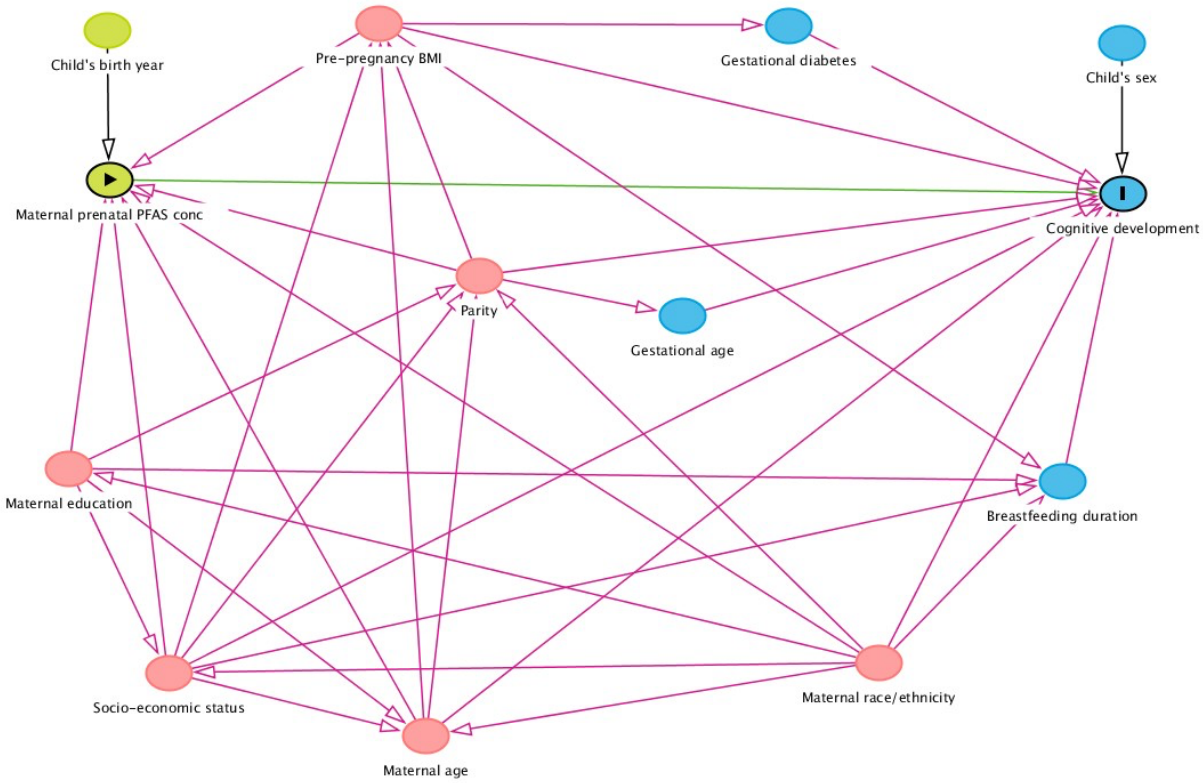

Figure 3.2 Directed acyclic graph used to identify potential confounders of association between prenatal PFAS concentrations and child's cognitive development. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome.

To examine longitudinal changes in MSEL Composite scores and T-scores of four subscales over the four assessment time points within subjects in relation to prenatal maternal serum PFAS concentrations, we used population-averaged generalized estimating equations (GEE) models with a linear link and autoregressive correlation structure, adjusting for the same covariate set as before (Ballinger 2004; Liang and Zeger 1986). In these models, age at assessment (expressed in months) was centered at 24 and included in the model in two terms; one for a main effect and the other for an interaction term with the log 2-transformed and centered PFAS exposure, with the interaction term considered statistically significant when $p < 0.10$. The GEE models used in this study can handle not only correlated data within individuals but also incomplete outcome data (e.g., Composite and subscale scores) by using all available observations (Hubbard et al. 2010). Therefore, as a sensitivity analysis, we ran the GEE models using the MARBLES baseline population that additionally include 78 children with incomplete MSEL outcomes.

To examine the trajectories of MSEL Composite scores over the four assessment time points within groups with distinct trajectories of the outcome, we performed a group-based trajectory analysis by characterizing the patterns of repeated outcomes (Nagin 2005). Due to the small sample size, we limited the number of trajectory groups to two, which were characterized by difference in the child's Composite score patterns from 6 to 36 months of age, and determined the trajectory shape of each group based on the Bayesian information criterion (Raftery 1995). We then classified children with decreasing and/or lower scores into a low-score group and those with increasing and/or higher scores into a high-score group. To estimate relative risks (RRs) of the low-score group compared to the high-score group in association with prenatal maternal

serum PFAS concentrations, we used Poisson regression models with robust error variance, adjusting for the same covariate set as before.

To examine the combined effect of PFAS on child cognitive development, we carried out a principal component analysis (PCA), a "variable reduction" strategy that can mitigate multicollinearity issues (Kaiser 1958). PCA has been used to identify patterns of exposure from chemical mixtures, including PFAS, in association with child neurodevelopment due to its versatility and ease of application (Hoffman et al. 2010; Skogheim et al. 2020; Spratlen et al. 2020; Stafoggia et al. 2017). From a PCA with varimax rotation using six PFAS, we selected the first and second principal components (PC-1 and PC-2), which each had eigenvalues greater than one. We included them in regression models as independent variables (Kaiser 1960).

Previous studies reported effect modification of associations between prenatal PFAS and cognitive development of infants or young children by child's sex (Goudarzi et al. 2016; Harris et al. 2018; Niu et al. 2019). Breastfeeding was reported to not only serve as an important PFAS exposure route for infants (Mogensen et al. 2015; Mondal et al. 2012) but also affect child cognitive development in infancy (McCrory and Murray 2013; Morrow-Tlucak et al. 1988). Thus, for each of these candidate effect modifiers (i.e., child's sex (female, male) and breastfeeding duration (< 12 months, ≥ 12 months)), we performed effect modification analyses by refitting regression models that included interaction terms involving the candidate effect modifier with PFAS (and, for the longitudinal analyses, with the age of assessment terms, as well). PFDA and PFUnDA were excluded from effect modification analyses because more than half of the samples had concentrations equal to or less than the LOD and the concentration range was narrow. As our study population included children diagnosed with neurodevelopmental concerns (i.e., ASD and Non-TD), we performed sensitivity analyses by restricting the multiple

linear regression and longitudinal GEE analyses to the TD children (n = 93). Except for interactions specified above in the GEE analyses, the level of statistical significance was set at p-value < 0.05 for the rest.

3.3. Results

3.3.1. Participant characteristics and MSEL scores

Of the 140 mother-child pairs included in the current study, 59% of children were males and 91% were full-term birth children (Table 3.1). Approximately 20% of mothers were obese before pregnancy, 19% had gestational diabetes, and 54% were multiparous at study enrollment. The majority of mothers were non-Hispanic white (51%) and were high school graduate or had some college credit without a degree in higher education (45%). There was no difference in population characteristics between this study population (n = 140) and the MARBLES baseline population (n = 218) enrolled during the same study period (Table S3.1).

Mean MSEL Composite scores differed among several participant characteristics (Table 3.1). At 12 and 24 months of age, females had higher scores than males. At 12, 24, and 36 months of age, children whose mothers had a bachelor's or higher degree had higher scores than those whose mothers did not have at least a bachelor's degree. At 24 months of age, children born to mothers who were older than 35 years at delivery had higher scores than those born to mothers younger than 35 years. At 36 months of age, children of mothers who were nulliparous or primiparous had higher scores than those of multiparous mothers.

When comparing the MSEL Composite scores and T-scores of four subscales across the four assessment time points, the mean scores of Composite and Visual Reception were lower at 24 months than other time points (Figure 3.3). At 12 months of age, the mean scores of Fine

Motor and Receptive Language were higher and lower than other time points, respectively. The number of children whose Composite scores fell below 2 SD of the normative mean (i.e., Composite < 70, indicating low cognitive function) was 0 for 6 months, 2 for 12 months, 18 for 24 months, and 13 for 36 months.

Table 3.1 MSEL Composite scores at 6, 12, 24, and 36 months by participant characteristics for children administered to the MSEL at all four time points.

Characteristic [a]	All (n = 140)		MSEL Composite							
			6 months		12 months		24 months		36 months	
	n	%	Mean (SD)	p [b]	Mean (SD)	p [b]	Mean (SD)	p [b]	Mean (SD)	p [b]
Child sex										
Female	57	41	99 (12)	0.99	104 (11)	0.01	99 (17)	0.05	105 (19)	0.12
Male	83	59	98 (12)		98 (15)		92 (18)		99 (22)	
Child's birth year										
2009-2010	46	33	96 (11)	0.15	97 (16)	0.22	94 (18)	0.39	102 (23)	0.77
2011-2013	50	36	101 (12)		101 (12)		92 (18)		99 (21)	
2014-2015	44	31	99 (11)		102 (13)		98 (17)		104 (19)	
Gestational age at delivery										
≤ 37 weeks	12	9	98 (15)	0.66	100 (18)	0.69	92 (16)	0.58	96 (20)	0.17
> 37 weeks	128	91	99 (12)		100 (13)		95 (18)		102 (21)	
Maternal age at delivery										
< 35 years	72	51	98 (12)	0.31	99 (14)	0.20	92 (19)	0.05	99 (21)	0.12
≥ 35 years	68	49	99 (12)		102 (13)		97 (17)		104 (21)	
Parity										
≤ 1	61	44	99 (12)	0.88	101 (16)	0.87	98 (17)	0.14	106 (19)	0.03
>1	76	54	98 (12)		100 (12)		92 (18)		98 (21)	
Maternal pre-pregnancy BMI										
Normal/underweight	71	51	98 (11)	0.84	101 (14)	0.33	98 (17)	0.04	103 (20)	0.52
Overweight	41	29	100 (11)		98 (15)		90 (20)		99 (24)	
Obese	28	20	98 (14)		102 (12)		92 (15)		102 (19)	
Gestational diabetes										
Yes	27	19	95 (11)	0.08	99 (14)	0.22	100 (14)	0.16	108 (16)	0.04
No	113	81	99 (12)		101 (14)		93 (19)		100 (22)	
Maternal race/ethnicity										
Non-Hispanic white	71	51	99 (12)	0.80	101 (14)	0.27	95 (16)	0.98	102 (20)	0.83
Hispanic	33	24	98 (10)		101 (13)		93 (20)		99 (22)	
Other [c]	36	26	98 (13)		98 (12)		94 (19)		103 (22)	
Maternal education										
Less than college degree	63	45	99 (12)	0.60	97 (12)	0.05	88 (19)	0.01	96 (21)	0.01
Bachelor's degree	46	33	100 (12)		105 (14)		100 (15)		106 (21)	
Graduate or professional degree	31	22	97 (11)		99 (14)		99 (17)		106 (19)	
Homeownership										
Yes	83	59	99 (12)	0.62	101 (13)	0.21	96 (15)	0.17	102 (20)	0.56
No	54	39	98 (12)		98 (14)		92 (21)		101 (23)	
Breastfeeding duration										
< 12 months	77	55	98 (11)	0.59	102 (13)	0.05	95 (18)	0.94	103 (22)	0.23
≥ 12 months	57	41	100 (12)		98 (14)		93 (18)		99 (20)	

[a] Missing information (n): parity (3), homeownership (3), breastfeeding duration (6).

[b] p-value from the Wilcoxon rank-sum test or the Kruskal-Wallis test.

[c] Includes Black (3%), Asian (20%), and others (3%).

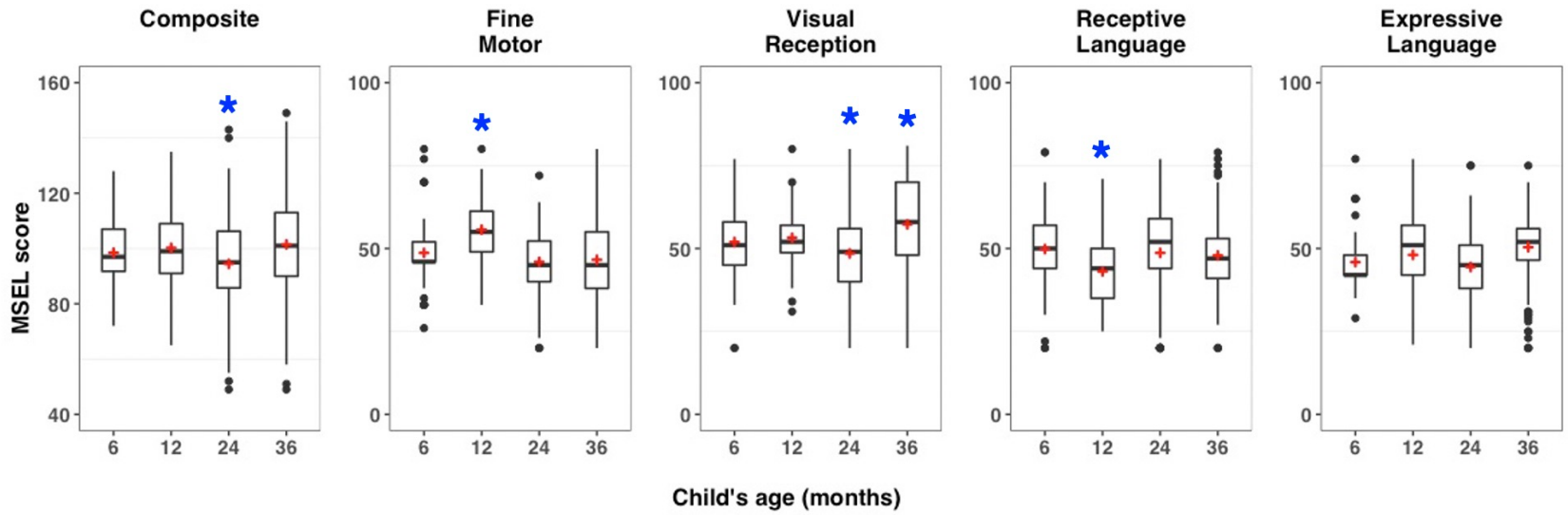

Figure 3.3 Distributions of MSEL Composite scores and T-scores of four subscales at 6, 12, 24, and 36 months of age for 140 children included in the current study. Red crosses denote mean values at each time point. Blue asterisks denote differences in mean scores with other time points identified by the postestimation test with Bonferroni correction following the linear mixed model at the significance level of 0.05. Composite scores have a normative mean of 100 and an SD of 15 and T-scores of each subscale have a normative mean of 50 and an SD of 10.

3.3.2. Prenatal maternal serum PFAS concentrations

PFOA, PFOS, PFHxS, and PFNA were detected in more than 99% of the samples, while the detection frequency of PFDA, PFUnDA, PFDoDA, MeFOSAA, and EtFOSAA was 85%, 62%, 31%, 53%, and 4%, respectively (Table 3.2). PFOS had the highest median (2.8 ng/mL), followed by PFOA (0.9 ng/mL), PFNA (0.5 ng/mL), and PFHxS (0.4 ng/mL). The medians of PFDA, PFUnDA, and MeFOSAA were at the LOD of 0.1 ng/mL. Due to the narrow concentration ranges for PFDA and PFUnDA, the results for these two PFAS should be interpreted with caution.

As combinations of six PFAS, two PCs were identified (Table 3.3). The first principal component (PC-1) had moderate positive loadings mainly on PFOA, PFOS, PFHxS, and PFNA, explaining approximately 55% of the total variance (0.46 for PFOA, 0.50 for PFOS, 0.62 for PFHxS, 0.37 for PFNA, 0.07 for PFDA, and -0.12 for PFUnDA). The second principal component (PC-2) had positive loadings mainly on PFUnDA, PFDA, and PFNA as well as negative loadings on PFHxS, accounting for about 17% of the total variance (0.13 for PFOA, 0.07 for PFOS, -0.28 for PFHxS, 0.33 for PFNA, 0.55 for PFDA, and 0.70 for PFUnDA).

Table 3.2 Distribution of nine PFAS concentrations (ng/mL) in 224 maternal serum samples collected from 140 mother-child pairs.

PFAS [a]	% detect	GM (ng/mL)	Percentiles (ng/mL)				
			5th	25th	50th	75th	95th
PFOA	100.0	0.88	0.30	0.60	0.90	1.25	2.20
PFOS	100.0	2.82	1.00	1.90	2.80	4.10	7.00
PFHxS	99.6	0.45	0.10	0.30	0.40	0.70	1.60
PFNA	100.0	0.48	0.20	0.30	0.50	0.70	1.10
PFDA	84.8	0.16	<LOD	0.10	0.10	0.20	0.40
PFUnDA	61.6	0.14	<LOD	<LOD	0.10	0.10	0.30
PFDoDA	31.3	0.11	<LOD	<LOD	<LOD	0.10	0.10
MeFOSAA	53.1	0.19	<LOD	<LOD	0.10	0.20	0.80
EtFOSAA	3.6	0.10	<LOD	<LOD	<LOD	<LOD	<LOD

[a] Limit of detection for all nine PFAS was 0.1 ng/mL.

Abbreviation: geometric mean (GM), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-ethyl-perfluoro-octane sulfonamido) acetate (EtFOSAA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA)

Table 3.3 Principal component and its component-loading weights for PFAS.

PFAS	Our study		Skogheim et al., 2020	
	PC-1	PC-2	PC-1	PC-2
PFOA	0.46	0.13	0.44	0.09
PFOS	0.50	0.07	0.49	0.04
PFHxS	0.62	-0.28	0.48	-0.06
PFNA	0.37	0.33	0.13	0.48
PFDA	0.07	0.55	-0.01	0.61
PFUnDA	-0.12	0.70	-0.08	0.62
PFHpS	-	-	0.56	-0.06

Abbreviation: principal component (PC), perfluoroheptane sulfonate (PFHpS)

3.3.3. Associations between prenatal maternal PFAS exposure and child's cognitive development

Prenatal maternal serum PFOA was inversely associated with Composite scores at 24 months (β = -5.22, 95% CI: -8.27, -2.17) and 36 months of age (β = -5.18, 95% CI: -9.46, -0.91) (Figure 3.4). PFOA was also inversely associated with all four subscale scores at 24 and 36 months of age, except for Receptive Language at 36 months and Visual Reception at 24 months (see Table S3.2 for βs and 95% CIs). Receptive Language scores at 24 months were inversely associated with PFNA (β = -2.79, 95% CI: -5.38, -0.20) and PC-1 (β = -1.53, 95% CI: -3.04, -0.01). When assessing longitudinal changes in MSEL scores over the four assessment time points in GEE models, PFOA was inversely associated with scores on the Composite (β = -0.19, *p*-value for interaction = 0.01) and three subscales (β = -0.12 for Fine Motor, β = -0.14 for Visual Reception, β = -0.08 for Expressive Language; *p*-value for interaction < 0.06) (Table 3.4). When the GEE analyses were expanded to the MARBLES baseline population (Table S3.3) or when restricting timepoint-stratified multiple linear regression and longitudinal GEE analyses to the TD children (Figure S3.1 and Table S3.4, respectively), overall effect estimates slightly moved toward a positive direction, but the trends were similar.

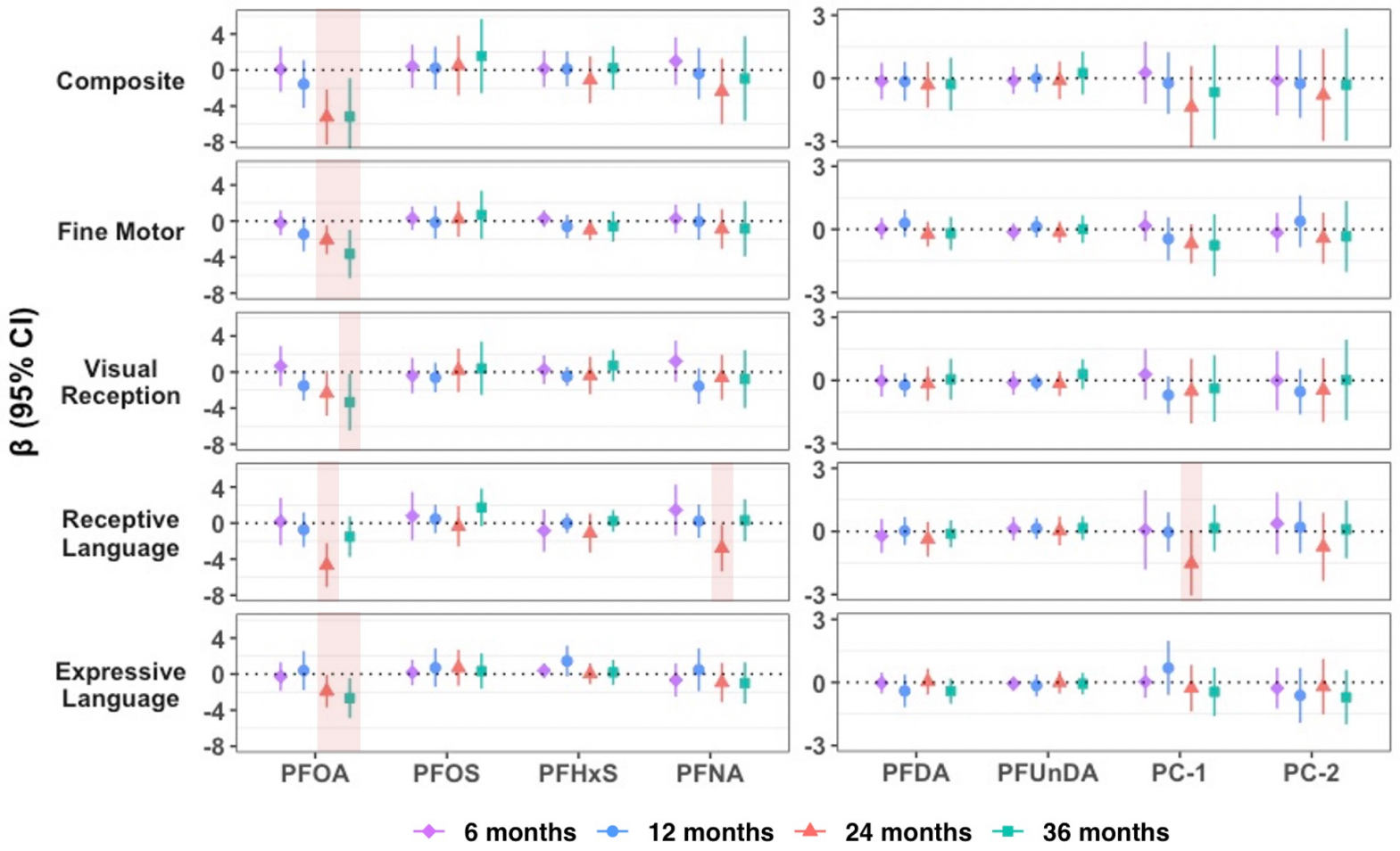

Figure 3.4 Adjusted mean differences (β) in MSEL Composite scores and T-scores of subscales of children at 6, 12, 24, and 36 months of age in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS and two PCs. Models were adjusted for child′s sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration. Red shaded areas represent associations with a *p*-value < 0.05.

Table 3.4 Longitudinal changes (β) in MSEL Composite scores and T-scores of subscales of children over the four assessment time points in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS and two PCs in generalized estimating equations.

MSEL	PFOA β (95% CI) [a]	PFOS β (95% CI) [a]	PFHxS β (95% CI) [a]	PFNA β (95% CI) [a]
Composite	-0.19 (-0.33, -0.04)[#]	0.02 (-0.12, 0.17)	-0.02 (-0.11, 0.07)	-0.09 (-0.27, 0.08)
Fine Motor	-0.12 (-0.20, -0.03)[#]	0.00 (-0.09, 0.09)	-0.04 (-0.09, 0.02)	-0.06 (-0.17, 0.05)
Visual Reception	-0.14 (-0.26, -0.03)[#]	0.00 (-0.11, 0.12)	-0.01 (-0.07, 0.06)	-0.08 (-0.21, 0.05)
Receptive Language	-0.07 (-0.16, 0.02)	0.03 (-0.06, 0.11)	0.01 (-0.06, 0.09)	-0.05 (-0.15, 0.05)
Expressive Language	-0.08 (-0.16, 0.00)[#]	0.02 (-0.06, 0.09)	-0.01 (-0.06, 0.04)	-0.03 (-0.12, 0.06)
MSEL	**PFDA β (95% CI) [a]**	**PFUnDA β (95% CI) [a]**	**PC-1 β (95% CI) [a]**	**PC-2 β (95% CI) [a]**
Composite	-0.01 (-0.06, 0.04)	0.01 (-0.03, 0.05)	-0.04 (-0.13, 0.04)	-0.02 (-0.12, 0.09)
Fine Motor	-0.01 (-0.04, 0.01)	0.00 (-0.02, 0.02)	-0.04 (-0.08, 0.01)	-0.02 (-0.08, 0.04)
Visual Reception	0.00 (-0.04, 0.04)	0.01 (-0.02, 0.04)	-0.04 (-0.10, 0.03)	0.00 (-0.08, 0.07)
Receptive Language	0.00 (-0.03, 0.03)	0.00 (-0.02, 0.02)	-0.01 (-0.07, 0.05)	-0.01 (-0.07, 0.05)
Expressive Language	-0.01 (-0.04, 0.01)	0.00 (-0.02, 0.02)	-0.02 (-0.06, 0.02)	-0.01 (-0.07, 0.04)

[a] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration. Regression coefficients (β) were derived from interaction terms between continuous age in months (centered at 24) and continuous log 2-transformed PFAS concentrations, after including the two main effects and their interaction term in the GEE model.

[#] *p*-value for interaction between age at assessment and PFAS concentrations < 0.10

When analyses were stratified by child's sex (Figure 3.5), at 12 months of age, higher PFHxS was associated with decreased Composite scores among females (β = -1.54, 95% CI: -3.29, 0.22), but with increased Composite scores among males (β = 3.48, 95% CI: 0.00, 6.96; *p*-value for interaction = 0.01). With respect to longitudinal changes in MSEL scores in GEE models, only among females, decreased Composite scores were associated with higher PFOA (β = -0.25, *p*-value for interaction = 0.02) over the four time points (Table S3.5). When analyses were stratified by breastfeeding duration, the associations of Composite scores with PFOA (*p*-value for interaction = 0.03), PFHxS (*p*-value for interaction = 0.09), and PFNA (*p*-value for interaction = 0.10) were modified at 12 months of age, showing inverse associations among children who were breastfed < 12 months and positive associations among children who were breastfed ≥ 12 months (Figure 3.5).

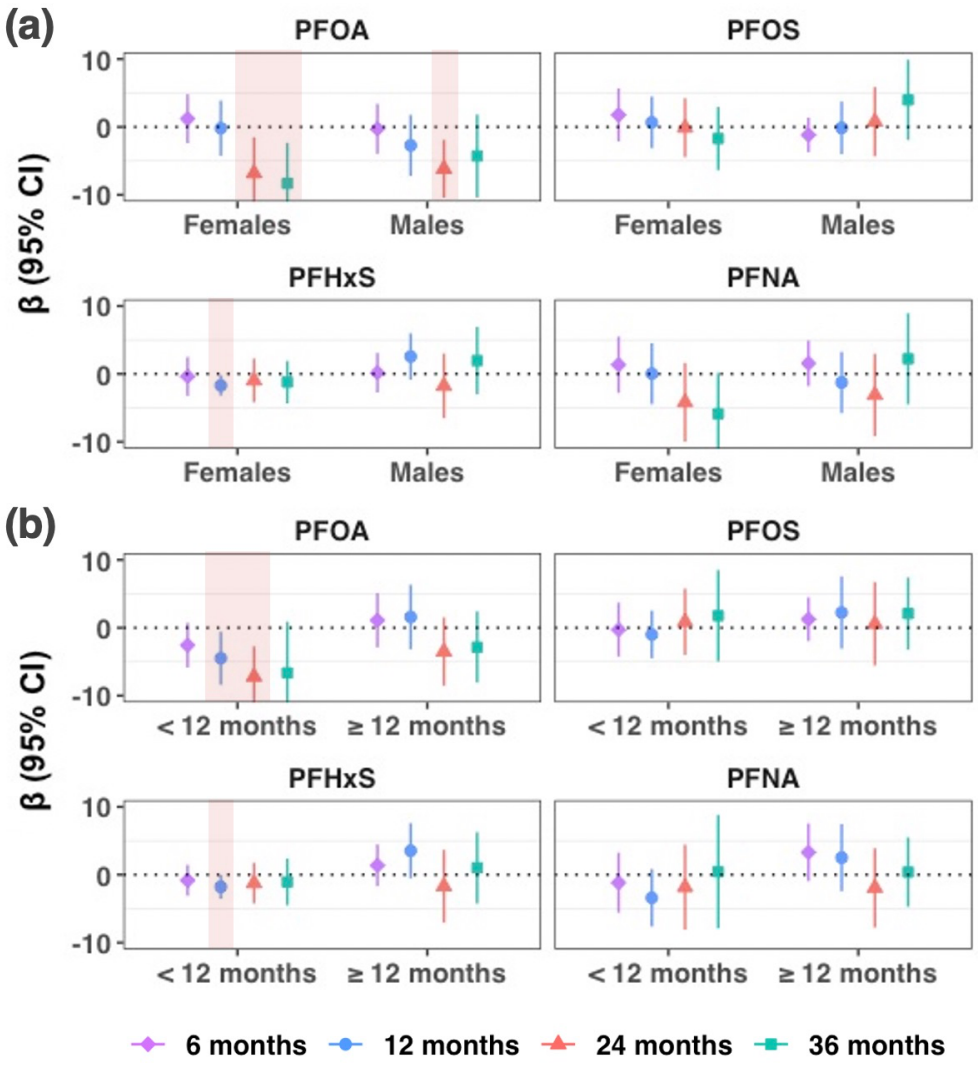

Figure 3.5 Effect modification analysis: Adjusted mean differences (β) in MSEL Composite scores of children at 6, 12, 24, and 36 months of age in association with log 2-transformed prenatal maternal serum concentrations of four PFAS stratified by (a) child's sex (females = 57, males = 83) and (b) breastfeeding duration (< 12 months = 77, ≥ 12 months = 57). Models were adjusted for child′s sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration, and missing covariates were not imputed. Red shaded areas represent associations with a *p*-value < 0.05.

3.3.4. Trajectories of child's cognitive developmental scores and their associations with prenatal maternal PFAS exposure

Two different groups were identified from the trajectory analysis. The low-score group (n = 40) showed lower and/or decreasing MSEL Composite scores over time, including 17 children with ASD, 10 Non-TD children, and 13 TD children (Figure 3.6). On the other hand, the high-score group (n = 100) showed higher and/or increasing scores over time, including 12 children with ASD, 8 Non-TD children, and 80 TD children. The trajectory of mean Composite scores for high-score group fell within 1 SD of the normative mean, while that for low-score group fell between 1 SD and 2 SD of the normative mean at 24 and 36 months of age. Compared to the high-score group, the low-score group included more males (73% versus 54%), more mothers who were younger than 35 years of age at delivery (65% versus 46%), less mothers with gestational diabetes (8% versus 24%), and more mothers who did not have at least a bachelor's degree (68% versus 36%) (Table S3.6). Having lower and/or decreasing Composite scores was associated with increasing prenatal maternal serum PFOA concentrations (RR = 1.49, 95% CI: 1.09, 2.03) (Table 3.5).

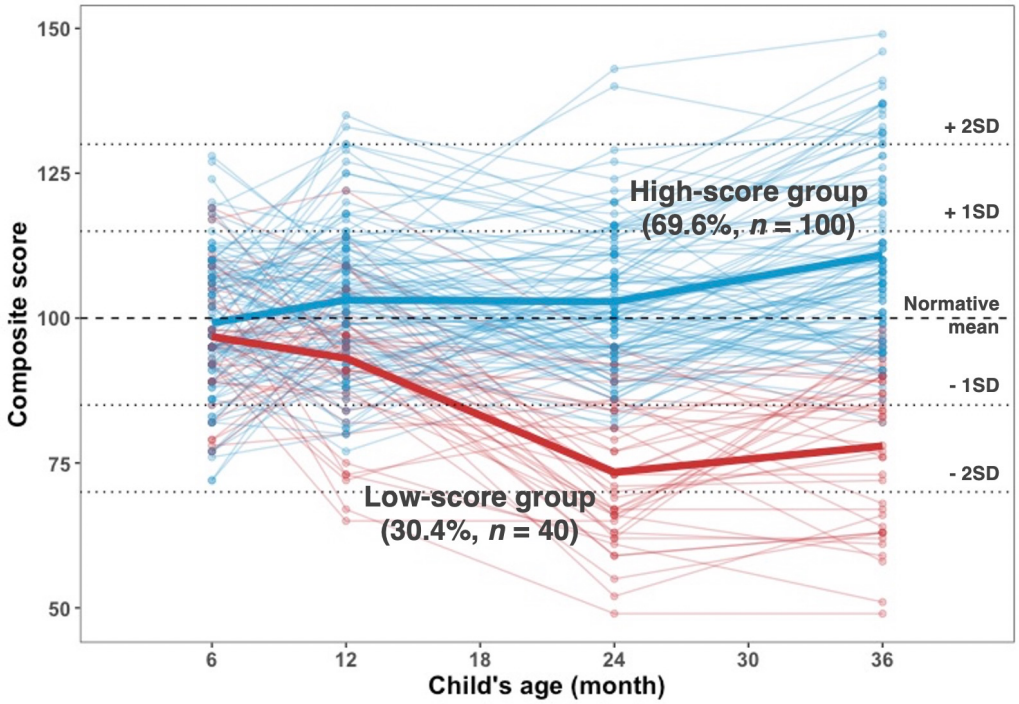

Figure 3.6 Trajectories of child's Composite scores from 6 to 36 months of age for low- and high -score groups. Solid lines represent trajectories of group mean, and fine lines and dots represent t rajectories of individuals.

Table 3.5 Adjusted relative risk (RR) and 95% confidence interval (CI) for the low-score group versus the high-score group in association with prenatal maternal serum PFAS concentrations and two primary principal components (PC-1 and PC-2).

Log 2-transformed PFAS or **two PCs**	**Low-score vs. high-score group RR [a] (95% CI)**
PFOA	1.49 (1.09, 2.03)*
PFOS	0.92 (0.70, 1.21)
PFHxS	1.10 (0.90, 1.35)
PFNA	1.19 (0.83, 1.72)
PFDA	1.02 (0.91, 1.15)
PFUnDA	0.95 (0.89, 1.02)
PC-1	1.12 (0.94, 1.33)
PC-2	0.97 (0.79, 1.20)

[a] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration.

* p-value < 0.05

3.4. Discussion

To better understand the potential effect of prenatal maternal exposure to PFAS on child cognitive development, we used prenatal maternal serum PFAS concentrations and child's MSEL scores repeatedly evaluated at four time points (i.e., 6, 12, 24, 36 months of age) in the MARBLES cohort study and examined both cross-sectional associations at each time point and longitudinal associations over four assessment time points. To our knowledge, this is the first study that reported cross-sectional and longitudinal associations and showed the trajectories of child cognitive development between low- and high-score groups. From cross-sectional associations at specific time points, PFOA was consistently associated with reduced scores on almost all subscales and the Composite at 24 and 36 months of age (Figure 3.4). From analyses of longitudinal changes over the four time points using GEE models, we observed prenatal PFOA to be associated with declining performance on the MSEL Composite and subscale scores (Table 3.4). When assessing trajectories of Composite scores from 6 to 36 months of age, PFOA was associated with having lower and/or decreasing Composite scores. From effect modification analyses, we observed that the associations of PFOA, PFHxS, and PFNA with Composite scores were modified by breastfeeding duration at 12 months of age, in which higher exposures were associated with reduced scores among children who were breastfed < 12 months and with increased scores among children who were breastfed ≥ 12 months.

Our finding should be interpreted in the context of mixed epidemiological literature on prenatal maternal exposure to PFAS and child cognitive development before or at 3 years of age. For example, Fei et al. and Goudarzi et al. reported no convincing association of prenatal maternal PFOA or PFOS concentrations with mental or motor development of infants at 6 and 18 months of age in the Danish National Birth Cohort and a Japanese birth cohort, respectively (Fei

et al. 2008; Goudarzi et al. 2016). Chen et al. observed that higher PFOS concentrations in cord blood were adversely associated with the overall development, especially with gross-motor and fine-motor domains, among 2-year-old children in a Taiwanese population (Chen et al. 2013). In contrast, Spratlen et al. reported better mental development of children at 3 years of age, but not at 1 and 2 years, in association with prenatal exposure to PFOA and PFHxS (Spratlen et al. 2020). The previous studies used one to three assessment time points before 3 years of age, which were mostly similar to those of our studies, but they only examined cross-sectional associations. To assess the child cognitive development, Fei et al. used mother's responses in questionnaires, mostly consisting of yes/no questions, while other studies, including the present study, used performance-based assessment tools, such as the Bayley Scales of Infant Development (Goudarzi et al. 2016; Spratlen et al. 2020) and Comprehensive Developmental Inventory for Infants and Toddlers (Chen et al. 2013). Thus, the differences in the assessment time points and tools across studies may affect the comparability of the results.

Beyond 3 years of age, a Norwegian cohort reported that the first principal component, mainly loaded with PFOA, PFOS, PFHxS, and perfluoroheptane sulfonate, was associated with decreased scores of nonverbal working memory among children at 3.5 years of age (Skogheim et al. 2020). The two principal components they selected showed very similar PFAS loading weights with our study (Table 3.3). A prospective cohort in Shanghai reported that prenatal plasma concentrations of PFNA and PFDA were associated with increased risk of developmental problems in personal-social skills among 4-year-old children (Niu et al. 2019). In contrast, in a U.S. birth cohort study, prenatal exposure to PFOA and MeFOSAA was associated with improved cognitive functions among 3- to 6-year-old children (Harris et al. 2018). As a child grows, we cannot exclude the possibility that increased interactions with siblings or other

children in childcare settings contribute to changes in the child cognitive development over time (Bontinck et al. 2018; Rutter 1985). Longer follow-up of the MARBLES cohort would allow us to evaluate delayed or persistent effects of prenatal PFAS exposures on child cognitive development.

We observed sex-specific associations between prenatal exposure to certain PFAS and cognitive development: negative associations among females and positive associations among males for PFHxS at 12 months of age. Although the mechanisms underlying the sex-specific associations are unknown, PFAS exposure during gestation may alter fetal thyroid and sex hormone levels, which can adversely affect cognitive functions in later life, in sexually dimorphic manner (Collaer and Hines 1995; de Cock et al. 2014; Itoh et al. 2016; Nian et al. 2020; Yao et al. 2019). Furthermore, effect modification by child's sex for PFHxS may relate to the higher efficiency of placental transfer in females than in males. The mean ratios of cord serum to maternal serum among male (n = 26) and female (n = 24) infants were 0.71 and 1.15, respectively (Liu et al. 2011). Our findings are in line with the results from previous epidemiological studies investigating sex-specific associations between prenatal PFAS exposure and child's neurobehaviors. For example, Goudarzi et al. observed that prenatal PFOA was associated with decreased scores of the mental developmental index only among 6-month-old female infants (Goudarzi et al. 2016). Skogheim et al. reported that PFAS principal components were associated with decreased scores of verbal working memory and language skills among females than males at 3.5 years of age (Skogheim et al. 2020). Niu et al. reported that 4-year-old females had higher risk of having problems in developing personal-social skills in association with prenatal PFOA, PFOS, PFNA, PFDA, PFUnDA, and PFDoDA, compared to males at the same age (Niu et al. 2019).

Strengths of this study include adjustment for covariates prospectively collected during the study period and repeated child's cognitive developmental scores. The repeated measures in the same children over time allowed us to examine cross-sectional and longitudinal associations between prenatal maternal serum PFAS and child cognitive scores as well as trajectories of child's scores by low- and high-score groups. The MSEL used in this study is known to be a reliable and valid assessment tool for child cognitive development and covers four subscales of child's cognitive function.

However, limitations should be also noted. Approximately one-third of the children included in the current study were diagnosed with ASD or had other neurodevelopmental concerns, such as broader autism phenotype and attention-deficit/hyperactivity disorder, which can result in lower MSEL scores. Thus, our findings should be interpreted cautiously because their generalizability to the general population may be limited. Although we performed sensitivity analyses after restricting to those children who were typically developed at 36 months, it should be noted that this does not entirely address the issue of these children being at high risk for other neurodevelopmental outcomes that are usually diagnosed at a later age. Another limitation of this study is selection bias. Though the MARBLES study has a relatively high retention rate (84%) (Hertz-Picciotto et al. 2018b), only 64% of our MARBLES baseline population completed all four MSEL tests from 6 to 36 months of age and were used in the analyses. Therefore, missing outcome measures of the rest of the children may have influenced our results. Child's postnatal environmental factors, such as breastfeeding duration, the intellectual home environment, and attendance at day-care centers, can influence cognitive development of infants and young children (McCrory and Murray 2013; Morrow-Tlucak et al. 1988; National Institute of Child Health and Human Development Early Child Care Research

Network 2000; Yeates et al. 1983). We adjusted for breastfeeding duration as well as maternal education as a surrogate for the intellectual environment in the regression models and considered effect modification by breastfeeding duration, but we did not account for child's attendance at day-care centers nor did we have an assessment of the home environment during this time period. Because cognitive abilities are malleable in infancy and toddlerhood and child's postnatal environments can influence cognitive development, further studies should account for child's postnatal environmental factors. Longer follow-up into mid-childhood and beyond would also contribute to an understanding of the longer-term impacts of prenatal PFAS exposures.

CHAPTER 4: PERFLUOROOCTANOATE AND PERFLUOROOCTANE SULFONATE IN UMBILICAL CORD BLOOD AND CHILD COGNITIVE DEVELOPMENT: HAMAMATSU BIRTH COHORT FOR MOTHERS AND CHILDREN (HBC STUDY)

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Oh J, Shin HM, Nishimura T, SR Mohammad, Takahashi N, Tsuchiya K. (under review). Perfluorooctanoate and perfluorooctane sulfonate in umbilical cord blood and child cognitive development: Hamamatsu Birth Cohort for Mothers and Children (HBC Study).

4.1. Introduction

4.1.1. Background

Per- and polyfluoroalkyl substances (PFAS) are a class of man-made fluorine-containing chemicals widely applied to water- and stain-resistant coatings for consumer products such as non-stick cookware, food packaging, fabric, and carpeting (ATSDR 2018). Humans are exposed to PFAS through ingestion of contaminated food, water, and house dust or by use of PFAS-containing products (Sunderland et al. 2019; Trudel et al. 2008). In general, perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) are two predominant long-chain PFAS found in humans. They have been detected in blood from North American, European, and Asian general populations (CDC 2019; Harada et al. 2011; Jian et al. 2018) as well as in pregnant women (Brantsaeter et al. 2013; Kingsley et al. 2018; Manzano-Salgado et al. 2016; Ode et al. 2013; Sagiv et al. 2015; Tian et al. 2018; Tsai et al. 2018). PFOA and PFOS could be transferred from a pregnant woman to a fetus via the placenta (Ma et al. 2021) because they are frequently detected in cord blood (Fromme et al. 2010; Hanssen et al. 2010; Jian et al. 2018; Kato et al. 2014; Manzano-Salgado et al. 2015; Monroy et al. 2008; Ode et al. 2013).

Prenatal exposure to PFOA and PFOS is reported to have neurotoxic and behavioral effects on offspring in animal studies (Mariussen 2012). Prenatal PFOS exposure led to delayed development of neuromotor skills in mice (Fuentes et al. 2007), decreased exploratory activity and increased number of inactive periods in male mice (Onishchenko et al. 2011), and increased locomotor activity and decreased habituation in male rats (Butenhoff et al. 2009). For PFOA, male mice that were prenatally exposed showed higher exploratory behaviors than female mice along with increased activity in the home cage (Onishchenko et al. 2011).

4.1.2. Statement of research problem

A number of epidemiological studies investigated associations between prenatal exposure to PFOA and PFOS and neurodevelopment in the first few years of child's life, but those that assessed child neurodevelopment at multiple ages within the same population are limited (Carrizosa et al. 2021; Fei et al. 2008; Goudarzi et al. 2016; Spratlen et al. 2020). These studies assessed child neurodevelopment at two, three, or five time points throughout infancy and early childhood and reported varying results. For example, a Japanese study observed that prenatal maternal PFOA level was associated with decreased Mental Developmental Index (MDI) scores in female children at 6 months of age but not at 18 months (Goudarzi et al. 2016), while a Danish study reported that prenatal maternal PFOS level was associated with slightly delayed sitting or language skills at 18 months but not at 6 months (Fei et al. 2008). In a United States (U.S.) study, cord blood PFOA level was associated with higher MDI scores at 3 years of age but not at 1, 2, 4, or 6 years (Spratlen et al. 2020). A Spanish study observed that prenatal maternal PFOS level was associated with decreased motor development in children at 14 months of age but increased cognitive development at 4-5 years (Carrizosa et al. 2021). As child neurodevelopment fluctuates during early life (Huttenlocher 2009), examining associations between prenatal exposure to PFOA and PFOS and neurodevelopment assessed at multiple time points and longitudinally is needed to gain a comprehensive understanding of their effects on child neurodevelopment. None of the above studies investigated longitudinal associations, but our recent study examined longitudinal associations as well as cross-sectional associations in a cohort of children having a first-degree relative with autism spectrum disorder (ASD) (Oh et al. 2021b). We observed that prenatal maternal PFOA level was inversely associated with child cognitive function at 24 and 36 months of age and with longitudinal changes from 6 to 36 months of age.

4.1.3. Objectives

To support the plausibility of these findings in a different population, the present study employed a cohort that is representative of the general population with a lower risk of ASD and examined associations of cord blood PFOA and PFOS with child cognitive development. We investigated cross-sectional associations more frequently than in our previous study and longitudinal associations across all assessment time points.

4.2. Methods

4.2.1. Study population

Participants were drawn from the Hamamatsu Birth Cohort Study for Mothers and Children (HBC Study), an ongoing prospective birth cohort study in Japan initiated in 2007 (Tsuchiya et al. 2010). Pregnant women were enrolled at one of two research sites (Hamamatsu University Hospital or Kato Maternity Clinic) during the first or second trimester of pregnancy and gave birth at Hamamatsu University Hospital. Approximately 98% of the enrolled women were Japanese. Participants provided written informed consent prior to enrolling in the study. Based on the National Statistics of the Japanese Government, mother-child participants are considered representative of the general Japanese population with regard to mother's age, socioeconomic status, child's birthweight, and gestational age at delivery (Takagai et al. 2016). Details of the study design, study population, recruitment, and data collection are described elsewhere (Takagai et al. 2016; Tsuchiya et al. 2010).

The HBC study included 1,138 mothers and 1,258 children born between December 2007 and March 2012. Of 1,258 live births, 1,244 cord blood samples were collected. Due to limited

funding, 600 cord blood samples were randomly selected for quantification of PFOA and PFOS. Among these, one child was excluded due to a diagnosis of Down syndrome with a profound level of neurodevelopmental delay and heart failure. Another child was excluded due to a sample processing error and three were excluded because PFOA or PFOS concentrations were below the method detection limit (MDL). Therefore, a total of 595 children born to 553 mothers were included in this study (Figure 4.1).

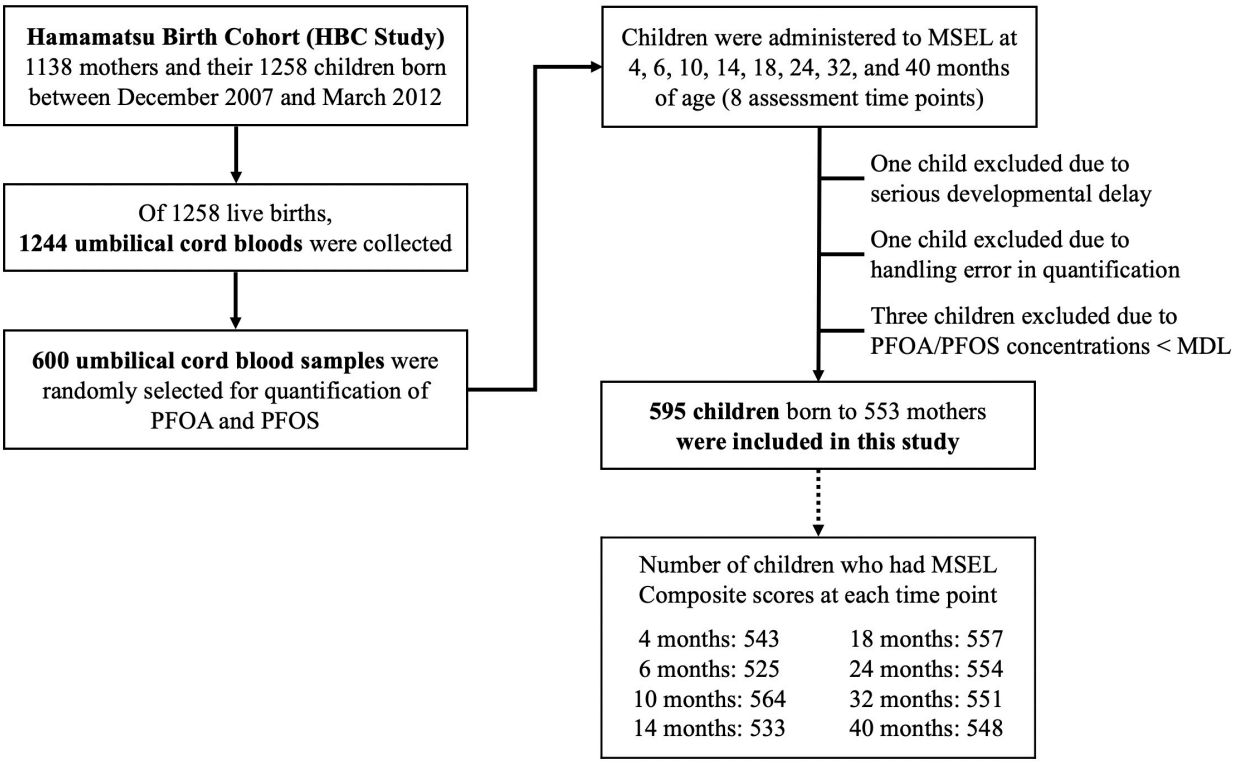

Figure 4.1 Study design and flow diagram for selecting study population.

4.2.2. Cord blood sample collection and PFAS quantification

Umbilical cord blood was collected immediately after birth from the umbilical vein and was stored at room temperature for 30 minutes. It was then centrifuged at 3,500 rpm for 10 minutes and serum was separated to Eppendorf tubes and stored at -80 °C. PFOA and PFOS concentration in serum was quantified at Shimadzu Techno-Research, Inc. (Kyoto, Japan) using high-performance liquid chromatography (Shimadzu Corporation) coupled with a tandem mass spectrometer (AB Sciex) followed by solid phase extraction. Detailed information on sample preparation and analytical methods are available in Table S4.1.

The MDL was 0.003 ng/mL for both PFOA and PFOS. Minimum values of the measured PFOA and PFOS concentrations were approximately 60 times greater than the MDL and replacement of values may bias the results of statistical analyses; therefore, three children whose sample concentrations were below the MDL were excluded (Figure 4.1). Quality assurance and quality control of the analytical methods were assessed by international round robin tests for determination of PFOA and PFOS in serum organized by the German External Quality Assessment Scheme.

4.2.3. Assessment of child cognitive development

The Mullen Scales of Early Learning (MSEL) was used to evaluate cognitive development, a specific aspect of neurodevelopment, in children at 4, 6, 10, 14, 18, 24, 32, and 40 months of age. MSEL is a performance-based assessment tool to evaluate cognitive abilities of infants and young children comprised of four subscales (Fine Motor, Visual Reception, Receptive Language, Expressive Language) (Mullen 1997). Raw scores of the subscales are standardized to a T-score with a mean of 50 and a standard deviation (SD) of 10, which is a

useful index to discern deviations from normative cognitive development. The T-scores of the four subscales are combined to calculate an Early Learning Composite (Composite) score with a mean of 100 and a SD of 15 (Swineford et al. 2015; Turner-Brown et al. 2013). Higher scores on the Composite and four subscales indicate better cognitive abilities. However, the U.S. version of normative data did not appear to correspond with Japanese T-scores, especially in language domains; therefore, we used the HBC Study samples to generate a Japanese version of standardized T-scores and Composite scores in accordance with the original procedure described by Mullen (Nishimura et al. 2016). All assessors were blinded to prior assessments and participants' demographic information.

4.2.4. Statistical analysis

Potential confounders were selected *a priori* based on a directed acyclic graph (Figure S4.1) (Hernán et al. 2004). We performed univariate analyses using the Wilcoxon rank-sum test or the Kruskal-Wallis test to compare MSEL Composite scores at each assessment time point with respect to potential confounders. Variables that were associated with MSEL Composite scores at one or more time points ($p < 0.05$) were selected as covariates: child's sex (female, male); child's birth year (2008, 2009, 2010, 2011); gestational age at delivery (< 38, ≥ 38 weeks); mother's age at delivery (< 30, 30 – 35, > 35 years); parity (0, 1 – 4); mother's educational history (≤ 12, 12 – 14, > 14 years); household income prior to delivery (< 4, 4 – 6, > 6 million Japanese yen [JPY]); and breastfeeding duration (< 6, 6 – 14, > 14 months). Child's birth year was selected as a covariate to adjust for the potential selection bias associated with calendar time and PFOA and PFOS decrease over time.

For regression analyses, cord blood PFOA and PFOS concentrations were: 1) log 2-transformed to lessen the impacts of outliers; and 2) categorized into tertiles with the lowest tertile as a reference group. To examine cross-sectional associations between PFOA and PFOS concentrations and MSEL Composite and T-scores of the four subscales at each assessment time point, we performed multiple linear regression analyses while using continuous or tertile-based PFOA and PFOS concentrations and then estimated regression coefficients (βs) and 95% confidence intervals (CIs). For mothers who delivered more than one child, we used clustered sandwich variance estimators to adjust for within-family correlations. To investigate associations between continuous or tertile-based PFOA and PFOS concentrations and longitudinal changes in MSEL Composite and subscale scores, we fit generalized estimating equation (GEE) models with a linear link and an autoregressive correlation structure to deal with correlation of the repeated outcomes as well as within-subject missing data (Ballinger 2004; Hubbard et al. 2010; Liang and Zeger 1986). GEE models additionally included age at assessment (in months) and its interaction term with continuous or tertile-based PFOA or PFOS concentrations.

Based on epidemiological evidence that the associations between prenatal PFAS exposure and child neurodevelopment were modified by child's sex (Goudarzi et al. 2016; Harris et al. 2018; Niu et al. 2019; Oh et al. 2021b), we performed effect modification analyses using Composite and subscale scores at each assessment time point. We stratified the multiple linear regression models by child's sex and examined whether regression coefficient directions differed between females and males. We also evaluated interactions between child's sex and continuous PFOA or PFOS concentrations in the regression models.

As exploratory analyses, we performed quantile regression analyses at each time point to examine associations between continuous PFOA and PFOS concentrations and MSEL

Composite scores at their different percentiles. Quantile regression allows us to characterize different exposure effects across the outcome distribution while linear regression only estimates mean effects (Koenker and Hallock 2001; Wei et al. 2019). We obtained regression coefficients at the 10th (lowest), 30th, 50th, 70th, and 90th (highest) percentiles, and their standard errors, via bootstrapping. For sensitivity analyses, we ran the multiple linear regression models using continuous and tertile-based PFOA and PFOS concentrations after excluding 25 children who were born preterm (< 37 weeks of pregnancy).

Statistical analyses were performed using STATA/IC version 16.1 (StataCorp LLC, College Station, TX, USA). A p-value < 0.05 was considered statistically significant.

4.3. Results

4.3.1. Demographic characteristics and MSEL scores

Among the 595 mother-child pairs, 48% of children were females and 81% were born after 38 weeks of pregnancy; 46% of mothers were nulliparous before the current pregnancy and 67% had normal pre-pregnancy weight (Table 4.1). Approximately 25% of mothers stopped breastfeeding before 6 months postpartum, while 36% were still breastfeeding after 14 months postpartum.

Means and SDs of MSEL Composite and subscales scores for 595 children were close to the normative mean and SD, respectively (Table S4.2). Composite scores at each assessment time point differed across some demographic characteristics (Table 4.1). Compared to males, females had higher scores at each timepoint from 14 to 40 months of age and children born in different calendar years had different scores at each timepoint from 4 to 24 months. Children whose gestational age at delivery was ≥ 38 weeks showed higher scores at each time point from

4 to 40 months of age compared to those whose gestational age was < 38 weeks. Children born to nulliparous women had higher scores at 10 and 40 months of age compared to those born to parous women. Children whose mother had > 14 years of education showed the highest 40-month scores compared to those with less education. Children born to families in the highest income group had higher scores at 24, 32, and 40 months of age compared to those born to lower-income families. Children who were breastfed more than 6 months showed higher scores at 6, 14, 18, 32, and 40 months of age compared to those who were breastfed less than 6 months.

Table 4.1 MSEL Composite scores for 595 children from 4 to 40 months of age by demographic characteristics.

Characteristic	All (*n* = 595)		MSEL Composite score, mean (standard deviation)							
	n	%	4 months (*n* = 543)	6 months (*n* = 525)	10 months (*n* = 564)	14 months (*n* = 533)	18 months (*n* = 557)	24 months (*n* = 554)	32 months (*n* = 551)	40 months (*n* = 548)
Child's sex										
Female	287	48	100 (14)	99 (15)	99 (16)	**100 (13)**	**103 (16)**	**102 (11)**	**103 (13)**	**104 (13)**
Male	308	52	100 (16)	101 (16)	98 (14)	**98 (13)**	**99 (14)**	**97 (12)**	**98 (13)**	**98 (14)**
Child's birth year										
2008	168	28	**99 (14)**	**99 (13)**	**99 (13)**	**98 (12)**	**99 (15)**	**97 (10)**	98 (12)	99 (13)
2009	143	24	**98 (13)**	**96 (13)**	**94 (15)**	**97 (14)**	**102 (16)**	**100 (11)**	101 (14)	101 (14)
2010	181	30	**95 (13)**	**99 (15)**	**100 (16)**	**101 (14)**	**103 (15)**	**101 (12)**	101 (14)	102 (14)
2011	103	17	**111 (16)**	**108 (17)**	**100 (16)**	**98 (12)**	**101 (14)**	**99 (13)**	101 (13)	101 (14)
Gestational age at delivery										
< 38 weeks	115	19	**95 (14)**	**94 (14)**	**92 (14)**	**95 (12)**	**97 (13)**	**96 (10)**	**97 (12)**	**97 (13)**
≥ 38 weeks	480	81	**101 (15)**	**101 (15)**	**100 (15)**	**100 (14)**	**102 (15)**	**100 (12)**	**101 (14)**	**102 (14)**
Mother's age at delivery										
< 30 years	203	34	101 (16)	101 (14)	**99 (15)**	**101 (13)**	101 (15)	99 (11)	100 (13)	100 (14)
30 – 35 years	211	35	99 (15)	101 (16)	**100 (15)**	**98 (14)**	101 (15)	100 (11)	100 (14)	101 (14)
> 35 years	181	30	99 (14)	98 (15)	**96 (14)**	**97 (13)**	102 (14)	99 (12)	100 (13)	101 (14)
Parity										
0	273	46	101 (15)	101 (15)	**101 (15)**	99 (14)	100 (15)	100 (12)	101 (14)	**102 (14)**
1 – 4	322	54	99 (15)	99 (15)	**97 (15)**	99 (13)	102 (15)	99 (11)	99 (13)	**99 (13)**
Mother's pre-pregnancy body mass index										
Underweight	121	20	99 (14)	98 (15)	96 (14)	97 (13)	101 (15)	100 (11)	100 (13)	101 (14)
Normal	397	67	100 (16)	100 (16)	99 (15)	100 (14)	101 (15)	100 (11)	101 (14)	101 (14)
Overweight/obese	77	13	160 (14)	99 (15)	99 (15)	97 (14)	101 (13)	97 (11)	97 (12)	97 (13)
Mother's educational history										
<12 years	195	33	101 (15)	100 (15)	99 (16)	100 (14)	102 (15)	99 (11)	100 (13)	**99 (14)**
12 – 14 years	196	33	99 (15)	101 (14)	99 (14)	97 (13)	100 (14)	99 (12)	99 (13)	**100 (13)**
> 14 years	204	34	100 (15)	99 (17)	97 (14)	99 (13)	102 (15)	100 (11)	102 (14)	**103 (14)**
Household income prior to delivery										
< 4 million JPY	136	23	101 (15)	100 (14)	100 (15)	100 (13)	101 (16)	**98 (11)**	**99 (12)**	**99 (13)**

4 – 6 million JPY	240	40	98 (14)	100 (15)	98 (15)	99 (14)	100 (15)	**98 (11)**	**99 (14)**	**99 (14)**
> 6 million JPY	219	37	100 (16)	99 (16)	98 (15)	98 (13)	102 (15)	**101 (11)**	**103 (14)**	**104 (13)**
Breastfeeding duration										
< 6 months	147	25	99 (15)	**97 (15)**	96 (14)	**95 (12)**	**98 (13)**	98 (11)	**97 (13)**	**97 (13)**
6 – 14 months	231	39	101 (15)	**102 (15)**	99 (15)	**100 (13)**	**100 (15)**	100 (12)	**101 (12)**	**101 (14)**
> 14 months	217	36	99 (15)	**100 (15)**	99 (16)	**100 (14)**	**104 (16)**	100 (12)	**101 (14)**	**103 (14)**

Note: *p*-values < 0.05 from the Wilcoxon rank-sum test or the Kruskal-Wallis test are highlighted in bold.

4.3.2. Cord blood PFOA and PFOS concentrations

Distributions of PFOA and PFOS concentrations in 595 cord blood samples were similar, although PFOA showed a slightly wider interquartile range (0.8 to 1.8 ng/mL) versus PFOS (0.9 to 1.7 ng/mL) (Figure 4.2). PFOA and PFOS concentrations had minimum values of 0.2 ng/mL, but PFOA skewed more to the right, with a greater maximum value (10.0 ng/mL) than PFOS (7.1 ng/mL). Median PFOA and PFOS concentrations were the same at 1.2 ng/mL.

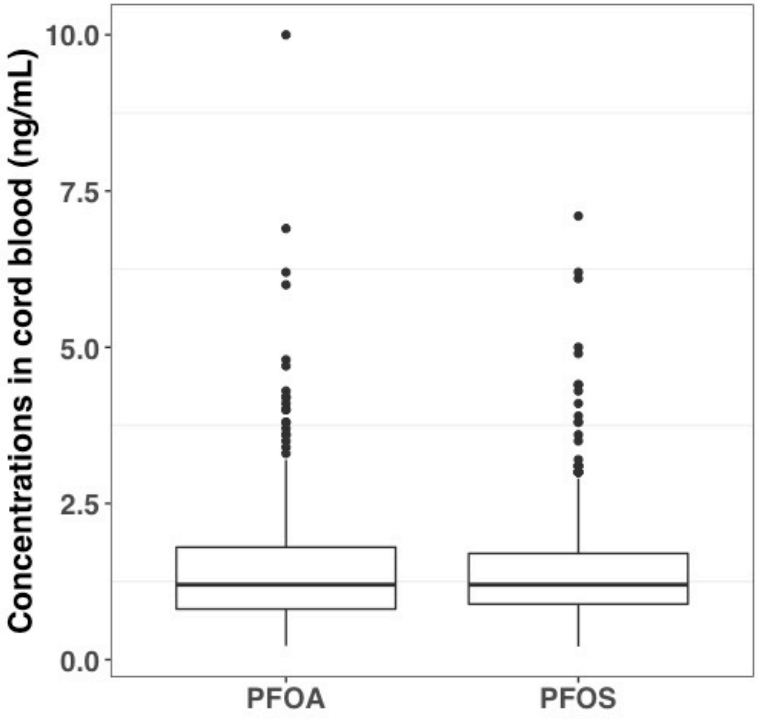

Figure 4.2 Distribution of PFOA and PFOS concentrations in 595 cord blood samples.

4.3.3. Associations between cord blood PFOA and PFOS concentrations and child cognitive development

When cord blood PFOA and PFOS continuous concentrations were used in the regression models, PFOA was associated with decreased Composite scores at 18 months of age (per 2-fold increase in concentration: β = -2.30, 95% CI: -3.96, -0.63) (Figure 4.3). With regard to subscale scores, PFOA was positively associated with Fine Motor scores at 4 months (β = 1.02, 95% CI: 0.09, 1.96) but was inversely associated with Visual Reception scores at 10 months (β = -1.46, 95% CI: -2.65, -0.26) and Receptive Language scores at 10 months (β = -1.63, 95% CI: -2.88, -0.38). PFOS was positively associated with Expressive Language scores at 24 months (β = 1.39, 95% CI: 0.25, 2.54) and at 32 months (β = 1.22, 95% CI: 0.17, 2.26).

When categorizing PFOA and PFOS concentrations into tertiles with the lowest tertile used as a reference group in the regression, the 3rd tertile for PFOA was inversely associated with Composite (β = -4.56, 95% CI: -8.04, -1.09), Fine Motor (β = -2.69, 95% CI: -4.74, -0.63), and Visual Reception (β = -2.44, 95% CI: -4.80, -0.08) scores at 18 months of age compared to the 1st tertile (Figure 4.4). The 3rd tertile for PFOA was also inversely associated with 6-month Fine Motor scores (β = -2.74, 95% CI: -4.87, -0.62) and 10-month Visual Reception scores (β = -3.25, 95% CI: -5.68, -0.82). Compared to the 1st tertile, the 2nd and 3rd tertiles for PFOA were associated with decreased Receptive Language scores at 10 months of age (β = -2.34 for the 2nd tertile, β = -2.75 for the 3rd tertile). On the other hand, the 2nd tertile for PFOS was positively associated with Composite (β = 2.22, 95% CI: 0.01, 4.44) and Fine motor scores (β = 1.90, 95% CI: 0.01, 3.78) at 24 months of age and with Expressive Language scores at 32 months (β = 2.34, 95% CI: 0.38, 4.29) and at 40 months (β = 2.10, 95% CI: 0.24, 3.96).

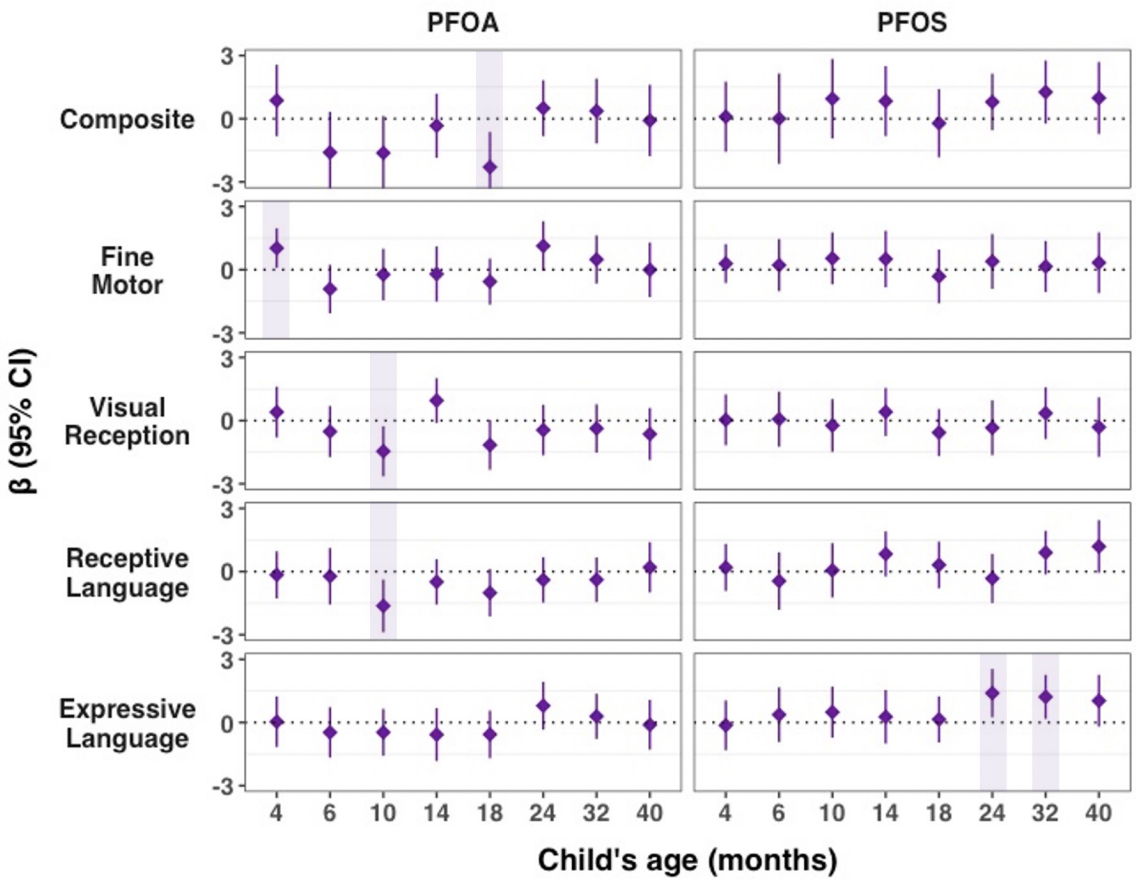

Figure 4.3 Estimated mean differences (β) in MSEL Composite and subscale scores at 4, 6, 10, 14, 18, 24, 32, and 40 months of age per 2-fold increase in cord blood PFOA and PFOS concentrations. Multiple linear regression models were adjusted for child's sex and birth year, gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Shaded areas indicate estimates with a p-value < 0.05.

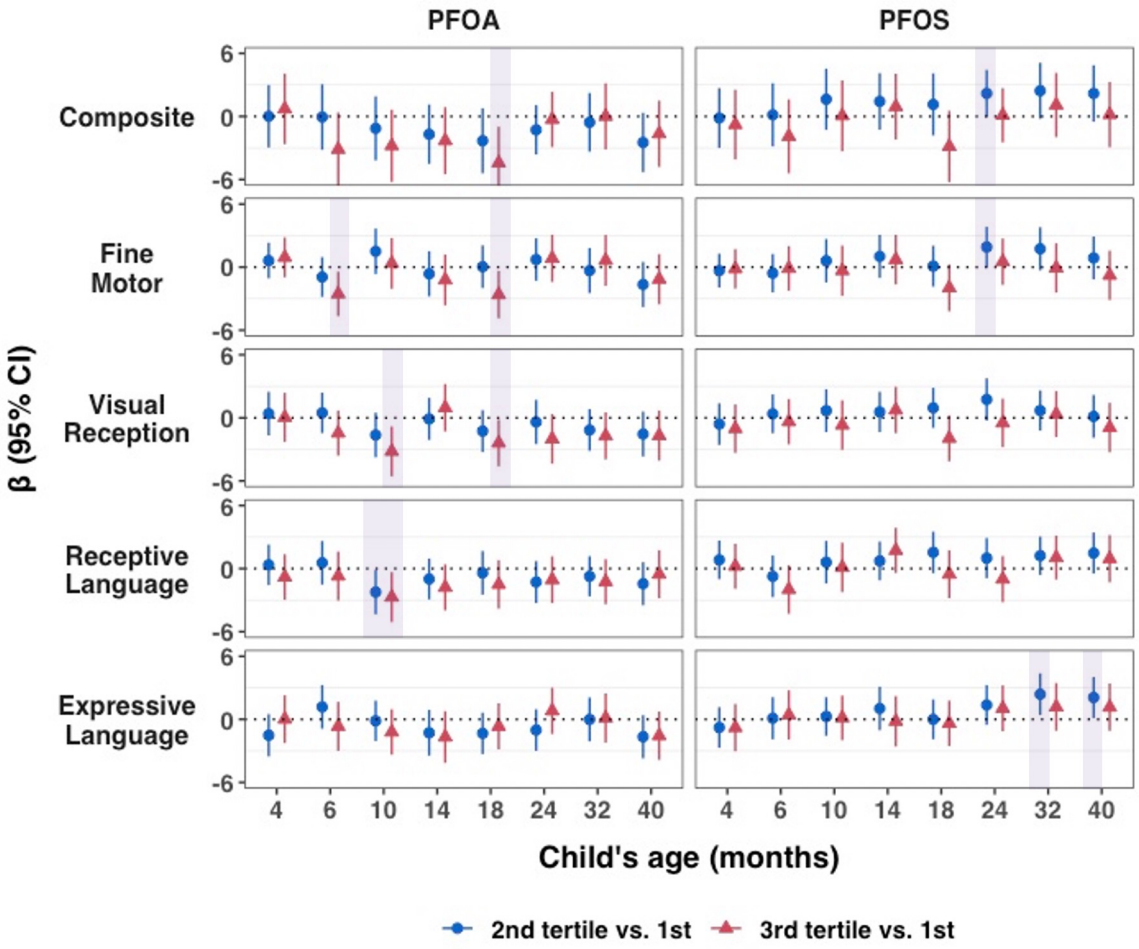

Figure 4.4 Estimated mean differences (β) in MSEL Composite and subscale scores at 4, 6, 10, 14, 18, 24, 32, and 40 months of age in association with the 2nd and 3rd tertile cord blood PFOA and PFOS concentrations versus the 1st tertile. Multiple linear regression models were adjusted for child's sex and birth year, gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Shaded areas indicate estimates with a *p*-value < 0.05.

When longitudinal associations from 4 to 40 months of age were examined using the GEE models, Fine Motor scores were inversely associated with continuous and the 3rd tertile (versus the 1st tertile) PFOA (β = -0.04 for continuous, β = -0.08 for the 3rd tertile) and PFOS (β = -0.06 for continuous, β = -0.11 for the 3rd tertile) (Table 4.2). Receptive Language scores were positively associated with continuous and the 3rd tertile PFOA (β = 0.05 for continuous, β = 0.07 for the 3rd tertile) and PFOS (β = 0.06 for continuous, β = 0.08 for the 3rd tertile). Additionally, the 2nd tertile PFOS was positively associated with Expressive Language scores (β = 0.07).

Table 4.2 Longitudinal changes (β) in MSEL Composite and subscale scores from 4 to 40 months of age in association with log 2-transformed or tertile-based cord blood PFOA and PFOS concentrations.

MSEL	PFAS exposure measures	β [a] (95% CI)	
		PFOA	PFOS
Composite	Continuous	0.02 (-0.02, 0.07)	0.03 (-0.02, 0.08)
	2nd tertile vs. 1st	-0.01 (-0.11, 0.09)	0.08 (-0.01, 0.18)
	3rd tertile vs. 1st	0.04 (-0.07, 0.14)	0.03 (-0.07, 0.13)
Fine Motor	Continuous	**-0.04 (-0.07, 0.00)**	**-0.06 (-0.10, -0.02)**
	2nd tertile vs. 1st	-0.07 (-0.13, 0.00)	0.03 (-0.04, 0.10)
	3rd tertile vs. 1st	**-0.08 (-0.15, -0.01)**	**-0.11 (-0.18, -0.05)**
Visual Reception	Continuous	-0.02 (-0.05, 0.01)	-0.02 (-0.06, 0.01)
	2nd tertile vs. 1st	-0.05 (-0.12, 0.01)	0.03 (-0.04, 0.09)
	3rd tertile vs. 1st	-0.05 (-0.12, 0.01)	-0.03 (-0.10, 0.03)
Receptive Language	Continuous	**0.05 (0.01, 0.08)**	**0.06 (0.02, 0.09)**
	2nd tertile vs. 1st	0.01 (-0.05, 0.08)	0.05 (-0.01, 0.12)
	3rd tertile vs. 1st	**0.07 (0.01, 0.14)**	**0.08 (0.01, 0.14)**
Expressive Language	Continuous	0.02 (-0.02, 0.05)	0.02 (-0.02, 0.05)
	2nd tertile vs. 1st	0.01 (-0.05, 0.08)	**0.07 (0.01, 0.13)**
	3rd tertile vs. 1st	0.03 (-0.04, 0.10)	0.02 (-0.05, 0.09)

a GEE models include a continuous or tertile exposure variable, continuous age (in months), and their interaction term with adjustment for child's sex and birth year, gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Regression coefficients represent the estimates of the interaction term (exposure × age).

Note: *p*-values < 0.05 from the interaction terms (exposure × age) are highlighted in bold.

When conducting effect modification analyses by child's sex, PFOA was associated with decreased Composite, Visual Reception, and Receptive Language scores at 18 months among females (Figure S4.2). In addition, PFOA was inversely associated with Composite and Expressive Language scores at 6 months, Receptive Language scores at 10 months, and Visual Reception scores at 40 months among females. On the contrary, PFOA was positively associated with 4-month Fine Motor scores and 18-month Visual Reception scores among males. The associations between PFOA and Composite and subscale scores, except for Fine Motor, were modified at 18 and 40 months, in a negative direction among females and a positive direction among males. Similar effect modification was observed for the associations of PFOA and PFOS with Composite and Fine Motor scores at 14 months.

Quantile regression analyses revealed that PFOA was inversely associated with the 90th percentile Composite scores at 10 months of age (Figure S4.3). At 18 months, PFOA was also inversely associated with the 10th and 70th percentile Composite scores. PFOS was positively associated with the 90th percentile Composite scores at 24, 32, and 40 months of age. When excluding 25 children who were born preterm, the cross-sectional associations for both continuous and tertile-based concentrations did not change (Figure S4.4).

4.4. Discussion

In the present study, we examined associations between cord blood PFOA and PFOS concentrations with child cognitive development from 4 to 40 months of age in the HBC Study, representative of the Japanese general population. We observed that PFOA was associated with decreased 18-month Composite and 10-month Visual Reception and Receptive Language scores, while PFOS was associated with increased Expressive Language scores at later ages (24, 32, and

40 months) (Figure 2 and Figure 3). From 4 to 40 months of age, PFOA and PFOS showed negative associations with longitudinal changes in Fine Motor scores and positive associations with longitudinal changes in Receptive Language scores (Table 2). We observed effect modification by child's sex for associations between PFOA and Composite, Visual Reception, and Receptive and Expressive Language scores at 18 and 40 months of age, with lower scores among females and higher scores among males (Figure S3). The associations of Composite and Fine Motor scores at 14 months of age with both PFOA and PFOS were modified by sex, with a similar trend.

Previous epidemiological studies that examined child neurodevelopment at multiple time points in association with prenatal exposure to PFOA and PFOS reported mixed associations within each population. In the Denmark National Birth Cohort, prenatal maternal serum PFOS was associated with delayed gross motor and expressive language skills at 18 months of age, but they did not observe any association at 6 months (Fei et al. 2008). A Japanese birth cohort study observed that prenatal maternal serum PFOA level was inversely associated with 6-month MDI scores (which represent cognitive, language, and personal and social development) only among females, but it was not associated with 18-month scores (Goudarzi et al. 2016). In a U.S. birth cohort, cord blood PFOA level was positively associated with cognitive outcomes at 3 years of age, but not at 1, 2, 4, and 6 years of age (Spratlen et al. 2020). A Spanish birth cohort reported that prenatal maternal plasma PFOS level was positively associated with verbal subscales at 4 to 5 years of age but not at 14 months or 7 years (Carrizosa et al. 2021). The mixed associations among previous studies might be due to differences in study population characteristics and assessment tools, varying time points for neurodevelopment testing and blood sample collection, and different exposure metrices (cord blood, prenatal maternal serum, or plasma).

We can compare the present study results to our previous work (Oh et al. 2021b) because both studies used the MSEL to assess child cognitive development at similar ages. In our previous study, prenatal maternal PFOA was inversely associated with Composite and subscale scores at 24 and 36 months as well as their longitudinal changes from 6 to 36 months of age. However, in the present study cord blood PFOA was associated with decreased Composite scores only at 18 months, and longitudinal analyses did not find convincing associations. These inconsistent results may be partly explained by differences in exposure matrices and study population characteristics. Of note, the present study used cord blood to represent prenatal exposure to PFOA and PFOS, but our previous study used average concentrations measured in maternal serum collected in the 1st, 2nd, and 3rd trimesters of pregnancy. Though PFOA and PFOS concentrations in maternal serum and cord serum are moderately- to highly-correlated (Kim et al. 2011a; Kim et al. 2011b; Liu et al. 2011; Manzano-Salgado et al. 2015), cord blood concentrations may better represent true fetal exposure versus maternal blood concentrations given different transfer efficiencies of PFAS through placenta. Furthermore, our previous study included one-third of children diagnosed with ASD or other neurodevelopmental concerns, which resulted in greater variability in Composite scores especially at 24 and 36 months of age (Oh et al. 2021b), whereas the present study consisted of children without severe neurodevelopmental concerns and with less variability in Composite scores.

In this study, child's sex modified associations between PFOA and 18- and 40-month Composite and subscale scores, except for Fine Motor, and associations between PFOS and 14-month Composite and Fine Motor scores. Overall, sex-specific associations were negative among females and were positive among males, consistent with several previous studies. In a Japanese study, prenatal maternal PFOA level was associated with decreased MDI scores only

among 6-month-old females (Goudarzi et al. 2016). A Chinese birth cohort study reported that prenatal maternal PFOA and PFOS levels were associated with increased risk of developmental problems in personal-social skills only among 4-year-old females (Niu et al. 2019). However, other studies observed associations in the opposite direction. In a U.S. birth cohort, cord blood PFOS was positively associated with 2-year MDI scores only among females (Spratlen et al. 2020). A Spanish birth cohort study observed that associations between prenatal maternal PFOA concentration and 4- to 5-year verbal working memory were positive among females and were negative among males (Carrizosa et al. 2021). As epidemiological evidence on sex-specific associations remains inconclusive, further studies are needed to confirm our findings.

A major strength of our study is repeated measures of child cognitive development using a reliable and standardized assessment instrument. Throughout infancy and toddlerhood, children were administered the MSEL at eight assessment points, enabling us to discern changes in associations between prenatal exposure to PFOA and PFOS and cognitive function and to perform longitudinal analyses. Quantified PFOA and PFOS concentrations in cord blood may better represent gestational exposure versus those in maternal prenatal serum. Compared to our previous study (Oh et al. 2021b), the present study has a larger sample size and a more general population with lower risks of neurodevelopmental concerns. However, this study also has several limitations. Among many PFAS, only PFOA and PFOS were selected and quantified, such that a comparison of our results with those of previous studies was limited. Furthermore, as the current study used the Composite and T-scores standardized to the HBC Study population, the generalization of our findings to other study populations should be made with caution.

CHAPTER 5: LONGITUDINAL CHANGES IN MATERNAL SERUM CONCENTRATIONS OF PER- AND POLYFLUOROALKYL SUBSTANCES FROM PREGNANCY TO TWO YEARS POSTPARTUM

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Oh J, Bennett DH, Tancredi D, Calafat A, Schmidt RJ, Hertz-Picciotto I, Shin HM. (Under review). Longitudinal Changes in Maternal Serum Concentrations of Per- and Polyfluoroalkyl Substances from Pregnancy to Two Years Postpartum.

5.1. Introduction

5.1.1. Background

Per- and polyfluoroalkyl substances (PFAS) are a class of synthetic chemicals that exhibit both hydrophilic and hydrophobic properties and thus are widely used in various consumer and industrial applications, such as coatings of cookware, textile, carpet and food packaging materials, and fire-fighting foams (Prevedouros et al. 2006). Humans are exposed to certain PFAS primarily via ingestion of contaminated food and water as well as non-dietary dust ingestion (De Silva et al. 2021; Sunderland et al. 2019; Trudel et al. 2008). Since the early 2000s, serum concentrations of the two most studied PFAS, perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), have decreased in the United States (U.S.), following regulatory and voluntary phase-out, while those of other long-alkyl chain PFAS showed increasing or unclear trends (Ding et al. 2020; Dong et al. 2019; Olsen et al. 2017). However, widespread detection of common long-alkyl chain PFAS in drinking water across the U.S. suggests continued exposure of the general population to these compounds (Hu et al. 2016).

Prenatal and early-life exposure to PFAS is of particular concern due to PFAS's potential adverse effects on child's health. In laboratory animals, PFAS are shown to have liver toxicity, metabolic toxicity, reproductive and developmental toxicity, neurotoxicity, and immunotoxicity (DeWitt 2015; Fenton et al. 2021). Epidemiologic studies reported that prenatal or lactational exposure to PFAS was associated with reduced fetal or infant growth (Chen et al. 2021; Jin et al. 2020; Kashino et al. 2020), immune dysfunction (Liew et al. 2018), neurodevelopmental disorders (Lenters et al. 2019; Oh et al. 2021a; Oh et al. 2021b; Vuong et al. 2021), and thyroid disruption (Kim et al. 2011a; Preston et al. 2020). PFAS have been commonly detected in blood of pregnant women (Berg et al. 2014; Bjerregaard-Olesen et al. 2016a; Brantsaeter et al. 2013;

Glynn et al. 2012; Kato et al. 2014; Kingsley et al. 2018; Ode et al. 2013; Sagiv et al. 2015; Tian et al. 2018; Tsai et al. 2018), the placenta (Bangma et al. 2020; Zhang et al. 2013), cord blood (Kim et al. 2011a; Kim et al. 2011b; Liu et al. 2011; Monroy et al. 2008), and breast milk (Cariou et al. 2015; Thomsen et al. 2010; Zheng et al. 2021). Moderate to high correlations of PFAS concentrations in maternal serum with those in paired cord serum and breast milk demonstrated that PFAS are transported from mother to child through the placenta during pregnancy and through breast milk during lactation (Beesoon et al. 2011; Fromme et al. 2010; Gützkow et al. 2012; Kim et al. 2011a; Kim et al. 2011b; Liu et al. 2011; Manzano-Salgado et al. 2015; Monroy et al. 2008; Ode et al. 2013).

5.1.2. Statement of research problem

Changes in maternal PFAS concentrations may differ not only between pregnancy and postnatal periods but also among individual PFAS. Several studies quantified PFAS in serial blood samples collected from the same women during pregnancy or early postpartum period (Buck Louis et al. 2019; Chen et al. 2021; Glynn et al. 2012; Kato et al. 2014). In these studies, maternal concentrations of several long-alkyl chain PFAS, including PFOA, PFOS, and perfluorononanoate (PFNA), decreased at different rates during pregnancy. Only one of the studies collected postnatal samples and observed declines in PFOA concentrations between 3 weeks and 3 months postpartum, while PFOS and PFNA concentrations did not change (Glynn et al. 2012). However, little is known about changes in maternal PFAS concentrations over time from pregnancy to postnatal periods, especially late postpartum when mothers are expected to cease exclusive breastfeeding (Davis et al. 2018). As PFAS are less likely to be transferred from mother to child after the start of the weaning period (Mogensen et al. 2015), maternal PFAS

concentration changes during late postpartum period can be used as a baseline change to be compared with those in earlier periods.

5.1.3. Objective

In the present study, we quantified ten PFAS in 251 blood serum samples prospectively collected from 42 mothers during the 1st, 2nd, and 3rd trimesters of pregnancy and 3, 6, and 24 months after delivery. Then, we separately examined changes in serum PFAS concentrations and their potential determinants for three sub-periods: (1) during pregnancy (from 1st to 3rd trimesters), (2) early postpartum (from delivery to 6 months postpartum) and (3) late postpartum (from 6 to 24 months postpartum).

5.2. Methods

5.2.1. Study population

This current study includes participants drawn from the MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns) study. Launched in 2006, MARBLES is a prospective birth cohort study that enrolls pregnant women who previously had a child who developed autism spectrum disorder (ASD) (Hertz-Picciotto et al. 2018b). The MARBLES families are primarily recruited from those who receive state-funded services for ASD in Northern California. Mothers are eligible if they have a child or other first degree relative with ASD, are pregnant and 18 years old or older, speak, read, and understand English, and live within 2.5 hours of the Davis/Sacramento region at the time of enrollment. Details of study design, study population, eligibility criteria, and data collection are available elsewhere (Hertz-Picciotto et al. 2018b). Any information or biological specimens were collected after completing informed consent. This

study was approved by the institutional review boards for the State of California and the University of California Davis (UC Davis). The analysis of coded samples at the Centers for Disease Control and Prevention (CDC) laboratory did not constitute engagement in human subjects' research.

The MARBLES study started collecting serum in 2009, thus we included 217 pregnancies from 208 mothers who provided at least one blood sample with sufficient volume since 2009 and conceived their baby by 2014, which resulted in 453 blood samples. Among them, we selected 42 mothers who prospectively provided six blood samples during the 1st, 2nd, and 3rd trimesters of pregnancy and at 3, 6, and 24 months after delivery. One of the 42 mothers did not provide the 1st trimester sample but was included in this study. We used 251 maternal blood samples collected from 42 mothers for statistical analyses and 453 samples collected from 217 unique pregnancies for sensitivity analyses (Figure 5.1).

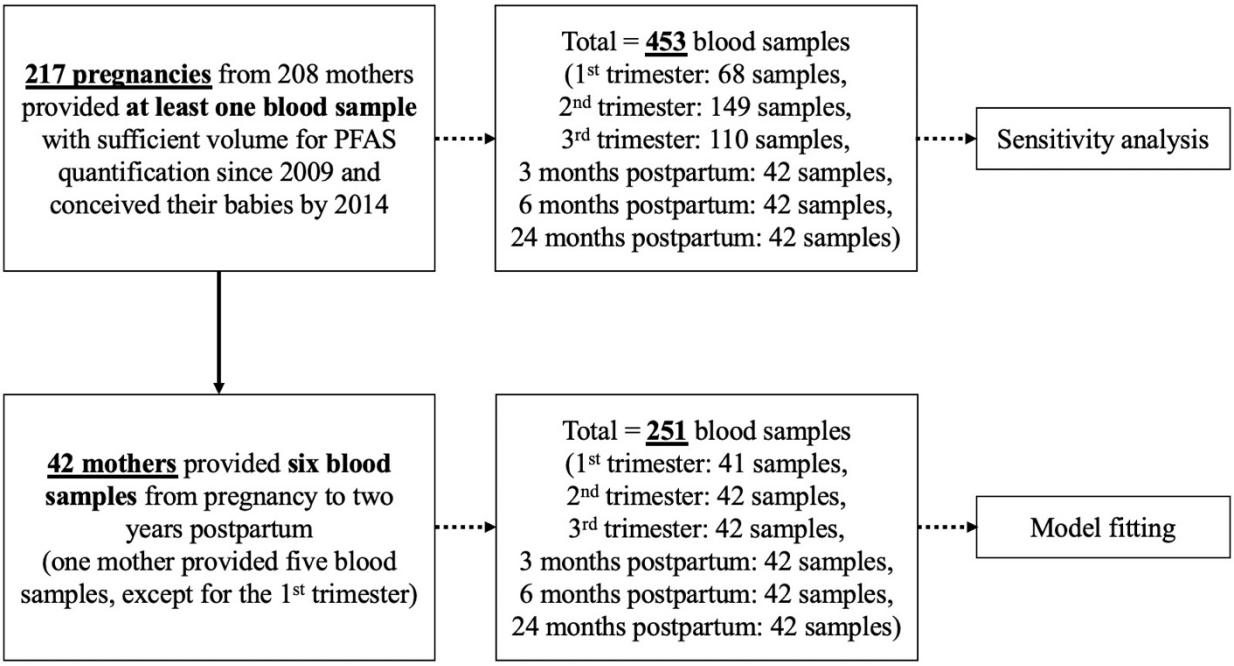

Figure 5.1 Numbers of blood serum samples collected at six collection time points (1st, 2nd, and 3rd trimesters of pregnancy and 3, 6, and 24 months postpartum) from MARBLES mothers. Note that postpartum blood serum samples were selected for only 42 mothers who provided six samples from pregnancy to two years postpartum. Ten PFAS were quantified in 453 blood serum samples.

5.2.2. Serum sample collection and PFAS quantification

Maternal blood was collected at home visits conducted during pregnancy and the first year after delivery and at visits to UC Davis Medical Investigations of Neurodevelopmental Disorders (MIND) Institute at two years postpartum.(Hertz-Picciotto et al. 2018b) After collection, whole blood was centrifuged to separate serum, stored at -80 °C and shipped to the CDC for PFAS quantification.

PFAS in maternal serum were quantified using online solid-phase extraction coupled to reversed-phase high-performance liquid chromatography-isotope dilution tandem mass spectrometry, as described elsewhere (Kato et al. 2011a). Ten PFAS quantified include linear PFOA (n-PFOA), linear PFOS isomer (n-PFOS), branched PFOS isomers (Sm-PFOS), perfluorohexane sulfonate (PFHxS), PFNA, perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA), and 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA). Blank samples and low- and high-concentration quality control samples were analyzed with the study samples. The study included 25 blind duplicate samples that were analyzed for quality assurance, their median coefficient of variation ranged from 0% to 11% depending on the PFAS. The limit of detection (LOD) for all PFAS was 0.1 ng/mL; PFAS concentrations below the LOD were replaced with a value of the LOD divided by the square root of two (Hornung and Reed 1990).

5.2.3. Potential determinants

Based on the literature review, we considered various maternal prenatal, perinatal, and demographic factors that were prospectively collected during our study period as potential determinants of maternal serum PFAS concentrations. Prenatal and perinatal factors included

parity, maternal body mass index (BMI) at pre-pregnancy (kg/m^2), maternal weight gain during pregnancy (kg), birthweight (kg), and total breastfeeding duration (month). Information on exclusive breastfeeding duration was not collected. Demographic factors included child's birth year (year), maternal age at delivery (year), maternal birthplace (U.S., non-U.S.), maternal race/ethnicity (non-Hispanic white, Hispanic/Asian/multiracial), maternal education (no bachelor's degree, bachelor's degree or higher), and homeownership (owner, non-owner).

5.2.4. Statistical analysis

All statistical analyses were performed using R version 4.1.0 (R Foundation for Statistical Computing, Vienna, Austria). For eight PFAS detected in greater than 50% of all samples, we computed pairwise Spearman correlation coefficients among the six points. We computed intraclass correlation coefficients (ICCs) and 95% confidence intervals (CIs) using ICCest() function in R to assess within-subject variability of ln-transformed PFAS concentrations (Fleiss 2011). An ICC is a ratio of between-subject variance to the sum of between- and within-subject variance, and a higher ICC indicates smaller within-subject variability (Rosner 2015). We calculated maternal serum concentration ratios by dividing the PFAS concentrations at each point (i.e., 2nd and 3rd trimesters of pregnancy, 3, 6, and 24 months postpartum) by the 1st trimester concentration. For one mother who did not provide the 1st trimester sample, the concentrations at each point were divided by the 2nd trimester concentration. In order to account for right-skewed distributions, PFAS concentrations and concentration ratios were natural log (ln)-transformed in subsequent regression analyses.

To examine changes in maternal PFAS serum concentrations from pregnancy to a few years postpartum, we used only five PFAS detected in more than 99% of the whole study

samples (i.e., n-PFOS, Sm-PFOS, PFHxS, n-PFOA, and PFNA). We grouped our study samples into three sub-periods: (1) pregnancy included samples from the 1st, 2nd, and 3rd trimesters of pregnancy, (2) early postpartum included samples from 3rd trimester and 3 and 6 months postpartum, and (3) late postpartum included samples from 6 and 24 months postpartum. For each sub-period, we investigated univariate associations between mean concentrations of each compound and *a priori* selected potential determinants by performing the Spearman correlation test for continuous variables and the Wilcoxon rank-sum test for binary variables.

Based on the univariate analyses, we included covariates that were associated with PFAS concentrations in all sub-periods ($p < 0.05$). Accordingly, child's birth year (centered to 2012), maternal age at delivery, parity, and maternal birthplace were adjusted in the final models. Breastfeeding duration was additionally included in the late postpartum models but not in the early postpartum models because approximately 88% of the mothers breastfed their child longer than 6 months. Then, we fitted the covariate-adjusted linear mixed models with random intercepts for maternal-child dyads to estimate changes in maternal serum PFAS concentrations for each sub-period. Given our small sample size, we performed parametric bootstrapping with 1000 replications and computed 95% bias corrected and accelerated confidence intervals (CIs) using bootMer() function in R (Bates et al. 2015). We calculated percent changes in maternal serum PFAS concentrations per one-unit increase in time as well as each covariate using the following equation [$(e^{\beta} - 1) \times 100$, where β is a regression coefficient for time and each covariate].

As a sensitivity analysis, we used ln-transformed concentration ratios of PFAS at each sample collection point to those at the 1st trimester of pregnancy as a dependent variable in the linear mixed models to account for different initial concentrations. We also ran the final linear

mixed models using samples from 217 unique pregnancies with at least one blood sample during the whole study period. We restricted this sensitivity analysis to pregnancy and early postpartum models because the additional samples from 175 pregnancies were only collected during pregnancy (Figure 5.1).

Maternal plasma volume increases approximately 45% throughout the pregnancy and returns to pre-pregnancy levels within 6 weeks postpartum (Aguree and Gernand 2019; Lund and Donovan 1967). To account for the dilution effect in pregnancy and lactation models, we additionally adjusted for maternal BMI at pre-pregnancy, maternal weight gain during pregnancy, and child's birthweight, which are potential predictors for plasma volume expansion (Faupel-Badger et al. 2007; Spiegelman et al. 2020). To evaluate whether changes in maternal serum PFAS concentration differ by breastfeeding duration, we divided the mothers into two groups: women who breastfed their child longer or shorter than 12 months. For each group, we used an early postpartum model that was fitted with 3-, 6-, and 24-month postpartum samples and compared the monthly percent changes of PFAS. We also included interaction terms between time from conception and a binary breastfeeding duration variable and examined *p*-values for the interaction term.

5.3. Results

5.3.1. Population characteristics

Approximately half of the mothers gave birth in the later study period (2014-2015), and overall, their average age at delivery was 34.9 years (range: 22.4 to 42.8) (Table 5.1). On average, their pre-pregnancy BMI was 25.1 kg/m^2 (range: 18.9 to 39.9) and they gained 14.4 kg of weight during pregnancy (range: 1.4 to 26.1). More than half of the mothers were non-

Hispanic white (57%), born in the U.S. (67%), had a bachelor's degree or higher (67%) and owned a home (67%). After delivery, the mothers breastfed their child for an average of 14.0 months (range: 2.8 to 36.7).

Table 5.1 Demographic and other characteristics of the study participants (n = 42).

Characteristics	***n***	**%**
Child's birth year		
2009	2	5%
2010	7	17%
2011	6	14%
2012	2	5%
2013	5	12%
2014	10	24%
2015	10	24%
Maternal race/ethnicity		
Non-Hispanic white	24	57%
Hispanic & other [a]	18	43%
Maternal birthplace		
United States	28	67%
Other	14	33%
Maternal education		
No bachelor's degree	14	33%
Bachelor's degree or higher	28	67%
Homeownership		
Non-owner	14	33%
Owner	28	67%
	Mean ± SD	**Range**
Maternal age at delivery (year)	34.9 ± 4.3	22.4 - 42.8
Maternal BMI at pre-pregnancy (kg/m^2)	25.1 ± 4.8	18.9 - 39.9
Maternal weight gain during pregnancy (kg)	14.4 ± 5.4	1.4 - 26.1
Birthweight (kg)	3.4 ± 0.5	1.6 - 4.3
Parity	2.1 ± 1.4	1 - 7
Breastfeeding duration (month)	14.0 ± 8.4	2.8 - 36.7

[a] Other race/ethnicity includes Asian (21%) and multiracial (2%).

5.3.2. Maternal serum PFAS concentrations

During the whole study period, n-PFOS, Sm-PFOS, n-PFOA, and PFNA were detected in all study samples, and PFHxS, PFDA, PFUnDA, and MeFOSAA were detected in 99%, 81%, 57%, and 52% of the samples, respectively (Table 5.2). PFDoDA and EtFOSAA were detected in less than 50% of the study samples. The medians of n-PFOS, Sm-PFOS, n-PFOA, PFHxS, and PFNA were 2.0, 0.7, 0.3, 0.7, and 0.4 ng/mL, respectively, while those of PFDA, PFUnDA, and MeFOSAA were similar to the LOD (i.e., 0.1 ng/mL). Compared to the 1st trimester of pregnancy, the GMs of concentration ratios for eight PFAS ranged from 0.79 to 1.01 during pregnancy and from 0.57 to 0.92 during a postnatal period (Table S5.1).

Concentrations of the eight PFAS detected in more than 50% of the samples showed moderate to high positive correlations across the six sample collection time points (r_{sp} = 0.56 to 0.97) (Table S5.2). PFAS concentrations during the 1st, 2nd, and 3rd trimesters of pregnancy were highly correlated with each other (r_{sp} = 0.62 to 0.97) as well as with those at 3 and 6 months postpartum (r_{sp} = 0.57 to 0.96) and were relatively moderately correlated with those at 24 months postpartum (r_{sp} = 0.58 to 0.85). The ICCs of eight PFAS ranged from 0.63 to 0.92 for the whole study period, 0.67 to 0.94 for pregnancy, 0.65 to 0.97 for early postpartum, and 0.60 to 0.88 for late postpartum, indicating relatively small within-subject variability of maternal PFAS serum concentrations (Figure S5.1). Among three sub-periods, the smallest ICCs were observed during late postpartum.

Table 5.2 Distribution of PFAS concentrations in 251 serum samples collected from 42 mothers during the 1st, 2nd, and 3rd trimesters of pregnancy and 3, 6, and 24 months after delivery.

PFAS [a]	% detect	Whole period			1Trim	2Trim	3Trim	3Mon	6Mon	24Mon
		Percentiles (ng/mL)								
		25th	50th	75th	50th	50th	50th	50th	50th	50th
n-PFOS	100	1.40	2.00	2.60	2.20	2.40	2.00	1.95	0.95	1.50
Sm-PFOS	100	0.50	0.70	1.10	0.90	0.85	0.70	0.75	0.70	0.60
PFHxS	99	0.20	0.30	0.50	0.40	0.40	0.40	0.30	0.20	0.30
n-PFOA	100	0.50	0.70	1.00	1.00	0.85	0.80	0.60	0.55	0.50
PFNA	100	0.30	0.40	0.60	0.60	0.50	0.40	0.40	0.40	0.30
PFDA	81	0.10	0.10	0.20	0.20	0.20	0.10	0.10	0.10	0.10
PFUnDA	57	<LOD	0.10	0.10	0.10	0.10	0.10	0.10	<LOD	0.10
PFDoDA	26	<LOD	<LOD	0.10	0.07	<LOD	<LOD	<LOD	<LOD	<LOD
MeFOSAA	52	<LOD	0.10	0.10	0.10	0.10	<LOD	<LOD	0.10	<LOD
EtFOSAA	2	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] The limit of detection is 0.1 ng/mL for all PFAS.

Note: One of the 42 mothers did not provide the 1st trimester sample but was included in this study.

Abbreviation: geometric mean (GM), perfluorooctanoate (PFOA), perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), perfluorononanoate (PFNA), perfluorodecanoate (PFDA), perfluoroundecanoate (PFUnDA), perfluorododecanoate (PFDoDA), 2-(N-ethyl-perfluorooctane sulfonamido) acetate (EtFOSAA), 2-(N-methyl-perfluorooctane sulfonamido) acetate (MeFOSAA)

5.3.3. Univariate associations of maternal serum PFAS concentrations with potential determinants

Among the five PFAS detected in more than 99% of the whole study samples, child's birth year was negatively correlated with maternal serum n-PFOS, Sm-PFOS, n-PFOA, PFHxS, and PFNA concentrations during all sub-periods (r_{sp} = -0.73 to -0.35), except for PFNA during pregnancy (Table S5.3). Mother's age at delivery was positively correlated with n-PFOS and PFHxS during pregnancy (r_{sp} = 0.31), n-PFOA during early postpartum (r_{sp} = 0.37), and n-PFOS, Sm-PFOS, PFHxS, n-PFOA and PFNA during late postpartum (r_{sp} = 0.32 to 0.53). Parity was negatively correlated with n-PFOA during pregnancy and early postpartum (r_{sp} = -0.39 and -0.33, respectively) and PFHxS during early postpartum and late postpartum (r_{sp} = -0.36 and -0.50, respectively). Breastfeeding duration was negatively correlated with n-PFOA during late postpartum (r_{sp} = -0.51). During all three sub-periods, mothers who were born in the U.S. had lower PFNA concentrations than those who were not.

5.3.4. Percent changes in maternal PFAS serum concentrations over time and by potential determinants

Maternal serum concentrations of n-PFOS changed -3.1% per month (95% CI: -4.6%, -1.5%) during pregnancy and -1.0% (95% CI: -1.6%, -0.6%) during late postpartum (Figure 5.2). Sm-PFOS decreased with similar rates during pregnancy (-3.4%; 95% CI: -4.7%, -2.2%) and late postpartum (-1.2%; 95% CI: -1.7%, -0.6%), but further changed -1.3% per month (95% CI: -2.1%, -0.5%) during early postpartum. PFHxS changed -5.6% per month (95% CI: -7.3%, -3.7%) only during early postpartum. n-PFOA changed -4.0% per month (95% CI: -4.8%, -3.4%) during pregnancy and -4.5% (95% CI: -5.3%, -3.7%) during early postpartum. PFNA changed -

4.4% per month (95% CI: -5.3%, -3.5%) during pregnancy, -1.1% (95% CI: -2.0%, -0.3%) during early postpartum, and -0.8% (95% CI: -1.3%, -0.2%) during late postpartum.

During all three sub-periods, n-PFOS, Sm-PFOS, PFHxS, n-PFOA, and PFNA concentrations changed -18.5% to -7.4% per child's birth year, and mothers who were born outside the U.S. had 58.4% to 66.4% higher PFNA than the U.S.-born mothers (Table 5.3). Mothers who were born outside the U.S. had 36.5% and 48.9% higher concentrations of n-PFOS and PFHxS during pregnancy compared to the U.S.-born mothers. With increasing parity, n-PFOA concentrations during pregnancy changed -11.4%, and PFHxS concentrations during early postpartum and late postpartum changed -13.2% and -15.1%, respectively. During late postpartum, n-PFOA concentrations changed 4.8% per year with increasing maternal age at delivery. With increasing breastfeeding duration, n-PFOA and PFNA concentrations changed -2.8% and -1.5% per month, respectively.

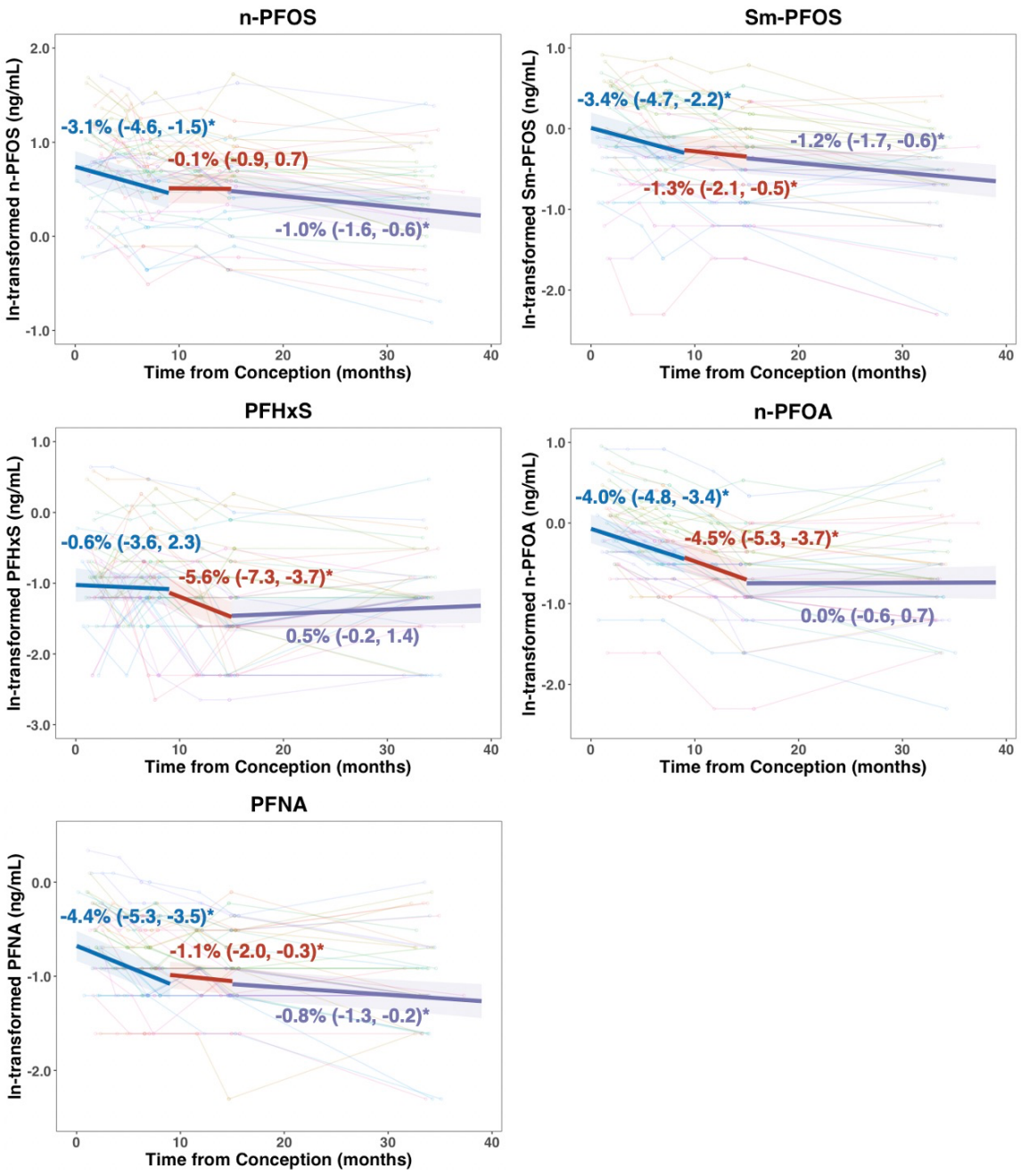

Figure 5.2 Adjusted monthly percent changes and 95% CIs in maternal serum PFAS concentrations during pregnancy, early postpartum, and late postpartum. Thick lines in blue, red, and purple represent adjusted mean concentrations during pregnancy, early postpartum (from delivery to 6 months postpartum), and late postpartum (from 6 to 24 months postpartum), respectively, and shaded areas represent corresponding 95% CIs. Thin lines and dots represent individual trajectory of PFAS concentrations. Asterisk represents significant changes in PFAS concentrations over time. All models were adjusted for child's birth year, maternal age at delivery, parity, and maternal birthplace. Late postpartum models were additionally adjusted for breastfeeding duration.

Table 5.3 Adjusted percent changes (95% CI) in maternal serum PFAS concentrations per one-unit increase of each potential determinants for each sub-period.

Potential determinants [a]	n-PFOS	Sm-PFOS	PFHxS	n-PFOA	PFNA
Pregnancy					
Child's birth year (year)	**-11.9 (-16.8, -6.4)**	**-18.5 (-24.8, -12.3)**	**-14.9 (-21.1, -7.5)**	**-8.9 (-14.9, -2.0)**	**-7.4 (-12.8, -2.0)**
Maternal age at delivery (year)	0.7 (-2.3, 3.9)	-0.3 (-4.0, 3.5)	-0.3 (-4.0, 3.6)	2.3 (-1.1, 5.5)	-2.1 (-5.1, 1.1)
Parity	2.6 (-6.3, 12.6)	-3.9 (-14.3, 7.8)	-4.2 (-16.6, 8.9)	**-11.4 (-20.8, -1.5)**	5.6 (-3.1, 15.8)
Maternal birthplace					
United States	Ref	Ref	Ref	Ref	Ref
Non-United States	**36.5 (3.3, 85.0)**	-23.9 (-45.6, 11.3)	**48.9 (4.4, 120.5)**	10.1 (-22.3, 54.1)	**60.8 (21.6, 116.9)**
Early postpartum					
Child's birth year (year)	**-10.1 (-15.4, -4.5)**	**-17.9 (-23.2, -12.0)**	**-18.0 (-24.5, -10.9)**	**-9.5 (-15.6, -2.7)**	**-9.0 (-13.9, -3.8)**
Maternal age at delivery (year)	0.6 (-2.6, 4.0)	0.4 (-2.9, 4.1)	-0.1 (-4.2, 4.6)	3.0 (-0.4, 6.8)	-1.7 (-4.3, 1.4)
Parity	2.3 (-6.9, 13.7)	-4.0 (-13.3, 6.4)	**-13.2 (-23.6, -2.2)**	-9.2 (-18.9, 1.1)	5.9 (-2.9, 15.3)
Maternal birthplace					
United States	Ref	Ref	Ref	Ref	Ref
Non-United States	30.5 (-1.4, 71.0)	-20.3 (-42.0, 8.7)	39.0 (-8.3, 110.0)	8.6 (-22.1, 48.6)	**58.4 (22.0, 105.8)**
Late postpartum					
Child's birth year (year)	**-8.2 (-14.8, -1.1)**	**-16.0 (-22.0, -8.9)**	**-12.1 (-19.6, -4.6)**	**-7.9 (-14.4, -0.7)**	**-9.8 (-15.5, -3.8)**
Maternal age at delivery (year)	2.4 (-1.4, 6.2)	1.2 (-2.6, 5.3)	1.5 (-2.9, 6.1)	**4.8 (1.2, 8.4)**	0.2 (-3.1, 3.6)
Parity	5.0 (-6.3, 17.4)	-1.0 (-10.9, 10.9)	**-15.1 (-25.5, -3.5)**	-3.6 (-13.4, 7.2)	7.6 (-2.6, 18.1)
Breastfeeding duration (month)	-0.4 (-2.1, 1.4)	-1.0 (-2.8, 0.7)	-1.4 (-3.2, 0.6)	**-2.8 (-4.6, -1.1)**	**-1.5 (-3.0, 0.0)**
Maternal birthplace					
United States	Ref	Ref	Ref	Ref	Ref
Non-United States	36.3 (-4.2, 93.1)	-17.1 (-39.8, 18.2)	4.4 (-3.8, 120.1)	28.4 (-8.7, 76.2)	**66.4 (22.4, 126.3)**

[a] *p*-values were from linear mixed models that mutually adjusted for time from conception and potential determinants for each sub-period, where the null hypothesis is the regression coefficients of each potential determinant equals to zero. Estimates with *p*-values less than 0.05 are highlighted in bold.

When using ln-transformed concentration ratios of PFAS at each time point to those at the 1st trimester of pregnancy as a sensitivity analysis, most monthly percent changes remained similar (Figure S5.2). When using samples from 217 unique pregnancies with at least one blood sample, monthly percent changes during pregnancy and early postpartum were also similar, except that those for n-PFOS, Sm-PFOS, n-PFOA, and PFNA during pregnancy slightly decreased (Figure S5.3). When additionally adjusting for maternal BMI at pre-pregnancy, maternal weight gain during pregnancy and child's birthweight in pregnancy and early postpartum models to account for maternal plasma volume expansion, monthly percent changes did not change (Table S5.4).

When stratifying postnatal samples (i.e., 3, 6, and 24 months) by breastfeeding duration, n-PFOA and PFNA concentrations decreased only among the mothers who breastfed longer than 12 months (p-value for interaction ≤ 0.01) (Table S5.5). Concentrations of n-PFOS and Sm-PFOS changed -1.3% and -1.6% per month among the mothers who breastfed longer than 12 months and -0.8% and -1.0% per month among the mothers who breastfed shorter than 12 months, respectively.

5.4. Discussion

In this study, we quantified ten PFAS in prospectively collected maternal blood during the 1st, 2nd, and 3rd trimesters of pregnancy and at 3, 6, and 24 months after delivery and examined changes in PFAS concentrations for three sub-periods (i.e., pregnancy, early postpartum, and late postpartum). Maternal serum concentrations of n-PFOS, Sm-PFOS, n-PFOA, and PFNA decreased 3% to 4% per month during pregnancy, those of Sm-PFOS, PFHxS, n-PFOA, and PFNA declined 1% to 6% per month during early postpartum, and those of n-

PFOS, Sm-PFOS, and PFNA declined 1% per month during late postpartum. We also explored prenatal, perinatal, and demographic factors affecting maternal serum PFAS concentrations. In all sub-periods, mothers who gave birth in a later study period had lower n-PFOS, Sm-PFOS, PFHxS, n-PFOA, and PFNA concentrations, and mothers who were born outside the U.S. had higher PFNA concentrations. During late postpartum, we identified that higher n-PFOA and PFNA concentrations were associated with shorter breastfeeding duration, which was confirmed by stratified analysis.

Decreases in our maternal serum PFAS concentrations from pregnancy to postnatal periods are consistent with those from other studies that repeatedly measured PFAS concentrations during pregnancy and/or early postpartum within the same women. Two previous studies collected three maternal blood samples during the 1st, 2nd, and 3rd trimesters of pregnancy and observed decreases in most of the PFAS concentrations, including PFOS, PFOA, PFNA, PFDA, PFUnDA, and PFDoDA, while PFHxS concentrations did not change, as in the current study (Buck Louis et al. 2019; Chen et al. 2021). Kato et al. collected two maternal blood samples at 16 weeks of pregnancy and at delivery from 71 women (Kato et al. 2014). They observed 25 to 43% decreases in unadjusted GM serum concentrations of PFOS and PFOA (sum of linear and branched isomers), PFHxS, and PFNA between 16 weeks of pregnancy and delivery, and their GM concentration ratios were 0.71 for PFOS, 0.79 for PFHxS, 0.70 for PFOA, and 0.77 for PFNA. We did not quantify PFAS in serum collected shortly after delivery, but the GM concentration ratios of Kato et al. were comparable to those between the 1st trimester of pregnancy and 3 months postpartum in our study (0.85 for n-PFOS, 0.79 for Sm-PFOS, 0.73 for PFHxS, 0.66 for n-PFOA, and 0.78 for PFNA) (Table S5.1). Glynn et al. serially collected maternal blood samples during the 1st and 3rd trimesters of pregnancy and at 3 weeks and 3

months after delivery from 19 women (Glynn et al. 2012). They observed decreases in mean serum concentrations of PFOS (sum of linear and branched isomers), PFOA and PFNA between the 1st and 3rd trimester of pregnancy and of PFOA between 3 weeks and 3 months postpartum.

Although we did not use direct evidence of placental transfer of PFAS such as concentrations in umbilical cord blood, declines in maternal serum PFAS concentrations during pregnancy may explain placental transfer from mother to fetus because PFAS concentrations in maternal serum have been shown to be moderately to highly correlated with those in cord blood(Beesoon et al. 2011; Fromme et al. 2010; Gützkow et al. 2012; Kim et al. 2011a; Kim et al. 2011b; Liu et al. 2011; Manzano-Salgado et al. 2015; Monroy et al. 2008; Ode et al. 2013) and fetal tissues.(Mamsen et al. 2019) Specifically, Mamsen et al. observed that PFOS, PFOA, and PFNA in maternal serum were positively correlated with those in placenta, fetal liver, lung, heart, and adipose tissue, while they did not observe significant correlations for PFHxS, PFDA, and PFUnDA.(Mamsen et al. 2019) Previous studies also suggested that maternal blood volume expansion during pregnancy can also explain decreases in PFAS concentration(Chen et al. 2021; Glynn et al. 2012) because blood volume increases approximately 45% throughout the pregnancy.(Lund and Donovan 1967) However, we observed that changes in maternal serum PFAS concentrations during pregnancy remained similar after adjusting for potential predictors of maternal blood volume expansion (Table S5.4).

Breastfeeding is a major PFAS excretion route for lactating mothers (Mogensen et al. 2015; Mondal et al. 2014; Thomsen et al. 2010). We observed that maternal serum concentrations of n-PFOS, PFHxS, n-PFOA, and PFNA decreased from delivery to 6 months postpartum and that longer breastfeeding duration was negatively associated with n-PFOA and PFNA after 6 months postpartum. Moreover, postnatal n-PFOA and PFNA concentrations

decreased only among mothers who breastfed their child longer than 12 months. This finding suggests that breastfeeding may be an important exposure route for nursing infants and thus those who are longer breastfed may experience higher postnatal exposure to PFOA and PFNA. In a study that quantified PFOA and PFOS in breast milk serially collected during one year of breastfeeding, PFOA showed 94% of decrease, which was 2.5 times higher than PFOS.(Thomsen et al. 2010) Several mother-child studies observed higher concentration ratios of breast milk to maternal serum for PFOA and PFNA compared to PFOS and PFHxS.(Liu et al. 2011; Zheng et al. 2021) We also observed that Sm-PFOS, but not n-PFOS, decreased during early postpartum, and our differences in monthly percent changes between two PFOS isomers were greater during early postpartum compared to pregnancy and late postpartum. Although previous studies reported higher placental transfer efficiency of Sm-PFOS than n-PFOS,(Beesoon et al. 2011; Gützkow et al. 2012) little is known about the differences in lactational transfer rates of PFOS isomers, thus further studies are needed to confirm our findings. This study did not collect individual exclusive breastfeeding duration data and did not quantify PFAS concentrations in breast milk and child serum during early postpartum. Therefore, future studies may benefit by collecting exclusive breastfeeding duration and PFAS measurement in breast milk to gain insight on lactational transfer of PFAS during early postpartum.

Changes in maternal serum PFHxS concentrations from pregnancy to early postpartum were different from other PFAS. Maternal PFHxS concentrations did not decrease during pregnancy, despite the possibility of placental transfer and plasma volume expansion. These results can be explained by a longer half-life of PFHxS. The half-life of PFHxS ranged from 5.3 to 15.5 years and is longer than PFOA (2.3 to 3.8 years) and PFOS (3.4 to 5.4 years) (Bartell et al. 2010; Li et al. 2018; Olsen et al. 2007; Russell et al. 2015; Wong et al. 2014; Worley et al.

2017). On the other hand, we observed that maternal PFHxS decreased with the highest percent change during early postpartum but was not associated with breastfeeding duration during late postpartum. As observed for PFOA, maternal serum concentrations of PFHxS appeared to stabilize after 6 months postpartum. Several previous studies reported that longer breastfeeding duration was associated with lower PFHxS in the serum of mothers of 2- to 5-year-old children (Kim et al. 2020; Mondal et al. 2014) or higher PFHxS in the serum of exclusively breastfed 2- to 4-month-old infants (Gyllenhammar et al. 2018). However, another study observed decreases in child's serum PFHxS concentrations during the first year of life, suggesting that early postnatal exposure to PFHxS may relate to sources other than breast milk (Mogensen et al. 2015). As the evidence on the lactational transfer of PFHxS is still inconclusive, further studies are needed.

From pregnancy through two years postpartum, maternal serum PFAS concentrations were negatively associated with child's birth year, ranging from 7% to 19% decreases per year. Previous studies examined temporal trends of PFAS in the serum of pregnant women (Berg et al. 2014; Bjerregaard-Olesen et al. 2016b; Sagiv et al. 2015; Tsai et al. 2018) as well mothers of 2 to 5 years old (Kim et al. 2020). Because there were nationwide efforts to phase out PFOS, PFOA and related compounds in early 2000s, the studies dealing with trends after 2000 reported decreased serum PFAS concentrations over calendar years. Our percent changes in PFOA, PFOS, and PFHxS concentrations were comparable to those of Kim et al., who investigated similar periods (i.e., 2009 - 2017) in the same study region (Kim et al. 2020). We also observed that mothers who were born outside the U.S. had 58% to 66% higher PFNA concentrations than the U.S.-born mothers throughout the whole study period. Similarly, Park et al. observed lower PFNA concentrations in midlife women (45-56 years old) born in the U.S. than those who are

not (Park et al. 2019). Other European studies also reported differences in PFAS concentrations of pregnant women according to country of birth (Bjerregaard-Olesen et al. 2016a; Manzano-Salgado et al. 2016; Ode et al. 2013). This suggests that the countries of birth can affect a mother's diet and lifestyle, which can further result in different patterns of exposure to PFAS (De Silva et al. 2021; Glynn et al. 2020).

To our knowledge, this is the first study that examined changes in maternal serum PFAS concentrations from pregnancy to two years postpartum by using repeated serum samples within the same women and adjusting for relevant covariates for each sub-period. However, some limitations should be noted. The relatively small sample size may have limited our ability to examine the determinants of PFAS concentrations that were identified in other studies (Berg et al. 2014; Bjerregaard-Olesen et al. 2016a; Brantsaeter et al. 2013; Kato et al. 2014; Kingsley et al. 2018; Ode et al. 2013; Sagiv et al. 2015; Tian et al. 2018; Tsai et al. 2018). Another limitation includes lack of maternal blood samples during a pre-pregnancy period and at delivery. Aversion and morning sickness may affect changes in diet and associated PFAS exposure between a pre-pregnancy period and the first trimester for some mothers. We used maternal serum PFAS concentrations at the 3rd trimester of pregnancy as surrogates of those at delivery, but there may be additional decreases in certain PFAS concentrations due to blood loss at delivery, which was not considered in this study (Prasertcharoensuk et al. 2000). Furthermore, the mothers in this study population were recruited from those who received state-funded services for ASD and had longer breastfeeding duration compared to those in the National Health and Nutrition Examination Survey (Davis et al. 2018). As this study population may not be generalizable, our results should be interpreted with caution.

CHAPTER 6: SUMMARY AND CONCLUSIONS

6.1. Summary of findings

This research aimed to (1) investigate effects of prenatal exposure to per- and polyfluoroalkyl substances (PFAS) on child neurodevelopment on or before 3 years of age and (2) evaluate longitudinal changes in maternal PFAS concentrations from pregnancy to two years postpartum. I used data from MARBLES (***M***arkers of ***A***utism ***R***isk in ***B***abies – ***L***earning ***E***arly ***S***igns), a prospective cohort study that follows pregnant women who already delivered a child with autism spectrum disorder (ASD).

Chapter 2 examined associations of prenatal PFAS exposure with the risk of ASD using maternal serum samples collected during pregnancy. Prenatal maternal perfluorooctanoate (PFOA) and perfluorononanoate (PFNA) concentrations were associated with increased risk of ASD. Among the mothers who were older than 35 years at delivery, prenatal maternal PFOA, perfluorooctane sulfonate (PFOS), perfluorohexane sulfonate (PFHxS), PFNA, and the principal component loaded with the four PFAS were more strongly associated with increased ASD risk, compared to those who were younger than 35 years.

Chapter 3 examined associations between prenatal maternal serum PFAS concentrations and child cognitive development assessed using Mullen Scales of Early Learning (MSEL) at 6, 12, 24, and 36 months of age. Prenatal maternal PFOA concentrations were associated with decreased scores of MSEL Composite and almost all subscales at 24 and 36 months of age. Prenatal maternal PFOA was also associated with longitudinal decreases in MSEL Composite and subscale scores and with lower and/or decreasing trajectories of Composite scores over the four assessment time points.

To support the findings from Chapter 3, Chapter 4 used a Japanese population and investigated associations between cord blood PFOA and PFOS concentrations and child

cognitive development assessed at 4, 6, 10, 14, 18, 24, 32, and 40 months of age using MSEL. PFOA was inversely associated with 18-month Composite and 10-month Visual Reception and Receptive Language scores. From 4 to 40 months of age, PFOA and PFOS were associated with decreased longitudinal changes in Fine Motor scores and increased changes in Receptive Language scores.

Chapter 5 investigated longitudinal changes in PFAS concentrations during pregnancy and early and late postpartum periods using maternal blood samples collected during the 1st, 2nd, and 3rd trimesters of pregnancy and at 3, 6, and 24 months after delivery. During pregnancy, maternal serum concentrations of linear PFOS (n-PFOS), branched PFOS (Sm-PFOS), linear PFOA (n-PFOA), and PFNA changed -4% to -3% per month. During early postpartum, maternal serum concentrations of Sm-PFOS, PFHxS, n-PFOA, and PFNA changed -6% to -1% per month. During late postpartum, maternal serum concentrations of n-PFOS, Sm-PFOS, and PFNA changed -1% per month.

6.2. Conclusions

Chapter 2 revealed that prenatal exposure to PFOA and PFNA may increase the risk of developing ASD in this high-risk ASD cohort. I also observed that individual as well as combined PFAS showed different effects on ASD risk by advanced maternal age at delivery. PFAS concentrations in this study population were similar or slightly lower than those in the National Health and Nutrition Examination Survey (NHANES), which represent the general population of the United States (U.S.). This study also clinically diagnosed the child ASD using gold standard instruments. The results of this study provided evidence in support of adverse effects of prenatal PFAS exposure on the development of ASD.

Chapter 3 showed that prenatal exposure to PFOA was associated with decreased scores of cognitive functions throughout infancy and toddlerhood among children who had an older sibling diagnosed with ASD. At 12 months of age, I observed that associations of cognitive development with PFHxS were modified by child's sex and that associations of cognitive development with PFOA, PFHxS, and PFNA were modified by breastfeeding duration. This study repeatedly assessed cognitive development in the same children using a reliable and valid assessment tool. Therefore, the results of this study are comparable to mixed results of the previous epidemiological studies that used different assessment age and tools. This is the first study that investigated longitudinal changes and trajectories of child cognitive development in association with prenatal PFAS exposure.

Chapter 4 revealed no convincing associations between cord blood PFOA and PFOS concentrations and cognitive development in infancy and toddlerhood in this prospective Japanese birth cohort. I only found suggestive evidence for an association between PFOA and decreased cognitive functions at 18 months of age. The results of Chapter 4 were comparable to those of Chapter 3 because both studies used the similar ages and the same assessment tools. Compared to Chapter 3, this study used the Japanese general population with a larger sample size and with a lower risk of ASD or other neurodevelopmental concerns.

Chapter 5 showed that decreases in serum concentrations of n-PFOS, Sm-PFOS, n-PFOA, and PFNA during pregnancy, Sm-PFOS, PFHxS, n-PFOA, and PFNA during early postpartum, and n-PFOS, Sm-PFOS, and PFNA during late postpartum. Throughout the whole study period, later child's birth year was associated with decreased concentrations of n-PFOS, Sm-PFOS, PFHxS, n-PFOA, and PFNA which appear to reflect regulations and manufacturing changes for these compounds. Longer breastfeeding duration was associated with decreased n-

PFOA and PFNA concentrations during late postpartum. Maternal serum PFAS concentration profiles from pregnancy to two years postpartum may improve understanding of pregnancy and lactational transfers from mother to child. Furthermore, our findings might be useful for reconstructing reliable pregnancy or early-life PFAS exposure for case-control or cross-sectional epidemiologic studies for which only postpartum PFAS serum concentrations are available.

6.3. Recommendations for future studies

The MARBLES study is a high-risk ASD cohort, thus the MARBLES children have elevated risk for developing ASD or other neurodevelopmental disorders. Therefore, to confirm our findings on the associations between prenatal PFAS exposure and ASD risk, future research based on general populations without high-risk genetic predisposition is warranted. Similarly, population-based studies are needed to confirm our findings on the associations between prenatal PFAS exposure and child cognitive development because a large fraction of the MARBLES children had developmental delays or cognitive deficits. Furthermore, as the MARBLES study has a relatively small sample size, further studies should be conducted in larger populations, especially to confirm our findings on the effect modification by maternal age at delivery in Chapter 2 and by child's sex and breastfeeding duration in Chapter 3.

A prenatal period is highly weighted in terms of prenatal origins of ASD, and the etiology of ASD can be explained by genetic and environmental factors and their interactions. Therefore, future studies that used integrated approaches, such as gene and environment interaction, may significantly improve understanding of the etiology of ASD. On the other hand, child's cognitive abilities fluctuate during their early life and can be affected by postnatal environmental factors, such as breastfeeding duration, intellectual home environments, and interactions with parents,

siblings, or other children. Therefore, future epidemiological studies should account for these postnatal factors. Furthermore, to better understand underlying mechanisms of PFAS exposure on child neurodevelopment, future studies that evaluate the potential mediation effect of thyroid hormones on the associations between prenatal PFAS exposure and child neurodevelopment are warranted.

To assess the combined effects of exposure to a PFAS mixture on child neurodevelopment, I used a principal component analysis (PCA) which is a variable reduction strategy to mitigate multicollinearity issues. Other than PCA, many statistical methods have been developed to evaluate the mixture effects of environmental chemicals, including elastic net regression, weighted quantile sum regression, and Bayesian kernel machine regression (Lazarevic et al. 2019). Therefore, future studies using various statistical approaches to study the mixture effects of prenatal PFAS exposure on child neurodevelopment are warranted.

REFERENCES

3M, 1999. The science of organic fluorochemistry. US Environmental Protection Agency. OPPT-2002-0043-0006.

Aguree, S., Gernand, A.D., 2019. Plasma volume expansion across healthy pregnancy: a systematic review and meta-analysis of longitudinal studies. BMC pregnancy and childbirth. 19, 1-11.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental

Disorders (DSM-5®). American Psychiatric Pub.

ATSDR, 2018. Toxicological Profile for Perfluoroalkyls. Draft for Public Comment. Agency for Toxic Substances and Disease Registry. Atlanta, GA.

Austin, M.E., Kasturi, B.S., Barber, M., Kannan, K., MohanKumar, P.S., MohanKumar, S.M., 2003. Neuroendocrine effects of perfluorooctane sulfonate in rats. Environmental health perspectives. 111, 1485-1489.

Ballinger, G.A., 2004. Using generalized estimating equations for longitudinal data analysis. Organizational research methods. 7, 127-150.

Bangma, J., Eaves, L.A., Oldenburg, K., Reiner, J.L., Manuck, T., Fry, R.C., 2020. Identifying risk factors for levels of per-and polyfluoroalkyl substances (PFAS) in the placenta in a high-risk pregnancy cohort in North Carolina. Environmental science & technology. 54, 8158-8166.

Banik, A., Kandilya, D., Ramya, S., Stünkel, W., Chong, Y., Dheen, S., 2017. Maternal factors that induce epigenetic changes contribute to neurological disorders in offspring. Genes. 8, 150.

Barrett, E.S., Mbowe, O., Thurston, S.W., Butts, S., Wang, C., Nguyen, R., Bush, N., Redmon, J.B., Sheshu, S., Swan, S.H., 2019. Predictors of Steroid Hormone Concentrations in Early Pregnancy: Results from a Multi-Center Cohort. Maternal and child health journal. 23, 397-407.

Bartell, S.M., Calafat, A.M., Lyu, C., Kato, K., Ryan, P.B., Steenland, K., 2010. Rate of decline in serum PFOA concentrations after granular activated carbon filtration at two public water systems in Ohio and West Virginia. Environmental health perspectives. 118, 222-228.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. Journal of Statistical Software. 67, 48.

Beesoon, S., Webster, G.M., Shoeib, M., Harner, T., Benskin, J.P., Martin, J.W., 2011. Isomer profiles of perfluorochemicals in matched maternal, cord, and house dust samples:

manufacturing sources and transplacental transfer. Environmental health perspectives. 119, 1659-1664.

Begley, T., Hsu, W., Noonan, G., Diachenko, G., 2008. Migration of fluorochemical paper additives from food-contact paper into foods and food simulants. Food additives and contaminants. 25, 384-390.

Begley, T., White, K., Honigfort, P., Twaroski, M., Neches, R., Walker, R., 2005. Perfluorochemicals: potential sources of and migration from food packaging. Food additives and contaminants. 22, 1023-1031.

Benford, D., De Boer, J., Carere, A., Di Domenico, A., Johansson, N., Schrenk, D., Schoeters, G., De Voogt, P., Dellatte, E., 2008. Opinion of the scientific panel on contaminants in the food chain on perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and their salts. EFSA Journal. 1-131.

Berbel, P., Mestre, J.L., Santamaria, A., Palazón, I., Franco, A., Graells, M., González-Torga, A., de Escobar, G.M., 2009. Delayed neurobehavioral development in children born to pregnant women with mild hypothyroxinemia during the first month of gestation: the importance of early iodine supplementation. Thyroid. 19, 511-519.

Berg, V., Nøst, T.H., Hansen, S., Elverland, A., Veyhe, A.-S., Jorde, R., Odland, J.Ø., Sandanger, T.M., 2015. Assessing the relationship between perfluoroalkyl substances, thyroid hormones and binding proteins in pregnant women; a longitudinal mixed effects approach. Environment international. 77, 63-69.

Berg, V., Nøst, T.H., Huber, S., Rylander, C., Hansen, S., Veyhe, A.S., Fuskevåg, O.M., Odland, J.Ø., Sandanger, T.M., 2014. Maternal serum concentrations of per-and polyfluoroalkyl substances and their predictors in years with reduced production and use. Environment international. 69, 58-66.

Berg, V., Sandanger, T.M., Hanssen, L., Rylander, C., Nøst, T.H., 2021. Time trends of perfluoroalkyl substances in blood in 30-year old Norwegian men and women in the period 1986–2007. Environmental Science and Pollution Research. 1-11.

Berntsen, H.F., Bjørklund, C.G., Audinot, J.-N., Hofer, T., Verhaegen, S., Lentzen, E., Gutleb, A.C., Ropstad, E., 2017. Time-dependent effects of perfluorinated compounds on viability in cerebellar granule neurons: Dependence on carbon chain length and functional group attached. Neurotoxicology. 63, 70-83.

Bjerregaard-Olesen, C., Bach, C.C., Long, M., Ghisari, M., Bech, B.H., Nohr, E.A., Henriksen, T.B., Olsen, J., Bonefeld-Jørgensen, E.C., 2016a. Determinants of serum levels of perfluorinated alkyl acids in Danish pregnant women. International journal of hygiene and environmental health. 219, 867-875.

Bjerregaard-Olesen, C., Bach, C.C., Long, M., Ghisari, M., Bossi, R., Bech, B.H., Nohr, E.A., Henriksen, T.B., Olsen, J., Bonefeld-Jørgensen, E.C., 2016b. Time trends of

perfluorinated alkyl acids in serum from Danish pregnant women 2008–2013. Environment international. 91, 14-21.

Björklund, J.A., Thuresson, K., De Wit, C.A., 2009. Perfluoroalkyl compounds (PFCs) in indoor dust: concentrations, human exposure estimates, and sources. Environmental science & technology. 43, 2276-2281.

Bland, J.M., Altman, D.G., 1995. Multiple significance tests: the Bonferroni method. Bmj. 310, 170.

Bontinck, C., Warreyn, P., Demurie, E., Bruyneel, E., Boterberg, S., Roeyers, H., 2018. Social interactions between 24-month-old children and their older sibling with autism spectrum disorder: Characteristics and association with social-communicative development. Journal of autism and developmental disorders. 48, 4118-4137.

Brantsaeter, A., Whitworth, K., Ydersbond, T., Haug, L., Haugen, M., Knutsen, H., Thomsen, C., Meltzer, H., Becher, G., Sabaredzovic, A., 2013. Determinants of plasma concentrations of perfluoroalkyl substances in pregnant Norwegian women. Environment international. 54, 74-84.

Braun, J.M., 2017. Early-life exposure to EDCs: role in childhood obesity and neurodevelopment. Nature Reviews Endocrinology. 13, 161-173.

Braun, J.M., Kalkbrenner, A.E., Just, A.C., Yolton, K., Calafat, A.M., Sjödin, A., Hauser, R., Webster, G.M., Chen, A., Lanphear, B.P., 2014. Gestational exposure to endocrine-disrupting chemicals and reciprocal social, repetitive, and stereotypic behaviors in 4-and 5-year-old children: the HOME study. Environmental health perspectives. 122, 513-520.

Buck Louis, G., Yeung, E., Kannan, K., Maisog, J., Zhang, C., Grantz, K.L., Sundaram, R., 2019. Patterns and variability of endocrine disrupting chemicals during pregnancy: implications for understanding the exposome of normal pregnancy. Epidemiology. 30, S65.

Buck, R.C., Franklin, J., Berger, U., Conder, J.M., Cousins, I.T., De Voogt, P., Jensen, A.A., Kannan, K., Mabury, S.A., van Leeuwen, S.P., 2011. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integrated environmental assessment and management. 7, 513-541.

Burns, T.G., King, T.Z., Spencer, K.S., 2013. Mullen Scales of Early Learning: The utility in assessing children diagnosed with autism spectrum disorders, cerebral palsy, and epilepsy. Applied Neuropsychology: Child. 2, 33-42.

Butenhoff, J.L., Ehresman, D.J., Chang, S.-C., Parker, G.A., Stump, D.G., 2009. Gestational and lactational exposure to potassium perfluorooctanesulfonate (K+ PFOS) in rats: developmental neurotoxicity. Reproductive toxicology. 27, 319-330.

Butenhoff, J.L., Kennedy Jr, G.L., Frame, S.R., O'Connor, J.C., York, R.G., 2004. The reproductive toxicology of ammonium perfluorooctanoate (APFO) in the rat. Toxicology. 196, 95-116.

Cariou, R., Veyrand, B., Yamada, A., Berrebi, A., Zalko, D., Durand, S., Pollono, C., Marchand, P., Leblanc, J.-C., Antignac, J.-P., 2015. Perfluoroalkyl acid (PFAA) levels and profiles in breast milk, maternal and cord serum of French women and their newborns. Environment international. 84, 71-81.

Carrizosa, C., Murcia, M., Ballesteros, V., Costa, O., Manzano-Salgado, C.B., Ibarluzea, J., Iñiguez, C., Casas, M., Andiarena, A., Llop, S., 2021. Prenatal perfluoroalkyl substance exposure and neuropsychological development throughout childhood: The INMA Project. Journal of Hazardous Materials. 416, 125185.

CDC, 2019. Fourth Report on Human Exposure to Environmental Chemicals, Updated Tables, January 2019. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Atlanta, GA.

Chaste, P., Leboyer, M., 2012. Autism risk factors: genes, environment, and gene-environment interactions. Dialogues in clinical neuroscience. 14, 281.

Chen, L., Tong, C., Huo, X., Zhang, J., Tian, Y., Cohort, S.B., 2021. Prenatal exposure to perfluoroalkyl and polyfluoroalkyl substances and birth outcomes: A longitudinal cohort with repeated measurements. Chemosphere. 267, 128899.

Chen, M.-H., Ha, E.-H., Liao, H.-F., Jeng, S.-F., Su, Y.-N., Wen, T.-W., Lien, G.-W., Chen, C.-Y., Hsieh, W.-S., Chen, P.-C., 2013. Perfluorinated compound levels in cord blood and neurodevelopment at 2 years of age. Epidemiology. 800-808.

Chen, W., Qian, L., Shi, J., Franklin, M., 2018. Comparing performance between log-binomial and robust Poisson regression models for estimating risk ratios under model misspecification. BMC medical research methodology. 18, 63.

Collaer, M.L., Hines, M., 1995. Human behavioral sex differences: a role for gonadal hormones during early development? Psychological bulletin. 118, 55.

Das, K.P., Grey, B.E., Rosen, M.B., Wood, C.R., Tatum-Gibbs, K.R., Zehr, R.D., Strynar, M.J., Lindstrom, A.B., Lau, C., 2015. Developmental toxicity of perfluorononanoic acid in mice. Reproductive toxicology. 51, 133-144.

Davis, K.E., Li, X., Adams-Huet, B., Sandon, L., 2018. Infant feeding practices and dietary consumption of US infants and toddlers: National Health and Nutrition Examination Survey (NHANES) 2003–2012. Public health nutrition. 21, 711-720.

de Cock, M., de Boer, M.R., Lamoree, M., Legler, J., van de Bor, M., 2014. Prenatal exposure to endocrine disrupting chemicals in relation to thyroid hormone levels in infants–a Dutch prospective cohort study. Environmental Health. 13, 106.

De Silva, A.O., Armitage, J.M., Bruton, T.A., Dassuncao, C., Heiger-Bernays, W., Hu, X.C., Kärrman, A., Kelly, B., Ng, C., Robuck, A., 2021. PFAS exposure pathways for humans and wildlife: a synthesis of current knowledge and key gaps in understanding. Environmental Toxicology and Chemistry. 40, 631-657.

DeWitt, J.C., 2015. Toxicological effects of perfluoroalkyl and polyfluoroalkyl substances. Springer.

DeWitt, J.C., Peden-Adams, M.M., Keller, J.M., Germolec, D.R., 2012. Immunotoxicity of perfluorinated compounds: recent developments. Toxicologic pathology. 40, 300-311.

Ding, N., Harlow, S.D., Batterman, S., Mukherjee, B., Park, S.K., 2020. Longitudinal trends in perfluoroalkyl and polyfluoroalkyl substances among multiethnic midlife women from 1999 to 2011: The Study of Women′ s Health Across the Nation. Environment international. 135, 105381.

Donauer, S., Chen, A., Xu, Y., Calafat, A.M., Sjodin, A., Yolton, K., 2015. Prenatal exposure to polybrominated diphenyl ethers and polyfluoroalkyl chemicals and infant neurobehavior. The Journal of pediatrics. 166, 736-742.

Dong, Z., Wang, H., Yu, Y.Y., Li, Y.B., Naidu, R., Liu, Y., 2019. Using 2003–2014 US NHANES data to determine the associations between per-and polyfluoroalkyl substances and cholesterol: Trend and implications. Ecotoxicology and environmental safety. 173, 461-468.

Esler, A.N., Bal, V.H., Guthrie, W., Wetherby, A., Weismer, S.E., Lord, C., 2015. The autism diagnostic observation schedule, toddler module: standardized severity scores. Journal of Autism and Developmental Disorders. 45, 2704-2720.

Faupel-Badger, J.M., Hsieh, C.-C., Troisi, R., Lagiou, P., Potischman, N., 2007. Plasma volume expansion in pregnancy: implications for biomarkers in population studies. Cancer Epidemiology and Prevention Biomarkers. 16, 1720-1723.

Fei, C., McLaughlin, J.K., Lipworth, L., Olsen, J., 2008. Prenatal exposure to perfluorooctanoate (PFOA) and perfluorooctanesulfonate (PFOS) and maternally reported developmental milestones in infancy. Environmental health perspectives. 116, 1391-1395.

Fenton, S.E., Ducatman, A., Boobis, A., DeWitt, J.C., Lau, C., Ng, C., Smith, J.S., Roberts, S.M., 2021. Per-and polyfluoroalkyl substance toxicity and human health review: Current state of knowledge and strategies for informing future research. Environmental toxicology and chemistry. 40, 606-630.

Fisher, M., Arbuckle, T.E., Liang, C.L., LeBlanc, A., Gaudreau, E., Foster, W.G., Haines, D., Davis, K., Fraser, W.D., 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. Environmental Health. 15, 59.

Fleiss, J.L., 2011. Design and analysis of clinical experiments. John Wiley & Sons.

Fraga, M.F., Esteller, M., 2007. Epigenetics and aging: the targets and the marks. Trends in Genetics. 23, 413-418.

Frazier, T.W., Thompson, L., Youngstrom, E.A., Law, P., Hardan, A.Y., Eng, C., Morris, N., 2014. A twin study of heritable and shared environmental contributions to autism. Journal of autism and developmental disorders. 44, 2013-2025.

Fromme, H., Mosch, C., Morovitz, M., Alba-Alejandre, I., Boehmer, S., Kiranoglu, M., Faber, F., Hannibal, I., Genzel-Boroviczény, O., Koletzko, B., 2010. Pre-and postnatal exposure to perfluorinated compounds (PFCs). Environmental science & technology. 44, 7123-7129.

Fuentes, S., Colomina, M.T., Vicens, P., Franco-Pons, N., Domingo, J.L., 2007. Concurrent exposure to perfluorooctane sulfonate and restraint stress during pregnancy in mice: effects on postnatal development and behavior of the offspring. Toxicological Sciences. 98, 589-598.

Glynn, A., Berger, U., Bignert, A., Ullah, S., Aune, M., Lignell, S., Darnerud, P.O., 2012. Perfluorinated alkyl acids in blood serum from primiparous women in Sweden: serial sampling during pregnancy and nursing, and temporal trends 1996–2010. Environmental science & technology. 46, 9071-9079.

Glynn, A., Kotova, N., Dahlgren, E., Lindh, C., Jakobsson, K., Gyllenhammar, I., Lignell, S., Nälsén, C., 2020. Determinants of serum concentrations of perfluoroalkyl acids (PFAAs) in school children and the contribution of low-level PFAA-contaminated drinking water. Environmental Science: Processes & Impacts. 22, 930-944.

Göckener, B., Weber, T., Rüdel, H., Bücking, M., Kolossa-Gehring, M., 2020. Human biomonitoring of per-and polyfluoroalkyl substances in German blood plasma samples from 1982 to 2019. Environment International. 145, 106123.

Goudarzi, H., Nakajima, S., Ikeno, T., Sasaki, S., Kobayashi, S., Miyashita, C., Ito, S., Araki, A., Nakazawa, H., Kishi, R., 2016. Prenatal exposure to perfluorinated chemicals and neurodevelopment in early infancy: The Hokkaido Study. Science of the Total Environment. 541, 1002-1010.

Gützkow, K.B., Haug, L.S., Thomsen, C., Sabaredzovic, A., Becher, G., Brunborg, G., 2012. Placental transfer of perfluorinated compounds is selective–a Norwegian Mother and Child sub-cohort study. International journal of hygiene and environmental health. 215, 216-219.

Gyllenhammar, I., Benskin, J.P., Sandblom, O., Berger, U., Ahrens, L., Lignell, S., Wiberg, K., Glynn, A., 2018. Perfluoroalkyl acids (PFAAs) in serum from 2–4-month-old infants: Influence of maternal serum concentration, gestational age, breast-feeding, and contaminated drinking water. Environmental science & technology. 52, 7101-7110.

Hallmayer, J., Cleveland, S., Torres, A., Phillips, J., Cohen, B., Torigoe, T., Miller, J., Fedele, A., Collins, J., Smith, K., 2011. Genetic heritability and shared environmental factors among twin pairs with autism. Archives of general psychiatry. 68, 1095-1102.

Hanssen, L., Röllin, H., Odland, J.Ø., Moe, M.K., Sandanger, T.M., 2010. Perfluorinated compounds in maternal serum and cord blood from selected areas of South Africa: results of a pilot study. Journal of environmental monitoring. 12, 1355-1361.

Harada, K.H., Hitomi, T., Niisoe, T., Takanaka, K., Kamiyama, S., Watanabe, T., Moon, C.-s., Yang, H.-r., Hung, N.N., Koizumi, A., 2011. Odd-numbered perfluorocarboxylates predominate over perfluorooctanoic acid in serum samples from Japan, Korea and Vietnam. Environment international. 37, 1183-1189.

Harris, M.H., Oken, E., Rifas-Shiman, S.L., Calafat, A.M., Ye, X., Bellinger, D.C., Webster, T.F., White, R.F., Sagiv, S.K., 2018. Prenatal and childhood exposure to per-and polyfluoroalkyl substances (PFASs) and child cognition. Environment international. 115, 358-369.

Haug, L.S., Huber, S., Becher, G., Thomsen, C., 2011. Characterisation of human exposure pathways to perfluorinated compounds—comparing exposure estimates with biomarkers of exposure. Environment international. 37, 687-693.

Henrichs, J., Bongers-Schokking, J.J., Schenk, J.J., Ghassabian, A., Schmidt, H.G., Visser, T.J., Hooijkaas, H., de Muinck Keizer-Schrama, S.M., Hofman, A., Jaddoe, V.V., 2010. Maternal thyroid function during early pregnancy and cognitive functioning in early childhood: the generation R study. The Journal of Clinical Endocrinology & Metabolism. 95, 4227-4234.

Hernán, M.A., Hernández-Díaz, S., Robins, J.M., 2004. A structural approach to selection bias. Epidemiology. 615-625.

Hertz-Picciotto, I., Schmidt, R.J., Krakowiak, P., 2018a. Understanding environmental contributions to autism: Causal concepts and the state of science. Autism Research. 11, 554-586.

Hertz-Picciotto, I., Schmidt, R.J., Walker, C.K., Bennett, D.H., Oliver, M., Shedd-Wise, K.M., LaSalle, J.M., Giulivi, C., Puschner, B., Thomas, J., 2018b. A prospective study of environmental exposures and early biomarkers in autism spectrum disorder: design, protocols, and preliminary data from the MARBLES study. Environmental health perspectives. 126, 117004.

Hoffman, K., Webster, T.F., Weisskopf, M.G., Weinberg, J., Vieira, V.M., 2010. Exposure to polyfluoroalkyl chemicals and attention deficit/hyperactivity disorder in US children 12–15 years of age. Environmental health perspectives. 118, 1762-1767.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of nondetectable values. Applied occupational and environmental hygiene. 5, 46-51.

Hu, X.C., Andrews, D.Q., Lindstrom, A.B., Bruton, T.A., Schaider, L.A., Grandjean, P., Lohmann, R., Carignan, C.C., Blum, A., Balan, S.A., 2016. Detection of poly-and perfluoroalkyl substances (PFASs) in US drinking water linked to industrial sites, military fire training areas, and wastewater treatment plants. Environmental science & technology letters. 3, 344-350.

Hubbard, A.E., Ahern, J., Fleischer, N.L., Van der Laan, M., Satariano, S.A., Jewell, N., Bruckner, T., Satariano, W.A., 2010. To GEE or not to GEE: comparing population average and mixed models for estimating the associations between neighborhood risk factors and health. Epidemiology. 467-474.

Huttenlocher, P.R., 2009. Neural plasticity. Harvard University Press.

Inoue, K., Okada, F., Ito, R., Kato, S., Sasaki, S., Nakajima, S., Uno, A., Saijo, Y., Sata, F., Yoshimura, Y., 2004. Perfluorooctane sulfonate (PFOS) and related perfluorinated compounds in human maternal and cord blood samples: assessment of PFOS exposure in a susceptible population during pregnancy. Environmental health perspectives. 112, 1204-1207.

Itoh, S., Araki, A., Mitsui, T., Miyashita, C., Goudarzi, H., Sasaki, S., Cho, K., Nakazawa, H., Iwasaki, Y., Shinohara, N., 2016. Association of perfluoroalkyl substances exposure in utero with reproductive hormone levels in cord blood in the Hokkaido Study on Environment and Children's Health. Environment international. 94, 51-59.

Janecka, M., Mill, J., Basson, M., Goriely, A., Spiers, H., Reichenberg, A., Schalkwyk, L., Fernandes, C., 2017. Advanced paternal age effects in neurodevelopmental disorders—review of potential underlying mechanisms. Translational psychiatry. 7, e1019.

Jeddy, Z., Hartman, T.J., Taylor, E.V., Poteete, C., Kordas, K., 2017. Prenatal concentrations of Perfluoroalkyl substances and early communication development in British girls. Early human development. 109, 15-20.

Jian, J.-M., Chen, D., Han, F.-J., Guo, Y., Zeng, L., Lu, X., Wang, F., 2018. A short review on human exposure to and tissue distribution of per-and polyfluoroalkyl substances (PFASs). Science of the Total Environment. 636, 1058-1069.

Jian, J.-M., Guo, Y., Zeng, L., Liang-Ying, L., Lu, X., Wang, F., Zeng, E.Y., 2017. Global distribution of perfluorochemicals (PFCs) in potential human exposure source–a review. Environment international. 108, 51-62.

Jin, H., Mao, L., Xie, J., Zhao, M., Bai, X., Wen, J., Shen, T., Wu, P., 2020. Poly-and perfluoroalkyl substance concentrations in human breast milk and their associations with postnatal infant growth. Science of The Total Environment. 713, 136417.

Johansson, N., Eriksson, P., Viberg, H., 2009. Neonatal exposure to PFOS and PFOA in mice results in changes in proteins which are important for neuronal growth and synaptogenesis in the developing brain. Toxicological Sciences. 108, 412-418.

Johansson, N., Fredriksson, A., Eriksson, P., 2008. Neonatal exposure to perfluorooctane sulfonate (PFOS) and perfluorooctanoic acid (PFOA) causes neurobehavioural defects in adult mice. Neurotoxicology. 29, 160-169.

Kaiser, H.F., 1958. The varimax criterion for analytic rotation in factor analysis. Psychometrika. 23, 187-200.

Kaiser, H.F., 1960. The application of electronic computers to factor analysis. Educational and psychological measurement. 20, 141-151.

Kalkbrenner, A.E., Schmidt, R.J., Penlesky, A.C., 2014. Environmental chemical exposures and autism spectrum disorders: a review of the epidemiological evidence. Current problems in pediatric and adolescent health care. 44, 277-318.

Kashino, I., Sasaki, S., Okada, E., Matsuura, H., Goudarzi, H., Miyashita, C., Okada, E., Ito, Y.M., Araki, A., Kishi, R., 2020. Prenatal exposure to 11 perfluoroalkyl substances and fetal growth: A large-scale, prospective birth cohort study. Environment international. 136, 105355.

Kato, K., Basden, B.J., Needham, L.L., Calafat, A.M., 2011a. Improved selectivity for the analysis of maternal serum and cord serum for polyfluoroalkyl chemicals. Journal of chromatography A. 1218, 2133-2137.

Kato, K., Wong, L.-Y., Chen, A., Dunbar, C., Webster, G.M., Lanphear, B.P., Calafat, A.M., 2014. Changes in serum concentrations of maternal poly-and perfluoroalkyl substances over the course of pregnancy and predictors of exposure in a multiethnic cohort of Cincinnati, Ohio pregnant women during 2003–2006. Environmental science & technology. 48, 9600-9608.

Kato, K., Wong, L.Y., Jia, L.T., Kuklenyik, Z., Calafat, A.M., 2011b. Trends in exposure to polyfluoroalkyl chemicals in the U.S. Population: 1999-2008. Environ Sci Technol. 45, 8037-8045.

Kim, K., Bennett, D.H., Calafat, A.M., Hertz-Picciotto, I., Shin, H.-M., 2020. Temporal trends and determinants of serum concentrations of per-and polyfluoroalkyl substances among Northern California mothers with a young child, 2009–2016. Environmental research. 186, 109491.

Kim, S., Choi, K., Ji, K., Seo, J., Kho, Y., Park, J., Kim, S., Park, S., Hwang, I., Jeon, J., 2011a. Trans-placental transfer of thirteen perfluorinated compounds and relations with fetal thyroid hormones. Environmental science & technology. 45, 7465-7472.

Kim, S.-K., Lee, K.T., Kang, C.S., Tao, L., Kannan, K., Kim, K.-R., Kim, C.-K., Lee, J.S., Park, P.S., Yoo, Y.W., 2011b. Distribution of perfluorochemicals between sera and milk from the same mothers and implications for prenatal and postnatal exposures. Environmental pollution. 159, 169-174.

Kingsley, S.L., Eliot, M.N., Kelsey, K.T., Calafat, A.M., Ehrlich, S., Lanphear, B.P., Chen, A., Braun, J.M., 2018. Variability and predictors of serum perfluoroalkyl substance concentrations during pregnancy and early childhood. Environmental research. 165, 247-257.

Kissa, E., 2001. Fluorinated surfactants and repellents. CRC Press.

Koenker, R., Hallock, K.F., 2001. Quantile regression. Journal of economic perspectives. 15, 143-156.

Kudo, N., Mizuguchi, H., Yamamoto, A., Kawashima, Y., 1999. Alterations by perfluorooctanoic acid of glycerolipid metabolism in rat liver. Chemico-biological interactions. 118, 69-83.

Lau, C., Butenhoff, J.L., Rogers, J.M., 2004. The developmental toxicity of perfluoroalkyl acids and their derivatives. Toxicology and applied pharmacology. 198, 231-241.

Lau, C., Thibodeaux, J.R., Hanson, R.G., Rogers, J.M., Grey, B.E., Stanton, M.E., Butenhoff, J.L., Stevenson, L.A., 2003. Exposure to perfluorooctane sulfonate during pregnancy in rat and mouse. II: postnatal evaluation. Toxicological Sciences. 74, 382-392.

Lazarevic, N., Barnett, A.G., Sly, P.D., Knibbs, L.D., 2019. Statistical methodology in studies of prenatal exposure to mixtures of endocrine-disrupting chemicals: a review of existing approaches and new alternatives. Environmental health perspectives. 127, 026001.

Lee, I., Viberg, H., 2013. A single neonatal exposure to perfluorohexane sulfonate (PFHxS) affects the levels of important neuroproteins in the developing mouse brain. Neurotoxicology. 37, 190-196.

Lee, Y.J., Lee, H.-G., Yang, J.-H., 2013. Perfluorooctane sulfonate-induced apoptosis of cerebellar granule cells is mediated by ERK 1/2 pathway. Chemosphere. 90, 1597-1602.

Lenters, V., Iszatt, N., Forns, J., Čechová, E., Kočan, A., Legler, J., Leonards, P., Stigum, H., Eggesbø, M., 2019. Early-life exposure to persistent organic pollutants (OCPs, PBDEs, PCBs, PFASs) and attention-deficit/hyperactivity disorder: A multi-pollutant analysis of a Norwegian birth cohort. Environment international. 125, 33-42.

Li, Y., Fletcher, T., Mucs, D., Scott, K., Lindh, C.H., Tallving, P., Jakobsson, K., 2018. Half-lives of PFOS, PFHxS and PFOA after end of exposure to contaminated drinking water. Occupational and environmental medicine. 75, 46-51.

Liang, K.-Y., Zeger, S.L., 1986. Longitudinal data analysis using generalized linear models. Biometrika. 73, 13-22.

Lien, G.-W., Huang, C.-C., Shiu, J.-S., Chen, M.-H., Hsieh, W.-S., Guo, Y.-L., Chen, P.-C., 2016. Perfluoroalkyl substances in cord blood and attention deficit/hyperactivity disorder symptoms in seven-year-old children. Chemosphere. 156, 118-127.

Liew, Z., Goudarzi, H., Oulhote, Y., 2018. Developmental exposures to perfluoroalkyl substances (PFASs): an update of associated health outcomes. Current environmental health reports. 5, 1-19.

Liew, Z., Ritz, B., von Ehrenstein, O.S., Bech, B.H., Nohr, E.A., Fei, C., Bossi, R., Henriksen, T.B., Bonefeld-Jørgensen, E.C., Olsen, J., 2014. Attention deficit/hyperactivity disorder and childhood autism in association with prenatal exposure to perfluoroalkyl substances: a nested case–control study in the Danish National Birth Cohort. Environmental health perspectives. 123, 367-373.

Lindstrom, A.B., Strynar, M.J., Libelo, E.L., 2011. Polyfluorinated compounds: past, present, and future. Environmental science & technology. 45, 7954-7961.

Liu, J., Li, J., Liu, Y., Chan, H.M., Zhao, Y., Cai, Z., Wu, Y., 2011. Comparison on gestation and lactation exposure of perfluorinated compounds for newborns. Environment international. 37, 1206-1212.

Liu, R.C., Hurtt, M.E., Cook, J.C., Biegel, L.B., 1996. Effect of the peroxisome proliferator, ammonium perfluorooctanoate (C8), on hepatic aromatase activity in adult male CrI: CD BR (CD) Rats. Toxicological Sciences. 30, 220-228.

Long, M., Ghisari, M., Kjeldsen, L., Wielsøe, M., Nørgaard-Pedersen, B., Mortensen, E.L., Abdallah, M.W., Bonefeld-Jørgensen, E.C., 2019. Autism spectrum disorders, endocrine disrupting compounds, and heavy metals in amniotic fluid: a case-control study. Molecular autism. 10, 1.

Lubin, J.H., Colt, J.S., Camann, D., Davis, S., Cerhan, J.R., Severson, R.K., Bernstein, L., Hartge, P., 2004. Epidemiologic evaluation of measurement data in the presence of detection limits. Environmental health perspectives. 112, 1691-1696.

Lund, C.J., Donovan, J.C., 1967. Blood volume during pregnancy: significance of plasma and red cell volumes. American journal of obstetrics and gynecology. 98, 393-403.

Lyall, K., Schmidt, R.J., Hertz-Picciotto, I., 2014. Maternal lifestyle and environmental risk factors for autism spectrum disorders. International journal of epidemiology. 43, 443-464.

Lyall, K., Yau, V.M., Hansen, R., Kharrazi, M., Yoshida, C.K., Calafat, A.M., Windham, G., Croen, L.A., 2018. Prenatal maternal serum concentrations of per-and polyfluoroalkyl substances in association with autism spectrum disorder and intellectual disability. Environmental health perspectives. 126, 017001.

Ma, D., Lu, Y., Liang, Y., Ruan, T., Li, J., Zhao, C., Wang, Y., Jiang, G., 2021. A Critical Review on Transplacental Transfer of Per-and Polyfluoroalkyl Substances: Prenatal Exposure Levels, Characteristics, and Mechanisms. Environmental Science & Technology.

Maenner, M.J., Shaw, K.A., Baio, J., 2020. Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2016. MMWR Surveillance Summaries. 69, 1.

Mamsen, L.S., Björvang, R.D., Mucs, D., Vinnars, M.-T., Papadogiannakis, N., Lindh, C.H., Andersen, C.Y., Damdimopoulou, P., 2019. Concentrations of perfluoroalkyl substances (PFASs) in human embryonic and fetal organs from first, second, and third trimester pregnancies. Environment international. 124, 482-492.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.-J., Ballester, F., Basterrechea, M., Grimalt, J.O., Jiménez, A.-M., Kraus, T., Schettgen, T., Sunyer, J., 2015. Transfer of perfluoroalkyl substances from mother to fetus in a Spanish birth cohort. Environmental research. 142, 471-478.

Manzano-Salgado, C.B., Casas, M., Lopez-Espinosa, M.-J., Ballester, F., Martinez, D., Ibarluzea, J., Santa-Marina, L., Schettgen, T., Vioque, J., Sunyer, J., 2016. Variability of perfluoroalkyl substance concentrations in pregnant women by socio-demographic and dietary factors in a Spanish birth cohort. Environment international. 92, 357-365.

Mariussen, E., 2012. Neurotoxic effects of perfluoroalkylated compounds: mechanisms of action and environmental relevance. Archives of toxicology. 86, 1349-1367.

McCrory, C., Murray, A., 2013. The effect of breastfeeding on neuro-development in infancy. Maternal and child health journal. 17, 1680-1688.

Miranda, A., Sousa, N., 2018. Maternal hormonal milieu influence on fetal brain development. Brain and behavior. 8, e00920.

Modesto, T., Tiemeier, H., Peeters, R.P., Jaddoe, V.W., Hofman, A., Verhulst, F.C., Ghassabian, A., 2015. Maternal mild thyroid hormone insufficiency in early pregnancy and attention-deficit/hyperactivity disorder symptoms in children. JAMA pediatrics. 169, 838-845.

Mogensen, U.B., Grandjean, P., Nielsen, F., Weihe, P., Budtz-Jørgensen, E., 2015. Breastfeeding as an exposure pathway for perfluorinated alkylates. Environmental science & technology. 49, 10466-10473.

Mondal, D., Lopez-Espinosa, M.-J., Armstrong, B., Stein, C.R., Fletcher, T., 2012. Relationships of perfluorooctanoate and perfluorooctane sulfonate serum concentrations between mother–child pairs in a population with perfluorooctanoate exposure from drinking water. Environmental health perspectives. 120, 752-757.

Mondal, D., Weldon, R.H., Armstrong, B.G., Gibson, L.J., Lopez-Espinosa, M.-J., Shin, H.-M., Fletcher, T., 2014. Breastfeeding: a potential excretion route for mothers and implications for infant exposure to perfluoroalkyl acids. Environmental health perspectives. 122, 187-192.

Monroy, R., Morrison, K., Teo, K., Atkinson, S., Kubwabo, C., Stewart, B., Foster, W.G., 2008. Serum levels of perfluoroalkyl compounds in human maternal and umbilical cord blood samples. Environmental research. 108, 56-62.

Morreale de Escobar, G., Jesús Obregón, M.a., Escobar del Rey, F., 2000. Is neuropsychological development related to maternal hypothyroidism or to maternal hypothyroxinemia? The Journal of Clinical Endocrinology & Metabolism. 85, 3975-3987.

Morrow-Tlucak, M., Haude, R.H., Ernhart, C.B., 1988. Breastfeeding and cognitive development in the first 2 years of life. Social science & medicine. 26, 635-639.

Mullen, E.M., 1997. Mullen scales of early learning. Western Psychological Services.

Nagin, D.S., 2005. Group-based modeling of development. Harvard University Press.

National Institute of Child Health and Human Development Early Child Care Research Network, 2000. The relation of child care to cognitive and language development. Child Development. 960-980.

Nian, M., Luo, K., Luo, F., Aimuzi, R., Huo, X., Chen, Q., Tian, Y., Zhang, J., 2020. Association between Prenatal Exposure to PFAS and Fetal Sex Hormones: Are the Short-chain PFAS Safer? Environmental Science & Technology.

Nishimura, T., Takei, N., Tsuchiya, K.J., Asano, R., Mori, N., 2016. Identification of neurodevelopmental trajectories in infancy and of risk factors affecting deviant development: a longitudinal birth cohort study. International journal of epidemiology. 45, 543-553.

Niu, J., Liang, H., Tian, Y., Yuan, W., Xiao, H., Hu, H., Sun, X., Song, X., Wen, S., Yang, L., 2019. Prenatal plasma concentrations of Perfluoroalkyl and polyfluoroalkyl substances and neuropsychological development in children at four years of age. Environmental Health. 18, 53.

Nøst, T.H., Vestergren, R., Berg, V., Nieboer, E., Odland, J.Ø., Sandanger, T.M., 2014. Repeated measurements of per-and polyfluoroalkyl substances (PFASs) from 1979 to 2007 in males from Northern Norway: assessing time trends, compound correlations and relations to age/birth cohort. Environment international. 67, 43-53.

Ode, A., Rylander, L., Lindh, C.H., Källén, K., Jönsson, B.A., Gustafsson, P., Olofsson, P., Ivarsson, S.A., Rignell-Hydbom, A., 2013. Determinants of maternal and fetal exposure and temporal trends of perfluorinated compounds. Environmental Science and Pollution Research. 20, 7970-7978.

Oh, J., Bennett, D.H., Calafat, A.M., Tancredi, D., Roa, D.L., Schmidt, R.J., Hertz-Picciotto, I., Shin, H.-M., 2021a. Prenatal exposure to per-and polyfluoroalkyl substances in association with autism spectrum disorder in the MARBLES study. Environment International. 147, 106328.

Oh, J., Schmidt, R.J., Tancredi, D., Calafat, A.M., Roa, D.L., Hertz-Picciotto, I., Shin, H.-M., 2021b. Prenatal exposure to per-and polyfluoroalkyl substances and cognitive development in infancy and toddlerhood. Environmental Research. 196, 110939.

Okada, E., Sasaki, S., Saijo, Y., Washino, N., Miyashita, C., Kobayashi, S., Konishi, K., Ito, Y.M., Ito, R., Nakata, A., 2012. Prenatal exposure to perfluorinated chemicals and relationship with allergies and infectious diseases in infants. Environmental research. 112, 118-125.

Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. Environmental health perspectives. 115, 1298-1305.

Olsen, G.W., Mair, D.C., Lange, C.C., Harrington, L.M., Church, T.R., Goldberg, C.L., Herron, R.M., Hanna, H., Nobiletti, J.B., Rios, J.A., Reagen, W.K., Ley, C.A., 2017. Per- and polyfluoroalkyl substances (PFAS) in American Red Cross adult blood donors, 2000-2015. Environ Res. 157, 87-95.

Onishchenko, N., Fischer, C., Ibrahim, W.N.W., Negri, S., Spulber, S., Cottica, D., Ceccatelli, S., 2011. Prenatal exposure to PFOS or PFOA alters motor function in mice in a sex-related manner. Neurotoxicity research. 19, 452-461.

Ozonoff, S., Goodlin-Jones, B.L., Solomon, M., 2005. Evidence-based assessment of autism spectrum disorders in children and adolescents. Journal of Clinical Child and Adolescent Psychology. 34, 523-540.

Ozonoff, S., Young, G.S., Belding, A., Hill, M., Hill, A., Hutman, T., Johnson, S., Miller, M., Rogers, S.J., Schwichtenberg, A., 2014. The broader autism phenotype in infancy: when does it emerge? Journal of the American Academy of Child & Adolescent Psychiatry. 53, 398-407. e392.

Ozonoff, S., Young, G.S., Carter, A., Messinger, D., Yirmiya, N., Zwaigenbaum, L., Bryson, S., Carver, L.J., Constantino, J.N., Dobkins, K., 2011. Recurrence risk for autism spectrum disorders: a Baby Siblings Research Consortium study. Pediatrics. 128, e488-e495.

Park, S.K., Peng, Q., Ding, N., Mukherjee, B., Harlow, S.D., 2019. Determinants of per-and polyfluoroalkyl substances (PFAS) in midlife women: evidence of racial/ethnic and geographic differences in PFAS exposure. Environmental research. 175, 186-199.

Paul, A.G., Jones, K.C., Sweetman, A.J., 2009. A first global production, emission, and environmental inventory for perfluorooctane sulfonate. Environmental science & technology. 43, 386-392.

Pop, V.J., Brouwers, E.P., Vader, H.L., Vulsma, T., Van Baar, A.L., De Vijlder, J.J., 2003. Maternal hypothyroxinaemia during early pregnancy and subsequent child development: a 3-year follow-up study. Clinical endocrinology. 59, 282-288.

Pop, V.J., Kuijpens, J.L., van Baar, A.L., Verkerk, G., van Son, M.M., de Vijlder, J.J., Vulsma, T., Wiersinga, W.M., Drexhage, H.A., Vader, H.L., 1999. Low maternal free thyroxine concentrations during early pregnancy are associated with impaired psychomotor development in infancy. Clinical endocrinology. 50, 149-155.

Prasertcharoensuk, W., Swadpanich, U., Lumbiganon, P., 2000. Accuracy of the blood loss estimation in the third stage of labor. International Journal of Gynecology & Obstetrics. 71, 69-70.

Preston, E.V., Webster, T.F., Henn, B.C., McClean, M.D., Gennings, C., Oken, E., Rifas-Shiman, S.L., Pearce, E.N., Calafat, A.M., Fleisch, A.F., 2020. Prenatal exposure to per- and polyfluoroalkyl substances and maternal and neonatal thyroid function in the project viva cohort: a mixtures approach. Environment international. 139, 105728.

Prevedouros, K., Cousins, I.T., Buck, R.C., Korzeniowski, S.H., 2006. Sources, fate and transport of perfluorocarboxylates. Environmental science & technology. 40, 32-44.

Raftery, A.E., 1995. Bayesian model selection in social research. Sociological methodology. 111-163.

Reistad, T., Fonnum, F., Mariussen, E., 2013. Perfluoroalkylated compounds induce cell death and formation of reactive oxygen species in cultured cerebellar granule cells. Toxicology letters. 218, 56-60.

Richardson, D.B., Ciampi, A., 2003. Effects of exposure measurement error when an exposure variable is constrained by a lower limit. American journal of epidemiology. 157, 355-363.

Román, G.C., Ghassabian, A., Bongers-Schokking, J.J., Jaddoe, V.W., Hofman, A., De Rijke, Y.B., Verhulst, F.C., Tiemeier, H., 2013. Association of gestational maternal hypothyroxinemia and increased autism risk. Annals of neurology. 74, 733-742.

Rosner, B., 2015. Fundamentals of biostatistics. Cengage learning.

Russell, M.H., Waterland, R.L., Wong, F., 2015. Calculation of chemical elimination half-life from blood with an ongoing exposure source: The example of perfluorooctanoic acid (PFOA). Chemosphere. 129, 210-216.

Rutter, M., 1985. Family and school influences on cognitive development. Journal of child psychology and psychiatry. 26, 683-704.

Sagiv, S.K., Rifas-Shiman, S.L., Webster, T.F., Mora, A.M., Harris, M.H., Calafat, A.M., Ye, X., Gillman, M.W., Oken, E., 2015. Sociodemographic and perinatal predictors of early pregnancy per-and polyfluoroalkyl substance (PFAS) concentrations. Environmental science & technology. 49, 11849-11858.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C.M., Reichenberg, A., 2014. The familial risk of autism. Jama. 311, 1770-1777.

Sato, I., Kawamoto, K., Nishikawa, Y., Tsuda, S., Yoshida, M., Yaegashi, K., Saito, N., Liu, W., Jin, Y., 2009. Neurotoxicity of perfluorooctane sulfonate (PFOS) in rats and mice after single oral exposure. The Journal of toxicological sciences. 34, 569-574.

Seacat, A.M., Thomford, P.J., Hansen, K.J., Olsen, G.W., Case, M.T., Butenhoff, J.L., 2002. Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. Toxicological Sciences. 68, 249-264.

Seals, R., Bartell, S.M., Steenland, K., 2011. Accumulation and clearance of perfluorooctanoic acid (PFOA) in current and former residents of an exposed community. Environmental health perspectives. 119, 119-124.

Shelton, J.F., Tancredi, D.J., Hertz-Picciotto, I., 2010. Independent and dependent contributions of advanced maternal and paternal ages to autism risk. Autism Research. 3, 30-39.

Shin, H.-M., Bennett, D.H., Calafat, A.M., Tancredi, D., Hertz-Picciotto, I., 2020. Modeled prenatal exposure to per-and polyfluoroalkyl substances in association with child autism spectrum disorder: A case-control study. Environmental Research. 109514.

Shin, H.-M., Vieira, V.M., Ryan, P.B., Detwiler, R., Sanders, B., Steenland, K., Bartell, S.M., 2011. Environmental fate and transport modeling for perfluorooctanoic acid emitted from the Washington Works Facility in West Virginia. Environmental science & technology. 45, 1435-1442.

Skogheim, T.S., Villanger, G.D., Weyde, K.V.F., Engel, S.M., Surén, P., Øie, M.G., Skogan, A.H., Biele, G., Zeiner, P., Øvergaard, K.R., 2020. Prenatal exposure to perfluoroalkyl substances and associations with symptoms of attention-deficit/hyperactivity disorder and cognitive functions in preschool children. International journal of hygiene and environmental health. 223, 80-92.

Spiegelman, J., Meng, M.-L., Haythe, J., Goffman, D., 2020. Cardiovascular Physiology of Pregnancy and Clinical Implications. Cardio-Obstetrics: A Practical Guide to Care for Pregnant Cardiac Patients.

Spratlen, M.J., Perera, F.P., Lederman, S.A., Rauh, V.A., Robinson, M., Kannan, K., Trasande, L., Herbstman, J., 2020. The association between prenatal exposure to perfluoroalkyl substances and childhood neurodevelopment. Environmental Pollution. 114444.

Stafoggia, M., Breitner, S., Hampel, R., Basagaña, X., 2017. Statistical approaches to address multi-pollutant mixtures and multiple exposures: the state of the science. Current environmental health reports. 4, 481-490.

Stein, C.R., Wolff, M.S., Calafat, A.M., Kato, K., Engel, S.M., 2012. Comparison of polyfluoroalkyl compound concentrations in maternal serum and amniotic fluid: a pilot study. Reproductive Toxicology. 34, 312-316.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly-and perfluoroalkyl substances

(PFASs) and present understanding of health effects. Journal of exposure science & environmental epidemiology. 29, 131-147.

Swineford, L.B., Guthrie, W., Thurm, A., 2015. Convergent and divergent validity of the Mullen Scales of Early Learning in young children with and without autism spectrum disorder. Psychological Assessment. 27, 1364.

Takagai, S., Tsuchiya, K.J., Itoh, H., Kanayama, N., Mori, N., Takei, N., 2016. Cohort profile: Hamamatsu birth cohort for mothers and children (HBC Study). International journal of epidemiology. 45, 333-342.

Thomsen, C., Haug, L.S., Stigum, H., Frøshaug, M., Broadwell, S.L., Becher, G., 2010. Changes in concentrations of perfluorinated compounds, polybrominated diphenyl ethers, and polychlorinated biphenyls in Norwegian breast-milk during twelve months of lactation. Environmental science & technology. 44, 9550-9556.

Tian, Y., Zhou, Y., Miao, M., Wang, Z., Yuan, W., Liu, X., Wang, X., Wang, Z., Wen, S., Liang, H., 2018. Determinants of plasma concentrations of perfluoroalkyl and polyfluoroalkyl substances in pregnant women from a birth cohort in Shanghai, China. Environment international. 119, 165-173.

Tordjman, S., Somogyi, E., Coulon, N., Kermarrec, S., Cohen, D., Bronsard, G., Bonnot, O., Weismann-Arcache, C., Botbol, M., Lauth, B., 2014. Gene× Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. Frontiers in psychiatry. 5, 53.

Trudel, D., Horowitz, L., Wormuth, M., Scheringer, M., Cousins, I.T., Hungerbühler, K., 2008. Estimating consumer exposure to PFOS and PFOA. Risk Analysis: An International Journal. 28, 251-269.

Tsai, M.-S., Miyashita, C., Araki, A., Itoh, S., Bamai, Y., Goudarzi, H., Okada, E., Kashino, I., Matsuura, H., Kishi, R., 2018. Determinants and Temporal Trends of Perfluoroalkyl Substances in Pregnant Women: The Hokkaido Study on Environment and Children's Health. International journal of environmental research and public health. 15, 989.

Tsuchiya, K., Matsumoto, K., Suda, S., Miyachi, T., Itoh, H., Kanayama, N., Hirano, K., Ohzeki, T., Takei, N., Team, H.S., 2010. Searching for very early precursors of autism spectrum disorders: the Hamamatsu Birth Cohort for Mothers and Children (HBC). Journal of developmental origins of health and disease. 1, 158-173.

Turner-Brown, L.M., Baranek, G.T., Reznick, J.S., Watson, L.R., Crais, E.R., 2013. The First Year Inventory: a longitudinal follow-up of 12-month-old to 3-year-old children. Autism. 17, 527-540.

von Ehrenstein, O.S., Fenton, S.E., Kato, K., Kuklenyik, Z., Calafat, A.M., Hines, E.P., 2009. Polyfluoroalkyl chemicals in the serum and milk of breastfeeding women. Reproductive toxicology. 27, 239-245.

Vuong, A.M., Webster, G.M., Yolton, K., Calafat, A.M., Muckle, G., Lanphear, B.P., Chen, A., 2021. Prenatal exposure to per-and polyfluoroalkyl substances (PFAS) and neurobehavior in US children through 8 years of age: The HOME study. Environmental Research. 195, 110825.

Vuong, A.M., Yolton, K., Webster, G.M., Sjödin, A., Calafat, A.M., Braun, J.M., Dietrich, K.N., Lanphear, B.P., Chen, A., 2016. Prenatal polybrominated diphenyl ether and perfluoroalkyl substance exposures and executive function in school-age children. Environmental research. 147, 556-564.

Wang, Y., Rogan, W.J., Chen, P.-C., Lien, G.-W., Chen, H.-Y., Tseng, Y.-C., Longnecker, M.P., Wang, S.-L., 2014. Association between maternal serum perfluoroalkyl substances during pregnancy and maternal and cord thyroid hormones: Taiwan maternal and infant cohort study. Environmental health perspectives. 122, 529-534.

Wang, Z., Zhang, T., Wu, J., Wei, X., Xu, A., Wang, S., Wang, Z., 2021. Male reproductive toxicity of perfluorooctanoate (PFOA): Rodent studies. Chemosphere. 270, 128608.

Wei, Y., Kehm, R.D., Goldberg, M., Terry, M.B., 2019. Applications for quantile regression in epidemiology. Current Epidemiology Reports. 6, 191-199.

Weng, H.-Y., Hsueh, Y.-H., Messam, L.L.M., Hertz-Picciotto, I., 2009. Methods of covariate selection: directed acyclic graphs and the change-in-estimate procedure. American journal of epidemiology. 169, 1182-1190.

Werling, D.M., Geschwind, D.H., 2013. Sex differences in autism spectrum disorders. Current opinion in neurology. 26, 146.

White, I.R., Royston, P., Wood, A.M., 2011. Multiple imputation using chained equations: issues and guidance for practice. Statistics in medicine. 30, 377-399.

Wong, F., MacLeod, M., Mueller, J.F., Cousins, I.T., 2014. Enhanced elimination of perfluorooctane sulfonic acid by menstruating women: evidence from population-based pharmacokinetic modeling. Environmental science & technology. 48, 8807-8814.

Worley, R.R., Moore, S.M., Tierney, B.C., Ye, X., Calafat, A.M., Campbell, S., Woudneh, M.B., Fisher, J., 2017. Per-and polyfluoroalkyl substances in human serum and urine samples from a residentially exposed community. Environment international. 106, 135-143.

Yahia, D., Abd El-Nasser, M., Abedel-Latif, M., Tsukuba, C., Yoshida, M., Sato, I., Tsuda, S., 2010. Effects of perfluorooctanoic acid (PFOA) exposure to pregnant mice on reproduction. The Journal of toxicological sciences. 35, 527-533.

Yao, Q., Shi, R., Wang, C., Han, W., Gao, Y., Zhang, Y., Zhou, Y., Ding, G., Tian, Y., 2019. Cord blood Per-and polyfluoroalkyl substances, placental steroidogenic enzyme, and cord blood reproductive hormone. Environment international. 129, 573-582.

Yeates, K.O., MacPhee, D., Campbell, F.A., Ramey, C.T., 1983. Maternal IQ and home environment as determinants of early childhood intellectual competence: A developmental analysis. Developmental Psychology. 19, 731.

Yuan, G., Peng, H., Huang, C., Hu, J., 2016. Ubiquitous occurrence of fluorotelomer alcohols in eco-friendly paper-made food-contact materials and their implication for human exposure. Environmental science & technology. 50, 942-950.

Zhang, T., Sun, H., Lin, Y., Qin, X., Zhang, Y., Geng, X., Kannan, K., 2013. Distribution of poly-and perfluoroalkyl substances in matched samples from pregnant women and carbon chain length related maternal transfer. Environmental science & technology. 47, 7974-7981.

Zheng, G., Schreder, E., Dempsey, J.C., Uding, N., Chu, V., Andres, G., Sathyanarayana, S., Salamova, A., 2021. Per-and Polyfluoroalkyl Substances (PFAS) in Breast Milk: Concerning Trends for Current-Use PFAS. Environmental Science & Technology.

Zoeller, R., Rovet, J., 2004. Timing of thyroid hormone action in the developing brain: clinical observations and experimental findings. Journal of neuroendocrinology. 16, 809-818.

Zou, G., 2004. A modified poisson regression approach to prospective studies with binary data. American journal of epidemiology. 159, 702-706.

APPENDIX: SUPPLEMENTAL MATERIAL

Table S2.1 Median (25th, 75th percentiles) of the prenatal maternal PFAS [a] serum concentrations and the principal component scores in relation to population characteristics in ASD and TD groups (n = 173).

Characteristics	Total	PFOA	PFOS	PFHxS	PFNA	PC-1
Child's sex						
Female	71	0.8 (0.6, 1.2)	2.9 (1.7, 3.8)	0.4 (0.3, 0.6)	0.5 (0.4, 0.7)	-0.1 (-1.2, 0.9)
Male	102	1.0 (0.7, 1.4)	3.0 (2.3, 4.6)	0.5 (0.3, 0.8)	0.5 (0.4, 0.6)	0.3 (-0.6, 1.3)
p-value [c]		0.09	**0.07**	**0.02**	0.42	0.05
Child's birth year						
2009-2010	61	1.1 (0.9, 1.4)	3.9 (3.0, 5.5)	0.5 (0.3, 0.6)	0.6 (0.4, 0.7)	0.7 (0.0, 1.4)
2011-2013	57	1.0 (0.7, 1.7)	2.9 (2.2, 4.9)	0.5 (0.4, 0.9)	0.6 (0.4, 0.7)	0.5 (-0.6, 1.7)
2014-2015	55	0.7 (0.5, 0.9)	2.2 (1.4, 2.9)	0.4 (0.3, 0.6)	0.4 (0.3, 0.5)	-0.7 (-1.9, 0.0)
p-value		**<0.001**	**<0.001**	0.06	**<0.001**	**<0.001**
Gestational age at delivery						
≤ 37 weeks	10	0.6 (0.5, 0.9)	3.2 (2.2, 6.2)	0.3 (0.2, 1.2)	0.4 (0.3, 0.6)	-0.6 (-1.4, 1.2)
> 37 weeks	163	1.0 (0.7, 1.4)	2.9 (2.1, 4.3)	0.5 (0.3, 0.7)	0.5 (0.4, 0.7)	0.1 (-0.7, 1.1)
p-value		**0.04**	0.70	0.42	0.31	0.46
Maternal age at delivery						
< 35 years	93	0.8 (0.6, 1.3)	2.8 (1.9, 4.1)	0.4 (0.3, 0.6)	0.4 (0.3, 0.6)	-0.1 (-1.4, 0.9)
≥ 35 years	80	1.0 (0.8, 1.4)	3.4 (2.3, 4.8)	0.5 (0.3, 0.7)	0.5 (0.4, 0.7)	0.4 (-0.4, 1.1)
p-value		**0.05**	**0.03**	0.14	**0.03**	**0.02**
Maternal BMI at pre-pregnancy						
Normal/ underweight	83	0.9 (0.7, 1.2)	2.9 (2.3, 4.4)	0.5 (0.3, 0.6)	0.5 (0.4, 0.6)	0.1 (-0.7, 0.9)
Overweight	50	0.9 (0.6, 1.1)	2.7 (1.5, 3.8)	0.4 (0.3, 0.6)	0.5 (0.3, 0.8)	-0.1 (-1.6, 0.8)
Obese	40	1.2 (0.8, 1.6)	3.6 (2.3, 5.9)	0.5 (0.4, 0.7)	0.6 (0.4, 0.7)	0.8 (-0.2, 1.4)
p-value		**0.03**	**0.02**	0.17	0.32	**0.04**
Gestational diabetes						
Yes	32	0.8 (0.6, 1.2)	3.0 (1.8, 5.0)	0.5 (0.3, 0.6)	0.5 (0.4, 0.6)	0.0 (-1.0, 1.2)
No	140	1.0 (0.7, 1.3)	3.0 (2.2, 4.4)	0.4 (0.3, 0.7)	0.5 (0.4, 0.7)	0.1 (-0.7, 1.0)
p-value		0.17	0.72	0.97	0.97	0.51
Maternal race/ethnicity						
Non-Hispanic white	94	0.9 (0.7, 1.3)	3.2 (2.3, 4.9)	0.5 (0.3, 0.7)	0.5 (0.4, 0.6)	0.1 (-0.8, 1.1)
Hispanic	41	0.9 (0.6, 1.4)	2.7 (1.8, 3.7)	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	0.1 (-0.8, 0.9)
Other [d]	38	0.9 (0.7, 1.3)	2.9 (1.7, 4.2)	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	0.1 (-0.7, 1.3)
p-value		0.99	0.08	0.44	0.07	0.99
Maternal education						
High school, some college	90	0.9 (0.6, 1.3)	2.8 (2.1, 3.9)	0.4 (0.3, 0.7)	0.5 (0.4, 0.6)	0.0 (-0.8, 0.8)
Bachelor's degree	50	1.0 (0.7, 1.5)	3.6 (2.3, 5.3)	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	0.7 (-0.8, 1.4)
Graduate or professional	33	0.8 (0.7, 1.2)	3.1 (2.1, 3.9)	0.5 (0.3, 0.6)	0.5 (0.4, 0.7)	0.1 (-0.7, 0.9)
p-value		0.50	0.17	0.82	0.35	0.35
Homeownership						
Yes	96	0.9 (0.7, 1.4)	3.1 (2.3, 4.6)	0.5 (0.3, 0.7)	0.6 (0.4, 0.7)	0.3 (-0.6, 1.2)
No	75	0.8 (0.6, 1.3)	2.8 (1.7, 4.1)	0.4 (0.3, 0.6)	0.4 (0.4, 0.6)	-0.1 (-1.1, 0.8)
p-value		0.19	0.19	0.58	**0.01**	**0.04**
Parity						
≤ 1	72	1.1 (0.8, 1.6)	3.2 (2.2, 5.0)	0.5 (0.4, 0.7)	0.6 (0.4, 0.7)	0.4 (-0.3, 1.4)
> 1	99	0.9 (0.6, 1.1)	2.8 (2.1, 3.9)	0.4 (0.3, 0.6)	0.5 (0.4, 0.6)	-0.1 (-1.1, 0.7)
p-value		**0.01**	0.14	**0.02**	0.17	**0.02**
Maternal vitamin intake in the first month of pregnancy						
Yes	88	0.9 (0.6, 1.3)	2.8 (2.1, 3.8)	0.4 (0.3, 0.6)	0.5 (0.4, 0.7)	0.0 (-1.0, 0.9)
No	84	1.0 (0.7, 1.3)	3.2 (2.4, 5.0)	0.4 (0.3, 0.7)	0.5 (0.4, 0.6)	0.3 (-0.6, 1.1)
p-value		0.34	**0.05**	0.83	0.79	0.29

Breastfeeding duration (months)						
Q1 (< 5.0)	42	1.0 (0.8, 1.3)	3.2 (2.5, 4.5)	0.5 (0.3, 0.7)	0.5 (0.4, 0.7)	0.2 (-0.2, 1.4)
Q2 (5.0 - < 9.9	41	0.9 (0.6, 1.3)	3.0 (1.5, 3.9)	0.4 (0.3, 0.6)	0.5 (0.3, 0.6)	0.1 (-1.6, 0.9)
Q3 (9.9 - < 15.0)	40	0.9 (0.6, 1.3)	2.6 (2.1, 4.3)	0.4 (0.3, 0.7)	0.5 (0.3, 0.6)	-0.1 (-1.2, 0.7)
Q4 (> 15.0)	40	1.0 (0.6, 1.6)	3.0 (2.3, 4.7)	0.4 (0.3, 0.6)	0.6 (0.4, 0.7)	0.5 (-0.7, 1.7)
p-value		0.39	0.35	0.60	0.11	0.21

[a] PFDA, PFUnDA, PFDoDA, MeFOSAA, and EtFOSAA that were detected in fewer than 85% of the samples were excluded in this table.

[b] Missing information (*n*): gestational diabetes (1), homeownership (2), parity (2), maternal vitamin intake in the first month of pregnancy (1), breastfeeding duration (10).

[c] *p*-value from the Wilcoxon rank-sum test for binary variables and the Kruskal-Wallis test for other categorical variables. *p*-values less than 0.05 are highlighted in bold.

[d] Includes Black/African American (3%), Asian (16%), and multiracial (3%).

Table S2.2 Summary statistics of maternal PFAS concentrations of each trimester of pregnancy among 40 mothers who provided blood samples during all trimesters.

PFAS	1st trimester				2nd trimester				3rd trimester				*p*-value [b]
	GM [a]	Percentiles			GM	Percentiles			GM	Percentiles			
		5th	50th	95th		5th	50th	95th		5th	50th	95th	
PFOA	0.92	0.45	0.95	2.30	0.82	0.35	0.85	2.00	0.76	0.35	0.80	1.75	0.18
PFOS	3.00	1.15	3.05	6.00	2.88	1.10	3.15	6.05	2.56	0.80	2.70	5.40	0.44
PFHxS	0.41	0.10	0.40	1.70	0.44	0.20	0.40	1.75	0.41	0.10	0.40	1.50	0.97
PFNA	0.54	0.20	0.55	1.10	0.47	0.20	0.50	0.95	0.44	0.20	0.40	0.90	0.13
PFDA	0.17	<LOD	0.20	0.35	0.18	<LOD	0.20	0.30	0.15	<LOD	0.10	0.30	0.89
PFUnDA	0.14	<LOD	0.10	0.30	0.13	<LOD	0.10	0.20	0.14	<LOD	0.10	0.25	0.72
PFDoDA	0.11	<LOD	<LOD	0.10	0.11	<LOD	<LOD	0.15	0.11	<LOD	<LOD	0.10	0.96
MeFOSAA	0.23	<LOD	<LOD	1.25	0.19	<LOD	0.10	0.95	0.19	<LOD	<LOD	0.95	0.53
EtFOSAA	-	<LOD	<LOD	<LOD	-	<LOD	<LOD	<LOD	-	<LOD	<LOD	<LOD	0.98

[a] *p*-value from the Wilcoxon rank-sum test to compare the median of the PFAS concentrations from different trimesters.

Table S2.3 Spearman's correlation coefficients among four maternal PFAS concentrations of each trimester of pregnancy from 40 mothers who provided blood samples during all trimesters.

	PFOA-1T	PFOA-2T	PFOA-3T	PFOS-1T	PFOS-2T	PFOS-3T	PFHxS-1T	PFHxS-2T	PFHxS-3T	PFNA-1T	PFNA-2T	PFNA-3T
PFOA-1T	1.00											
PFOA-2T	0.95	1.00										
PFOA-3T	0.94	0.98	1.00									
PFOS-1T	0.49	0.42	0.49	1.00								
PFOS-2T	0.36	0.37	0.43	0.86	1.00							
PFOS-3T	0.48	0.46	0.54	0.94	0.87	1.00						
PFHxS-1T	0.65	0.65	0.68	0.47	0.37	0.48	1.00					
PFHxS-2T	0.23	0.18	0.24	0.29	0.45	0.29	0.30	1.00				
PFHxS-3T	0.66	0.69	0.73	0.53	0.55	0.57	0.87	0.46	1.00			
PFNA-1T	0.50	0.42	0.44	0.48	0.49	0.48	0.51	0.26	0.43	1.00		
PFNA-2T	0.48	0.48	0.49	0.37	0.46	0.42	0.50	0.22	0.47	0.94	1.00	
PFNA-3T	0.52	0.51	0.54	0.46	0.53	0.55	0.53	0.26	0.53	0.90	0.94	1.00

Note: Machine-observed concentrations were used for PFAS concentrations below the limit of detection. 1T stands for the first trimester, 2T for the second trimester, and 3T for the third trimester of pregnancy.

Table S2.4 Spearman's correlation coefficients among prenatal maternal PFAS concentrations of 204 mother-child pairs.

	PFOA	PFOS	PFHxS	PFNA	PFDA	PFUnDA	PFDoDA	MeFOSAA	EtFOSAA
PFOA	1.00								
PFOS	0.62**	1.00							
PFHxS	0.46**	0.51**	1.00						
PFNA	0.64**	0.65**	0.36**	1.00					
PFDA	0.43**	0.52**	0.22**	0.62**	1.00				
PFUnDA	0.28**	0.30**	0.13*	0.53**	0.55**	1.00			
PFDoDA	-0.10	-0.03	0.08	0.11	0.02	-0.03	1.00		
MeFOSAA	0.14*	0.36**	0.16**	0.03	0.02	-0.15*	-0.07	1.00	
EtFOSAA	-0.02	-0.02	-0.07	0.04	0.03	0.01	0.04	0.11	1.00

Note: Machine-observed concentrations were used for PFAS concentrations below the limit of detection. Correlation coefficients of PFUnDA, PFDoDA, MeFOSAA, and EtFOSAA should be interpreted cautiously due to their low detection frequency (60%, 33%, 55%, 4%, respectively).

** p-value < 0.01, * p-value < 0.05

Table S2.5 Sensitivity analysis I: adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (n = 57) versus TD (n = 116) in association with prenatal maternal PFAS concentrations after excluding the top 2.5 percentiles.

PFAS [a]	Log 2-transformed PFAS RR [b] (95% CI)	Untransformed PFAS RR [b] (95% CI)
PFOA	1.17 (0.84, 1.64)	1.37 (0.88, 2.15)
PFOS	0.97 (0.72, 1.32)	1.00 (0.87, 1.15)
PFHxS	0.90 (0.77, 1.04)	1.04 (0.62, 1.76)
PFNA	1.14 (0.79, 1.63)	1.69 (0.64, 4.46)

[a] Four PFAS detected in more than 99% of the study samples were individually included in the Poisson regression model.

[b] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

Table S2.6 Sensitivity analysis II: adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (n = 57) versus TD (n = 116) in association with prenatal maternal PFAS concentrations and principal component scores.

PFAS [a] or principal component score	Log 2-transformed PFAS RR [a] (95% CI)	Untransformed PFAS RR [a] (95% CI)
a) Additionally adjusted for potential confounders [b]		
PFOA	1.21 (0.89, 1.63)	1.32 (1.03, 1.71)
PFOS	0.97 (0.74, 1.28)	0.98 (0.89, 1.08)
PFHxS	0.88 (0.77, 1.00)	0.89 (0.53, 1.47)
PFNA	1.25 (0.88, 1.76)	1.91 (1.09, 3.35)
PC-1	1.03 (0.86, 1.22)	1.10 (0.96, 1.27)
b) Using the highest PFAS concentration instead of average		
PFOA	1.18 (0.89, 1.58)	1.30 (1.04, 1.63)
PFOS	0.97 (0.74, 1.28)	0.98 (0.89, 1.08)
PFHxS	0.88 (0.77, 1.01)	0.91 (0.53, 1.56)
PFNA	1.27 (0.94, 1.72)	1.79 (1.12, 2.85)
PC-1	1.03 (0.87, 1.22)	1.10 (0.96, 1.26)
c) Using randomly selected one PFAS concentration instead of average		
PFOA	1.19 (0.90, 1.59)	1.30 (1.04, 1.63)
PFOS	0.94 (0.71, 1.24)	0.98 (0.89, 1.08)
PFHxS	0.87 (0.76, 1.00)	0.91 (0.53, 1.56)
PFNA	1.17 (0.86, 1.61)	1.79 (1.12, 2.85)
PC-1	1.01 (0.85, 1.20)	1.10 (0.96, 1.26)

[a] Final Poisson regression models were adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[b] Additionally adjusted for maternal pre-pregnancy BMI (normal/underweight, overweight, obese), parity (≤ 1, > 1), maternal age at delivery (< 35 years, ≥ 35 years), and maternal race/ethnicity (non-Hispanic white, Hispanic, other).

Table S2.7 Sensitivity analysis III: adjusted relative risk (RR) and 95% confidence interval (CI) for ASD (n = 57) versus TD (n = 116) in association with prenatal maternal untransformed PFAS concentrations stratified by child's sex and maternal age at delivery.

Untransformed PFAS	**RR** (95% CI)		***p*-value** [a]
Child's sex [b]	**Females** [c]	**Males** [d]	
PFOA	1.16 (0.88, 1.54)	1.47 (0.99, 2.19)	1.00
PFOS	1.06 (0.88, 1.28)	1.00 (0.91, 1.09)	0.79
PFHxS	0.65 (0.12, 3.47)	1.27 (0.81, 2.00)	0.35
PFNA	1.75 (0.85, 3.60)	1.62 (0.79, 3.35)	0.22
PC-1	1.12 (0.95, 1.31)	1.13 (0.95, 1.33)	0.41
Maternal age at delivery [e]	**< 35 years** [f]	**≥ 35 years** [g]	
PFOA	1.06 (0.76, 1.54)	2.90 (1.65, 5.12)	0.01
PFOS	0.83 (0.67, 1.04)	1.10 (0.96, 1.27)	0.03
PFHxS	0.60 (0.30, 1.32)	1.51 (0.74, 3.08)	0.15
PFNA	1.25 (0.63, 2.57)	4.05 (1.48, 11.1)	0.04
PC-1	0.95 (0.77, 1.20)	1.52 (1.19, 1.95)	0.03

[a] p-value for interaction term of PFAS with each potential effect modifier (potential effect modifiers and an interaction term were added as additional terms in the regression model, along with other covariates).

[b] Adjusted for child's birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[c] Females (n = 71; TD = 54, ASD = 17), [d] Males (n = 102; TD = 62, ASD = 40).

[e] Adjusted for child's sex and birth year, homeownership, maternal education, and maternal vitamin intake in the first month of pregnancy.

[f] < 35 years (n = 93; TD = 62, ASD = 31), [g] ≥ 35 years (n = 80; TD = 54, ASD = 26).

Note: underlined RR estimates had the ratio of the upper to lower 95% confidence limits larger than 10, indicating the instability of the results from this model.

Table S3.1 Characteristics of the study population included in the current study and the MARBLES baseline population.

Characteristic	Current study [a] (*n* = 140)		MARBLES [b] (*n* = 218)		*p*-value [c]
	n	%	*n*	%	
Child sex					
Female	57	41%	92	42%	0.78
Male	83	59%	126	58%	
Child's birth year					
2009-2010	46	33%	77	35%	0.77
2011-2013	50	36%	70	32%	
2014-2015	44	31%	71	33%	
Gestational age at delivery					
≤ 37 weeks	12	9%	13	6%	0.35
> 37 weeks	128	91%	205	94%	
Maternal age at delivery					
< 35 years	72	51%	118	54%	0.52
≥ 35 years	68	49%	97	44%	
Maternal pre-pregnancy BMI					
Normal/underweight	71	51%	100	46%	0.66
Overweight	41	29%	64	29%	
Obese	28	20%	51	23%	
Gestational diabetes					
Yes	27	19%	37	17%	0.61
No	113	81%	179	82%	
Maternal race/ethnicity					
Non-Hispanic white	71	51%	114	52%	0.76
Hispanic	33	24%	53	24%	
Other [d]	36	26%	48	22%	
Maternal education					
Less than college degree	63	45%	113	52%	0.33
Bachelor's degree	46	33%	57	26%	
Graduate or professional degree	31	22%	45	21%	
Homeownership					
Yes	83	59%	117	54%	0.32
No	54	39%	95	44%	
Parity					
0	2	1%	2	1%	0.90
1	59	42%	92	42%	
> 1	76	54%	121	56%	
Breastfeeding duration					
< 12 months	77	55%	131	60%	0.19
≥ 12 months	57	41%	72	33%	

[a] Missing information in the current study population (*n*): homeownership (3), parity (3), breastfeeding duration (6).
[b] Missing information in the MARBLES baseline population (*n*): maternal age at delivery (3), maternal pre-pregnancy BMI (3), gestational diabetes (2), maternal race/ethnicity (3), maternal education (3), homeownership (6), parity (3), breastfeeding duration (15).
[c] *p*-value from the Pearson's chi-squared test comparing the current study population and MARBLES baseline population.
[d] Includes Black, Asian, and multiracial.

Table S3.2 Adjusted mean differences (β) in MSEL Composite scores and T-scores of subscales of children at 6, 12, 24, and 36 months of age in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS and two PCs.

PFAS	MSEL	6 months β (95% CI) [a]	12 months β (95% CI) [a]	24 months β (95% CI) [a]	36 months β (95% CI) [a]
PFOA	Composite	0.1 (-2.4, 2.6)	-1.6 (-4.2, 1.1)	**-5.2 (-8.3, -2.2)**	**-5.2 (-9.5, -0.9)**
	Fine Motor	-0.2 (-1.6, 1.2)	-1.5 (-3.4, 0.5)	**-2.1 (-3.7, -0.5)**	**-3.7 (-6.3, -1.0)**
	Visual Reception	0.7 (-1.6, 2.9)	-1.5 (-3.2, 0.1)	-2.3 (-4.8, 0.2)	**-3.3 (-6.5, -0.2)**
	Receptive Language	0.2 (-2.4, 2.8)	-0.7 (-2.7, 1.2)	**-4.7 (-7.1, -2.2)**	-1.5 (-3.8, 0.7)
	Expressive Language	-0.3 (-1.9, 1.3)	0.4 (-1.8, 2.6)	**-1.9 (-3.7, -0.1)**	**-2.7 (-4.9, -0.4)**
PFOS	Composite	0.4 (-2.0, 2.8)	0.2 (-2.2, 2.6)	0.5 (-2.8, 3.8)	1.5 (-2.6, 5.7)
	Fine Motor	0.3 (-1.0, 1.6)	-0.2 (-2.0, 1.7)	0.2 (-1.7, 2.2)	0.7 (-2.0, 3.4)
	Visual Reception	-0.4 (-2.4, 1.6)	-0.6 (-2.2, 1.0)	0.2 (-2.2, 2.6)	0.4 (-2.5, 3.4)
	Receptive Language	0.8 (-1.9, 3.5)	0.5 (-1.1, 2.0)	-0.4 (-2.6, 1.9)	1.7 (-0.4, 3.8)
	Expressive Language	0.2 (-1.3, 1.6)	0.7 (-1.4, 2.8)	0.7 (-1.3, 2.7)	0.3 (-1.6, 2.3)
PFHxS	Composite	0.1 (-1.9, 2.2)	0.1 (-1.8, 2.1)	-1.1 (-3.7, 1.5)	0.2 (-2.2, 2.6)
	Fine Motor	0.3 (-0.7, 1.2)	-0.6 (-1.9, 0.7)	-1.0 (-2.1, 0.2)	-0.6 (-2.3, 1.1)
	Visual Reception	0.3 (-1.3, 1.9)	-0.5 (-1.5, 0.6)	-0.4 (-2.5, 1.7)	0.7 (-1.0, 2.5)
	Receptive Language	-0.8 (-3.2, 1.5)	0.0 (-1.1, 1.1)	-1.1 (-3.3, 1.0)	0.2 (-1.0, 1.4)
	Expressive Language	0.4 (-0.4, 1.2)	1.5 (-0.3, 3.2)	0.0 (-1.1, 1.2)	0.2 (-1.2, 1.6)
PFNA	Composite	1.0 (-1.7, 3.6)	-0.4 (-3.2, 2.4)	-2.4 (-6.0, 1.3)	-0.9 (-5.6, 3.7)
	Fine Motor	0.3 (-1.3, 1.8)	-0.1 (-2.1, 2.0)	-0.9 (-3.1, 1.3)	-0.8 (-3.9, 2.2)
	Visual Reception	1.2 (-1.1, 3.5)	-1.6 (-3.5, 0.4)	-0.6 (-3.1, 1.9)	-0.8 (-4.0, 2.4)
	Receptive Language	1.4 (-1.4, 4.3)	0.2 (-1.7, 2.1)	**-2.8 (-5.4, -0.2)**	0.3 (-2.0, 2.6)
	Expressive Language	-0.7 (-2.5, 1.2)	0.5 (-1.9, 2.9)	-0.9 (-3.1, 1.2)	-1.0 (-3.3, 1.3)
PFDA	Composite	-0.1 (-1.0, 0.7)	-0.1 (-1.1, 0.8)	-0.3 (-1.4, 0.8)	-0.3 (-1.5, 1.0)
	Fine Motor	0.0 (-0.5, 0.5)	0.3 (-0.4, 0.9)	-0.2 (-0.8, 0.4)	-0.2 (-1.0, 0.6)
	Visual Reception	0.0 (-0.8, 0.7)	-0.2 (-0.8, 0.3)	-0.2 (-1.0, 0.6)	0.1 (-0.9, 1.0)
	Receptive Language	-0.2 (-1.0, 0.6)	0.0 (-0.6, 0.7)	-0.4 (-1.2, 0.5)	0.1 (-0.8, 0.5)
	Expressive Language	0.0 (-0.5, 0.5)	-0.4 (-1.2, 0.4)	0.0 (-0.6, 0.7)	-0.4 (-1.0, 0.2)
PFUnDA	Composite	-0.1 (-0.7, 0.5)	0.0 (-0.7, 0.7)	-0.1 (-1.0, 0.8)	0.3 (-0.8, 1.3)
	Fine Motor	-0.1 (-0.5, 0.3)	0.1 (-0.4, 0.6)	-0.1 (-0.6, 0.4)	0.0 (-0.7, 0.7)
	Visual Reception	-0.1 (-0.7, 0.4)	-0.1 (-0.5, 0.3)	-0.1 (-0.7, 0.4)	0.3 (-0.4, 1.0)
	Receptive Language	0.1 (-0.4, 0.7)	0.1 (-0.4, 0.6)	0.0 (-0.7, 0.7)	0.2 (-0.4, 0.7)
	Expressive Language	-0.1 (-0.4, 0.3)	-0.1 (-0.7, 0.4)	0.0 (-0.5, 0.5)	-0.1 (-0.6, 0.5)
PC-1	Composite	0.3 (-1.2, 1.8)	-0.2 (-1.7, 1.2)	-1.4 (-3.3, 0.6)	-0.7 (-2.9, 1.6)
	Fine Motor	0.2 (-0.6, 0.9)	-0.4 (-1.5, 0.6)	-0.7 (-1.6, 0.3)	-0.8 (-2.2, 0.7)
	Visual Reception	0.3 (-0.9, 1.5)	-0.7 (-1.6, 0.2)	-0.5 (-2.1, 1.0)	-0.4 (-2.0, 1.2)
	Receptive Language	0.1 (-1.8, 2.0)	0.0 (-1.0, 0.9)	**-1.5 (-3.0, 0.0)**	0.2 (-1.0, 1.3)
	Expressive Language	0.0 (-0.7, 0.8)	0.7 (-0.6, 2.0)	-0.3 (-1.4, 0.8)	-0.4 (-1.6, 0.7)

	Composite	-0.1 (-1.8, 1.6)	-0.3 (-1.9, 1.4)	-0.8 (-3.0, 1.4)	-0.3 (-3.0, 2.4)
	Fine Motor	-0.2 (-1.1, 0.8)	0.4 (-0.8, 1.6)	-0.4 (-1.6, 0.8)	-0.3 (-2.0, 1.4)
PC-2	Visual Reception	0.0 (-1.4, 1.4)	-0.5 (-1.6, 0.5)	-0.5 (-2.0, 1.1)	0.0 (-1.9, 1.9)
	Receptive Language	0.4 (-1.1, 1.9)	0.2 (-1.0, 1.4)	-0.7 (-2.4, 0.9)	0.1 (-1.3, 1.5)
	Expressive Language	-0.3 (-1.2, 0.7)	-0.6 (-1.9, 0.7)	-0.2 (-1.5, 1.1)	-0.7 (-2.0, 0.6)

[a] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration.

Note: Bolded numbers indicate p-value < 0.05

Table S3.3 Sensitivity analysis: Longitudinal changes (β) in MSEL Composite scores and T-scores of subscales of children over the four assessment time points in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS in generalized estimating equations for the MARBLES baseline population (n = 218 [a]).

MSEL	PFOA β (95% CI) [b]	PFOS β (95% CI) [b]	PFHxS β (95% CI) [b]
Composite	-0.11 (-0.26, 0.03)	0.00 (-0.13, 0.14)	-0.02 (-0.12, 0.08)
Fine Motor	-0.08 (-0.16, 0.00)[#]	-0.03 (-0.11, 0.05)	-0.06 (-0.12, 0.01)
Visual Reception	-0.12 (-0.23, -0.02)[#]	0.00 (-0.10, 0.10)	0.00 (-0.07, 0.06)
Receptive Language	-0.03 (-0.12, 0.06)	0.00 (-0.08, 0.09)	0.02 (-0.05, 0.09)
Expressive Language	-0.02 (-0.10, 0.06)	0.00 (-0.08, 0.07)	0.01 (-0.05, 0.07)
MSEL	**PFNA β (95% CI) [b]**	**PFDA β (95% CI) [b]**	**PFUnDA β (95% CI) [b]**
Composite	-0.04 (-0.20, 0.12)	0.00 (-0.05, 0.05)	0.02 (-0.01, 0.06)
Fine Motor	-0.05 (-0.14, 0.05)	-0.01 (-0.03, 0.02)	0.00 (-0.02, 0.03)
Visual Reception	-0.05 (-0.16, 0.07)	0.01 (-0.03, 0.04)	0.02 (-0.01, 0.04)
Receptive Language	-0.03 (-0.13, 0.07)	0.00 (-0.03, 0.03)	0.01 (-0.01, 0.03)
Expressive Language	-0.02 (-0.11, 0.07)	-0.01 (-0.04, 0.02)	0.00 (-0.02, 0.02)

[a] 194 mother-child pairs were used in the GEE models, excluding those pairs who had missing covariates (not imputed because of missing outcomes) and had one-point MSEL score only (due to the characteristics of autoregressive correlation structure).

[b] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration. Regression coefficient (β) was derived from interaction term between continuous age in months (centered at 24) and continuous log 2-transformed PFAS concentrations, after including the two main effects and their interaction term in the GEE model.

[#] p-value for interaction between age at assessment and PFAS concentrations < 0.10

Table S3.4 Sensitivity analysis: Longitudinal changes (β) in MSEL Composite scores and T-scores of subscales of children over the four assessment time points in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS in generalized estimating equations after restricting to the TD children (*n* = 93).

MSEL	PFOA β (95% CI) [a]	PFOS β (95% CI) [a]	PFHxS β (95% CI) [a]
Composite	-0.15 (-0.30, -0.01)[#]	-0.02 (-0.17, 0.14)	-0.08 (-0.23, 0.07)
Fine Motor	-0.11 (-0.21, -0.01)[#]	-0.03 (-0.13, 0.08)	-0.08 (-0.18, 0.02)
Visual Reception	-0.12 (-0.25, 0.01)[#]	-0.01 (-0.14, 0.11)	-0.02 (-0.14, 0.10)
Receptive Language	-0.07 (-0.15, 0.02)	-0.03 (-0.11, 0.06)	-0.03 (-0.12, 0.06)
Expressive Language	-0.04 (-0.11, 0.04)	0.03 (-0.05, 0.11)	-0.03 (-0.11, 0.05)
MSEL	**PFNA** **β (95% CI) [a]**	**PFDA** **β (95% CI) [a]**	**PFUnDA** **β (95% CI) [a]**
Composite	-0.03 (-0.21, 0.15)	0.02 (-0.03, 0.07)	0.03 (-0.01, 0.07)
Fine Motor	-0.03 (-0.17, 0.11)	0.01 (-0.02, 0.04)	0.01 (-0.02, 0.04)
Visual Reception	-0.06 (-0.20, 0.09)	0.02 (-0.02, 0.07)	0.03 (-0.01, 0.06)[#]
Receptive Language	-0.04 (-0.13, 0.06)	0.00 (-0.03, 0.04)	0.01 (-0.02, 0.03)
Expressive Language	0.04 (-0.05, 0.13)	0.01 (-0.02, 0.03)	0.01 (-0.01, 0.03)

[a] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration. Regression coefficient (β) was derived from interaction term between continuous age in months (centered at 24) and continuous log 2-transformed PFAS concentrations, after including the two main effects and their interaction term in the GEE model.

[#] *p*-value for interaction between age at assessment and PFAS concentrations < 0.10

Table S3.5 Effect modification analysis: Longitudinal changes (β) in MSEL Composite scores and T-scores of subscales of children over the four assessment time points in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of four PFAS stratified by child's sex and breastfeeding duration in generalized estimating equations.

PFAS (ng/mL)	**β (95% CI)** [a]		***p*-value for interaction** [b]
Child's sex	**Females** (*n* = 57)	**Males** (*n* = 83)	
PFOA	-0.25 (-0.45, -0.04)[#]	-0.08 (-0.30, 0.13)	0.28
PFOS	-0.11 (-0.31, 0.09)	0.14 (-0.07, 0.34)	0.10
PFHxS	-0.04 (-0.20, 0.12)	0.01 (-0.16, 0.18)	0.68
PFNA	-0.13 (-0.37, 0.11)	0.02 (-0.22, 0.26)	0.38
Breastfeeding duration	**12 months** (*n* = 77)	**≥ 12 months** (*n* = 57)	
PFOA	-0.15 (-0.37, 0.06)	-0.16 (-0.36, 0.03)	0.95
PFOS	0.01 (-0.18, 0.20)	-0.02 (-0.24, 0.21)	0.87
PFHxS	-0.02 (-0.15, 0.10)	-0.05 (-0.21, 0.12)	0.84
PFNA	0.00 (-0.23, 0.24)	-0.16 (-0.37, 0.06)	0.32

[a] Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration, and missing covariates were not imputed. Regression coefficient (β) was derived from interaction term between continuous age in months (centered at 24) and continuous log 2-transformed PFAS concentrations, after including the two main effects and their interaction term in the GEE model.

[b] *p*-value for interaction among age at assessment, PFAS concentrations, and potential effect modifiers (child's sex or breastfeeding duration)

[#] *p*-value for interaction between age at assessment and PFAS concentrations < 0.10

Table S3.6 Characteristics of the study participants by trajectory group.

Characteristic [a]	Low-score group (n = 40)		High-score group (n = 100)		p-value [b]
	n	%	n	%	
Child sex					
Female	11	28%	46	46%	0.04
Male	29	73%	54	54%	
Child's birth year					
2009-2010	13	33%	33	33%	0.48
2011-2013	17	43%	33	33%	
2014-2015	10	25%	34	34%	
Gestational age at delivery					
≤ 37 weeks	4	10%	8	8%	0.70
> 37 weeks	36	90%	92	92%	
Maternal age at delivery					
< 35 years	26	65%	46	46%	0.04
≥ 35 years	14	35%	54	54%	
Maternal pre-pregnancy BMI					
Normal/underweight	17	43%	54	54%	0.03
Overweight	18	45%	23	23%	
Obese	5	13%	23	23%	
Gestational diabetes					
Yes	3	8%	24	24%	0.03
No	37	93%	76	76%	
Maternal race/ethnicity					
Non-Hispanic white	18	45%	53	53%	0.67
Hispanic	10	25%	23	23%	
Other [c]	12	30%	24	24%	
Maternal education					
Less than college degree	27	68%	36	36%	< 0.01
Bachelor's degree	7	18%	39	39%	
Graduate or professional degree	6	15%	25	25%	
Homeownership					
Yes	20	50%	63	63%	0.16
No	19	48%	35	35%	
Parity					
0	0	0%	2	2%	0.34
1	14	35%	45	45%	
> 1	25	63%	51	51%	
Breastfeeding duration					
< 12 months	23	58%	54	54%	0.82
≥ 12 months	16	40%	41	41%	

[a] Missing information (n): homeownership (3), parity (3), breastfeeding duration (6).

[b] p-value from the Pearson's chi-squared test comparing the low- and high-score groups.

[c] Includes Black, Asian, and multiracial.

Table S4.1 Details on sample preparation and analytical methods.

1. Pretreatment method
2 ng of internal standard was added to 0.5 mL of serum sample, 1 mL of methanol was added to remove proteins, and 10 mL of water and 0.1 mL of formic acid were added. The supernatant of this sample solution was loaded to solid phase (Oasis WAX). Then, target substances were eluted by 5 mL of methanol containing 0.1% ammonia. The eluent was concentrated to less than 0.5 mL using gentle stream of nitrogen gas. Measurement solution was obtained after adjusting to 0.5 mL of sample volume.
2. Measurement
Perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS) were measured with a liquid chromatograph tandem mass spectrometer (LC/MS/MS). The measurement condition was shown in the Table S4.1-1.
3. Validation of analytical method
1) Calibration curve: The calibration curve ranged from 0.01 to 30 ng/mL with more than 0.999 coefficient of determination (r^2) value. 2) Calculation of Instrumental Detection Limit (IDL): The standard solution with the lowest concentration used for the calibration curve was repeatedly measured for 7 times. The measured values were calculated according to the "Guidelines for conducting a survey on the environment of chemical substances (2015)", which applied the definition of Currie (1997). 3) Calculation of Method Detection Limit (MDL) and Method Quantification Limit (MQL): A test solution in which the internal standard and the native standard of about 5 times of IDL were added to a blank sample was pretreated in the same method as the actual sample. This operation was repeated for 7 times, and MDL and MQL were calculated according to the definition of Currie (1997) as in IDL. MDL were 3 pg/mL for both PFOS and PFOA. 4) Recovery: A test solution in which the internal standard and 20 pg native standard were added to a blank sample was subjected to the same pretreatment as the actual sample. This procedure was repeated for 7 times, and the range of recovery ratio for PFOS was 68.4 to 113.2% and that for PFOA was 67.8 to 109.2%.
4. Round robin test
Participated in German External Quality Assessment Scheme (G-EQUAS) 59, an international round robin test and the result was within the tolerance for the analysis of PFOA and PFOS in serum.
5. References
Currie, L.A. (1997) Detection: International update, and some emerging di-lemmas involving calibration, the blank, and multiple detection decisions. Chemometrics and Intelligent Laboratory Systems, 37: 151-181.

Table S4.1-1. LC/MS/MS measurement condition for PFOA and PFOS.

Equipment	LC：LC-20A Prominence（Shimadzu Corporation） MS：API 4000 (AB SCIEX)		
LC conditions			
Column	Inertsil ODS-SP (2.1 mm (i.d.)×150 mm, 3μm) (GL Sciences)		
Mobile phase A	10mM ammonium acetate		
Mobile phase B	acetonitrile		
Gradient	0-2 min	A：65%	B：35%
	2-8 min	A：65→50%	B：35→50%
	8-10.5 min	A：50%	B：50%
	10.5-15.5 min	A：50→20%	B：50→80%
	15.5-20.5 min	A：20%	B：80%
	20.5-30 min	A：65%	B：35%
Mobile phase flow rate	0.2 mL/min		
Column temperature	40°C		
Sample injection volume	10μL		
MS/MS condition			
Ionization method	ESI Negative (MRM：Multiple Reaction Monitoring)		
Monitor ion（*m/z*）			
Measured substance	For quantification		For confirmation
PFOS	498.9→80.0		498.9→99.0
PFOA	412.9→369.0		412.9→169.0
Internal standard substance			
$^{13}C_4$-PFOS	502.9→80.0		502.9→99.0
$^{13}C_4$-PFOA	416.9→372.0		416.9→169.0

Table S4.2 Distribution of MSEL Composite and subscale scores in 595 children from 4 to 40 months of age.

MSEL	Age (months)	*n*	Mean	SD	Min	Max
Composite	4	543	100	15	58	143
	6	525	100	15	46	139
	10	564	98	15	59	136
	14	533	99	13	64	138
	18	557	101	15	58	142
	24	554	99	11	74	134
	32	551	100	13	61	139
	40	548	101	14	59	138
Fine Motor	4	556	48	9	20	71
	6	545	47	9	26	76
	10	574	48	11	20	76
	14	553	49	10	20	77
	18	576	50	10	20	80
	24	579	49	10	20	74
	32	557	50	10	20	80
	40	558	51	10	20	80
Visual Reception	4	552	48	10	20	67
	6	542	48	9	22	80
	10	575	48	10	20	75
	14	553	49	9	20	77
	18	577	49	10	20	80
	24	579	50	10	20	80
	32	555	49	10	20	76
	40	556	51	10	20	75
Receptive Language	4	547	48	10	20	67
	6	533	49	10	29	77
	10	573	48	10	20	78
	14	535	50	9	20	80
	18	564	49	10	22	80
	24	561	50	10	20	68
	32	556	50	9	20	80
	40	558	50	10	20	80
Expressive Language	4	554	48	10	20	68
	6	535	49	10	20	80
	10	570	48	9	25	78
	14	550	50	10	20	70
	18	570	49	9	20	74
	24	568	50	10	20	80
	32	559	50	10	20	80
	40	554	50	10	20	79

Abbreviation: sample size (*n*), standard deviation (SD), minimum (min), maximum (max)

Note: Normative mean and SD of Composite scores are 100 and 15, respectively. Normative mean and SD of subscale scores are 50 and 10, respectively.

Table S5.1 Geometric means (GMs) and 95% confidence intervals (CIs) of maternal serum concentration ratios of eight PFAS at each sample collection point compared to the 1st trimester of pregnancy.

	GM (95% CI)			
	n-PFOS	**Sm-PFOS**	**PFHxS**	**n-PFOA**
1Trim	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
2Trim	0.98 (0.91, 1.06)	0.90 (0.85, 0.96)	0.98 (0.84, 1.14)	0.88 (0.84, 0.92)
3Trim	0.84 (0.78, 0.91)	0.83 (0.77, 0.90)	1.01 (0.85, 1.19)	0.81 (0.77, 0.85)
3Mon	0.85 (0.79, 0.90)	0.79 (0.75, 0.83)	0.73 (0.64, 0.84)	0.66 (0.62, 0.70)
6Mon	0.82 (0.77, 0.88)	0.74 (0.69, 0.80)	0.65 (0.56, 0.76)	0.57 (0.52, 0.62)
24Mon	0.68 (0.61, 0.75)	0.60 (0.54, 0.66)	0.72 (0.61, 0.86)	0.57 (0.49, 0.65)
	PFNA	**PFDA**	**PFUnDA**	**MeFOSAA**
1Trim	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)	1.00 (1.00, 1.00)
2Trim	0.86 (0.81, 0.90)	0.96 (0.85, 1.07)	0.94 (0.86, 1.03)	0.94 (0.84, 1.06)
3Trim	0.79 (0.74, 0.85)	0.91 (0.81, 1.03)	0.89 (0.81, 0.99)	0.83 (0.76, 0.91)
3Mon	0.78 (0.73, 0.83)	0.85 (0.73, 0.98)	0.88 (0.79, 0.99)	0.82 (0.74, 0.92)
6Mon	0.72 (0.67, 0.77)	0.86 (0.77, 0.97)	0.85 (0.76, 0.96)	0.87 (0.76, 1.00)
24Mon	0.62 (0.55, 0.71)	0.75 (0.64, 0.88)	0.92 (0.83, 1.03)	0.83 (0.69, 0.97)

Note: For one mother who did not provide the 1st trimester sample, the concentration at each point were divided by the 2nd trimester concentration.

Table S5.2 Spearman correlation coefficients of maternal serum PFAS concentrations across the six collection time points.

	1Trim	2Trim	3Trim	3Mon	6Mon	24Mon		1Trim	2Trim	3Trim	3Mon	6Mon	24Mon
n-PFOS							**Sm-PFOS**						
1Trim	1.00						**1Trim**	1.00					
2Trim	0.83	1.00					**2Trim**	0.96	1.00				
3Trim	0.83	0.78	1.00				**3Trim**	0.94	0.93	1.00			
3Mon	0.85	0.80	0.83	1.00			**3Mon**	0.96	0.96	0.96	1.00		
6Mon	0.84	0.83	0.79	0.83	1.00		**6Mon**	0.94	0.94	0.94	0.96	1.00	
24Mon	0.77	0.74	0.74	0.73	0.79	1.00	**24Mon**	0.85	0.85	0.84	0.85	0.85	1.00
PFHxS							**n-PFOA**						
1Trim	1.00						**1Trim**	1.00					
2Trim	0.62	1.00					**2Trim**	0.94	1.00				
3Trim	0.69	0.76	1.00				**3Trim**	0.95	0.97	1.00			
3Mon	0.78	0.70	0.81	1.00			**3Mon**	0.94	0.91	0.92	1.00		
6Mon	0.71	0.62	0.73	0.82	1.00		**6Mon**	0.87	0.85	0.86	0.92	1.00	
24Mon	0.64	0.68	0.73	0.75	0.73	1.00	**24Mon**	0.72	0.71	0.70	0.80	0.87	1.00
PFNA							**PFDA**						
1Trim	1.00						**1Trim**	1.00					
2Trim	0.92	1.00					**2Trim**	0.74	1.00				
3Trim	0.89	0.94	1.00				**3Trim**	0.72	0.81	1.00			
3Mon	0.87	0.87	0.85	1.00			**3Mon**	0.59	0.65	0.77	1.00		
6Mon	0.88	0.89	0.87	0.89	1.00		**6Mon**	0.73	0.66	0.77	0.77	1.00	
24Mon	0.74	0.76	0.73	0.82	0.84	1.00	**24Mon**	0.70	0.58	0.58	0.56	0.67	1.00
PFUnDA							**MeFOSAA**						
1Trim	1.00						**1Trim**	1.00					
2Trim	0.70	1.00					**2Trim**	0.74	1.00				
3Trim	0.66	0.75	1.00				**3Trim**	0.84	0.83	1.00			
3Mon	0.58	0.77	0.75	1.00			**3Mon**	0.70	0.71	0.77	1.00		
6Mon	0.57	0.63	0.70	0.79	1.00		**6Mon**	0.71	0.82	0.86	0.81	1.00	
24Mon	0.58	0.69	0.77	0.71	0.73	1.00	**24Mon**	0.66	0.58	0.68	0.62	0.60	1.00

Note: *p*-values of all the Spearman correlation coefficients were less than 0.001. One of the 42 mothers did not provide the 1st trimester sample but was included in this study.

Table S5.3 Univariate associations between maternal serum PFAS concentrations and potential determinants.

Potential determinants	r_{sp} or median (25th, 75th percentiles) [a]				
	n-PFOS	**Sm-PFOS**	**PFHxS**	**n-PFOA**	**PFNA**
Pregnancy					
Child's birth year	**-0.52**	**-0.68**	**-0.53**	**-0.43**	-0.27
Maternal age at delivery	**0.31**	0.18	**0.31**	0.28	0.09
Maternal BMI at pre-pregnancy	0.03	0.10	0.02	-0.10	0.25
Maternal weight gain during pregnancy	-0.22	-0.19	-0.19	-0.10	-0.13
Birthweight	0.04	0.07	-0.26	0.02	0.07
Parity	-0.00	0.04	-0.24	**-0.39**	-0.20
Maternal race/ethnicity					
Non-Hispanic white	2.25 (1.65, 3.40)	0.90 (0.80, 1.70)	0.40 (0.30, 0.52)	0.83 (0.52, 1.13)	0.40 (0.37, 0.60)
Hispanic & other [b]	2.20 (1.83, 2.95)	0.70 (0.52, 1.23)	0.40 (0.28, 0.58)	0.87 (0.67, 0.99)	0.65 (0.45, 0.70)
Maternal birthplace					
United States	2.10 (1.30, 2.85)	0.90 (0.70, 1.65)	0.37 (0.28, 0.47)	0.75 (0.53, 1.10)	0.40 (0.37, 0.55)
Other	2.80 (2.02, 3.20)	0.75 (0.60, 1.23)	0.48 (0.34, 0.71)	0.92 (0.81, 1.16)	**0.67 (0.61, 0.76)**
Maternal education					
Less than bachelor's degree	2.15 (1.45, 2.98)	0.90 (0.72, 0.98)	0.34 (0.28, 0.42)	0.68 (0.53, 0.94)	0.42 (0.40, 0.68)
Bachelor's degree or higher	2.25 (1.78, 3.20)	0.85 (0.60, 1.72)	0.45 (0.30, 0.63)	0.92 (0.67, 1.22)	0.53 (0.39, 0.70)
Homeownership					
Non-owner	2.20 (2.02, 2.75)	0.90 (0.72, 1.45)	0.40 (0.30, 0.46)	0.83 (0.57, 1.00)	0.40 (0.38, 0.63)
Owner	2.30 (1.65, 3.32)	0.85 (0.60, 1.63)	0.40 (0.30, 0.65)	0.87 (0.65, 1.14)	0.58 (0.40, 0.72)
Early postpartum					
Child's birth year	**-0.45**	**-0.70**	**-0.60**	**-0.45**	**-0.37**
Maternal age at delivery	0.24	0.21	0.26	**0.37**	0.14
Maternal BMI at pre-pregnancy	-0.02	0.09	0.08	-0.12	0.23
Maternal weight gain during pregnancy	**-0.31**	-0.20	-0.23	-0.07	-0.13
Birthweight	-0.07	0.07	-0.17	-0.06	-0.04
Parity	-0.08	-0.03	**-0.36**	**-0.33**	-0.16
Maternal race/ethnicity					
Non-Hispanic white	1.85 (1.45, 2.47)	0.80 (0.60, 1.22)	0.30 (0.27, 0.40)	0.63 (0.43, 0.90)	0.37 (0.30, 0.52)
Hispanic & other [b]	2.05 (1.35, 2.32)	0.55 (0.50, 0.90)	0.38 (0.17, 0.55)	0.63 (0.52, 0.76)	0.53 (0.38, 0.63)
Maternal birthplace					
United States	1.80 (1.25, 2.40)	0.70 (0.55, 1.25)	0.27 (0.22, 0.40)	0.57 (0.42, 0.87)	0.37 (0.30, 0.47)
Other	2.10 (2.00, 2.53)	0.65 (0.43, 0.90)	0.45 (0.25, 0.78)	0.72 (0.57, 0.87)	**0.55 (0.47, 0.66)**

Maternal education					
Less than bachelor's degree	1.65 (1.22, 2.45)	0.75 (0.52, 0.88)	0.27 (0.18, 0.38)	0.57 (0.41, 0.73)	0.38 (0.34, 0.62)
Bachelor's degree or higher	2.10 (1.75, 2.40)	0.70 (0.50, 1.33)	0.40 (0.26, 0.70)	0.70 (0.52, 1.01)	0.45 (0.33, 0.61)
Homeownership					
Non-owner	2.00 (1.43, 2.55)	0.75 (0.52, 1.12)	0.32 (0.24, 0.43)	0.58 (0.43, 0.84)	0.37 (0.30, 0.43)
Owner	2.05 (1.45, 2.40)	0.70 (0.50, 1.22)	0.33 (0.19, 0.60)	0.68 (0.49, 0.92)	0.48 (0.36, 0.63)
Late postpartum					
Child's birth year	**-0.50**	**-0.73**	**-0.42**	**-0.35**	**-0.41**
Maternal age at delivery	**0.35**	**0.32**	0.30	**0.53**	**0.34**
Maternal BMI at pre-pregnancy	0.03	0.06	0.06	-0.09	0.07
Parity	0.05	0.07	**-0.50**	-0.20	-0.08
Breastfeeding duration	-0.12	-0.25	-0.18	**-0.51**	-0.28
Maternal race/ethnicity					
Non-Hispanic white	1.95 (1.58, 2.23)	0.80 (0.57, 1.05)	0.25 (0.20, 0.35)	0.57 (0.39, 0.82)	0.35 (0.30, 0.52)
Hispanic & other [b]	1.95 (1.42, 2.38)	0.55 (0.40, 0.88)	0.30 (0.20, 0.45)	0.52 (0.35, 0.70)	0.40 (0.31, 0.64)
Maternal birthplace					
United States	1.90 (1.35, 2.25)	0.80 (0.50, 1.05)	0.25 (0.20, 0.32)	0.50 (0.30, 0.70)	0.35 (0.28, 0.45)
Other	2.15 (1.52, 2.55)	0.55 (0.40, 0.92)	**0.42 (0.25, 0.73)**	0.62 (0.41, 0.78)	**0.48 (0.35, 0.65)**
Maternal education					
Less than bachelor's degree	1.95 (1.45, 2.53)	0.70 (0.52, 0.80)	0.25 (0.20, 0.29)	0.45 (0.30, 0.64)	0.38 (0.30, 0.56)
Bachelor's degree or higher	1.90 (1.50, 2.23)	0.65 (0.40, 1.05)	0.35 (0.24, 0.46)	0.55 (0.44, 0.82)	0.35 (0.30, 0.61)
Homeownership					
Non-owner	1.85 (1.52, 2.00)	0.75 (0.52, 0.98)	0.30 (0.25, 0.35)	0.45 (0.33, 0.74)	0.32 (0.25, 0.44)
Owner	2.05 (1.45, 2.32)	0.65 (0.40, 1.00)	0.25 (0.15, 0.46)	0.55 (0.35, 0.76)	0.38 (0.30, 0.65)

[a] Estimates whose corresponding *p*-values were less than 0.05 (from the Spearman correlation test or the Wilcoxon rank-sum test) are highlighted in bold.

[b] Other race/ethnicity includes Asian (21%) and multiracial (2%).

Table S5.4 Percent changes in maternal serum PFAS concentrations over time after additionally adjusting for maternal BMI at pre-pregnancy, maternal weight gain during pregnancy and child's birthweight in pregnancy and early postpartum models.

	Monthly percent change (95% CI) [a]	
	Pregnancy	Early postpartum
n-PFOS	**-3.1 (-4.5, -1.5)**	-0.1 (-0.9, 0.7)
Sm-PFOS	**-3.4 (-4.6, -2.1)**	**-1.3 (-2.1, -0.5)**
PFHxS	-0.6 (-3.6, 2.4)	**-5.6 (-7.4, -3.8)**
n-PFOA	**-4.1 (-4.8, -3.3)**	**-4.5 (-5.2, -3.6)**
PFNA	**-4.4 (-5.4, -3.4)**	**-1.1 (-1.9, -0.2)**

[a] All models were adjusted for child's birth year, maternal age at delivery, parity, maternal birthplace, maternal BMI at pre-pregnancy, maternal weight gain during pregnancy and child's birthweight. Estimates with *p*-values less than 0.05 are highlighted in bold.

Table S5.5 Percent changes in maternal serum PFAS concentrations over time stratified by breastfeeding duration.

	Monthly percent change (95% CI) [a]		Interaction [b]
	Breastfeeding duration < 12 months (n = 23)	Breastfeeding duration ≥ 12 months (n = 19)	
n-PFOS	**-0.8 (-1.3, -0.4)**	**-1.3 (-1.8, -0.8)**	0.21
Sm-PFOS	**-1.0 (-1.5 -0.5)**	**-1.6 (-2.1, -1.0)**	0.11
PFHxS	0.3 (-0.6, 1.2)	0.0 (-1.0, 0.8)	0.22
n-PFOA	0.1 (-0.5, 0.9)	**-1.1 (-1.7, -0.5)**	0.01
PFNA	-0.3 (-0.8, 0.1)	**-1.7 (-2.2, -1.0)**	0.00

[a] All models were adjusted for child's birth year, maternal age at delivery, parity, and maternal birthplace. Estimates with corresponding *p*-values less than 0.05 are highlighted in bold.
[b] *p*-value for interaction term of time from conception with binary breastfeeding duration variable (breastfeeding duration and an interaction term were added as additional terms in the model, along with other covariates).

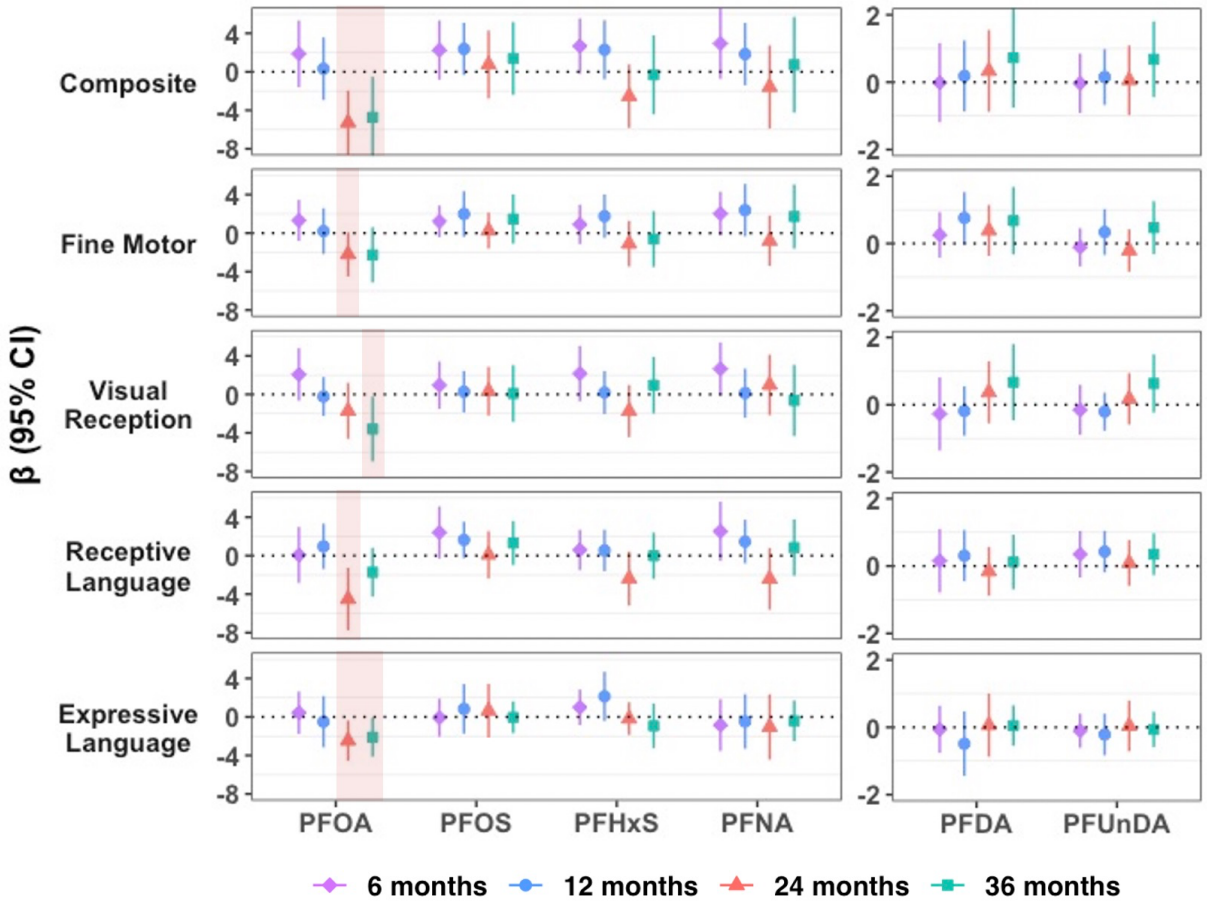

Figure S3.1 Sensitivity analysis: Adjusted mean differences (β) in MSEL Composite scores and T-scores of subscales of children at 6, 12, 24, and 36 months of age in association with a unit increase in log 2-transformed prenatal maternal serum concentrations of six PFAS after restricting to the TD children (n = 93). Models were adjusted for child's sex, parity, maternal pre-pregnancy BMI, gestational diabetes, maternal education, and breastfeeding duration. Red shaded areas represent associations with a p-value < 0.05.

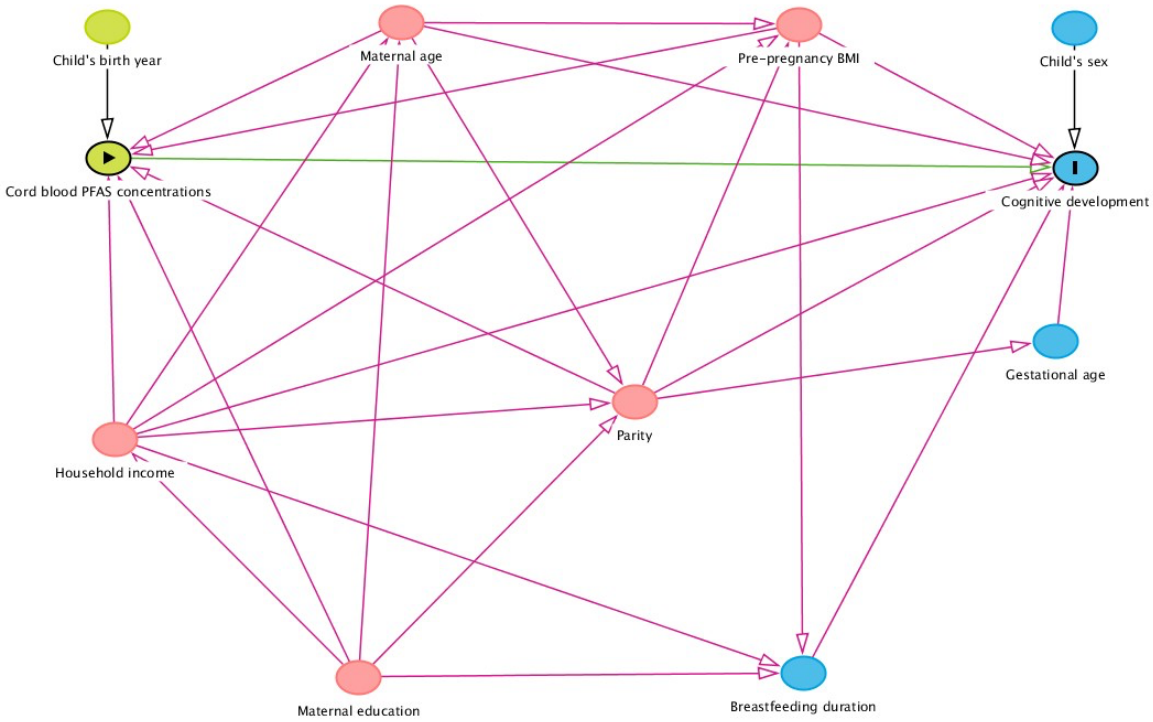

Figure S4.1 Directed acyclic graph used to identify potential confounders for the associations between cord blood PFAS concentrations and child cognitive development. Green circles represent ancestors of the exposure, blue circles ancestors of the outcome, pink circles ancestors of both exposure and outcome.

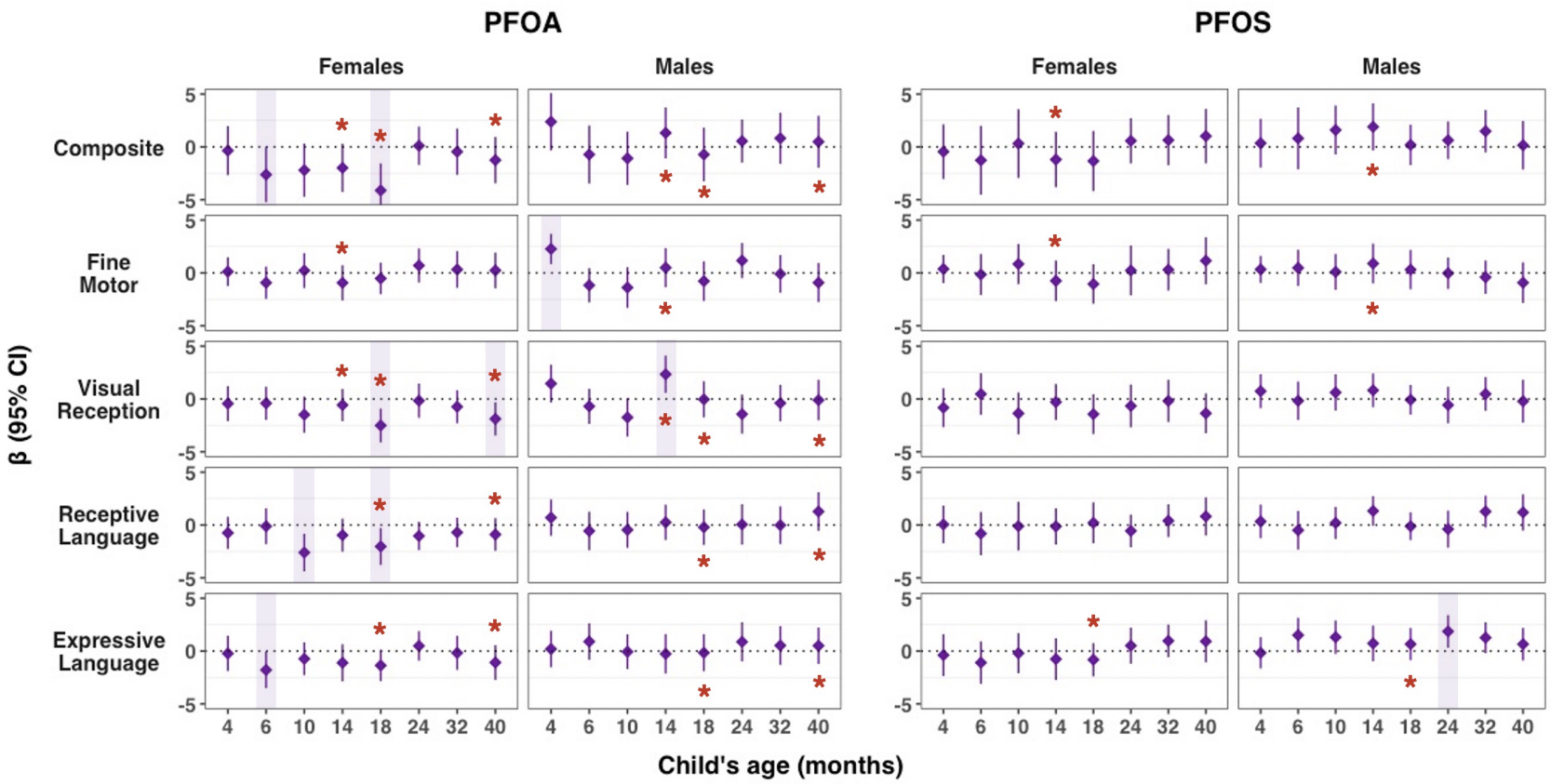

Figure S4.2 Cross-sectional associations between log 2-transformed cord blood PFOA and PFOS concentrations and MSEL Composite and subscale scores at 4, 6, 10, 14, 18, 24, 32, and 40 months of age stratified by child's sex (females = 287, males = 308). Multiple linear regression models were adjusted for child's birth year and gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Shaded areas indicate estimates with a *p*-value < 0.05. Red asterisks indicate that a *p*-value for interaction terms (exposure × child's sex) is less than 0.10.

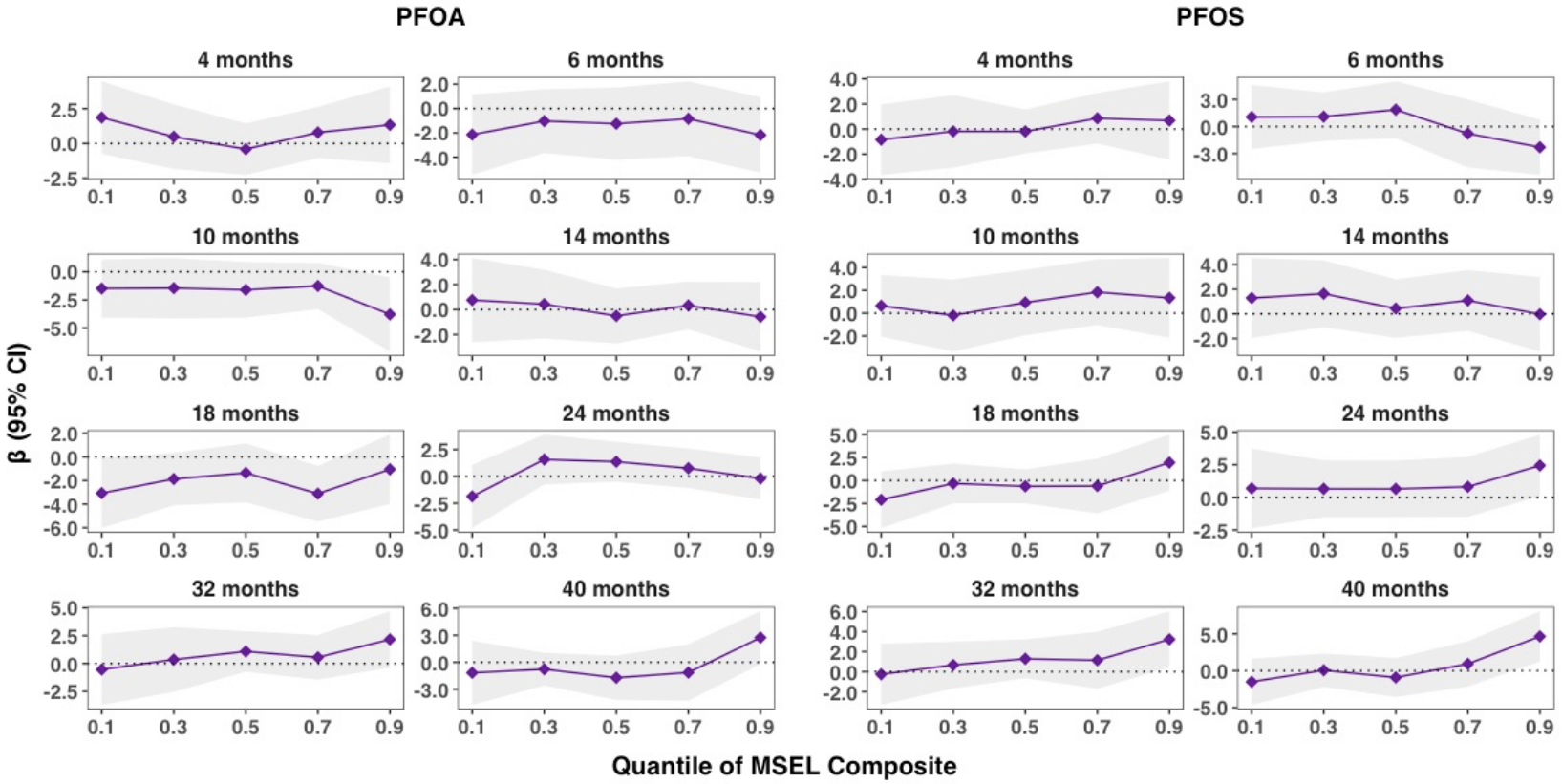

Figure S4.3 Cross-sectional associations between log 2-transformed cord blood PFOA and PFOS concentrations and quantiles of MSEL Composite scores at 4, 6, 10, 14, 18, 24, 32, and 40 months of age. Quantile regression models were adjusted for child's sex, birth year, and gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Shaded areas indicate 95% confidence intervals of the estimates.

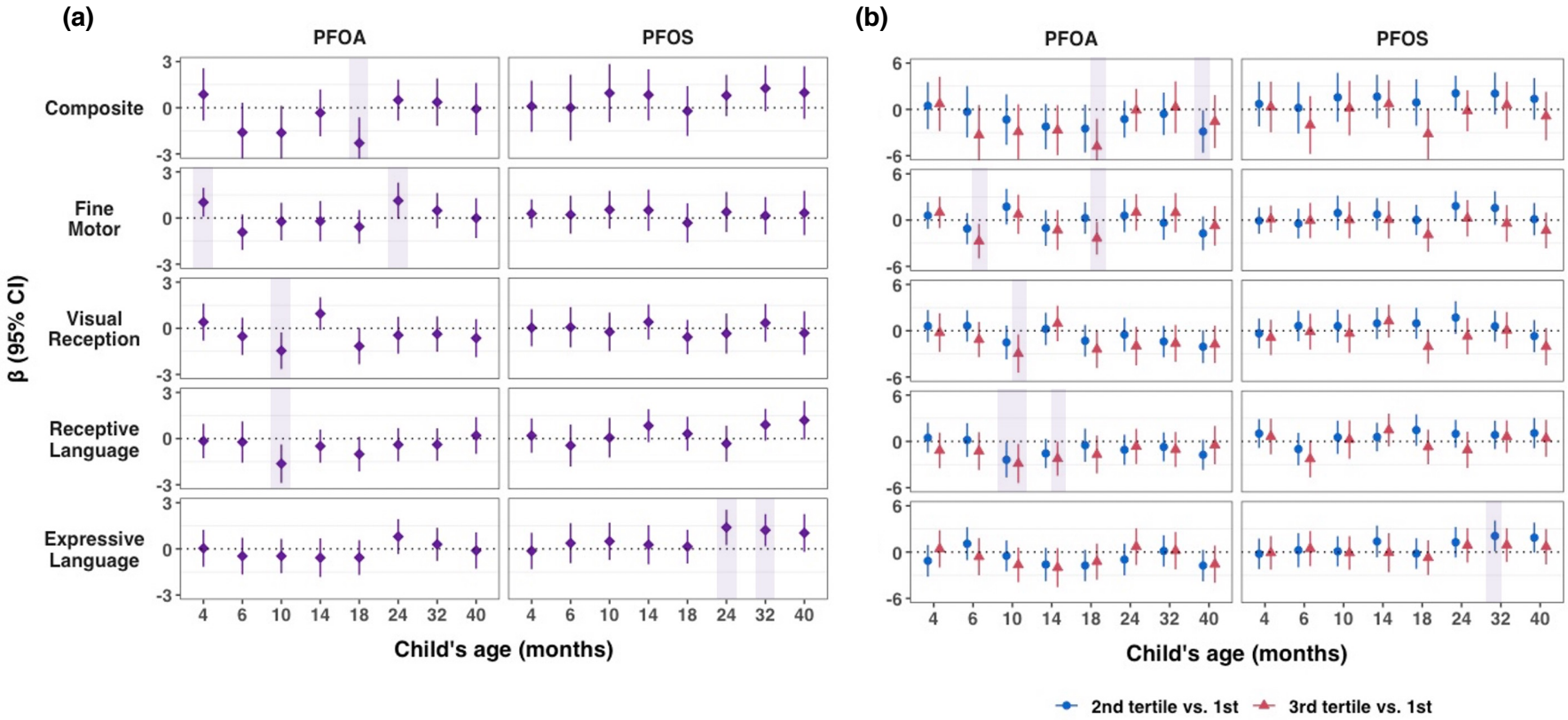

Figure S4.4 Sensitivity analysis: Estimated mean differences (β) in MSEL Composite and subscale scores at 4, 6, 10, 14, 18, 24, 32, and 40 months of age in association with (a) log 2-transformed and (b) tertile-based cord blood PFOA and PFOS concentrations, after excluding 25 children who were born preterm (< 37 weeks of pregnancy). Multiple linear regression models were adjusted for child's sex and birth year, gestational age, maternal age at delivery, parity, household income, maternal educational history, and breastfeeding duration. Shaded areas indicate estimates with a *p*-value < 0.05.

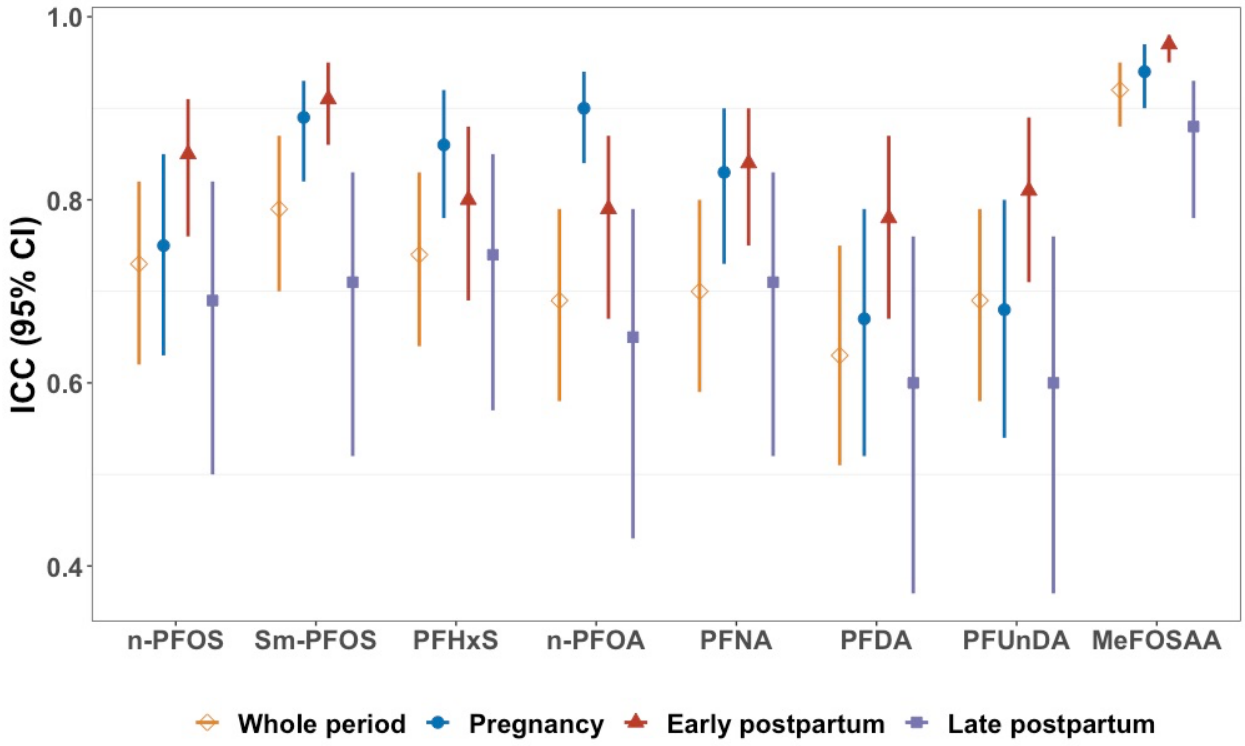

Figure S5.1 Intraclass correlation coefficients (ICC) and 95% confidence intervals (CIs) of ln-transformed PFAS concentrations in maternal serum samples collected during the whole study period and three sub-periods (i.e., pregnancy, early postpartum, and late postpartum).

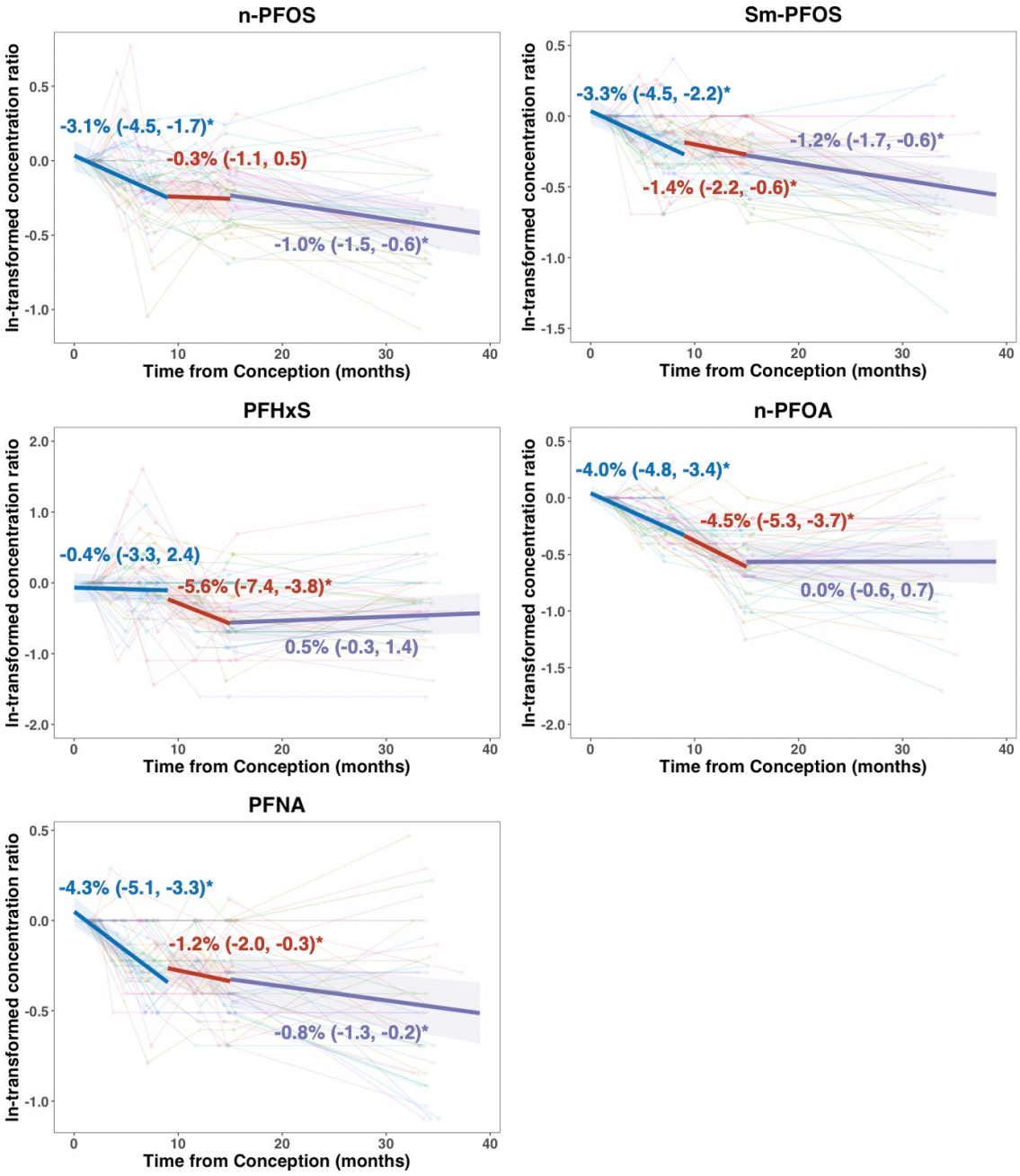

Figure S5.2 Sensitivity analysis: Percent changes in maternal serum PFAS concentrations over time using ln-transformed concentration ratios of PFAS at each sample collection points to those at the 1st trimester of pregnancy as dependent variable. Thick lines in blue, red, and purple represent adjusted mean concentrations during pregnancy, early postpartum, and late postpartum, respectively, and shaded areas represent corresponding 95% CIs. Thin lines and dots represent individual trajectory of PFAS concentrations. Asterisk represents significant changes in PFAS concentrations over time. All models were adjusted for child's birth year, maternal age at delivery, parity, and maternal birthplace. Late postpartum models were additionally adjusted for breastfeeding duration.

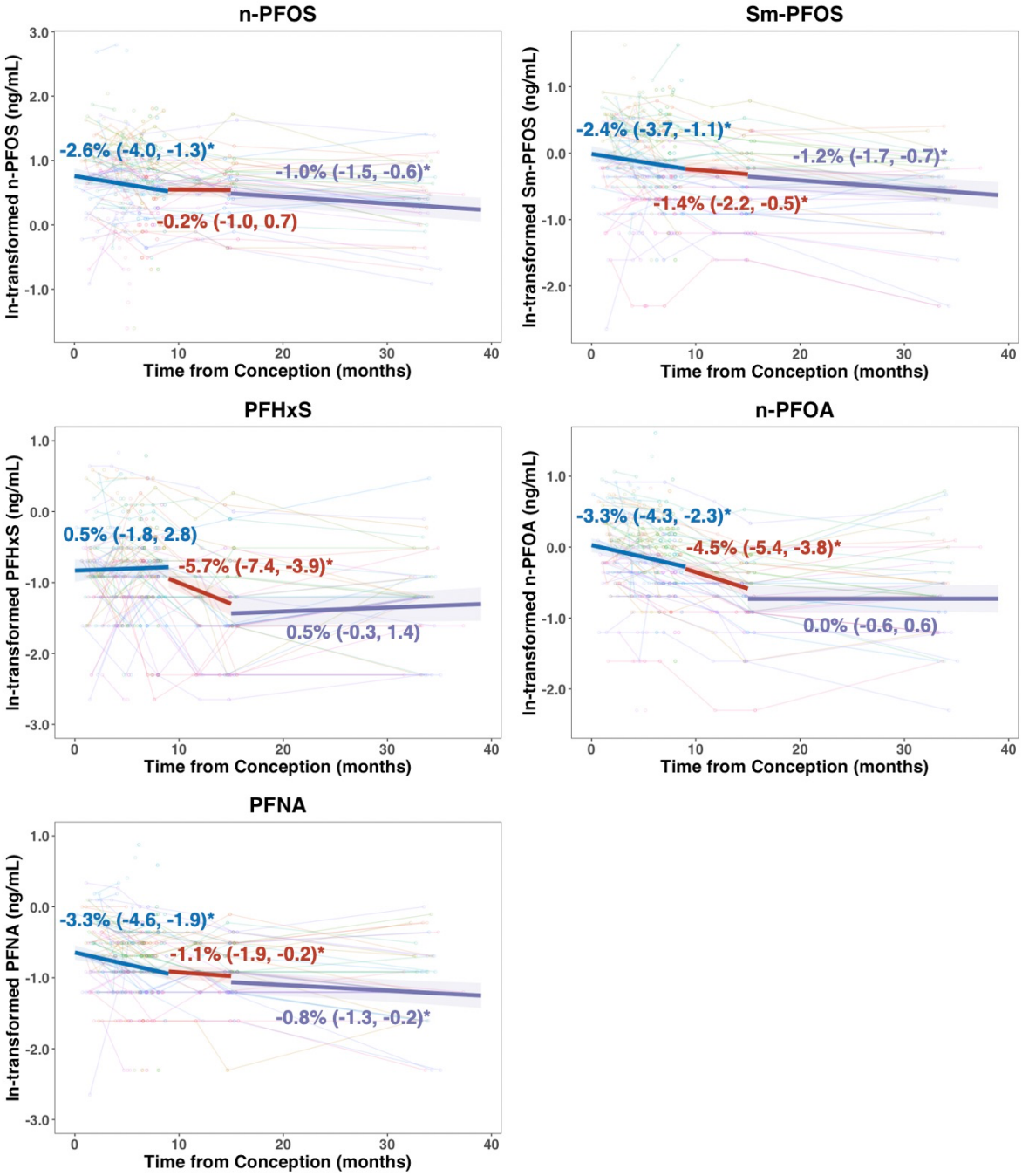

Figure S5.3 Sensitivity analysis: Percent changes in maternal serum PFAS concentrations over time after including 217 unique pregnancies that provided at least one blood sample during the whole study period. Thick lines in blue, red, and purple represent adjusted mean concentrations during pregnancy, early postpartum, and late postpartum, respectively, and shaded areas represent corresponding 95% CIs. Thin lines and dots represent individual trajectory of PFAS concentrations. Asterisk represents significant changes in PFAS concentrations over time. All models were adjusted for child's birth year, maternal age at delivery, parity, and maternal birthplace. Late postpartum models were additionally adjusted for breastfeeding duration.