THE ROLE OF THE PLACENTA-BRAIN AXIS IN CHILDREN'S NEURODEVELOPMENT: AN INTERDISCIPLINARY APPROACH TO UNDERSTANDING PLACENTAL ORIGINS OF BRAIN DEVELOPMENT INTO ADOLESCENCE

Carmen Amelia Marable

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Approved by:

Rebecca C. Fry

T. Michael O'Shea

Carmen Marsit

Jessica Cohen

Mohanish Deshmukh

ABSTRACT

Carmen Amelia Marable: The Role of the Placenta-Brain Axis in Children's Neurodevelopment: An Interdisciplinary Approach to Understanding Placental Origins Of Brain Development into Adolescence
(Under the direction of Rebecca C. Fry)

Neurodevelopmental dysfunction and neurologic disorders may have placental origins. During pregnancy, exposure to *in utero* stressors alters placental growth and formation resulting in fetal malprogramming. This disruption of placenta and fetal growth is associated with increased risk of neurodevelopmental dysfunction and neurologic disorders. Children born prematurely (<37 weeks of gestation) are at increased risk for neurodevelopmental disorders. This dissertation focuses on neurodevelopmental disability among children who are born extremely preterm (< 28 weeks of gestation). Included research leverages the unique extremely low gestational age newborns (ELGAN) cohort. Using transdisciplinary scientific approaches, I investigated the complex relationships that exist among the placenta, the neonatal brain, and the maturing brain into the critical adolescent phase of those born extremely prematurely.

My research provided a novel assessment of the transcriptomic architecture of the placenta in relation to ELGAN brain development. First, I examined the association between placenta mRNA expression and abnormalities in the cerebral white matter during the neonatal period. Next, findings were followed with additional studies of the relationship between the expression of inflammation and immune response-related placental transcripts as they related to adolescent regional brain volumes and neurodevelopmental disability at age 15. Lastly, I investigated the relationship between neonatal inflammatory markers and brain volume in

ELGAN adolescence. Hence, findings from these studies provide novel molecular insight into how physiological changes in the placenta are associated with structural or cognitive neurodevelopment. The research is unique in its longitudinal focus on brain development spanning the neonatal to adolescent periods of life. The results of my work demonstrate that inflammation in the placenta is tied to brain structure in the neonate, neurodevelopmental disability, and brain volume later in life. Taken together this work supports that children born extremely preterm with dysregulated placental transcriptomic expression represent a population of individuals particularly susceptible to cerebral white matter damage, reduced brain volumes, or cognitive deficits.

To my grandparents and parents. I could not have imagined this opportunity without you setting the standard and showing me through action that success is what we make it to be. Thank you for your prayers, immeasurable wisdom, and sacrifices.

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LIST OF ABBREVIATIONS

ADHD	attention deficit/hyperactivity disorder
AKT	protein kinase B
ASD	Autism Spectrum Disorder
ATP1A1	ATPase Na+/K+ transporting subunit alpha 1
ATRX	alpha thalassemia/mental retardation syndrome X-linked
BDNF	brain-derived neurotrophic Factor
BPA	Bisphenol A
BPS	Bisphenol S
CpG methylation	DNA methylation of cytosine
CST5	Cystatin D
DA	Dopamine
DOHaD	Developmental Origins of Health and Disease
ECHO	Environmental influences on Child Health Outcome
eIF2	eukaryotic translation Initiation Factor 2
EIF4G1	eukaryotic translation initiation factor 4 gamma 1
ELGAN	extremely low gestational age newborn
EP	Extremely preterm; <28 weeks' gestation
FC	Fold change
FKPB5	FK506 binding protein 5
GP6	Glycoprotein VI Platelet
GSK3B	glycogen synthase kinase 3 beta
HFS1	Heat shock transcription factor 1
HIF1A	Hypoxia-inducible factor 1-alpha

HPA	Hypothalamic–pituitary–adrenal
HSPA5	Heat shock protein A5
ID	Intellectual Disability
IGF2	Insulin growth factor 2
ILK	Integrin-linked kinase
IL1R1	Interlukin 1 receptor 1
Il-6	Interleukin 6
IPA	Ingenuity Pathway Analysis
IQ	Intellectual Quotient
LAMB1	laminin subunit beta 1
LARP1	La ribonucleoprotein 1
LPA	Latent Profile Analysis
MBD1	methyl-CpG binding domain protein 1
MRI	Magnetic resonance imaging
MUC1	mucin 1, cell surface associated
mTOR	Mammalian target of rapamycin
NCTB	National Institutes of Health Cognition Toolbox Battery
NF-κB	Nuclear factor kappa B
NR3C1	nuclear receptor subfamily group 3Cmember 1
OCT4	Octamer-binding transcription factor 4
PDLIM2	PDZ and LIM Domain 2
PI3K	phosphatidylinositol 3-kinase
POHaD	Placental Origins of Health and Disease
PTTPRR	protein tyrosine phosphatase receptor type R

qMRI	Quantitative brain MRI
ROI	Region of interest
RXR	Retinoid X receptor
SFARI	Simons Foundation Autism Research Initiative
SVA	Surrogate variable analysis
TBV	Total brain volume
TCF4	Transcription factor 4
VDR	Vitamin D receptor
WASI-II	Wechsler Abbreviated Scale of Intelligence-II
5-HT	Serotonin

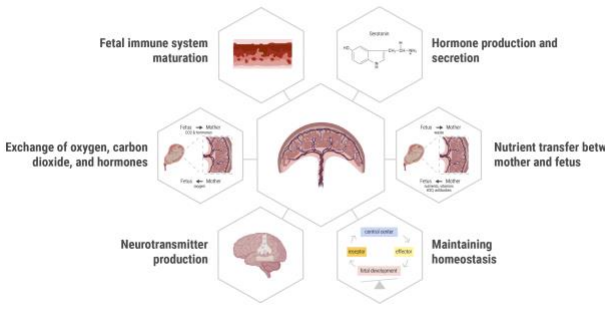
GENERAL INTRODUCTION

The Placenta-Brain Axis

Overview and Introduction of the Placenta

The placenta is a transient organ formed from fetal trophoblasts, essential during pregnancy to facilitate fetal growth and development.[1, 2] In doing so, the placenta carries out a plethora of indispensable functions throughout pregnancy (**Figure 1**). First, the placenta is responsible for transferring nutrients between the mother and the fetus.[3] As an endocrine organ, the placenta acts as a barrier, synthesizing and secreting hormones to maintain pregnancy and protect the fetus from adverse exposure to toxicants and stressors.[4] The placenta also shapes the maternal immune environment, by regulating maternal immune functions and secreting immune factors into fetal circulation, which ultimately aid in the maturation of the fetal immune system. [5]

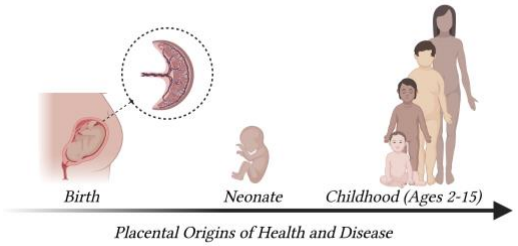
FIGURE 1: Important roles of the placenta in mediating physiological adaptations.



Lastly, the placenta assists mechanistically in maintaining homeostasis. [6] Being able to recognize and promptly respond to alterations in the *in utero* environment, such as inflammation, maternal stress, exposure to endogenous chemicals, or infection is an intrinsic property of the placenta's adaptability. [7] The aforementioned *in utero* stressors may prompt fetal programming and lead to the development of adverse health outcomes. [7]

An effort to understand and Identify the early life origins that are tied to later life development of adverse health outcomes is described as the developmental origins of health and disease (DOHaD) paradigm. [8] Although understanding DOHaD relationships can be complex, it may be more palatable to focus on a foundational organ that was present during development such as the placenta. Thus, exploring DOHaD through the lens of the placenta can be considered the placental origins of health and disease (POHaD) as shown in **Figure 2**. How the placenta responds to *in utero* stressors may dictate the trajectory of fetal development.

FIGURE 2: Placental origins of health and disease (POHaD).



The Placenta and Fetal Brain

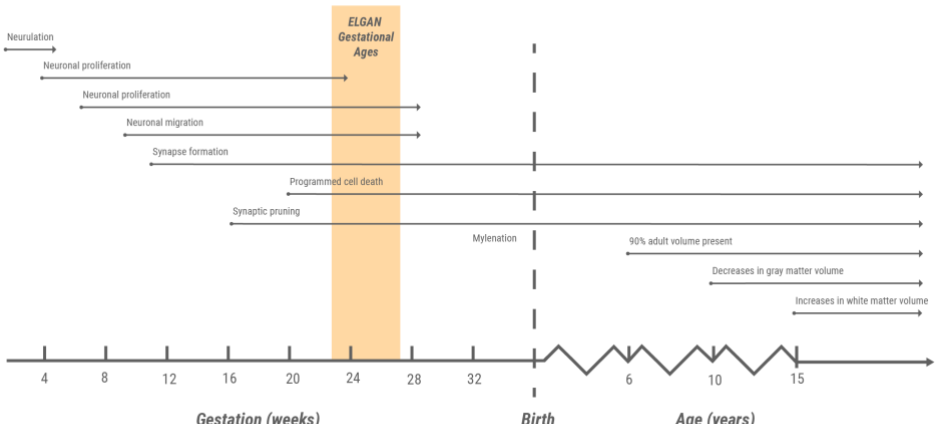
Increasing evidence has linked disruptions in placental growth and development to disrupted neurodevelopment. The basis for this linkage is the fact that the placenta is involved in sensing the *in utero* environment, controlling hormones, neurotransmitters and inflammation-related signaling molecules and is thus a critical organ implicated in brain development of the fetus. This concept is known as the placenta-brain axis, which will be the guiding focus moving forward. [9] Notably, the relevance of the placenta extends beyond pregnancy and into fetal programming, where placental changes are also able to guide fetal brain development. [7] The placenta is also responsible for producing circulating neurotransmitters that influence brain development such as dopamine (DA), norepinephrine/epinephrine, and serotonin (5-HT). [10] Several studies have highlighted the role of placenta-derived serotonin for normal brain development. [11-16] Alterations to serotonin signaling during this critical window of development during pregnancy increases the risk of psychiatric disorders across the lifespan. Taking a deep

dive into understanding the significance of the placenta-brain axis may guide us to identify key events that contribute to the origination of altered fetal brain development.

Brain Development in the Fetus and Child

Human brain development begins shortly after conception, continuing through childhood and into late adolescence. [17, 18] Fetal brain development begins with the differentiation of neural progenitor cells in the third week of gestation. [18] Contributions to brain development range from key initiating cellular and molecular events to epigenetic modifications responding to both the natural and built environments. Disruption of any of these contributing factors can fundamentally alter neural development. Throughout pregnancy, brain structure continues to change **(Figure 3)**. By week 10 of gestation, which is also the end of the embryonic period, distinct brain regions are characterized, and the central nervous system is established. [18] Changes in brain structure of the prenatal neural system are reflective of changes occurring at cellular and molecular levels. [17]

FIGURE 3: Stages of brain development from gestation to late adolescence.

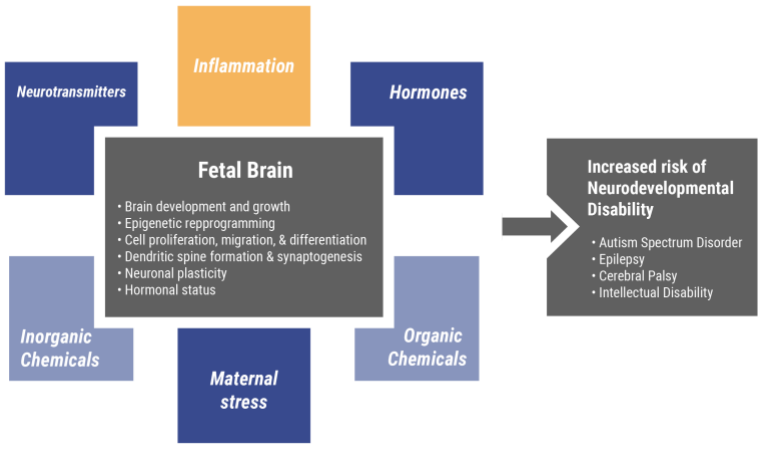


Processes such as the facilitation of neuronal migration, division, and organization as well as synapse development occur between the 23rd and 27th weeks of gestation. [19, 20] Perturbations during the facilitation of these vulnerable processes needed for brain maturation can lead to postnatal consequences. [21] Outwardly, the foundation of the brain is assembled prenatally, but brain function continues to develop after birth. During the preschool-aged period, the brain increases four-fold in size, and by age six it reaches approximately 90% of adult volume. [22, 23] However, structural changes in grey and white matter continue through late childhood and into adolescence, namely decreases in grey matter volume and increases in white matter volume. [18, 24] These structural changes are often paired with corresponding functional changes that can be evidenced in behavior. [18]

Potential Mechanisms Linking Placental Biology and Later Life Outcomes

The placenta supports fetal development through the production of hormones, proteins needed to metabolize endogenous molecules, neurotransmitters, and growth factors. [25, 26] Still, the contributions these placental processes provide to separate but linked biological domains of the fetus, and their later life neurodevelopment, are poorly understood. In a typically developing pregnancy, these processes are undisturbed throughout gestation. Studies have investigated disruptions in neurotransmitter production, inflammatory

FIGURE 4: Potential mechanisms linking dysfunction in placental biology that may affect various aspects of child brain development.



signaling, cellular toxicity, hormone changes, and epigenetic alterations as potential mechanisms that may influence the fetal brain and later-life neural outcomes (**Figure 4**).

A better understanding of potential contributing mechanisms will allow us to think critically about the biology that links the mother and the offspring via the placenta and make more informed preventative decisions concerning children's brain development.

Despite the placenta being the likely sole source of select neurotransmitters during initial brain formation, the placenta's ability to regulate fetal brain development through neurotransmitter production is not commonly acknowledged.[10] The placenta produces dopamine, epinephrine/norepinephrine, and serotonin which are able to circulate and influence the fetal brain. [10] Dysfunction of the neurotransmitter system is described as an imbalance of the excitation and inhibition of these neurotransmitters during key stages of development. [27, 28] An overwhelming amount of evidence supports the finding that maternal serotonin is unable to cross the blood-placenta barrier. [11-14] Studies suggest that the placenta is responsible for the direct synthesis of serotonin. [11-14] One biologically plausible reason for the linkage between placental disruption in serotonin levels and fetal brain development may be in part due to serotonin's role in stimulating neuronal migration, cell division, cell differentiation, and synaptogenesis during fetal brain development. [29]

In support of the connection between serotonin levels and fetal brain development, the placenta has been shown to be the potential sole provider of serotonin; both hyposerotonemia and hyperserotonemia were able to disrupt early neural programming. [30] Such alterations in the placental production of serotonin have been hypothesized to increase the risk of autism spectrum disorder (ASD). [29, 31] Alterations in the serotonergic neurotransmitter system may serve as a biological substrate of ASD, through the regulation of serotonergic overgrowth and maturation

of target brain regions. [32, 33] For instance, hyperserotonemia can trigger the impairment of sensory, cognitive, and motor abilities which collectively contribute to ASD and other neurodevelopmental disorders. [10, 29]

Dopamine is an excitatory neurotransmitter that is also be produced by the placenta. [10, 30] In the placenta, dopamine plays a crucial role in early brain development, and its receptors are present prior to mature synaptic contact formation. Additionally, dopamine receptors can modulate neurogenesis, differentiation, and neuronal migration. There are two major classes of dopamine receptors, D-1 like and D-2 like. The lack of balance in the dopamine system has been linked to developmental neurological disorder like ASD. One example of such imbalance is a recent study highlighting an influx in total placental dopamine concentration following developmental exposure to bisphenol A (BPA) and bisphenol S (BPS). [30]

Prenatal exposure to environmental toxicants and maternal inflammation may represent risk factors for neural development and neurological disorders.[34] Additionally, in the event the mother is exposed to neurotoxin/toxicants(s) during pregnancy and more specifically during a critical window of development, this may increase the risk of decreased dopamine neurons later in life in the offspring.[34, 35]

Inflammatory signals during pregnancy can be passed from mother to fetus via the placenta.[36] Moreover, maternal inflammation during this time can alter placental function and has been associated with an increased risk of neurodevelopmental disorders in children. [35] Placental inflammatory pathology is associated with subsequent child neurodevelopment at eight months and four years of age. These associations were evidenced by a 2.15-fold increased risk of low motor scores and a 1.51-fold increased risk of low mental scores at eight months following assessment using the Bayley Scales of Infant development, and a .13-fold increased risk of IQ of

70-84 at four years of age assessed using the Stanford-Binet IQ scale. [37] Another study has implicated the role of maternal-fetal inflammation in acute placental inflammation and its association with neurodevelopmental diseases. [38]

Although understudied, acute placental inflammation has been shown to mechanistically impact placental inflammation and long-term child health. [38] Acute placental inflammation can be further characterized as maternal inflammatory responses and fetal inflammatory responses, depending on the source of inflammation. While extensively examined, the association between acute placental inflammation remains uncertain as studies have mixed results ranging from an increased risk of lower mental development and low-performance IQ to no associations being found. [39-42]

Placenta-borne oxidative stress may cause apoptosis of syncytiotrophoblasts which are the placenta's epithelial covering needed for placental growth and differentiation, but it can also lead to elevated reactive oxygen species in the developing fetal brain. [43, 44] Dying syncytiotrophoblasts release inflammatory molecules into maternal circulation. [45] This in turn promotes systemic maternal inflammation. [45] Clinical studies have linked maternal and fetal inflammation to brain damage, such as cerebral white matter damage. [46]

By week nine of gestation, the placenta becomes the primary source of maternal estradiol and progesterone. [4] These steroid hormones are essential for maintaining pregnancy. Maternal stress can modulate the levels of these steroids present in maternal blood and placenta, impacting birth outcomes. A fetal consequence of experiencing too much stress during pregnancy includes a higher risk for preterm birth and low birth weight. [4] Excessive stress can disrupt placental biology, however distinct mechanisms responsible for healthy versus problematic outcomes as a result of stress remain unclear. [4] Maternal stress negatively impacting pregnancy outcomes is

thought to be primarily driven by glucocorticoid release. [47] However, stress also incites progesterone secretion. Progesterone metabolites are able to modulate the hypothalamic–pituitary–adrenal (HPA) axis function, but the role of progesterone and its metabolites in humans is not well characterized. [48] Allopregnanolone, a progesterone metabolite is neuroprotective as it aids in the premature secretion of oxytocin, helping to maintain pregnancy and minimize the risk of preterm birth.[49] Ultimately, the placenta's endocrine function is an important factor in better understanding and identifying potential mechanisms that may link placental dysfunction and later life neurologic outcomes.

Various approaches have been deployed to assess the role of epigenetic modifications as links between placental biology and later life outcomes. Placental gene expression and the expression of epigenetic modifiers such as miRNA and DNA methylation of cytosine (CpG methylation) could mechanistically underlie the relationship between placental dysfunction and developing impairments in children's neurodevelopment. Biological dysfunction that may influence placental malprograming can be explored using epigenomics and genomics. Placental CpG methylation has been identified as a major mechanistic vehicle for the placenta to respond to changing conditions during pregnancy that may alter long-term health outcomes. This relationship is supported by reports of placental CpG methylation in association with exposure to newborn neurobehavior. [50-55] Previously published work by researchers in the extremely low gestational age newborn (ELGAN) study group, showed a relationship between CpG methylation of placental inflammation genes and neurocognition in ELGAN children at age 10. [56] Many of the identified genes, such as brain-derived neurotrophic factor (*BDNF*) regulate both HPA axis function and placental function. The HPA-axis is a major component of the neuroendocrine system. Genes found within the HPA-axis signaling pathway play a role in placental physiology

and impact fetal development and the HPA-axis pathway has received significant attention with respect to the placenta-brain axis. For example, disruptions in the HPA-axis pathway can adversely affect learning, memory, and the development of psychological disorders. Published and preliminary data support that mRNA alterations of critical genes in the placenta predict neurodevelopmental disability later in life. [36, 57-60]

Neurodevelopmental Impairment

Neurodevelopmental disorders are a collective group of conditions resulting from impaired growth and development affecting the brain. These disorders may impact learning, memory, behavior, language, and self-control, which result in a range of impairments portrayed as delays and or deficits presented early in a child's development, continuing throughout the individual's life. In the United States, each year roughly 15 million children are born prior to 37 weeks' gestation, [61] this often involves complications of major organ systems such as the brain, which has been evidenced by early difficulties in neural development (i.e., intraventricular hemorrhaging, echolucency, and cerebral palsy). Resultingly, a great focus on understanding the developmental progression of neurodevelopment in extremely preterm (EP; <28 weeks' gestation) children may help identify contributing factors. Cerebral palsy (CP), epilepsy, autism spectrum disorder (ASD), and intellectual disability (ID) are four major neurodevelopmental disorders that I will discuss in greater detail in **Chapter 3**.

Autism Spectrum Disorder

Autism Spectrum Disorder is one of the most prevalent early-onset neurological and developmental disorders, diagnosed globally in roughly 1 in 100 children [62] and in about 1 in 44 children within the United States. [63] Since 2000, the prevalence of ASD has increased 178%, which is likely due to earlier diagnosis and increased awareness. [64] This neurodevelopmental disorder is quite complex and is thought to be the result of multiple neuropathological and

etiological mechanisms. [65] Thus, in the absence of a biological marker, autism spectrum disorder has been characterized by repetitive behaviors, restricted interests, and communication and social impairments. [66] Although there is no single cause that leads to ASD, several prenatal and perinatal risk factors have been studied and identified. For example, children born premature (< 37 weeks' gestation) are at an increased risk of health complications including abnormal brain development, and the risk of ASD. Studies found an association between ASD susceptibility and lower gestational age. [67] Because of this, there is a need to investigate potential associations between children born extremely preterm and the presence of ASD symptoms and diagnoses.

Over 20 years ago, adolescents and adults with ASD were reported to have increased brain volumes. [68] Since then, subsequent reports have identified evidence of brain overgrowth in children with ASD even earlier during childhood, following an autism spectrum disorder at three to four years of age when compared to age-matched typically developing children. [69] The aforementioned reports may be due to increasingly earlier detection and diagnosis of autism spectrum disorder.

Intellectual Disability

Intellectual disability is the most prominent developmental disability, affecting 1-3% of the global population and 6.5 million people in the United States.[70] Historically, the term "global developmental delay" and "intellectual disability" have been characterized as being genetically and phenotypically heterogeneous. Briefly, global developmental delay is often limited to younger children (typically 5 years of age or younger) who fail to reach developmental milestones within the expected age range, in two or more developmental domains. [71] The five developmental domains include cognition, gross or fine motor skills, speech and language, emotional maturity, and social competence. [71, 72] Intellectual disability (ID) involves problems affecting both cognitive functioning (i.e. learning and reasoning) and adaptive functioning (i.e.

communication and practical skills) that may originate later in childhood, once the intellectual quotient (IQ) testing is deemed more reliable. [73] However, when more severe delays are present across multiple developmental domains, individuals are often diagnosed with ID at earlier ages.[73]

Similar to ASD, there are a vast number of intellectual disabilities with varied etiologies. For this reason, I will focus specifically on impairments in cognitive functioning. Cognition is one of the four domains (cognition, sensation, movement, emotion) highlighted by the National Institutes of Health (NIH) Toolbox for the Assessment of Neurological and Behavioral Function (NIH-TB). The NIH-TB is unique in that it uniformly measures and captures these domains in the form of a 30-minute battery assessment. The NIH Toolbox Cognition Battery (NTCB) has been validated in a sample of 476 participants ranging from 3 to 85 years of age and includes seven assessments that capture Executive Function, Episodic Memory, Language, Processing Speed, Working Memory, and Attention. [74] These assessments are significant for success in school and work, health and wellness, and independence in daily functioning. [74] It is important to note that children born extremely preterm (EP) are at an increased risk of differential impairment as it relates to cognitive outcomes beginning early in childhood that persist into adolescence, [75, 76] such as executive function, [77-80] attention, [81, 82] general intelligence, [83-85] processing speed, [80, 86-89] and language. [90, 91]

Cognitive impairment is the most prevalent developmental disability among EP-born individuals. Most prominently, EP-related brain injury during the neonatal period such as white matter damage has been linked to deficits in school-aged cognitive outcomes. [92, 93] Cranial ultrasound and structural brain magnetic resonance imaging (MRI) have been used to provide insight into the neurological underpinnings of major neurological disorders including cognitive impairment. [94-98] When compared to children born full-term, previous MRI studies have

evidenced that children born EP have brain volume differences that persist into early childhood, [99] brain region-specific vulnerabilities following injury, [100] and smaller brain volume. [101]

Because neurodevelopmental differences for children born EP persist longitudinally in comparison to children born full-term, a better understanding of how early life factors may contribute to the risk of cognitive impairment is needed. There are currently only two large studies that investigate cognitive outcomes of individuals born EP after structural brain changes occur. [102, 103] Collectively, the literature suggests that further evaluations devoted to identifying and understanding early life factors in association with later life functional and structural brain development of EP-born individuals into adolescence are needed. With an innovative focus on the placental origins of health and disease and the placenta-brain axis, I hope to address these concerns through my dissertation research.

Key Scientific Research Gaps

Throughout pregnancy, brain structure continues to change and develop, and the pace of brain maturation may vary between regions, [104] suggesting some brain regions may be more vulnerable than others to alterations. The intrauterine environment largely regulates brain development via genetic control and with EP-born individuals, the brain maturation sequence may subsequently be affected depending on the nature and timing of perinatal insults. [105] There are major gaps in the literature surrounding systems-level mechanistic interaction between the brain and placenta. My studies will identify critical genes in the placenta that underlie MRI-derived brain outcomes including reduced brain volumes and neurological deficits and disorders, shifting the current research paradigms. Additionally, I will also investigate the role of neonatal inflammation as a potential antecedent of brain volume alterations later in life, to capture multiple temporal contributions of ELGAN neurodevelopment. I hope to address and add scientific value to the following understudied areas that exist within the scientific literature:

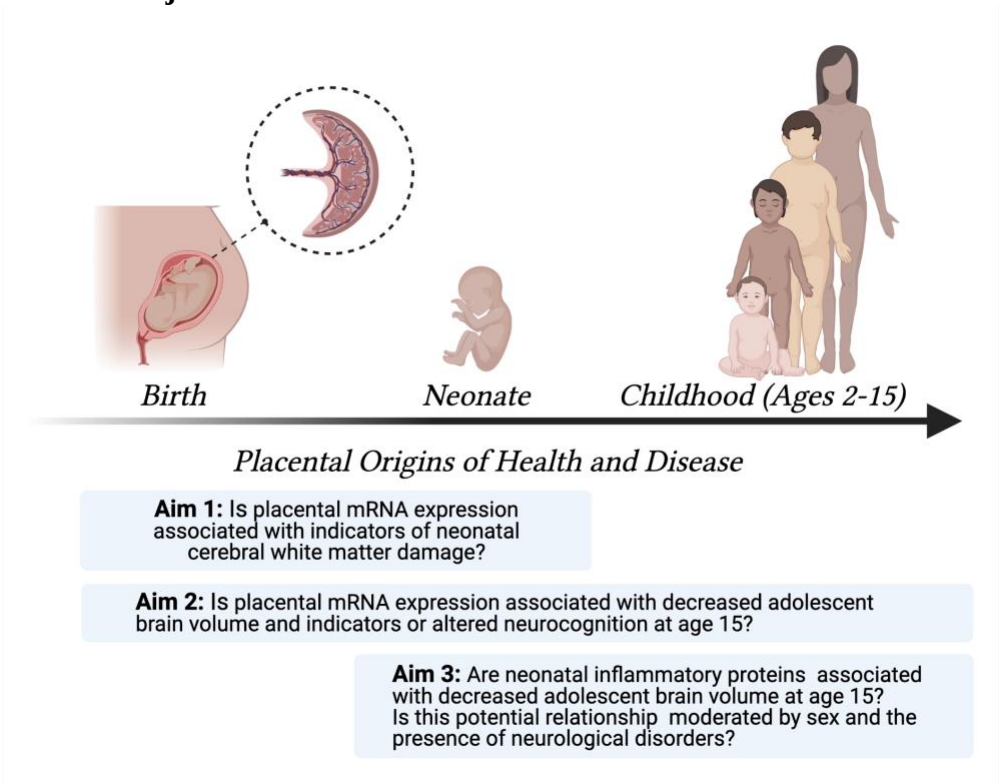
1. Understanding linkages between the molecular machinery in the placenta and brain damage in the neonate.
2. Understanding linkages between the molecular machinery in the placenta and brain volume in adolescents.
3. Understanding linkages between the molecular machinery in the placenta and neurocognitive outcomes in adolescents.
4. **Understanding sex-specific differences in the placenta-brain relationships**
5. **Understanding longitudinal or temporal trends in brain development and their linkage to the placenta.**
6. **Identifying specific genes and pathways in the placenta that are predictive and associated with white matter damage, brain volume alterations, and neurocognitive outcomes in adolescents.**

Project Overview

The research presented in this dissertation focuses on identifying initiators of brain damage and alterations in neurodevelopment, with a specific focus on highlighting molecular processes that may be implicated in decreasing the risk of brain damage and alterations in neurodevelopment, among vulnerable preterm infants born prior to 28 weeks of gestation.

A central goal of this dissertation is to address current gaps in the literature on the functional and structural neurodevelopment of children born extremely prematurely (less than 28 weeks' gestation). By exploring alterations in the human placenta as a potential influencer of neurodevelopmental disability and brain structure later in life, and the relationship between early-life inflammation, adolescent brain volume, and neurological disorders, we can better understand these understudied fundamental gaps in knowledge of what factors mechanistically influence the neurodevelopment of children born extremely preterm (**Figure 5**).

FIGURE 5: Project overview.



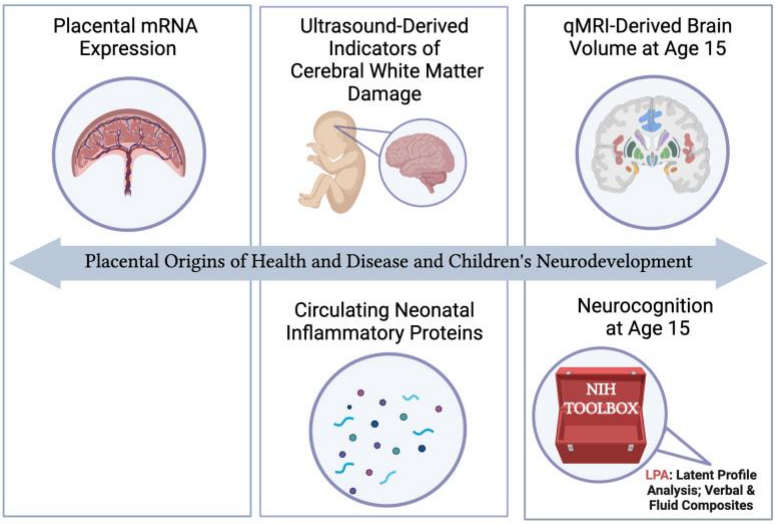
In addition, this work hypothesizes that placental gene expression will be able to provide molecular insight into *in utero* changes that lead to later life alterations and adverse effects in children's neurodevelopment. Previously in the extremely low gestational age newborn (ELGAN) cohort (N = 1506), we have found associations between placental genes related to stress response with both cognitive impairments among children born extremely preterm. [56] *Second*, our group has described strong associations between neonatal inflammation and both cognitive impairment, [57, 106-112] and reduced brain volume. [113] Third, inflammatory processes in the placenta are associated with neurodevelopmental disability later in life.[36, 114]

This project employs a multi-disciplinary approach, coupling placental biology, genomic assays, protein assessment of neonatal inflammation and later life clinically-assessed child neurological measures, to examine these hypotheses. Moreover, this work investigates an under-

studied mechanism, namely the disruption of key biological pathways in the placenta, as a driver of neurodevelopmental disability in children. Prior work from the ELGAN researchers have highlighted key findings that form a foundation for my research. For example, reduced brain volume in children born prematurely in comparison to children born full-term, [99] increased brain region development vulnerabilities in children born prematurely, [100] children born prematurely continue to be at significant risk for moderate-to-severe neurocognitive impairment into young adulthood, [75] and evidence of brain volume differences between preterm and full-term groups persisting into early adulthood are foundational to this research. [94] Findings from this work will provide mechanistic associations linking the placenta and early life inflammation to developmental deficits in the brain.

The primary research of my dissertation is detailed in Chapters 1,2 and 3. Together these studies employ the integration of several novel datasets including (1) transcriptome-wide data from the placenta; (2) neonatal blood protein biomarkers of early life inflammation; (3) cranial neonatal ultrasound measures of white matter damage; (4) neurodevelopmental disability assessments; and (5) qMRI-derived data on brain volume. My research has an emphasis on brain development

FIGURE 6: Overview of datasets used in Aims 1-3.



spanning birth to adolescence and utilizes sophisticated approaches to identify relationships between these neonatal, 2-year, 10-year, and 15-year outcomes (**Figure 6**).

Chapter 1 investigates the differential expression of 37,268 placental mRNA transcripts, in association with neonatal cranial ultrasound-derived measures of ventriculomegaly and echolucency in a longitudinal birth cohort using both transcriptome-wide data from the placenta and cranial neonatal ultrasound measures of white matter damage. This is detailed as the first of my three aims titled, "Examine the potential association between differential gene expression in the placenta and neonatal abnormalities in the cerebral white matter." My primary hypothesis for Aim 1 is that placental transcripts that encode for proteins that are involved in proinflammatory processes are highly expressed in the placentas of ELGANs who developed ultrasound-defined indicators of white matter damage.

Chapter 2 assesses the expression of 37,268 placental mRNA transcripts, in two independent associations with (1) qMRI-derived regional brain volumes and (2) measures of neurodevelopmental disability using the NIH Toolbox Cognition Battery (NTCB) at age 15. The rationale for conducting two independent analyses is to foundationally capture both structural and functional relationships. In this aim, I set out to evaluate the relationship between placenta transcriptomics and adolescent brain volumes at age 15 in the ELGAN cohort. The first hypothesis for this aim was that disrupted placental gene expression will be associated with regional adolescent brain volumes. This second aim also includes identifying promising indicators of neurocognitive disability at age 15 in relation to placental transcriptomic data. Then, determine whether placental proteomics mediates the relationship between placental transcriptomics and neurodevelopment measurements at age 15. I

hypothesized that disrupted placental gene expression would be associated with various indicators.

Lastly, Chapter 3 evaluates neonatal blood concentrations of circulating inflammatory protein biomarkers assessed in the first two postnatal weeks, reflecting sustained inflammation, in association with qMRI-derived brain volumes at age 15, and the presence of neurodevelopmental neurological disorders. This aim was to estimate the relationship, in a subsample of the ELGAN cohort, between early life circulating inflammation proteins, regional brain volumes for numerous anatomical structures at age 15, and the presence of neurological disorders. The hypotheses of this aim were that elevated circulating inflammation-related proteins are associated with decreased total brain volume and decreased regional brain volumes. We further hypothesize that these associations will be stronger for those ELGANs with neurological disorders.

The ELGAN study was established as a prospective multicenter birth cohort to assess the health effects of children born 23-27 weeks' gestation from 2002-2004. Prior to beginning my research, various aspects of the impact of low gestational age on fetal, neonatal, school-aged, and age-10 development were uncovered using data from this birth cohort, including the assessment of brain volumes in association with intelligence quotient (IQ) in full-term, preterm, and extremely preterm populations at various cross-sectional time-points; [115-117]

Specific to the ELGAN cohort, EP-born children with white matter damage (WMD), compared to children without WMD, were 3-4 times more likely to have intellectual deficits; [118] at age two, 40% of the children had at least one neurodevelopmental impairment; after comparing standardized test performance at ages two and 10, many children showed less impairment at age 10; the likelihood of developing cerebral palsy was greater for ELGAN

children who were exposed to more than one day of inflammation during the neonatal period, [117] and more likely to have attention deficits at 2 years of life, [111, 112] than children without inflammation; [118] placental CpG methylation of EP-born children was associated with cognitive impairment at age 10; [59] and our group has established that changes in white matter, cerebellum, and brainstem volume in children born prematurely are associated with lower IQ at age 10. [119]

My research expands on this collection of studies by investigating placental transcriptomics in association with white matter damage within the first month of life (**Aim 1**), structural and cognitive development at age 15 (**Aim 2**), and examining the relationship between sustained neonatal inflammation, brain volume at age 15, and the presence of neurological disorders **(Aim 3)**. Integrating epidemiologic approaches, neurodevelopmental assessments, and brain imaging, the significance of this work lies in providing a comprehensive overview of EP-born children's neurodevelopment assessing interactions between placental omics, neonatal inflammation, and ultrasound-derived white matter damage, brain volume and neurodevelopment at age 15, and neurological disorders captured from age 2 to age 15. The proposed research investigates an understudied mechanism, namely placental disruption of key biological pathways as a driver of neurodevelopmental disability in children born extremely. The findings from this work will provide a mechanistic foundation to identify potential biomarkers and antecedents of neurodevelopmental disability among EP-born children.

CHAPTER 1: PLACENTAL TRANSCRIPTIONAL SIGNATURES ASSOCIATED WITH CEREBRAL WHITE MATTER DAMAGE [1]

Background

Each year in the United States alone, more than 380,000 infants are born prematurely (<37 weeks gestational age). [61] Globally, this number reaches approximately 15 million infants.[120] Preterm birth predisposes the child to structural brain abnormalities, such as cerebral white matter injury, as well as functional disorders, such as neurodevelopmental impairments.[121] More specifically, infants who are born prematurely have a 1.3 and 2.64 relative risk of developing autism spectrum disorder (ASD) and attention deficit/hyperactivity disorder (ADHD), respectively. [121, 122]. The relative risks for these adverse outcomes are even larger for those who are born extremely prematurely (<28 weeks gestational age). [123] Premature birth is associated with reduced volume in both cerebral white and gray matter, and the period of greatest vulnerability to white matter injury is 23-32 weeks. [124] Preterm brains are particularly susceptible to cerebral white matter damage, characterized by diffuse white matter damage with aberrant regeneration of oligodendrocytes and disturbances of myelination. Among individuals born preterm, white matter damage is strongly associated with neurodevelopmental impairment, but specific treatment methods are not available for this disease. Among individuals born extremely

[1] A variation of this chapter previously appeared as an article in Frontiers in Neuroscience. The original citation is as follows: Marable, C.A., Roell, K., Kuban, K., O'Shea, T.M., Fry, R.C. "Placental transcriptional signatures associated with cerebral white matter damage in the neonate", *Frontiers in Neuroscience* 16, no. 1 (October 2022): 189.

preterm, neonatal cerebral white matter damage has been associated with an increased risk of autism spectrum disorder, [125] cognitive impairment, epilepsy, and cerebral palsy. [126]

Increasing evidence suggests that the placenta plays a critical role in programming early life origins of disease, including neurodevelopmental impairment. [56, 59, 127] During fetal development, the placenta serves as a critical transient organ and a conduit between the mother, the environment, and the fetus. Supporting the connection between the placenta and brain, placental inflammation has been associated with white matter damage in preterm neonates. [128] Inflammatory signals derived from the placenta including cytokines, chemokines, can influence the developing neurons of the fetal brain. [129] Gene expression in the placenta has been associated with intellectual and social impairment predictability, [127] and genome-wide CpG methylation has been related to cognitive impairment. [59] In addition, epigenetic processes such as DNA methylation and genome-wide gene and miRNA expression in the placenta have been related to infant neurodevelopment. [130] Due to the complexity of capturing robust RNA information from the placenta, few studies have investigated the relationship between mRNA abundance and neurodevelopment. One potential molecular mechanism that could underlie the association between altered expression of placental mRNA transcripts and white matter damage in the neonate is systemic and neuroinflammation in the fetus that disrupts oligodendrocytes development and impairs myelination specifically in the preterm brain. [92, 131]

Study Objectives

In the present study we set out to examine the relationship between genome-wide transcript levels in the placenta, and cerebral white matter damage in the Extremely Low for Gestational Age Newborn (ELGAN) cohort. We hypothesized that transcripts that encode for proteins that are involved in proinflammatory processes would be highly expressed in the placentas of ELGANs who developed ultrasound-defined indicators of white matter damage.

This research supports and contributes to increasing the understanding of the "developmental origins of health and disease" hypothesis by examining *in utero* transcriptomic changes in association with neonatal white matter damage.

Materials and Methods

ELGAN Study Cohort

The ELGAN study enrolled participants from 2002 to 2004 at 14 participating medical centers across the U.S. and was approved by the Institutional Review Board at each participating center. The eligibility criterion for the ELGAN study was gestational age less than 28 weeks. Study procedures have been comprehensively described.[113] The current analysis is based on a subsample of 279 children with data on at least two neonatal cranial ultrasounds, demographic data, and placental mRNA data.

Placental mRNA Expression Data

The methods for placental DNA and RNA extraction have been detailed in prior publications. [132, 133] The methodology for the RNA sequencing is detailed in Eaves e*t al*. [134]. Briefly, the AllPrep DNA/RNA/miRNA Universal kit was used to extract (Qiagen) RNA molecules ≥18 nucleotides and RNA quality was assessed using a LabChip (Perkin Elmer, MA, USA) instrument and RNA integrity numbers (RIN) determined. The isolated placental RNA samples from ELGANs were used to measure genome-wide mRNA expression profiles using the QuantSeq 3′ mRNA-Seq Library Prep Kit (Illumina). Libraries were pooled and sequenced (single-end 50 bp) on one lane of the Illumina Hiseq 2500 and the count of sequencing reads per mRNA were aligned to the GENCODE database v3 [135] and organized using Salmon. [136] This process yields 37,268 unique human RNA transcripts, including protein-coding and non-coding RNAs. The resulting summarized count data were then used in data processing and statistical analyses. Additional quality control steps were included to optimize the final results including:

(1) filtering out non-detectable transcripts (detailed below); (2) incorporating surrogate variables in the statistical modeling approach to account for other sources of heterogeneity (detailed below); and (3) confirming previously published placental mRNA transcripts were present in the data. Specifically, Eaves e*t al* [134] compared the mRNA data from the present study with the most abundant mRNAs from an independent placental whole genome RNA-sequencing study [137], resulting in a 96% overlap between the mRNA transcripts and the mRNA transcripts. Details of this analysis can be found in Eaves e*t al.* [134]

Maternal and Newborn Characteristics

Maternal characteristics were previously ascertained via interview of the mother within a few days of delivery. Data collected during this interview included maternal race, education, and eligibility for public assistance, as well as the mother's height and weight prior to the ELGAN pregnancy. Maternal medical records were reviewed by trained research assistants to collect information about pre-natal maternal health, pregnancy complications, medications taken during pregnancy, and medical treatments provided to mothers. The gestational age estimates were based on a hierarchy of the quality of available information. Prioritized were estimates based on the dates of embryo retrieval or intrauterine insemination or fetal ultrasound before the 14th week (62%). When these were not available, reliance was placed sequentially on a fetal ultrasound at 14 or more weeks (29%), last menstrual period without fetal ultrasound (7%), and gestational age recorded in the log of the neonatal intensive care unit (1%).[133]

Cranial Ultrasounds

During study participants' initial hospitalizations (typically, the first 3-4 postnatal months), ultrasounds were collected as a component of routine clinical care. Extensive efforts, described elsewhere, were directed towards enhancing the reliability of radiologists' interpretations of ultrasound findings interpretations of cerebral white matter injury, as indicated

by either ventriculomegaly or echolucency,[138, 139] which are the outcomes in this study. A minimum of two sonologists read the scans and for any scans that were not 100% concordant, a third sonologist resolved the discrepancy. [19,20]

Data Processing & Analytical Methods

To identify the placental mRNAs associated with cerebral white matter damage, mRNA sequencing data was processed using R (v 3.6.2) (cran.r-project.org/). Similar to our previously published genome-wide mRNA analyses, [134] mRNA count data were normalized by median signal intensity using algorithms within DESeq2 (v1.24.0) to produce variance-stabilized expression counts.[140] Differential mRNA expression analysis was performed with an exclusion criterion that account for: (1) low expression values; (2) sample outliers identified through principal component analysis (PCA); and (3) missing demographic information. Prior to differential gene expression testing, batch effect and cell type differences were accounted for using surrogate variable analysis (SVA) within the SVA R package (v3.32.1). [141] This resulted in a total of n = 11,981 mRNA analysis-ready transcripts. In these models, the dependent variables were white matter damage measures of ventriculomegaly or echolucency and the independent variables were each of the 11,981 mRNA expression levels.

Known and potential hidden confounders for gene expression were estimated using the SVA approach[141] to identify significant (FDR-corrected $p<0.1$) associations between mRNA transcripts and white matter damage variables. Models were also adjusted for potential confounders defined as variables that are associated with both placental gene expression and white matter damage. These included: maternal age (years; continuous), maternal education (12, 13-15, <= 16 years; categorical), insurance status (Medicaid/no Medicaid; binary), fetal sex (male/female; binary), newborn gestational age at delivery (days; continuous), and newborn birth weight z scores (< -2, < -1, >= -1; categorical). In the primary model (Model 1), race was

excluded from the analysis, as self-reported race does not represent a biological variable. Its inclusion has been met with controversy.[142] Nevertheless, to address this potential confounder, a secondary analysis (Model 2) included the aforementioned variables as well as race (White, Black, Other; categorical) and ethnicity (Hispanic/non- Hispanic; binary).

Pathway and Protein-Protein Network Enrichment Analyses

We examined the established functional relationships among the proteins encoded by the identified genes and their biological pathways using the Ingenuity Pathway Analysis (IPA, Ingenuity Systems®, Redwood City, CA) and STRING v10.0. [143, 144] Enriched canonical pathways were defined as those containing more cerebral white matter damage-associated mRNAs than expected by random chance, based on a BH-corrected p-value calculated from a right-tailed Fisher's Exact Text. The IPA relationships were only considered if they had been experimentally observed. Pathways with enrichment BH-corrected p-values < 0.05 were considered significant.[145] To understand the mechanisms of transcriptional regulation underlying the observed changes in gene expression of mRNAs, the upstream regulator analysis (URA) in IPA to identify transcriptional regulators of those genes. URA uses information about the direction of the gene expression to provide an activation z-score to measure the likelihood of certain molecules to serve as regulators based on statistically significant matched patterns of down- and up-regulation using IPA libraries. The URA is also able to predict activation state of a putative regulator, which can be either activated or inhibited. Analysis was restricted to only include genes, RNAs, and proteins. We analyzed and reported canonical pathways, diseases and disorders, and molecular and cellular functions from IPA and protein domains from STRING enriched among these gene sets. To capture ASD-implicated proteins in our study, protein lists were cross-referenced with the Simons Foundation Autism Research Initiative (SFARI) gene database. [146]

Data Availability

Raw and processed placental molecular sequencing data have been submitted to National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) repository and are publicly available under GEO series GSE154829.[147]

Results

Summary of Study Participants

Among surviving ELGAN participants (n = 1506), 379 (25.2%) had the required demographic data, placental mRNA data, and ultrasound data. General characteristics of the study participants from the full ELGAN cohort and the study sub-cohort are summarized in **Table 1.1**. Among the sub-cohort analyzed in the present study, a total of 110 (29%) self-identified as Black and 232 (61.2%) as White. Two hundred and one (53%) were male and 178 (47%) were female. The majority of infants were born between 25-26 weeks. Overall, 54 (14.2%) had white matter damage defined as either ventriculomegaly, echolucency or both as determined via ultrasound assessed during the neonatal period (birth-two months of life). Demographic characteristics were largely similar between the full ELGAN cohort and the study sub-cohort for race and ethnicity, sex and gestational age.

TABLE 1.1: Maternal demographics, pregnancy characteristics, and neonatal outcomes among study participants.

Maternal and pregnancy characteristics		[Full ELGAN cohort]	[Study sub-cohort]
N (%)		*n=1506*	*n=379*
Racial identity (self-reported)	Black	427 (28.4)	110 (29.0)
	White	866 (57.5)	232 (61.2)
	Other	187 (12.4)	35 (9.23)
	Missing	26 (1.73)	2 (0.53)
Hispanic ethnicity (self-reported)	Yes	179 (11.9)	32 (8.44)
	No	1313 (87.2)	347 (91.6)
	Missing	14 (0.93)	0 (0%)
Sex	Female	707 (46.9)	178 (47.0)
	Male	799 (53.1)	201 (53.0)
Gestational age completed weeks (days)	23-24 (161-168)	409 (27.2)	88 (23.2)
	25-26 (175-182)	661 (43.9)	163 (43.0)
	27 (189)	436 (29.0)	128 (33.8)
White matter disease (ventriculomegaly and/or echolucency)	Yes	230 (15.3)	54 (14.2)
	No	1225 (81.3)	325 (85.8)
	Missing	51 (3.39)	0 (0)
Ventriculomegaly	Yes	172 (11.4)	45 (11.9)
	No	1283 (85.2)	334 (88.1)
	Missing	51 (3.39)	0 (0)
Echolucency	Yes	113 (7.50)	25 (6.60)
	No	1342 (89.1)	354 (93.4)
	Missing	51 (3.39)	0 (0)

Distributions of select characteristics among study participants of the Extremely Low Gestational Age Newborns Cohort, 2002–2004. Maternal demographic data, pregnancy characteristics, and data on birth outcomes are presented for the ELGAN subjects used in each analysis. Data are presented as the number (%) of subjects in the cohort. *Note that some subjects have both ventriculomegaly and echolucency, so the number of subjects with ventriculomegaly and or echolucency is not intended to be equivalent to the summation of ventriculomegaly and echolucency.

Placental mRNA Transcripts Among ELGANs Are Associated with White Matter Damage

A total of 659 genes were identified in the placenta that showed an association of their expression levels with either echolucency or ventriculomegaly. A common set of 244 of the 659 genes or 37% were associated with both echolucency and ventriculomegaly (**Figure 1.1**). Of these 244 common genes, 242 (99%) showed increased expression and two showed decreased expression (**Figure 1.2, Supplemental Table 1.1a**).

FIGURE 1.1: Venn diagram of differentially expressed genes in the placenta in relation to cerebral white matter damage. Venn diagram demonstrating differentially expressed placenta genes in relation to echolucency, ventriculomegaly or both. A total of 625 genes displayed associations with echolucency, 278 genes displayed associations with ventriculomegaly, and 244 genes were displayed associations with both echolucency and ventriculomegaly

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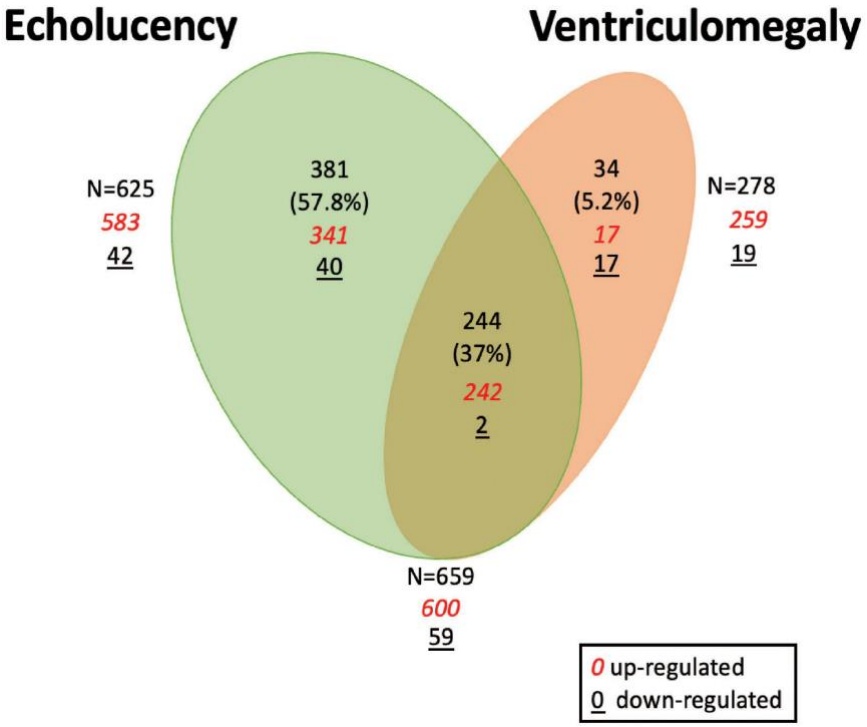
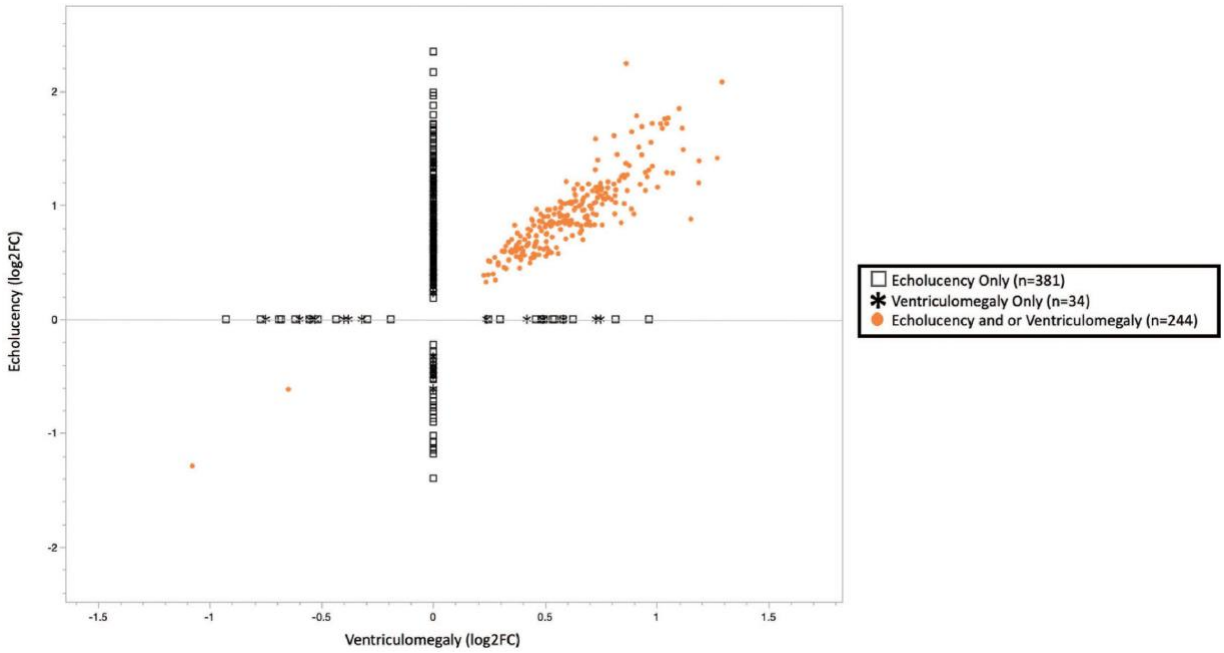


FIGURE 1.2: Plot of cerebral white matter damage-associated genes (*n* = 659). Similar patterns of gene expression were identified for the common gene set (*n* = 244), 242 or 99.2% genes had increased expression while 2 or 0.8% had decreased expression. While 63% of the echolucency and ventriculomegaly-associated genes displayed distinct expression levels. Log2-fold changes in mRNA expression associated with ventriculomegaly vs. echolucency. Data points colored in orange indicate genes significantly upregulated or downregulated (FDR *p*-value < 0.1) associated with both ventriculomegaly and or echolucency, data points denoted by an open square indicate genes significantly (FDR *p*-value < 0.1) associated with echolucency, and data points denoted by a star indicate genes significantly (FDR *p*-value < 0.1) associated with ventriculomegaly.



Echolucency was associated with the altered expression of 625 genes in placental tissue (**Figure 1.1, Supplemental Table 1.1a**). Of these genes, the majority (n=583) displayed increased expression in relation to echolucency, while 42 genes displayed decreased expression (**Figure 1.1, Supplemental Table 1.1a**). Of these 625 echolucency-associated genes, 381 were unique (e.g. not identified in relation to ventriculomegaly) to this form of white matter damage. For these 381 echolucency unique genes, the majority (n=341 or 89.5%) displayed increased expression and 40 displayed decreased expression (**Figure 1.2, Supplemental Table 1.1a**).

Ventriculomegaly was associated with the altered expression of a total of 278 genes in placental tissue (**Figure 1.1, Supplemental Table 1.1a**). Of these, the majority (n=259 or 93%) displayed increased expression, while 19 genes displayed decreased expression (**Figure 1.1, Supplemental Table 1.1a**). Of the 278 ventriculomegaly-associated genes, 34 were unique (e.g. not identified in relation to echolucency) to this form of white matter damage. For these 34 ventriculomegaly unique genes, 17 displayed increased expression and 17 displayed decreased expression (**Figure 1.2, Supplemental Table 1.1a**).

In an alternate approach, statistical models that included self-reported race and ethnicity were run (Model 2). Similar to Model 1, this resulted in a total of 659 placental genes with altered expression. In relation to both indicators of white matter damage, a total of 231 (35%) genes were associated with altered expression (**Supplemental Table 1.1a**). There was a 93% (n=226) overlap between Model 1 (n=244) and Model 2 (n=231) detailed in **Supplemental Table 1.1a**.

Biological Pathway, Canonical Pathway, and Molecular and Cellular Function Enrichment Analysis Reveal Placental Signatures of White Matter Damage

Analysis of the common set of 244 genes resulted in the identification of the enrichment of numerous key canonical pathways (p-values ≤ 0.05). The top five included: eukaryotic translation Initiation Factor 2 (eIF2) Signaling, Mammalian target of rapamycin (*mTOR*) Signaling, Regulation of eIF4 and p70S6K Signaling, DNA Double-Strand Break Repair by Homologous Recombination, and Interleukin 6 (Il-6) (**Table 1.2, Supplemental Table 1.1b**). Interleukin 1 receptor 1 (*IL1R1*), which displayed increased expression in relation to both forms of white matter damage, is one of the genes identified as a part of the IL-6 pathway (**Supplemental Table 1.1b)**. Specifically, *IL1R1* displayed a log2 fold change (FC) of +0.56 in relation to echolucency and a log2 FC of +0.41 in relation to ventriculomegaly. Diseases and

disorders that were enriched among the common set of white matter damage-associated genes, include those related to organismal injury and abnormalities, cancer, endocrine system disorders, neurological disease, inflammatory response, and inflammatory and immunological disease (p-values ≤ 0.01) (**Supplemental Table 1.2a)**. A notable gene identified from the diseases and disorders analysis included heat shock protein A5 (*HSPA5*), which is expressed in response to a range of cellular stressors, (**Supplemental Table 1.2a**). Specifically, *HSPA5* displayed a log2 fold change (FC) of +0.65 in relation to echolucency and a log2 FC of +0.42 in relation to ventriculomegaly (**Supplemental Table 1.2a**). Molecular and cellular functions that were enriched among common set of the white matter damage-associated genes included RNA damage and repair, cell death and survival among others (**Supplemental Table 1.2b**).

TABLE 1.2: Top canonical pathways enriched among the 244 common genes.

Canonical Pathways	p-value	Downregulated	Upregulated	Encoded Proteins
EIF2 Signaling	5.40E-13	0/212 (0%)	20/212 (8%)	EIF3I, FAU, HSPA5, PIK3C2A, PPP1R15A, PTBP1, RALA*, RALB, RAP1B, RPL12, RPL13A, RPL24, RPLP2, RPS10, RPS11, RPS8, RPSA
mTOR Signaling	4.37E-06	0/204 (0%)	12/204 (6%)	DGKZ, EIF3I, FAU, PIK3C2A, RALA*, RALB, RAP1B, RHOQ, RPS10, RPS11, RPS8, RPSA
Regulation of eIF4 and p70S6K Signaling	5.85E-06	0/175 (0%)	11/175 (6%)	EIF3I, FAU, ITGA1, PIK3C2A, RALA*, RALB, RAP1B, RPS10, RPS11, RPS8, RPSA
DNA Double-Strand Break Repair by Homologous Recombination	3.48E-07	0/14 (0%)	5/14 (29%)	ATRX, BRCA2*, MRE11, RAD50
Hereditary Breast Cancer Signaling	4.73E-06	0/139 (0%)	9/139 (6%)	ARID1A, BRCA2*, MRE11, PIK3C2A, RAD50, RALA*, RALB, RAP1B, XPC*
IL-6 Signaling	1.16E-04	0/128 (0%)	8/128 (6%)	IL1R1, MAPKAPK2, MCL1, PIK3C2A, PTPN11, RALA*, RALB, RAP1B

*Note ---ASD genes within the study are denoted by an *.*

The 381 echolucency unique genes were enriched for numerous canonical pathways including the follow top five: Vitamin D receptor (VDR) and retinoid X receptor (RXR) Activation, Glycoprotein VI Platelet (*GP6*) Signaling Pathway, Role of octamer-binding transcription factor 4 (*OCT4*) in Mammalian Embryonic Stem Cell Pluripotency, Retinoic Acid Receptor RAR Activation, and Growth Hormone Signaling, (**Table 1.3, Supplemental Table 1.1c**). For these canonical pathways, the following gene expression patterns were observed: within the Growth Hormone Signaling Pathway, a decrease in insulin like growth factor 2 (*IGF2*). Specifically, *IGF2* displayed a log2 fold change (FC) of -0.77 in relation to echolucency. Also, from the Growth Hormone Signaling Pathway was insulin like growth factor binding protein 3 (*IGFBP3*) which displayed a log2 fold change (FC) of +.0.83 in relation to echolucency. (**Supplemental Table 1.1c**). A total of 25 diseases and disorders were enriched among the echolucency unique genes including the following: organismal injury and abnormalities, cancer, endocrine system disorders, neurological disease, and inflammatory and immunological disease (p-values ≤ 0.05) (**Supplemental Table 1.3a**). There were 21 molecular and cellular functions that were enriched within the distinct set of echolucency-associated genes including cellular growth and proliferation, cell death and survival among others (**Supplemental Table 1.3c**).

TABLE 1.3: Top canonical pathways enriched among the 381 echolucency unique genes.

Canonical Pathways	p-value	Downregulated	Upregulated	Encoded Proteins
VDR/RXR Activation	2.50E-03	1/77 (1.3%)	5/77 (6.5%)	IGFBP3, KLF4, MED1, PRKCA*, PRKCZ, SPP1
GP6 Signaling Pathway	6.80E-03	1/124 (0.8%)	6/124 (4.8%)	COL4A1, LAMA5, LAMB1*, LAMC1, LAMC3, PRKCA, PRKCZ
Role of OCT4 in Mammalian Embryonic Stem Cell Pluripotency	8.27E-03	0/45 (0%)	4/45 (8.9%)	IGF2BP1, NR2F1, REST, SPP1
RAR Activation	8.40E-03	0/195 (0%)	9/195 (4.6%)	BRD7, IGFBP3, MED1, NR2F1*, PRKCA*, PRKCZ, PRMT2, SMARCC1, SMARCE1
Growth Hormone Signaling	8.66E-03	1/71 (1.4%)	4/71 (5.6%)	CSH1/CSH2, IGF2, IGFBP3, PRKCA*, PRKCZ

*Note ---ASD genes within the study are denoted by an *.*

Pathway analysis of the 34 ventriculomegaly unique genes highlighted the following top five canonical pathways, Tumor protein P53 (p53) Signaling, MicroRNA Biogenesis Signaling Pathway, Integrin-linked kinase (ILK) Signaling, phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) Signaling, and Integrin Signaling (**Table 1.4, Supplemental Table 1.1d**). Within the P53 Pathway, a decrease in the expression of glycogen synthase kinase 3 beta (*GSK3B*) (log2 FC: -0.43) was observed. Organismal injury and abnormalities, cancer, endocrine system disorders, neurological disease, inflammatory response, and immunological disease (p-values ≤ 0.05) were significantly enriched in the ventriculomegaly unique genes (**Supplemental Table 1.3b**). Among the set of unique ventriculomegaly genes, we identified 21 enriched molecular and cellular functions that included cellular function and maintenance, cell-to-cell signaling and interaction and others (Enter info for unique gene sets (**Supplemental Table 1.3d**).

TABLE 1.4: Top canonical pathways enriched in the 34 ventriculomegaly unique genes.

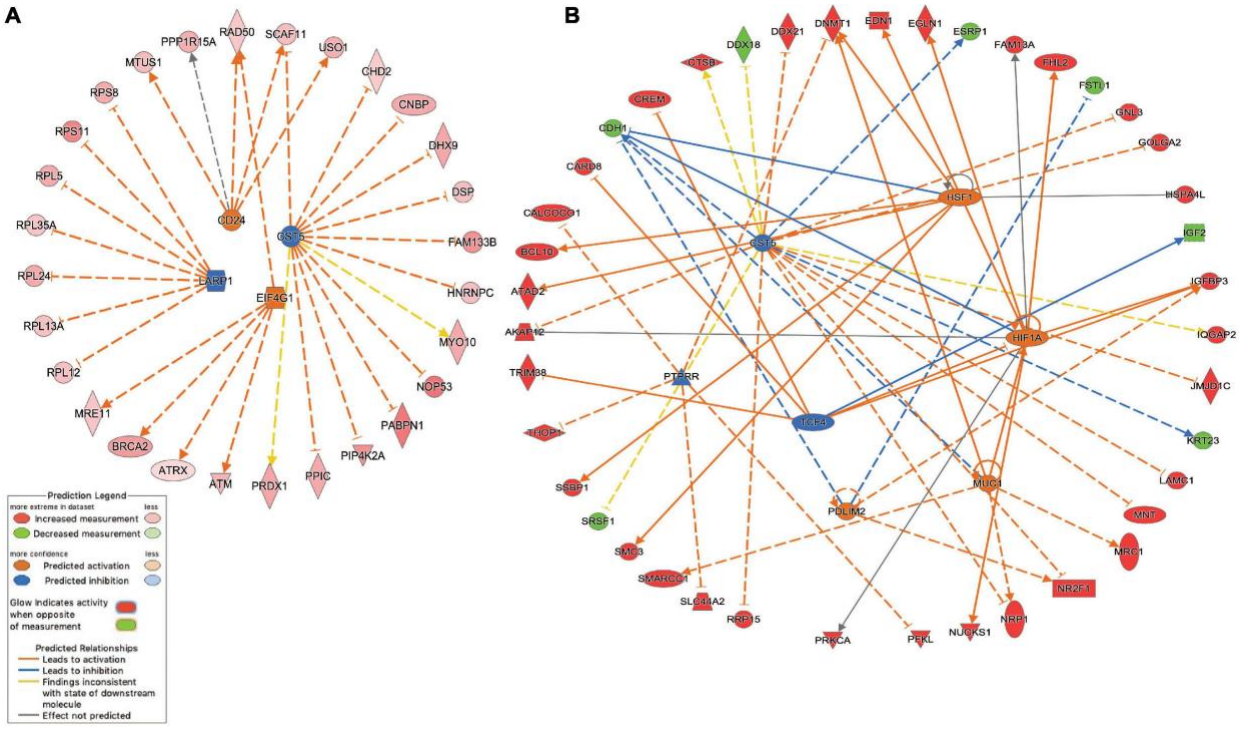
Canonical Pathways	p-value	Downregulated	Upregulated	Encoded Proteins
P53 Signaling	4.96E-04	2/98 (2.0%)	1/98 (1%)	GSK3B, ST13, STAG1*
MicroRNA Biogenesis Signaling Pathway	2.99E-03	1/183 (0.5%)	2/183 (1.1%)	DDX5, HSP90AB1, ST12
ILK Signaling	3.63E-03	2/196 (1.0%)	1/196 (0.5%)	GSBK3B, ITGB2, RHOBTB1
PI3K/AKT Signaling	3.73E-03	1/198 (0.5%)	2/198 (1%)	GSK3B, HSP90AB1, ITGB2
Integrin Signaling	4.11E-03	2/205 (1%)	1/205 (0.5%)	GSBK3B, ITGB2, RHOBTB1

*Note ---ASD genes within the study are denoted by an *.*

Potential Regulatory Mechanisms Underlying White Matter-Associated Gene Expression Changes

Network analysis identified 64 protein-protein interactions among the 244 common white matter damage-associated genes (**Supplemental Figure 1.1**). The identified network has significantly more interactions than expected by chance (protein-protein interaction enrichment p-value: 2.11e-11). To gain an understanding of transcriptional regulators that may underly the expression of the common gene set, IPA upstream regulator analysis was performed, identifying 131 significant upstream regulators (p-value<0.05 (**Supplemental Table 1.4a**). Among those upstream regulators is eukaryotic translation initiation factor 4 gamma 1 (*EIF4G1)* and CD24 molecule *(CD24)* were identified as predictively activated regulators (*z*-score = 2.24 and 2.00, respectively). In contrast, La ribonucleoprotein 1 (*LARP1)* and cystatin D (*CST5)* were identified as predictively inhibited regulators (*z*-scores = -2.65 and -2.50, respectively). **Figure 1.4a** highlights these four upstream regulators and their target genes.

FIGURE 1.3: Inhibited/activated upstream regulators and their target genes. (A) An integrated network *among* the common set of *cerebral white matter associated genes* (*n* = 244) *that includes* La ribonucleoprotein 1 (*LARP1)*, CD24 molecule *(CD24)*, Cystatin D (*CST5)*, and eukaryotic translation initiation factor 4 gamma 1 (*EIF4G1);* **(B)** An integrated network *among the echolucency unique genes (n = 381*) that includes *CST5*, Hypoxia-inducible factor 1-alpha (*HIF1A*), mucin 1, cell surface associated (*MUC1*), PDZ and LIM Domain 2 (*PDLIM2*), *PTPRR*, and transcription factor 4 (*TCF4*) *1.* All the target genes were differentially expressed based on the meta-analysis. For example, the activation of *EIF4G1* leads to the overexpression (indicated by the orange arrow line) of alpha thalassemia/mental retardation syndrome X-linked (*ATRX*) (indicated by the red color). For other indicators, please refer to the Prediction Legend.



Network analysis identified 106 protein-protein interactions among the 381 echolucency unique-associated genes (**Supplemental Figure 1.2**). The identified network has significantly more interactions than expected by chance (protein-protein interaction enrichment p-value: 0.004). Additionally, 241 significant upstream regulators were identified from the echolucency unique gene set (**Figure 1.4b**). These included Cystatin D (*CST5)*, protein tyrosine phosphatase receptor type R (*PTTPRR*), and transcription factor 4 (*TCF4*) (*z*-scores = -2.21, -2.22, and -2.24,

respectively) were predicted to be inhibited relative to echolucency, while mucin 1, cell surface associated (*MUC1*), heat shock transcription factor 1(*HFS1*), Hypoxia-inducible factor 1-alpha (*HIF1A*) and PDZ and LIM Domain 2 (*PDLIM2*) were predicted to be activated (*z*-scores = 2.19, 2.43, 2.39, and 2.0 respectively). Another notable upstream regulator identified from the echolucency gene set is estrogen receptor 1 (ESR1) (**Supplemental Table 1.4b)**.

Network analysis identified two protein-protein interactions among the 34 ventriculomegaly unique genes (**Supplemental Figure 1.2**). The identified network did not have significantly more interactions than expected by chance (protein-protein interaction enrichment p-value: 0.55). A total of 52 significant upstream regulators were identified from the ventriculomegaly unique genes (p-value<0.05). Due to the small number, predicted activation and inhibition of upstream regulator information was unavailable for the ventriculomegaly unique gene set (**Supplemental Table 1.4c)**.

ASD and Endocrine System Disorder Associated Biological Processes Identified Among the White Matter Damage-Associated Genes.

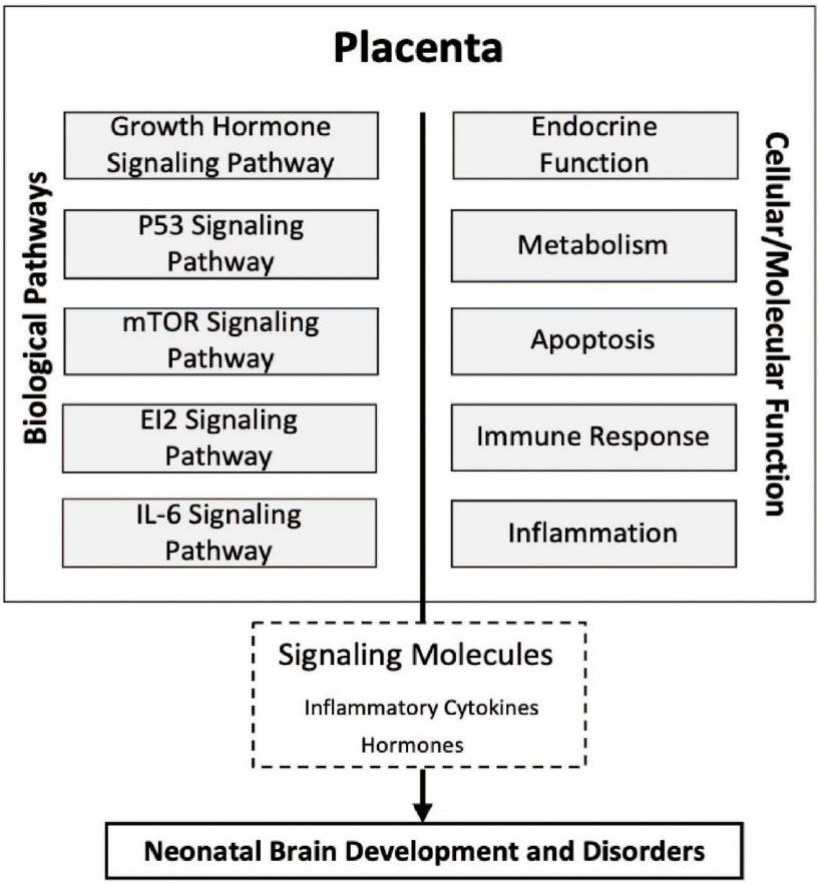
To identify whether any of the white matter damage-associated genes from the present study (n=659) have known alterations in neurodevelopmental disease states, we compared these genes to a list of n=1075 autism spectrum disorder (ASD)-associated genes obtained from the SFARI database. Numerous (n=63, 3.8%) ASD-associated genes were found including common (n=24/244), echolucency unique (n=31/381), and ventriculomegaly unique (n=1/34) genes. These genes included laminin subunit beta 1(LAMB1), ATPase Na+/K+ transporting subunit alpha 1 (*ATP1A1*), alpha thalassemia/mental retardation syndrome X-linked (*ATRX*), and methyl-CpG binding domain protein 1 (*MBD1*) (**Supplemental Table 1.1a**).

Furthermore, to identify whether any of the white matter damage-associated genes from the present study (n=659) have known alterations in the endocrine system, we analyzed these

genes within the IPA database. A total of 170 endocrine system disorder genes were differentially expressed in the placenta in relation to the common gene set (n=71/244) and also the echolucency unique genes (n=99/381) (**Supplemental Table 1.1a**). Interestingly, these genes also included the ASD-associated genes *IGFBP3*, *ATRX*, *LAMB1* and *MBD1* (**Supplemental Table 1.1a**).

Taken together, the results of the present study highlight the association of the expression of genes involved in the eIF2, mTOR, IL-6, growth hormone, and P53 signaling within the placenta as a contributor to white matter damage. These pathways are broadly tied to apoptosis, inflammation, immune response, hormone disruption, and metabolism. The dysregulation of these cellular and molecular processes within these placental pathways *in utero* may modify the release of signaling molecules such as inflammatory cytokines and hormones that ultimately lead to neonatal brain development and disorders in children born extremely preterm intriguing (**Figure 1.4**).

FIGURE 1.4: Conceptual diagram of the study findings. Relationships among molecular processes associated with cerebral white matter damage that may provide insight into the physiologic state of the placentas, transcriptomic pathways, and neonatal brain development. Five novel signaling pathways eukaryotic translation initiation factor 2 (eIF2), interleukin-6 (IL-6), and mammalian target of rapamycin (mTOR), growth hormone, and tumor protein P53 (p53) in relation to ventriculomegaly associated cerebral white matter brain damage relatively early in life. Many of the cellular/molecular processes are associated with more than one signaling pathway and there may be shared overlap of many of the genes within a given pathway.



Discussion

Strong evidence supports that placental physiology is tied to neurodevelopment later in life, a concept known as the placenta-brain-axis. [9] Nevertheless, linkages between the placenta, placental gene expression, and white matter damage in the neonate are understudied. To address this, we examined whether transcript levels of specific genes in the placenta are related to two forms of white matter damage in the neonate. The white matter damage was identified by two

ultrasound-defined indicators of cerebral white matter damage in the ELGAN cohort. In addition to supporting our hypothesis that inflammation-related genes in the placenta would be associated with white matter damage, the transcriptomic signatures highlighted other biological processes in the placenta. These include immune response, apoptosis, metabolism, and hormone-related signaling in relation to echolucency and ventriculomegaly. These results are clinically relevant as cerebral white matter injury occurs in 12% of ELGAN survivors and is associated strongly with ASD[125], cerebral palsy [148] and cognitive impairment.[126]

Expression of a total of 659 genes in the placenta was associated with echolucency, and/or ventriculomegaly. Of these genes, a set of 244 genes displayed expression levels that were related to both forms of white matter damage. The small overlap (37%) between echolucency and ventriculomegaly-associated transcripts was not unexpected as there may be etiologic pathways that are not shared by these two indicators of white matter damage. The transcripts that were part of the common gene set encode proteins involved in proinflammatory processes and were highly expressed in the placentas of individuals who had cerebral white matter damage. Among the differentially expressed transcripts were those involved broadly in the Interleukin 1 (IL-1) and Interleukin 6 (IL -6) pathways. These pathways which are critical for the modulation of the immune system and coordinating cell-mediated immune responses.[149] The identification of these pathways support our *a priori* hypothesis that inflammation-associated genes would be dysregulated in the placenta of ELGANs with white matter damage.

Interestingly, inflammation in the placenta has been tied to neurodevelopment later in life.[128] In addition to inflammation-related genes in the common gene set, we also found genes in the eIF2 and mTOR signaling pathways that were enriched. These pathways are broadly tied to apoptosis, and immune response. Specifically, the mTOR pathway is critical for cellular

growth, metabolism and apoptosis.[150] Apoptosis in the placenta has been tied to hypoxia, oxidative stress and neurodevelopmental outcomes.[151, 152] The altered expression of these pathways may point to improper placental growth and invasion which would directly impact placental development and function. [153]

By uncoupling the echolucency and ventriculomegaly-specific gene expression we observed the enrichment of apoptosis-related pathways in the placenta as they relate specifically to ventriculomegaly. Among the pathways enriched in the ventriculomegaly unique gene set was the P53 signaling pathway. This pathway which is related to mTOR and plays an intrinsic role in apoptotic and metabolic processes. In support of these data, alterations during central nervous system (CNS) development such as overexpression and inhibition of P53 pathway genes contribute to cerebellar defects.[154] In the present analysis we observed a decrease in *GSK3B*, which is a negative regulator of glucose mobilization essential to apoptotic pathways as well as energy metabolism, and inflammation.[155] Alterations such as this within the P53 pathway may be present as a response to DNA damage or oxidative stress in the placenta.

Additionally, the uncoupling of the placental gene expression signatures in relation to echolucency and ventriculomegaly may help to reveal genes and pathways that are related to one form of white matter damage but not the other. For example, among the pathways enriched in the echolucency unique gene set was growth hormone signaling. This pathway involves genes including *IGF2* and *IGFBP3* which displayed decreased expression in the placentae in relation to echolucency in the present study. These alterations may be involved in changes in fetal growth.[156] Further support for our findings are that associations have also been found between systemic inflammation measured via *IGF* family proteins relative to white matter injury. [157]

Interestingly, when analyzing the complete gene set of white matter damage-associated genes, genes involved in ASD and the endocrine system were identified. The latter is consistent with the known influence on neurodevelopment of several hormones produced by the placenta, such as corticotropin-releasing hormone (CRH), [158] estradiol, [159] and allopregnanolone. [160] Previous studies within the ELGAN cohort have highlighted the associations between differential methylation in placenta of hypothalamic-pituitary-adrenal (HPA) axis genes, such as nuclear receptor subfamily group 3cMember 1 (*NR3C1*), FK506 binding protein 5 (*FKPB5*), and brain-derived neurotrophic Factor (*BDNF*), and cognitive impairment in the offspring. [56] Dysregulation of many of these genes within the HPA axis may be associated with crucial biological functions of the placenta like nutrient transfer and cellular proliferation, and hormone production. [161] For example, *BDNF* promotes both regulation of CRH. [56, 162, 163] Subsequently, characterizing the dysregulation of hormones in the placenta is an important area of investigation since prenatal hormone dysregulation may contribute to alterations in fetal neurodevelopment and ultimately ASD development.

These data are among the first to highlight the expression of these critical pathways in placentas collected from infants born extremely prematurely (< 28 week'' gestation) who subsequently developed white matter damage. Still, this study is not without limitations. First, the ELGAN cohort includes the limited range of gestational ages in the study sample, which could limit the generalizability of the study results. Second, the present research focused on mRNA levels only in the placenta. In the future, the analysis of proteomic data could increase understanding of the relationship between molecular processes in the placenta and brain structure and function later-in-life. Fourth, neonatal cranial ultrasound identifies only macroscopic white matter damage and is less sensitive than magnetic resonance imaging for

detection of white matter damage. [164] Nevertheless, in the ELGAN study, the presence of either echolucency or ventricular enlargement was strongly associated with subsequent development of cerebral palsy, [165] epilepsy, [166] and cognitive impairment [166] and were the ultrasound lesions most predictive of neurodevelopmental impairments. Furthermore, we understand that the scope of neurodevelopment among ELGANs and other infants who survive preterm birth cannot be accounted for solely by the presence of structural brain abnormalities. To address this, future research will incorporate functional measures of neurodevelopment data as an outcome measurement in relation to the placental epigenomic signatures identified here and other later-in-life neurodevelopmental outcomes.

Conclusions

Using a unique placental repository comprising genome-wide transcript levels, we have integrated the transcript level changes of gene expression within a framework of biological pathways aimed at uncovering the physiologic state of the placenta. These data point to the gene regulation of hormones and inflammatory molecules that may have influenced the developing brain of the fetus. The results highlight biological pathways in the placenta that are associated with white matter damage among children born extremely premature. In terms of a mechanism that may underlie the observed association between the increased expression of these placental transcripts and white matter damage in the neonate, inflammatory molecules such as cytokines and hormones are known to disrupt oligodendrocytes development and impair myelination in the preterm brain. [92, 131] In addition, the presence of placental inflammation and alterations in the associated placental transcripts may be indicators of placental toxicity and dysregulated placental development. The pathways identified in the present study may represent targets for disease intervention, through the enhanced understanding of perinatal factors that influence their expression in the placenta.

CHAPTER 2: THE PLACENTAL TRANSCRIPTOME PREDICTS ADOLESCENT BRAIN VOLUME AND FUNCTION

Background

Intellectual disability is identified in 1.4% (95% CI: 1.2–1.7%) of children in the US ranging from 3 to 17 years of age. [167, 168] Children born extremely preterm (EP; less than 28 weeks' gestation) are at even greater risk for intellectual disability. Heightened risk for intellectual disability among children born EP is evidenced by their increased prevalence of neonatal white matter damage and deficits in school-aged cognitive outcomes. [92, 93] These outcomes have been tied to circulating neonatal inflammation-related proteins early in life [106] as well as reduced brain volume. [117]

Brain structure is influenced throughout the course of pregnancy. During the *in utero* period, a range of key events occur within the intrauterine environment that influence fetal brain development. Processes such as neuronal migration and synapse development occur between the 23rd and 27th weeks of gestation [19, 20]. Perturbations during the establishment of these vulnerable processes can affect brain maturation and result in postnatal consequences. [21] Inflammatory signals including cytokines and chemokines, present in the intrauterine environment, can influence the developing neurons of the fetal brain. [129] The placenta is a source of various signals that influence neurodevelopmental programming. These signals can transform early brain development as well as long-term neurodevelopmental outcomes. Thus, while it is a transient organ, the placenta is a critical mediator between maternal and environmental signals and the developing fetus. This relationship has been characterized as the placenta-brain axis.

Within the framework of the placenta-brain axis, few studies explore the linkage between disrupted placental pathways evidenced by placental gene expression (or the expression of epigenetic modifiers) in relation to neurocognitive impairments. [169] Links between the placenta and adolescent brain development are understudied.

Altered brain maturation in individuals born very preterm (less than 32 weeks' gestation) has been demonstrated by grey matter volume deficits persisting into adolescence and adulthood. [170] Among the developmental disabilities, cognitive impairment is one of the most prevalent developmental disabilities among EP-born individuals. [89, 102, 167, 168] Some variance seen in cognitive impairment is related to brain volume/structure. Brain volume is a well-documented proxy for studying neurodevelopment as structural brain magnetic resonance imaging (MRI) may provide insight into neurological substrates that underlie neurocognitive impairments. [94-98, 171] Structural changes are often paired with corresponding functional changes that can be evidenced in performance on neurodevelopmental assessments and in behavior. [18, 115-117]

Research from the Extremely Low Gestational Age Newborns (ELGAN) Study indicates that preterm newborns are at an increased risk for brain damage. [172] This may be largely due to easily disturbed developmental processes and the lack of protection against the disturbances in the form of adequate amounts of placenta/maternal-provided neurotrophins during the vulnerable window of brain development. [173] The assessment of mRNA expression within the placenta may provide a window to better understand factors that influence developmental programming. Supporting this linkage between the placenta and neurodevelopment, gene expression in the placenta has been associated with the likelihood of intellectual impairment, [127] and brain-derived neurotrophic factor (BDNF) is a key neurotrophin as well as a hypothalamus-pituitary-adrenal (HPA) axis gene identified through placental DNA (CpG) methylation in association with EP

cognitive impairment at age 10. However, literature exploring the relationship between altered placental gene expression and brain volume and function during adolescence has not been documented. This study focuses on the relationship between placental mRNA expression, brain function and volume in adolescence within ELGANs.

Study Objective

The ELGAN Study is a multi-center longitudinal cohort study of 1506 newborns born before 28 weeks. The present study employs a subset of ELGAN participants with data on placenta gene expression and brain volumes at 15 years of age. Our analyses consider the parallel relationship of placental mRNA expression with regional brain MRI volumes and placental mRNA expression with cognition at 15 years. Through targeted analysis of mRNA gene expression that encodes for inflammation and immune response as it relates to brain MRI volumes and indicators of neurocognitive impairment, we hope to provide molecular insights into early placental biological processes that might influence adolescent brain structure and function.

This analysis will address a fundamental gap in the understanding of placental mechanistic origins that underlie neurodevelopmental disability and reduced brain volume as measured via magnetic resonance imaging (MRI) in ELGAN children. The focus is on inflammation and immune response pathways at the level of messenger RNA (mRNA) expression in the placenta. The central hypothesis is that the elevated expression of critical inflammation and immune response pathways in the placenta is associated with structural neurodevelopment and neurocognition later in life. The proposed study is innovative; we are aware of no previous study of this hypothesis. The research includes the assessment of the association between placental genes and MRI brain volumes in 14 segmented brain regions along with three outcomes of neurocognition. While the precise mechanism is unknown as to how

placental epigenetic variation and inflammation may be influencing neurodevelopment, here we will analyze the transcriptomic architecture that exists between the placenta and the maturing brains of those born prematurely into the critical adolescent phase. Between childhood and adolescence, frontal gray matter volume decreases and white matter volume increases have been visualized via structural MRI techniques. [174] Successful completion of the proposed aim(s) will shed light on mechanisms linking the placenta and the maturing brain among individuals born extremely preterm.

Our understanding of gene-environment impacts during pregnancy that may target structural brain changes and neurodevelopmental delays and disorders can be enhanced through investigation of fundamental placental signaling during critical windows of fetal brain development in association with later life changes in brain volume and cognitive assessments. Identifying key genes in the placenta relative to neurodevelopmental disability and altered brain structure can help us determine the placentas' role in child neurodevelopment. Because key placental genes have already been associated with cognition of children born EP, [56, 169, 175] findings from the present study will not only extend those findings longitudinally to capture adolescent neurocognition but will also expand to include multiple measures of neurocognition in hopes of identifying the most promising indicators of neurodevelopmental disability at age 15 in relation to placental transcriptomic data. This study enriches the literature in the field of neurobiology by providing evidence of the longitudinal correlative interplay between the endocrine system at birth and the nervous system at age 15, consistent with the concept that alterations in one system influence changes in another system. [176]

Materials and Methods

Participants

Participants The ELGAN study enrolled participants from 2002 to 2004 at 14 participating medical centers across the U.S. and was approved by the Institutional Review Board at each participating center. The eligibility criterion for the ELGAN study was gestational age of less than 28 weeks. Study procedures have been comprehensively described elsewhere. [113] The 15-year assessment was carried out for a total of 700 (58%) participants among the 1198 ELGAN study participants who survived to 15 years of age, of whom 681 (57%) accompanied their parent/guardian for a cognitive assessment, and 465 (66%) assented to undergo brain MRI. The current study includes two parallel analyses (1) focused on adolescent regional brain volumes based on a subsample of 146 children that also had placental mRNA data and the other focused on adolescent neurocognition based on two subsamples (Verbal and Fluid Composite (n=288) and Latent Profile Analysis (n=277)) of children that also had placental mRNA data.

Maternal and Newborn Characteristics

Maternal characteristics were self-reported at the time of the child's birth and included maternal race and ethnicity, age, education, health insurance status, supplemental nutrition assistance, and marital/partnered status. A cumulative composite variable representing social stress was derived from these latter factors. Four variables were used as a proxy indicator of maternal social disadvantage, which increased by one point for maternal education less than high school, receipt of government-provided supplemental nutritional assistance, lack of private health insurance, and single marital/unpartnered status. [177] Newborn characteristics (gestational age, sex, birthweight) and medical variables were identified by review of medical records, [172] and structural neonatal brain injuries (echolucent lesions of white matter damage, ventriculomegaly) were identified by cranial ultrasound. [138]

Placental mRNA Expression Data

Placental DNA and RNA extraction have previously been detailed, [132, 133] and a comprehensive description of RNA sequencing methodology has been described in Eaves e*t al.* [134] To summarize, RNA molecules ≥18 nucleotides were extracted (AllPrep DNA/RNA/miRNA Universal Kit; Qiagen), RNA quality was assessed (LabChip), and RNA integrity numbers (RIN) were assigned. Genome-wide mRNA expression profiles were then measured from the isolated placental RNA samples (QuantSeq 3′ mRNA-Seq Library Prep Kit; Illumina). The mRNA libraries were then pooled and sequenced (single-end 50 bp) onto one lane (Hiseq 2500; Illumina), the number of sequencing reads per mRNA were aligned to the GENCODE database v3 [135] and organized (Salmon) [136] resulting in 37,268 unique human RNA transcripts (protein-coding and non-coding RNAs) used in data processing and statistical analyses. Surrogate variables were incorporated in the statistical modeling approach to account for other sources of heterogeneity (detailed below), and non-detectable transcripts were filtered out (detailed below) as additional quality control steps. For this study, we analyzed genes that were characterized under the biological process terms of the Gene Ontology (GOBP) lists for inflammatory response (n=854) and immune response (n=1894) from the Molecular Signatures Database (mSigDB) (https://www.gsea-msigdb.org/gsea/msigdb/).

Cognitive Assessments at Age 15

Neuropsychological assessment. A comprehensive description of the ELGAN NIH Toolbox Cognition Battery (NTCB) data set has been described in great detail. **[178] [179]** The NTCB **[180]** assessment includes 7 measurements to generate estimates of more specific cognitive abilities (e.g., executive function, processing speed), including 5 measures of "fluid" cognition, Pattern Comparison Processing Speed (processing speed), List Sorting Working Memory (verbal working memory), Picture Sequence Memory (episodic memory), Flanker Inhibitory Control and

Attention (sustained attention/inhibition), and Dimensional Change Card Sort (set switching/cognitive flexibility), and 2 measures of verbal or "crystallized cognition," Picture Vocabulary (receptive vocabulary) and Oral Reading Recognition (oral reading). A General Cognition Composite score was generated in response to all 7 tests. In line with a recent study [134] which yielded a different breakdown of factor-based composites specific to adolescents than the composite breakdown in the original NTCB, such that "verbal" cognition is now a 3-subset measure including the Picture Vocabulary, Oral Reading Recognition, and List Sorting Working Memory tests, and the second factor ("fluid cognition") is now a 4-subset measure that includes the Flanker Inhibitory Control and Attention, Dimensional Change Card Sort, and Pattern Comparison Processing Speed tests. These resulting factors of "verbal cognition" and "fluid cognition" will be referred to hereafter as the Verbal Composite Score and Fluid Composite Score respectively.

Latent profile analysis (LPA) of IQ and NTCB scores. An LPA was conducted on the Wechsler Abbreviated Scale of Intelligence-II (WASI-II) verbal and nonverbal [181], and the 7 National Institutes of Health Cognition Toolbox Battery (NCTB) [182] measures using Mplus 7.11, [183] providing a maximum likelihood estimation. [184] By latent profile analysis, [184] subtests scores from the WASI-II and NCTB were used to classify participants' level of cognitive functioning into normal, low-normal, and impaired subgroups. LPA may provide better predictive value for future cognitive and adaptive function, [185] and has been argued to provide a more sensitive assessment of cognitive function than IQ alone. [179, 184]

Brain MRI Acquisition and Processing

As described in detail in **Chapter 3**, age 15 quantitative brain MRI (qMRI) attained for 465 participants at 12 ELGAN-ECHO participating sites was used to classify study participants' brain volumetry based on fine-scale brain partitioning using FreeSurfer v7.1.0 under Linux

RHEL-7 environment. More specifically, qMRI images were acquired on Siemens Prisma 3T MRI scanners at 6 participating sites, a Philips 3T MRI scanner was used at 3 participating sites, 2 participating sites used GE 3T MRI scanners, and a single participating site used 1.5T MRI scanner with different imaging parameters, so these 24 1.5T datasets were not included in this analysis to avoid potential data inconsistencies. Brain regional volumes of cerebral cortex, cerebral white matter, cerebellar cortex, cerebellar white matter, brain stem, thalamus, lateral ventricle, putamen, hippocampus, ventral diencephalon, caudate, corpus callosum, pallidum, amygdala, and accumbens area were extracted but ventral diencephalon, lateral ventricle, and accumbens area were excluded from the investigation as they have no functional relevance of interest to the present analysis.

Statistical Analysis

To analyze associations between placental mRNA expression levels and 15-year brain volume and function, linear regression was used for all models, with the exception of the association between placental mRNA expression levels and LPA where ordinal logistic regression was used. Covariates included in all models included sex, birthweight, and maternal socioeconomic risk. For the measure of socioeconomic risk, we used the summative index of maternal social disadvantage, increasing by one point for each of the following: lack of private health insurance, maternal education less than high school, receipt of government-provided supplemental nutritional assistance, and single marital/unpartnered status. Additional covariates used in brain volume inclusive analyses also included study site, and total brain volume (TBV), except when assessing for total brain volume where TBV adjusting was omitted.

These analyses were carried out for both the GOBP-derived inflammation genes and the GOBP-derived immune response genes. TNFα included in the set of markers, which resulted in a smaller dataset for brain volume (n = 146), verbal and fluid composite (n=288), and LPA

(n=277) analyses respectively. To account for multiple testing in the individual marker analyses we used $p < 0.05$ to declare a significant relationship and an FDR-adjusted threshold of $p < 0.05$. Pearson correlation coefficients were also generated for all linear models during scatterplot plot generation. An ANOVA was generated during boxplot generation for logistic regression-based analysis. All analyses were conducted in R v4.1.2.[186]

Pathway Analysis

Established functional relationships among the proteins encoded by the identified genes and their biological pathways were examined using the Reactome Pathway Database (https://reactome.org). [187] Specifically, Pathway Browser version 3.7 and Reactome database release 83. We analyzed and reported significant pathways, from Reactome enriched among these gene sets (BH-corrected p-values < 0.05).

Results

Of 146 participants included in brain volume assessment, 66 (45.2%) were female and 80 (54.8%) were male. The average gestational age of study participants was 26.1 weeks. While the majority of participants' mothers had a college degree or higher 68 (46.6%), 38 (26%) had educational attainment of a high school diploma. The average maternal age was 29.4 (5.71%). The majority of participants had private insurance 221 (68.4%) and were born to married mothers. A minority of participants received food stamps, 13 (9.00%). Of the participants included in the neurocognitive outcome assessment (Verbal and Fluid Composite (n=288); LPA (n=277)), general demographic information was similar to that observed for the brain volume subset (**Table 2.1**).

TABLE 2.1: Study participants' demographics.

N (%)	Brain Volume n = 146	Neurocognitive Outcomes			
		Verbal and Fluid Composite n = 288	LPA n =277		
			Impaired n = 137	Low Normal n = 42	High Normal n = 98
Sex					
Female	66 (45.2%)	134 (46.5%)		128 (46.2%)	
Male	80 (54.8%)	154 (53.5%)		149 (53.8%)	
Birthweight Z-score					
< -2	8 (5.50%)	18 (6.30%)		16 (6.50%)	
< -1	20 (13.7%)	32 (11.1%)		31 (11.2%)	
- 1 to 1	118 (80.0%)	238 (82.6%)		228 (82.3%)	
GA (weeks.days)					
Mean (SD)	26.1 (1.21)	26.0 (1.31)		26.0 (1.31)	
Race					
White	104 (71.2%)	193 (67.0%)		184 (66.4%)	
Black	31 (21.2%)	73 (25.3%)		71 (25.6%)	
Other	11 (7.5%)	22 (7.70%)		22 (7.9%)	
Hispanic Ethnicity					
Yes	7 (4.80%)	16 (5.60%)		16 (5.80%)	
No	139 (95.2%)	272 (94.4%)		261 (94.2%)	
Socioeconomic Factors					
Maternal Education					
high school diploma or less	38 (26.0%)	96 (33.3%)		93 (33.6%)	
At least some college	37 (25.3%)	61 (21.2%)		59 (21.3%)	
College degree or greater	68 (46.6%)	123 (42.7%)		117 (42.2%)	
Missing	3 (2.10%)	8 (2.80%)		8 (2.90%)	
Maternal Age (years.days)					
Mean (SD)	29.4 (6.71)	30.0 (6.73)		29.9 (6.73)	
Insurance					
Private Insurance	115 (78.8%)	203 (70.5%)		193 (69.7%)	
Medicaid	30 (20.5%)	82 (28.5%)		81 (29.2%)	
Missing	1 (0.70%)	3 (1.00%)		3 (1.10%)	
Marital Status					
Not Married	46 (31.5%)	107 (37.2%)		103 (37.2%)	
Married	100 (68.5%)	181 (62.8%)		174 (62.8%)	
Food Stamps					
No	132 (90.4%)	258 (89.6%)		248 (89.5%)	
Yes	13 (8.90%)	27 (9.38%)		26 (9.39%)	
Missing	1 (0.70%)	3(1.02%)		3(1.11%)	

Maternal demographic data, pregnancy characteristics, and data on birth outcomes are presented for the ELGAN subjects used in each analysis. Data are presented as the number (%) of subjects in the cohort.

Placental mRNA expression in Relation to Brain Volumes

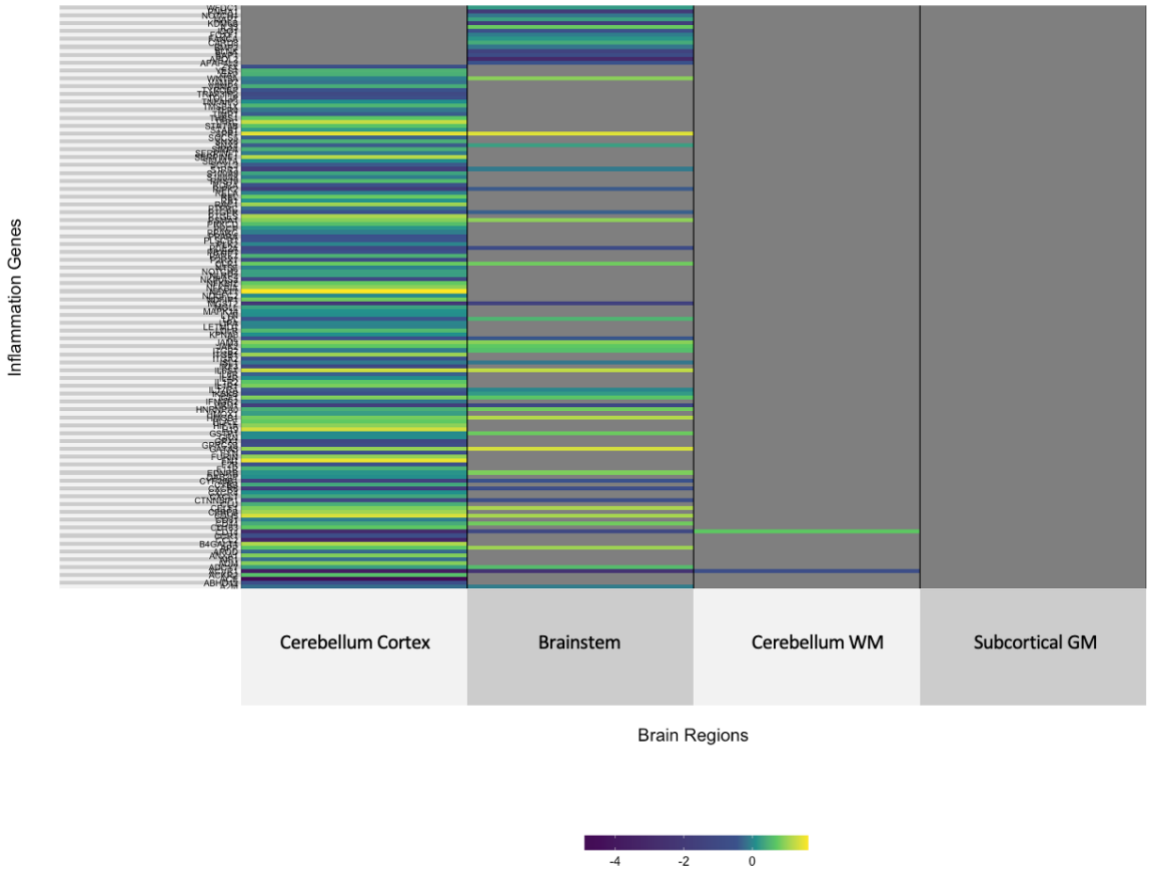
A total of 368 inflammation-related genes were analyzed for the association of their expression levels relative to volumes for 14 brain regions. Four brain regions were identified where mRNA expression passed FDR correction. Specifically, 113 genes displayed decreased expression in relation to cerebellum cortex volume, two genes displayed decreased expression in relation to cerebellum white matter, 52 genes displayed decreased expression in relation to brainstem volume, and a single gene displayed decreased expression in relation to subcortical grey matter (**Table 2.2**). Across these four brain regions, a total of 149 unique genes displayed associations between expression and brain volume. These data are graphically displayed in **Figure 2.1**.

TABLE 2.2: Placental mRNAs that encode for inflammation in relation to brain volumes.

Brain Region	Genes (N) P value ≤ .05 (FDR ≤ .05)	Upregulated (+)/ Downregulated P value ≤ .05 (FDR ≤ .05)
Total brain tissue	11	8+, 3 -
Cerebral cortex	18	18+
Cerebral white matter	10	6 +, 4 -
Corpus Callosum	37	37 -
Cerebellum cortex	**191 (133)**	**191 - , (133 -)**
Cerebellum white matter	**145 (2)**	**145 -, (2 -)**
Brainstem	**139 (52)**	**139 -, (52 -)**
Subcortical gray matter	**94 (1)**	**94 - , (1 -)**
Thalamus	25	24 -
Putamen	47	1+, 46 -
Hippocampus	30	30 -
Amygdala	6	2 +, 4 -
Caudate	38	38 -
Pallidum	35	35 -

Note— All were adjusted for sex, birthweight, SES risk, Study site, and total brain volume (TBV), except for Total Brain Volume where TBV adjusting was omitted. Significantly associated brain regions are in bold.

FIGURE 2.1: Significant placental mRNAs that encode for inflammation in relation to brain volumes.



Note— GM is grey matter and WM is white matter.

A total of 785 immune response-related genes were analyzed for the association of their expression levels relative to volumes for 14 brain regions. Five brain regions were identified where mRNA expression passed FDR correction. Specifically, two genes displayed decreased expression in relation to corpus callosum volume, 295 genes displayed decreased expression in relation to cerebellum cortex, 95 genes displayed decreased expression in relation to brainstem volume, and a single gene displayed decreased expression in relation to cerebellum white matter (**Table 2.3**). One gene displayed increased expression with cerebral cortex volume and three genes displayed increased expression with cerebellum cortex volume. Across these five brain

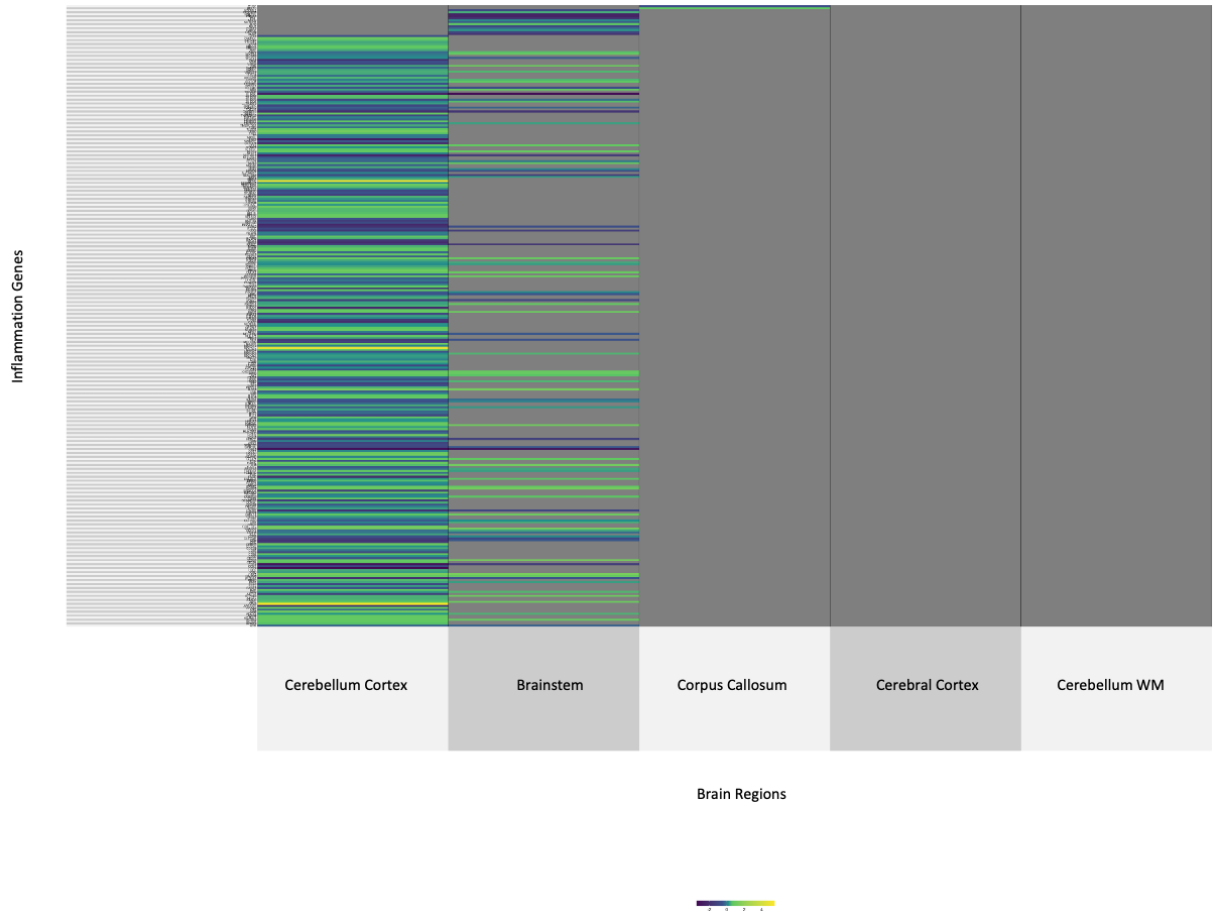
regions, a total of 313 unique genes displayed associations between expression and brain volume. These data are graphically displayed in **Figure 2.2**.

TABLE 2.3: Placental mRNAs that encode for immune response in relation to brain volumes.

Brain Region	Genes(N) P value ≤ .05 (FDR ≤ .05)	Upregulated (+)/ Downregulated P value ≤ .05 (FDR ≤ .05)
Total brain tissue	24	12+, 12 -
Cerebral cortex	**48 (1)**	**6 +, 42 - (1 +)**
Cerebral white matter	16	6 +,10-
Corpus Callosum	**78 (2)**	**75+, 3- (2-)**
Cerebellum cortex	**408 (298)**	**3+, 405 -(3+, 295 -)**
Cerebellum white matter	**299 (1)**	**6+, 294- (1-)**
Brainstem	**308 (95)**	**1+, 307 - (95-)**
Subcortical gray matter	195	195 -
Thalamus	49	1+, 48-
Putamen	95	1+ ,94-
Hippocampus	37	1+, 36 -
Amygdala	15	1+, 14 -
Caudate	79	79 -
Pallidum	60	2+, 58 -

Note— All were adjusted for sex, birthweight, SES risk, Study site, and total brain volume (TBV), except for Total Brain Volume where TBV adjusting was omitted.

FIGURE 2.2: Placental mRNAs that encode for immune response in relation to brain volumes.



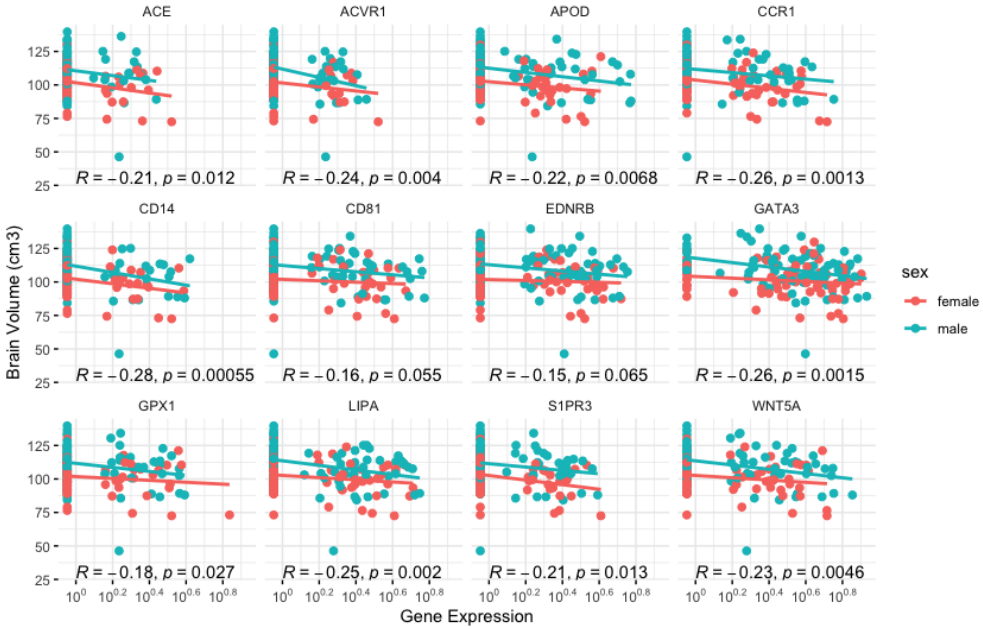
Note— GM is grey matter and WM is white matter.

Associations between mRNA expression and brain volume were most enriched for cerebellum cortex and brainstem for both inflammation and immune response-focused analyses. A single gene overlapped between the set of significantly associated inflammation genes (n=235) and immune response genes (n=298) for cerebellum cortex (**Supplemental Table 2.1**). The expression of the top 12 significant genes and their relationship with brainstem volume are detailed for inflammation and immune response in **Figure 2.3A** and **Figure 2.4A**. There were 23 overlapping genes between the set of significantly associated inflammation genes (n=52) and immune response genes (n=95) for brainstem. The expression of the top 12 significant genes and

their relationship with brainstem volume are detailed for inflammation and immune response in **Figure 2.3B** and **Figure 2.4B**.

FIGURE 2.3 (A-B): Significant genes that encode for inflammation associated with placental mRNA expression in relation to (a) cerebellum cortex and (b) brainstem.

A



B

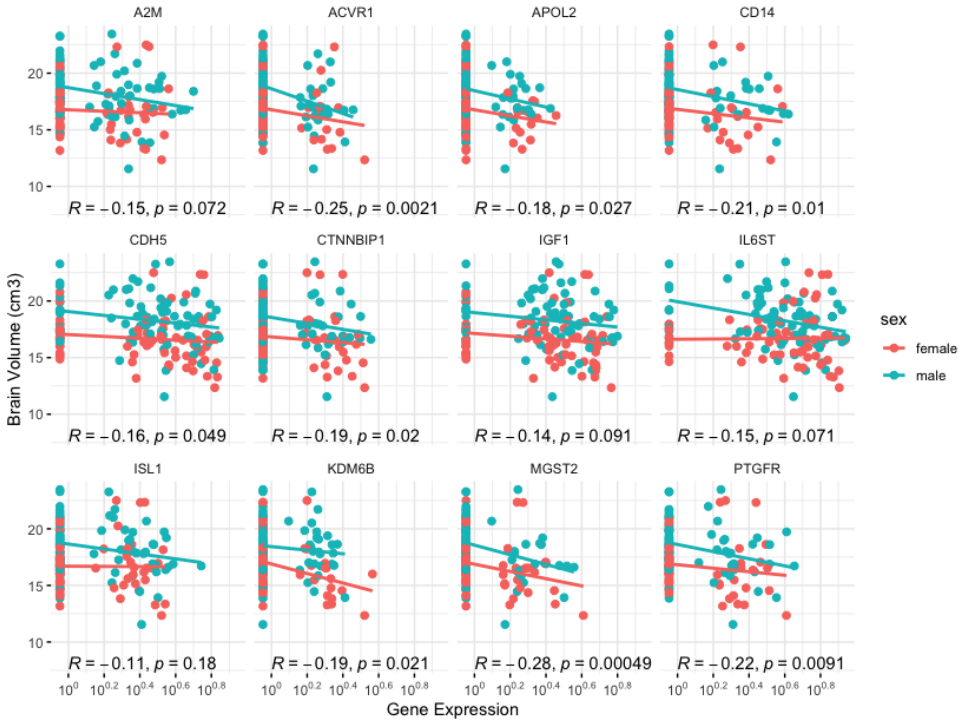
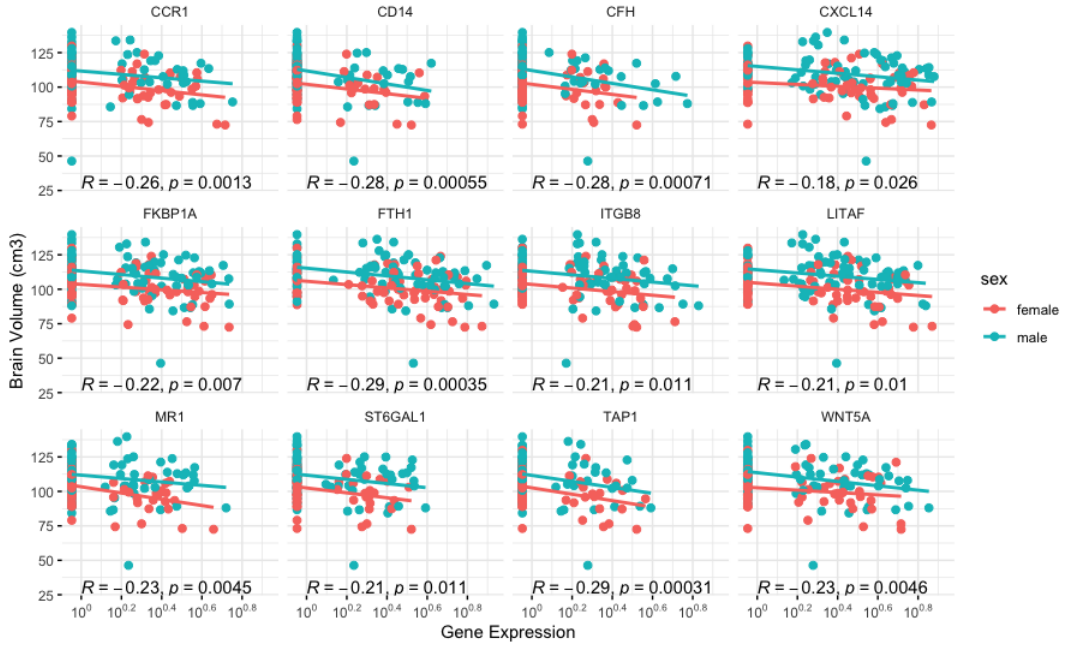
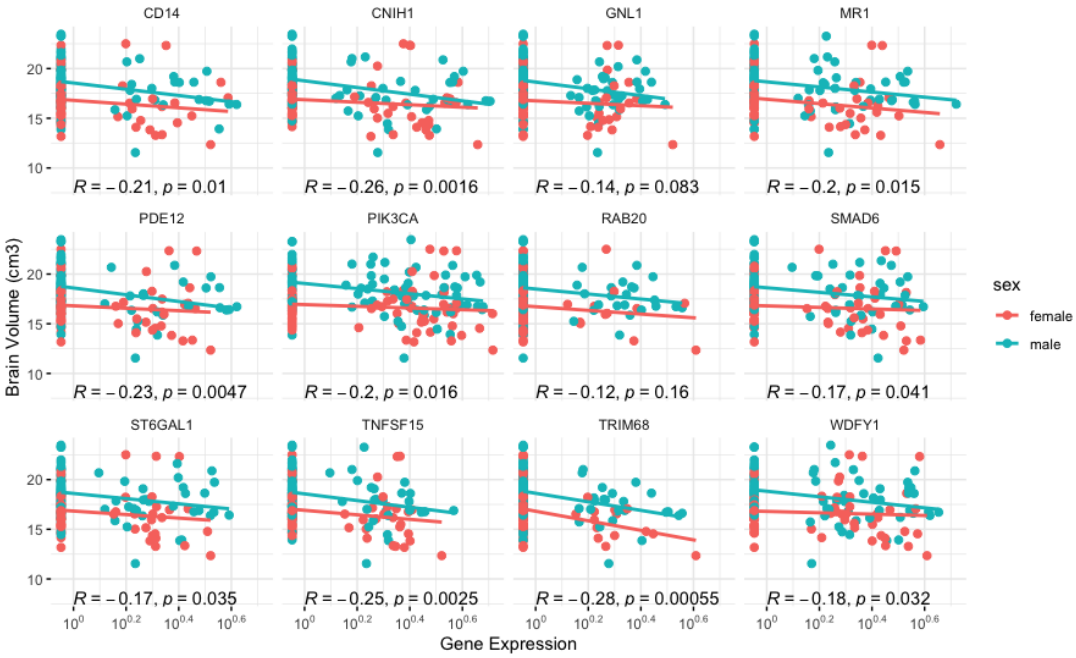


FIGURE 2.4 (A-B): Significant genes that encode for immune response associated with placental mRNA expression in relation to (a) cerebellum cortex and (b) brainstem.

A



B



Placental mRNA expression in Relation to Neurocognition

When inflammation mRNAs were analyzed in relation to Verbal Composite Scores, none of the genes passed FDR correction. However, a total of 38 genes displayed decreased expression ($p \leq .05$). Similarly, when inflammation mRNAs were analyzed in relation to Fluid Composite Scores, none of the genes passed FDR correction. However, a total of three genes displayed decreased expression, and four genes displayed increased expression ($p \leq .05$). When inflammation mRNAs were analyzed in relation to LPA, 137 genes passed FDR correction and displayed increased expression when comparing the impaired LPA participants to the low normal LPA participants. (**Table 2.4**). None of the inflammation-related genes passed FDR correction when comparing the high normal LPA participants to the low normal LPA participants. However, a total of seven genes displayed increased expression ($p \leq .05$) (**Table 2.4**).

TABLE 2.4: Placental mRNAs that encode for inflammation and immune response in relation to neurocognition.

Composite Score	**Genes(N)** P value ≤ .05 (FDR ≤ .05)	**Upregulated (+)/ Downregulated (-)** P value ≤ .05 (FDR ≤ .05)
Inflammation		
Verbal Composite	38	38 -
Fluid Composite	7	4+, 3 -
LPA- Impaired	193 (137)	193 + (137 +)
LPA-High Normal	7	7+
Immune Response		
Verbal Composite	73	1 +, 72 -
Fluid Composite	12	7+, 5 -
LPA- Impaired	380 (212)	380 + (212+)
LPA-High Normal	24	23+, 1 -

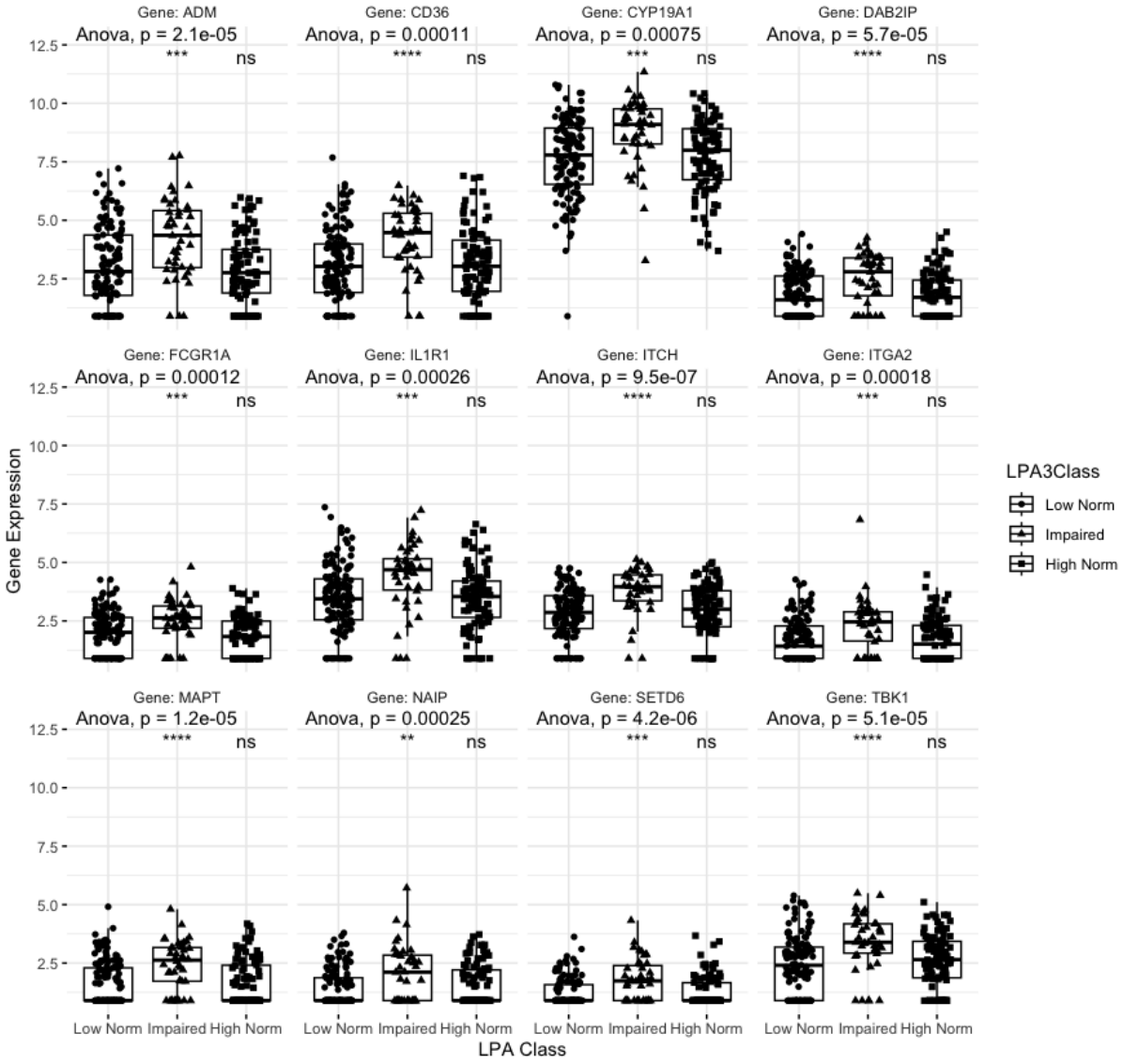
Note— All were adjusted for sex, birthweight, and SES risk.

The analysis of immune response mRNAs in relation to Verbal Composite Scores resulted in zero FDR-corrected genes. A total of 73 genes reached significance ($p \leq .05$) in relation to Verbal Composite Scores. Of which, 72 genes displayed decreased expression and a single gene displayed increased expression. A total of 73 genes reached significance ($p \leq .05$) in relation to Fluid Composite Scores. Of which, 12 genes displayed decreased expression and a single gene displayed increased expression. Five of those genes displayed decreased expression and seven genes displayed increased expression. When immune response mRNAs were analyzed in relation to LPA, 212 genes passed FDR correction and displayed increased expression when comparing the impaired LPA participants to the low normal LPA participants. (**Table 2.4**). None of the immune response-related genes passed FDR correction when comparing the high normal LPA participants to the low normal LPA participants. However, a total of 23 genes displayed increased expression and a single gene expressed decreased expression ($p \leq .05$) (**Table 2.4**).

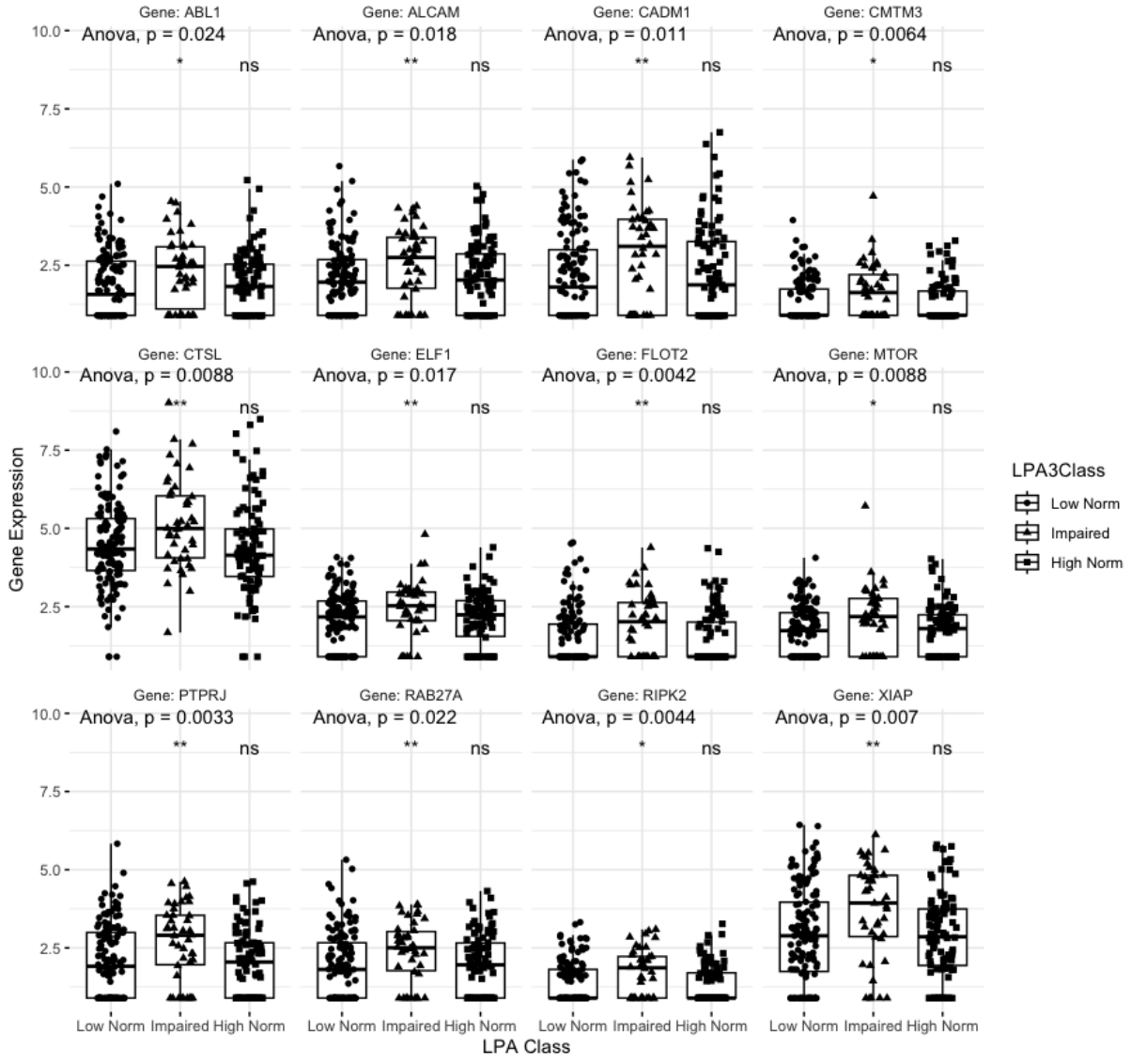
Associations between mRNA expression and neurocognition were significantly enriched for study participants with impaired LPA in both inflammation and immune response-focused analyses. There were 64 overlapping genes between the set of significantly associated inflammation genes (n=137) and immune response genes (n=212) for impaired LPA (**Supplemental Table 2.1**). The expression of the top 12 significant genes and their relationship with LPA classification are detailed for inflammation and immune response in **Figure 2.5(A-B)**.

FIGURE 2.5 (A-B): (A) Inflammation or (B) immune response genes in relation to neurocognition measure assessed through LPA.

A



B



Pathway Analysis of Placental mRNA expression in Relation to Brain Volumes

Pathway-based analysis was performed on inflammation-associated genes identified for cerebellum cortex and brainstem as they resulted in a sufficient number of significant genes. A total of 112 pathways were identified for cerebellum cortex that passed FDR correction (**Supplemental Table 2.2**). Among the most significantly enriched pathways were Signal Transduction (n= 88 genes; 0.003 = FDR p-value), Interleukin-1 Family Signaling (n= 23 genes;

<0.001 = FDR p-value), and Apoptosis (n= 12 genes; <0.001 = FDR p-value) (**Table 2.5**). A total of 52 pathways were identified for brainstem that passed FDR correction (**Supplemental Table 2.3**). Among the most significantly enriched pathways were Insulin-like Growth Factor-2 mRNA Binding Proteins (IGF2BPs/IMPs/VICKZs) bind RNA (n= 5 genes; <0.001 = FDR p-value), MAPK1/MAPK3 signaling (n=6 genes; 0.05= FDR p-value), and Apoptosis (n= 5 genes; 0.03= FDR p-value) (**Table 2.5**).

TABLE 2.5: Significant pathways that encode for inflammation associated with placental mRNA expression in relation to cerebellum cortex volume, brainstem volume, and impaired LPA at age 15.

		Entities		
	Pathway	found	p-value	FDR
Cerebellum Cortex	Signal Transduction	88	<0.001	0.003
	Interleukin-1 Family Signaling	23	<0.001	<0.001
	Apoptosis	12	<0.001	0.008
Brainstem	Insulin-like Growth Factor-2 mRNA Binding Proteins	5	<0.001	<0.001
	MAPK1/MAPK3 signaling	6	0.006	0.05
	Apoptosis	5	0.003	0.03
Impaired LPA	Regulated Necrosis	7	<0.001	0.002
	NOD1/2 Signaling Pathway	9	<0.001	<0.001
	Growth hormone receptor signaling	3	0.006	0.04

Pathway-based analysis was performed on immune response-associated genes identified for cerebellum cortex and brainstem as they resulted in a sufficient number of significant genes. A total of 302 pathways were identified for cerebellum cortex that passed FDR correction (**Supplemental Table 2.4**). Among the most significantly enriched pathways were TNFR1-induced NF-kB signaling pathway (n= 4 genes; <0.03 = FDR p-value), Apoptosis (n= 14 genes; 0.005= FDR p-value), and Axon guidance (n= 38 genes; <0.001 = FDR p-value) (**Table 2.6**). A

total of 46 pathways were identified for brainstem that passed FDR correction (**Supplemental Table 2.5**). Among the most significantly enriched pathways were Cytokine Signaling in Immune system (n= 31 genes; <0.001 = FDR p-value), TRAF6 mediated NF-kB activation (n= 3 genes; 0.006= FDR p-value), and Defective Intrinsic Pathway for Apoptosis (n= 4 genes; 0.009= FDR p-value) (**Table 2.6**).

TABLE 2.6: Significant pathways that encode for immune response associated with placental mRNA expression in relation to cerebellum cortex volume, brainstem volume, and impaired LPA at age 15.

		Entities		
Inflammation Gene Set	Pathway name	found	p-value	FDR
Cerebellum Cortex	TNFR1-induced NF-kB signaling pathway	4	0.01	0.03
	Apoptosis	14	<0.001	0.005
	Axon guidance	38	<0.001	<0.001
Brainstem	Cytokine Signaling in Immune system	31	<0.001	<0.001
	TRAF6 mediated NF-kB activation	4	<0.001	0.006
	Defective Intrinsic Pathway for Apoptosis	4	<0.001	0.009
Impaired LPA	Cytokine Signaling in Immune system	74	<0.001	<0.001
	MAP kinase activation	10	<0.001	<0.001
	TNF signaling	3	<0.001	<0.001

Pathway Analysis of Placental mRNA expression in Relation to Neurocognition

Pathway-based analysis was performed on inflammation-associated genes identified for study participants with impaired LPA, resulting in a sufficient number of significant genes. A total of 130 pathways were identified that passed FDR correction (**Supplemental Table 2.6**). Among the most significantly enriched pathways were Regulated Necrosis (n= 7 genes; 0.002 = FDR p-value), NOD1/2 Signaling Pathway (n= 9 genes; <0.001 = FDR p-value), and Growth hormone receptor signaling (n= 3 genes; 0.04 = FDR p-value) (**Table 2.5**).

Pathway-based analysis was performed on immune response-associated genes identified for study participants with impaired LPA and resulted in a sufficient number of significant genes. A total of 163 pathways were identified that passed FDR correction (**Supplemental Table 2.7**). Among the most significantly enriched pathways were Cytokine Signaling in Immune system (n= 74 genes; 0.002 = FDR p-value), MAP kinase activation (n= 10 genes; <0.001 = FDR p-value), and TNF signaling (n= 3 genes; <0.001 = FDR p-value) (**Table 2.6**).

Discussion

There is strong evidence that the placenta plays a critical role in fetal brain development and later brain function. In prior work, we have shown that the expression level of specific mRNAs within the placenta are associated with white matter damage in the neonate. [188] In addition, the ELGAN team has shown that factors that control mRNA expression (i.e., CpG methylation) in the placenta are associated with later life cognition. [56, 59] It remains unstudied whether mRNAs in the placenta are associated with later-in-life brain structure and function. In the present study, we address this by investigating the relationship between mRNAs in the placenta and both brain volume and neurocognition in adolescents who were born extremely preterm as part of the ELGAN Study. Based upon the findings of our team and others, [36, 60, 117, 175, 188-190] we elected to focus on targeted sets of genes that are involved in inflammation and immune response. Our *a priori* hypothesis was that elevated expression of placental genes related to inflammation and immune response would be related to decreased structural development and an indicator of decreased or impaired cognition. There are three major findings from this work. First, the elevated expression of genes that encode proteins that play a role in inflammatory response as well as immune response were associated with lower brain volume at age 15. Second, elevated expression of inflammation and immune-associated genes was associated with increased risk for impaired cognition. Finally, while genes related to

inflammation and immune response were identified, the results also highlight other pathways including hormone regulation, apoptosis, and imprinting.

Elevated expression of inflammatory and immune response genes in the placenta was associated with decreased volume in select brain regions. Specifically, reductions were observed in the cerebellum cortex, brainstem, and cerebellum white matter. In the ELGAN cohort, early life signatures of inflammation are associated with structural brain alterations later in life. [117, 188, 190] As one example, placental inflammatory signatures are associated with ultrasound-identified cerebral white matter damage in the neonate. [188] Also, high expression of neonatal circulating inflammatory markers were associated with decreased brain volume at age 10. [117] We have recently expanded upon the relationship between neonatal circulatory inflammatory markers and large brain regions (grey matter, white matter, and cerebellum and brainstem) at age 10 to show that neonatal inflammation is associated with decreased brain volumes at age 15 including regionally segmented subcortical grey matter (see Chapter 3). Thus, the data from the present study are in line with these findings of increased inflammation in relation to decreased brain volume, but highlight a novel aspect that now indicates inflammatory signatures are present prior to the first few weeks of life (i.e. the placenta) and are strongly associated with decreased brain volume at age 15. [189, 191, 192] These results support the placenta-brain axis but also the developmental origins of disease. Studies of volumetric-based analyses have found correlations between decreased cerebellar volume following white matter injury, which is a recognized complication of preterm birth. [193-195] In the present analysis, we highlight an association between the increased expression of inflammation and immune response-related placental genes and reduced cerebellum grey matter volume assessed at age 15. Nuclear factor kappa B (NF-κB) is an inducible transcription factor and a notable contributor to the regulation of immune response,

inflammation, cell growth and survival, development, and release of neuroinflammatory cytokines. However, NF-κB's relationship with structural and cognitive brain alterations is still understudied. [196] Future research should investigate the perinatal antecedents of placental inflammation. These could include chemical exposures, maternal nutrition, and non-chemical stressors including maternal medications and social environment.

We show here that elevated inflammation and immune response gene expression is associated with an increased risk of cognitive impairment in ELGAN cohort participants at age 15. Broadly, the neurocognitive measurements analyzed here assessed working memory, information processing, and a more sensitive characterization of cognition using measures of executive function in addition to IQ. Prior work from the ELGAN cohort has identified linkages between placental CpG methylation and LPA at age 10, [56] and umbilical cord inflammation and neurological outcomes at age 10. [60] As has been found in other preterm cohorts, multifaceted deficits have been reported regarding the cognitive development of children born preterm. Among these deficits are difficulties in each domain of cognition, including language, executive function, learning and memory, perceptual-motor function, and complex attention. A high prevalence of delays and changes was observed in a Brazilian birth cohort when assessing their longitudinal neurodevelopmental trajectories from four to eight months to 24 months of age. [197] In another cohort, extreme prematurity, particularly in males born to parents of low education was associated with worse neurodevelopmental impairment status at ages two, five, and eight years of age, assessed using a composite score inclusive of cognitive, neurological, visual, and auditory functions. [198] Similar to many of these studies, we are capturing cognition across different time points within the ELGAN cohort where cognition has been assessed, [179] but we are still one of the only extremely preterm birth cohorts with placental omic data, and this is the first

study within our group to assess mRNA expression of implicated mechanistic antecedents to adolescent measures of cognition. While the results support our a priori hypothesis that inflammation and immune response genes would be associated with our brain structure and neurocognitive, we also found that pathways present in other tissues are known to be involved in tumorigenesis.

Although this is a very comprehensive study with several novel aspects relating mRNA in the placenta to brain structure and function in the adolescent, it is not without limitations. The results of this study are based solely on a cohort of children born extremely preterm. Thus, the results may not be generalizable to cohorts that were born at term. Sample attrition and "missingness" (both in the placenta sample and the brain MRI sample) limited the overall sample size of our analysis.

Conclusions

While the data point to placental inflammation as a driver of brain volume and function later in life, the perinatal antecedents that drive placental inflammation and subsequent immune responses are largely unknown. Finally, it is currently not known whether there are any moderators which would identify potential methods for public health protections against adverse neurodevelopmental outcomes in children. In summary, this is one of the first studies to identify linkages between mRNA expression in the placenta, and brain volume and neurocognition at age 15. These findings lay the foundation for solution-oriented research that will identify methods to reduce placenta inflammation and reduce risk of adverse neurodevelopmental outcomes.

CHAPTER 3: ASSOCIATION OF EARLY LIFE INFLAMMATION, ADOLESCENT BRAIN VOLUME AND NEUROLOGIC DISORDERS

Background

Extremely low gestational age newborns (ELGANs) are born <28 weeks gestation and are at increased risk for neurodevelopment abnormalities. Neurodevelopment abnormalities including epilepsy, cerebral palsy (noted in 5%–10 %), and cognitive, attentional, behavioral, and socialization disturbances (noted in 25%–50%). [121, 122, 199-202] A probable intermediate between being born preterm and increased neurodevelopmental risks is brain structure and size, since individuals born preterm have reduced brain regional volumes at school age as compared to children born full term. [96, 203, 204]

Reduced brain volume in children born very preterm (≤ 32 weeks gestation) has been associated with impaired neurocognition. [98] Specifically, reduced volumes in cerebral white matter (WM), gray matter (GM), cerebellum, and subcortical structures are correlated with less favorable cognition, psychological, and educational outcomes in adolescents both preterm and extremely preterm. [116, 205-214] Our team has recently shown that in adolescents born extremely preterm, lower brain volume was associated with neurologic disorders. [215] Given the relationships between brain volumes and neurologic status, examination of factors that influence brain volumes is of significance.

Compared to extensive studies that relate brain volume with cognitive functions, [209, 216-219] fewer studies have examined the antecedents of abnormal brain development in children who were born extremely preterm. [119, 220, 221] Perinatal factors such as social risk, birthweight, and

gestational age, may be associated with neonatal brain volumes. [101, 220] Identification of early life factors associated with brain structural and functional disorders is a critical step in designing interventions to decrease the risk of these disorders. The ELGAN Study was initiated to address this knowledge gap. In prior analyses from the ELGAN study, neonatal systemic inflammation was associated with an increased risk of cerebral palsy and cognitive impairment, [222] as well as decreased brain volumes in children at age 10. [119] To date, no studies have evaluated associations between neonatal systemic inflammation and regional brain volumes in adolescence.

Study Objective

The aim of this study was to investigate the relationship between early life circulating inflammation proteins and total and regional brain volumes in ELGAN study participants at age 15. We further aimed to test whether sex or the presence of neurological disorders modified the inflammation-brain volume relationships. We hypothesized that elevated circulating inflammation-related proteins would be associated with decreased total and regional brain volumes and that these associations would differ by sex and in the presence of a neurological disorder.

Materials and Methods

Study Participants

The ELGAN Study is a multicenter observational study of the risk of neuroanatomical and neurofunctional disorders in extremely premature infants. [223] From 2002 to 2004, 1249 mothers and 1506 infants who were born less than 28 weeks of gestation were enrolled. At age 15, 1198 surviving adolescents were targeted for recruitment. Of these 1198 participants, 701 re-enrolled for follow-up study at age 15 years where they underwent comprehensive neurocognitive assessment and brain MRI (463/701, 66%). Enrollment in the ELGAN prospective study was approved by the institutional review boards of all 12 participating

institutions. Parental consent was obtained for all participants and each participant assented to undergo brain MRI acquisitions. A total of 323 subjects had data for neonatal inflammation, brain MRI image processing, and whether a neurological disorder had been identified (**Supplemental Figure 3.1**). All 323 participants included in the MRI analyses were imaged with a Siemens Prisma 3T system (Siemens, Erlangen, Germany) at six participating sites (UNC-Chapel Hill, Wake Forest, Boston Children's Hospital, Beaumont Medical Center, Baystate Medical Center, and Yale University).

Neonatal Blood Protein Measurements and Inflammation Status Assignment

As detailed previously [119, 224] whole blood was collected on filter paper (Schleicher & Schuell 903, GE Healthcare, Chicago, IL) on postnatal day 1 (range, 1-3 days), 7 (range, 5-8 days), and 14 (range, 12-15 days). Inflammatory-associated proteins were measured in the Laboratory of Genital Tract Biology, Brigham and Women's Hospital. [119] Six inflammation-related proteins previously reported to be associated with structural and functional neurologic outcomes in ELGAN Study were analyzed in the present study including interleukin 6 (IL-6); tumor necrosis factor-alpha (TNFα); intercellular adhesion molecule-1 (ICAM-1); interleukin 8 (IL-8); serum amyloid A (SAA); and C-reactive protein (CRP). [119] Associations between these proteins and neurological outcomes in ELGAN are summarized in **Supplemental Table 3.1**. Protein abundance levels were categorized into four quartiles (**Supplemental Figure 3.2**). Quartile one represented the lowest protein concentrations while quartile four represented the highest increase in protein concentration. Sustained inflammation was classified as reaching the fourth quartile for at least two of the 3 measurements made in the first 2 postnatal weeks. If sustained inflammation was present for 0-1 proteins out of the six, ELGANs were categorized into the low inflammation group. If sustained inflammation was present for 2-3 proteins out of the six, ELGANs were categorized into the moderate inflammation group. If sustained

inflammation was present for 4+ proteins out of the six, ELGANs were categorized into the high inflammation group (**Supplemental Figure 3.2**). These inflammatory risk groups were used in the study of the association between neonatal inflammation and brain regional volumes at age 15.

MRI Image Acquisition and Analysis at Age 15

A total of 463 participants at 12 ELGAN-ECHO participating sites had brain MRI acquired at age 15. While 6 sites contributed to the MRIs included in the present study, for the 15 year assessment of the ELGAN cohort, MRIs were collected at all 12 participating sites. MRI images were acquired on Siemens Prisma 3T MRI scanner at 6 participating sites, Philips 3T MRI scanner at 3 participating sites, and GE 3T MRI scanner at 2 participating sites. One participating site used 1.5T MRI scanner with different imaging parameters; to avoid potential data inconsistencies, these 1.5T images (n=24) were not included in this analysis. A dual-echo turbo-spin-echo (DE-TSE) and a single-echo turbo-spin-echo (triple-TSE), referred to together as a triple turbo-spin-echo (Tri-TSE) pulse sequence was used for image acquisition, with typical imaging parameters as field-of-view = 240 x 240 x 160 mm^3, voxel size = 0.5 x 0.5 x 2 mm^3, tEeff1,2 = 12 ms and 102 ms, tRlong = 10 s, tRshort = 0.5 s, echo train length = 10 for SE-TSE, and 20 for DE-TSE, with a 7.57 min total scan time.

Our team previously reported global scale brain qMRI results in adolescent ELGAN study participants.[215] The study herein deals with finer-scale brain partitioning using FreeSurfer v7.1.0 under Linux RHEL-7 environment. Brain regional volumes of cerebral cortex, cerebral white matter, cerebellar cortex, cerebellar white matter, brain stem, thalamus, lateral ventricle, putamen, hippocampus, ventral diencephalon, caudate, corpus callosum, pallidum, amygdala, and accumbens area were extracted and investigated. Bi-hemisphere brain regional volumes were examined. An image processing pipeline was constructed using MATLAB R2018b (MathWorks,

Natick, MA) and was used to generate qMRI maps of the proton density (PD), and the relaxation times (T1 and T2), as well as synthetic magnetization prepared rapid gradient echo (MP-RAGE) images. Such synthetic MP-RAGE images have high image white-to-gray matter contrast and are therefore suitable for multi-region brain segmentation using the FreeSurfer package. These three primary qMRI parameter maps were used with a Bloch equation-based method to produce synthetic MP-RAGE images [225]; the synthetic Bloch equation parameters were TR = 2500 ms; TE = 2.15 ms; TI = 1000 ms; $\alpha = 6^{\circ}$; $N_{3D} = 160$; ES = 10.2 ms; $B_1 = 1; RP = 1$. Compared to directly acquired MP-RAGE images, synthetic MP-RAGEs are less vulnerable to degradation from magnetic field inhomogeneities caused by dental hardware (braces), which are common in adolescents. Synthetic MP-RAGE images were interpolated to 1 mm isotropic resolution and used for further segmentation with Freesurfer.

Data Harmonization

Data harmonization was performed for all original brain regional volume data extracted by FreeSurfer using Combat. [226] Each of the six participating sites in the present study and their associated scanners were regarded as unwanted sources of non-biological variance and assigned with categorical batch ids. The original volume data was stored in a data matrix with rows representing regional volume features and columns representing participants as input into the Combat model. The Combat model was run under its default setting with empirical Bayes procedure and parametric priors. The output of the model is the harmonized data with the same dimension as the input original data.

Identification of Neurological Disorders

Cerebral palsy was identified using a standard neurological evaluation when participants were about two years of age. [201] Autism spectrum disorder and epilepsy were identified when children were ~10 years of age using rigorous multi-step processes as previously described. [215,

[227, 228] Cognitive impairments were identified using the Wechsler Abbreviated Scale of Intelligence – II (WASI-II), [178, 181, 200, 201, 227, 229] In the current analysis, children were classified as having a neurological disorder if they had one or more of the following: cerebral palsy at two years of age, [201] ASD at age 10, [227] epilepsy at age 10, [200] or a full-scale IQ <85 at age 15 [178].

Statistical Analysis

Multiple regression was used to assess associations between neonatal inflammation category, as previously defined, and brain region volumes. Models were run with brain region volume as the dependent variable and inflammation category, as the predictor variable. These models adjusted for sex, socioeconomic status, birthweight z-score, and participation site. Because inflammation quartiles were stratified by gestational age, this variable was not included as a covariate in multivariable regression. For the measure of socioeconomic status, we used a summative index of maternal social disadvantage, increasing by one point for each of the following: maternal education less than high school, lack of private health insurance, receipt of government-provided supplemental nutritional assistance, and single marital/unpartnered status. For comparisons of brain volume between sexes, two variations in analyses were performed. One method used average volumes that were unadjusted by participant brain volume. A second method normalized each brain region to the participants' total brain volume resulting in a proportion. To test for moderation, sex-stratified regression analyses were conducted to characterize the relationship between neonatal inflammation status and brain region volumes within sex stratum. An addition moderation analysis was performed where the relationship between neonatal inflammation status and brain region volumes was assessed within neurologic status stratum. P significance was set at <0.05 for all studies. All analyses were conducted in R v4.1.2.[186]

Data Availability

Brain volume data and the code used for the analyses are available at https://dataverse.unc.edu/dataverse/frylab.

Results

ELGAN Study Participants

A total of 323 adolescent ELGAN-ECHO participants were included in the present study. Inclusion criteria were availability of data for the following: neonatal blood inflammation-related proteins, MRI at 15 years of age, and presence or absence of a major neurologic disorder through 15 years of age. General characteristics of the study participants are summarized in **Table 3.1**. A total of 156 (48.3%) of the ELGAN participants were female and 167 (51.7%) were male. Two hundred sixteen (66.9%) self-identified as White, 69 (21.4%) self-identified as Black, and 33 (10.2%) self-identified as other. The average gestational age of the adolescent ELGAN participants was 26.1 weeks and the average maternal age at the time of birth was 29.4 years. Eighty-nine (31%) of the sample had a major neurological disorder. Across neonatal inflammation categories (i.e., low, moderate, high), there were significant differences in birthweight Z score, race, and neurological status ($p < 0.05$) (**Table 3.1**). Of the 89 participants with a major neurological disorder, 49 (34%) were male and 40 (28%) were female. Of the 198 ELGAN adolescents who did not have a major neurological disorder, 95 (66%) were male and 103 (72%) were female. (**Table 3.1**).

TABLE 3.1: Study participant'' demographics stratified according to their inflammation categories as low, moderate, or high.

	Overall (N= 323)	low (N = 241)	moderate (N = 62)	high (N =20)	*P* value
Inflammation Summary					**<0.001**
Mean (SD)	0.95 (1.34)	0.28 (0.45)	2.39 (0.49)	4.60 (0.75)	
Sex					0.91
Female	156 (48.3%)	118 (49.0%)	29 (46.8%)	9 (45.0%)	
Male	167 (51.7%)	123 (51.0%)	33 (53.2%)	11 (55.0%)	
Birthweight Z-score					**0.01**
< -2	21 (6.50%)	13 (5.39%)	8 (12.9%)	0 (0.00%)	
< -1	40 (12.4%)	24 (10.0%)	13 (21.0%)	3 (15.0%)	
<= 1	113 (35.0%)	84 (34.9%)	18 (29.0%)	11 (55.0%)	
>1	149 (46.1%)	120 (49.8%)	23 (37.1%)	6 (30.0%)	
GA (weeks.days)					0.47
Mean (SD)	26.1 (1.21)	26.1 (1.20)	25.9 (1.19)	26.2 (1.34)	
Neurological Disorder					**0.03***
ND -	198 (61.3%)	155 (64.3%)	28 (45.2%)	15 (75%)	
Female	103 (52.0%)	84 (54.2%)	12 (42.9%)	7 (46.7%)	
Male	95 (47.9%)	71 (45.8%)	16 (57.1%)	8 (53.3%)	
ND+	89 (27.6%)	58 (24.1%)	27 (43.5%)	4 (20.0%)	
Female	40 (44.9%)	26 (44.8%)	13 (48.1%)	1 (25.0%)	
Male	49 (55.1%)	32 (55.2%)	14 (51.9%)	3 (75.0%)	
Missing	36 (11.1%)	28 (11.6%)	7 (11.3%)	1 (5.00%)	
Race					**0.001**
White	216 (66.9%)	168 (69.7%)	36 (58.1%)	12 (60.0%)	
Black	69 (21.4%)	43 (17.8%)	23 (37.1%)	3 (15.0%)	
Other	33 (10.2%)	28 (11.6%)	3 (4.8%)	2 (10.0%)	
Missing	5 (1.55%)	2 (0.83%)	0 (0.00%)	3 (15.0%)	
Hispanic Ethnicity					0.48
No	293 (90.7%)	218 (90.5%)	58 (93.5%)	17 (85.0%)	
Yes	30 (9.29%)	23 (9.54%)	4 (6.45%)	3 (15.0%)	
Maternal Education					0.27
<=12	113 (35.0%)	85 (35.3%)	24 (38.7%)	4 (20.0%)	
13-15	72 (22.3%)	48 (19.9%)	16 (25.8%)	8 (40.0%)	
16+	131 (40.6%)	103 (42.7%)	20 (32.3%)	8 (40.0%)	
Missing	7 (2.17%)	5 (2.07%)	2 (3.23%)	0 (0.00%)	
Pre-pregnancy BMI					0.32
Underweight	27 (8.36%)	18 (7.47%)	7 (11.3%)	2 (10.0%)	
Normal	149 (46.1%)	115 (47.7%)	27 (43.5%)	7 (35.0%)	
Overweight	62 (19.2%)	49 (20.3%)	11 (17.7%)	2 (10.0%)	
Obese	73 (22.6%)	51 (21.2%)	13 (21.0%)	9 (45.0%)	
Missing	11 (3.7%)	8 (3.3%)	3 (6.5%)	0 (0.00%)	
Maternal Age (years.days)					0.72
Mean (SD)	29.4 (6.71)	29.2 (6.59)	30.0 (7.12)	29.0 (7.06)	
Public Insurance					0.26
Insurance	221 (68.4%)	169 (70.1%)	36 (58.1%)	16 (80.0%)	
Medicaid	98 (30.3%)	69 (28.6%)	25 (40.3%)	4 (20.0%)	

Note. —Gestational Age is abbreviated as GA, the absence or presence of a major Neurological Disorder is ND+ or ND- respectively, standard deviation (SD), and body mass index (BMI). As notated by the *, ND does''t account for sex it is just used in inflammation status and ND analyses.

As shown in **Supplemental Figure 3.1**, of the 465 MRI images (representing 462 unique participants), 353 were included in the brain segmentation analysis. Of the 110 participants that were excluded for brain segmentation analysis, 24 were scanned on a 1.5T MRI. In addition, 86 participants were excluded due to triple-TSE data inconsistencies, syn-MP-RAGE failed FreeSurfer segmentation, segmentation inaccuracies due to (motion, low image quality, and extreme anatomy. Additionally, among the 353 participants, 31 did not have neonatal blood protein data or neurologic disorder data collected. Therefore, a total of 323 participants were included in the final analyses.

Adolescent Brain Volume

Segmental brain volumes were assessed for 16 brain regions at age 15 in the ELGANs. These included: total brain, cerebral cortex, cerebral white matter, corpus callosum, cerebellum cortex, cerebellum white matter, brainstem, lateral ventricle, ventral diencephalon, and subcortical grey matter (thalamus, putamen, hippocampus, caudate, pallidum, amygdala, and accumbens). Brain anatomical structures of interest and their anatomical location are found in **Figure 3.1**. Corresponding volumetric details for the sample (*N=323*) are reported in **Table 3.2**. Volumes of brain regions ranged from 1-480 cm^3 (accumbens-cerebral cortex) relative to total brain (1041 cm^3).

FIGURE 3.1: Brain regions of interest in adolescents born extremely preterm.

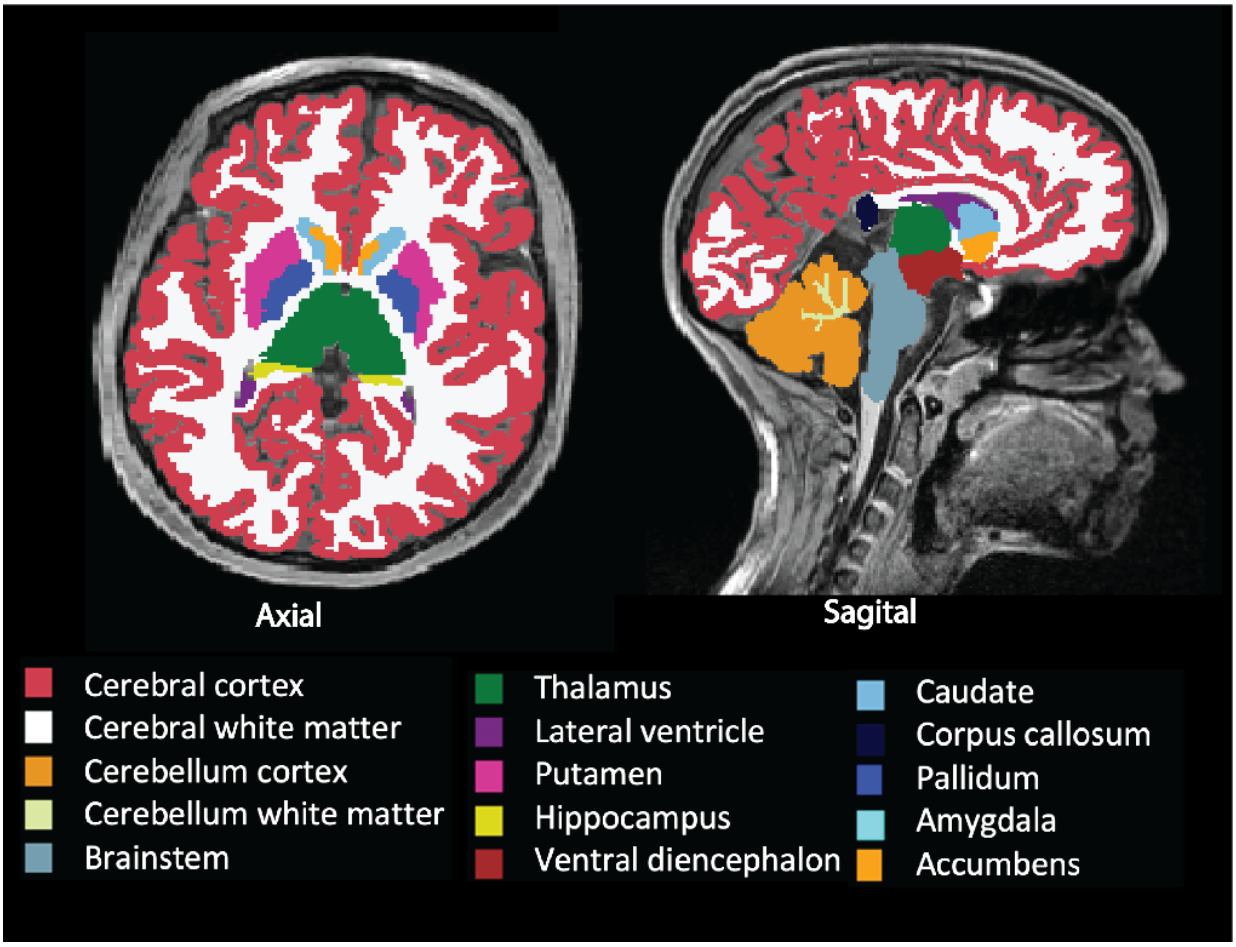


TABLE 3.2: Brain volumes (cm^3) in ELGAN adolescents (N=323) overall and stratified by sex.

		female	male
	N=323 N (SD)	N=156 N (SD)	N=167 N (SD)
Cerebral cortex	480 (57.3)	458 (51.3)	501 (54.8)
Cerebral white matter	358 (56.0)	337 (47.3)	379 (56.1)
Corpus callosum	2.84 (0.55)	2.80 (0.52)	2.88 (0.58)
Cerebellum cortex	105 (15.5)	99.3 (14.8)	110 (14.5)
Cerebellum white matter	20.5 (3.46)	20.0 (3.46)	21.0 (3.40)
Brainstem	17.4 (2.48)	16.7 (2.36)	18.1 (2.40)
Lateral ventricle	20.0 (14.0)	18.5 (11.8)	21.4 (15.7)
Subcortical grey matter	6.77 (0.87)	6.48 (0.77)	7.04 (0.86)
Ventral diencephalon	52.7 (6.25)	50.1 (5.40)	55.1 (6.06)
Thalamus	13.3 (1.72)	12.7 (1.48)	13.8 (1.75)
Putamen	9.78 (1.79)	9.18 (1.68)	10.3 (1.72)
Hippocampus	7.29 (0.86)	6.93 (0.75)	7.62 (0.82)
Caudate	6.65 (1.03)	6.41 (0.96)	6.87 (1.05)
Pallidum	2.87 (0.60)	2.75 (0.59)	2.98 (0.60)
Amygdala	2.80 (0.46)	2.61 (0.38)	2.99 (0.45)
Accumbens	1.28 (0.29)	1.22 (0.28)	1.34 (0.28)
Total brain volume	1041 (128)	987 (108)	1091 (124)

Note. — Standard deviation (SD), Extremely Low Gestational Age Newborn (ELGAN). All comparisons except corpus callosum and lateral ventricle were significant at $p < 0.05$ when unadjusted by total brain volume.

Neonatal Inflammatory Status is Associated with Brain Volume at Age 15

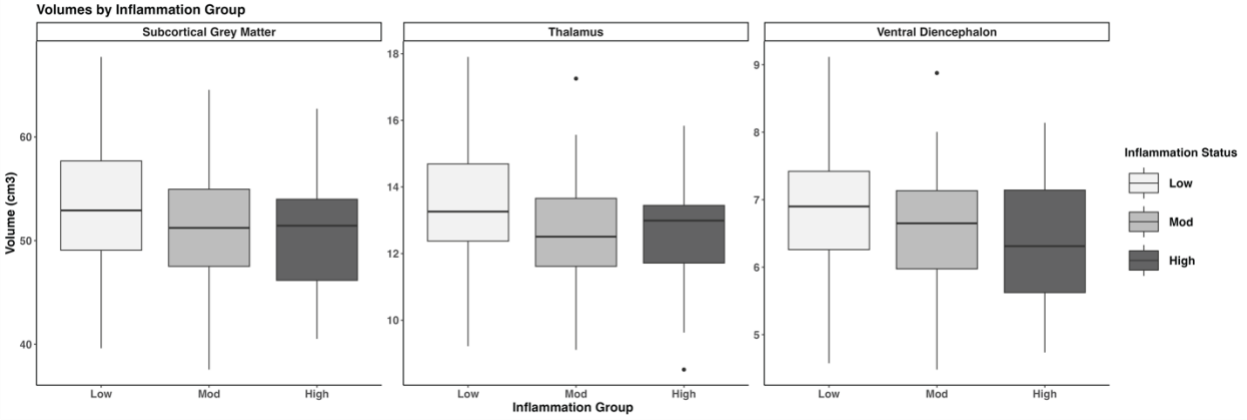
Neonatal inflammation categorization is detailed in **Supplemental Figure 3.2**. The severity of neonatal inflammation (i.e., low, moderate, or high) was analyzed in relation to brain volumes using multiple linear regression modeling adjusting for confounders. Pairwise comparisons of the brain volumes according to their inflammation groups (i.e., low versus moderate, low versus high) were performed. Brain volumes of participants who had both moderate or high neonatal inflammation groups were reduced compared to participants with low neonatal inflammation for the following brain regions: subcortical grey matter, thalamus, and ventral diencephalon (**Table 3.3, Figure 3.2**). Additionally, some segmental volumes were significantly reduced only for participants with moderate or high inflammation compared to participants with low inflammation status. Specifically, ELGANs with moderate inflammation displayed reduced volumes relative to those with low inflammation specifically for the following regions: cerebral cortex and cerebral white matter. ELGANs with high inflammation displayed reduced brain volumes relative to those with low inflammation specifically for the cerebellum cortex, cerebellum white matter, brainstem, and amygdala (**Table 3.3**). No significant association was observed between neonatal inflammatory status and volumes for the following regions: cerebellum cortex, cerebellum white matter, caudate, accumbens, putamen, hippocampus, pallidum, corpus callosum, and lateral ventricles.

TABLE 3.3: Brain volumes (cm^3) as they relate to neonatal inflammation categories (low, moderate, high) in ELGAN adolescents (n=323).

Brain Region	Brain volume (low)	Brain volume (moderate)	Δ (low and moderate)	P value	Cohen's d	Brain volume (high)	Δ (low and high)	P value	Cohen's d
Cerebral cortex	483 (56.3)	470 (62.6)	-12.5	**0.02**	0.22*	482 (51.3)	0.64	0.46	0.01
Cerebral white matter	362 (56.6)	348 (55.0)	-13.5	**0.03**	0.24*	349 (48.7)	12.8	0.12	0.23*
Cerebellum cortex	106 (13.9)	103 (17.5)	3.01	0.40	0.20*	92.4 (22.0)	13.8	**<0.001**	0.94‡
Cerebellum white matter	20.8 (3.13)	20.1 (4.03)	0.71	0.31	0.21*	19.3 (4.90)	1.46	0.07	0.44†
Corpus callosum	2.87 (0.54)	2.77 (0.60)	0.11	0.32	0.19	2.69 (0.50)	0.18	0.20	0.34†
Brainstem	17.7 (2.30)	17.0 (2.63)	0.64	0.13	0.27*	15.70 (3.38)	1.96	**<0.001**	0.82‡
Lateral ventricle	19.9 (14.9)	19.6 (10.24)	0.34	0.98	0.02	22.4 (14.5)	-2.44	0.43	-0.16
Subcortical gray matter	53.3 (6.14)	51.0 (6.39)	2.33	**0.01**	0.38†	50.2 (5.84)	3.08	**0.01**	0.50†
Ventral diencephalon	6.86 (0.85)	6.56 (0.86)	0.30	**0.01**	0.36†	6.35 (0.90)	0.51	**0.002**	0.60‡
Thalamus	13.5 (1.71)	12.7 (1.62)	0.75	**0.002**	0.44†	12.7 (1.86)	0.72	**0.04**	0.42†
Putamen	9.90 (1.80)	9.46 (1.89)	0.44	0.15	0.24*	9.31 (1.14)	0.59	0.11	0.33*
Hippocampus	7.34 (0.84)	7.16 (0.85)	0.19	0.12	0.22*	7.05 (1.06)	0.30	0.07	0.34*
Caudate	6.74 (1.01)	6.40 (1.02)	0.34	0.08	0.34*	6.25 (1.13)	0.49	0.07	-0.48†
Pallidum	2.90 (0.59)	2.75 (0.69)	0.16	0.09	0.25*	2.79 (0.36)	0.12	0.29	0.21*
Amygdala	2.82 (0.46)	2.79 (0.45)	0.03	0.40	0.06	2.69 (0.41)	0.13	**0.05**	0.28*
Accumbens	1.29 (0.30)	1.25 (0.26)	0.05	0.61	0.17	1.25 (0.22)	0.04	0.74	0.15
Total brain volume	1049 (127)	1015 (135)	33.5	**0.02**	0.26*	1018 (103)	30.3	0.09	0.24*

Note. — Standard deviation (SD), Extremely Low Gestational Age Newborn (ELGAN). MRI-derived brain volumetrics (cm3) as it relates to neonatal inflammatory groups (e.g., low, moderate or high), for total brain matter, cerebral matter; total, deep, and cortical grey matter; white matter; and cerebellum and brain stem. Bold p values denote associations that are significant following FDR correction (pFDR < 0.05). Effects are considered to be small when Cohe''s d is between 0.2 or 0.3 *, medium effects are assumed for values around 0.5†, and values of Cohe''s d larger than 0.8 ‡would depict large effects.

FIGURE 3.2: Box plots of brain regions where volumes were significantly associated with neonatal inflammation status. Neonates were classified into inflammation categories as low, moderate, or high.



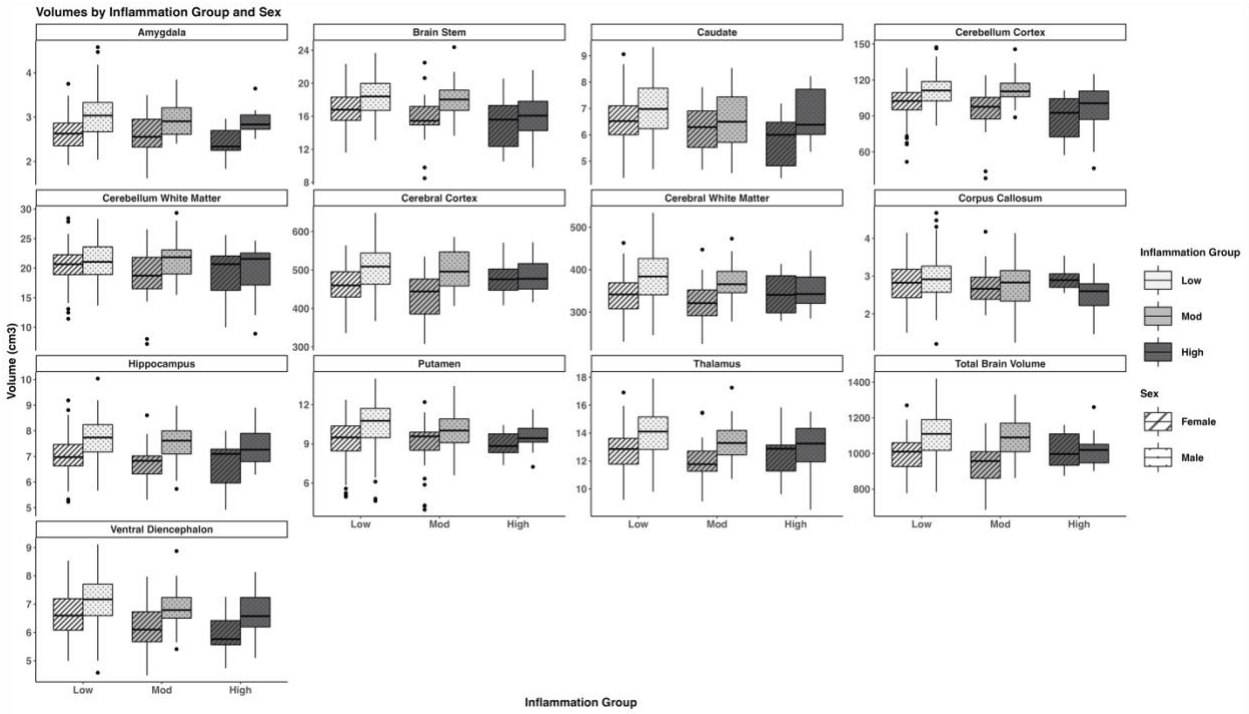
Neonatal Inflammatory Status is Associated with Brain Volume at Age 15 in a Sex-Specific Manner

A total of 118 of the females in our ELGAN adolescent cohort were classified as having low inflammation, 29 had moderate inflammation, and 9 had high inflammation (**Table 3.1**). For ELGAN adolescent males, 123 were classified as having low inflammation, 33 had moderate inflammation, and 11 had high inflammation. There were no differences in the number of males or females who belonged to the neonatal inflammation groups (*p-value =0.9)* (**Table 3.1**).

Neonatal inflammation-brain volume relationships differed for males as compared to females. (**Figure 3.3, Supplemental Tables 3.3 and 3.4**). Specifically, in relation to high neonatal inflammation, a reduction in cerebral white matter, corpus callosum, putamen, and total brain volumes was observed in males but not females (**Figure 3.3, Supplemental Tables 3.3 and** 3.**4**). Both males and females displayed reductions in brain volumes of the cerebellum cortex, brainstem, ventral diencephalon and in relation to high inflammation. Females, but not males, showed decreased brain volumes relative to moderate

Females, but not males, showed decreased brain volumes relative to moderate inflammation in the brainstem, cerebral cortex, cerebral white matter, cerebellum cortex, cerebellum white matter, thalamus, ventral diencephalon, and total brain (**Supplemental Tables 3.3 and** 3.**4**).

FIGURE 3.3: Brain volumes (cm^3) as they relate to neonatal inflammation categories (low, moderate, high) in ELGAN adolescent males and females (n=323).

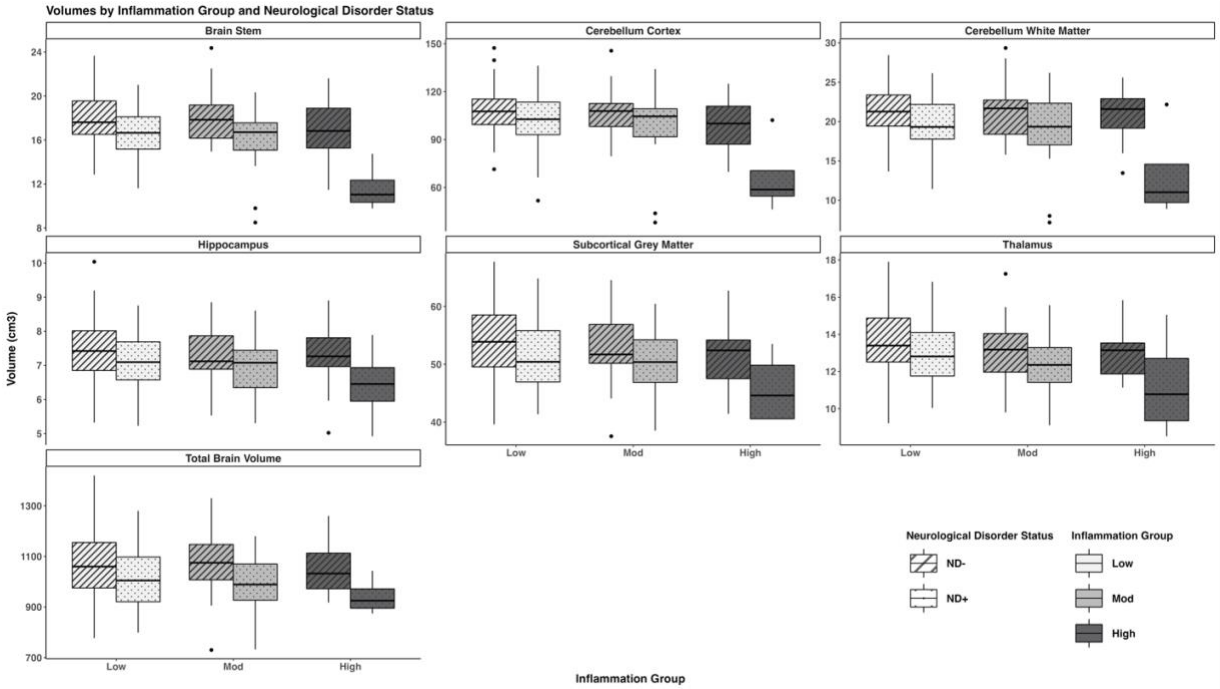


Note. —Moderate inflammation is abbreviated as Mod

Neonatal Inflammatory Status is Associated with Brain Volume Among Individuals with a Neurologic Disorder

To evaluate relationships among neonatal inflammation, brain volumes, and the presence or absence of a neurodevelopmental disorders, we analyzed relationships between neonatal inflammation and segmental brain volumes when stratified by the presence or absence of a major neurological disorder. There was a negative association between high neonatal inflammation and brain volume among participants with a neurological disorder for the thalamus, hippocampus, subcortical grey, cerebellum cortex, cerebellum white matter, brainstem, and total brain (**Figure 3.4, Supplemental Tables 3.5 and 3.6, Supplemental Figure 3.4**). ELGAN adolescents without a neurological disorder only showed an association between neonatal inflammation and brain volume in the cerebellum cortex (**Supplemental Tables 3.5 and 3.6).**

FIGURE 3.4: Brain volumes stratified by neonatal inflammation groups and by typical vs atypical neurodevelopment. Neonates were classified into inflammation categories as low, moderate, or high.



Note. —Moderate inflammation is abbreviated as Mod.

Discussion

This study investigated a component of the developmental origins of health and disease (DOHaD) hypothesis by focusing on the relationship between levels of neonatal inflammation in the first few days of life and segmental brain volume at age 15 in ELGANs. Four major findings were identified: *first,* we identified specific regions of the brain that were larger in males than females even when adjusting for total brain volume; *second,* adolescents with both moderate and high neonatal inflammation displayed decreased brain volumetry for specific brain regions; *third,* there were sex-specific associations between high neonatal inflammation and brain volume where the reductions were stronger for males; and *fourth,* the neonatal inflammation-brain volume associations were stronger among ELGANs with a major neurological disorder. These results support the strong linkage between neonatal inflammation and adolescent brain volume in individuals born extremely preterm and the role of sex and presence of neurologic disorders as moderators.

Within ELGAN as well as across other published studies, sex-specific brain volumetric differences have been reported. [174, 215, 230-233] Specifically, we previously found larger white matter, grey matter, and cerebral spinal fluid volumes for males than females at age 10 years. [215] In the present analysis, we extend the prior work using region-specific volumes as a proportion of total brain volume by participant. The present analysis highlights larger volumes of deep grey matter nuclei and other parcellated brain region volumes for ELGAN males than females at age 15. [174, 215, 230-233] While sex-based differences in brain volume have been reported previously, there is a lack of standardization in adjustment for total brain volume by participant. These differences limit cross-study interpretation and support the need for both unadjusted and adjusted analyses. Taken together, the sex-specific findings in the ELGAN adolescents are highly

informative representing one of the first studies to investigate brain volume in teens who were born extremely prematurely. [94, 203, 234]

We have previously reported an association between neonatal inflammation and brain volumes in ELGAN children at age 10 including gray matter and brain stem/cerebellum volumes. [119] In the present study, we build on this work highlighting a direct relationship between increased neonatal inflammation and decreased regional brain volumes in ELGAN adolescents. Specifically, increased neonatal inflammation (both moderate and high) was associated with decreased brain volume within the subcortical grey matter, thalamus, and ventral diencephalon. To our knowledge, an association between neonatal inflammation and reduced regional brain volumes of extremely preterm adolescents has not been reported in the literature. Interestingly, no significant association was observed between neonatal inflammatory status and volumes of the cerebellum cortex, cerebellum white matter, caudate, accumbens, putamen, hippocampus, pallidum, corpus callosum, and lateral ventricles suggesting the potential robustness of these brain regions to early-life inflammation as compared to other regions.

The present analysis highlighted stronger relationships between high neonatal inflammation and brain volume reductions for males versus females. These data suggest that the male brain may be more vulnerable to the effects of neonatal inflammation. Similarly, prenatal famine is associated with a greater reduction in brain volume among males, as compared to females. [235] While the mechanisms for this sex-specific association are likely complex, one potential mechanism for this vulnerability could be sexual epigenetic dimorphism of the placenta. Our team has identified differential DNA methylation, miRNA expression, and mRNA expression in the male or female-derived placenta. [134, 236] An example of a key pathway that could link neonatal inflammation to brain volume and underlie sex-differences is nuclear factor

kappa B (NF-κB). The NFkB pathway is a key regulator of response to inflammatory and immunomodulatory factors and has been linked to perinatal exposures as well as ELGAN health outcomes assessed longitudinally. [237] Sex-based differences in the expression of the NF-κB pathway and other inflammatory and immune-responsive pathways in the placenta could influence differential fetal responses to inflammatory-related insults. Similarly, sex differences in the expression of potential neuroprotective molecules, such as brain-derived neurotrophic factor, could contribute to attenuated effects of neonatal inflammation. [238]

The relationship between neonatal inflammation and brain volume was found almost exclusively among individuals with a major neurologic disorder. This finding is consistent with a causal pathway linking neonatal inflammation to neurologic impairments, mediated by disrupted brain development, as reflected by decreased brain volumes. While there was a higher proportion of males than females with a major neurological disorder (34% versus 28%), this difference cannot fully explain the observed inflammation-brain volume relationship. The reduction in volume of specific brain regions for individuals with a major neurological disorder is likely a result of the intimate relationship between brain volume and neurological disorders.[94-98] Future analyses of ELGAN data will investigate the specific relationships among regional brain volumes and other brain MRI characteristics and complex neurological disorders.

This study is unique in its identification of neonatal inflammation-brain volume relationships within specific brain structures, and the modification by sex and neurologic disorders among ELGANs. Strengths of the study include a large multi-center sample of adolescents born extremely preterm, the assessment of 15 brain regions including multiple deep gray matter regions, and the availability of biomarker data as a measure of neonatal systemic inflammation. [198, 239]. Another strength of the study is that multiple brain cortical and

subcortical anatomical regions were evaluated with the inclusion of high-quality synthetic images. Since the study sample was comprised of individuals born extremely preterm, the results might not be generalizable to adolescents born at term gestation. However, the presented analyses are self-consistent as they reveal internal differential effects. The sample size for the high neonatal inflammation group was small thus limiting the precision of estimated group differences. We acknowledge that there is likely some inherent selection bias related to exclusion of study participants whose MRI data were not considered usable for the analyses presented here. In our experience, participants with a major neurological disorder had greater difficulty limiting their movement during MRI scans and thus accounted for a disproportionate share of the scans that were not considered usable. [240] Given the strong association between neonatal inflammation and brain volume, almost exclusively among individuals with a major neurological disorder, the true estimate of the association is likely underestimated. Future research would aim to optimize MRI protocols [240] for ELGAN participants with neurological disorders to enhance their inclusion. Finally, future research could employ a methodology identifying inflammation-volume relationships using an individually normalized analysis accounting for the proportion of a subregion to the total brain. These results would more precisely highlight the relationship between an individual's neonatal inflammation and region-specific brain volumetry.

Conclusions

In summary, our findings highlight the importance of brain imaging and quantitative measurements in children born extremely preterm. As a significant proportion of the ELGAN cohort showed neonatal inflammation and brain volumetric differences at adolescence, further studies including biological and structural brain imaging assessments, as well as cognitive and neuropsychological assessments will be warranted as the cohort ages. The results from this study suggest that interventions to prevent or attenuate perinatal inflammation might improve

neurodevelopmental outcomes of infants born extremely preterm. A potential focus of solution-oriented research could be the prevention of exposure to environmental factors that initiate neonatal inflammation such as exposure to chemicals during pregnancy, or poor nutrition. [241-243] The data from the present study are of significant value to this population and the neuroscience research community in general as they underscore the value of measurements of neonatal inflammation, and regional brain volumes in those born extremely preterm.

CHAPTER 4: SUMMARY OF FINDINGS

An Interdisciplinary Approach Provides a Comprehensive Overview and Mechanistic Insight to Better Understand Placental Origins of Brain Development into Adolescence

As highlighted throughout my dissertation, the research that I have presented addresses a critical primary goal of the ELGAN Study to identify initiators of brain damage and alterations in neurodevelopment. Another goal is to identify molecular processes that may be implicated in decreasing the risk of brain damage and alterations in neurodevelopment, among vulnerable preterm infants born prior to 28 weeks of gestation. The *significance* of this dissertation lies in its investigation of the human placenta as driving increased susceptibility to neurodevelopmental disability later in life among ELGANs. Here, I explore the placenta-brain axis by investigating placental gene expression signals as a novel mechanism and risk factor for cerebral white matter damage (**Aim 1**), reduced brain volume (**Aim 2A**), and neurodevelopmental disability susceptibility (**Aim 2B**). Placing emphasis on the key scientific research gaps in bold, I hope to have added scientific value to the following understudied areas that exist within the literature:

1. Understanding linkages between the molecular machinery in the placenta and brain damage in the neonate. **(Aim 3)**
2. Understanding linkages between the molecular machinery in the placenta and brain volume in adolescents. **(Aim 2A)**
3. Understanding linkages between the molecular machinery in the placenta and neurocognitive outcomes in adolescents. **(Aim 2B)**

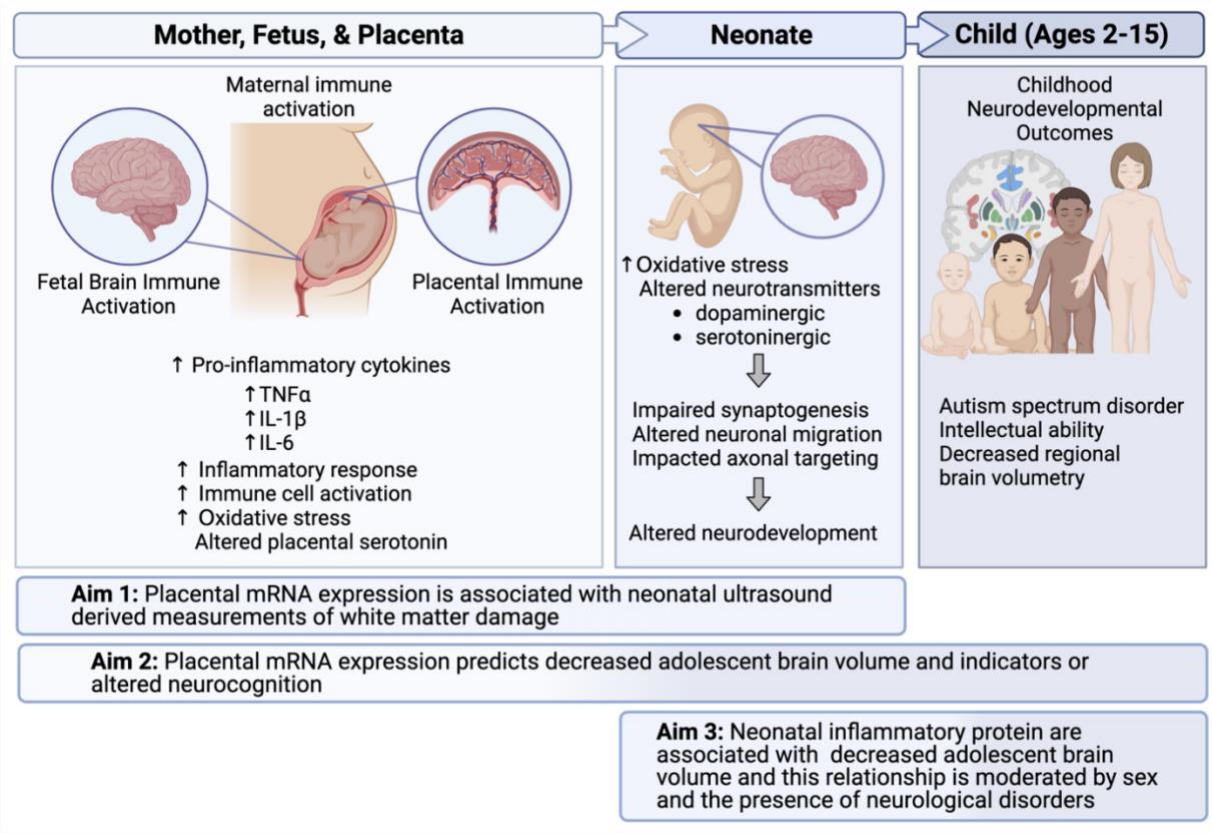
4. **Understanding sex-specific differences in the placenta-brain relationships (Aim 3)**
5. **Understanding longitudinal or temporal trends in brain development and their linkage to the placenta. (Aims 1-3)**
6. **Identifying specific genes and pathways in the placenta that are predictive and associated with white matter damage, brain volume alterations, and neurocognitive outcomes in adolescents. (Aims 1 and 2)**

Both published work and the data presented here support that mRNA alterations of critical genes in the placenta predict neurodevelopmental disability later in life. [244-247] Prior studies have highlighted genes which initiate inflammation or alter fetal growth are involved in fetal response to stressors. [135, 136, 248-252] My work, particularly in **Aims 1 and 2,** also suggests that modulation of these inflammation and fetal growth-related genes in the placenta, may be critical in the development of neurodevelopmental disability among individuals born extremely preterm. The associations found using mRNA expression as a biomarker of exposure predictor variable are biologically plausible in relation to the four significant outcomes identified in **Aims 1 and 2**. Specifically, these outcomes included: ultrasound-derived measurements of cerebral white matter in the neonate, reduced cerebellum cortex, reduced brainstem volume, and altered neurocognition, as indicated by being classified in the LPA-impaired group. The identified mRNAs may be a proxy for the physiologic state of the placentas following a maternal insult. Many of the cellular/molecular processes were found to be associated with more than one signaling pathway and had shared overlap of many of the genes within a given pathway. Of note, only the cerebellum cortex and brainstem volume were significantly associated with both inflammation and immune response-related genes, suggesting some brain regions may be more vulnerable than others to alterations.

The precise mechanism is unknown as to how placental epigenetic variation and inflammation may be influencing neurodevelopment. In these studies, I analyzed the transcriptomic architecture that exists between the placenta, neonate, and the maturing brains of those born prematurely into the critical adolescent phase, and also captured the relationship between markers of sustained inflammation in the neonate in relation to adolescent regional brain volume in **Aim 3**. Because there is also a known association between brain volume and neurodevelopmental disability, [113, 253, 254] I looked at the presence of one or more of four major neurologic disorders (cerebral palsy at two years of age, ASD at age 10, epilepsy at age 10, or a full-scale IQ <85 at age 15). Neonatal inflammation was associated with reductions in brain volumes at age 15 for the total brain and subcortical gray matter in the thalamus, ventral diencephalon, and caudate. Moreover, the relationship between neonatal inflammation and brain volumes was stronger for ELGANs with a major neurologic disorder than those without. There are known brain differences between men and women. [255, 256] Relative to my research, men generally have larger brain volumes as well as surface area, and higher cortical thickness in comparison to females. [255] As a result, I also examined whether sex modified the relationship between neonatal inflammation and brain volumes. Males display stronger neonatal inflammation-brain volume relationships than females. These data highlight the long-lasting effects of neonatal inflammation on the developing brain and the importance of neonatal inflammation-preventative research. Similar to **Aim 2A,** these findings also suggest that some brain regions may be more vulnerable than others to alterations.

Findings from my dissertation research are summarized in **Figure 4.1**

FIGURE 4.1: Summary of findings from my dissertation.



Future Research Directions

In line with the investigations of placental mRNA expression in association with age 15 brain volume and neurocognition, additional studies of interest could be to extend the placental -omic data to include miRNAs and/or CpG methylation data as predictor variables. This expansion would potentially aid in identifying additional -omic biomarkers of decreased brain volume and altered neurocognition for children born extremely preterm within the ELGAN Cohort. Additionally, taking a closer look at other potential perinatal antecedents other than inflammation, such as neurotransmitters, hormones, organic chemicals, inorganic chemicals including metals, and maternal stress as mentioned in **Figure 4** may help us uncover and better understand potential mechanisms biologically linking placental dysfunction to various aspects of child brain development. Examples could include longitudinal measures of chemical exposures of interest, namely umbilical cord metals and urinary concentrations of metal, in relation to brain volume and function. This approach will allow researchers to not only test for potential interactions, but they will be able to evaluate the impacts of fetal development and more specifically neurodevelopment that may not have been captured by the placental omics or maternal measurements previously provided.

Conclusions

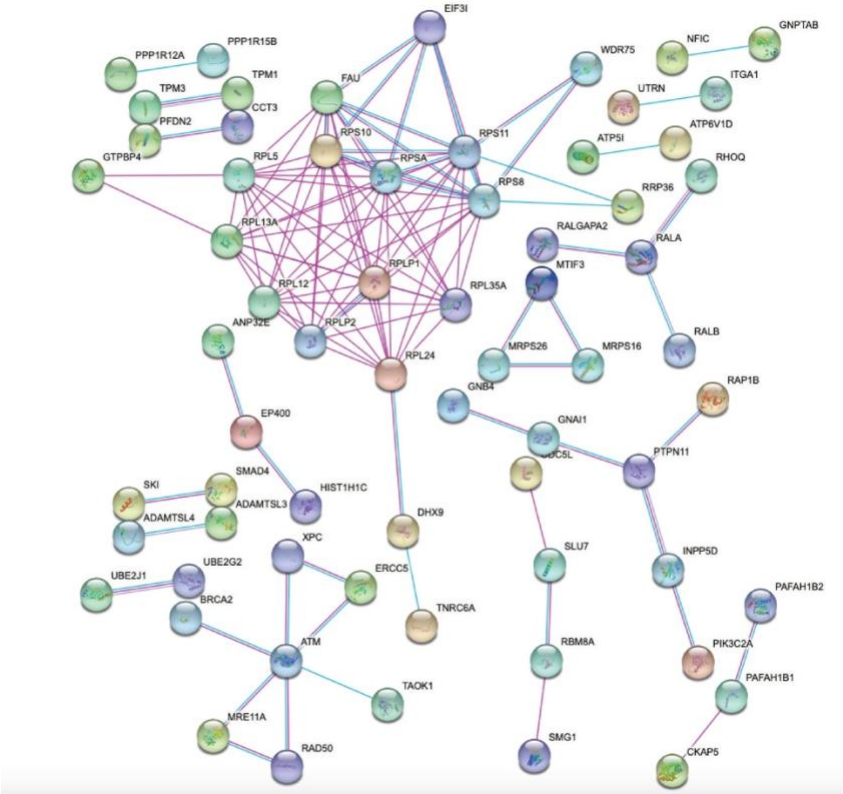
To conclude, my research explored child neurodevelopment during the first few weeks of life and at age 15 and examined the role of inflammation through the lens of the placenta. This multi-disciplinary effort coupled placental biology, genomic assays, protein assessment of neonatal inflammation, and later life clinically-assessed child neurological measures, to study the placenta-brain axis. In **Aim1**, I assessed the links between the placenta and ultrasound-derived white matter outcomes as well as the placenta and MRI-derived brain outcomes including brain volume and neurodevelopmental disability later in life. The strategy and design used are novel

relative to any published literature on this topic. Because of this, I was able to identify mRNA gene expression patterns in the placenta as they relate to brain volume and altered neurocognition in **Aim 2**. Lastly, in **Aim 3** I identified a subpopulation of study participants with one or more major neurological disorders (cerebral palsy at two years of age, ASD at age 10, epilepsy at age 10, or a full-scale IQ <85 at age 15) who were more susceptible to the effects of increased neonatal inflammation and displayed decreased adolescent brain volume.

Taken together, providing a unique assessment spanning birth to adolescence, I have identified novel biological connections among inflammation/immune mRNAs in the placenta, circulating inflammatory proteins in the neonate, brain damage in the neonate, and brain structure/function in adolescents born extremely preterm. This work has strong potential to provide novel mechanistic insights at the population level to guide future etiological studies for intervention that may ultimately help reduce neurodevelopmental disability among children born preterm.

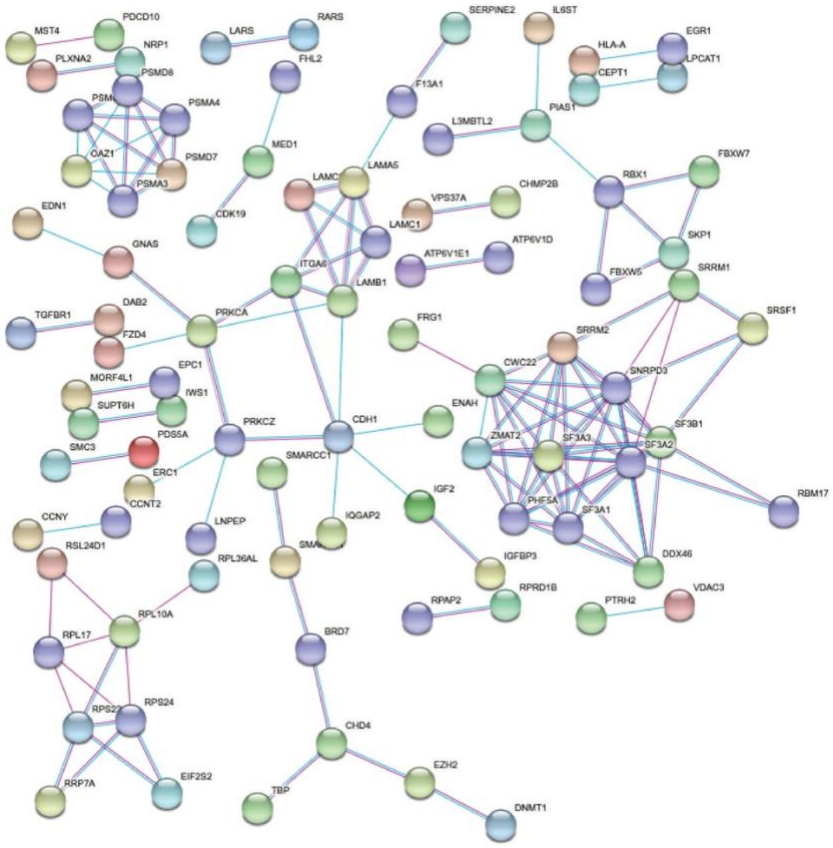
APPENDIX A: SUPPLEMENTAL MATERIAL FOR CHAPTER 1

SUPPLEMENTAL FIGURE 1.1: Network analysis of the common gene set (n=244) in the placenta. Interactome of gene–gene interactions representing joint contribution to a shared function. Protein interaction network analysis indicates the genes belonging to the EIF2, mTOR, and IL-6 Signaling pathways playing a central role in the interactome of placenta that are associated with cerebral white matter damage.



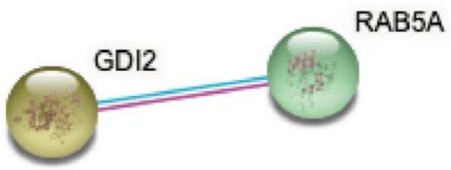
All 244 genes were used as input for STRING analysis and a network of 64 proteins was built (Supplemental Table 1a). Shown are the details of protein-protein interactions based on highest confidence (0.9) evidence from experimental protein-protein interaction (purple lines) and curated (blue lines) databases. Proteins are indicated by nodes labeled with the encoding gene symbol. Nodes of disconnected proteins were hidden. The network is enriched in interactions (p = 4.2E-10)

SUPPLEMENTAL FIGURE 1.2: Network analysis of the echolucency unique genes (n=381) in the placenta. Interactome of gene–gene interactions representing joint contribution to a shared function. Protein interaction network analysis indicates the genes belonging to the growth hormone pathway playing a central role in the interactome of placenta that are associated with cerebral white matter damage.



All 381 genes were used as input for STRING analysis and a network of 106 proteins was built (Supplemental Table 1a). Shown are the details of protein-protein interactions based on highest confidence (0.9) evidence from experimental protein-protein interaction (purple lines) and curated (blue lines) databases. Proteins are indicated by nodes labeled with the encoding gene symbol. Nodes of disconnected proteins were hidden. The network is enriched in interactions ($p = 0.00405$)

SUPPLEMENTAL FIGURE 1.3: Network analysis of the ventriculomegaly unique genes (n=34) in the placenta. Interactome of gene–gene interactions representing joint contribution to a shared function. Protein interaction network analysis indicates the genes belonging to the clathrin-mediated endocytosis signaling pathway playing a central role in the interactome of placenta that are associated with cerebral white matter damage.



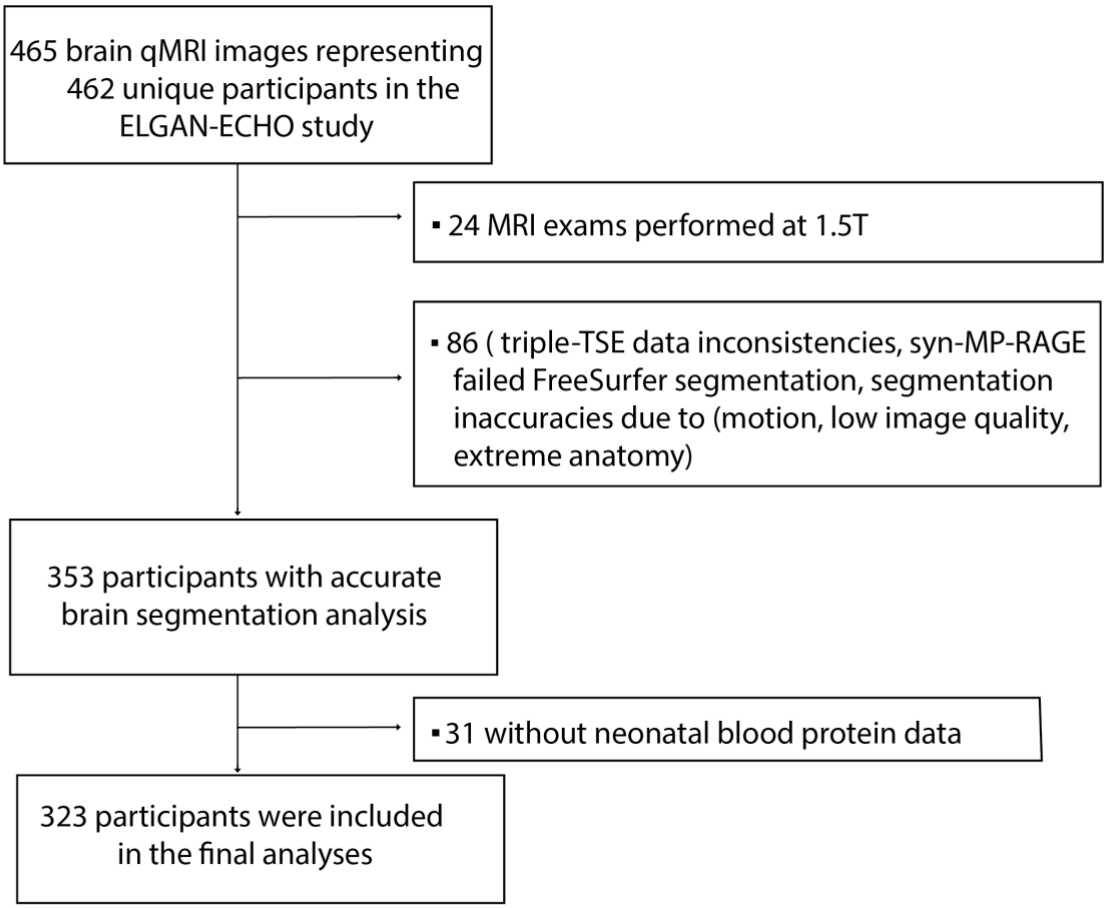
All 381 genes were used as input for STRING analysis and a network of two proteins was built (Supplemental Table 1a). Shown are the details of protein-protein interactions based on highest confidence (0.9) evidence from experimental protein-protein interaction (purple lines) and curated (blue lines) databases. Proteins are indicated by nodes labeled with the encoding gene symbol. Nodes of disconnected proteins were hidden. The network is enriched in interactions ($p = 0.569$)

APPENDIX B: SUPPLEMENTAL MATERIAL FOR CHAPTER 3

SUPPLEMENTAL TABLE 3.1: Inflammation-related proteins associated with structural and functional neurologic outcomes in ELGAN

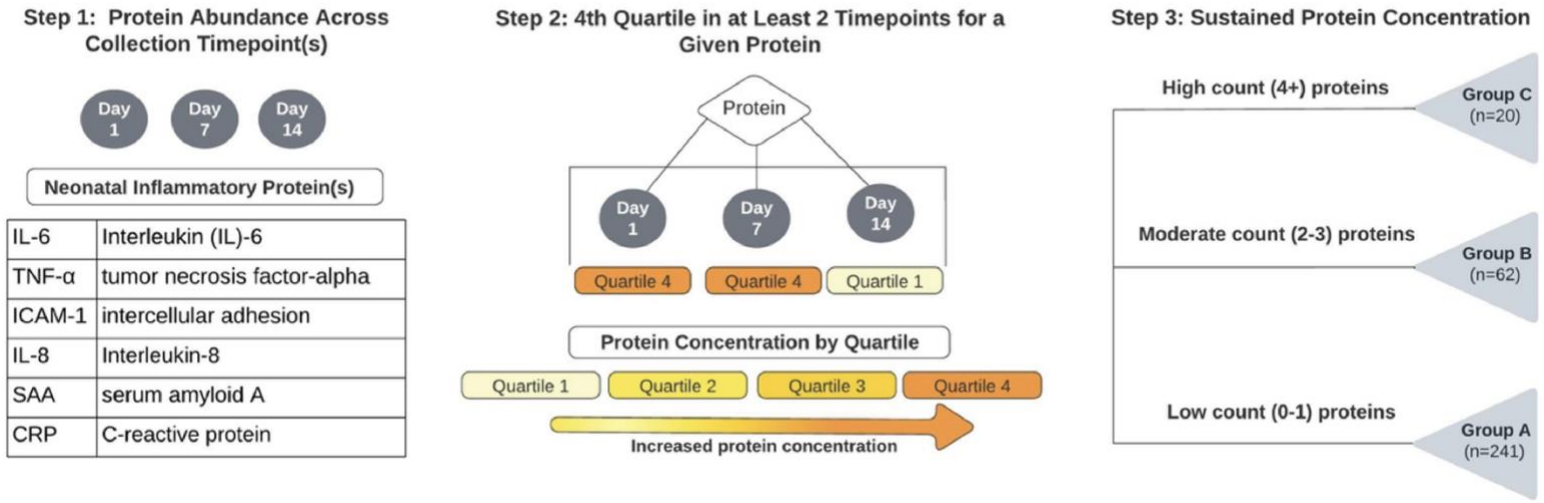
Protein	Protein Name	Associated Outcome	Neurodevelopment association in ELGAN
IL-6	Interleukin (IL) -6	Ventriculomegaly (when in the NICU) and microcephaly at age 2 year; latent profile analysis of cognition; indicator of brain damage	Leviton et al. (2016); (O'Shea et al. 2013; Korzeniewski et al. 2018; Leviton et al. 2018)
TNF-α	Tumor necrosis factor-alpha	Mental Development Index (MDI) of the Bayley-II < 55 at age 2	(Leviton et al. 2016; O'Shea et al. 2013; Korzeniewski et al. 2018)
ICAM -1	Intercellular adhesion molecule -1	Ventriculomegaly (when in the NICU) and microcephaly at age 2 year; indicator of brain damage; Mental Development Index (MDI) of the Bayley-II < 55 and Psychomotor Development Index (PDI) < 55 at age 2	(Leviton et al. 2016; O'Shea et al. 2013; Korzeniewski et al. 2018; Leviton et al. 2018)
IL -8	Interleukin (IL) -8	Ventriculomegaly (when in the NICU) and microcephaly at age 2 year; indicator of brain damage	(Leviton et al. 2016; Korzeniewski et al. 2018; Leviton et al. 2018)
SAA	Serum amyloid A	Mental Development Index (MDI) of the Bayley-II < 55 at age 2	(O'Shea et al. 2013; Korzeniewski et al. 2018)
CRP	C-reactive protein	Ventriculomegaly (when in the NICU) and microcephaly at age 2 year; Mental Development Index (MDI) of the Bayley-II < 55 at age 2	(O'Shea et al. 2013; Korzeniewski et al. 2018)

SUPPLEMENTAL FIGURE 3.1: Flowchart of participant selection.



Note. —Flowchart of participant selection from each of the 6 participating study sites. ELGAN-ECHO = Extremely Low Gestational Age Newborns–Environmental Influences on Child Health Outcomes, qMRI = quantitative MRI, TSE = turbo spin echo.

SUPPLEMENTAL FIGURE 3.2: Diagram of neonatal inflammation categorization. Neonates were classified into inflammation categories as low, moderate, or high.

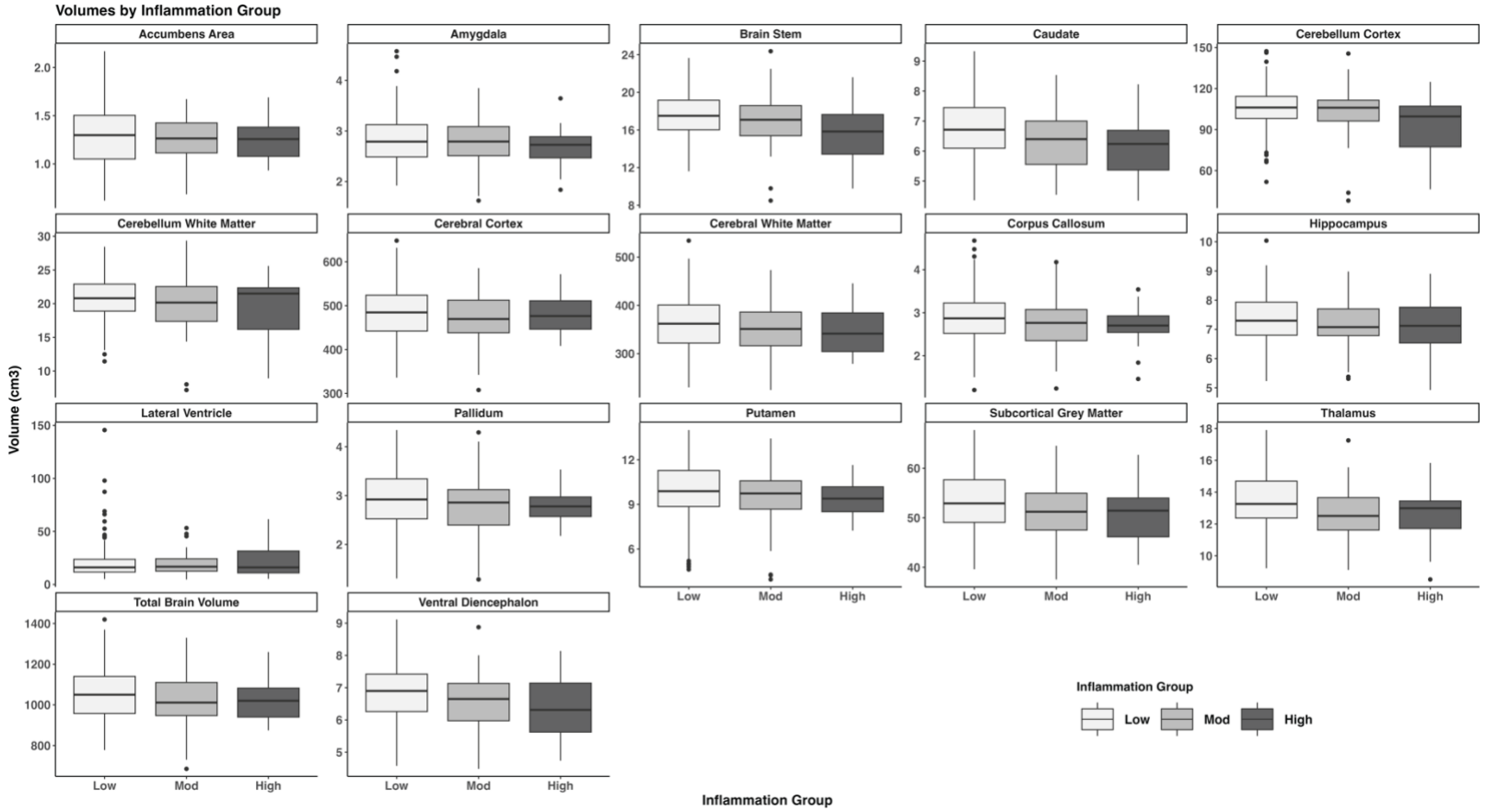


SUPPLEMENTAL TABLE 3.2: Adjusted brain volumes (cm^3) in ELGAN adolescents (N=323) overall and stratified by sex. The values below represent the mean brain volume proportions after adjusting for each participant's brain region by their total brain volume.

Brain Region	Overall (N=323)	female (N=241	male (N=62)	p.overall
Cerebral cortex	46.2 (1.84)	46.4 (1.82)	46.0 (1.83)	**0.04**
Cerebral white matter	34.3 (2.05)	34.1 (2.19)	34.6 (1.90)	**0.03**
Cerebellum cortex	10.1 (1.22)	10.1 (1.27)	10.1 (1.19)	0.91
Cerebellum white matter	1.98 (0.28)	2.03 (0.30)	1.93 (0.26)	**0.001**
Corpus callosum	0.27 (0.05)	0.28 (0.04)	0.26 (0.05)	**<0.001**
Brainstem	1.68 (0.18)	1.69 (0.20)	1.66 (0.17)	0.15
Lateral ventricle	1.92 (1.24)	1.88 (1.17)	1.95 (1.30)	0.62
Subcortical gray matter				
Ventral diencephalon	0.65 (0.06)	0.66 (0.06)	0.65 (0.05)	0.06
Thalamus	1.28 (0.10)	1.29 (0.10)	1.27 (0.10)	0.10
Putamen	0.94 (0.14)	0.93 (0.16)	0.95 (0.12)	0.32
Hippocampus	0.70 (0.07)	0.71 (0.07)	0.70 (0.07)	0.61
Caudate	0.64 (0.08)	0.65 (0.07)	0.63 (0.08)	**0.02**
Pallidum	0.28 (0.05)	0.28 (0.05)	0.27 (0.04)	0.25
Amygdala	0.27 (0.04)	0.27 (0.04)	0.28 (0.04)	**0.01**
Accumbens	0.12 (0.03)	0.12 (0.03)	0.12 (0.02)	0.92

Note. — Subcortical grey matter regions include ventral diencephalon, thalamus, putamen, hippocampus, caudate, pallidum, amygdala, and accumbens. Extremely Low Gestational Age Newborn (ELGAN).

SUPPLEMENTAL FIGURE 3.3: Box plots of volumes of 16 brain compartments and total brain volume (n=323) in relation to neonatal inflammation categories (low, moderate, or high.



Note. —Moderate inflammation is abbreviated as Mod.

SUPPLEMENTAL TABLE 3.3: Brain volumes (cm^3) as they relate to neonatal inflammation categories in ELGAN adolescent males. Neonates were classified into inflammation categories as low, moderate, or high.

Brain Region	Brain volume (low)	Brain volume (moderate)	Δ (low and moderate)	P value	Cohen's d	Brain volume (high)	Δ (low and high)	P value	Cohen's d
Cerebral cortex	503 (56.3)	500 (51.5)	3.12	0.25	0.06	486 (49.0)	17.08	0.20	0.31*
Cerebral white matter	383 (58.7)	371 (45.1)	11.7	0.11	0.21*	351 (49.0)	32.29	**0.02**	0.56 †
Cerebellum cortex	111 (13.5)	112 (11.6)	-0.97	0.43	-0.07	95.4 (24.3)	15.09	**0.002**	1.03
Cerebellum white matter	21.1 (3.22)	21.3 (3.40)	-0.25	0.73	-0.08	19.4 (5.06)	1.69	0.09	0.50 †
Corpus callosum	2.93 (0.55)	2.82 (0.66)	0.12	0.65	0.21*	2.49 (0.53)	0.44	**0.02**	0.81‡
Brainstem	18.3 (2.29)	18.0 (2.11)	0.31	0.59	0.14	15.9 (3.45)	2.40	**0.001**	1.00
Lateral ventricle	21.6 (17.2)	22.0 (11.3)	-0.33	0.62	-0.02	18.5 (9.55)	3.01	0.78	0.18
Subcortical gray matter	55.7 (6.16)	53.5 (5.43)	2.24	0.06	0.37*	52.3 (5.47)	3.42	0.07	0.56 †
Ventral diencephalon	7.12 (0.88)	6.88 (0.74)	0.24	0.19	0.28*	6.64 (0.87)	0.48	**0.03**	0.55 †
Thalamus	14.0 (1.78)	13.4 (1.44)	0.64	0.08	0.38*	13.0 (1.98)	1.05	0.07	0.58 †
Putamen	10.5 (1.79)	10.0 (1.47)	0.49	0.12	0.28*	9.56 (1.18)	0.94	**0.05**	0.53 †
Hippocampus	7.66 (0.83)	7.54 (0.79)	0.12	0.31	0.14	7.43 (0.87)	0.23	0.51	0.28*
Caudate	6.97 (1.03)	6.56 (1.08)	0.41	0.15	0.39*	6.70 (1.06)	0.27	0.62	0.26*
Pallidum	3.02 (0.60)	2.87 (0.63)	0.15	0.17	0.24*	2.91 (0.38)	0.11	0.47	0.19
Amygdala	3.00 (0.47)	2.97 (0.38)	0.03	0.33	0.08	2.91 (0.31)	0.09	0.41	0.20*
Accumbens	1.36 (0.30)	1.28 (0.25)	0.08	0.27	0.29*	1.29 (0.16)	0.07	0.71	0.25*
Total brain volume	1099 (129)	1083 (106)	15.7	0.26	0.13	1026(103)	73.31	**0.02**	0.58 †

Note. — Standard deviation (SD) is reported in parenthesis, Extremely Low Gestational Age Newborn (ELGAN). MRI-derived brain volumetrics (cm3) as it relates to neonatal inflammatory groups (e.g., low, moderate, or high). Bold p values denote associations that are significant following FDR correction (pFDR < 0.05). Effects are considered to be small when Cohe''s d is between 0.2 or 0.3 *, medium effects are assumed for values around 0.5†, and values of Cohe''s d larger than 0.8 ‡would depict large effects.

SUPPLEMENTAL TABLE 3.4: Brain volumes (cm^3) as they relate to neonatal inflammation categories in ELGAN adolescent females. Neonates were classified into inflammation categories as low, moderate, or high.

Brain Region	Brain volume (low)	Brain volume (moderate)	(low and moderate)	P value	Cohen's d	Brain volume (high)	(low and high)	P value	Cohen's d
Cerebral cortex	461 (48.2)	436 (57.4)	25.2	**0.02**	0.50†	477 (56.6)	-15.8	0.63	-0.32*
Cerebral white matter	340 (44.8)	322 (54.1)	17.7	**0.05**	0.38*	346 (51.2)	-7.26	0.76	-0.16
Cerebellum cortex	102 (12.7)	93.6 (18.4)	7.95	**0.05**	0.57†	88.6 (19.6)	12.91	**0.02**	0.97‡
Cerebellum white matter	21.5 (3.02)	18.6 (4.25)	1.84	**0.05**	0.56†	19.2 (4.99)	1.24	0.28	0.39*
Corpus callosum	2.81 (0.53)	2.71 (0.51)	0.10	0.42	0.18	2.94 (0.33)	-0.13	0.40	-0.25
Brainstem	17.0 (2.09)	15.9 (2.73)	1.09	0.05	0.49†	15.4 (3.49)	1.53	**0.05**	0.69†
Lateral ventricle	18.2 (11.8)	17.0 (8.27)	1.26	0.88	0.11	27.0 (18.4)	-8.81	0.07	-0.71†
Subcortical gray matter	50.8 (5.02)	48.1 (6.27)	2.67	0.06	0.51†	47.7 (5.51)	3.10	0.07	0.61†
Ventral diencephalon	6.59 (0.73)	6.20 (0.85)	0.40	**0.01**	0.53†	6.00 (0.82)	0.59	**0.03**	0.81‡
Thalamus	13.0 (1.40)	11.9 (1.49)	0.92	**0.004**	0.65†	12.4 (1.77)	0.42	0.31	0.29
Putamen	9.28 (1.60)	8.84 (2.14)	0.44	0.42	0.26*	9.01 (1.07)	0.27	0.58	0.17
Hippocampus	7.01 (0.72)	6.72 (0.71)	0.29	0.18	0.41*	6.58 (1.13)	0.43	**0.04**	0.58†
Caudate	6.51 (0.94)	6.21 (0.93)	0.29	0.42	0.31*	5.70 (0.99)	0.80	**0.02**	0.85‡
Pallidum	2.79 (0.56)	2.61 (0.74)	0.18	0.28	0.30*	2.64 (0.30)	0.15	0.24	0.28
Amygdala	2.62 (0.36)	2.59 (0.45)	0.04	0.75	0.09	2.42 (0.37)	0.20	**0.05**	0.56†
Accumbens	1.22 (0.28)	1.20 (0.27)	0.02	0.70	0.06†	1.20 (0.28)	0.02	0.98	0.06
Total brain tissue	997 (102)	938 (125)	58.4	**0.009**	0.55†	1009 (107)	-13.3	0.95	-0.13

Note. — Standard deviation (SD) is reported in parenthesis, Extremely Low Gestational Age Newborn (ELGAN). MRI-derived brain volumetrics (cm3) as it relates to neonatal inflammatory groups (e.g., low, moderate, or high). Bold p values denote associations that are significant following FDR correction (pFDR < 0.05). Effects are considered to be small when Cohe''s d is between 0.2 or 0.3 *, medium effects are assumed for values around 0.5†, and values of Cohe''s d larger than 0.8 ‡would depict large effects.

SUPPLEMENTAL TABLE 3.5: Brain volumes (cm^3) as they relate to neonatal inflammation categories in ELGAN subjects without a major neurologic disorder. Neonates were classified into inflammation categories as low, moderate, or high.

Brain Region	Brain volume (low)	Brain volume (moderate)	Δ (low and moderate)	P value	Cohen's d	Brain volume (high)	Δ (low and high)	P value	Cohen's d
Cerebral cortex	490 (57.0)	494(57.4)	-3.75	0.85	-0.07	490 (49.0)	0.54	0.74	0.01
Cerebral white matter	368 (58.0)	367 (51.1)	0.93	0.66	0.02	361 (45.0)	7.64	0.51	0.14
Cerebellum cortex	108 (12.7)	108 (14.0)	-0.16	0.87	-0.01	98.5 (17.0)	9.29	**0.01**	0.71†
Cerebellum white matter	21.3 (3.0)	21.2 (3.57)	0.13	0.92	0.04	20.7 (3.40)	0.57	0.65	0.19
Corpus callosum	2.92 (0.52)	2.87 (0.61)	0.05	0.86	0.10	2.75 (0.46)	0.17	0.45	0.33*
Brainstem	18.0 (2.31)	18.1 (2.40)	-0.16	0.67	-0.07	16.7 (2.94)	1.21	0.11	0.51†
Lateral ventricle	18.7 (12.2)	20.6 (11.9)	-1.88	0.31	-0.16	22.3 (14.1)	-3.59	0.16	-0.29*
Subcortical gray matter	54.0 (6.34)	53.1 (5.85)	0.88	0.42	0.14	51.5 (5.50)	2.42	0.22	0.39*
Ventral diencephalon	6.92 (0.85)	6.80 (0.80)	0.11	0.55	0.13	6.66 (0.79)	0.26	0.28	0.31*
Thalamus	13.6 (1.74)	13.2 (1.66)	0.44	0.11	0.25*	13.1 (147)	0.48	0.47	0.28*
Putamen	10.1(1.82)	9.94 (1.61)	0.15	0.56	0.08	9.47 (112)	0.62	0.16	0.35*
Hippocampus	7.40 (0.85)	7.33 (0.81)	0.07	0.41	0.09	7.27 (0.99)	0.12	0.87	0.14
Caudate	6.82 (0.98)	6.77 (0.91)	0.05	0.62	0.05	6.39 (1.11)	0.42	0.42	0.43*
Pallidum	2.99 (0.61)	2.94 (0.63)	0.05	0.66	0.08	2.82 (0.39)	0.16	0.41	0.27*
Amygdala	2.82 (0.45)	2.88 (0.48)	-0.06	0.78	-0.12	2.70 (0.40)	0.12	0.15	0.27*
Accumbens	1.32 (0.30)	1.30 (0.25)	0.02	0.59	0.07	1.24 (0.21)	0.08	0.84	0.27*
Total brain tissue	1064 (129)	1068 (122)	-4.49	0.87	-0.04	1048 (96)	15.7	0.52	0.13

Note. — Standard deviation (SD) is reported in parenthesis, Extremely Low Gestational Age Newborn (ELGAN). MRI-derived brain volumetrics (cm3) as it relates to neonatal inflammatory groups (e.g., low, moderate, or high). Bold p values denote associations that are significant following FDR correction (pFDR < 0.05). Effects are considered to be small when Cohen's d is between 0.2 or 0.3 *, medium effects are assumed for values around 0.5†, and values of Cohen's d larger than 0.8 ‡would depict large effects.

SUPPLEMENTAL TABLE 3.6: Brain volumes (cm^3) as they relate to neonatal inflammation categories in ELGAN subjects with a major neurologic disorder. Neonates were classified into inflammation categories as low, moderate, or high.

Brain Region	Brain volume (low)	Brain volume (moderate)	Δ (low and moderate)	P value	Cohen's d	Brain volume (high)	Δ (low and high)	P value	Cohen's d
Cerebral cortex	462 (52.2)	456 (54.0)	6.06	0.43	0.12	470 (55.1)	-7.84	0.30	-0.15
Cerebral white matter	343 (51.6)	340 (47.9)	3.52	0.46	0.07	321 (48.4)	22.4	0.11	0.44*
Cerebellum cortex	102 (15.8)	99.6 (20.3)	2.32	0.83	0.13	66.5 (24.5)	35.5	**<0.001**	2.17‡
Cerebellum white matter	19.6 (3.16)	19.3 (4.48)	0.30	0.65	0.08	13.3 (6.07)	6.33	**<0.001**	1.88‡
Corpus callosum	2.73 (0.63)	2.74 (0.60)	-0.02	0.92	-0.03	2.38 (0.61)	0.34	0.49	0.55†
Brainstem	16.8 (2.12)	16.1 (2.63)	0.62	0.13	0.27	11.6 (2.19)	5.12	**<0.001**	2.41‡
Lateral ventricle	120.0 (11.6)	19.8 (9.13)	0.23	0.73	0.02	26.8 (16.8)	-6.82	0.52	-0.57†
Subcortical gray matter	51.5 (5.66)	49.9 (5.62)	1.53	0.21	0.27	45.8 (6.39)	5.65	**0.012**	0.99‡
Ventral diencephalon	6.70 (0.86)	6.38 (0.84)	0.32	**0.01**	0.38*	5.37 (0.59)	1.33	**<0.001**	1.58‡
Thalamus	13.0 (1.59)	12.4 (1.41)	0.55	0.09	0.36*	11.3 (2.88)	1.69	**0.005**	1.01‡
Putamen	9.47 (1.64)	9.39 (1.70)	0.08	0.76	0.05	8.45 (0.93)	1.02	0.31	0.63†
Hippocampus	7.09 (0.76)	6.97 (0.84)	0.12	0.34	0.16	6.43 (1.22)	0.66	**0.006**	0.83‡
Caudate	6.54 (1.13)	6.11 (0.95)	0.43	0.07	0.40*	5.74 (1.37)	0.80	0.06	0.7†
Pallidum	2.75 (0.52)	2.76 (0.62)	-0.01	0.89	-0.02	2.64 (0.29)	0.11	0.62	0.21
Amygdala	2.74 (0.48)	2.75 (0.35)	-0.01	0.79	-0.02	2.75 (0.51)	-0.01	0.56	-0.01
Accumbens	1.23 (0.25)	1.22 (0.26)	0.02	0.67	0.06	1.35 (0.27)	-0.11	0.38	-0.45†
Total brain tissue	1006 (112)	988 (116)	17.8	0.28	0.16	942 (73.9)	63.6	**0.03**	0.57†

Note. — Standard deviation (SD) is reported in parenthesis, Extremely Low Gestational Age Newborn (ELGAN). MRI-derived brain volumetrics (cm3) as it relates to neonatal inflammatory groups (e.g., low, moderate, or high). Bold p values denote associations that are significant following FDR correction (pFDR < 0.05). Effects are considered to be small when Cohen's d is between 0.2 or 0.3 *, medium effects are assumed for values around 0.5†, and values of Cohen's d larger than 0.8 ‡would depict large effects.

REFERENCES

1. Gude, N. M.; Roberts, C. T.; Kalionis, B.; King, R. G., Growth and function of the normal human placenta. *Thromb Res* **2004,** *114* (5-6), 397-407.

2. Knöfler, M.; Haider, S.; Saleh, L.; Pollheimer, J.; Gamage, T.; James, J., Human placenta and trophoblast development: key molecular mechanisms and model systems. *Cell Mol Life Sci* **2019,** *76* (18), 3479-3496.

3. Díaz, P.; Powell, T. L.; Jansson, T., The role of placental nutrient sensing in maternal-fetal resource allocation. *Biol Reprod* **2014,** *91* (4), 82.

4. Vuppaladhadiam, L.; Lager, J.; Fiehn, O.; Weiss, S.; Chesney, M.; Hasdemir, B.; Bhargava, A., Human Placenta Buffers the Fetus from Adverse Effects of Perceived Maternal Stress. *Cells* **2021,** *10* (2).

5. Smith, A. L.; Bole Aldo, P.; Racicot, K. E., Chapter 17 - Placental regulation of immune functions. In *Reproductive Immunology*, Mor, G., Ed. Academic Press: 2021; pp 335-348.

6. Burton, G. J.; Fowden, A. L., The placenta: a multifaceted, transient organ. *Philos Trans R Soc Lond B Biol Sci* **2015,** *370* (1663), 20140066.

7. Myatt, L., Placental adaptive responses and fetal programming. *J Physiol* **2006,** *572* (Pt 1), 25-30.

8. Mandy, M.; Nyirenda, M., Developmental Origins of Health and Disease: the relevance to developing nations. *Int Health* **2018,** *10* (2), 66-70.

9. Behura, S. K.; Dhakal, P.; Kelleher, A. M.; Balboula, A.; Patterson, A.; Spencer, T. E., The brain-placental axis: Therapeutic and pharmacological relevancy to pregnancy. *Pharmacol Res* **2019,** *149*, 104468-104468.

10. Rosenfeld, C. S., The placenta-brain-axis. *J Neurosci Res* **2021,** *99* (1), 271-283.

11. Bonnin, A.; Levitt, P., Fetal, maternal, and placental sources of serotonin and new implications for developmental programming of the brain. *Neuroscience* **2011,** *197*, 1-7.

12. Bonnin, A.; Goeden, N.; Chen, K.; Wilson, M. L.; King, J.; Shih, J. C.; Blakely, R. D.; Deneris, E. S.; Levitt, P., A transient placental source of serotonin for the fetal forebrain. *Nature* **2011,** *472* (7343), 347-50.

13. Laurent, L.; Deroy, K.; St-Pierre, J.; Côté, F.; Sanderson, J. T.; Vaillancourt, C., Human placenta expresses both peripheral and neuronal isoform of tryptophan hydroxylase. *Biochimie* **2017,** *140*, 159-165.

14. Huang, W. Q.; Zhang, C. L.; Di, X. Y.; Zhang, R. Q., Studies on the localization of 5-hydroxytryptamine and its receptors in human placenta. *Placenta* **1998,** *19* (8), 655-61.

15. Muller, C. L.; Anacker, A. M.; Rogers, T. D.; Goeden, N.; Keller, E. H.; Forsberg, C. G.; Kerr, T. M.; Wender, C.; Anderson, G. M.; Stanwood, G. D.; Blakely, R. D.; Bonnin, A.; Veenstra-VanderWeele, J., Impact of Maternal Serotonin Transporter Genotype on Placental Serotonin, Fetal Forebrain Serotonin, and Neurodevelopment. *Neuropsychopharmacology* **2017,** *42* (2), 427-436.

16. Tuteja, G.; Chung, T.; Bejerano, G., Changes in the enhancer landscape during early placental development uncover a trophoblast invasion gene-enhancer network. *Placenta* **2016,** *37*, 45-55.

17. Konkel, L., The Brain before Birth: Using fMRI to Explore the Secrets of Fetal Neurodevelopment. *Environ Health Perspect* **2018,** *126* (11), 112001.

18. Stiles, J.; Jernigan, T. L., The Basics of Brain Development. *Neuropsychology Review* **2010,** *20* (4), 327-348.

19. Rahimi-Balaei, M.; Bergen, H.; Kong, J.; Marzban, H., Neuronal Migration During Development of the Cerebellum. *Frontiers in Cellular Neuroscience* **2018,** *12*.

20. Hwang, H. M.; Ku, R. Y.; Hashimoto-Torii, K., Prenatal Environment That Affects Neuronal Migration. *Frontiers in Cell and Developmental Biology* **2019,** *7*.

21. Kolb, B.; Gibb, R., Brain plasticity and behaviour in the developing brain. *J Can Acad Child Adolesc Psychiatry* **2011,** *20* (4), 265-76.

22. Reiss, A. L.; Abrams, M. T.; Singer, H. S.; Ross, J. L.; Denckla, M. B., Brain development, gender and IQ in children. A volumetric imaging study. *Brain* **1996,** *119 (Pt 5)*, 1763-74.

23. Iwasaki, N.; Hamano, K.; Okada, Y.; Horigome, Y.; Nakayama, J.; Takeya, T.; Takita, H.; Nose, T., Volumetric quantification of brain development using MRI. *Neuroradiology* **1997,** *39* (12), 841-6.

24. Bray, S.; Krongold, M.; Cooper, C.; Lebel, C., Synergistic Effects of Age on Patterns of White and Gray Matter Volume across Childhood and Adolescence. *eNeuro* **2015,** *2* (4).

25. Napso, T.; Yong, H. E. J.; Lopez-Tello, J.; Sferruzzi-Perri, A. N., The Role of Placental Hormones in Mediating Maternal Adaptations to Support Pregnancy and Lactation. *Front Physiol* **2018,** *9*.

26. Stern, C.; Schwarz, S.; Moser, G.; Cvitic, S.; Jantscher-Krenn, E.; Gauster, M.; Hiden, U., Placental Endocrine Activity: Adaptation and Disruption of Maternal Glucose Metabolism in Pregnancy and the Influence of Fetal Sex. *Int J Mol Sci* **2021,** *22* (23).

27. Rubenstein, J. L.; Merzenich, M. M., Model of autism: increased ratio of excitation/inhibition in key neural systems. *Genes Brain Behav* **2003,** *2* (5), 255-67.

28. Yarlagadda, A.; Acharya, G.; Kasaraneni, J.; Hampe, C. S.; Clayton, A. H., Placental Barrier and Autism Spectrum Disorders: The Roles of Prolactin and Dopamine in the Developing Fetal Brain-Part II. *Innov Clin Neurosci* **2019,** *16* (11-12), 36-39.

29. Yang, C. J.; Tan, H. P.; Du, Y. J., The developmental disruptions of serotonin signaling may involved in autism during early brain development. *Neuroscience* **2014,** *267*, 1-10.

30. Mao, J.; Jain, A.; Denslow, N. D.; Nouri, M. Z.; Chen, S.; Wang, T.; Zhu, N.; Koh, J.; Sarma, S. J.; Sumner, B. W.; Lei, Z.; Sumner, L. W.; Bivens, N. J.; Roberts, R. M.; Tuteja, G.; Rosenfeld, C. S., Bisphenol A and bisphenol S disruptions of the mouse placenta and potential effects on the placenta-brain axis. *Proc Natl Acad Sci U S A* **2020,** *117* (9), 4642-4652.

31. Sato, K., Placenta-derived hypo-serotonin situations in the developing forebrain cause autism. *Med Hypotheses* **2013,** *80* (4), 368-72.

32. Hranilovic, D.; Bujas-Petkovic, Z.; Vragovic, R.; Vuk, T.; Hock, K.; Jernej, B., Hyperserotonemia in adults with autistic disorder. *J Autism Dev Disord* **2007,** *37* (10), 1934-40.

33. Whitaker-Azmitia, P. M., Serotonin and brain development: role in human developmental diseases. *Brain Res Bull* **2001,** *56* (5), 479-85.

34. Carvey, P. M.; Chang, Q.; Lipton, J. W.; Ling, Z., Prenatal exposure to the bacteriotoxin lipopolysaccharide leads to long-term losses of dopamine neurons in offspring: a potential, new model of Parkinson's disease. *Front Biosci* **2003,** *8*, s826-37.

35. Goeden, N.; Velasquez, J.; Arnold, K. A.; Chan, Y.; Lund, B. T.; Anderson, G. M.; Bonnin, A., Maternal Inflammation Disrupts Fetal Neurodevelopment via Increased Placental Output of Serotonin to the Fetal Brain. *J Neurosci* **2016,** *36* (22), 6041-9.

36. Tomlinson, M. S.; Lu, K.; Stewart, J. R.; Marsit, C. J.; O'Shea, T. M.; Fry, R. C., Microorganisms in the Placenta: Links to Early-Life Inflammation and Neurodevelopment in Children. *Clin Microbiol Rev* **2019,** *32* (3).

37. Chen, C.; Lu, D.; Xue, L.; Ren, P.; Zhang, H.; Zhang, J., Association between Placental Inflammatory Pathology and Offspring Neurodevelopment at 8 Months and 4 and 7 Years of Age. *The Journal of Pediatrics* **2020,** *225*, 132-137.e2.

38. Goldstein, J. A.; Gallagher, K.; Beck, C.; Kumar, R.; Gernand, A. D., Maternal-Fetal Inflammation in the Placenta and the Developmental Origins of Health and Disease. *Frontiers in Immunology* **2020,** *11*.

39. Liu, C.; Chen, Y.; Zhao, D.; Zhang, J.; Zhang, Y., Association Between Funisitis and Childhood Intellectual Development: A Prospective Cohort Study. *Front Neurol* **2019,** *10*, 612.

40. Xiao, D.; Zhu, T.; Qu, Y.; Gou, X.; Huang, Q.; Li, X.; Mu, D., Maternal chorioamnionitis and neurodevelopmental outcomes in preterm and very preterm neonates: A meta-analysis. *PLoS One* **2018,** *13* (12), e0208302.

41. Straughen, J. K.; Misra, D. P.; Divine, G.; Shah, R.; Perez, G.; VanHorn, S.; Onbreyt, V.; Dygulska, B.; Schmitt, R.; Lederman, S.; Narula, P.; Salafia, C. M., The association between placental histopathology and autism spectrum disorder. *Placenta* **2017,** *57*, 183-188.

42. Pappas, A.; Kendrick, D. E.; Shankaran, S.; Stoll, B. J.; Bell, E. F.; Laptook, A. R.; Walsh, M. C.; Das, A.; Hale, E. C.; Newman, N. S.; Higgins, R. D., Chorioamnionitis and early childhood outcomes among extremely low-gestational-age neonates. *JAMA Pediatr* **2014,** *168* (2), 137-47.

43. Aouache, R.; Biquard, L.; Vaiman, D.; Miralles, F., Oxidative Stress in Preeclampsia and Placental Diseases. *Int J Mol Sci* **2018,** *19* (5).

44. Barron, A.; McCarthy, C. M.; O'Keeffe, G. W., Preeclampsia and Neurodevelopmental Outcomes: Potential Pathogenic Roles for Inflammation and Oxidative Stress? *Molecular Neurobiology* **2021,** *58*, 2734 - 2756.

45. Ji, L.; Brkić, J.; Liu, M.; Fu, G.; Peng, C.; Wang, Y. L., Placental trophoblast cell differentiation: physiological regulation and pathological relevance to preeclampsia. *Mol Aspects Med* **2013,** *34* (5), 981-1023.

46. Buonocore, G.; Tataranno, M. L.; Perrone, S., Oxidative Stress in Fetal and Neonatal Brain Development. *Pediatric Research* **2011,** *70* (5), 118-118.

47. Sze, Y.; Gill, A. C.; Brunton, P. J., Sex-dependent changes in neuroactive steroid concentrations in the rat brain following acute swim stress. *J Neuroendocrinol* **2018,** *30* (11), e12644-e12644.

48. Wilsterman, K.; Gotlieb, N.; Kriegsfeld, L. J.; Bentley, G. E., Pregnancy stage determines the effect of chronic stress on ovarian progesterone synthesis. *Am J Physiol Endocrinol Metab* **2018,** *315* (5), E987-e994.

49. Brunton, P. J.; Russell, J. A.; Hirst, J. J., Allopregnanolone in the brain: protecting pregnancy and birth outcomes. *Prog Neurobiol* **2014,** *113*, 106-36.

50. Nugent, B. M.; Bale, T. L., The omniscient placenta: Metabolic and epigenetic regulation of fetal programming. *Front Neuroendocrinol* **2015,** *39*, 28-37.

51. Bromer, C.; Marsit, C. J.; Armstrong, D. A.; Padbury, J. F.; Lester, B., Genetic and epigenetic variation of the glucocorticoid receptor (NR3C1) in placenta and infant neurobehavior. *Dev Psychobiol* **2013,** *55* (7), 673-83.

52. Conradt, E.; Lester, B. M.; Appleton, A. A.; Armstrong, D. A.; Marsit, C. J., The roles of DNA methylation of NR3C1 and 11β-HSD2 and exposure to maternal mood disorder in utero on newborn neurobehavior. *Epigenetics* **2013,** *8* (12), 1321-9.

53. Marsit, C. J.; Maccani, M. A.; Padbury, J. F.; Lester, B. M., Placental 11-beta hydroxysteroid dehydrogenase methylation is associated with newborn growth and a measure of neurobehavioral outcome. *PLoS One* **2012,** *7* (3), e33794.

54. Paquette, A. G.; Houseman, E. A.; Green, B. B.; Lesseur, C.; Armstrong, D. A.; Lester, B.; Marsit, C. J., Regions of variable DNA methylation in human placenta associated with newborn neurobehavior. *Epigenetics* **2016,** *11* (8), 603-13.

55. Lesseur, C.; Paquette, A. G.; Marsit, C. J., Epigenetic Regulation of Infant Neurobehavioral Outcomes. *Med Epigenet* **2014,** *2* (2), 71-79.

56. Meakin, C. J.; Martin, E. M.; Santos, H. P., Jr.; Mokrova, I.; Kuban, K.; O'Shea, T. M.; Joseph, R. M.; Smeester, L.; Fry, R. C., Placental CpG methylation of HPA-axis genes is associated with cognitive impairment at age 10 among children born extremely preterm. *Horm Behav* **2018,** *101*, 29-35.

57. Leviton, A.; Kuban, K. C.; Allred, E. N.; Fichorova, R. N.; O'Shea, T. M.; Paneth, N., Early postnatal blood concentrations of inflammation-related proteins and microcephaly two years later in infants born before the 28th post-menstrual week. *Early Hum Dev* **2011,** *87* (5), 325-30.

58. Bangma, J. T.; Kwiatkowski, E.; Psioda, M.; Santos, H. P., Jr.; Hooper, S. R.; Douglass, L.; Joseph, R. M.; Frazier, J. A.; Kuban, K. C. K.; O'Shea, T. M.; Fry, R. C., Early life antecedents of positive child health among 10-year-old children born extremely preterm. *Pediatr Res* **2019,** *86* (6), 758-765.

59. Tilley, S. K.; Martin, E. M.; Smeester, L.; Joseph, R. M.; Kuban, K. C. K.; Heeren, T. C.; Dammann, O. U.; O'Shea, T. M.; Fry, R. C., Placental CpG methylation of infants born extremely preterm predicts cognitive impairment later in life. *PLoS One* **2018,** *13* (3), e0193271.

60. Tilley, S. K.; Joseph, R. M.; Kuban, K. C. K.; Dammann, O. U.; O'Shea, T. M.; Fry, R. C., Genomic biomarkers of prenatal intrauterine inflammation in umbilical cord tissue predict later life neurological outcomes. *PloS one* **2017,** *12* (5), e0176953-e0176953.

61. Purisch, S. E.; Gyamfi-Bannerman, C., Epidemiology of preterm birth. *Semin Perinatol* **2017,** *41* (7), 387-391.

62. Taghizadeh, N.; Davidson, A.; Williams, K.; Story, D., Autism spectrum disorder (ASD) and its perioperative management. *Pediatric Anesthesia* **2015,** *25* (11), 1076-1084.

63. Barbaresi, W.; Cacia, J.; Friedman, S.; Fussell, J.; Hansen, R.; Hofer, J.; Roizen, N.; Stein, R. E.; Vanderbilt, D.; Sideridis, G., Clinician Diagnostic Certainty and the Role of the Autism Diagnostic Observation Schedule in Autism Spectrum Disorder Diagnosis in Young Children. *JAMA pediatrics* **2022,** *176* (12), 1233-1241.

64. Salari, N.; Rasoulpoor, S.; Rasoulpoor, S.; Shohaimi, S.; Jafarpour, S.; Abdoli, N.; Khaledi-Paveh, B.; Mohammadi, M., The global prevalence of autism spectrum disorder: a comprehensive systematic review and meta-analysis. *Italian Journal of Pediatrics* **2022,** *48* (1), 1-16.

65. Bailey, A.; Phillips, W.; Rutter, M., Autism: towards an integration of clinical, genetic, neuropsychological, and neurobiological perspectives. *J Child Psychol Psychiatry* **1996,** *37* (1), 89-126.

66. Trull, T. J.; Vergés, A.; Wood, P. K.; Jahng, S.; Sher, K. J., The structure of Diagnostic and Statistical Manual of Mental Disorders (text revision) personality disorder symptoms in a large national sample. *Personality Disorders: Theory, Research, and Treatment* **2012,** *3* (4), 355.

67. Chang, Y. S.; Chen, L. W.; Yu, T.; Lin, S. H.; Kuo, P. L., Preterm birth and weight-for-gestational age for risks of autism spectrum disorder and intellectual disability: A nationwide population-based cohort study. *J Formos Med Assoc* **2022**.

68. JOSEPH PIVEN, Understanding Other Minds: Perspectives From Autism. *American Journal of Psychiatry* **1995,** *152* (9), 1392-a-1393.

69. Courchesne, E.; Karns, C. M.; Davis, H. R.; Ziccardi, R.; Carper, R. A.; Tigue, Z. D.; Chisum, H. J.; Moses, P.; Pierce, K.; Lord, C.; Lincoln, A. J.; Pizzo, S.; Schreibman, L.; Haas, R. H.; Akshoomoff, N. A.; Courchesne, R. Y., Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* **2001,** *57* (2), 245-54.

70. Centers for Disease Control and Prevention (CDC) Addressing Gaps in Health Care for Individuals with Intellectual Disabilities. https://www.cdc.gov/grand-rounds/pp/2019/20191015-intellectual-disabilities.html (accessed January 24).

71. Vasudevan, P.; Suri, M., A clinical approach to developmental delay and intellectual disability. *Clin Med (Lond)* **2017,** *17* (6), 558-561.

72. Curtin, M.; Browne, J.; Staines, A.; Perry, I. J., The Early Development Instrument: an evaluation of its five domains using Rasch analysis. *BMC Pediatrics* **2016,** *16* (1), 10.

73. Gary Nelson Intellectual Disability & Global Developmental Delay. https://www.medicalhomeportal.org/diagnoses-and-conditions/intellectual-disability-and-global-developmental-delay#:~:text=The%20term%20%E2%80%9Cglobal%20developmental%20delay,and%20adaptive%20or%20functional%20components. (accessed January 24).

74. Weintraub, S.; Dikmen, S. S.; Heaton, R. K.; Tulsky, D. S.; Zelazo, P. D.; Bauer, P. J.; Carlozzi, N. E.; Slotkin, J.; Blitz, D.; Wallner-Allen, K.; Fox, N. A.; Beaumont, J. L.; Mungas, D.; Nowinski, C. J.; Richler, J.; Deocampo, J. A.; Anderson, J. E.; Manly, J. J.; Borosh, B.; Havlik, R.; Conway, K.; Edwards, E.; Freund, L.; King, J. W.; Moy, C.; Witt, E.; Gershon, R. C., Cognition assessment using the NIH Toolbox. *Neurology* **2013,** *80* (11 Suppl 3), S54-64.

75. Anderson, P. J., Neuropsychological outcomes of children born very preterm. *Semin Fetal Neonatal Med* **2014,** *19* (2), 90-6.

76. Johnson, S.; Marlow, N., Early and long-term outcome of infants born extremely preterm. *Arch Dis Child* **2017,** *102* (1), 97-102.

77. Burnett, A. C.; Scratch, S. E.; Anderson, P. J., Executive function outcome in preterm adolescents. *Early Hum Dev* **2013,** *89* (4), 215-20.

78. Mulder, H.; Pitchford, N. J.; Hagger, M. S.; Marlow, N., Development of executive function and attention in preterm children: a systematic review. *Dev Neuropsychol* **2009,** *34* (4), 393-421.

79. Taylor, H. G.; Clark, C. A., Executive function in children born preterm: Risk factors and implications for outcome. *Semin Perinatol* **2016,** *40* (8), 520-529.

80. Brydges, C. R.; Landes, J. K.; Reid, C. L.; Campbell, C.; French, N.; Anderson, M., Cognitive outcomes in children and adolescents born very preterm: a meta-analysis. *Dev Med Child Neurol* **2018,** *60* (5), 452-468.

81. Anderson, P. J.; De Luca, C. R.; Hutchinson, E.; Spencer-Smith, M. M.; Roberts, G.; Doyle, L. W., Attention problems in a representative sample of extremely preterm/extremely low birth weight children. *Dev Neuropsychol* **2011,** *36* (1), 57-73.

82. Wilson-Ching, M.; Molloy, C. S.; Anderson, V. A.; Burnett, A.; Roberts, G.; Cheong, J. L.; Doyle, L. W.; Anderson, P. J., Attention difficulties in a contemporary geographic cohort of adolescents born extremely preterm/extremely low birth weight. *J Int Neuropsychol Soc* **2013,** *19* (10), 1097-108.

83. Johnson, S.; Fawke, J.; Hennessy, E.; Rowell, V.; Thomas, S.; Wolke, D.; Marlow, N., Neurodevelopmental disability through 11 years of age in children born before 26 weeks of gestation. *Pediatrics* **2009,** *124* (2), e249-57.

84. Kerr-Wilson, C. O.; Mackay, D. F.; Smith, G. C.; Pell, J. P., Meta-analysis of the association between preterm delivery and intelligence. *J Public Health (Oxf)* **2012,** *34* (2), 209-16.

85. Orchinik, L. J.; Taylor, H. G.; Espy, K. A.; Minich, N.; Klein, N.; Sheffield, T.; Hack, M., Cognitive outcomes for extremely preterm/extremely low birth weight children in kindergarten. *J Int Neuropsychol Soc* **2011,** *17* (6), 1067-79.

86. Anderson, P.; Doyle, L. W., Neurobehavioral outcomes of school-age children born extremely low birth weight or very preterm in the 1990s. *Jama* **2003,** *289* (24), 3264-72.

87. Mulder, H.; Pitchford, N. J.; Marlow, N., Processing speed and working memory underlie academic attainment in very preterm children. *Arch Dis Child Fetal Neonatal Ed* **2010,** *95* (4), F267-72.

88. Rose, S. A.; Feldman, J. F., Memory and processing speed in preterm children at eleven years: a comparison with full-terms. *Child Dev* **1996,** *67* (5), 2005-21.

89. Marlow, N.; Wolke, D.; Bracewell, M. A.; Samara, M., Neurologic and developmental disability at six years of age after extremely preterm birth. *N Engl J Med* **2005,** *352* (1), 9-19.

90. Vohr, B., Speech and language outcomes of very preterm infants. *Semin Fetal Neonatal Med* **2014,** *19* (2), 78-83.

91. Barre, N.; Morgan, A.; Doyle, L. W.; Anderson, P. J., Language abilities in children who were very preterm and/or very low birth weight: a meta-analysis. *J Pediatr* **2011,** *158* (5), 766-774.e1.

92. Back, S. A.; Miller, S. P., Brain injury in premature neonates: A primary cerebral dysmaturation disorder? *Ann Neurol* **2014,** *75* (4), 469-86.

93. Hintz, S. R.; Vohr, B. R.; Bann, C. M.; Taylor, H. G.; Das, A.; Gustafson, K. E.; Yolton, K.; Watson, V. E.; Lowe, J.; DeAnda, M. E.; Ball, M. B.; Finer, N. N.; Van Meurs, K. P.; Shankaran, S.; Pappas, A.; Barnes, P. D.; Bulas, D.; Newman, J. E.; Wilson-Costello, D. E.; Heyne, R. J.; Harmon, H. M.; Peralta-Carcelen, M.; Adams-Chapman, I.; Duncan, A. F.; Fuller, J.; Vaucher, Y. E.; Colaizy, T. T.; Winter, S.; McGowan, E. C.; Goldstein, R. F.; Higgins, R. D., Preterm Neuroimaging and School-Age Cognitive Outcomes. *Pediatrics* **2018,** *142* (1).

94. Thompson, D. K.; Matthews, L. G.; Alexander, B.; Lee, K. J.; Kelly, C. E.; Adamson, C. L.; Hunt, R. W.; Cheong, J. L. Y.; Spencer-Smith, M.; Neil, J. J.; Seal, M. L.; Inder, T. E.; Doyle, L. W.; Anderson, P. J., Tracking regional brain growth up to age 13 in children born term and very preterm. *Nature Communications* **2020,** *11* (1), 696.

95. Nagy, Z.; Ashburner, J.; Andersson, J.; Jbabdi, S.; Draganski, B.; Skare, S.; Böhm, B.; Smedler, A. C.; Forssberg, H.; Lagercrantz, H., Structural correlates of preterm birth in the adolescent brain. *Pediatrics* **2009,** *124* (5), e964-72.

96. Ment, L. R.; Kesler, S.; Vohr, B.; Katz, K. H.; Baumgartner, H.; Schneider, K. C.; Delancy, S.; Silbereis, J.; Duncan, C. C.; Constable, R. T.; Makuch, R. W.; Reiss, A. L., Longitudinal brain volume changes in preterm and term control subjects during late childhood and adolescence. *Pediatrics* **2009,** *123* (2), 503-11.

97. Volpe, J. J., Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. *Lancet Neurol* **2009,** *8* (1), 110-24.

98. Inder, T. E.; Warfield, S. K.; Wang, H.; Hüppi, P. S.; Volpe, J. J., Abnormal cerebral structure is present at term in premature infants. *Pediatrics* **2005,** *115* (2), 286-94.

99. Matthews, L. G.; Inder, T. E.; Pascoe, L.; Kapur, K.; Lee, K. J.; Monson, B. B.; Doyle, L. W.; Thompson, D. K.; Anderson, P. J., Longitudinal Preterm Cerebellar Volume: Perinatal and Neurodevelopmental Outcome Associations. *Cerebellum* **2018,** *17* (5), 610-627.

100. Thompson, D. K.; Warfield, S. K.; Carlin, J. B.; Pavlovic, M.; Wang, H. X.; Bear, M.; Kean, M. J.; Doyle, L. W.; Egan, G. F.; Inder, T. E., Perinatal risk factors altering regional brain structure in the preterm infant. *Brain* **2007,** *130* (Pt 3), 667-77.

101. Alexander, B.; Kelly, C. E.; Adamson, C.; Beare, R.; Zannino, D.; Chen, J.; Murray, A. L.; Loh, W. Y.; Matthews, L. G.; Warfield, S. K.; Anderson, P. J.; Doyle, L. W.; Seal, M. L.; Spittle, A. J.; Cheong, J. L. Y.; Thompson, D. K., Changes in neonatal regional brain volume associated with preterm birth and perinatal factors. *Neuroimage* **2019,** *185*, 654-663.

102. Linsell, L.; Johnson, S.; Wolke, D.; O'Reilly, H.; Morris, J. K.; Kurinczuk, J. J.; Marlow, N., Cognitive trajectories from infancy to early adulthood following birth before 26 weeks of gestation: a prospective, population-based cohort study. *Arch Dis Child* **2018,** *103* (4), 363-370.

103. Doyle, L. W.; Cheong, J. L.; Burnett, A.; Roberts, G.; Lee, K. J.; Anderson, P. J., Biological and Social Influences on Outcomes of Extreme-Preterm/Low-Birth Weight Adolescents. *Pediatrics* **2015,** *136* (6), e1513-20.

104. Huttenlocher, P. R.; Dabholkar, A. S., Regional differences in synaptogenesis in human cerebral cortex. *J Comp Neurol* **1997,** *387* (2), 167-78.

105. Peterson, B. S., Brain imaging studies of the anatomical and functional consequences of preterm birth for human brain development. *Ann N Y Acad Sci* **2003,** *1008*, 219-37.

106. Kuban, K. C.; O'Shea, T. M.; Allred, E. N.; Fichorova, R. N.; Heeren, T.; Paneth, N.; Hirtz, D.; Dammann, O.; Leviton, A., The breadth and type of systemic inflammation and the risk of adverse neurological outcomes in extremely low gestation newborns. *Pediatr Neurol* **2015,** *52* (1), 42-8.

107. Kuban, K. C.; O'Shea, T. M.; Allred, E. N.; Paneth, N.; Hirtz, D.; Fichorova, R. N.; Leviton, A., Systemic inflammation and cerebral palsy risk in extremely preterm infants. *J Child Neurol* **2014,** *29* (12), 1692-8.

108. Leviton, A.; Allred, E. N.; Dammann, O.; Engelke, S.; Fichorova, R. N.; Hirtz, D.; Kuban, K. C.; Ment, L. R.; O'Shea T, M.; Paneth, N.; Shah, B.; Schreiber, M. D., Systemic inflammation, intraventricular hemorrhage, and white matter injury. *J Child Neurol* **2013,** *28* (12), 1637-45.

109. Leviton, A.; Fichorova, R. N.; O'Shea, T. M.; Kuban, K.; Paneth, N.; Dammann, O.; Allred, E. N., Two-hit model of brain damage in the very preterm newborn: small for gestational age and postnatal systemic inflammation. *Pediatr Res* **2013,** *73* (3), 362-70.

110. Leviton, A.; Kuban, K.; O'Shea, T. M.; Paneth, N.; Fichorova, R.; Allred, E. N.; Dammann, O., The relationship between early concentrations of 25 blood proteins and cerebral white matter injury in preterm newborns: the ELGAN study. *J Pediatr* **2011,** *158* (6), 897-903.e1-5.

111. O'Shea, T. M.; Joseph, R. M.; Kuban, K. C.; Allred, E. N.; Ware, J.; Coster, T.; Fichorova, R. N.; Dammann, O.; Leviton, A., Elevated blood levels of inflammation-related proteins are associated with an attention problem at age 24 mo in extremely preterm infants. *Pediatr Res* **2014,** *75* (6), 781-7.

112. O'Shea, T. M.; Allred, E. N.; Kuban, K. C.; Dammann, O.; Paneth, N.; Fichorova, R.; Hirtz, D.; Leviton, A., Elevated concentrations of inflammation-related proteins in postnatal blood predict severe developmental delay at 2 years of age in extremely preterm infants. *J Pediatr* **2012,** *160* (3), 395-401.e4.

113. O'Shea, T. M.; Allred, E. N.; Dammann, O.; Hirtz, D.; Kuban, K. C. K.; Paneth, N.; Leviton, A.; Investigators, E. s., The ELGAN study of the brain and related disorders in extremely low gestational age newborns. *Early human development* **2009,** *85* (11), 719-725.

114. Venkatesh, K. K.; Leviton, A.; Hecht, J. L.; Joseph, R. M.; Douglass, L. M.; Frazier, J. A.; Daniels, J. L.; Fry, R. C.; O'Shea, T. M.; Kuban, K. C. K., Histologic chorioamnionitis and risk of neurodevelopmental impairment at age 10 years among extremely preterm infants born before 28 weeks of gestation. *Am J Obstet Gynecol* **2020,** *223* (5), 745.e1-745.e10.

115. Pietschnig, J.; Penke, L.; Wicherts, J. M.; Zeiler, M.; Voracek, M., Meta-analysis of associations between human brain volume and intelligence differences: How strong are they and what do they mean? *Neurosci Biobehav Rev* **2015,** *57*, 411-32.

116. Cheong, J. L.; Anderson, P. J.; Roberts, G.; Burnett, A. C.; Lee, K. J.; Thompson, D. K.; Molloy, C.; Wilson-Ching, M.; Connelly, A.; Seal, M. L.; Wood, S. J.; Doyle, L. W., Contribution of brain size to IQ and educational underperformance in extremely preterm adolescents. *PLoS One* **2013,** *8* (10), e77475.

117. Kuban, K. C. K.; Jara, H.; O'Shea, T. M.; Heeren, T.; Joseph, R. M.; Fichorova, R. N.; Alshamrani, K.; Aakil, A.; Beaulieu, F.; Horn, M.; Douglass, L. M.; Frazier, J. A.; Hirtz, D.; Rollins, J. V.; Cochran, D.; Paneth, N., Association of Circulating Proinflammatory and Anti-inflammatory Protein Biomarkers in Extremely Preterm Born Children with Subsequent Brain Magnetic Resonance Imaging Volumes and Cognitive Function at Age 10 Years. *J Pediatr* **2019,** *210*, 81-90.e3.

118. O'Shea, T. M.; Kuban, K. C.; Allred, E. N.; Paneth, N.; Pagano, M.; Dammann, O.; Bostic, L.; Brooklier, K.; Butler, S.; Goldstein, D. J.; Hounshell, G.; Keller, C.; McQuiston, S.; Miller, A.; Pasternak, S.; Plesha-Troyke, S.; Price, J.; Romano, E.; Solomon, K. M.; Jacobson, A.; Westra, S.; Leviton, A., Neonatal cranial ultrasound lesions and developmental delays at 2 years of age among extremely low gestational age children. *Pediatrics* **2008,** *122* (3), e662-9.

119. Kuban, K. C. K.; Jara, H.; O'Shea, T. M.; Heeren, T.; Joseph, R. M.; Fichorova, R. N.; Alshamrani, K.; Aakil, A.; Beaulieu, F.; Horn, M.; Douglass, L. M.; Frazier, J. A.; Hirtz, D.; Rollins, J. V.; Cochran, D.; Paneth, N.; Investigators, E. S., Association of Circulating Proinflammatory and Anti-inflammatory Protein Biomarkers in Extremely Preterm Born Children with Subsequent Brain Magnetic Resonance Imaging Volumes and Cognitive Function at Age 10 Years. *J Pediatr* **2019,** *210*, 81-90 e3.

120. Dimes, M. o. Fighting Premature Birth: The Prematurity Campain. https://www.marchofdimes.org/mission/prematurity-campaign.aspx (accessed August 26, 2021).

121. Bhutta, A. T.; Cleves, M. A.; Casey, P. H.; Cradock, M. M.; Anand, K. J., Cognitive and behavioral outcomes of school-aged children who were born preterm: a meta-analysis. *Jama* **2002,** *288* (6), 728-37.

122. Wang, C.; Geng, H.; Liu, W.; Zhang, G., Prenatal, perinatal, and postnatal factors associated with autism: A meta-analysis. *Medicine* **2017,** *96* (18).

123. Hirschberger, R. G.; Kuban, K. C. K.; O'Shea, T. M.; Joseph, R. M.; Heeren, T.; Douglass, L. M.; Stafstrom, C. E.; Jara, H.; Frazier, J. A.; Hirtz, D.; Rollins, J. V.; Paneth, N.; Investigators, E. S., Co-occurrence and Severity of Neurodevelopmental Burden (Cognitive Impairment, Cerebral Palsy, Autism Spectrum Disorder, and Epilepsy) at Age Ten Years in Children Born Extremely Preterm. *Pediatr Neurol* **2018,** *79*, 45-52.

124. Back, S. A., White matter injury in the preterm infant: pathology and mechanisms. *Acta Neuropathol* **2017,** *134* (3), 331-349.

125. Dean, D. C., 3rd; Travers, B. G.; Adluru, N.; Tromp do, P. M.; Destiche, D. J.; Samsin, D.; Prigge, M. B.; Zielinski, B. A.; Fletcher, P. T.; Anderson, J. S.; Froehlich, A. L.; Bigler, E. D.; Lange, N.; Lainhart, J. E.; Alexander, A. L., Investigating the Microstructural Correlation of White Matter in Autism Spectrum Disorder. *Brain Connect* **2016,** *6* (5), 415-33.

126. Campbell, H.; Check, J.; Kuban, K. C. K.; Leviton, A.; Joseph, R. M.; Frazier, J. A.; Douglass, L. M.; Roell, K.; Allred, E. N.; Fordham, L. A.; Hooper, S. R.; Jara, H.; Paneth, N.; Mokrova, I.; Ru, H.; Santos, H. P., Jr.; Fry, R. C.; O'Shea, T. M., Neonatal Cranial Ultrasound Findings among Infants Born Extremely Preterm: Associations with Neurodevelopmental Outcomes at 10 Years of Age. *J Pediatr* **2021,** *237*, 197-205.e4.

127. Santos Jr, H. P.; Bhattacharya, A.; Joseph, R. M.; Smeester, L.; Kuban, K. C. K.; Marsit, C. J.; O'Shea, T. M.; Fry, R. C., Evidence for the placenta-brain axis: multi-omic kernel aggregation predicts intellectual and social impairment in children born extremely preterm. *Molecular Autism* **2020,** *11* (1), 97.

128. Korzeniewski, S. J.; Romero, R.; Cortez, J.; Pappas, A.; Schwartz, A. G.; Kim, C. J.; Kim, J. S.; Kim, Y. M.; Yoon, B. H.; Chaiworapongsa, T.; Hassan, S. S., A "multi-hit" model of neonatal white matter injury: cumulative contributions of chronic placental inflammation, acute fetal inflammation and postnatal inflammatory events. *J Perinat Med* **2014,** *42* (6), 731-43.

129. Yockey, L. J.; Iwasaki, A., Interferons and Proinflammatory Cytokines in Pregnancy and Fetal Development. *Immunity* **2018,** *49* (3), 397-412.

130. Lester, B. M.; Marsit, C. J., Epigenetic mechanisms in the placenta related to infant neurodevelopment. *Epigenomics* **2018,** *10* (3), 321-333.

131. Van Steenwinckel, J.; Schang, A. L.; Sigaut, S.; Chhor, V.; Degos, V.; Hagberg, H.; Baud, O.; Fleiss, B.; Gressens, P., Brain damage of the preterm infant: new insights into the role of inflammation. *Biochem Soc Trans* **2014,** *42* (2), 557-63.

132. Santos, H. P., Jr.; Bhattacharya, A.; Martin, E. M.; Addo, K.; Psioda, M.; Smeester, L.; Joseph, R. M.; Hooper, S. R.; Frazier, J. A.; Kuban, K. C.; O'Shea, T. M.; Fry, R. C., Epigenome-wide DNA methylation in placentas from preterm infants: association with maternal socioeconomic status. *Epigenetics* **2019,** *14* (8), 751-765.

133. Addo, K. A.; Bulka, C.; Dhingra, R.; Santos, H. P., Jr; Smeester, L.; O'Shea, T. M.; Fry, R. C., Acetaminophen use during pregnancy and DNA methylation in the placenta of the extremely low gestational age newborn (ELGAN) cohort. *Environmental Epigenetics* **2019,** *5* (2).

134. Eaves, L. A.; Phookphan, P.; Rager, J. E.; Bangma, J.; Santos, H. P., Jr.; Smeester, L.; O'Shea, T. M.; Fry, R. C., A role for microRNAs in the epigenetic control of sexually dimorphic gene expression in the human placenta. *Epigenomics* **2020,** *12* (17), 1543-1558.

135. Hodyl, N. A.; Stark, M. J.; Osei-Kumah, A.; Clifton, V. L., Prenatal programming of the innate immune response following in utero exposure to inflammation: a sexually dimorphic process? *Expert Rev Clin Immunol* **2011,** *7* (5), 579-92.

136. Stark, M. J.; Hodyl, N. A.; Wright, I. M.; Clifton, V. L., Influence of sex and glucocorticoid exposure on preterm placental pro-oxidant-antioxidant balance. *Placenta* **2011,** *32* (11), 865-70.

137. Saben, J.; Zhong, Y.; McKelvey, S.; Dajani, N. K.; Andres, A.; Badger, T. M.; Gomez-Acevedo, H.; Shankar, K., A comprehensive analysis of the human placenta transcriptome. *Placenta* **2014,** *35* (2), 125-31.

138. Kuban, K.; Adler, I.; Allred, E. N.; Batton, D.; Bezinque, S.; Betz, B. W.; Cavenagh, E.; Durfee, S.; Ecklund, K.; Feinstein, K.; Fordham, L. A.; Hampf, F.; Junewick, J.; Lorenzo, R.; McCauley, R.; Miller, C.; Seibert, J.; Specter, B.; Wellman, J.; Westra, S.; Leviton, A., Observer variability assessing US scans of the preterm brain: the ELGAN study. *Pediatr Radiol* **2007,** *37* (12), 1201-1208.

139. O'Shea, T. M.; Volberg, F.; Dillard, R. G., Reliability of interpretation of cranial ultrasound examinations of very low-birthweight neonates. *Dev Med Child Neurol* **1993,** *35* (2), 97-101.

140. Love, M. I.; Huber, W.; Anders, S., Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology* **2014,** *15* (12), 550.

141. Leek, J. T.; Storey, J. D., Capturing heterogeneity in gene expression studies by surrogate variable analysis. *PLoS Genet* **2007,** *3* (9), 1724-35.

142. Ioannidis, J. P. A.; Powe, N. R.; Yancy, C., Recalibrating the Use of Race in Medical Research. *JAMA* **2021,** *325* (7), 623-624.

143. IPA, Q. *Bioinformatics Software and Services*, Ingenuity Systems.

144. Szklarczyk, D.; Franceschini, A.; Wyder, S.; Forslund, K.; Heller, D.; Huerta-Cepas, J.; Simonovic, M.; Roth, A.; Santos, A.; Tsafou, K. P.; Kuhn, M.; Bork, P.; Jensen, L. J.; von Mering, C., STRING v10: protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res* **2015,** *43* (Database issue), D447-52.

145. Clark, J.; Eaves, L. A.; Gaona, A. R.; Santos, H. P.; Smeester, L.; Bangma, J. T.; Rager, J. E.; O'Shea, T. M.; Fry, R. C., Pre-pregnancy BMI-associated miRNA and mRNA expression signatures in the placenta highlight a sexually-dimorphic response to maternal underweight status. *Sci Rep* **2021,** *11* (1), 15743.

146. Abrahams, B. S.; Arking, D. E.; Campbell, D. B.; Mefford, H. C.; Morrow, E. M.; Weiss, L. A.; Menashe, I.; Wadkins, T.; Banerjee-Basu, S.; Packer, A., SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). *Mol Autism* **2013,** *4* (1), 36.

147. NCBI, Geo gene expression omnibus. Placental genomicand epigenomic signatures in infants borns at extremely lowgestational age. 2020.

148. Kuban, K. C.; Allred, E. N.; O'Shea, T. M.; Paneth, N.; Pagano, M.; Dammann, O.; Leviton, A.; Du Plessis, A.; Westra, S. J.; Miller, C. R.; Bassan, H.; Krishnamoorthy, K.; Junewick, J.; Olomu, N.; Romano, E.; Seibert, J.; Engelke, S.; Karna, P.; Batton, D.; O'Connor, S. E.; Keller, C. E., Cranial ultrasound lesions in the NICU predict cerebral palsy at age 2 years in children born at extremely low gestational age. *J Child Neurol* **2009,** *24* (1), 63-72.

149. Hirano, T., IL-6 in inflammation, autoimmunity and cancer. *International Immunology* **2020,** *33* (3), 127-148.

150. Saxton, R. A.; Sabatini, D. M., mTOR Signaling in Growth, Metabolism, and Disease. *Cell* **2017,** *168* (6), 960-976.

151. Sharp, A. N.; Heazell, A. E. P.; Crocker, I. P.; Mor, G., Placental apoptosis in health and disease. *Am J Reprod Immunol* **2010,** *64* (3), 159-169.

152. Marks, K.; Vincent, A.; Coutinho, E., Maternal-Autoantibody-Related (MAR) Autism: Identifying Neuronal Antigens and Approaching Prospects for Intervention. *J Clin Med* **2020,** *9* (8), 2564.

153. Raguema, N.; Moustadraf, S.; Bertagnolli, M., Immune and Apoptosis Mechanisms Regulating Placental Development and Vascularization in Preeclampsia. *Front Physiol* **2020,** *11*, 98-98.

154. Mendrysa, S. M.; Ghassemifar, S.; Malek, R., p53 in the CNS: Perspectives on Development, Stem Cells, and Cancer. *Genes Cancer* **2011,** *2* (4), 431-42.

155. Kulikov, R.; Boehme, K. A.; Blattner, C., Glycogen synthase kinase 3-dependent phosphorylation of Mdm2 regulates p53 abundance. *Mol Cell Biol* **2005,** *25* (16), 7170-80.

156. Velegrakis, A.; Sfakiotaki, M.; Sifakis, S., Human placental growth hormone in normal and abnormal fetal growth. *Biomed Rep* **2017,** *7* (2), 115-122.

157. Hansen-Pupp, I.; Hövel, H.; Löfqvist, C.; Hellström-Westas, L.; Fellman, V.; Hüppi, P. S.; Hellström, A.; Ley, D., Circulatory insulin-like growth factor-I and brain volumes in relation to neurodevelopmental outcome in very preterm infants. *Pediatric Research* **2013,** *74* (5), 564-569.

158. Kassotaki, I.; Valsamakis, G.; Mastorakos, G.; Grammatopoulos, D. K., Placental CRH as a Signal of Pregnancy Adversity and Impact on Fetal Neurodevelopment. *Frontiers in Endocrinology* **2021,** *12*.

159. McCarthy, M. M., Estradiol and the developing brain. *Physiol Rev* **2008,** *88* (1), 91-124.

160. Vacher, C.-M.; Lacaille, H.; O'Reilly, J. J.; Salzbank, J.; Bakalar, D.; Sebaoui, S.; Liere, P.; Clarkson-Paredes, C.; Sasaki, T.; Sathyanesan, A.; Kratimenos, P.; Ellegood, J.; Lerch, J. P.; Imamura, Y.; Popratiloff, A.; Hashimoto-Torii, K.; Gallo, V.; Schumacher, M.; Penn, A. A., Placental endocrine function shapes cerebellar development and social behavior. *Nature Neuroscience* **2021,** *24* (10), 1392-1401.

161. Kawamura, K.; Kawamura, N.; Sato, W.; Fukuda, J.; Kumagai, J.; Tanaka, T., Brain-Derived Neurotrophic Factor Promotes Implantation and Subsequent Placental Development by Stimulating Trophoblast Cell Growth and Survival. *Endocrinology* **2009,** *150* (8), 3774-3782.

162. Gao, L.; Lv, C.; Xu, C.; Li, Y.; Cui, X.; Gu, H.; Ni, X., Differential Regulation of Glucose Transporters Mediated by CRH Receptor Type 1 and Type 2 in Human Placental Trophoblasts. *Endocrinology* **2012,** *153* (3), 1464-1471.

163. Lu, B.; Nagappan, G.; Lu, Y., BDNF and synaptic plasticity, cognitive function, and dysfunction. *Handb Exp Pharmacol* **2014,** *220*, 223-50.

164. Ciambra, G.; Arachi, S.; Protano, C.; Cellitti, R.; Caoci, S.; Di Biasi, C.; Gualdi, G.; De Curtis, M., Accuracy of transcranial ultrasound in the detection of mild white matter lesions in newborns. *Neuroradiol J* **2013,** *26* (3), 284-289.

165. Kuban, K. C.; Allred, E. N.; O'Shea, T. M.; Paneth, N.; Pagano, M.; Dammann, O.; Leviton, A.; Du Plessis, A.; Westra, S. J.; Miller, C. R.; Bassan, H.; Krishnamoorthy, K.; Junewick, J.; Olomu, N.; Romano, E.; Seibert, J.; Engelke, S.; Karna, P.; Batton, D.; O'Connor, S. E.; Keller, C. E.; investigators, E. s., Cranial ultrasound lesions in the NICU predict cerebral palsy at age 2 years in children born at extremely low gestational age. *J Child Neurol* **2009,** *24* (1), 63-72.

166. Campbell, H.; Check, J.; Kuban, K. C. K.; Leviton, A.; Joseph, R. M.; Frazier, J. A.; Douglass, L. M.; Roell, K.; Allred, E. N.; Fordham, L. A.; Hooper, S. R.; Jara, H.; Paneth, N.; Mokrova, I.; Ru, H.; Santos, H. P., Jr.; Fry, R. C.; O'Shea, T. M., Neonatal Cranial Ultrasound Findings among Infants Born Extremely Preterm: Associations with Neurodevelopmental Outcomes at 10 Years of Age. *J Pediatr* **2021**.

167. National Center for Health Statistics, National health interview survey, 2020 survey description. *National Center for Health Statistics* **2021**.

168. National Center for Health Statistics, National health interview survey, 2019 survey description. *National Center for Health Statistics* **2020**.

169. Freedman, A. N.; Eaves, L. A.; Rager, J. E.; Gavino-Lopez, N.; Smeester, L.; Bangma, J.; Santos, H. P.; Joseph, R. M.; Kuban, K. C.; O'Shea, T. M.; Fry, R. C., The placenta epigenome–brain axis: placental epigenomic and transcriptomic responses that preprogram cognitive impairment. *Epigenomics* **2022,** *14* (15), 897-911.

170. Karolis, V. R.; Froudist-Walsh, S.; Kroll, J.; Brittain, P. J.; Tseng, C.-E. J.; Nam, K.-W.; Reinders, A. A. T. S.; Murray, R. M.; Williams, S. C. R.; Thompson, P. M.; Nosarti, C., Volumetric grey matter alterations in adolescents and adults born very preterm suggest accelerated brain maturation. *NeuroImage* **2017,** *163*, 379-389.

171. Inder, T. E.; Anderson, N. J.; Spencer, C.; Wells, S.; Volpe, J. J., White matter injury in the premature infant: a comparison between serial cranial sonographic and MR findings at term. *AJNR Am J Neuroradiol* **2003,** *24* (5), 805-9.

172. O'Shea, T. M.; Allred, E. N.; Dammann, O.; Hirtz, D.; Kuban, K. C.; Paneth, N.; Leviton, A., The ELGAN study of the brain and related disorders in extremely low gestational age newborns. *Early Hum Dev* **2009,** *85* (11), 719-25.

173. Sahay, A.; Kale, A.; Joshi, S., Role of neurotrophins in pregnancy and offspring brain development. *Neuropeptides* **2020,** *83*, 102075.

174. Giedd, J. N., Structural magnetic resonance imaging of the adolescent brain. *Ann N Y Acad Sci* **2004,** *1021*, 77-85.

175. Bangma, J. T.; Hartwell, H.; Santos, H. P.; O'Shea, T. M.; Fry, R. C., Placental programming, perinatal inflammation, and neurodevelopment impairment among those born extremely preterm. *Pediatric Research* **2021,** *89* (2), 326-335.

176. Hsiao, E. Y.; Patterson, P. H., Placental regulation of maternal-fetal interactions and brain development. *Dev Neurobiol* **2012,** *72* (10), 1317-26.

177. Santos, H. P.; Bhattacharya, A.; Martin, E. M.; Addo, K.; Psioda, M.; Smeester, L.; Joseph, R. M.; Hooper, S. R.; Frazier, J. A.; Kuban, K. C.; O'Shea, T. M.; Fry, R. C., Epigenome-wide DNA methylation in placentas from preterm infants: association with maternal socioeconomic status. *Epigenetics* **2019,** *14* (8), 751-765.

178. Joseph, R. M.; Hooper, S. R.; Heeren, T.; Santos Jr, H. P.; Frazier, J. A.; Venuti, L.; Foley, A.; Rollins, C. K.; Kuban, K. C. K.; Fry, R. C.; O'Shea, T. M.; Investigators, f. t. E. S., Maternal social risk, gestational age at delivery, and cognitive outcomes among adolescents born extremely preterm. *Paediatric and Perinatal Epidemiology* **2022,** *36* (5), 654-664.

179. O'Shea, T. M.; Register, H. M.; Joe, X. Y.; Jensen, E. T.; Joseph, R. M.; Kuban, K. C.; Frazier, J. A.; Washburn, L.; Belfort, M.; South, A. M., Growth During Infancy After Extremely Preterm Birth: Associations with Later Neurodevelopmental and Health Outcomes. *The Journal of Pediatrics* **2023,** *252*, 40-47. e5.

180. Akshoomoff, N.; Newman, E.; Thompson, W. K.; McCabe, C.; Bloss, C. S.; Chang, L.; Amaral, D. G.; Casey, B. J.; Ernst, T. M.; Frazier, J. A.; Gruen, J. R.; Kaufmann, W. E.; Kenet, T.; Kennedy, D. N.; Libiger, O.; Mostofsky, S.; Murray, S. S.; Sowell, E. R.; Schork, N.; Dale, A. M.; Jernigan, T. L., The NIH Toolbox Cognition Battery: results from a large normative developmental sample (PING). *Neuropsychology* **2014,** *28* (1), 1-10.

181. Wechsler, D., Wechsler Abbreviated Scale of Intelligence–Second Edition (WASI-II). **2011**.

182. Akshoomoff, N.; Beaumont, J. L.; Bauer, P. J.; Dikmen, S. S.; Gershon, R. C.; Mungas, D.; Slotkin, J.; Tulsky, D.; Weintraub, S.; Zelazo, P. D., VIII. NIH Toolbox Cognition Battery (CB): composite scores of crystallized, fluid, and overall cognition. *Monographs of the Society for Research in Child Development* **2013,** *78* (4), 119-132.

183. Muthén, L.; Muthén, B., Mplus User's Guide, 7th edn, 1998–2012. *Muthén & Muthén: Los Angeles, CA* **2012**.

184. Heeren, T.; Joseph, R. M.; Allred, E. N.; O'Shea, T. M.; Leviton, A.; Kuban, K. C. K., Cognitive functioning at the age of 10 years among children born extremely preterm: a latent profile approach. *Pediatr Res* **2017,** *82* (4), 614-619.

185. Chung, P. J.; Opipari, V. P.; Koolwijk, I., Executive function and extremely preterm children. *Pediatric Research* **2017,** *82* (4), 565-566.

186. R Core Team *R: A language and environment for statistical computing.* , R Foundation for Statistical Computing: Vienna, Austria, 2021.

187. Jassal, B.; Matthews, L.; Viteri, G.; Gong, C.; Lorente, P.; Fabregat, A.; Sidiropoulos, K.; Cook, J.; Gillespie, M.; Haw, R., The reactome pathway knowledgebase. *Nucleic acids research* **2020,** *48* (D1), D498-D503.

188. Marable, C. A.; Roell, K.; Kuban, K.; O'Shea, T. M.; Fry, R. C., Placental transcriptional signatures associated with cerebral white matter damage in the neonate. *Frontiers in Neuroscience* **2022,** *16*.

189. Swartz, J. R.; Carranza, A. F.; Tully, L. M.; Knodt, A. R.; Jiang, J.; Irwin, M. R.; Hostinar, C. E., Associations between peripheral inflammation and resting state functional connectivity in adolescents. *Brain Behav Immun* **2021,** *95*, 96-105.

190. Leviton, A.; Allred, E. N.; Fichorova, R. N.; O'Shea, T. M.; Fordham, L. A.; Kuban, K. K. C.; Dammann, O., Circulating biomarkers in extremely preterm infants associated with ultrasound indicators of brain damage. *Eur J Paediatr Neurol* **2018,** *22* (3), 440-450.

191. Bennet, L.; Dhillon, S.; Lear, C. A.; van den Heuij, L.; King, V.; Dean, J. M.; Wassink, G.; Davidson, J. O.; Gunn, A. J., Chronic inflammation and impaired development of the preterm brain. *Journal of Reproductive Immunology* **2018,** *125*, 45-55.

192. Dammann, O.; Leviton, A., Intermittent or sustained systemic inflammation and the preterm brain. *Pediatric Research* **2014,** *75* (3), 376-380.

193. Tam, E. W.; Ferriero, D. M.; Xu, D.; Berman, J. I.; Vigneron, D. B.; Barkovich, A. J.; Miller, S. P., Cerebellar development in the preterm neonate: effect of supratentorial brain injury. *Pediatr Res* **2009,** *66* (1), 102-6.

194. Mercuri, E.; He, J.; Curati, W. L.; Dubowitz, L. M.; Cowan, F. M.; Bydder, G. M., Cerebellar infarction and atrophy in infants and children with a history of premature birth. *Pediatr Radiol* **1997,** *27* (2), 139-43.

195. Messerschmidt, A.; Brugger, P. C.; Boltshauser, E.; Zoder, G.; Sterniste, W.; Birnbacher, R.; Prayer, D., Disruption of cerebellar development: potential complication of extreme prematurity. *AJNR Am J Neuroradiol* **2005,** *26* (7), 1659-67.

196. Dresselhaus, E. C.; Meffert, M. K., Cellular Specificity of NF-κB Function in the Nervous System. *Front Immunol* **2019,** *10*, 1043.

197. Valentini, N. C.; de Borba, L. S.; Panceri, C.; Smith, B. A.; Procianoy, R. S.; Silveira, R. C., Early Detection of Cognitive, Language, and Motor Delays for Low-Income Preterm Infants: A Brazilian Cohort Longitudinal Study on Infant Neurodevelopment and Maternal Practice. *Frontiers in Psychology* **2021,** *12*.

198. van Beek, P. E.; van der Horst, I. E.; Wetzer, J.; van Baar, A. L.; Vugs, B.; Andriessen, P., Developmental Trajectories in Very Preterm Born Children Up to 8 Years: A Longitudinal Cohort Study. *Front Pediatr* **2021,** *9*, 672214.

199. Joseph, R. M.; O'Shea, T. M.; Allred, E. N.; Heeren, T.; Hirtz, D.; Jara, H.; Leviton, A.; Kuban, K. C., Neurocognitive and Academic Outcomes at Age 10 Years of Extremely Preterm Newborns. *Pediatrics* **2016,** *137* (4).

200. Douglass, L. M.; Heeren, T. C.; Stafstrom, C. E.; DeBassio, W.; Allred, E. N.; Leviton, A.; O'Shea, T. M.; Hirtz, D.; Rollins, J.; Kuban, K., Cumulative Incidence of Seizures and Epilepsy in Ten-Year-Old Children Born Before 28 Weeks' Gestation. *Pediatr Neurol* **2017,** *73*, 13-19.

201. Kuban, K. C.; Allred, E. N.; O'Shea, M.; Paneth, N.; Pagano, M.; Leviton, A., An algorithm for identifying and classifying cerebral palsy in young children. *J Pediatr* **2008,** *153* (4), 466-72.

202. Volpe, J. J., Dysmaturation of Premature Brain: Importance, Cellular Mechanisms, and Potential Interventions. *Pediatr Neurol* **2019,** *95*, 42-66.

203. Nosarti, C.; Nam, K. W.; Walshe, M.; Murray, R. M.; Cuddy, M.; Rifkin, L.; Allin, M. P., Preterm birth and structural brain alterations in early adulthood. *Neuroimage Clin* **2014,** *6*, 180-91.

204. de Kieviet, J. F.; Zoetebier, L.; van Elburg, R. M.; Vermeulen, R. J.; Oosterlaan, J., Brain development of very preterm and very low-birthweight children in childhood and adolescence: a meta-analysis. *Dev Med Child Neurol* **2012,** *54* (4), 313-23.

205. Kelly, C. E.; Thompson, D. K.; Cheong, J. L.; Chen, J.; Olsen, J. E.; Eeles, A. L.; Walsh, J. M.; Seal, M. L.; Anderson, P. J.; Doyle, L. W.; Spittle, A. J., Brain structure and neurological and behavioural functioning in infants born preterm. *Dev Med Child Neurol* **2019,** *61* (7), 820-831.

206. Hintz, S. R.; Vohr, B. R.; Bann, C. M.; Taylor, H. G.; Das, A.; Gustafson, K. E.; Yolton, K.; Watson, V. E.; Lowe, J.; DeAnda, M. E.; Ball, M. B.; Finer, N. N.; Van Meurs, K. P.; Shankaran, S.; Pappas, A.; Barnes, P. D.; Bulas, D.; Newman, J. E.; Wilson-Costello, D. E.; Heyne, R. J.; Harmon, H. M.; Peralta-Carcelen, M.; Adams-Chapman, I.; Duncan, A. F.; Fuller, J.; Vaucher, Y. E.; Colaizy, T. T.; Winter, S.; McGowan, E. C.; Goldstein, R. F.; Higgins, R. D.; Health, S. s. g. o. t. E. K. S. N. I. o. C.; Human Development Neonatal Research, N., Preterm Neuroimaging and School-Age Cognitive Outcomes. *Pediatrics* **2018,** *142* (1).

207. Brossard-Racine, M.; du Plessis, A. J.; Limperopoulos, C., Developmental cerebellar cognitive affective syndrome in ex-preterm survivors following cerebellar injury. *Cerebellum* **2015,** *14* (2), 151-64.

208. Taylor, H. G.; Filipek, P. A.; Juranek, J.; Bangert, B.; Minich, N.; Hack, M., Brain volumes in adolescents with very low birth weight: effects on brain structure and associations with neuropsychological outcomes. *Dev Neuropsychol* **2011,** *36* (1), 96-117.

209. Northam, G. B.; Liégeois, F.; Chong, W. K.; Wyatt, J. S.; Baldeweg, T., Total brain white matter is a major determinant of IQ in adolescents born preterm. *Ann Neurol* **2011,** *69* (4), 702-11.

210. Lind, A.; Haataja, L.; Rautava, L.; Valiaho, A.; Lehtonen, L.; Lapinleimu, H.; Parkkola, R.; Korkman, M.; Group, P. S., Relations between brain volumes, neuropsychological assessment and parental questionnaire in prematurely born children. *Eur Child Adolesc Psychiatry* **2010,** *19* (5), 407-17.

211. Martinussen, M.; Flanders, D. W.; Fischl, B.; Busa, E.; Lohaugen, G. C.; Skranes, J.; Vangberg, T. R.; Brubakk, A. M.; Haraldseth, O.; Dale, A. M., Segmental brain volumes and cognitive and perceptual correlates in 15-year-old adolescents with low birth weight. *J Pediatr* **2009,** *155* (6), 848-853 e1.

212. Parker, J.; Mitchell, A.; Kalpakidou, A.; Walshe, M.; Jung, H. Y.; Nosarti, C.; Santosh, P.; Rifkin, L.; Wyatt, J.; Murray, R. M.; Allin, M., Cerebellar growth and behavioural & neuropsychological outcome in preterm adolescents. *Brain* **2008,** *131* (Pt 5), 1344-51.

213. Nosarti, C.; Giouroukou, E.; Healy, E.; Rifkin, L.; Walshe, M.; Reichenberg, A.; Chitnis, X.; Williams, S. C.; Murray, R. M., Grey and white matter distribution in very preterm adolescents mediates neurodevelopmental outcome. *Brain* **2008,** *131* (Pt 1), 205-17.

214. Allin, M.; Matsumoto, H.; Santhouse, A. M.; Nosarti, C.; AlAsady, M. H.; Stewart, A. L.; Rifkin, L.; Murray, R. M., Cognitive and motor function and the size of the cerebellum in adolescents born very pre-term. *Brain* **2001,** *124* (Pt 1), 60-6.

215. McNaughton, R.; Pieper, C.; Sakai, O.; Rollins, J. V.; Zhang, X.; Kennedy, D. N.; Frazier, J. A.; Douglass, L.; Heeren, T.; Fry, R. C.; O'Shea, T. M.; Kuban, K. K.; Jara, H.; Investigators, F. t. E.-E. S.; Rollins, J. V.; Shah, B.; Singh, R.; Vaidya, R.; Marter, L. V.; Martin, C.; Ware, J.; Rollins, C.; Cole, C.; Perrin, E.; Sakai, C.; Bednarek, F.; Frazier, J.; Ehrenkranz, R.; Benjamin, J.; Montgomery, A.; O'Shea, T. M.; Washburn, L.; Gogcu, S.; Bose, C.; Warner, D.; O'Shea, T. M.; Engelke, S.; Higginson, A.; Higginson, J.; Bear, K.; Poortenga, M.; Pastyrnak, S.; Karna, P.; Paneth, N.; Lenski, M.; Schreiber, M.; Hunter, S.; Msall, M.; Batton, D.; Klarr, J.; Lee, Y. A.; Obeid, R.; Christianson, K.; Klein, D.; Wagner, K.; Pimental, M.; Hallisey, C.; Coster, T.; Dolins, M.; Mittleman, M.; Haile, H.; Rohde, J.; Nylen, E.; Neger, E.; Mattern, K.; Ma, C.; Toner, D.; Vitaro, E.; Venuti, L.; Powers, B.; Foley, A.; Sacco, T.; Williams, J.; Romano, E.; Henry, C.; Hiatt, D.; Peters, N.; Brown, P.; Ansusinha, E.; Smith, J.;

Yang, N.; Bose, G.; Wereszczak, J.; Bernhardt, J.; Adams, J.; Wilson, D.; Darden-Saad, N.; Williams, B.; Jones, E.; Sutton, D.; Rathbun, J.; Fagerman, S.; Boshoven, W.; Johnson, J.; James, B.; Miras, K.; Solomon, C.; Weiland, D.; Yoon, G.; Ramoskaite, R.; Wiggins, S.; Washington, K.; Martin, R.; Prendergast, B.; Lynch, E.; Kring, B.; Smith, A.; McQuiston, S.; Butler, S.; Wilson, R.; McGhee, K.; Lee, P.; Asgarian, A.; Sadhwani, A.; Henson, B.; Keller, C.; Walkowiak, J.; Barron, S.; Miller, A.; Dessureau, B.; Wood, M.; Damon-Minow, J.; Romano, E.; Mayes, L.; Tsatsanis, K.; Chawarska, K.; Kim, S.; Dieterich, S.; Bearrs, K.; Waldrep, E.; Friedman, J.; Hounshell, G.; Allred, D.; Helms, R.; Whitley, L.; Stainback, G.; Bostic, L.; Jacobson, A.; McKeeman, J.; Meyer, E.; Price, J.; Lloyd, M.; Plesha-Troyke, S.; Scott, M.; Solomon, K. M.; Brooklier, K.; Vogt, K., Quantitative MRI Characterization of the Extremely Preterm Brain at Adolescence: Atypical versus Neurotypical Developmental Pathways. *Radiology* **2022,** *304* (2), 419-428.

216. Lemola, S.; Oser, N.; Urfer-Maurer, N.; Brand, S.; Holsboer-Trachsler, E.; Bechtel, N.; Grob, A.; Weber, P.; Datta, A. N., Effects of gestational age on brain volume and cognitive functions in generally healthy very preterm born children during school-age: A voxel-based morphometry study. *PLOS ONE* **2017,** *12* (8), e0183519.

217. Cho, H. J.; Jeong, H.; Park, C.-A.; Son, D. W.; Shim, S.-Y., Altered functional connectivity in children born very preterm at school age. *Sci Rep* **2022,** *12* (1), 7308.

218. Kvanta, H.; Bolk, J.; Strindberg, M.; Jiménez-Espinoza, C.; Broström, L.; Padilla, N.; Ådén, U., Exploring the distribution of grey and white matter brain volumes in extremely preterm children, using magnetic resonance imaging at term age and at 10 years of age. *PLOS ONE* **2021,** *16* (11), e0259717.

219. Taylor, H. G.; Filipek, P. A.; Juranek, J.; Bangert, B.; Minich, N.; Hack, M., Brain volumes in adolescents with very low birth weight: Effects on brain structure and associations with neuropsychological outcomes. *Developmental Neuropsychology* **2011,** *36*, 96-117.

220. Parikh, N. A.; Lasky, R. E.; Kennedy, K. A.; McDavid, G.; Tyson, J. E., Perinatal factors and regional brain volume abnormalities at term in a cohort of extremely low birth weight infants. *PLoS One* **2013,** *8* (5), e62804.

221. Laptook, A. R.; O' Shea, T. M.; Shankaran, S.; Bhaskar, B.; Network, a. t. N. N., Adverse Neurodevelopmental Outcomes Among Extremely Low Birth Weight Infants With a Normal Head Ultrasound: Prevalence and Antecedents. *Pediatrics* **2005,** *115* (3), 673-680.

222. Kuban, K. C. K.; Heeren, T.; O'Shea, T. M.; Joseph, R. M.; Fichorova, R. N.; Douglass, L.; Jara, H.; Frazier, J. A.; Hirtz, D.; Taylor, H. G.; Rollins, J. V.; Paneth, N., Among Children Born Extremely Preterm a Higher Level of Circulating Neurotrophins Is Associated with Lower Risk of Cognitive Impairment at School Age. *J Pediatr* **2018,** *201*, 40-48.e4.

223. O'Shea, T. M.; Allred, E. N.; Dammann, O.; Hirtz, D.; Kuban, K. C.; Paneth, N.; Leviton, A.; Investigators, E. s., The ELGAN study of the brain and related disorders in extremely low gestational age newborns. *Early Hum Dev* **2009,** *85* (11), 719-25.

224. Leviton, A.; Allred, E. N.; Yamamoto, H.; Fichorova, R. N., Relationships among the concentrations of 25 inflammation-associated proteins during the first postnatal weeks in the blood of infants born before the 28th week of gestation. *Cytokine* **2012,** *57* (1), 182-90.

225. Noth, U.; Hattingen, E.; Bahr, O.; Tichy, J.; Deichmann, R., Improved visibility of brain tumors in synthetic MP-RAGE anatomies with pure T1 weighting. *NMR Biomed* **2015,** *28* (7), 818-30.

226. Fortin, J. P.; Cullen, N.; Sheline, Y. I.; Taylor, W. D.; Aselcioglu, I.; Cook, P. A.; Adams, P.; Cooper, C.; Fava, M.; McGrath, P. J.; McInnis, M.; Phillips, M. L.; Trivedi, M. H.; Weissman, M. M.; Shinohara, R. T., Harmonization of cortical thickness measurements across scanners and sites. *Neuroimage* **2018,** *167*, 104-120.

227. Joseph, R. M.; O'Shea, T. M.; Allred, E. N.; Heeren, T.; Hirtz, D.; Paneth, N.; Leviton, A.; Kuban, K. C., Prevalence and associated features of autism spectrum disorder in extremely low gestational age newborns at age 10 years. *Autism Res* **2017,** *10* (2), 224-232.

228. Hirschberger, R. G.; Kuban, K. C. K.; O'Shea, T. M.; Joseph, R. M.; Heeren, T.; Douglass, L. M.; Stafstrom, C. E.; Jara, H.; Frazier, J. A.; Hirtz, D.; Rollins, J. V.; Paneth, N., Co-occurrence and Severity of Neurodevelopmental Burden (Cognitive Impairment, Cerebral Palsy, Autism Spectrum Disorder, and Epilepsy) at Age Ten Years in Children Born Extremely Preterm. *Pediatr Neurol* **2018,** *79*, 45-52.

229. McCrimmon, A.; Climie, E., Test Review: D. Wechsler Wechsler Individual Achievement Test-Third Edition. Aan antonio, TX: NCS Pearson, 2009. *Canadian Journal of School Psychology* **2011,** *26*, 148-156.

230. Lenroot, R. K.; Giedd, J. N., Sex differences in the adolescent brain. *Brain Cogn* **2010,** *72* (1), 46-55.

231. De Bellis, M. D.; Keshavan, M. S.; Beers, S. R.; Hall, J.; Frustaci, K.; Masalehdan, A.; Noll, J.; Boring, A. M., Sex Differences in Brain Maturation during Childhood and Adolescence. *Cerebral Cortex* **2001,** *11* (6), 552-557.

232. Giedd, J. N.; Raznahan, A.; Mills, K. L.; Lenroot, R. K., Review: magnetic resonance imaging of male/female differences in human adolescent brain anatomy. *Biology of Sex Differences* **2012,** *3* (1), 19.

233. Leonard, C. M.; Towler, S.; Welcome, S.; Halderman, L. K.; Otto, R.; Eckert, M. A.; Chiarello, C., Size matters: cerebral volume influences sex differences in neuroanatomy. *Cereb Cortex* **2008,** *18* (12), 2920-31.

234. Nosarti, C.; Al-Asady, M. H. S.; Frangou, S.; Stewart, A. L.; Rifkin, L.; Murray, R. M., Adolescents who were born very preterm have decreased brain volumes. *Brain* **2002,** *125* (7), 1616-1623.

235. de Rooij, S. R.; Caan, M. W.; Swaab, D. F.; Nederveen, A. J.; Majoie, C. B.; Schwab, M.; Painter, R. C.; Roseboom, T. J., Prenatal famine exposure has sex-specific effects on brain size. *Brain* **2016,** *139* (Pt 8), 2136-42.

236. Martin, E.; Smeester, L.; Bommarito, P. A.; Grace, M. R.; Boggess, K.; Kuban, K.; Karagas, M. R.; Marsit, C. J.; O'Shea, T. M.; Fry, R. C., Sexual epigenetic dimorphism in the human placenta: implications for susceptibility during the prenatal period. *Epigenomics* **2017,** *9* (3), 267-278.

237. Leviton, A.; Gressens, P.; Wolkenhauer, O.; Dammann, O., Systems approach to the study of brain damage in the very preterm newborn. *Front Syst Neurosci* **2015,** *9*, 58.

238. Spratt, E. G.; Granholm, A. C.; Carpenter, L. A.; Boger, H. A.; Papa, C. E.; Logan, S.; Chaudhary, H.; Boatwright, S. W.; Brady, K. T., Pilot Study and Review: Physiological Differences in BDNF, a Potential Biomarker in Males and Females with Autistic Disorder. *Int Neuropsychiatr Dis J* **2015,** *3* (1), 19-26.

239. Brumbaugh, J. E.; Hansen, N. I.; Bell, E. F.; Sridhar, A.; Carlo, W. A.; Hintz, S. R.; Vohr, B. R.; Colaizy, T. T.; Duncan, A. F.; Wyckoff, M. H.; Baack, M. L.; Rysavy, M. A.; DeMauro, S. B.; Stoll, B. J.; Das, A.; Higgins, R. D., Outcomes of Extremely Preterm Infants With Birth Weight Less Than 400 g. *JAMA Pediatr* **2019,** *173* (5), 434-445.

240. Pua, E. P. K.; Barton, S.; Williams, K.; Craig, J. M.; Seal, M. L., Individualised MRI training for paediatric neuroimaging: A child-focused approach. *Developmental Cognitive Neuroscience* **2020,** *41*, 100750.

241. Padula, A. M.; Monk, C.; Brennan, P. A.; Borders, A.; Barrett, E. S.; McEvoy, C. T.; Foss, S.; Desai, P.; Alshawabkeh, A.; Wurth, R.; Salafia, C.; Fichorova, R.; Varshavsky, J.; Kress, A.; Woodruff, T. J.; Morello-Frosch, R., A review of maternal prenatal exposures to environmental chemicals and psychosocial stressors-implications for research on perinatal outcomes in the ECHO program. *J Perinatol* **2020,** *40* (1), 10-24.

242. Mattison, D. R., Environmental exposures and development. *Curr Opin Pediatr* **2010,** *22* (2), 208-18.

243. Prasad, J. D.; Gunn, K. C.; Davidson, J. O.; Galinsky, R.; Graham, S. E.; Berry, M. J.; Bennet, L.; Gunn, A. J.; Dean, J. M., Anti-Inflammatory Therapies for Treatment of Inflammation-Related Preterm Brain Injury. *Int J Mol Sci* **2021,** *22* (8).

244. Bangma, J. T.; Kwiatkowski, E.; Psioda, M.; Santos, H. P., Jr.; Hooper, S. R.; Douglass, L.; Joseph, R. M.; Frazier, J. A.; Kuban, K. C. K.; O'Shea, T. M.; Fry, R. C., Early life antecedents of positive child health among 10-year-old children born extremely preterm. *Pediatr Res* **2019**.

245. Leviton, A.; Kuban, K.; Allred, E.; Fichorova, R. N.; O'Shea, T.; Paneth, N.; Investigators, E. S., Early postnatal blood concentrations of inflammation-related proteins and microcephaly two years later in infants born before the 28th post-menstrual week. *Early Hum. Dev* **2011,** *87* (5), 325-330.

246. Tilley, S. K.; Joseph, R. M.; Kuban, K. C. K.; Dammann, O. U.; O'Shea, T. M.; Fry, R. C., Genomic biomarkers of prenatal intrauterine inflammation in umbilical cord tissue predict later life neurological outcomes. *PLoS One* **2017,** *12* (5), e0176953.

247. Tomlinson, M. S.; Bommarito, P. A.; Martin, E. M.; Smeester, L.; Fichorova, R. N.; Onderdonk, A. B.; Kuban, K. C. K.; O'Shea, T. M.; Fry, R. C., Microorganisms in the human placenta are associated with altered CpG methylation of immune and inflammation-related genes. *PLoS One* **2017,** *12* (12), e0188664.

248. Walker, M. G.; Fitzgerald, B.; Keating, S.; Ray, J. G.; Windrim, R.; Kingdom, J. C., Sex-specific basis of severe placental dysfunction leading to extreme preterm delivery. *Placenta* **2012,** *33* (7), 568-71.

249. Tarrade, A.; Panchenko, P.; Junien, C.; Gabory, A., Placental contribution to nutritional programming of health and diseases: epigenetics and sexual dimorphism. *J Exp Biol* **2015,** *218* (Pt 1), 50-8.

250. Pilsner, J. R.; Hall, M. N.; Liu, X.; Ilievski, V.; Slavkovich, V.; Levy, D.; Factor-Litvak, P.; Yunus, M.; Rahman, M.; Graziano, J. H.; Gamble, M. V., Influence of prenatal arsenic exposure and newborn sex on global methylation of cord blood DNA. *PLoS One* **2012,** *7* (5), e37147.

251. Clifton, V. L., Review: Sex and the human placenta: mediating differential strategies of fetal growth and survival. *Placenta* **2010,** *31 Suppl*, S33-9.

252. Sen, A.; Heredia, N.; Senut, M. C.; Hess, M.; Land, S.; Qu, W.; Hollacher, K.; Dereski, M. O.; Ruden, D. M., Early life lead exposure causes gender-specific changes in the DNA methylation profile of DNA extracted from dried blood spots. *Epigenomics* **2015,** *7* (3), 379-93.

253. Heeren, T.; Joseph, R. M.; Allred, E. N.; O'Shea, T. M.; Leviton, A.; Kuban, K. C. K., Cognitive functioning at the age of 10 years among children born extremely preterm: a latent profile approach. *Pediatric Research* **2017,** *82* (4), 614-619.

254. Tomlinson, M. S.; Santos, H. P.; Stewart, J. R.; Joseph, R.; Leviton, A.; Onderdonk, A. B.; Kuban, K. C. K.; Heeren, T.; O'Shea, T. M.; Fry, R. C., Neurocognitive and social-communicative function of children born very preterm at 10 years of age: Associations with microorganisms recovered from the placenta parenchyma. *J Perinatol* **2020,** *40* (2), 306-315.

255. Ritchie, S. J.; Cox, S. R.; Shen, X.; Lombardo, M. V.; Reus, L. M.; Alloza, C.; Harris, M. A.; Alderson, H. L.; Hunter, S.; Neilson, E.; Liewald, D. C. M.; Auyeung, B.; Whalley, H. C.; Lawrie, S. M.; Gale, C. R.; Bastin, M. E.; McIntosh, A. M.; Deary, I. J., Sex Differences in the Adult Human Brain: Evidence from 5216 UK Biobank Participants. *Cerebral Cortex* **2018,** *28* (8), 2959-2975.

256. Xin, J.; Zhang, Y.; Tang, Y.; Yang, Y., Brain Differences Between Men and Women: Evidence From Deep Learning. *Frontiers in Neuroscience* **2019,** *13*.